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Cancer du foie au Cambodge : état des lieux épidémiologiques, description des médecines traditionnelles utilisées et évaluation d'espèces médicinales sélectionnées

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« Providing contextual data ! », ces quelques mots prononcés par un *reviewer* pointilleux m'ont donné du fil à retordre. Et pourtant, il est vrai que sortis de leur contexte, beaucoup de choses peuvent vouloir tout dire, et ne rien dire. Ainsi, un simple listing des personnes qui m'ont aidé, ne serait pas adéquat pour exprimer mes remerciements. Pour cela, les différents personnages qui ont contribué à donner vie à cette thèse doivent être replacés dans leur contexte.

Tout a commencé, le 28 mars 1983, une belle soirée de printemps... Non, en fait, non. On ne va pas remonter aussi loin. Sinon, je suis reparti pour trois ans...

Voici, plutôt…

C'est après 3 ans d'activité professionnelle, ou plutôt d'inactivité cérébrale que je décidais de rempiler pour mes premiers amours : la science et la nature.

A 30 ans, une femme et une fille étaient venues s'ajouter au paysage de mes rêves, et j'avais décidé de quitter une situation confortable pour me bousculer de nouveau et partir à la découverte des racines de ma famille et de la nature cambodgienne. Le but n'était pas le simple dépaysement, il était plus lointain. La thèse. Oui, la thèse ! Elle devait être réalisée. Après de nombreux échecs, je ne pouvais me résoudre à abandonner.

Tout commença lors d'une rencontre impromptue en pleine cambrousse cambodgienne. **Eric** et **Geneviève** (un grand MERCI à tous les deux de m'avoir accueilli parmi vous, de m'avoir soutenu, d'avoir tout le temps répondu à mes innombrables sollicitations, et d'avoir suggéré, facilité et amélioré ce travail de thèse) deux personnages habitués au déracinement et aux charmes de la biodiversité, prospectaient dans le coin. Moi, qui papillonnais seul au milieu de la forêt, je me faisais une joie de les rencontrer. Le contact fut rapidement établi, et les idées lancées. Malheureusement sans source de financement, rien n'était possible. Dès lors, je me mis à la recherche d'une bourse atypique pour thésard poussiéreux mais motivé. La mécanique devait pouvoir se relancer, c'est certain. Et après plusieurs mois à scruter le net sans relâche, le

miracle se produisit. La **Fondation pour la Recherche Médicale**, dont je ne pourrais jamais assez remercier **les donateurs**, m'offrit la possibilité de débuter cette nouvelle aventure.

Quelques formalités administratives plus tard, nous étions repartis, ma famille et moi, au Cambodge. Le **Professeur Sokha Eav**, chef de service de l'unité de cancérologie de l'hôpital Calmette de Phnom Penh me permit d'entamer cette thèse sous les meilleurs auspices. Grâce à lui, toutes les portes s'ouvrirent devant moi. **Le service de cancérologie**, tout d'abord. Avec des médecins presque tous francophones, compétents et sympathiques. Puis **le service d'hépato-gastroentérologie**, avec des gens humainement bons, enthousiastes et chaleureux. Je ne peux me permettre d'oublier ce **local** de $4m^2$ dans lequel le trésor se trouvait. Des dossiers à foison, poussiéreux, rongés par les rats, mangés par l'humidité, recouverts de déjections, mais qui furent d'une importance capitale.

Une fois les informations récoltées, il fallut les analyser. Et il est évident que tout seul, je n'y serais pas arrivé aussi bien. L'aide de **Pascal**, **Stéphane** et **Teresa** fut déterminante.

Un premier papier dans les rails, et pourtant l'affaire n'était pas bouclée. Je me devais d'en savoir plus sur ces patients dont je n'avais vu que les dossiers. Il fallait que je mette un visage sur ces noms, que je donne une âme à cette identité. La prochaine étape me fit rencontrer des **patients** et des **familles** pleins de vie, de courage et de combattivité. A travers l'aide de mes **interprètes** (**Chhayrong** en particulier), je compris toute la difficulté à laquelle ils étaient confrontés.

Le choc était rude pour moi, je basculais dans un monde lourd et douloureux. Il me faisait rappeler que la seule chose certaine dans la vie, c'est que tout a une fin. Les bonnes comme les mauvaises, tout s'arrête à un moment. Il fallait vite reprendre de la force pour ne pas sombrer. Grâce à une communauté exceptionnelle, je pus mettre de côté ces émotions, et retrouver un peu de vigueur. Avec **Florence**, **Philippe**, **Audrey, José**, **Eve, Guillaume et Dominique** tout d'abord. Puis avec **Simon** et **Clément** par la suite. Je n'oublierais jamais nos soirées improvisées ou organisées, nos balades et voyages singuliers, notre confiante liberté, nos quelques moments d'égarement et de confidence. Et puis, il y eut le temps des retrouvailles avec mes amis de longue date. Quelle fut ma joie de partager de grands moments à la fois décalés et entiers avec **Julien**

C., Julien T. et **Claire**. Je ne vous remercierais jamais assez d'avoir été et de continuer à être auprès de moi.

Revigoré par cette chaleur humaine, je décidais de me remettre au charbon et enfin revenir au cœur de mes passions. L'ethnobotanique est une discipline à la fois simple et complexe. Ce paradoxe s'explique par son caractère ancestral. Nous avons tous une âme d'ethnobotaniste, puisque nos aïeux nous l'ont léguée. Seulement, en tant que science, elle se complexifie au regard des nombreuses domaines dont elle se nourrit.

Une approche botanique tout d'abord : ici, je me dois de remercier tout particulièrement **Mme Hul** pour son expertise et sa gentillesse. Une approche anthropologique ensuite : les nombreuses discussions avec **Elizabeth** et **Meriem** dans le domaine m'ont beaucoup apporté. Mais tout cela ne serait rien sans la participation de personnes qui ont accepté de livrer leur secret : les **médecins traditionnels**.

Cette période fut aussi riche que la précédente. Partir chaque jour à la rencontre de personnes à la fois savantes, mystérieuses voire même troublantes, me faisait comprendre l'importance de leur sauvegarde. Et puis, il y avait tout le travail derrière d'analyse des données. Des soirées qui s'éternisaient à essayer de classer les documents, les plantes, les photos et les mots.

Une bonne partie de ces moments de réflexion s'est faite dans un endroit incontournable pour moi : **l'Institut Français du Cambodge**. Alors que les bibliothéques locales étaient peu nombreuses, mal équipées, et guère accueillantes, la France, encore une fois, était là pour me sauver. C'est à l'étranger que l'on se rend compte de la beauté de nos institutions. Nous qui avons accès à tout, tout le temps et gratuitement. J'avais ainsi à disposition des locaux neufs, confortables et parfaitement aménagés, un endroit idéal pour travailler.

C'est d'ailleurs lors d'une des innombrables conférences de qualité organisées chaque année par l'Institut, que je découvris une personne d'influence.

Erik Orsenna était venu, ce soir-là, répondre à la question qui obsèdent tous bons chercheurs : « comment trouver ? ». Son argumentation se basait sur les histoires de Christophe Colomb et Louis Pasteur. Pour lui, sept points clés permettraient d'aider à y répondre : le savoir bien évidemment, mais aussi l'interdisciplinarité, la curiosité, l'audace, la chance, l'argent, et un

entourage bienveillant. Si je peux me permettre, j'aimerais rajouter quelques considérations importantes de mon point de vue. Tout d'abord, un chercheur doit être persévérant. On ne sait jamais quand on va trouver, ni même si on va trouver. Il ne faut jamais baisser les bras et continuer à travailler. Notre tour finira par arriver. Aussi, il doit être flexible et être capable de s'adapter à de nouveaux environnements et de nouvelles thématiques. La science est en constante évolution, le chercheur doit l'être aussi. Enfin, et c'est peut-être le plus important, il doit être profondément humain. Le chercheur ne peut plus réaliser ses recherches seul dans son coin. Il doit savoir communiquer, gérer des gens et créer un climat propice à l'épanouissement de chacun. Ce n'est que comme cela, qu'il trouvera.

Cette longue période en pays Khmer fut entrecoupée de séjours dans la capitale laotienne. Là-bas, Eric y avait installé son QG, et débauché celui qui devait devenir mon futur acolyte : **Thomas**. Grâce à lui, je pus rencontrer **Olivia**, ma colocatrice préférée, qui elle-même me présenta à toute la communauté d'expatriés français, des personnages parfois hauts en couleur. Bien évidemment, ce sont les institutions qui encore une fois nous ont permis d'exister dans ce pays ; et en particulier **la faculté de pharmacie de Vientiane** et tous ces formidables pharmaciens et pharmaciennes laotiens toujours prêts à nous aider. Mais aussi l'**IRD**, ses employés et tous ses chercheurs très ouverts avec lesquels il était toujours possible d'échanger.

Plus de 2 ans s'écoulèrent avant mon retour en France. La décision avait été prise quelques mois auparavant avec **Mohamed** et **Guillaume**. Grâce à eux, je découvris l'autre versant du devoir d'ethnopharmacologue : le travail de paillasse. En peu de temps, je fus formé à des techniques diverses et variées. Et surtout, j'embrassais de nouveau la joie de faire partie d'une équipe.

Fini, le labeur solitaire à l'autre bout du monde, j'étais maintenant entouré d'une flotte de chercheurs aussi compétents qu'attachants. Par ordre alphabétique (pour ne pas faire de jaloux !) : **Anne-Cécile, Assi, Aurélien, Chio, Cynthia, Fatima, Franck, Ikram, Ingrid, Jan, Joran, Justine, Karine, Mahamane, Manon, Marieke, Monivan, Pierre, Polo, Valérie C. et Valérie J.** Je vous remercie tous pour l'affection que vous m'avez portée, et pour les nombreux rires échangés.

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Enfin, d'autres personnes ont contribué à faire avancer le travail de thèse par leurs critiques constructives. **Bruno** et **Didier**, les membres de mon comité de thèse. **Pierre-Marie Preux**, **Marie-Geneviève Dijoux-Franca, Thierry Hennebelle, Marco Leonti et Nicolas Fabre,** les membres de mon jury de thèse.

Voilà pour la chronologie des faits. Chacun, à un moment donné, a joué un rôle dans la construction de cette thèse.

A côté de toutes ces personnes, trois figures se démarquent. Tout simplement, parce qu'elles n'ont jamais cessé d'être à mes côtés. Voici mon entourage bienveillant…

Mutti, tout d'abord. Après avoir parfaitement rempli ton devoir de mère, tu as été une grand-mère exemplaire. Tu es un modèle pour de nombreux parents. C'est grâce à toi, que j'ai pu me consacrer à 100% à l'écriture de cette thèse. Je te ne le dirais jamais assez. Merci maman.

Chakrya, ensuite. Tu as été la source de ce travail et tu m'as soutenu depuis le début de cette thèse. Tu ne m'as jamais freiné dans ce projet qui paraissait fou et risqué. Tu as mis de côté tes aspirations pour me permettre de concrétiser mes rêves. Merci d'avoir donné de ta personne pour me permettre de m'épanouir.

Mathilde, enfin. Depuis le jour de ta naissance, tu as été un enfant parfait. Douce, attachante, joyeuse, généreuse, indépendante, sage. Tu es ce que tout parent rêve d'avoir, et je remercie le ciel chaque jour de t'avoir mis auprès de moi. Grâce à ta personnalité hors norme, j'ai pu réaliser cette thèse en toute quiétude. Merci, ma fille, d'être ce que tu es.

SOMMAIRE

ABRÉVIATIONS

ABTS :	Acide 2,2'-azino-bis(3-éthylbenzothiazoline-6-sulphonique)
À d. :	A droite
À g. :	À gauche
AFP :	Alpha-fœtoprotéine
AgHbs :	Antigène Hbs
AMM :	Autorisation de Mise sur le Marché
ANG :	Anglais
Anti-VHC :	Anticorps anti-virus de l'hépatite C
AP :	Alcaloïdes pyrrolizidinique
BCLC :	Barcelona Clinic Liver Cancer
CHC :	Carcinome hépatocellulaire
CNMT :	Centre National de Médecine Traditionnelle
CT:	Tomodensitométrie (Computerized Tomography)
DPPH :	1,1-diphenyl-2-picrylhydrazyl
FR :	Français
HEP :	Hépatocyte
HSC :	Cellules stellaires hépatiques (Hepatic Stellate Cell)
KH :	Khmer
Mϕ :	Macrophage
MDCT :	Tomodensitomètre multidétecteur (Multidetector CT)
MRI :	Image par Résonance Magnétique (Magnetic Resonance Imaging)
MT :	Médecine Traditionnelle
NA :	Non Applicable
ND :	Non Documenté
NECHR :	Comité National d'Éthique pour la Recherche en Santé Publique (National Ethics Committee for Health Research)
RFA :	Ablation par radiofréquence (Radiofrequency Ablation)
US :	Ultrasonographie (Ultrasound)
TACE :	Chimioembolisation transartérielle (Transarterial Chemoembolization)
VEGF :	Facteur de croissance de l'endothélium vasculaire (Vascular Endothelial Growth Factor)
VHB :	Virus de l'hépatite B
VHC :	Virus de l'hépatite C

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RÉSUMÉ GRAPHIQUE

RÉSUMÉ

Le cancer du foie est le 6ème cancer le plus fréquent et le 2ème plus meurtrier dans le monde. Au Cambodge, en raison du contexte historique et économique, les données précises concernant cette pathologie manquent. A l'aide d'outils épidémiologiques, nous avons décrit les caractéristiques de 553 patients atteints de cancer du foie à l'hôpital Calmette à Phnom Penh, et ainsi mis en évidence l'importance de l'infection par les virus des hépatites B et C chez les sujets étudiés. Puis, nous avons documenté les connaissances de 42 de ces patients vis-à-vis de leur maladie. Nous avons détaillé leurs itinéraires thérapeutiques, mis en évidences des pratiques à risques (forte utilisation d'injections thérapeutiques et de techniques de dermabrasion), et le recours fréquent à des médecines dites traditionnelles. Nous avons ensuite tenté de comprendre les stratégies de prise en charge des patients souffrant de maladies hépatiques par les médecins traditionnels, et mis en évidence la variété des remèdes utilisés et l'importance de la perception khmère des propriétés des plantes. Enfin, à l'aide d'un modèle *in vitro* de culture de cellules cancéreuses hépatiques couplé à des outils d'analyse métabolomique, nous avons évalué 10 espèces médicinales, sélectionnées sur des critères bibliographiques et de terrain, et tenté d'identifier les composés potentiellement responsables de l'activité antiproliférative observée.

Mots-clés : activité antiproliférative ; Cambodge ; carcinome hépatocellulaire ; connaissance, attitude, pratique ; dossiers médicaux ; ethnopharmacologie ; hepG2 ; hépatite B ; hépatite C ; médecine traditionnelle ; métabolomique ; phytochimie ; plantes médicinales ; tradipraticiens.

INTRODUCTION GENERALE

1. LE CANCER DU FOIE : UN ENJEU DE SANTE PUBLIQUE

1.1. Les différents types de cancer du foie

La classification internationale des maladies (ICD, International Classification of Diseases) publiée par l'Organisation Mondiale de la Santé (OMS) distingue les différents types de tumeurs malignes du foie sur des critères histologiques[1] :

- Le carcinome hépatocellulaire (CHC) (C22.0) touchant les hépatocytes
- Le cholangiocarcinome (CCA) (C22.1) touchant les cellules des canaux biliaires intra-hépatiques
- L'hépatoblastome (C22.2) touchant des cellules embryonnaires du foie
- L'angiosarcome du foie (C22.3) touchant des cellules des vaisseaux hépatiques
- Les autres sarcomes du foie (C22.4)
- Les autres carcinomes du foie précisés (C22.7)
- Les tumeurs du foie, sans précision (C22.9)
- Les tumeurs malignes secondaires (métastases) ont été classées dans une catégorie à part (C78.7).

Dans la grande majorité des cas de cancers primitifs du foie, la forme histologique prédominante est le carcinome hépatocellulaire (env. 85% des cas), et le cholangiocarcinome (env. 15% des cas). Les autres cas de tumeurs malignes hépatiques sont considérés comme rares (Amon et al. 2005).

[1] Informations extraites du site internet: http://apps.who.int/classifications/icd10/browse/2014/en

Cependant, le nombre de cas de cholangiocarcinome varie grandement à l'échelle régionale. Ainsi, il peut représenter de 5 à 90% des cas de cancers primitifs du foie dans certaines régions du monde. Parmi celles-ci, la région de Pusan (Corée du Sud) et le district de Khon Kaen (Thaïlande) sont particulièrement touchées avec des taux respectifs de 20 et 90% (Shin et al. 2010).

1.2. Épidémiologie

Le cancer du foie est le 6ème cancer le plus fréquent et la 2ème cause de décès par cancer dans le monde (Ferlay et al. 2015). En 2012, les estimations portaient à 782 000 le nombre de nouveaux cas et 745 000 le nombre de morts causés par le cancer du foie à l'échelon mondial (Stewart and Wild 2014) (Figure 1 et 2).

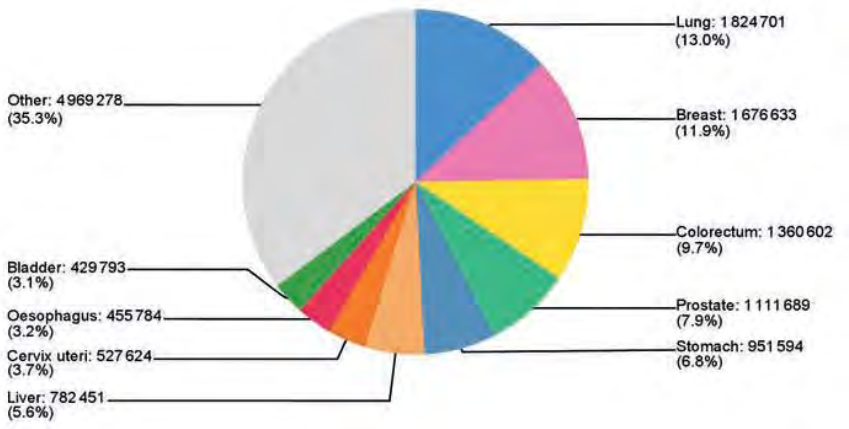

Figure 1 : Estimation en 2012 du nombre de cas de cancer, tous âges et sexes confondus à l'échelon mondial (extrait de Stewart and Wild, 2014)

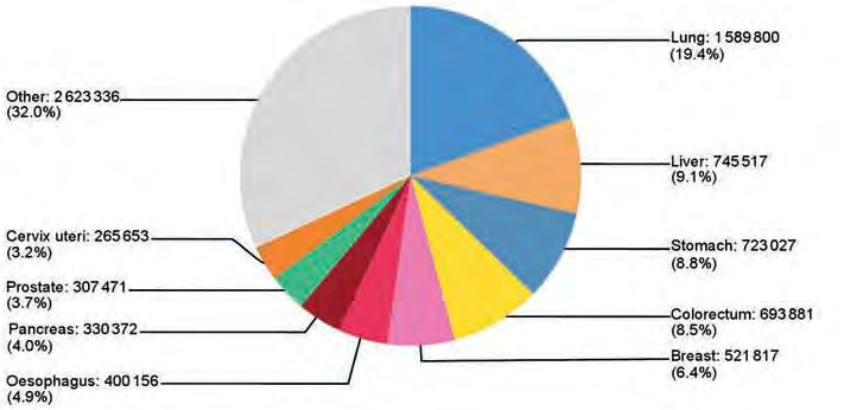

Figure 2 : Estimation en 2012 du nombre de décès par cancer, tous âges et sexes confondus à l'échelon mondial (extrait de Stewart and Wild, 2014)

Les hommes représentent la population la plus touchée par le cancer du foie, puisqu'ils sont 2 à 4 fois plus atteints par la maladie que les femmes (Bosch et al. 2004). A titre de comparaison, en 2012, 70.9% des nouveaux cas de cancer du foie et 69.9% des cas de décès par cancer du foie dans le monde touchaient le sexe masculin (Torre et al. 2015).

Le cancer du foie est principalement rencontré dans les pays du Sud, et plus particulièrement dans trois régions (Figure 3) :

- Asie de l'Est (Chine, Corée du Nord, Corée du Sud, Japon, Taïwan)
- Asie du Sud-Est (Birmanie, le Brunei, le Cambodge, l'Indonésie, le Laos, la Malaisie, les Philippines, Singapour, la Thaïlande, le Timor Oriental, et le Vietnam)
- Afrique subsaharienne (regroupe 48 pays répartis sur le continent africain au sud du sahara)

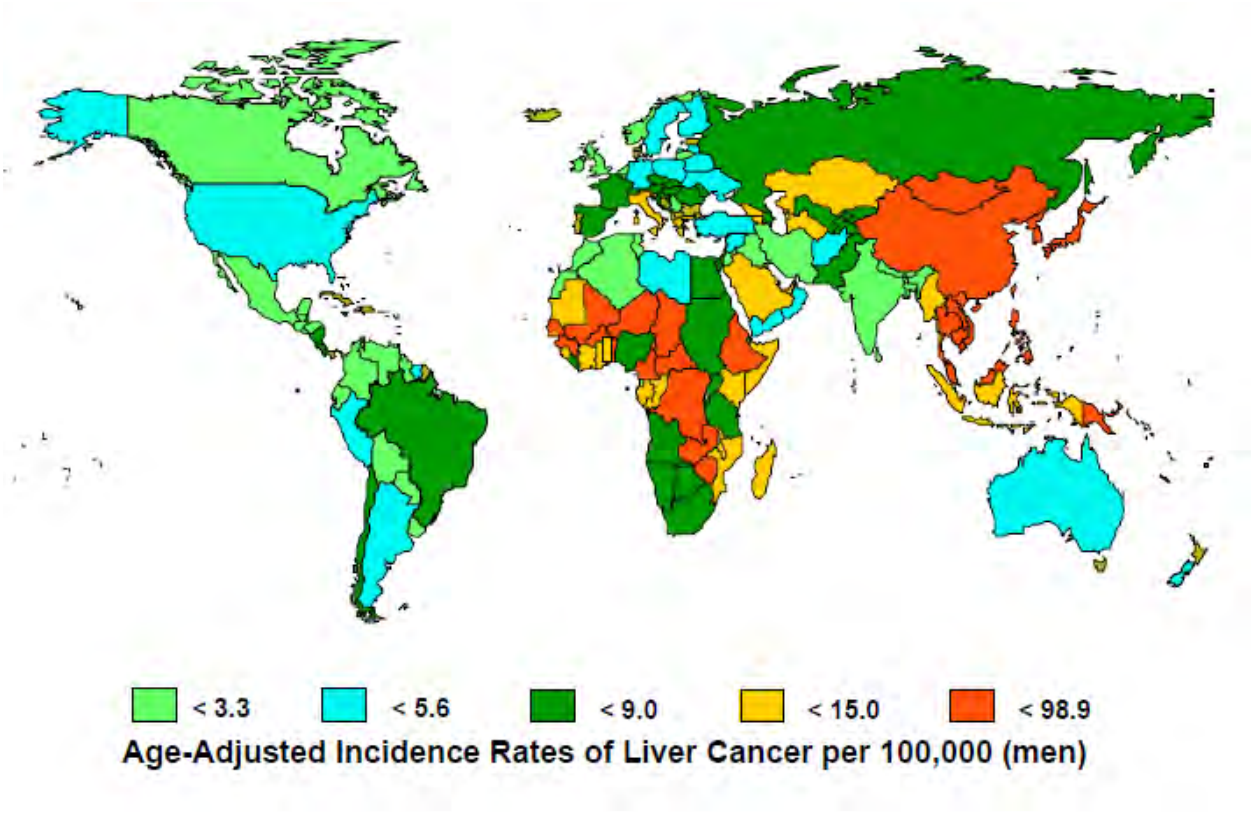

Figure 3 : Taux d'incidence ajusté à l'âge de cas de cancer du foie chez les hommes. Estimation de l'année 2000 (extrait de Bosch et al., 2004)

Près de 50% des nouveaux cas déclarés de cancer du foie dans le monde se trouvent en Chine. Alors que l'Asie du Sud-Est arrive seconde en terme d'incidence avec 29.4% des cas de cancer du foie (22.2% pour les hommes et 7.2% pour les femmes) (Torre et al. 2015).

La partie continentale de l'Asie du Sud-Est (Birmanie, Cambodge, Laos, Thaïlande, et Vietnam) est particulièrement exposée aux problèmes du cancer du foie (Moore et al. 2010). En 2008, le cancer du foie représentait le 2ème cancer le plus fréquent et la 2ème cause de décès par cancer dans cette région. Le Cambodge, le Laos, la Thaïlande et le Vietnam ont les taux d'incidence et de mortalité les plus élevés des pays d'Asie du Sud-Est (Kimman et al. 2012). Malheureusement, pour deux de ces quatre pays (Cambodge et Laos), aucune donnée nationale

n'étant disponible, les chiffres reportés sont basés sur une estimation globale des données des pays voisins[2].

Selon Globocan[2], le cancer du foie au Cambodge représente la première cause de décès par cancer tous sexes confondus, et le premier cancer le plus fréquent et le plus mortel chez les hommes. Au Laos, le cancer du foie serait le premier cancer le plus fréquent et le plus mortel toutes catégories de sexe et d'âge confondues.

1.3. Principaux facteurs de risque

A l'échelle mondiale, l'infection chronique par les virus de l'hépatite B et le virus de l'hépatite C représentent les facteurs de risques prédominants du carcinome hépatocellulaire, alors que l'infection par les parasites de la famille des Opisthorchiidae (*Opisthorchis viverrini* et *Clonorchis sinensis*) sont les facteurs de risque majoritaire du cholangiocarcinome. Au total, près de 80% des cancers du foie dans le monde sont causés par l'un de ces quatre agents infectieux (de Martel et al. 2012; Srivatanakul, Sriplung, and Deerasamee 2004).

D'autres facteurs de risque sont aussi responsables de l'apparition du cancer du foie, tel que l'alcoolisme chronique, les aflatoxines, les composés N-nitrosés, l'obésité, le diabète, les pesticides, le thorotrast (produit de contraste utilisé en radiodiagnostic), la cigarette, les métaux lourds, les contraceptifs oraux, et certaines maladies hépatiques : cirrhose, stéatohépatite non alcoolique, hépatite auto-immune, cholangite sclérosante primitive, lithiase hépatique, maladie de Wilson, etc. (Hamed and Ali 2013; Shin et al. 2010).

[2] Informations extraites du site internet : http://globocan.iarc.fr

1.3.1. Virus de l'hépatite B et C

En Asie du Sud-Est, près de 70% des cas de carcinomes hépatocellulaire seraient attribuables aux infections chroniques par le virus de l'hépatite B et/ou de l'hépatite C. Plus précisément, la part des carcinomes hépatocellulaires causée par le virus de l'hépatite B est environ 1,5 fois plus importante que celle causée par le virus de l'hépatite C (Kimman et al. 2012; Perz et al. 2006). Cela peut s'expliquer par la forte prévalence du virus de l'hépatite B (AgHbs = 8-12%) et une prévalence plus modérée du virus de l'hépatite C dans la région (Anti-VHC = 2-3%) (Mohd Hanafiah et al. 2013; Sievert et al. 2011; Xeuatvongsa et al. 2014).

La transmission du virus de l'hépatite B fait intervenir différentes voies, dont la transmission périnatale (mère à enfant) et la transmission horizontale chez les jeunes enfants (exposition à du sang infecté) qui représentent les modes de transmission prédominants dans les pays à forte endémicité (Gust 1996). Par ailleurs, d'autres modes de transmission sont aussi possibles tels que : les pratiques à risque d'injections, les rapports sexuels, les actes médicaux, chirurgicaux ou dentaires, les tatouages, l'utilisation de rasoir ou d'objets similaires contaminés par du sang (Custer et al. 2004).

Les modes de transmission du virus de l'hépatite C les plus fréquents sont l'injection de drogue par voie intraveineuse, toutes les pratiques à risque d'injections (réutilisation et mauvaise stérilisation du matériel d'injection), l'utilisation de matériel médical mal stérilisé, les transfusions de sang et les pratiques de tatouage. Dans une moindre mesure, le virus de l'hépatite C peut également se transmettre de la mère à l'enfant, lors de rapports sexuels, lors de rites de scarification, par la circoncision, l'acupuncture, le piercing, et lors de l'application de ventouse (Shepard, Finelli, and Alter 2005; Sievert et al. 2011).

1.3.2. *Opisthorchis viverrini*

Deux types de parasites (*Clonorchis sinensis* et *Opisthorchis viverrini*) sont responsables de l'apparition du cholangiocarcinome en Asie. Alors que *Clonorchis sinensis* est endémique à la Chine du sud, la Corée et le Vietnam du nord, *Opisthorchis viverrini* est endémique au Cambodge,

au Laos, à la Thaïlande et à la région centrale du Vietnam (Sithithaworn et al. 2012; Sripa et al. 2007). Selon le Centre International de Recherche contre le Cancer (CIRC), 6 millions de personnes sont infectées par ce parasite en Thaïlande, 2 millions au Laos et 600 000 au Cambodge (Figure 4) (IARC Working Group 2012). Ainsi, la région la plus touchée par l'infection à *Opisthorchis viverrini* est le Nord-Est de la Thaïlande et en particulier la région de Khon Kaen, où de nombreuses études épidémiologiques ont permis de mettre en évidence le lien entre l'infection à ce parasite et la survenue du cholangiocarcinome (Sripa et al. 2011).

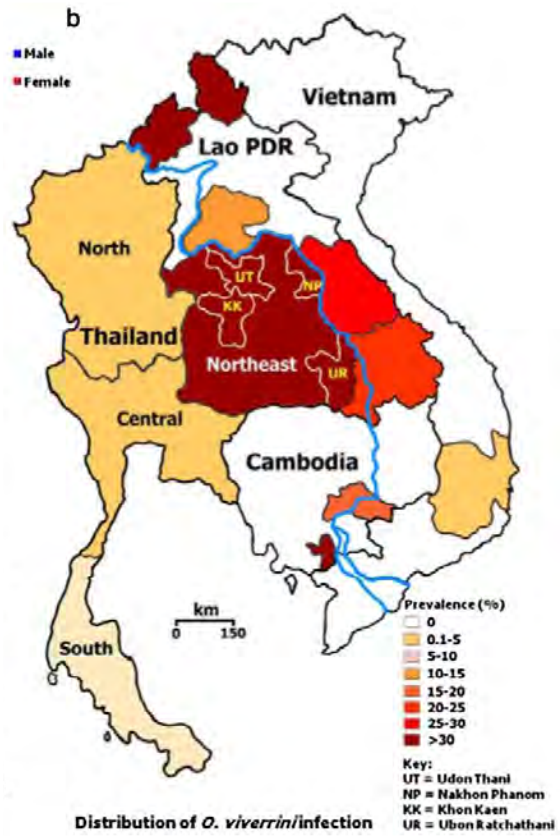

Figure 4 : Taux de prevalence de l'infection à *Opisthorchis viverrini* en Asie du Sud-Est (Sithithaworn et al. 2014)

Le mode de contamination par le parasite *Opisthorchis viverrini* se fait par l'ingestion de poissons crus de la famille des Cyprinidae, vivant en eau douce. Le cycle biologique du parasite comporte plusieurs étapes : (1) les œufs du parasite sont rejetés par l'homme dans ses excréments, (2) ils sont ensuite ingérés par un gastéropode du genre *Bythinia* sp. qui permet au parasite de passer sous forme larvaire , (3) ces cercaires sont relargués dans l'eau par le

mollusque, puis pénètrent et s'enkystent dans les muscles des poissons pour devenir des métacercaires, (4) lorsque le poisson est ingéré par l'homme, ces métacercaires prennent leur forme adulte et s'établissent dans les voies biliaires (Sripa et al. 2011).

Les habitudes alimentaires des populations locales, avec notamment la préparation de plats à base de poisson cru appelé « *koi-pla* » en Thailande et au Laos, ou « *pra-hok* » au Cambodge, sont la cause principale de la contamination humaine par ce parasite (Grundy-Warr et al. 2012; Sripa et al. 2007).

Ces facteurs de risque (VHB, VHB, *Opisthorchis viverrini*) jouant un rôle majeur dans l'apparition des cancers du foie en Asie, la lutte contre ces agents infectieux constitue un élément essentiel dans la prévention de la maladie. Ainsi, la vaccination contre l'hépatite B, les campagnes antiparasitaires et les programmes d'éducation sur les pratiques à risque sont à la base des recommandations internationales (Shin et al. 2010; Srivatanakul, Sriplung, and Deerasamee 2004).

1.4. Pathogénèse du cancer du foie

L'hépatocarcinogenèse est un processus multifactoriel et séquentiel qui fait intervenir différents mécanismes selon le type de facteur de risque impliqué (VHB, VHC, aflatoxine, alcool) (Figure 5). Ainsi, le virus de l'hépatite B induit le développement du carcinome hépatocellulaire par l'intermédiaire de voies directes et indirectes, puisqu'il est capable d'intégrer le génome de la cellule hôte et donc d'agir sur la croissance et la signalisation des cellules. Par contre, le virus de l'hépatite C n'intègre pas le génome de la cellule hôte et agit donc principalement d'une manière indirecte, en induisant une inflammation chronique, ainsi qu'une prolifération et une mort cellulaire (But, Lai, and Yuen 2008).

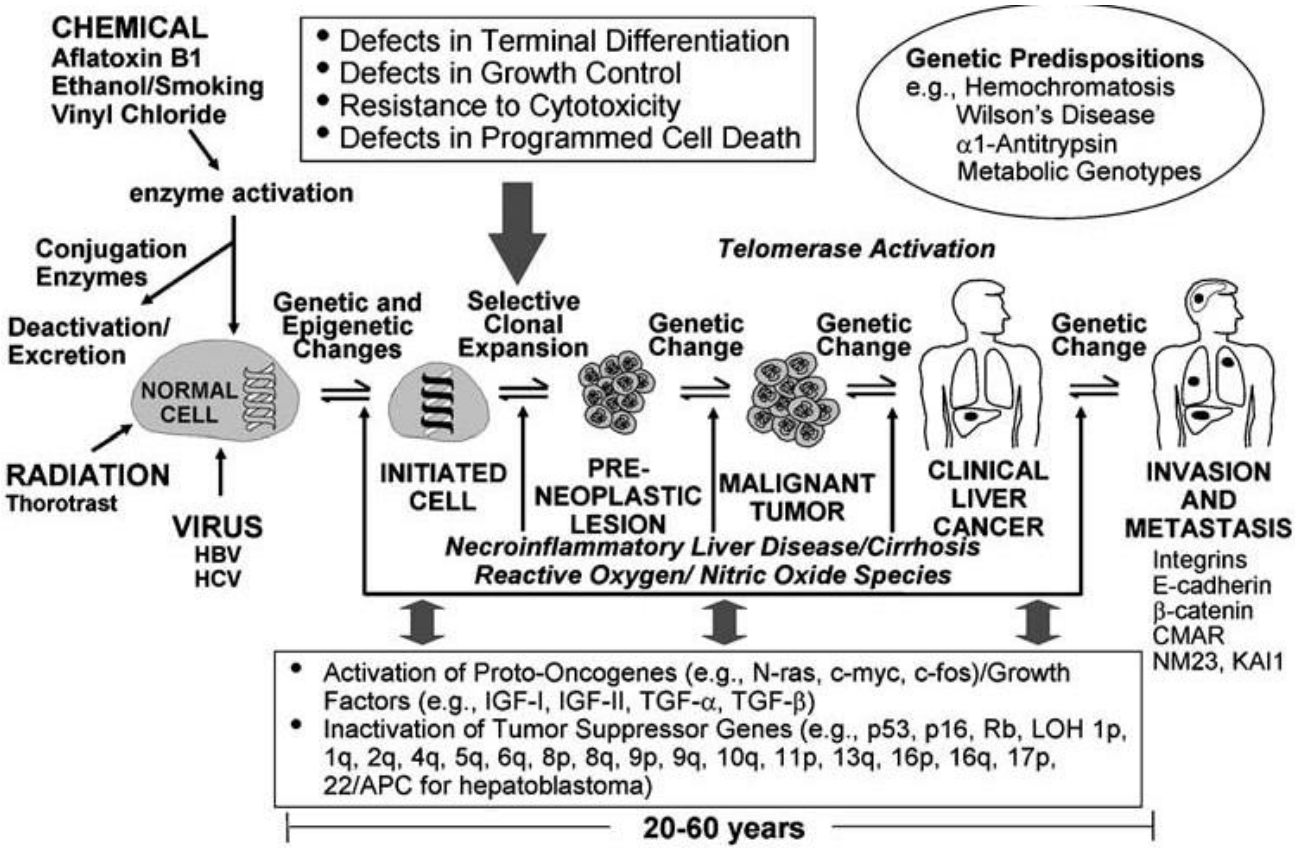

Figure 5 : Les différentes étapes de l'hépatocarcinogenèse (extrait de (Hussain et al. 2007)

Certaines voies et processus en cause dans la pathogénèse du cancer du foie sont communs aux divers facteurs de risque (Farazi and DePinho 2006). On peut citer notamment l'inactivation du gène p53, faisant partie de la famille des gènes suppresseurs de tumeur (Hussain et al. 2007), ainsi que l'inflammation chronique, le stress oxydatif et les cycles continus de nécrose et de régénération (Bartsch and Nair 2006; Farazi and DePinho 2006).

L'ensemble contribue à l'apparition de foyers d'hépatocytes dysplasiques, devenant ensuite des nodules identifiables à l'imagerie, qui peuvent eux-mêmes dégénérer en carcinome hépatocellulaire si les altérations génétiques sont suffisamment importantes pour entrainer la transformation et l'immortalisation de ces cellules hépatiques (Farazi and DePinho 2006).

Dans le cas du cholangiocarcinome, moins de données sont disponibles sur la pathogénèse de la maladie. Cependant, des facteurs communs à la genèse du carcinome hépatocellulaire ont été mis en évidence, tel que l'importance de l'inflammation (Palmer and Patel 2012). La cholangiocarcinogenèse, induite par l'infection avec le parasite *Opisthorchis viverrini*, repose sur ce processus inflammatoire chronique dont on peut distinguer trois éléments importants : les dommages mécaniques causés par l'activité de succion du parasite, les molécules secrétées par le parasite, et un processus immunopathologique spécifique (Sripa et al. 2007).

1.5. Dépistage et diagnostic

Dans la majorité des cas, les patients souffrant d'un CHC ne présentent pas de signes cliniques de la maladie. L'identification des populations à risque de développer la maladie et leur dépistage est donc d'une grande importance dans la prise en charge de la pathologie (Yu et al. 1997).

D'après (El-Serag et al. 2008), le dépistage du CHC est recommandé chez :

- les patients porteurs d'une infection chronique au virus de l'hépatite B et :
  -de race asiatique et d'un âge supérieur à 40ans
  -de race africaine et d'un âge supérieur à 20 ans
  -ayant une cirrhose avérée
  -ayant une histoire familiale de CHC.
- les patients atteints d'infection chronique à l'hépatite C, de cirrhose alcoolique, d'hémochromatose génétique, de cirrhose biliaire primitive, de déficit en alpha1 antitrypsine, de stéatose hépatique non alcoolique, ou d'hépatite auto-immune.

Les tests de dépistage les plus utilisés sont l'image échographique par la technique d'ultrasonographie (US) pour la recherche de nodules et la mesure du taux sérique d'alpha-fœtoprotéine (AFP). Cette dernière méthode étant peu spécifique et peu sensible, une fréquence

de surveillance tous les 6 mois par la technique des ultrasons est donc recommandée (Collier and Sherman 1998; European Association for Study of Liver and European Organisation for Research and Treatment of Cancer 2012).

Le diagnostic du CHC est principalement basé sur les techniques d'imagerie (tomodensitométrie [ou CT scan] et imagerie par résonance magnétique [IRM]) et la biopsie hépatique (Bruix and Sherman 2011). Les techniques d'imagerie médicale permettent de détecter la présence de nodules hépatiques et de déterminer leur taille. En fonction de ces résultats, d'autres méthodes de diagnostic sont à employer afin de confirmer ou non la présence d'un CHC. Selon les auteurs, la taille de nodule à considérer dans les algorithmes de décision varie de 1 à 2 cm (Bruix, Reig, and Sherman 2016; Bruix and Sherman 2011; El-Serag et al. 2008). Dans l'exemple d'algorithme donné (Figure 6), la détection d'un nodule inférieur à 1 cm doit être suivi par la réalisation d'échographie à intervalle régulier, alors que la détection d'un nodule de taille supérieur à 1 cm nécessite la réalisation d'examens complémentaires afin de confirmer le CHC.

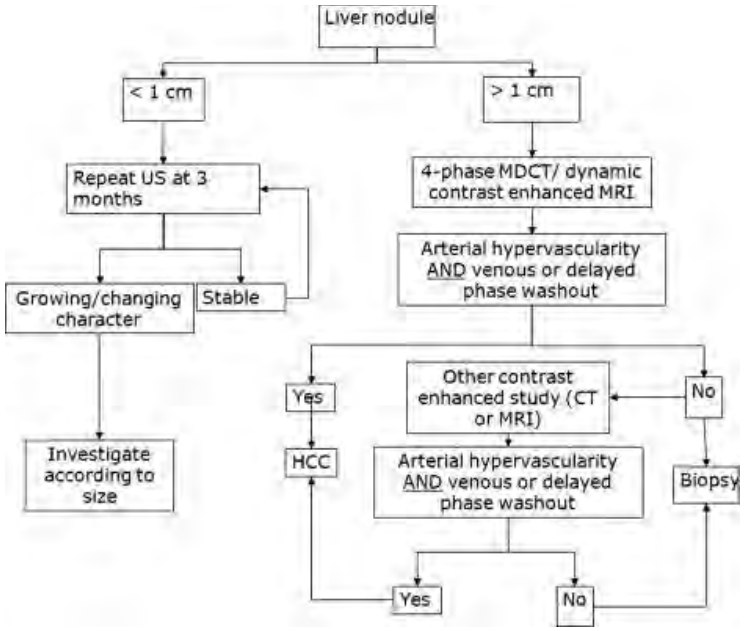

Figure 6 : Algorithme de diagnostic du CHC (extrait de (Bruix and Sherman 2011). (CT= Tomodensitométrie, MDCT= Tomodensitomètre multidétecteur, MRI= Image par Résonance Magnétique, US= Ultrasonographie)

De même que dans le cas du carcinome hépatocellulaire, le diagnostic du cholangiocarcinome se base principalement sur les techniques d'imagerie (tomodensitométrie [CT scan], imagerie par résonance magnétique [IRM] ou cholangiographie par résonance magnétique [Bili-IRM]). Bien que n'ayant pas prouvé son efficacité, le dépistage peut se réaliser chez les patients atteints de cholangite sclérosante primitive en recherchant la présence de nodules par des examens d'imagerie et en dosant les marqueurs tumoraux (CA 19-9, CA-125) (Khan et al. 2012).

Malheureusement, dans les pays en voie de développement et en particulier au Cambodge et au Laos, le dépistage est quasi-inexistant et le diagnostic se fait à des stades déjà avancés de la maladie (Eav et al. 2012).

1.6. Traitements

1.6.1. Traitement du carcinome hépatocellulaire

Différents types de traitements existent pour prendre en charge le patient atteint de carcinome hépatocellulaire. Le type de traitement à utiliser va dépendre de l'état du foie non tumoral, de la taille et de l'extension de la tumeur, et de l'état général du patient. L'âge et l'espérance de vie attendue suite au traitement doivent également être pris en compte dans la décision thérapeutique.

La classification BCLC (Barcelona Clinic Liver Cancer) sert de référence internationale pour aider les praticiens à décider du type de traitement à adopter en fonction de la présentation clinique du cancer du foie (Figure 7). Cependant, cette classification est remise en cause dans son application « sur le terrain » par plusieurs chirurgiens en Europe, notamment en Italie, et en Asie, en Chine et en Corée. Une étude récente menée au Pérou suggère que cet arbre décisionnel pourrait ne pas être adapté à toutes les populations de patients atteintes de cancer du foie,

notamment celle dans les pays en voie de développement, et devrait être contextualisée en fonction des observations cliniques faites parmi les populations (Ruiz et al. 2016).

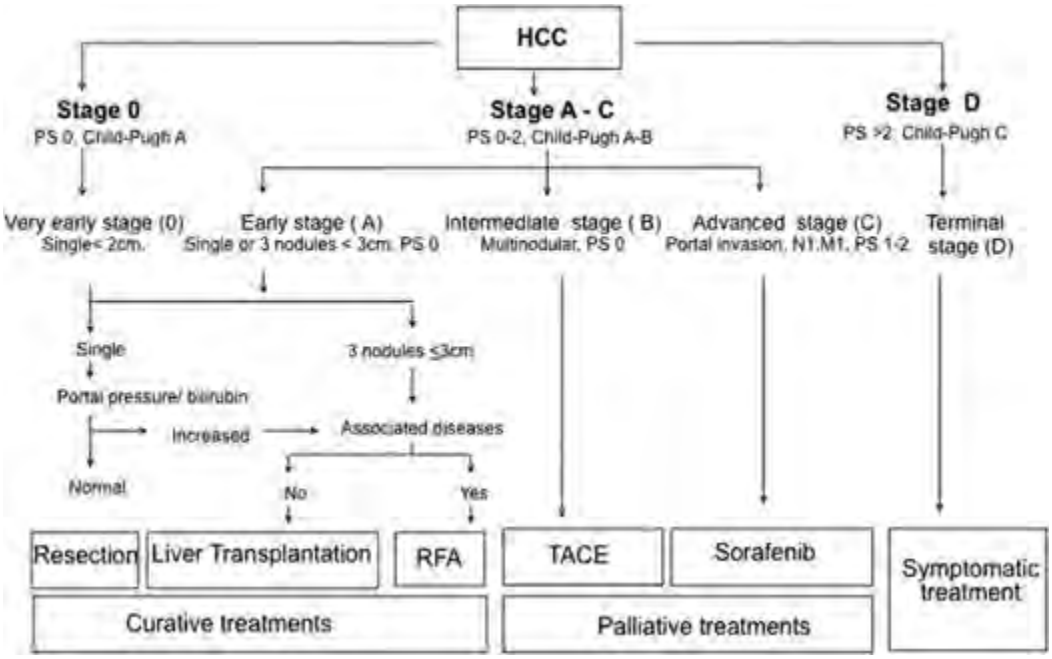

Figure 7 : Arbre décisionnel BCLC (extrait de (Bruix and Sherman 2011). (Child-Pugh= classification de la sévérité de la cirrhose, M= classification des métastases à distance du foyer cancéreux, N= classification des métastases ganglionnaires, PS= Performance Status [classification de l'état physique du patient cancéreux], RFA= Ablation par Radiofréquence, TACE= Chimioembolisation transartérielle)

Les méthodes de traitement à visée curative comprennent la transplantation hépatique, la résection hépatique (ou hépatectomie), et la destruction percutanée. Elles sont principalement indiquées pour les malades atteints de tumeur à taille limitée (Befeler and Bisceglie 2002).

La transplantation hépatique constitue la méthode de choix dans le traitement du carcinome hépatocellulaire, car elle agit sur la tumeur et sa cause. Malheureusement, la pénurie en greffons hépatiques impose une sélection stricte des patients pouvant y accéder.

La résection hépatique consiste à enlever chirurgicalement la portion de foie cancéreuse. Elle constitue le traitement de référence des patients sans cirrhose et sans foyers métastasiques.

La destruction percutanée, méthode simple et bien tolérée, est une alternative à la chirurgie. Elle consiste à introduire une aiguille dans la tumeur et à y appliquer un courant

électrique interne (par radiofréquence) afin de permettre sa nécrose thermique (Befeler and Bisceglie 2002).

Les méthodes de traitement à visée palliative comprennent la chimio-embolisation artérielle et les traitements médicamenteux (traitements symptomatiques, thérapies ciblées). Elles sont principalement indiquées pour les patients atteints d'un carcinome hépatocellulaire à un stade intermédiaire ou avancé.

La chimio-embolisation artérielle associe une chimiothérapie intra-artérielle (anthracycline ou sel de platine) administrée dans les artères nourricières du processus tumoral, et une embolisation permettant d'obstruer temporairement l'artère hépatique. Il en résulte une concentration du médicament anti-cancéreux dans la tumeur et une diminution du risque d'effets indésirables systémiques. C'est la méthode qui est retenue pour les patients atteints d'un carcinome hépatocellulaire à un stade intermédiaire et pour lesquels un traitement chirurgical ou par destruction percutané ne peut être proposé (Befeler and Bisceglie 2002; El-Serag et al. 2008).

Parmi les traitements médicamenteux, la chimiothérapie par voie systémique, les anti-androgènes, le tamoxifène, l'octréotide et l'interféron n'ont pas démontré leur efficacité dans le traitement du carcinome hépatocellulaire (Bruix and Llovet 2002). Jusqu'en 2007, aucun traitement médicamenteux spécifique n'était donc recommandé pour ces patients avec un CHC avancé. Le sorafenib (Nexavar®) inhibiteur de protéines-kinases, a été le premier à ouvrir la voie des thérapies ciblées. Son double mécanisme d'action, inhibant à la fois la prolifération et l'angiogénése tumorale, lui a permis d'obtenir une AMM en 2007 pour les patients atteints d'un carcinome hépatocellulaire avancé, avec fonction hépatique conservée (stade Child Pugh A), non éligibles à un traitement chirurgical ou locorégional, ou en échec à l'un de ces traitements (Cheng et al. 2009; Llovet et al. 2008). Toutefois, la survie globale des patients traités reste limitée (9,2 mois), et son coût important (env. 3500 euros par mois) en fait un médicament difficilement accessible aux populations des pays en voie de développement (Ferenci et al. 2010).

1.6.2. Traitement du cholangiocarcinome

Les patients atteints de cholangiocarcinome ont un pronostic défavorable, puisque le taux de survie de ces patients à cinq ans est d'environ 5 à 10% (Anderson et al. 2004).

De plus, les méthodes de traitements sont limitées et la chirurgie représente le seul traitement efficace de la maladie (Khan et al. 2012).

Parmi les thérapies curatives, la résection chirurgicale complète de la tumeur permet d'obtenir des taux de survie à 5 ans de 8 à 44%. L'autre méthode possible est une transplantation hépatique qui reste néanmoins une pratique controversée, et est réservée aux patients ayant un cholangiocarcinome à un stade débutant (Anderson et al. 2004).

Les méthodes de traitements palliatives comprennent la radiothérapie, la chimiothérapie, et la pose d'endoprothèse métallique (stent) afin d'effectuer un drainage biliaire. Parmi les chimiothérapies utilisées, le 5-Fluorouracile (5-FU) et la gemcitabine sont les deux médicaments anti-cancéreux les plus utilisés. Cependant, ces méthodes n'ont pas prouvé leur effet dans l'amélioration de la survie des patients atteints de cholangiocarcinome (Anderson et al. 2004; Blechacz and Gores 2008).

2. LES DIFFERENTS SYSTEMES MEDICAUX AU CAMBODGE

2.1. Présentation de la population cambodgienne et des systèmes médicaux

Figure 8 : Carte du Cambodge (extrait du site ezilon.com)

Le royaume du Cambodge est un pays de la péninsule indochinoise, couvrant une superficie de 181 000 km^2. Située entre les latitudes 10° et 15°N, la nation partage ses frontières avec le Laos (au nord), le Vietnam (au sud et à l'est), et la Thaïlande (à l'ouest). Ce pays au climat tropical est influencé par les vents de mousson et possède une saison pluvieuse marquée. On y distingue trois grandes régions géographiques (Legris and Blasco 1972):

- le centre du pays constitué de plaines où coule le lac Tonlé Sap et le fleuve du Mékong
- le sud-ouest dominé par la chaîne des Cardamones
- le nord du pays où de grandes étendues planes parsemées de collines constituent l'essentiel du paysage

D'une population estimée à 15 578 000 habitants[3], le Cambodge compte une large majorité de Khmers (plus de 90% de la population totale), auquel s'ajoutent des populations immigrées de pays voisins (Vietnamiens, Chinois), un groupe ethnique d'origine malayo-polynésienne (Chams[4]) et des communautés indigènes (Moul and Seng 2012). Le nombre de groupes indigènes vivant sur le territoire cambodgien varient, selon les auteurs, de 17 à 27 et comptent au total près de 150 000 personnes (Asian Development Bank 1999; Bourdier 2009).

Alors que les Khmers, les Chams, les Vietnamiens et les Chinois se rencontrent principalement dans les plaines du centre du pays et sur les berges du Mékong, la majorité des peuples indigènes (95%) se répartissent sur quatre provinces (Figure 8) situées dans les paysages vallonés du nord-est du Cambodge (Kratie, Mondulkiri, Ratanakiri et Stung Treng) (Moul and Seng 2012). Considérés comme les premiers occupants du pays, ou plus généralement de la région (les Jarai et les Tampuan ayant migré sur le territoire cambodgien à partir du Vietnam), ils appartiennent au groupe des « proto-indochinois » (Condominas and Haudricourt 1952). Traditionnellement, ils pratiquent l'essartage, la chasse, la pêche et la cueillette et s'adonnent à une forme de religion reposant sur le culte des génies (Bourdier 2009).

[3] Source OMS (2015) : http://www.who.int/countries/khm/fr/

[4] Les Chams forment un groupe distinct des communautés indigènes de par leur histoire et leur religion. Le royaume de Champa dominait le sud du Vietnam entre les II^{ème} et XVII^{ème} siècle. Les Chams étaient de culture hindouiste puis se sont convertis à l'Islam à partir du X^{ème} siècle.

La civilisation khmère a fait son apparition au VI^{ème} siècle de notre ère avec l'avènement de l'empire des Chen-La, anciennement vassal du royaume indianisé Fou-Nan. Au IXème siècle, le roi Jayavarman II inaugure la période dite angkorienne de la royauté khmère. C'est le début de l'âge d'or de la civilisation khmère qui durera jusqu'au XIII^{ème} siècle, avant de tomber dans un long déclin résultant de conflits internes et d'agressions extérieures (notamment siamoise). Au cours de leur histoire, les Khmers ont subi diverses influences. Tout d'abord, indienne avec notamment l'apparition de l'hindouisme dès le VI^{ème} siècle puis du bouddhisme au cours du XIII^{ème} siècle. Ensuite chinoise avec la migration de chinois sur le territoire cambodgien par vagues successives et régulières au cours des siècles. Et enfin, française avec la signature d'un traité de protectorat à la fin du 19ème et jusqu'au 20ème siècle (Guillou 2001).

En 2012, le Ministère de la Santé du Cambodge estimait que 40 à 50 % de la population du pays utilisait la médecine traditionnelle (WHO 2012). Cette médecine traditionnelle (MT) est plurielle puisque chaque groupe ethnique possède son propre système médical, plus ou moins influencé par les contacts interethniques. Ainsi, on peut individualiser la MT khmère, la MT chinoise et la MT vietnamienne, ainsi que la médecine traditionnelle des populations autochtones tel que les Bunong, les Jaraï, les Tampuan et les Brou (Ashwell and Walston 2008; Cheng et al. 2007). Si les trois premiers systèmes médicaux sont bien documentés, ceux des populations indigènes le sont beaucoup moins. À ce jour, seules les pharmacopées des Brou, des Kavet, des Lao et des Bunongs ont été, en partie, documentées (Audibert et al. 2015; Matras and Martin 1972a; Savajol, Toun, and Sam 2011). Au cours de cette thèse, un article portant sur les remèdes traditionnels utilisés par la communauté Bunong (peuple indigène majoritaire du Cambodge) pour traiter 11 affections les plus communes au sein de cette ethnie a été publié dans la revue *Journal of Ethnopharmacology* (Chassagne et al. 2016) (Annexe 1).

A côté de ces systèmes médicaux traditionnels, existe le système thérapeutique occidental (ou biomédecine) au sein duquel exercent des professionnels formés selon les critères du paradigme de la biologie. Ce système biomédical est présent dans les villes sous forme

d'hôpitaux publics ou de cliniques privés, et dans une moindre mesure dans les villages sous forme de centres de santé communaux ou de dispensaires (Crochet 2000; Dumas 2008).

2.2. Origine de la médecine traditionnelle khmère

Quelques épigraphies anciennes permettent de se rendre compte de l'importance de la santé à l'époque angkorienne (IX^{e} – XIII^{e} siècles). Dès la fin du IX^{e} siècle, sous le règne de la dynastie Jayavarman, des hôpitaux sont construits au côté des temples et des monastères. Puis, lors de l'accession au pouvoir du roi Jayavarman VII en 1181, le bouddhisme introduit au Cambodge devient religion d'état et sous son règne, 102 hôpitaux répartis sur tout le territoire sont créés (Chhem 2006; Coedes 1906). Pendant cette période, les chartes de fondation de ces hôpitaux furent gravées sur des pierres en sanskrit, dont certaines d'entre elles ont été retrouvées au cours du siècle dernier. L'inscription la plus connue est celle de Say-Fong découverte près de Vientiane au Laos (Finot 1903). Sur cette stèle sont détaillés le règlement, la liste du personnel et les fournitures de l'hôpital (Figure 9). Au total, 36 substances d'origine végétale, animale et minérale sont décrites, tel que le gingembre (*Zingiber officinale* Roscoe), le camphrier (*Cinnamomum camphora* (L.) J.Presl), le calophylle (*Calophyllum inophyllum* L.), le curcuma (*Curcuma longa* L.), et le jujubier (*Ziziphus jujuba* Mill.). Tous sont encore utilisés dans la médecine traditionnelle khmère d'aujourd'hui (Leti, David, and Cheng 2010).

Figure 9 : Stèle de Say-Fong découverte au début du XX^{e} siècle près de Vientiane au Laos (extrait de (Leti, David, and Cheng 2010)

2.3. Conception de la personne et de la maladie en MT khmère

Actuellement, le système médical khmer se base sur plusieurs théories médicales empruntées à :

- la médecine Ayurvédique (Ang 1992)
- au bouddhisme : une importance est donnée, dans la cause des maladies, aux fautes commises dans des existences antérieures (karma) (Leti, David, and Cheng 2010)
- la médecine traditionnelle chinoise (Ashwell and Walston 2008)
- aux traditions autochtones : existence d'esprits vitaux à l'intérieur du corps ou de génie autour du village qui ont la possibilité de provoquer la maladie si une faute a été commise (Chhem 2002)
- la médecine occidentale : en particulier la conception microbienne et les notions d'hygiène qui en découlent. Introduite par le corps médical français à l'époque du Protectorat, cette conception a été intégrée et transformée par les khmers et sert à désigner l'agent étiologique de la maladie. Ainsi, le terme « *mé rok* » signifiant « origine de la maladie » ou « initiateur de la maladie » est utilisé dans le langage courant pour représenter les microbes (Guillou 2001)

Dans la médecine khmère, de même que dans la médecine ayurvédique, le fonctionnement symbolique du corps est défini par la théorie humorale qui représente le concept médical le plus influent et le plus répandu en Asie du Sud-Est (Van Esterik 1988). Dans cette théorie, le corps est vu comme étant composé de quatre éléments : l'eau, la terre, le feu et l'air. Chez une personne en bonne santé, ces quatre composantes sont dans un état d'équilibre. Lorsqu'un déséquilibre survient, la maladie apparaît (Manderson 1981).

En médecine traditionnelle khmère, le feu est associé au chaud (*kedaeu*) et la terre au froid (*trocheak*) (Martin 1983). Le couple d'opposition chaud/froid joue un rôle essentiel dans la pathogénie et dans les différentes méthodes de traitement des maladies. En effet, un déséquilibre entre ces deux qualités peut provoquer l'apparition de maladies « chaudes » ou de maladies « froides », qui seront traitées respectivement par des traitements « froids » ou des

traitements « chauds ». L'exemple le plus connu est celui des désordres provoqués après l'accouchement. En période de post-partum, les femmes khmères se considèrent dans un état « froid », pour lequel elles nécessitent des aliments chauds (ex. : poivre, viande) et/ou des traitements chauds (ex. : préparation à base d'alcool) (Fishman, Evans, and Jenks 1988; Montesanti 2011). A l'opposé, la fièvre est considérée comme un état « chaud » que l'on doit contrebalancer avec des traitements « froids » (Kemp 1985; Van Esterik 1988).

L'autre élément impliqué dans le fonctionnement symbolique du corps, l'air (*ktchol*) est perçu comme le support permettant la circulation de certains fluides et matières du corps (sang, urine, bile, fèces) dans des canaux dénommés « *sasaï* », et celui-ci se décompose en différents vents[5], individualisés selon leur localisation corporelle et leur fonctionnalité (Guillou 2001). Un dérèglement d'un ou plusieurs de ces vents est source de maladie, dont les symptômes varient en fonction du type de vent qui est affecté (Piat 1965). Les troubles les plus fréquemment associés aux « mauvais vents » comprennent des vertiges, des courbatures, de la confusion mentale, des frissons, des maux de ventre, des céphalées, voire des paralysies ou une perte de conscience dans les cas les plus sévères (Muecke 1979).

Dans ce type de médecine, à l'instar des médecines ayurvédique, tibétaine et chinoise, l'action pharmacologique des plantes est intrinsèquement liée à leur saveur et leur goût (sucré, salé, amer, acide, astringent). Par exemple, l'amertume de *Azadirachta indica* A.Juss. lui confère des propriétés « refroidissantes » lui permettant ainsi de traiter la fièvre (Dy Phon 2000; Leti, David, and Cheng 2010; Martin 1983).

Également, la doctrine des signatures, se définissant comme une théorie dans laquelle les caractéristiques physiques des plantes expliquent leur usages thérapeutiques, est aussi largement employée au Cambodge (Bennett 2007). Ainsi, certaines plantes se voient attribuer des propriétés curatives en fonction de leur forme (le tubercule de la plante *Hydnophytum formicarum* Jack ressemble à des poumons et est donc utilisé dans le traitement de l'asthme), de leur odeur (la plante *Paederia foetida* L. ayant une odeur de flatulence humaine est utilisée dans

[5] En khmer, l'air et le vent portent le même nom et se disent « *ktchol* »

le traitement des maux de ventre), de leur sécrétion (de nombreuses plantes au latex blanc sont utilisés dans les problèmes liés à l'allaitement), et de leur couleur.

A côté de cette représentation du corps qui s'inspire de l'ayurvéda, il existe au sein de la médecine khmère, d'autres conceptions du fonctionnement symbolique du corps qui, sur la base d'une cosmologie bouddhique traditionnelle et des concepts associés (notion de karma, de faute morale en tant qu'origine de certaines pathologies), mettent en jeu les relations qu'entretient la personne avec certains esprits. Ceux-ci sont plus particulièrement évoqués dans l'apparition de certaines maladies mentales, crises d'épilepsie, confusions, et autres perturbations de la conscience (Lemoine and Eisenbruch 1997). Dans ce contexte, la maladie provient de trois sphères : celles des divinités, en haut ; des démons, en bas ; et des hommes, au milieu, tous considérés comme faisant partie de l'espace social du patient. Les désordres provenant du monde des humains comprennent les attaques de sorcellerie, les fautes karmiques provenant de vies antérieures. Ceux provenant des deux autres univers sont attribués soit aux esprits maléfiques, soit aux ancêtres.

Le corps du patient est lui-même considéré comme un représentant de cette série d'univers, et les problèmes de santé signent une perturbation dans les relations de la personne avec ces différentes entités, nécessitant alors la pratique de rituels de guérisons effectués par des guérisseurs spécialisés (Figure 10).

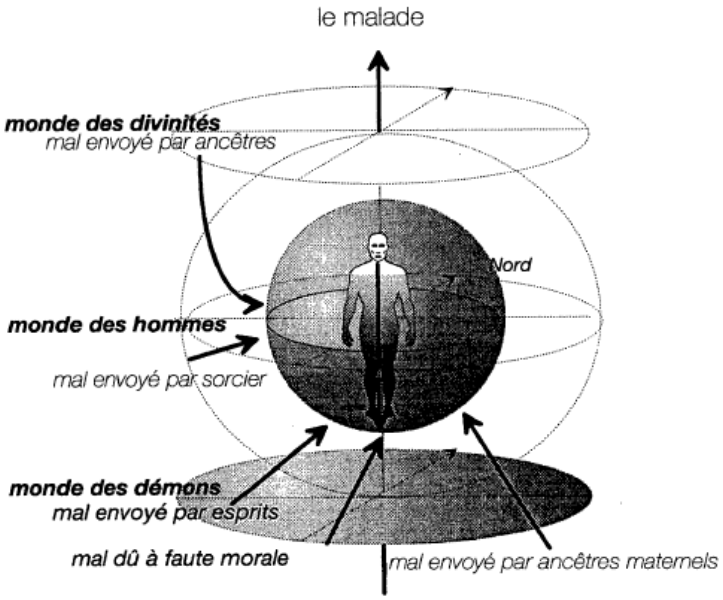

Figure 10 : Interaction de trois mondes dans la constitution de la maladie chez les khmers (extrait de (Lemoine and Eisenbruch 1997)

2.4. Médecine traditionnelle savante et populaire

Selon (Crochet 2000), dans le système médical traditionnel khmer, on peut distinguer :

- la médecine dite populaire, pratiquée par une population non-spécialiste, bien souvent féminine, et dont les connaissances se transmettent oralement. 48 à 67% des patients cambodgiens ont recours à cette médecine (Dumas 2008).
- la médecine dite savante, qui est l'apanage de spécialistes, majoritairement masculins, dont le savoir s'appuie sur des textes. Entre 3 et 10% des cambodgiens utilisent ce type de médecine (Dumas 2008).

2.4.1. Médecine traditionnelle populaire

La définition de la médecine traditionnelle populaire pose question notamment sur les liens qu'elle entretient avec la médecine traditionnelle savante. Alors que certains la considèrent

comme une expression culturelle authentique et autonome, d'autres l'appréhendent comme une dégénérescence des formes médicales traditionnelles savantes (Guillou 2001).

Au Cambodge, (Crochet 2000) définit la MT populaire comme étant une pratique d'automédication mettant en œuvre un « ensemble complexe de techniques alliant les remèdes de la pharmacopée locale aux drogues de l'industrie pharmaceutique ». Cette pratique peut aussi prendre la forme d'une spécialisation et d'un commerce, lorsque les acteurs de cette MT populaire décident de proposer leurs services, contre rémunération (Guillou 2001).

L'acteur principal de cette médecine populaire est la femme, mère au foyer, garante de la santé de ses enfants et des autres membres de la famille vivant sous son toit. Elles utilisent des ingrédients venant du jardin ou du « placard à cuisine », et traitent des affections courantes tels que la fièvre, les courbatures, les coupures, les brûlures, les désordres cutanés et les diarrhées. Ce savoir est transmis oralement aux filles du foyer en âge d'aider leurs parents (Crochet 2000).

Parmi les pratiques populaires les plus fréquemment utilisées, on peut distinguer :

- les soins basés sur la manipulation du patient.

Le « grattage du vent » (*kos ktchol*) est une technique de soins très répandue (Figure 11). Cette méthode, appelée « *coining* » en anglais, permet de traiter les déséquilibres liés au vent (*ktchol*) et consiste à gratter vivement le dos, le cou, le torse du patient jusqu'à l'apparition de rougeurs voire de saignements. Afin de réaliser l'acte thérapeutique, les khmers utilisent une pièce de monnaie ou une cuillère enduite d'un produit gras (huile, baume mentholé) (Crochet 2000; Guillou 2001).

Des variantes de cette méthode de dermabrasion reposant toujours sur le principe de la réactivation de l'équilibre des « vents » sont aussi utilisées, notamment une technique qui consiste à « attraper les vents » (*chop ktchol*) en pinçant le nez jusqu'à l'apparition de rougeurs (Piat 1965), ou encore l'application de ventouses chauffées (*baum ktchol*) sur le corps du patient (Guillou 2001).

Les massages (*massa*) sont pratiqués dans les mêmes indications que le « *kos ktchol* » et aussi pour améliorer le confort des personnes âgées (Guillou 2001). Par ailleurs, la réalisation

d'une brûlure de cigarettes (*och*) sur le corps du patient permet de rééquilibrer la balance chaud/froid (Richman et al. 2010; Sargent and Marcucci 1984).

Figure 11 : Pratique du "*baum ktchol*" (à g.), du "*kos ktchol*" (à d.), et instrument permettant la réalisation du "*kos ktchol*" (à d.)

- l'utilisation de substances d'origine naturelle : plantes et animaux principalement.

Plus d'une quarantaine d'espèces végétales ont été reportées comme faisant partie de la pharmacopée des femmes en médecine populaire (Crochet 2000). Ces plantes proviennent le plus souvent du potager ou de la cuisine. Ainsi, des arbres fruitiers et des légumes sont largement utilisés, comme par exemple, l'aubergine pour le traitement des dartres ou la sève de bananier pour la candidose buccale du nouveau-né (Crochet 2000). Des herbes aromatiques sont aussi employées, tel que le basilic sacré (*Ocimum tenuiflorum* L.) dans le traitement des douleurs, ou la « menthe oreille de cochon » en khmer (*Plectranthus amboinicus* (Lour.) Spreng.) dans le traitement de la toux (Martin 1971). Par ailleurs, des condiments alimentaires comme le piment ou le poivre considérés comme « chaud », peuvent être utilisés dans le traitement du post-partum (Richman et al. 2010).

Par ailleurs, le nid d'araignée est utilisé dans le traitement des plaies, le nid d'abeille est posé sur l'ombilic du nouveau-né et la sangsue est employée dans différentes indications (Crochet 2000).

- le recours à des produits manufacturés et pharmaceutiques

Ce sont les baumes mentholés (baume du tigre), et les spécialités pharmaceutiques comme les antibiotiques, les antalgiques (paracétamol), et les corticoïdes. Ces derniers sont distribués par des vendeurs à l'étalage qui ne possèdent bien souvent aucune formation médicale et qui conseillent à leurs clients des mélanges de médicaments (*psom tnam*) pour une durée de quelques jours (Crochet 2000).

2.4.2. Les acteurs de la médecine traditionnelle savante

A la différence de la MT populaire, la MT savante a été largement documentée depuis la fin du XXe siècle[6]. Elle se définit principalement à travers des acteurs qui vont chacun avoir des pratiques thérapeutiques et des rituels de guérison spécifiques, et qui partagent des univers sociaux, culturels et symboliques assez proches les uns des autres (Guillou 2001).

D'une manière générale, on peut distinguer trois types de thérapeutes traditionnels :

- les « *kruu* khmer » ou maître-guérisseur khmer.

Le terme « *kruu* » est utilisé pour désigner toutes personnes ayant un savoir. Ainsi, l'expression « *kruu banrien* », traduit littéralement par maître enseigner, identifie les instituteurs. Le terme « *kruu peit* », *kruu* hôpital, désigne les biomédecins. Traditionnellement, le terme « *kruu* » suffisait pour nommer un médecin traditionnel, puisqu'il n'y avait pas encore d'école ni de dispensaires dans les villages (Guillou 2001; Martin 1983).

Au sein même des « *kruu* khmer », différentes catégories ont été individualisées en fonction des spécialités et des techniques de soins utilisées (Martin 1983), de leur domaine

[6] Voir notamment les travaux réalisés par (Eisenbruch 1992; Guillou 2001; Lemoine and Eisenbruch 1997; Martin 1983).

d'intervention (action sur le monde des divinités et des démons) (Eisenbruch 1992), de leur statut socio-économique et de leur formation (Guillou 2001). La seule constante qui semble être retrouvée dans chaque catégorie est la prédominance masculine dans la fonction (Dumas 2008).

Le portrait traditionnel d'un « *kruu* khmer » est celui d'un agriculteur, chef de famille, recevant les malades dans sa maison d'une manière occasionnelle en fonction des demandes. C'est une figure emblématique du village à qui on accorde considération et respect (Martin 1983). Il est décrit à la fois comme botaniste, pharmacien, guérisseur et protecteur spirituel (Eisenbruch 1992; Guillou 2001; Martin 1983). Sa formation est longue et comprend divers enseignements professés par d'autres maîtres-guérisseurs de différentes provinces (Guillou 2001), et par des bonzes des pagodes environnantes desquels il tire une formation en science médicale (Martin 1983). Ses pratiques de soin sont variées et combinent généralement phytothérapie, rituels de guérison avec récitations de formules magiques, et réalisation de diagramme de protection (Guillou 2001; Lemoine and Eisenbruch 1997; Martin 1983). Enfin, il ne réclame pas de compensation financière, mais plutôt reçoit des offrandes diverses (riz, fruits, argent) selon les moyens du patient (Guillou 2001).

Bien que cette description du « *kruu khmer* » reste encore d'actualité, une évolution notable des pratiques a récemment été notée. Ainsi, (Guillou 2001) différencie le « *kruu* » traditionnel du « *kruu* » moderniste. Ce dernier est décrit comme se rapprochant du biomédecin dans sa pratique, puisqu'il accueille les patients dans un local dédié, perçoit des honoraires fixes et se concentre sur la prescription de remèdes phytothérapeutiques. Il bénéficie également d'une légitimité institutionnelle puisqu'il possède une autorisation ou une formation spécifique émanant d'une institution légale (institut de médecine bouddhique, centre de médecine traditionnelle).

- Les « *preah sang* » ou moines bouddhistes

Les bonzes sont des vecteurs essentiels de la vie culturelle, sociale et spirituelle au Cambodge. Ils peuvent aussi être amenés à prendre en charge des patients pour le traitement de leurs troubles. En effet, les bonzes possèdent un statut privilégié grâce à leur maitrise de l'écrit

et du pali, ils peuvent ainsi s'appuyer sur un corpus académique enseigné et transmis (Bertrand 1997).

Les cambodgiens ont recours aux bonzes guérisseurs dans le but de traiter des affections aigues ou chroniques, mais également pour contrer une succession négative d'évènements, ou encore attirer la chance afin de réussir un examen par exemple. D'autre part, les bonzes peuvent aussi accueillir et prendre en charge les malades mentaux au sein même de la pagode (Guillou 2001).

Les bonzes guérisseurs consultent lors de cérémonies collectives au cours desquelles différentes méthodes thérapeutiques sont employées tel que les bains d'eau lustrale, les paroles sacrées, les remèdes à base de plantes et le souffle thérapeutique. Ils peuvent également inscrire en pali des formules de guérison ou de protection sur différents supports (feuilles de bananier, plaque en métal, talisman) (Cheng et al. 2007).

Contrairement aux maitres-guérisseurs khmers, l'influence d'un bonze ne repose pas essentiellement sur son pouvoir de guérison mais également sur son charisme personnel et les réseaux qu'ils sont capables d'établir avec les hauts fonctionnaires au pouvoir. Ainsi, certains bonzes peuvent jouir d'une notoriété internationale (Guillou 2001).

- Les « *ruup* », ou médiums

Le terme « *ruup* » se définit par la forme, l'image, l'apparence. Il renvoie donc à la notion d'enveloppe physique à travers lequel un esprit va pouvoir s'exprimer (Guillou 2001).

A la différence des autres guérisseurs, le médium est une femme dont le savoir ne repose pas sur une transmission orale ou écrite mais possède un caractère subi. Ainsi, (Guillou 2001) décrit le cas d'une femme tombant fréquemment malade et ayant eu recours à divers thérapeutes sans succès. Cette personne finit par consulter un « *kruu khmer* » qui pose le diagnostic. Elle est « tenue par un esprit ». Ainsi, le seul moyen de guérison possible est alors d'accepter d'être habitée ponctuellement par cet esprit, et de lui rendre hommage par la réalisation d'un autel dédié. Cette femme deviendra donc medium malgré elle, et disposera du pouvoir de guérison conféré par cet esprit, puisque l'invocation de l'esprit permettra aux malades de rentrer en contact directement avec lui et de trouver les solutions à leurs problèmes.

Comme chez les maîtres-guérisseurs, les demandes sont variées. Les patients viennent traiter diverses affections tel qu'une perte d'énergie, un manque d'appétit, ou des maux de tête ; mais ils peuvent aussi consulter le medium dans le but de se faire prédire l'avenir ou de connaître la vérité sur certains événements passés (Guillou 2001). Selon (Dumas 2008), le medium est avant tout un spécialiste du diagnostic et non un spécialiste du traitement comme les autres guérisseurs : on vient le consulter pour connaître l'origine du malheur.

Par ailleurs, hormis une légitimité traditionnelle, le medium n'est pas autant respecté que les autres thérapeutes. En effet, leur action dépend d'une entité extérieure et non de leur propre faculté à guérir (Guillou 2001).

- D'autres tradipraticiens ont également été décrits tel que :

Les matrones ou accoucheuses traditionnelles (*yeay map*) qui jouent un rôle dans la préparation de l'accouchement et dans les soins post-partum (Dumas 2008).

Les sorciers (*tmop*) qui sont capables de provoquer la maladie en faisant usage de la magie noire (Eisenbruch 1992).

Les voyants (*kruu tiey*) qui sont capable de voir l'avenir (Martin 1983).

3. PHARMACOPEES TRADITIONNELLES ET PATHOLOGIES DU FOIE

3.1. Perceptions des maladies du foie

De nombreux systèmes de soins traditionnels prennent en charge les maladies du foie, et notamment la médecine traditionnelle chinoise, la médecine ayurvédique, la médecine traditionnelle japonaise (médecine Kampo), et les médecines traditionnelles africaines (Seeff et al. 2001).

Cependant, la principale difficulté du travail ethnopharmacologique est de faire le lien entre un système de pensée biomédical appliqué aux maladies du foie et une nosologie non biomédicale. En effet, ce qui est clairement défini comme une cirrhose ou un cancer du foie dans le système biomédical ne l'est certainement pas dans les autres systèmes de soins.

Par exemple, dans la nosologie chinoise, les symptômes de la jaunisse peuvent être liés à des problèmes de rate et d'estomac, sans que le foie en tant qu'organe ne soit mentionné. Dans un effort de modernisation et d'ajustement avec la médecine occidentale, les praticiens chinois modifièrent leur définition des maladies chroniques du foie et y intégrèrent dans les années 1970, les mots jaunisse, gonflement abdominal et douleur de l'hypochondre droit (Zhang and Schuppan 2014).

A l'inverse, dans un grand nombre de systèmes médicaux, les maladies du foie sont principalement identifiées par la coloration jaune des muqueuses, de la peau et des conjonctives. C'est notamment le cas en Inde (Sharma et al. 2012), au Togo (Kpodar et al. 2016) et au Rwanda (Mukazayire et al. 2011). D'un point de vue médical, l'ictère est dû à une augmentation de la bilirubinémie, qui peut se manifester dans des pathologies liées directement au foie (ex. :

hépatites virales, cirrhose, cholangiocarcinome, calcul biliaire), mais aussi au cours d'autres pathologies dans lesquelles le foie n'est pas directement impliqué (ex. : anémie hémolytique, tumeur du pancréas, paludisme) (Beers et al. 2008).

Enfin, dans notre propre culture française, le mot « crise de foie » peut désigner des affections hépatiques mais également d'autres problèmes tel que des désordres digestifs et des manifestations neurologiques (Pouchelle 2007).

Un des premiers objectifs de l'ethnopharmacologue sera donc, dans un système médical donné, de comprendre la nosologie des maladies du foie et les principes thérapeutiques associés.

3.2. Méthode d'évaluation de l'activité biologique

La diversité des affections hépatiques, de leurs causes et de leurs symptômes rend difficile la recherche d'un modèle d'étude « parfait » pour évaluer l'activité biologique des pharmacopées utilisées dans le traitement des maladies du foie. Cependant, plusieurs tests sont classiquement utilisés pour évaluer les plantes à visée hépatique. Ces tests peuvent être classés en deux grands groupes :

- Les tests *in vitro*

  Ils présentent l'avantage d'être peu couteux, faciles à mettre en œuvre, et ils permettent de cibler une activité spécifique. Leurs inconvénients majeurs résident dans le fait qu'ils ne rendent pas compte de l'absorption et de la métabolisation des molécules dans le corps humain et qu'ils se restreignent à l'étude d'une composante d'un système complexe (Houghton et al. 2007).

- Les tests *in vivo*

  Ils permettent d'évaluer l'effet physiopathologique de la matière étudiée, et offrent donc une vision globale de l'activité. Par contre, ils sont plus couteux, plus

compliqués à mettre en place et posent des problèmes éthiques (Atanasov et al. 2015).

D'après la littérature scientifique, les tests *in vivo* d'hépatoprotection et les tests *in vitro* d'activité antiproliférative sont les plus couramment utilisés (Tableau 1). Les premiers utilisent des modèles murins, sur lesquels une hépatotoxicité est provoquée par diverses substances (le tetrachlorure de carbone est le plus employé) et l'effet protecteur de l'extrait végétal est évalué. Les deuxièmes utilisent des modèles cellulaires d'hépatocarcinome humain sur lesquels on évalue l'activité antiproliférative des extraits. D'autres méthodes complémentaires peuvent être employées afin de déterminer les mécanismes d'action du produit testé. Pour les tests *in vitro* : induction de l'apoptose, arrêt du cycle cellulaire, morphologie cellulaire, etc. Pour les tests *in vivo* : activité anti-inflammatoire, effet anti-oxydant, propriété antitumorale, etc.

Tableau 1 : Tests d'activité pharmacologiques les plus couramment utilisés pour les plantes hépatotropes

Type	Activités pharmacologiques	Pathologies du foie ciblées	Détails des tests utilisés et références
In vivo	Anti-tumorale	Cancer du foie	Etude sur des rats ayant un CHC induit par du diéthyl nitrosamine (Boye et al. 2015). Etude sur des souris transplantées avec des cellules cancéreuses hépatiques (H22) (Chen et al. 2012)
	Hépatoprotection	Cirrhose Fibrose Hépatite Stéatose hépatique	Etude sur des rats/souris ayant une intoxication hépatique induite par le tétrachlorure de carbone (Ghaffari et al. 2013), la D-galactosamine (Luo et al. 2016), l'éthanol (Cao et al. 2015), l'aflatoxine B1 (Naaz, Javed, and Abdin 2007), et l'acétaminophène (Lin et al. 1996)
	Hépatoprotection	Cholestase hépatique	Etude sur des rats ayant une cholestase provoquée par l'α-naphthylisothiocyanate (Yan et al. 2015)
In vitro	Anti-angiogénique	Cancer du foie	Etude de l'inhibition du VEGF sur cellules endothéliales aortique bovines (Cheng et al. 2005)
	Anti-inflammatoire	Nombreuses	Etude sur macrophages murins sécrétant des cytokines pro-inflammatoires (Chen et al. 2007)
	Antimicrobien	Abcès du foie	Etude sur diverses bactéries (*E. coli*, *S. aureus,…*) (de Moura et al. 2004)
	Antioxydant	Nombreuses	Etude de réaction chimique avec des radicaux libres (DPPH, ABTS, superoxyde,..) ou d'inhibition de réaction oxydante (test au ß-caroténe, méthode du thiocyanate ferrique) (Zhou et al. 2013)
	Antiprolifératif	Cancer du foie	Etude sur cellules d'hépatocarcinome humain : HepG2 (Chaudhary et al. 2011), Hep3B (Carraz et al. 2015), PLC/PRF/5 (Wills and Asha 2009), et SMMC7721 (Hu et al. 2009)
	Anti-viral (Anti-VHB)	Hépatite B	Etude sur cellules MS-G2 produisant VHB (Lee et al. 2002)
	Anti-viral (Anti-VHC)	Hépatite C	Etude sur cellules modifiées génétiquement pour produire VHC (Ravikumar et al. 2011)
	Immunomodulation	Nombreuses	Etude sur cellules mononucléaires et neutrophiles périphériques humains (Ao et al. 2009)

La stratégie à adopter dans l'évaluation préclinique de plantes hépatotropes pourrait être la suivante (Butterweck and Nahrstedt 2012) :

- Un premier examen de l'extrait végétal sur un modèle *in vivo* afin de confirmer l'usage ethnopharmacologique relevé (c-à-d test d'hépatoprotection *in vivo*).
- Un fractionnement bioguidé de l'extrait végétal utilisant un modèle *in vitro* cohérent
- Une étude pharmacocinétique du composé isolé
- Une évaluation du composé isolé sur modèle *in vivo*
- Une approche des mécanismes d'action à l'aide de technique de pharmacologie moléculaire et/ou de tests biochimiques

Cette stratégie est particulièrement utile si le but de l'étude est d'aboutir à la mise sur le marché d'une molécule extraite de plantes. Dans le cadre du travail ethnopharmacologique, l'étude scientifique des médecines traditionnelles et notamment la validation de leur efficacité et de leur innocuité ne nécessite pas forcément l'isolement de la molécule active.

3.3. Plantes hépatotropes utilisées dans le monde

Parmi les plantes les plus citées dans le traitement des maladies du foie dans le monde, on trouve :

Le chardon-marie (***Silybum marianum* (L.) Gaertn.**, Compositae), plante originaire d'Europe du Sud et de l'Asie mineure, utilisée en Europe depuis le IV^{ème} siècle avant Jésus-Christ pour traiter les pathologies hépatobiliaires (Schuppan et al. 1999). Elle est actuellement disponible dans une spécialité pharmaceutique (Legalon®), indiquée dans le traitement des hépatopathies. Son activité est due à un mélange de flavonolignanes : la silymarine (Ghosh et al. 2011). La silymarine a démontré une activité hépatoprotectrice sur des modèles *in vivo* dont

l'hépatotoxicité a été induit par de l'éthanol, de l'acétaminophène, du tétrachlorure de carbone et un champignon toxique : *Amanita phalloïdes* (Ghosh et al. 2011). Ces flavonolignanes possèdent également des activités anti-fibrotique, antioxydante, anti-inflammatoire, cytoprotectrice, anti-angiogénique, et anti-tumorale (Zhang, Sun, and Wang 2013). Par ailleurs, plusieurs essais cliniques ont été entrepris et ils semblent montrer une amélioration des fonctions hépatiques chez des patients alcooliques chroniques, et un effet protecteur dans la réponse inflammatoire au virus de l'hépatite C (Ghosh et al. 2011).

La réglisse (***Glycyrrhiza glabra* L.**, Leguminosae), plante originaire du bassin méditerranéen, de Russie et d'Asie mineure, est connue pour ses propriétés hépatoprotectrices et plus particulièrement pour ses effets sur les hépatites virales. Elle est présente dans les pharmacopées européenne, chinoise, japonaise et indienne (Levy, Seeff, and Lindor 2004; Luk et al. 2007; Thyagarajan et al. 2002). L'acide glycyrrhizique (aussi appelé glycyrrhizine) présent dans la racine est responsable de l'activité pharmacologique (Asl and Hosseinzadeh 2008). Ses propriétés hépatoprotectrices ont été montrées sur modèles murins en présence de tétrachlorure de carbone, d'acétaminophène, et de D-galactosamine. De plus, la glycyrrhizine a montré une activité anti-virale *in vitro* contre le virus de l'hépatite A, le virus de l'hépatite B et le virus de l'hépatite C (Asl and Hosseinzadeh 2008). Des essais cliniques ont ainsi montré que la prise de glycyrrhizine pouvait prévenir l'apparition d'un CHC chez les patients atteints d'hépatites C chroniques (Hidaka et al. 2007; Levy, Seeff, and Lindor 2004). Cependant, des effets secondaires ont été observés à cause de l'activité minéralocorticoïde de la plante.

Plus spécifique aux régions tropicales et en particulier à l'Asie, ***Phyllanthus amarus* Schumach. & Thonn.** (Phyllanthaceae), est une plante très utilisée pour traiter la jaunisse et divers pathologies hépatiques. Elle est employée en Chine et en Inde où elle fait partie de diverses préparations hépatoprotectrices : Hepatomed®, Livfit®, Tefroliv® (Ghosh et al. 2011; Levy, Seeff, and Lindor 2004). Bien que des composés hépatoprotecteurs (phyllanthine, hypophyllanthine) aient été isolés de la plante, ce sont principalement des études sur la plante entière qui ont été réalisées. Ainsi, l'extrait aqueux et éthanolique de la plante a montré des effets

hépatoprotecteurs sur souris intoxiquées par de l'aflatoxine B1, du tétrachlorure de carbone et du nimésulide (Ghosh et al. 2011; Naaz, Javed, and Abdin 2007). (Rajeshkumar and Kuttan 2000) ont par ailleurs mis en évidence une augmentation de la durée de vie de rats atteints de CHC et traités par un extrait aqueux de la plante. Dans une analyse de 22 essais cliniques, (Liu, Lin, and McIntosh 2001) concluent à un effet anti-HBV et une amélioration des analyses biochimiques du foie de la plupart des études, et proposent d'utiliser la plante en combinaison avec les thérapeutiques conventionnels (ex. : interféron α).

Viennent ensuite : le rhizome de ***Picrorhiza kurroa*** **Royle ex Benth.** (Plantaginaceae) utilisé en médecine ayurvédique ; le rhizome de ***Curcuma longa*** **L.** (Zingiberaceae) utilisé en Asie et dont on a isolé le principe actif : la curcumine ; les fruits de ***Schisandra chinensis*** **(Turcz.) Baill.** (Schisandraceae) employés en Chine et au Japon, dont la schisandrine constitue la molécule bioactive ; ou encore la plante entière d'***Andrographis paniculata*** **(Burm.f.) Nees** (Acanthaceae) très utilisé en Asie et particulièrement en Inde, et dont l'andrographolide constitue le chef de file des molécules actives (Ghosh et al. 2011; Thabrew and Hughes 1996; Zhang, Sun, and Wang 2013).

3.4. Revue de la littérature en Asie du Sud-Est

Une revue de la littérature a été réalisée afin d'identifier les espèces employées dans les maladies du foie en Asie du Sud-Est, et plus particulièrement au Cambodge, et ainsi déterminer les espèces les plus citées.

3.4.1. Critères d'inclusion

Une recherche bibliographique aussi exhaustive que possible a été réalisée en consultant des ouvrages disponibles dans les bibliothéques cambodgiennes (Institut Français du Cambodge

à Phnom Penh, Bibliothèque Nationale du Cambodge à Phnom Penh, Centre d'Études Khmers à Siem Reap et Ecole française d'Extrême Orient à Siem Reap), laotiennes (Institut Français du Laos à Vientiane, Bibliothéque Nationale du Laos à Vientiane) et françaises (Bibliothèque du Centre Pompidou à Paris, Bibliothèque Universitaire des Langues et Civilisations à Paris). Les livres, thèses, mémoires, et rapports ayant trait à la médecine traditionnelle, à la botanique, ou à l'ethnobotanique en Asie du Sud-Est ont été analysés (Voir Annexe 2 pour une liste des documents de source cambodgienne analysés).

Des bases de données électroniques ont également été consultés : Pubmed, Google Scholar, Web of Science, en utilisant les mots-clés suivants : "traditional medicine", "medicinal plants", en combinaison avec des termes relatifs aux maladies du foie : "liver", "jaundice", "icterus", "hepatitis" ; et des noms de régions ou pays: "Southeast Asia", "Cambodia", "Laos", "Vietnam", "Thailand", "Southern China".

Pour chaque plante, différentes informations ont été relevées : nom de genre, nom d'espèce, nom de famille, partie de plantes utilisées, usages thérapeutiques, nom du pays dans lequel la plante est utilisée, nombre de références citant la plante. Les noms de plantes extraites de cette analyse ont ensuite été vérifiés avec la base de données The Plant List (http://www.theplantlist.org/), puis rentrés dans un fichier Excel.

3.4.2. Résultats de l'analyse

Au total, 45 publications parues entre 1930 et 2016 ont été analysées dont 23 livres, 18 articles scientifiques, 2 thèses de doctorat et 2 mémoires. 378 espèces de plantes appartenant à 112 familles botaniques ont été relevées.

Les références consultées sont de source cambodgienne (19 documents, 42,2%), laotienne (7, 15,6%), thaïlandaise (7, 15,6%), vietnamienne (5, 11,1%), et chinoise (3, 6,7%). Quatre publications ont pour sujet l'Asie du Sud-Est en général.

37 termes différents ont été utilisés pour qualifier les pathologies du foie dont : maladie du foie (267 citations, 48,5%), jaunisse (157, 28,5%), hépatite (104, 18,9%), ictère (18, 3,3%), cirrhose (13, 2,4%), hépatomégalie (11, 2%), ascite (10, 1,8%), et infection du foie (6, 1,1%).

Les familles les plus citées sont : les Leguminosae (39 plantes citées), les Compositae et les Rubiaceae (17), les Apocynaceae, Cucurbitaceae, Euphorbiaceae, Lamiaceae, et Poaceae (13).

Les genres botaniques les plus représentés sont : *Clerodendrum*, *Croton* et *Senna* (5 espèces citées pour chaque), suivis par *Caesalpinia*, *Ficus*, *Phyllanthus* et *Polygonum* (4).

Pour analyser ces données, deux indices ont été utilisées : le premier (I_1) correspond au nombre de publications (extraits de la revue bibliographique) qui mentionne l'usage d'une espèce donnée pour une pathologie du foie, et le deuxième (I_2) est le nombre de pays dans lequel est reporté l'usage d'une espèce donnée pour une pathologie du foie (Tableau 2). Pour une espèce donnée, ces deux indices permettent de définir un score (S) de convergence d'usage bibliographique et de diffusion géographique :

$$S = I_1 \times I_2$$

Au final, les espèces de plantes présentant le score le plus élevé sont : *Curcuma longa* L. (S=28), *Oroxylum indicum* (L.) Kurz (S=24), *Melastoma sanguineum* Sims (S=18), *Orthosiphon aristatus* (Blume) Miq. (S=18), *Senna alata* (L.) Roxb. (S=18), *Willughbeia edulis* Roxb. (S=16), et *Phyllanthus urinaria* L. et (S=16).

Ce score permet la sélection de plantes dont l'usage traditionnel dans les maladies du foie est culturellement et géographiquement répandu. Il serait donc intéressant d'approfondir l'étude de ces espèces afin de valider leur mode d'action dans le cadre des pathologies hépatiques, ainsi que de standardiser leur usage.

Tableau 2 : Espèces les plus citées (I_1 élevé) dans la littérature d'Asie du Sud-Est pour traiter les pathologies hépatiques

Nom et famille scientifique	Partie de plantes utilisées	Nombre de citations (I_1)	Noms des pays où la plante est utilisée	Nombre de pays (I_2)
Melastoma sanguineum Sims MELASTOMATACEAE	Fleur / Plante entière / Racine	9	Cambodge, Laos	2
Phyllanthus urinaria L. PHYLLANTHACEAE	Plante entière	8	Cambodge, Vietnam	2
Willughbeia edulis Roxb. APOCYNACEAE	Latex / Liane / Racine	8	Cambodge, Malaisie	2
Curcuma longa L. ZINGIBERACEAE	Rhizome	7	Cambodge, Chine, Thaïlande et Vietnam	4
Piper retrofractum L. PIPERACEAE	Fruit	7	Cambodge	1
Muntingia calabura L. MUNTINGIACEAE	Feuille / Fleur / Racine	6	Cambodge	1
Oroxylum indicum (L.) Kurz BIGNONIACEAE	Bois / Écorce / Graine / Racine	6	Cambodge / Chine / Laos / Vietnam	4
Orthosiphon aristatus (Blume) Miq. LAMIACEAE	Plante entière	6	Cambodge / Laos / Vietnam	3
Senna alata (L.) Roxb. LEGUMINOSAE	Bois / Branche / Feuille	6	Cambodge / Laos / Vietnam	3
Alstonia scholaris (L.) R. Br. APOCYNACEAE	Racine	5	Cambodge / Thaïlande	2
Cananga latifolia (Hook.f. & Thomson) Finet & Gagnep. ANNONACEAE	Bois	5	Cambodge	1
Cyperus rotundus L. CYPERACEAE	Racine	5	Cambodge / Chine	2

Nom et famille scientifique	Partie de plantes utilisées	Nombre de citations (I_1)	Noms des pays où la plante est utilisée	Nombre de pays (I_2)
Hydnophytum formicarum Jack RUBIACEAE	Rhizome	5	Cambodge	1
Momordica charantia L. CUCURBITACEAE	Fruit / Feuille	5	Cambodge / Indonésie / Thaïlande	3
Tamarindus indica L. LEGUMINOSAE	Bois / Écorce / Fruit mûr	5	Cambodge / Indochine	2

3.4.3 Discussion ethnopharmacologique sur les espèces les plus citées dans la bibliographie

Figure 12 : *Melastoma sp.*

***Melastoma sanguineum* Sims** est un arbuste de 1,5 à 3 mètres de hauteur de la famille des Melastomataceae, originaire des régions tropicales et subtropicales d'Asie du Sud-Est (Figure 12). La racine est utilisée traditionnellement dans les maladies du foie, la jaunisse et les hépatites au Cambodge et au Laos. Peu d'études ont décrit les composés chimiques et les activités biologiques de la plante (Lee et al. 2013). Seule l'activité anti-oxydante de ces fruits a été démontré en utilisant des tests *in vitro* FRAP (Ferric Reducing Antioxidant Power) et TEAC (Trolox Equivalent Antioxidant Capacity) (Fu et al. 2010). Au niveau du genre, l'extrait méthanolique de l'espèce *Melastoma malabathricum* L. a démontré une activité hépatoprotectrice *in vivo*, qui serait due aux propriétés anti-inflammatoires et antioxydantes de la plante (Mamat et al. 2013). Étant donné le faible nombre d'études scientifiques et le fort nombre de citations dans le traitement des maladies du foie, un examen

approfondi de la plante se révèle nécessaire pour confirmer son usage traditionnel, et comparer son activité aux autres espèces du même genre[7].

***Phyllanthus urinaria* L.** est une plante herbacée pantropicale de la famille des Phyllanthaceae pouvant aller jusqu'à 80 cm de hauteur (Figure 13). Dans notre étude bibliographique, la plante entière est utilisée pour soigner les maladies hépatobiliaires et la jaunisse au Cambodge et au Vietnam. En Inde, cette espèce est traditionnellement utilisée dans le traitement de l'hépatite B et de diverses affections du foie (Fang, Rao, and Tzeng 2008).

Figure 13 : *Phyllanthus urinaria*

(Patel et al. 2011) indique que *P. urinaria* est souvent confondu avec *P. amarus* et *P. niruri* en médecine ayurvédique du fait de leur morphologie similaire. L'effet hépatoprotecteur de ces différentes espèces a donc été comparé et il a été montré que toutes ces espèces possèdent des activités similaires et peuvent donc être utilisées d'une manière interchangeable (Prakash et al. 1995). L'extrait aqueux et hydrométhanolique de *P. urinaria* a par ailleurs prouvé une activité antiproliférative sur cellules cancéreuses hépatiques (Chudapongse, Kamkhunthod, and Poompachee 2010; Huang, Yang, and Pang 2004). L'usage de cette espèce en Asie du Sud-Est est donc corroboré par son utilisation pour les mêmes indications dans d'autres régions, et par son activité biologique *in vitro* et *in vivo*.

[7] Dans la littérature, seul l'espèce *M. sanguineum* est mentionné pour le traitement des maladies du foie. Dans la pratique, (Schmitt 2004) note que plusieurs plantes du même genre (*M. normale, M. saigonense*, *M. sanguineum*) portent une même dénomination vernaculaire et pourraient donc être utilisées d'une manière interchangeable par les tradipraticiens.

Figure 14 : *Willughbeia edulis* (en haut : fruits, en bas : feuilles)

***Willughbeia edulis* Roxb.** est une liane de la famille des Apocynaceae, endémique à l'Asie du Sud-Est et dont les fruits sont comestibles (Figure 14). La liane et la racine sont employées dans le traitement des maladies du foie, des hépatites et de la jaunisse au Cambodge et en Malaisie. À ce jour, aucune évaluation pharmacologique relative à ces effets hépatoprotecteurs n'a été réalisée. Seule son activité contre l'infection au virus de l'herpès (HSV-1) a été mesurée (Lipipun et al. 2003). L'activité de cette liane mériterait donc d'être évaluée *in vitro* et *in vivo* sur différents modèles hépatiques.

***Curcuma longa* L.** est une plante herbacée à rhizome charnu de la famille des Zingiberaceae, cultivée dans toute l'Asie et principalement en Inde et en Chine (Figure 15). Le rhizome est utilisé dans le traitement des hépatites, de la jaunisse et du cancer du foie dans de nombreux pays d'Asie du Sud-Est. Le principe actif de la plante, la curcumine, a démontré des effets protecteurs sur différents modèles murins d'hépatotoxicité (intoxication hépatique induite par l'acétaminophène, l'aflatoxine B1, l'éthanol et le tétrachlorure de carbone). L'effet hépatoprotecteur de la curcumine est dû à l'inhibition de la production de cytokines pro-inflammatoires et à l'activité antioxydante (Ghosh et al. 2011). Par ailleurs, la curcumine inhibe le développement et la progression du cancer, en agissant sur plusieurs stades de la carcinogénèse (initiation, progression et promotion). Plusieurs essais cliniques ont été réalisés ou sont actuellement en cours pour le traitement de diverses pathologies (cancer colorectal, cancer de la vésicule biliaire, cancer du pancréas, maladie d'Alzheimer, psoriasis). Aussi, une étude de phase I chez des patients présentant des lésions précancéreuses montrent l'absence de toxicité de la molécule

Figure 15 : Rhizome de *Curcuma longa*

(Hatcher et al. 2008). L'utilisation traditionnelle de cette espèce en Asie du Sud-Est est donc appuyée par une littérature abondante sur ses activités pharmacologiques.

Figure 16 : *Piper retrofractum* (extrait de Lim, 2012)

***Piper retrofractum* L.** est une plante herbacée de la famille des Piperaceae originaire d'Asie du Sud-Est, et cultivée en Chine, Indonésie et au Cambodge (Figure 16). Le fruit est employé au Cambodge pour le traitement de la jaunisse et des œdèmes consécutifs aux maladies du foie. L'extrait méthanolique et acétonique du fruit ont démontré une activité hépatoprotectrice sur des souris traitées par la D-galactosamine. L'extrait méthanolique de la tige possède une propriété antioxydante, antiinflammatoire, analgésique et diurétique. Enfin, l'extrait aqueux du fruit ne présente pas de toxicité aigüe ni subaiguë sur modèle murins (Lim 2012). Des essais complémentaires pourraient être envisagés sur le fruit pour déterminer son activité antiproliférative sur modèles d'hépatocarcinome humain *in vitro*.

Figure 17 : *Muntingia calabura* (extrait de florafaunaweb)

***Muntingia calabura* L.** est un arbre de 3 à 12 mètres de hauteur originaire d'Amérique du Sud, et la seule espèce appartenant au genre *Muntingia* de la famille des Muntingiaceae (Figure 17). La racine et les feuilles sont utilisées dans le traitement des maladies du foie au Cambodge. La plante est également employée en Amérique latine dans le traitement des pathologies hépatiques. Les extraits de feuilles de *M. calabura* ont montré une activité antioxydante, anti-inflammatoire, antipyrétique et antibactérienne (notamment sur des espèces du genre *Staphylococcus*) (Rofiee et al. 2015). L'extrait méthanolique de feuilles possède un effet hépatoprotecteur *in vivo* et antiproliférative *in vitro* sur différentes lignées cellulaires

cancéreuses (Mahmood et al. 2014). Puisqu'aucune étude n'a été réalisée sur modèles d'hépatocarcinome *in vitro*, cette activité pourrait être évaluée sur les feuilles et les racines.

Figure 18 : *Oroxylum indicum*

***Oroxylum indicum* (L.) Kurz** est un arbre de la famille des Bignoniaceae présent en Asie tropicale (Figure 18). L'écorce et le bois sont employées au Laos, au Cambodge, en Chine et au Vietnam pour soigner la jaunisse. Il est également utilisé en Inde pour traiter la jaunisse et les hépatites virales. Différentes extraits du tronc et de l'écorce ont montré une activité anti-inflammatoire, antimicrobienne et antioxydante (Dinda et al. 2015). L'extrait méthanolique, éthanolique et aqueux de l'écorce et du bois présente une activité hépatoprotectrice *in vivo* (Tripathy et al. 2011). Par ailleurs, deux composés isolés de la plante : l'oroxyline A et la baicaléine ont montré une activité antiproliférative *in vitro* sur cellules cancéreuses hépatiques (HepG2) (Hu et al. 2006; Kinjo et al. 2006). Etant donné son utilisation traditionnelle dans les hépatites virales, une étude de ces propriétés anti-HBV et anti-HCV pourrait être envisagée.

Figure 19 : *Orthosiphon aristatus*

***Orthosiphon aristatus* (Blume) Miq.** est une plante herbacée de la famille des Lamiaceae, originaire d'Asie du Sud-Est (Figure 19). La plante entière est utilisée dans le traitement des hépatites et de la jaunisse au Cambodge, au Laos et au Vietnam. Elle est inscrite à la pharmacopée européenne pour ses propriétés diurétiques. Différents extraits de la plante entière ont montré un effet analgésique, anti-inflammatoire, antipyrétique, antioxydant et diurétique (Ameer et al. 2012). Son activité hépatoprotectrice a également été prouvée sur modèles murins traités par du thioacétamide (Alshawsh et al. 2011). Une fraction de l'extrait aqueux de feuilles a été testée pour son activité sur cellules cancéreuses hépatiques (HepG2) et

montre un faible effet antiprolifératif (Salleh et al. 2011). Ces informations confirment l'usage traditionnel de cette espèce dans les maladies du foie.

Figure 20 : *Senna alata*

***Senna alata* (L.) Roxb.** est un arbuste pantropical appartenant à la famille des Leguminosae (Figure 20). La tige et les feuilles sont employées au Cambodge et au Vietnam pour traiter les hépatites et les ictères. Les feuilles sont aussi utilisées dans différentes régions du monde (Martinique, Ouganda, Togo) pour le traitement des maladies du foie et de la jaunisse (Hennebelle et al. 2009; Kpodar et al. 2016). Des extraits de feuille ont montré une activité analgésique, anticancéreuse, anti-inflammatoire, antioxydante et antimicrobienne. L'activité hépatoprotective d'extraits de feuilles et de fleurs a été prouvée *in vivo* (Lim 2014). Une activité cholérétique a également été montré (Hennebelle et al. 2009). Aucune étude n'étant été réalisée sur cellules cancéreuses hépatiques, des examens complémentaires pourraient être effectués afin de compléter les informations disponibles.

Au final, sur les 9 espèces de plantes les plus citées dans la littérature d'Asie du Sud-Est pour le traitement des maladies du foie, trois plantes (*Curcuma longa*, *Orthosiphon aristatus*, *Phyllanthus urinaria*) sont déjà bien connues et étudiées pour leurs propriétés relatives aux maladies du foie, quatre plantes (*Muntingia calabura*, *Oroxylum indicum*, *Piper retrofractum*, *Senna alata*) pourraient faire l'objet d'examens pharmacologiques complémentaires, et enfin deux plantes (*Melastoma sanguineum* et *Willughbeia edulis*) devraient être étudiées d'une manière approfondie sur le plan phytochimique et pharmacologique.

CHAPITRES

CHAPITRE 1 : ETUDE EPIDEMIOLOGIQUE RETROSPECTIVE DE DOSSIERS PATIENTS

1. Contexte et objectifs

Au début de notre étude, le virus de l'hépatite B était considéré comme facteur de risque prédominant du cancer du foie dans la région indochinoise. Au Cambodge, le cancer du foie est la première cause de décès par cancer tous sexes confondus dans le pays, ainsi que le premier cancer en termes de morbidité et de mortalité chez les hommes[8].

Ces données sont basées sur une projection estimative à partir de la situation observée dans les pays voisins, et ne s'appuient pas sur les données intrinsèques du pays. En effet, dû en grande partie à un contexte historique récent difficile et des moyens limités, le manque d'outils de surveillance épidémiologique et l'absence de registres des cancers ont empêché le recueil et l'analyse de ces informations.

Afin de déterminer les facteurs de risque majoritaires des patients atteints d'un cancer du foie au Cambodge et de préciser leurs profils sociodémographique, clinique et biologique, nos travaux se sont focalisés sur l'étude de dossiers médicaux de patients cancéreux de l'hôpital Calmette de Phnom Penh, l'un des deux seuls hôpitaux référents en cancérologie dans le pays[9].

[8] Informations extraites du site internet : http://globocan.iarc.fr

[9] L'autre hôpital spécialisé en cancérologie au Cambodge est l'hôpital de l'Amitié khméro-soviétique situé à Phnom Penh.

2. Matériels et Méthodes

Une étude d'épidémiologie clinique rétrospective des patients ayant développé un cancer du foie a été réalisée dans deux services de l'Hôpital Calmette : le service de cancérologie et le service d'hépato-gastroentérologie[10].

Un total de 553 dossiers patients extraits des archives de ces services ont été révisées pour une période couvrant pratiquement 13 ans (de janvier 2003 à mai 2015). Les informations disponibles incluaient des données sociodémographiques, des manifestations cliniques, des analyses biologiques, des examens d'imagerie, les méthodes de diagnostic, ainsi que les traitements alloués.

3. Principaux résultats

Sur les 553 dossiers étudiés, 511 patients ont développé un carcinome hépatocellulaire (CHC) et 42 un cholangiocarcinome (CCA). Parmi les 511 cas de CHC, 44,3% étaient infectés par le virus de l'hépatite B, 43% par celui de l'hépatite C et 43,2% ont mentionné boire de l'alcool régulièrement.

Par ailleurs, le diagnostic reposait essentiellement sur l'association de l'échographie et de la mesure de l'alpha-fœtoprotéine (AFP). Peu d'histoires cliniques faisaient intervenir des méthodes d'imagerie telles que la tomodensitométrie (CT scan) [15,8% des patients] et l'imagerie par résonance magnétique (IRM) [0,2%]. Les prélèvements de tissus biologiques comme la biopsie ne furent pas pratiqués [0%], alors que cela constitue pourtant une méthode de référence du diagnostic.

Enfin, les stratégies thérapeutiques employées comprenaient essentiellement le traitement palliatif des patients dans 84% des cas de CHC et 73,8% des cas de CCA. Seulement

[10] En Annexe 3 sont rassemblés les documents autorisant l'étude sur le territoire cambodgien. Ils ont été fournis par l'hôpital Calmette et par le Comité National d'Ethique pour la Recherche en Santé Publique (NECHR) du Cambodge

2% des patients ont été traités chirurgicalement (hépatectomie), et aucun n'a subi de transplantation hépatique.

4. Discussion et conclusion

Tout d'abord, les résultats de notre étude mettent en lumière le manque de moyens matériels et techniques disponibles dans les hôpitaux cambodgiens, du moins à l'Hôpital Calmette. Ces informations indiquent que la prise en charge des patients atteints de cancer du foie dans le pays ne suit pas les recommandations internationales, et est largement insuffisant par rapport aux standards internationaux.

Aussi, la proportion similaire de patients CHC porteurs du VHB et de patients porteurs du VHC suggère une importance proportionnelle du virus de l'hépatite C en tant que facteur de risque. Cette forte incidence peut s'expliquer par une contamination importante de la population par le virus de l'hépatite C, notamment à cause des pratiques de soins inappropriées, tel que la mauvaise stérilisation des équipements médicaux, l'absence de contrôle qualité et de recyclage, et l'utilisation de pratiques d'injections à risque.

Toutes ces informations devraient permettre aux autorités locales de mettre en place des études épidémiologiques plus poussées pour confirmer la forte prévalence du virus de l'hépatite C parmi les cas de cancer du foie au Cambodge, mais également d'améliorer le dépistage des populations à risque de développer un cancer du foie, ainsi que d'instaurer des mesures de prévention afin de diminuer la transmission des agents à risque et la consommation de boissons alcoolisées.

Ce travail s'est traduit par la rédaction d'un article soumis le 22 décembre 2015 à la revue scientifique internationale indexée *Oncology*, publié dans cette même revue le 2 Juin 2016 après révision, et intitulé :

Article 1. « A 13-Year Retrospective Study on Primary Liver Cancer in Cambodia: A Strikingly High Hepatitis C Occurrence among Hepatocellular Carcinoma Cases »

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L'article est présenté ci-dessous *in extenso* dans sa forme originale.

L'article est disponible en libre accès à l'adresse internet suivante :

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A 13-Year Retrospective Study on Primary Liver Cancer in Cambodia: A Strikingly High Hepatitis C Occurrence among Hepatocellular Carcinoma Cases

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Key Words
Hepatocellular carcinoma · Cholangiocarcinoma · Hepatitis B virus · Hepatitis C virus · *Opisthorchis viverrini* · Cambodia

Abstract
Objectives: Hepatocellular carcinoma (HCC) is the main type of primary liver cancer (PLC) worldwide, but cholangiocarcinoma (CCA) may be predominant in some specific regions of Southeast Asia. The aim of the present study was to delineate a pattern of Cambodian PLC patients attending the Calmette Hospital in the Cambodian capital Phnom Penh. ***Materials and Methods:*** A total of 553 medical charts diagnosing PLCs from January 2003 to May 2015 were obtained from both the Oncology and Hepato-Gastroenterology Departments of the Calmette Hospital. ***Results:*** HCC was the predominant type of PLC recorded, with 511 cases (92.4%), whereas CCA represented merely 7.6% (42 cases) of the overall series. Hepatitis B virus (HBV; 44.3%) and hepatitis C virus (HCV; 43%) infection rates were similar among the HCC patients, while small subsets of CCA patients were infected with HBV (15.4%) or HCV (11.5%). Most HCC (84%) and CCA (73.8%) patients received palliative treatment only. ***Conclusion:*** The present study indicates that HCC is the main form of primary hepatic neoplasm among PLC patients attending a hospital in Cambodia. HBV and HCV infections represented equivalent burdens and major contributing factors to HCC. Therefore, the implementation of prevention programs for these infectious agents should become a priority for health policy makers in the country.

The work was conducted in the Calmette Hospital, Phnom Penh, Cambodia.

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Introduction

Primary liver cancer (PLC) is associated with a high mortality rate; it is the second cause of cancer-related death worldwide [1]. Along with Sub-Saharan Africa, Eastern and Southeastern Asia represents a major region of incidence of the disease, ranking respectively first and second, both in terms of incidence and mortality [2]. In Cambodia, very little data about the cancer burden is currently available. According to the estimates available, 20% of all male cancers in the country are PLCs, making it the most frequent tumor in Cambodian men [3, 4].

Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) are the two main histologic types of PLC [5]. HCC is globally known as the leading form of liver cancer and accounts for up to 85% of incidences worldwide [6]. However, CCA represents the predominant form of PLC in some specific regions of Southeast Asia. For instance, CCA is responsible for 90% of the liver cancer cases monitored in the Khon Kaen province of Thailand [7–9].

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are considered to be the main etiological factors for HCC. These two infectious agents are estimated to be responsible for more than three quarters (78%) of the HCC cases worldwide [10]. In Southeast Asia, which is a highly endemic area for HBV, the major risk factor for HCC is chronic HBV infection [2, 11]. So far, the number of cases of HCC related to HCV infection in the region was poorly known and considered to be of marginal significance when compared with that linked to HBV [10]. Concerning the risk factor for CCA, it results largely from the infection with the liver fluke *Opisthorchis viverrini* endemic to Cambodia, Laos, Thailand, and Vietnam [8, 9, 12]. According to the International Agency for Research on Cancer (IARC), 6 million people are infected with *O. viverrini* in Thailand, 2 million in Laos, and 600,000 in Cambodia [13].

In Cambodia, hepatitis B surface antigen (HBsAg) seroprevalence in the general population is estimated at between 7.7 and 13%, whereas the hepatitis C antibody seroprevalence range is between 2.3 and 14.7% [4, 14–18]. Concerning the liver fluke, a high parasitic prevalence has been recorded in some provinces, especially in Kampong Cham, Kampong Thom, Kandal, Kratie, and Takeo provinces. In these regions, infestation rates among the general population range from 1.3 to 65.1% [19–22].

With the exception of a single study that aimed to describe the clinical and demographic features of Cambodian HCC patients at a single center [4], no data have been published on CCA and HCC tumor presentation in this country. Therefore, the present work was carried out with the objective of describing and comparing the clinico-epidemiological features of both CCA and HCC in Cambodian patients attending the Calmette Hospital (Phnom Penh, Cambodia) over a 13-year period.

Materials and Methods

The Department of Oncology of the Calmette Hospital, was opened in 2012. Prior to 2012, the Department of Hepato-Gastroenterology was in charge of attending to the PLC patients. We thus carried out a retrospective study of the archives of these two departments in order to collate the PLC medical charts that had been compiled over an almost 13-year period from January 2003 to May 2015.

A total of 553 medical charts of PLC patients were reviewed. The data provided on paper, such as medical reports, daily follow-up sheets, laboratory and imaging results, were extracted from the patients' files available in each department and recorded in Excel file version 2013 (Microsoft). Sociodemographic information, clinical manifestations, biological analyses, medical imaging, method of diagnosis, and treatment outcomes were also monitored and compiled into the database.

However, due to poor resources in the country, none of the files examined included all items listed above. As a consequence, serological parameters, biochemistry analyses, diagnostic imaging, and therapeutic options were not always available (ranging from 60% for the γ-glutamyl transpeptidase level to 100% for clinical features).

The diagnosis of HCC at the Calmette Hospital was based on clinical, biochemical, and imaging criteria. The three decisive features were: (1) the presence of liver cirrhosis and signs of neoplastic disease in an alcoholic patient or a patient chronically infected with HBV or HCV, (2) α-fetoprotein (AFP) >350 ng/ml, and (3) nodule size over 20 mm (by ultrasound examination). In other cases (AFP <350 ng/ml and/or nodule size ranging between 10 and 20 mm), the diagnosis was made using computed tomography showing a typical arterial pattern. Regarding clinical symptoms, ascites, portal hypertension, jaundice, liver mass at palpation, hepatomegaly, liver collateral circulation, splenomegaly, hemorrhages, bruises, encephalopathy, pain, and weight loss were the most frequent features found in patients. In the case of CCA patients, the diagnosis was predominantly made based on personal background (presence of primary sclerosing cholangitis), clinical features (jaundice, pale stool, dark urine, and pruritus), biochemical parameters (elevation in total bilirubin, direct bilirubin, and aminotransferases) suggesting a biliary obstruction, the presence of tumor markers (elevation in CEA and CA 19-9, and normal AFP) as well as on ultrasound examination (biliary ductal dilatation and related mass lesion) and on the results of the CT scan (bile duct dilatation and tumor mass).

Differential diagnosis between HCC and CCA was based on several criteria: personal background and risk factors (chronic HBV/HCV infection, alcohol consumption), clinical features (liver cirrhosis, signs of portal hypertension, signs of biliary obstruction, ascites), laboratory parameters (transaminases, bilirubin, AFP, CEA, CA 19-9), and imaging techniques (location and size of the mass, enhancement pattern).

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Table 1. Characteristics of the HCC and CCA patients

Patients characteristics	HCC patients	CCA patients
Overall subjects		
Total number	511 (92.4%)	42 (7.6%)
Age (n = 511/42), years		
Mean ± SD	58.1±11.9	62.3±11.9
Range	28–91	40–90
Sex (n = 511/42)		
Male	369	21
Female	142	21
Sex ratio	2.6	1
Symptoms and signs (n = 511/42)		
Abdominal pain	263 (51.4%)	25 (59.5%)
Ascites	340 (66.5%)	12 (28.6%)
Asthenia	132 (25.8%)	9 (21.4%)
Fever	69 (13.5%)	7 (16.7%)
Gastrointestinal bleeding	168 (32.9%)	1 (2.4%)
Hepatomegaly	226 (44.2%)	9 (21.4%)
Jaundice	131 (25.6%)	31 (73.8%)
Edema	37 (7.2%)	ND
Pruritus	6 (1.2%)	11 (26.2%)
Splenomegaly	278 (54.4%)	5 (11.9%)
Associated disorders and risk factors		
Alcohol drinking (n = 324/30)	140 (43.2%)	4 (13.3%)
Chronic HBV infection only (n = 467/26)	207 (44.3%)	4 (15.4%)
Chronic HCV infection only (n = 467/26)	201 (43.0%)	3 (11.5%)
Chronic HBV/HCV infection (n = 467/26)	20 (4.3%)	0
Cirrhosis (n = 460/24)	454 (98.7%)	9 (37.5%)
Diabetes (n = 457/35)	90 (17.6%)	4 (11.4%)
Hypertension (n = 456/35)	102 (20.0%)	7 (20.0%)

Unless otherwise indicated the values represent the number. Mean values are associated with the standard deviation (SD). The sex ratio was calculated as males:females. n = Total number of HCC patients/CCA patients with data available; ND = not determined.

Imaging modalities included mostly ultrasonography testing for 94.3% HCC patients and 94.1% CCA patients, while only 15.2% HCC patients and 58.8% CCA patients had a computed tomography scan. Neither liver biopsy nor magnetic resonance imaging was performed.

Regarding the clinical chemistry, laboratory analyses were performed in the Biochemistry Department of the Calmette Hospital, where the following parameters were determined: γ-glutamyl transpeptidase, alkaline phosphatase, aspartate and alanine aminotransferase, bilirubin, prothrombin time, hemoglobin, albumin, and AFP. It should be noted that, until 2014, the AFP level was indicated as '>350 ng/ml' for each value above 350 ng/ml in the biological report. Thus, no fully accurate data were available for this parameter.

Other information, which would have been useful for the study, including tumor grade, infection by *O. viverrini*, aflatoxin contamination, arsenic exposure, type of alcohol consumed, pack-year units for cigarette smoking, and associated disorders (except diabetes and hypertension), was not present in the medical records. Moreover, no precise information was given for the serological tests used in the diagnosis of HBV and HCV.

Different statistical analyses were performed according to the set of data analyzed. Data were recorded in Microsoft Excel 2013 (Microsoft) and later analyzed using SPSS Statistic software version 22.0 (IBM). Comparisons between groups (numerical data) or proportions (categorical data) were realized using the Student t test or Mann-Whitney test, ANOVA and the Fisher exact test as appropriate. Results of the analysis were considered statistically significant if the probability of occurrence by chance was less than 5%. The free and open-source QGIS software version 2.8.2 was used to elaborate repartition maps.

The study was carried out in accordance with the ethical principles of the Declaration of Helsinki and was approved by the National Ethics Committee for Health Research under the guidance of the Ministry of Health of Cambodia in January 2015.

Results

Descriptive Characteristics of the 553 Patients

Out of the 553 patients included, 511 (92.4%) had a diagnosis of HCC and 42 (7.6%) were diagnosed with CCA. The demographic characteristics of HCC and CCA

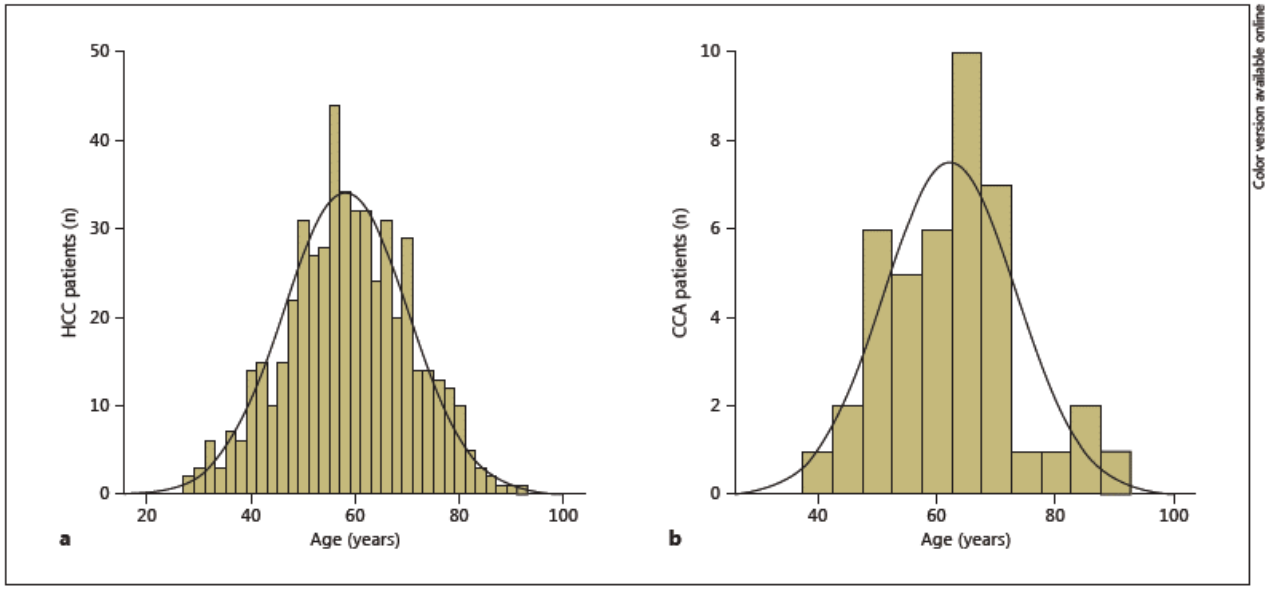

Fig. 1. Age distribution of the 511 HCC (**a**) and 42 CCA (**b**) patients admitted to the Calmette Hospital between 2003 and 2015.

patients as well as the clinical manifestations, risk factors, and associated disorders are given in table 1.

Of the HCC patients, a large majority (76.9%) were more than 50 years old. The distribution of the data was fairly symmetrical (i.e., skewness coefficient = –0.04). Of the 42 CCA patients, 81% were more than 50 years old (fig. 1).

A large subset of the HCC patients was farmers (n = 136; 33.6%), whereas a majority of CCA patients were housewives or unemployed (n = 14; 40%). Professions of the remaining HCC cases were, in decreasing order, either housewives/unemployed (n = 98; 24.2%), public sector employees (n = 87; 21.5%), or private sector employees (n = 84; 20.7%). Regarding CCA cases, 10 (28.6%) were farmers, 7 (20%) were private sector employees, and 4 (11.4%) were public sector employees. Following hospitalization, the average length of the medical stay was 6.5 and 5.9 days for HCC and CCA patients, respectively.

As mentioned above, information was not always available for the entire series. As a consequence, we mention the true numbers corresponding to each parameter, which is the total number of HCC or CCA cases with data recorded (tables 1, 2).

Interestingly, chronic HBV and HCV infections (i.e., HBsAg+ and anti-HCV+, respectively) showed similar rates in the HCC patient population, with 207 (44.3%) HBsAg+ cases and 201 (43%) anti-HCV+ cases. Moreover, 20 patients (4.3%) were infected both with HBV and HCV, while only 38 patients (8.1%) were neither infected with HBV nor with HCV.

The mean age of HBsAg+ and anti-HCV– patients (n = 207) was 52.1 ± 12.0 years, with an age range of 28–91 years. The mean age of HBsAg– and anti-HCV+ patients (n = 201) was 63.9 ± 12.0 years, with a range of 36–89 years.

Amongst HCC patients under 50 years, 99 (73.9%) were infected only with HBV (HBAgs+/anti-HCV–), whereas only 12 (9%) were infected with HCV alone (HBAgs–/anti-HCV+). Reciprocally, among the 229 HCC cases aged over 60 years, 53 (23.1%) were infected with HBV, and 133 (58.1%) were infected with HCV.

In CCA patients, serological results were available for only 26 patients. Among these, 19 (73.1%) were neither infected with HBV nor with HCV, 4 (15.4%) had chronic HBV infection, and 3 (11.5%) had chronic HCV infection.

As shown in table 2, an elevation of serum aspartate aminotransferase and alanine aminotransferase was found, respectively, in 98 and 61% of the HCC cases and in 89.7 and 46.2% for the CCA cases. Total and direct bilirubin levels were increased in 84.8 and 88.6% of the HCC cases and in 89.5 and 97.4% of the CCA patients. γ-Glutamyl transpeptidase and alkaline phosphatase levels were elevated for 82.3 and 73.2% of the HCC patients,

Table 2. Laboratory and radiologic findings of the HCC and CCA patients

Patient characteristics	HCC patients	CCA patients
Liver function test		
AST (R <38) (n = 461/39), IU/l	196.8 (14–3,359)	124.6 (24–351)
ALT (R <50) (n = 461/39), IU/l	77.1 (10–415)	69.1 (13–102)
Total bilirubin (R <10) (n = 446/38), mg/l	61.6 (4–1,069)	183.5 (6–485)
Direct bilirubin (R <2.5) (n = 446/38), mg/l	36.4 (1–897)	123.3 (2–401)
GGT (R <66) (n = 344/32), IU/l	246.8 (12–3,412)	284.7 (11–1,213)
ALP (35< R <123) (n = 306/29), IU/l	278.6 (36–1,928)	431.2 (85–1,152)
Prothrombin time (70< R <100) (n = 420/28), %	60.4 (5–100)	71.8 (26–100)
Hemoglobin (130< R <170) (n = 366/16), g/l	101.7 (25–171)	92.3 (32–139)
Albumin (38< R <51) (n = 392/23), g/l	28.8 (15–52)	29.3 (20–48)
AFP (R <10) (n = 366/19)		
<10 µg/l	53 (14.5%)	16 (84.2%)
10–100 µg/l	50 (13.7%)	2 (10.5%)
100–350 µg/l	32 (8.7%)	0
>350 µg/l	231 (63.1%)	1 (5.3%)
Imaging features		
Nodule size (n = 345/13), mm	64.1 (10–185)	39.8 (22–72)
Multinodular type (n = 319/15)	212 (66.5%)	6 (40%)
Therapy (n = 511/42)		
Palliative	429 (84%)	31 (73.8%)
Systemic/oral chemotherapy	71 (13.9%)	11 (26.2%)
Hepatectomy	11 (2.1%)	0
PEI/TOEC	0	0
Transplantation	0	0

Values represent mean (range) or number. n = Total number of HCC patients/CCA patients with data available; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = γ-glutamyltranspeptidase; PEI = percutaneous ethanol injection; R = reference values; TOEC = transarterial oil chemoembolization.

respectively, and for 90.6 and 93.1% of the CCA patients. Statistical analyses of biochemistry parameters were also performed (online suppl. table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000446398).

The AFP level was documented for 366 HCC patients and 19 CCA patients, with the corresponding percentage of 63.1 and 5.3% values above 350 ng/ml. High AFP levels (>350 ng/ml) among HCC patients are associated significantly with larger nodules ($p = 0.05$) and multifocal tumor ($p = 0.03$) when compared with low AFP expressers (<350 ng/ml). Moreover, statistical analyses between a normal AFP level (<10 ng/ml) and an abnormal AFP level (>10 ng/ml) in HCC patients indicate a significant association with the latter and chronic HCV infection ($p = 0.05$).

Palliative treatment was the only option for 429 (84%) HCC patients; only 11 (2.1%) patients had a hepatectomy, 10 (2.0%) patients were given oral chemotherapy with sorafenib, and 61 (11.9%) patients received tamoxifen. Of the CCA patients, 11 (26.2%) benefited from systemic chemotherapy (including GEMOX, FOLFOX, and FOLFIRI protocols), whereas 31 patients (73.8%) received palliative treatment only. Some of the most affluent patients decided, however, to benefit from surgical intervention outside the country. They represent approximately 5–10% of the patients attending the Calmette Hospital for liver tumor.

Clinicobiological Correlations

Age was an important feature structuring the series of patients with HCC. When considering the whole cohort in age quartiles (fig. 2a–e), significant differences related to age were found for HBsAg seroreactivity ($p < 0.0001$), sex ratio ($p = 0.0014$), nodule size ($p = 0.0016$), and alcohol consumption ($p < 0.0001$) that decrease with age, while the proportion of diabetes ($p = 0.0005$) and the anti-HCV seroreactivity increase with age ($p < 0.0001$). Finally, the prevalence of abdominal pain ($p = 0.0008$) and jaundice decrease with age ($p = 0.008$).

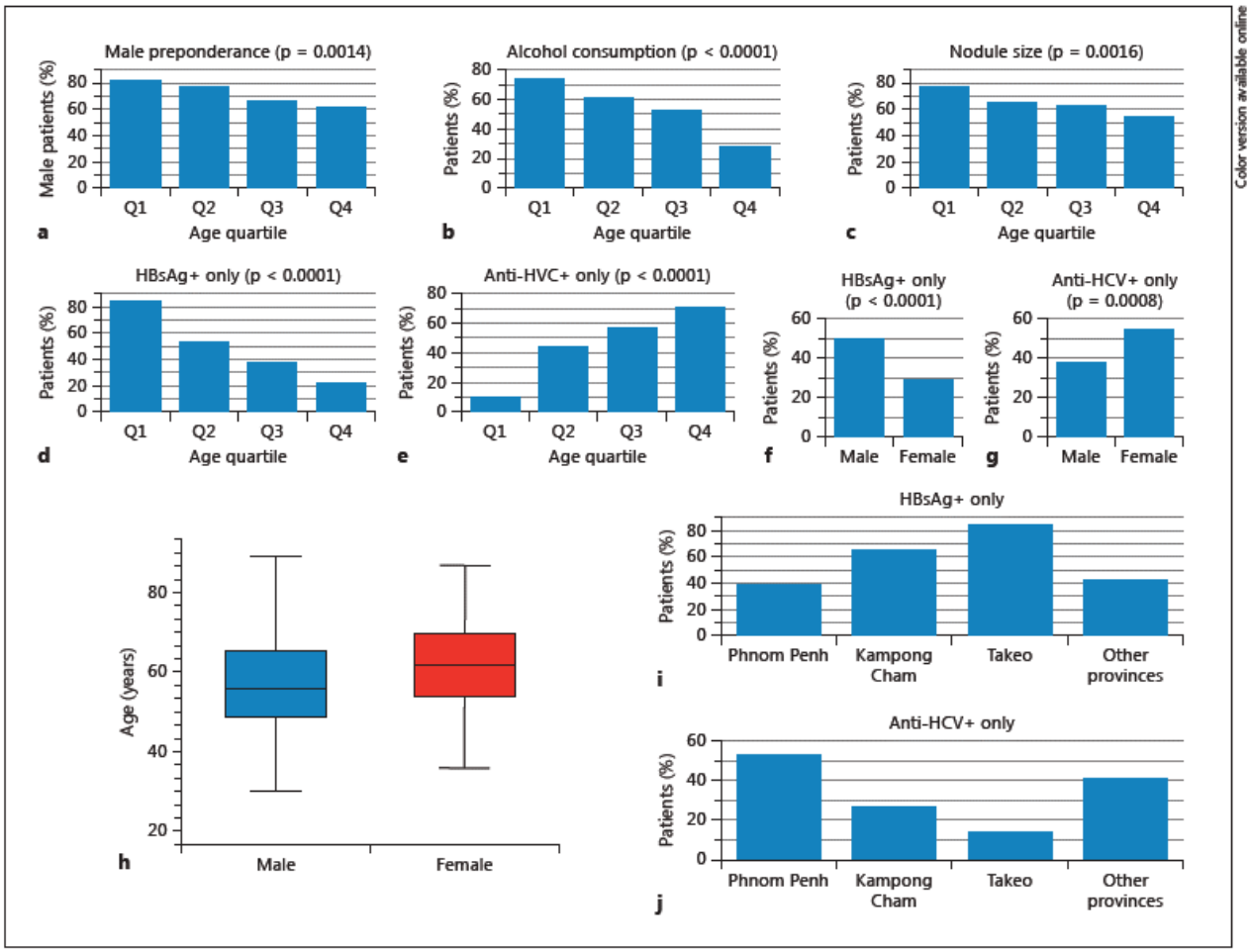

Fig. 2. Age quartile comparisons among HCC patients according to male preponderance (**a**), alcohol consumption (**b**), nodule size (**c**), HBsAg+ only (**d**), and anti-HCV only (**e**). Q1 = Quartile 1 (28–49 years old); Q2 = quartile 2 (50–57 years old); Q3 = quartile 3 (58–65 years old); Q4 = quartile 4 (66–91 years old). Gender comparisons among HCC patients: percentage of males and females with HBsAg+ only (**f**) and anti-HCV+ only (**g**). **h** Box plot representing age distribution in male (blue) and female (red) HCC patients (colors in the online version only). Geographical comparisons among HCC patients: bar charts displaying the percentage of HBV- (**i**) and HCV- (**j**) infected patients with HCC according to their province of residence.

In addition, we conducted gender comparisons amongst HCC patients (fig. 2f–h). The results indicate that men develop tumors 3 years earlier than women (56.8 vs. 61.5 years; $p < 0.0001$), hepatomegaly prevalence was higher in men (48.8 vs. 32.4%; $p = 0.001$), while arterial hypertension was more frequent among women (16.8 vs. 36.7%; $p < 0.0001$). Regarding lifestyle risk factors, alcohol consumption and tobacco smoking were, as expected, significantly higher in men than in women (50.6 vs. 17.9% and 48.9 vs. 5.6%; $p < 0.0001$ in both cases), whereas anti-HCV seroreactivity was more frequent in the female group (38.1 vs. 55.4%; $p = 0.0009$). Symmetrically, HBsAg seropositivity was more prevalent among males (50 vs. 29.4%; $p < 0.0001$).

We then compared the two monoinfected subsets of patients. The results show that the sex ratio was significantly higher in the HBV+ group than in the HCV+ group (4.4 vs. 1.8; $p < 0.0001$), the patients were younger (52.1 vs. 63.8 years; $p < 0.0001$), and alcohol consumption was more frequent in the HBV+ group (45.7 vs. 32.5%; $p = 0.04$), whereas diabetes mellitus was less frequent (15.5 vs. 25.6%; $p = 0.02$). Regarding biochemical features, serum

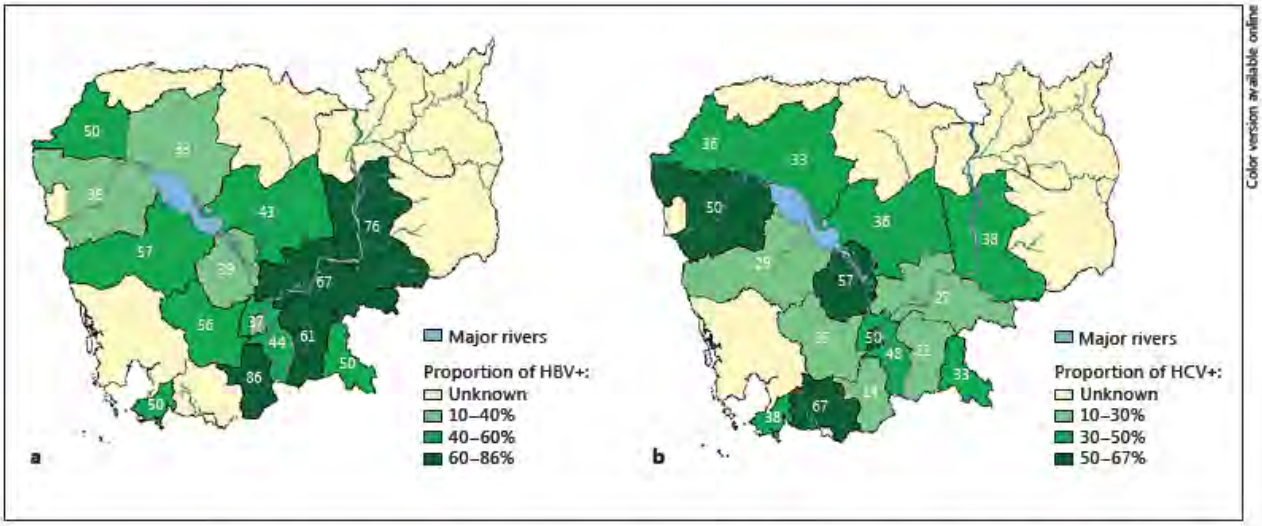

Fig. 3. Regional distribution of HBV- (**a**) and HCV- (**b**) infected patients with HCC attending the Calmette Hospital between 2003 and 2015. Numbers on the map indicate the percentage of HBV-HCC (**a**) and HCV-HCC (**b**) patients.

alkaline phosphatase and serum aspartate aminotransferase were significantly higher (302.8 vs. 218.7 and 213.5 vs. 170.4 IU/l; $p < 0.05$ in both cases), and tumor nodules were bigger in the HBV+ group (67.5 vs. 59.3 mm; $p = 0.04$) compared to the HCV+ group.

Frequency and Spatial Distribution of HCC and CCA Patients

Among the HCC patient population, the highest number of cases originated from the Phnom Penh province (n = 197; 38.6%) followed by the Kandal province (n = 59; 11.5%) and the Kampong Cham province (n = 46; 9%). In the case of CCA, Phnom Penh (n = 13; 30.9%), Kampong Cham, and Kandal provinces (both n = 6; 14.3%) exhibited the highest values (online suppl. fig. 1).

Concerning the characteristics of HCC patients for each province, we noticed that Phnom Penh and Kandal provinces display similar features, such as an older age of patients (a mean of 60.8 and 59.4 years, respectively), a male:female sex ratio of 2.5 and 2.3, respectively, and a high proportion of diabetes mellitus (30.2 and 25.9%, respectively). Conversely, patients from the Prey Veng and Takeo provinces, on the southern border, share comparable features of young age at diagnosis (a mean of 52.8 and 51.4 years, respectively), an elevated sex ratio (5.6 and 14, respectively), a high proportion of alcohol consumers (53 and 33%, respectively), and a high number of HBsAg+ cases (61 and 86%, respectively). Concerning the spatial distribution of HBsAg+ and anti-HCV+ HCC patients, we observed a great disparity of distribution of the two types of hepatitis viruses between different Cambodian provinces (fig. 2i, j, 3).

In Phnom Penh, the proportion of patients infected with HBV was significantly lower than in other provinces (39.8 vs. 50.5%, respectively, $p = 0.03$), whilst, in contrast, a significantly greater subset was infected with HCV (53.2 vs. 39.4%, respectively, $p = 0.004$). Similarly, Kampong Cham province displayed a strikingly different proportion of patients infected either with HBV or with HCV (66.7 and 27.3%, respectively) with significant differences in both subpopulations ($p = 0.006$ and $p = 0.02$, respectively). Finally, Takeo province showed the highest proportion of HBV+ patients (85.8%, $p = 0.004$) and the lowest proportion of HCV+ patients (14.3%, $p = 0.03$).

Discussion

This study aimed to characterize the forms and the etiological factors of PLC in Cambodia through a retrospective analysis of the cohort treated in the Calmette Hospital from 2003 and 2015. It is also the first review on CCA patients in the country and provides relevant information

about epidemiological features of HCC and CCA among Cambodian people.

Interestingly, we found a similar rate of chronic infection with HBV (44.3%) and HCV (43%) among HCC patients. This observation was unexpected, since the overall contribution of HBV infection to PLC is more than twice higher than that of HCV infection worldwide, 53 and 25%, respectively [10].

However, the infection rate found in our study cannot be extrapolated to the whole country, since the only available survey of PLC cases in Cambodia showed that the HBV and the HCV infection rate in HCC patients was 46.4 and 21.5%, respectively [4]. In this earlier study, the demographic and clinical characteristics of 281 cases diagnosed as primary HCC in the Khmer-Soviet Friendship Hospital (Phnom Penh, Cambodia) were described. The majority of the HCC patients were aged between 40 and 69 (74.4%) and the male:female ratio was 3. These results are consistent with our data, since we found that 74.3% of the HCC cases were aged between 40 and 69 years, and the male:female ratio was 2.6. Furthermore, the mean age of the HCV+ HCC cases was 10 years higher than those with HBV+ (61.4 vs. 48 years old), which is quite similar to our results (63.9 vs. 52.1 years old). Moreover, the male:female ratio for HBV+ HCC patients was also similar to that found in our survey (4.4 and 4.5, respectively). Finally, the male:female ratio for HCV+ HCC cases was different to that found in our study (4 and 1.8, respectively). These data could be explained by the small number of reported cases in the Khmer-Soviet Friendship Hospital (i.e., 45 patients) as described below.

In other Southeast Asian countries, a greater proportion of HBV infections compared to HCV infections is also consistently found among HCC patients. In some regions of Thailand, chronic HBV infection was identified in 49.6–80% of HCC cases and chronic HCV infection in 10.1–19.2% [23–25]. In Vietnam, a review estimated the HCC-HBV patients at 80–90% and the HCC-HCV patients at 1–5% [26]. In Malaysia, a recent study recorded HBV in 57.6% of HCC patients and discovered HCV in only 2.4% of the same cohort [27]. In the Asia-Pacific region, chronic HBV infection accounts for 70–80% of the HCC cases, with the exception of Japan, Pakistan, and Mongolia where chronic HCV infection is predominantly found (68, 45, and 40% of the HCC cases, respectively) [23, 28].

Many studies have already reported a large local variation in the prevalence of HBV and HCV infection, especially in Southeast Asia and Cambodia. For instance, the prevalence of HBsAg was reported to be 4.6% in Siem Reap province, 6.5% in Samlot City (Battambang province), 8% in Takeo province, 8.8% in Pailin City (Pailin province), 9.4% in Banteay Meanchey province, and 10.8% in Cambodian migrant workers from Thailand [17, 18, 29–31]. For HCV infection, the prevalence of anti-HCV was 2.3% among Cambodian migrant workers from Thailand, 5.8% in Siem Reap province, 6.5% in Takeo province, 12.3% in Pailin City, and 17% in Samlot City [15, 17, 18, 29]. These observations could explain the difference between the work of Narin et al. [4] and our survey. This previous study was conducted in the Khmer-Soviet Friendship Hospital where patients are usually coming from rural areas with lower socioeconomic status, while in our study, patients are mostly capital city dwellers and belong to a higher social class.

The high number of incidences attributed to HCV in our work may appear surprising. However, it is known that there is still an increasing impact of HCV infection on the HCC burden in Asia. A recent study, conducted among Southeast Asian migrants in the US, revealed that both HBV and HCV are major causes of HCC in these populations [32]. Moreover, a recent review has identified chronic hepatitis C as the true driving force for the increased incidence of HCC in Asia [33].

In Cambodia, unsafe injection practices, the absence of quality control and recycling, and the lack of proper sterilization of surgical instruments are known to be the main risk factors for HCV contamination [34, 35]. Therefore, inappropriate health-care practices plausibly represent the predominant source of HCV contamination in the country. Much of the data we recorded support these hypotheses. In this regard, it was clearly found that women with HCC show a higher rate of hepatitis C than men. This observation has been confirmed in a recent study on Asian migrants in the US [32], showing that female gender is a risk factor for HCV-related HCC. According to Vong et al. [35], Cambodian women are more likely to receive therapeutic injections than men and are, therefore, more exposed to unsafe medical practices. Moreover, in an urban area such as Phnom Penh Province, where access to health care is easier than anywhere else in the country, patients were significantly more affected by HCV infection than HBV infection. Lastly, HCC cases with chronic HCV infection were not distributed homogenously as it is apparently the case with HBV-infected patients. These erratic contaminations could result from unsafe practices employed by local practitioners as recently shown for the 2014 HIV infection outbreak in the village of Roka (Kandal province) [36].

The clinicopathological features of HBV- and HCV-related HCC cases were drastically different. HBV-infect-

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ed patients displayed a strong male predominance, younger age, tobacco and alcohol dependency, and an aggressive tumor presentation (liver functions, frequent hepatomegaly, nodule size) whereas HCV-infected cases displayed a lower male:female ratio, an older age, and, as expected, maturity-related comorbidities (diabetes mellitus and hypertension). Similar findings have already been reported from previous studies. The age difference at diagnosis is usually explained by the natural history of infection [37–39]. Indeed, HBV contamination occurs mainly in the perinatal period or in childhood, whereas exposure to HCV usually occurs in adulthood. Moreover, the different mechanism in hepatocarcinogenesis between HBV-HCC and HCV-HCC could also explain these results.

A large majority of the PLCs recorded at the hospital was diagnosed as HCC (92.4%), while only few CCA cases were reported (7.6%). Given the high prevalence of *O. viverrini* infection (i.e., the main risk factor of CCA in the region) in some Cambodian provinces, the number of CCA patients was expected to be more important [40]. In Thailand, the country with the populations presenting the highest rates of infestation by *O. viverrini*, the proportion of diagnosed CCA cases reaches 22.1% of all PLC in Bangkok, but ranges from 4% (Songkhla province in the south) to 82.1% (Khon Kaen province near the Laotian border in the north) [5]. Several limitations of the survey such as the lack of histopathological examination could explain the low proportion of CCA diagnosed in the Calmette Hospital.

As mentioned above, our study has several limitations. First, missing data have already been reported as an important issue in Cambodian hospitals [4]. This situation is primarily due to the high cost of medical investigations, which, in the absence of health insurance, have to be paid by the patients [3]. Second, precise biological data on risk factors associated with these diseases were not obtained (i.e., *O. viverrini* infection, quantification of HBV DNA and HCV RNA, investigation of viral replication level). The third limitation of the present study stems from the fact that we studied liver cancer in a single Cambodian health-care facility, which is only partly representative of the overall HCC and CCA epidemiology of the country. Indeed, some patients are treated in private clinics and are not referred to a public hospital; others prefer to go to foreign countries (Vietnam, Thailand, and Singapore), while poor people cannot afford the cost of treatment and are either treated in a provincial hospital or not at all. Another limitation of our work is that the diagnosis was based on clinical features, tumor markers, and imaging tests. No histopathological examination was performed, and thus there might be misdiagnosis, albeit in a rather limited number of cases given the very evocative clinical context of the diseases reported (viral serology, AFP level, ultrasonography and computed tomography outcome). Finally, our survey is characterized by shortcomings inherent to cross-sectional observational studies and as such cannot compete with case-control or prospective studies. However, it has the merit to provide preliminary insights to conduct more informative epidemiological research in the future.

In conclusion, PLC represents a worrying public health issue in Cambodia. In our study, chronic HBV and HCV infections are contributing evenly to the bulk of HCC cases (44.3 and 43%, respectively), while *O. viverrini* infection is suspected to contribute to the CCA cases. The number of incidences attributed to HCV in the present series was surprisingly high (>40%) for a continental Southeast Asian country suggesting that the epidemiology of HCC has changed locally during the last decades. This result should be taken seriously, because it is reminiscent of the current ongoing HCC catastrophe in Mongolia [41]. Another observation that should stimulate the implementation of further investigations, preventive measures, and public information is the association of alcohol consumption and the early development of HCC in men. This observation is often overlooked in scientific literature and should prompt surveys investigating mutagenic/carcinogenic properties of local alcoholic beverages [42]. Our findings should now be used by local authorities in order to improve the surveillance of populations at risk to develop HCC or CCA as well as to prevent the diseases by avoidance both of transmission of the relevant infectious agents and the consumption of carcinogenic compounds.

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Erratum

In the article by Mitchell et al., entitled 'Validation of a preclinical model of diethylnitrosamine-induced hepatic neoplasia in Yucatan miniature pigs' [Oncology 2016;91:90–100, DOI: 10.1159/000446074], there is an error in the Materials and Methods section. Under the subheading of Anesthesia, the text reads:

For all procedures, pigs were anesthetized via intramuscular injection with either tiletamine or zolazepam (6 mg/kg; Zoetis, Florham Park, N.J., USA) in a mixture of ketamine hydrochloride (15 mg/kg) and acepromazine maleate (0.15 mg/kg).

However, the text should read:
For all procedures, pigs were anesthetized via intramuscular injection with tiletamine/zolazepam (6 mg/kg; Zoetis, Florham Park, N.J., USA) or a mixture of ketamine hydrochloride (15 mg/kg) and acepromazine maleate (0.15 mg/kg).

CHAPITRE 2 : CONNAISSANCES ET PRATIQUES DES PATIENTS ATTEINTS DE CANCER DU FOIE

1. Contexte et objectifs

Dans le chapitre précédent, nous avons vu qu'une grande partie des patients atteints de cancer du foie au Cambodge étaient diagnostiqués à un stade avancé de la maladie. Un diagnostic de CHC réalisé tardivement réduit considérablement les options thérapeutiques disponibles pour le patient et le limite à la prise de traitements palliatifs. Étant donné l'importance de certains facteurs de risques dans la survenue de la pathologie et plus précisément de l'infection chronique au virus de l'hépatite B et C, la meilleure stratégie pour lutter contre la maladie semble être la mise en place de mesures de prévention afin d'éviter la contamination par ces virus. La vaccination contre l'hépatite B permet de réduire significativement les cas d'infection chronique, mais aucun vaccin n'est actuellement disponible pour la prévention de l'infection au virus de l'hépatite C, et de ce fait la seule possibilité serait de limiter cette transmission en réduisant toutes les pratiques dites « à risque » par le biais de mesures de santé publique adaptées.

Afin de mettre en place ces mesures de prévention au sein d'une population, il est nécessaire de mieux comprendre les connaissances de cette dernière vis-à-vis de la maladie, des

causes et des modes de transmission, et de détailler leurs pratiques thérapeutiques. En effet, les représentations liées à la pathologie ou l'utilisation de pratiques non adaptées peuvent constituer un obstacle majeur à l'instauration de ces mesures. Dans ce but, une enquête CAP (Connaissance, Attitude, Pratique) a été menée auprès de 42 patients dont les dossiers médicaux ont été étudiés dans le cadre de l'étude épidémiologique précédente (cf. chapitre 1).

2. Matériels et Méthodes

De mars à juin 2015, les patients ont été interrogés soit directement à l'hôpital Calmette soit à leur domicile à l'aide d'un questionnaire.

Le questionnaire administré était constitué de plusieurs parties :

- Données sociodémographiques (âge, sexe, lieu de résidence, niveau d'éducation, profession)
- Histoire médicale du patient focalisée sur les facteurs de risque du cancer du foie (infection chronique au virus de l'hépatite B et/ou C, historique de transfusion de sang, historique d'injections thérapeutiques, consommation d'alcool) et sa prévention (vaccination contre l'hépatite B)
- Noms donnés par le patient à sa pathologie
- Causes de la pathologie selon le patient
- Itinéraire thérapeutique du patient
- Pratiques thérapeutiques : type de médecine utilisé (moderne ou traditionnelle), détails des traitements utilisés, source de l'information conduisant à l'usage de ces traitements, efficacité perçue.

3. Principaux résultats

La moyenne d'âge des patients était de 62,4 ans, et 66,7% des patients étaient des hommes.

Au total, 35 (83,3%) personnes ont mentionné être infectées par le virus de l'hépatite B et/ou C, et 37 (88,1%) patients ont décrit avoir pratiqué une ou plusieurs injection(s) intraveineuse(s) à des fins thérapeutiques depuis leur enfance.

Sur les 42 patients interrogés, 18 (42,9%) ont mentionné avoir une cirrhose du foie et 17 (40,5%) ont parlé de cancer du foie.

Les principales causes de la maladie citées par les patients étaient : le partage de la nourriture (n=19, 45,2%), le sang (n=14, 33,3%) et l'alcool (n=9, 21,4%). Quelques personnes ont aussi mentionné des causes relatives aux perceptions culturelles physiologiques khmères (alimentation chaude, mauvais vent). Seulement sept et trois personnes respectivement ont cité les injections intraveineuses ou les virus de l'hépatite comme cause de la maladie.

Parmi les thérapeutiques utilisées, 35 personnes (83,3%) ont déclaré employer une association de médecine moderne et de médecine traditionnelle. La médecine traditionnelle consistait dans la plupart des cas, à l'utilisation de remèdes préparés à base de plantes (64,3%) et plus particulièrement de remèdes prescrits par les médecins traditionnels (35,7%). D'autres pratiques comme les méthodes de dermabrasion (52,4%) ont également été très citées.

4. Discussion et conclusion

Bien que les trois-quarts des patients soient capables de nommer la pathologie dont ils souffrent en employant un terme correspondant bien à la définition biomédicale du cancer du foie ou à ses manifestations symptomatiques, peu de personnes semblent conscientes des causes, et a fortiori des modes de transmission impliqués dans le développement du cancer. En particulier, les patients savent qu'ils sont porteurs d'infection virales mais ne les imputent pas à

leur état de santé actuel. De même, la plupart d'entre eux pratiquent des injections intraveineuses thérapeutiques mais ne les citent pas comme cause probable de survenue de leur pathologie.

De plus, la majorité des patients associent le partage de nourriture (pratique culturelle fréquente au Cambodge) entre personnes saines et personnes contaminées à la cause principale de leur maladie. Cette mauvaise perception des causes du cancer du foie pourrait être attribuée à la mécompréhension de messages délivrés par les campagnes de santé publique dans le cadre de la lutte contre l'hépatite A. En effet, les patients semblent considérer les hépatites A, B et C comme les 3 stades d'une même pathologie (Burke et al., 2011), et donc ils pourraient également confondre les modes de transmission et cause de ces différentes hépatites virales.

Par ailleurs, certaines pratiques thérapeutiques (dermabrasion) sont largement utilisées par les patients et ne sont pas perçues comme potentiellement contaminantes, bien que certains auteurs (Hurie et al., 1992) suspectent leur rôle dans la transmission de pathogènes.

Toutes ces données suggèrent que ces patients peuvent facilement s'infecter et infecter les autres avec le virus de l'hépatite B et/ou C.

Des campagnes d'éducation à la santé devraient donc être lancées urgemment afin de développer les connaissances des patients sur ces virus. Une formation spécifique destinée aux professionnels et personnels de santé devrait être également mise en place afin de limiter l'utilisation d'équipements médicaux non stérilisés et la réutilisation de matériels d'injection usagés. De plus, les messages délivrés devraient être replacés dans le contexte culturel cambodgien pour conduire à une meilleure compréhension et intégration des recommandations dans la vie quotidienne de la population.

L'autre fait marquant de cette étude est la place importante accordée aux médecines traditionnelles dans le traitement des patients. Des substances d'origine végétale et animale sont largement utilisées en complément des traitements de l'hôpital. Parmi les matières identifiées, le nid d'hirondelle, le corossolier (*Annona muricata*) et le moringa (*Moringa oleifera*) sont les plus citées. De plus, près d'un tiers des patients ont recours aux médecins traditionnels afin de prendre en charge leur maladie. Il nous a donc semblé pertinent de connaitre les pratiques et

méthodes de traitements employées par ces médecins traditionnels, afin de pouvoir formuler un avis sur le bien-fondé de l'utilisation de ces dernières (cf. chapitre 3).

Ce travail s'est traduit par la rédaction d'un article en cours de soumission dans la revue scientifique internationale indexée *Asian Pacific Journal of Cancer Prevention*, et intitulé :

Article 2. « Knowledge, attitudes and practices among liver cancer patients in Cambodia »
François Chassagne, Stéphane Bertani, Sokha Eav, Eric Deharo, Geneviève Bourdy.

L'article est présenté ci-dessous *in extenso* dans sa forme manuscrite.

Title:

Knowledge, attitudes, and practices among liver cancer patients in Cambodia

Running title :

KAP study toward liver cancer in Cambodia

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Statement conflict of interest:

The authors declare no conflict of interest.

Abstract

Objective:

To assess the knowledge, attitudes, and practices among liver cancer patients in Cambodia for public health recommendations.

Methods:

A descriptive study was conducted at the Calmette hospital in Phnom Penh from March to June 2015. Forty-two patients diagnosed with liver cancer in the hospital were included in the study. A questionnaire was administered to the patients in order to assess their knowledge, attitudes, and practices toward liver cancer. Socio-demographic data, medical history related to risk factors and prevention, as well as knowledge and practices regarding this disease were collected.

Results:

Hepatocellular carcinoma was the main type of liver cancer diagnosed among the interviewees. The majority of informants were males over 50 years old. Participants cited either liver cancer (40.5%) or cirrhosis (42.9%) to define their health condition. Most of patients (83.3%) reported to have either chronic infection with hepatitis B, hepatitis C, or both; but these viral infections were not clearly understood as a risk of developing liver cancer. A high percentage (88.1%) of interviewees had history of therapeutic injection, but this was poorly perceived as a risky practice conducive to viral hepatitis infection and then hepatic tumor development. Meanwhile, food sharing was cited as the main cause of liver cancer, revealing some misconceptions about the natural history of the disease. Regarding therapeutic practices, a large majority of informants (83.3%) claimed using traditional, complementary and alternative medicine for treating their disease that includes medicinal plants (mainly prescribed by traditional healers) (64.3%) and dermabrasive practices (52.4%).

Conclusion:

Given the lack of knowledge and awareness of patients toward the causes and routes of developing liver cancer, health education campaigns focusing notably on the mode of

transmission of viral hepatitis are urgently needed, in order to overcome the burden of liver cancer in Cambodia.

Keywords:

Liver cancer – hepatitis – health knowledge, attitudes, practices – complementary therapies – public health – Cambodia

1. Introduction

Liver cancer is the sixth most common neoplasm and the second leading cause of cancer-related death worldwide (Ferlay et al., 2015). More than 80% of the new cases occur in low- and middle-income countries, and the highest incidence rates are found in Eastern and South-Eastern Asia (Stewart and Wild, 2014). Liver cancer is also associated with poor prognosis and the median survival time with treatment is estimated at less than one year after diagnosis (Nordenstedt et al., 2010). The main risk factor for hepatocarcinogenesis is chronic infections with hepatitis B (HBV) and C (HCV) viruses, contributing for almost 80% of all liver cancer cases in the world (Perz et al., 2006). In Cambodia, chronic HCV and HBV infections are suspected to contribute equally to liver cancer cases, and inappropriate health-care practices have been proposed to be the major source of contamination of HCV (Narin et al., 2015; Chassagne et al., 2016). Others risk factors are alcohol consumption, exposure to aflatoxins, and liver fluke infection (Shin et al., 2010; Stewart and Wild, 2014).

Treatment options for liver cancer patients include curative treatments for patients diagnosed at early stage (e.g. surgical resection, liver transplantation) and palliative treatments for patients diagnosed at intermediate or advanced stages (Bruix and Sherman, 2011). In low-income countries, the majority of patients are diagnosed at advanced stage that makes the disease practically incurable and expensive to manage (Ferenci et al., 2010; Ruiz et al., 2016). One of the best strategies to lower the burden of the disease remains the implementation of primary prevention measures that focus primarily on the prevention of HBV and HCV infections and secondarily in reducing exposure to aflatoxins (Yang and Roberts, 2010).

In the case of HBV, perinatal and early childhood transmission represent the most common route of transmission, but later horizontal transmission caused by sexual or health-care-related transmission may also occur (WHO, 2015). A safe and effective vaccine that prevents chronic HBV infection in 95% of cases is available since 1982 (Lavanchy, 2004). Others general measures to reduce HBV transmission can be recommended such as the use of condom during sexual intercourse, the adoption of safe injection practices, as well as avoiding the sharing of personal care items (WHO, 2015).

Unfortunately, no vaccine against HCV is currently available. Thus, prevention of HCV infection should focus on the implementation of safe transfusion and injection practices (Shepard et al., 2005). Indeed, the modes of transmission most frequently cited are transfusions with contaminated blood from unscreened donors and unsafe therapeutic or recreational drug injection practices (WHO, 2016). Percutaneous exposures to blood or blood-derived body fluids, e.g. tattooing, body piercing, ritual scarification, circumcision, acupuncture, or cupping, have also demonstrated epidemiological association with HCV infection (Shepard et al., 2005).

As liver cancer is an aggressive disease with low survival rates, its primary causes have to be prevented through public health measures. However, some misperceptions and a general lack of knowledge about liver cancer and its determinants may contribute to an inappropriate self-care and a lax of adherence to primary and secondary preventive measures. To the best of our knowledge, no study has been conducted to assess the knowledge and to document lifestyle practices and treatment seeking behavior of Cambodian patients diagnosed with liver cancer. Therefore, the aim of this exploratory research was to provide further insights into the knowledge, attitudes, and practices of Cambodian people about liver cancer and its determinants in order to improve the designing of future education campaigns.

2. Materials and methods

The Calmette hospital (Phnom Penh, Cambodia) is one of the two cancer centers of Cambodia and provides *ad rem* conditions to assess the knowledge, attitudes, and practices toward liver cancer in Cambodia. Thus, a descriptive study was conducted at the departments of oncology and hepato-gastro-enterology of the Calmette hospital, where 42 adult patients with liver cancer were interviewed between March 2015 and June 2015.

Recruitment was made among patients diagnosed with liver cancer (hepatocellular carcinoma or cholangiocarcinoma) who attended the Calmette hospital at least one time during the period of the study. Medical records were first inputted into a database (Chassagne et al., 2016), and then, patients were interviewed directly depending on their availability or via phone call. Face-to-face interviews were performed at the hospital shortly before or right after medical consultation, or, when not possible, at the residence of patients. Questionnaire was administered with the help of a translator who was a native Cambodian speaker. Following the interview, medical practitioners translated the local name of liver disorders into biomedical terms (Chassagne et al., 2017). The exclusion criterion was liver cancer patients under 18 years old. Participants were not remunerated.

2.1. Definition

We used the definition provided by Bodeker and Burford (2006) about traditional, complementary and alternative medicine (TCAM) to construe non-conventional therapeutical practices. Since the term TCAM was not understood by Cambodian patients, it was translated as "traditional medicine" (*tnam khmer*) in Khmer language. Modern medicine was translated as "medicine from the hospital" (*tnam peit*), and encompassed drugs delivered at the hospital or at the pharmacy, which are mainly tablets and intravenous injections used as symptomatic treatments for anti-inflammatory, antalgic, antipyretic, antispasmodic, and diuretic purposes.

2.2. KAP questionnaire:

The questionnaire included different sections:

(a) Socio-demographic data (age, gender, place of residence, education level, profession).

(b) Knowledge of patients about risk factors and primary and secondary prevention measures [closed-ended questions]. These factors were identified based on a previous study (Chassagne et al., 2016).

(c) Description of the illness experienced at the time of the interview. Patients were asked to report their health conditions [open-ended questions].

(d) Assumed causes of the disease. Patients were asked to appoint putative causes and habits at the origin of their health condition [open-ended questions].

(e) Treatment seeking behavior. Questions aimed to determine the type of practitioners consulted (pharmacy, private clinic, hospital, traditional healer) by the informants at the onset of symptoms [open-ended questions].

(f) Therapeutic practices. Patients were invited to point out the types of treatment used (traditional medicine, modern medicine, or both) [closed-ended questions]; to evaluate their effectiveness [open-ended questions]; to provide details about traditional treatments/practices (if used) [open-ended questions]; and finally to precise their source of information [open-ended question].

2.3. Herbs and animal by products information

For each plant and/or animal products cited as a treatment by the informants, details on their vernacular name (local name), parts of plant/animal used, method of preparation, and administration were asked. In order to identify the scientific name of the plants, a voucher specimen was collected with the informants (or their relatives) after the survey and deposited at the herbarium of National Herbarium of Cambodia of Royal University of Phnom Penh (RUPP) for further determination.

2.4. Ethical considerations

Before their inclusion in the survey, patients (and their relatives) received information regarding the purpose and the conduct of the study. They were then given the choice to participate or not. For those who accepted to be interviewed, prior informed consents were

obtained. This study has received an ethical approval from the National Ethics Committee for Health Research under the guidance of the Ministry of Health of Cambodia in January 2015 (006NECHR2015).

It is important to consider the conservation status and the legality of harvesting natural species when reporting the use of protected and endangered wildlife (Nijman and Nekaris, 2016; Chassagne, 2017). In Cambodia, the 2002 Forestry Law of the Ministry of Agriculture and Fisheries has ruled to be illegal the hunting, trading, and possessing of 16 endangered and 76 rare animal species (Gray et al., 2017). This notably includes the mainland serow (*Capricornis sumatraensis*) reported herein, which has been indexed as a vulnerable species in the International Union for Conservation of Nature red list.

3. Results

3.1. Socio-demographic data and medical history related to risk factors and prevention

Among the 42 patients interviewed, 40 were diagnosed with hepatocellular carcinoma and two had a diagnosis of cholangiocarcinoma. Most of the patients were males (66.7%) and a large majority of them were over 50 years old (81%) (Table 1). Mean age of participants was 62.4±10.8, with an age range of 39 to 80 years old. A large subset of the population were employees either in the public or private sectors (59.5%), and only few of them were farmers (9.5%). Regarding education level, a high number of patients (66.7%) did not reach high school, and six of them (14.3%) were illiterate.

Of the 42 patients, 35 of them (83.3%) reported to be chronically infected with HBV and/or HCV (Table 2). Seven patients did not know their serological status. A high percentage of patients (88.1%) mentioned to have received one to several intravenous injections for therapeutic purposes from their childhood to the date of interview. None of them ever performed injection for recreational drug purpose. Only one patient reported to have been vaccinated against HBV.

3.2. Associated disorder denomination and *bona fide* origin of liver cancer

Regarding the name of the associated disorders cited by informants to best describe their health status, a wide range of terms was reported (12 items). Sixteen patients (38.1%) reported only one pathology, 18 (42.9%) two different ones, and seven (16.7%) three or four different names. One patient did not answer this question. Altogether, 18 patients (42.9%) reported cirrhosis and 17 of them (40.5%) mentioned liver cancer (Table 3). Three patients did not refer to any pathology or symptom related with liver disorders (e.g. abdominal pain, stomach disorders).

Among the 25 patients who listed two or more names of disorder, eight (32%) explained that their illness evolved in different stages with time. Four patients (16%) said that cirrhosis evolved in liver cancer, two of them (8%) that liver nodules evolved in liver cancer, and two others (8%) that liver mass evolved in liver cancer. Pathologies most frequently associated together

were: cirrhosis and ascites (n=4, 9.5%), cirrhosis and liver cancer (n=3, 7.1%), and cirrhosis and hepatitis (n=3, 7.1%).

Among the 39 patients who answered the question, 19 informants (45.2%) cited food sharing as the chief cause responsible for their liver cancer (Table 4). Of these 19 patients, 17 (89.5%) indicated that spoons, plates, and food shared with affected people could be the cause of the disease.

"Blood" was the secondary attributed cause of liver cancer, cited by 14 (33.3%) patients, and two of them added spontaneously that it was the same origin as the infection with human immunodeficiency virus. Alcohol consumption (n=9, 21.4%) was also cited, followed by intravenous injection (n=7, 16.7%), smoking (n=5, 11.9%), heredity (n=3, 7.1%), undernutrition (n=3, 7.1%), and sexual intercourse (n=3, 7.1%). Other potential origins of liver cancer were related with Khmer medical concepts and personal beliefs, such as: intake of hot food (n=2, 4.8%), bad wind (n=1, 2.4%), coins used for dermabrasive practices, as well as hard life conditions during Khmer Rouge regime (n=2, 4.8%). Four informants (9.5%) did not attribute their liver cancer to any potential cause.

3.3. Therapeutical strategy

Regarding their treatment seeking behavior, patients reported to consult primary private clinics or pharmacies in 53.8% of the cases and hospital in 46.2% of the cases. Whereas, when symptoms persisted or worsened, 79.5% of patients attended hospitals, 17.9% private clinics, and none pharmacies.

A large majority of patients interviewed (n=35, 83.3%) declared that they treated themselves with a combination of modern medicine and TCAM. The others (n=6, 14.3%) mentioned solely using modern medicine, and none of them reported relying only on TCAM. One patient did not answer this question. For those who declared to use both modern medicine and TCAM, they all reported to use TCAM as a secondary strategy after having employed primary modern medicine.

The most common type of TCAM used by patients (Table 5) was herbal medicine (64.3%), which was mainly prescribed by traditional healers in 57.9% of the cases. Other plants or plant-derived products (38.1%) were used in self-medication. Decoction of leaves of *Annona muricata* was used in 11.9% of the cases as a curative treatment for hepatic disorders. Decoction of leaves (7.1%) or seeds (4.8%) of *Moringa oleifera* were consumed to treat liver diseases and cancer in general. Ripe fruits of papaya (7.1%) were eaten for relieving constipation. Finally, the bark of *Azadirachta indica* (2.4%) and the whole plant of *Passiflora foetida* (2.4%) were prepared as a decoction and drunk as a "cold" treatment for liver diseases.

Dermabrasive practices such as coin rubbing (or coining) were frequently practiced among patients (40.5%) to alleviate symptoms associated to their condition: fever, fatigue, dizziness, headache, sore, in order to remedy to "wind illness".

Moreover, patients interviewed reported to use different types of dietary and food supplements (40.5%), such as supplemental nutrition drinks (e.g. Ensure®) (n=5, 11.9%), cow milk (n=3, 7.1%), and soy milk (n=3, 7.1%). About 7% of patients declared using vitamins.

Edible bird's nest (EBN) made from the hardened saliva of swiftlets was mentioned by 23.8% of the informants. EBN is boiled in water for few minutes then mixed with sugar, coconut juice or meat, and administered orally as a meal replacement and for giving energy. Bones of southern serow (*Capricornis sp.*) were also used by 7.1% of patients as a panacea. Bones were grounded in water, and the liquid obtained was swallowed. Finally, massage was mentioned for relieving sore muscles (16.7%), and cupping for treating headache (11.9%).

Concerning the respondents' main sources of information for TCAM, 29 patients out of 35 cited friends or family members (83%), 12 patients cited traditional healers (34.3%), three cited the plant sellers from the open market (8.5%) and two cited the internet and Facebook (5.7%).

When patients were asked about the most suitable type of treatment (modern medicine, traditional medicine, or both) to be used for their disease, a large majority of them mentioned only modern medicine (n=30, 71.4%) and the others cited both medicines (n=7, 16.7%). None of

them reported that traditional medicine alone was the best way to treat their disease. Five informants did not answer the question.

Modern medicine was especially prized because of its perceived effectiveness (n=22, 52.3%), whereas TCAM was claimed to lack scientific validation (n=4, 9.5%), having limited activity with no curative activity (n=3, 7.1%), as well as not being trustworthy (n=3, 7.1%). Two persons (4.8%) commented that the doctor's advised them to not take TCAM.

4. Discussion

Regarding the medical history of patients, 38.1% and 40.5% of cases reported to be chronically infected by HBV and HCV, respectively. Similar proportion were found among 553 medical records of patients analyzed in the same hospital (Chassagne et al., 2016). Moreover, 88.1% of the patients in our survey mentioned to have realized previously intravenous injections for therapeutic purposes, whereas the rate of therapeutic injection in the general population is estimated about 30% (Vong et al., 2005). Only one patient received HBV vaccination. This low number of vaccination is consistent with the age of the population interviewed (mean age years old), who were too old to benefit from the national immunization program launched in 2001 (Soeung et al., 2012). Altogether, these results suggest that the knowledge of patients regarding risk factors and prevention in their medical history is in accordance with the health situation in the country, and it can be hypothesized that the number of people practicing therapeutic injections is largely underestimated in Cambodia.

Most of the patients (92.9%) described their health condition with terms related to liver diseases, and about four-fifths reported to have cirrhosis or liver cancer. Some of them also described liver cancer as the final stage of an evolutionary process. These data are in accordance with the results obtained by Burke et al. (2011) among Cambodian immigrants and by Chassagne et al. (2017) among Khmer traditional healers. Therefore, it can be assumed that patients have a correct perception of their health status, and of the lengthy process leading to liver cancer.

In our study, patients did not seem to note the difference between the cause and the mode of transmission of the disease according biomedical concepts. Moreover, most of them cited food sharing as the cause of their pathology. Among American Cambodian and Southeast-Asian communities, food sharing is reported to be a common misperception of the HBV route of transmission (Mohamed et al., 2012; Taylor et al., 2009, 2005, 2002). Moreover, Chassagne et al. (2017) highlighted that the importance placed upon food sharing might reflect the public health messages delivered in the country on the prevention of different types of viral hepatitis (A, B, and C) reinterpreted by Cambodian people.

From our preliminary data, it can be hypothesized that there is a significant discrepancy between what informants say about their personal medical history, and what they know about the etiology of their pathology.

HBV and HCV, as well as intravenous injections were seldom identified as a risk factor by patients, while a high percentage of them reported to be infected with HBV and/or HCV, and mentioned to have realized one to several intravenous injections for therapeutic purposes.

The overuse of injections is a common practice in Cambodia as well as in others Asian countries. Patients and health staff believe that injections are more effective than oral treatments (Vong et al., 2005). However, these practices carry substantial risks such as the transmission of blood borne pathogens (e.g. HBV, HCV, and HIV) (Kane et al., 1999). Moreover, our data suggest that patients refer first to pharmacies or private clinics when symptoms appear. Vong et al. (2005) reported that private professionals might over-prescribe injectable medications as a way to attract patients. Since, these private providers are strongly suspected to expose patients to unsafe injection in the country (Goyet et al., 2014; Saphonn et al., 2017), they might play an important role in the contamination of patients.

Dermabrasive practices (especially coining) were seldomly cited in our survey as a possible cause of liver cancer. Coining consist of rubbing the edge of a coin firmly and repeatedly in a linear pattern on the skin until blood appear. This healing practice is highly prized by Cambodian and others Asian people (Buchwald et al., 1992; Burke et al., 2011; Richman et al., 2010; Tan and Mallika, 2011). But, because the same coin can be reused on different patients, some authors suggest that it is an unsafe practices that might play an important role in the transmission of hepatitis B and C viruses (Hurie et al., 1992).

Finally, “blood” was a term frequently used by patients to indicate the cause of liver cancer/cirrhosis. According to Burke et al. (2011), American Cambodian immigrants with hepatitis B describe their pathology in terms of bad blood, unclean blood, or contaminated blood, and attribute hepatitis to blood problems rather than the liver. Moreover, Caruana et al. (2005) noted that Cambodian people do not know how the different forms of hepatitis can be transmitted. Therefore, it does not seem that blood is perceived as a possible agent of contamination of the hepatitis virus.

A large majority of patients (83%) reported to use traditional medicine, especially medicinal plants (64.3%). To the best of our knowledge, only one study has focused on TCAM use among liver cancer patients in developing countries. In this survey, Rojas Rojas et al. (2016) reported the use of herbal medicine for 56.8% patients diagnosed with liver cancer in Peru. More generally, Ernst and Cassileth (1998) estimated the prevalence of TCAM use among cancer patients from 7 to 64% worldwide. In Southeast Asia, the use of TCAM for chronic diseases was reported to be 63.9% in Malaysia, 52.5% in Thailand and 22.7% in Singapore (Hasan et al., 2009; Jiaranaikajorn et al., 2002; Lee et al., 2004). Thus, the number reported in our study can be considered as high. Indeed, in our study, most of the patients are diagnosed at a late stage, when therapeutic options are very limited, and treatments mainly palliative. Therefore, the use of traditional medicine might represent the only alternative, when affordable modern symptomatic treatments (e.g. painkiller) found in drugstores have been proven to be inefficient and/or insufficient to alleviate pain or debilitating symptoms.

In the present survey, three natural products from non-conventional medicine have been reported to be widely used by liver cancer patients.

The most popular one is a preparation based on swiftlet's nest, which is made from the saliva of the bird. It is used as energy drinks (tonic) and as a meal replacement, and it is considered to be a health enhancer. Edible bird's nest (EBN) is well known in traditional Chinese medicine for their nutritional and medicinal properties (Ma and Liu, 2012). In Asian countries, swiftlet farming have been created to respond to the high demand for birds and reduce the consequences of overharvesting (Saengkrajang et al., 2013). Some studies have also reported the importance of the use of EBN among children and adult cancer patients in others countries of Southeast Asia (Lim et al., 2006; Shih et al., 2009). Indeed, it has been shown that the EBN, mainly composed of glycoproteins, amino acids, vitamins, and minerals, have anti-inflammatory, antioxidant, antimicrobial and immunostimulant activities (Ma and Liu, 2012; Marcone, 2005; Yida et al., 2015).

Annona muricata, another product frequently cited in this study, was already reported to be used in the treatment of liver cancer in Peru (Rojas Rojas et al., 2016). This species is also marketed as “cancer killer” tablets in different countries (Sun et al., 2014). Moreover, Hansra et al. (2014) reported the clinical benefit of using this species in a patient with breast cancer.

Finally, besides its highly nutritive quality, *Moringa oleifera* has also gained a high reputation as a treatment for cancer and liver diseases (Al-Asmari et al., 2015; Anwar et al., 2007).

Unfortunately, none of these products have been shown to possess a clinically proven anticancer effect and to increase patient’s lifetime in the case of liver cancer. Further studies should be carried out to determine their beneficial effectiveness in the treatment of liver cancer patients.

They were several limitations in this study. First, only a limited sample size was studied due to the difficulty to find people with liver cancer at the hospital, and because few people accepted to participate in the study (more than 150 people were asked to be included). Second, the survey was performed in a single institution (Calmette hospital), which does not reflect the entire population. Third, there is a potential for recall bias since some patients reported information retrospectively. Finally, because of the use of a translator, some questions and answers could be source of misinterpretation.

5. Conclusion

In recent years, vaccination campaigns in Cambodia have rapidly increased the hepatitis B vaccine coverage, with the hope to reduce the burden of chronic liver diseases (Soeung et al., 2012). Since there is no vaccination against HCV infection, a major health public concern should be the implementation of health education programs to limit the spread of HCV by alerting people about the risk of unsafe injections practices and dermabrasive methods. Also, significant efforts should be also made in the public and private sector, in order to guarantee a proper sterilization of medical equipment, and the use of disposable needles. This is especially urgent considering the high number of incidences attributed to HCV (Chassagne et al., 2016b). We hope

that this preliminary work will help to design further researches on liver cancer in Cambodia to confirm and complete our data.

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Table 1: Baseline socio-demographic features of the people investigated (n=42).

Feature	**Number of patients**	**Proportion (%)**
Gender		
Male	28	66.7
Female	14	33.3
Age classes (years old)		
[31-40]	1	2.4
[41-50]	7	16.7
[51-60]	8	19
[61-70]	18	42.9
[71-80]	8	19
Area of residence		
Metropolitan Phnom Penh	35	83.3
Province	7	16.7
Professional sector		
Farming	4	9.5
Private sector	17	40.5
Public sector	8	19
None	13	31

Academic education		
Elementary education	11	26.2
Lower secondary education	11	26.2
Upper secondary education	7	16.7
Third level education	3	7.1
None	6	14.3
No specify	4	9.5

Table 2: Bona fide risk factors and practices associated with liver cancer among people interviewed (n=42)

Associated risk factor	Number of patients	Proportion (%)
Chronic HBV infection	16	38.1
Chronic HCV infection	17	40.5
Dual HBV/HCV infection	2	4.8
Alcohol consumption	10	23.8
Intravenous injection	37	88.1
Transfusion	9	21.4
Intravenous drug addiction	0	0

Table 3: List of symptoms and liver disorders cited by the people interviewed (n=41)

Associated risk factor	**Number of patients**	**Proportion (%)**
Cirrhosis (*krenn thlaeum*)	18	42.9
Liver cancer (*moharik thlaeum*)	17	40.5
Liver nodule (*dos satch thlaeum*)	7	16.7
Ascites (*tiyek teuk*)	5	11.9
Liver mass (*dom thlaeum*)	5	11.9
Hepatitis (*relik thlaeum*)	4	9.5
HCV (*tchumnguh thlaeum poipet C*)	4	9.5
Stomach disorders (*tchumnguh krepair*)	4	9.5
Liver disease (*tchumnguh thlaeum*)	3	7.1
HBV (*tchumnguh thlaeum poipet B*)	2	4.8
Abdominal pain (*tchu pouh*)	1	2.4
Intestinal disorder (*tchumnguh pouvién*)	1	2.4

Table 4: Assumed cause of liver cancer among the people interviewed (n=39)

Assumed cause	**Number of patients**	**Proportion (%)**
Food sharing	19	45.2
Blood	14	33.3
Alcohol consumption	9	21.4
Intravenous injection	7	16.7
Smoking	5	11.9
Heredity	3	7.1
Khmer Rouge regime (notably plant consumption during the associated period)	3	7.1
Undernutrition	3	7.1
Sexual intercourse	3	7.1
HBV infection	2	4.8
Hot food	2	4.8
Poor hygiene	2	4.8
Bad wind	1	2.4
Blood transfusion		
Canned food		
Spicy food (chili pepper)		
Coin handling		

Drug consumption		
HCV infection		
Modern medicine		
Sharing toothbrush		
Sharing razor		
Stomach disorder		
Unknown	4	9.5

Table 5: TCAM used by patients interviewed for their illness (n=42)

Type of TCAM	Number of patients	Proportion (%)
1. Herbal	27	64.3
1.1. Mix of plants	15	35.7
1.2. Soursop (*Annona muricata* L.)	5	11.9
1.3. Moringa (*Moringa oleifera* Lam.)	5	11.9
1.4. Papaya (*Carica papaya* L.)	3	7.1
1.5. Neem tree (*Azadirachta indica* A.Juss.)	2	4.8
1.6. Passion fruit (*Passiflora edulis* Sims)	2	4.8
1.7. Ivy gourd (*Coccinia grandis* (L.) Voigt)	1	2.4
1.8. Bitter gourd (*Momordica charantia* L.)	1	2.4
1.9. Gale of wind (*Phyllanthus amarus* Schumach. & Thonn.)	1	2.4
2. Coining	17	40.5
3. Dietary and food supplements	17	40.5
3.1. Cow milk, soy milk	6	14.3
3.2. Nutritional drink (e.g. Ensure®)	5	11.9
3.3. Vitamins, calcium	3	7.1
3.4. Fresh coconut juice	1	2.4

3.5. Sugarcan juice	1	2.4
3.6. Porridge	1	2.4
4. Animal products	14	33.3
4.1. Edible bird nest	10	23.8
4.2. Caprine bones	3	7.1
4.3. Hornet	1	2.4
5. Massage	7	16.7
6. Cupping	5	11.9
7. Traditional Chinese medicine	1	2.4

CHAPITRE 3 : PRISE EN CHARGE DES PATIENTS ATTEINTS D'UNE PATHOLOGIE HEPATIQUE PAR LES MEDECINS TRADITIONNELS

1. Contexte et objectifs

Dans les chapitres précédents, nous avons vu que le cancer du foie constituait un problème majeur de santé publique au Cambodge, que les virus de l'hépatite B et C étaient respectivement responsables de 44,3 et 43 % des cas, et que sa prise en charge à l'hôpital se réduisait à la prescription de traitements palliatifs. Il a aussi été montré que les patients font appel à d'autres thérapeutiques en complément des traitements de l'hôpital, en utilisant à la fois des médicaments issus de la médecine occidentale et des traitements définis comme traditionnels. Ces thérapies traditionnelles incluent une variété de pratiques et de remèdes, et sont, soit pris en automédication soit prescrits par un médecin traditionnel.

Au Cambodge, les médecins traditionnels khmers (*kruu khmer* et moines bouddhistes[11]) exercent dans un cadre légal puisqu'ils sont reconnus par le gouvernement comme

[11] Les mediums ne possèdent pas de statut officiel et ne sont pas reconnus comme acteur de santé

professionnels de santé à part entière. Ainsi, le Centre National de Médecine Traditionnelle s'occupe de promouvoir la médecine traditionnelle, ainsi que de former et d'enregistrer ces tradithérapeutes. Cette formation leur permet d'acquérir des bases en médecine occidentale et dans d'autres domaines liés à la pharmacopée. Elle vient s'ajouter à leur expérience de terrain et à un apprentissage généralement transmis de père en fils. Enfin, seule la validation de cette formation leur permet de bénéficier d'une licence professionnelle et du droit officiel d'exercice.

Étant donné que plus d'un tiers des patients de notre étude (cf. chapitre 2) ont eu recours à un médecin traditionnel, le rôle du médecin traditionnel dans la prise en charge des patients souffrant de pathologie chronique du foie est donc crucial, puisqu'il peut permettre de soulager certains symptômes, et aider à améliorer le confort du patient.

Dans ce contexte, une enquête ethnopharmacologique a été conduite auprès de ces praticiens afin d'avoir une vue globale de leur connaissance sur les maladies du foie, ainsi que de décrire leurs pratiques de prise en charge des patients.

2. Matériels et Méthodes

De septembre 2015 à janvier 2016, trente-trois médecins traditionnels, exerçant dans la capitale ou à proximité (trois provinces visitées), ont été interrogés à l'aide d'un questionnaire.

Les informations relevées étaient les suivantes :

- Données sociodémographiques
- Formation et contexte d'exercice du médecin traditionnel
- Connaissance et perception des maladies du foie (types de maladie du foie connus, causes des maladies du foie, méthodes de diagnostic)
- Prise en charge de la maladie (recommandations hygiéno-diététiques et types de thérapeutiques prescrites).

En plus de ces informations, le détail des remèdes administrés a été recueilli. Ainsi, les noms de plantes composant chaque remède, et les parties de plantes utilisées ont été notées.

Des indications sur les méthodes de préparation et d'administration ont également été relevées. Et pour chaque plante mentionnée, un échantillon d'herbier a été réalisé et identifié botaniquement.

3. Principaux résultats

Parmi les 33 médecins traditionnels interrogés, 31 étaient des hommes dont l'âge était compris entre 32 et 73 ans. 26 pouvaient être classés dans la catégorie « *kruu khmer* » (cf. introduction), 6 étaient des moines bouddhistes et le dernier était un médium.

Les maladies du foie identifiées par les médecins traditionnels étaient principalement les hépatites virales A, B et C désignés par le terme khmer « *propet A* », « *propet B* », « *propet C* » pour 6, 8 et 8 tradipraticiens respectivement, et la cirrhose « *krenn thlaeum* » (ou foie dur) citée par 6 tradipraticiens.

Les principales causes des maladies du foie mentionnées par les médecins traditionnels étaient la nourriture contaminée (soit par des pesticides ou par le partage de nourriture), l'alcool et les microbes avec 10, 9 et 7 citations respectivement.

Afin de réaliser le diagnostic, les médecins traditionnels se basent principalement sur les examens médicaux apportés par le patient, et secondairement sur une symptomatologie caractéristique (jaunisse). Par ailleurs, chaque médecin traditionnel possède ses propres pratiques de soins. La plupart d'entre eux prescrivent un traitement général composé de plusieurs plantes (une douzaine en moyenne) et traitant différentes maladies du foie (ex. : cirrhose, hépatite, cancer du foie, abcès du foie). Ces traitements sont principalement préparés sous forme d'une décoction et administrés oralement.

Concernant les plantes composant ces remèdes, 83 espèces ont été identifiées. *Cananga latifolia*, *Andrographis paniculata*, *Smilax glabra*, *Gomphrena celosioides*, *Passiflora foetida*, *Phyllanthus amarus* et *Willughbeia edulis* étaient les plus citées.

Ces plantes sont soigneusement sélectionnées par les médecins traditionnels afin de combiner plusieurs propriétés pharmacologiques telles qu'explicitées dans la médecine khmère (cf. introduction). Ainsi, une majorité de plantes (33 espèces) dites « *trocheak* » (froides) sont utilisées pour traiter les maladies du foie perçues comme « *kedaeu* » (chaudes). Un nombre équivalent de plantes (34 espèces) est qualifié de « *psah* » (terme relatif à leurs propriétés curatives et cicatrisantes des infections et des inflammations) et possède, dans plus de la moitié des cas, des propriétés « *trocheak* » également. Ce terme « *psah* » fait aussi référence aux traitements antibiotiques (*tnam psah*, médicament *psah*) considérés comme un traitement curatif rapide et efficace. Par ailleurs, 18 plantes sont dites « *ktchol* » (vent/air) désignant leur effet sur la circulation interne des fluides (selles et urine), et donc leurs propriétés diurétiques et laxatives. Enfin dans une moindre mesure, certaines plantes sont qualifiées de « *mérok* » pour désigner leur effet contre l'origine de la maladie (par extension, les microbes).

4. Discussion et conclusion

Au total, 42 remèdes médicinaux différents ont été reportés par les 33 médecins traditionnels, incluant 83 espèces identifiées botaniquement. Une grande majorité des plantes utilisées dans ces remèdes sont connues dans d'autres pharmacopées pour leur action sur le foie, et nombre d'entre elles ont également démontré leur action pharmacologique comme hépatoprotectrice, antimicrobienne et anti-inflammatoire. Ces informations suggèrent que les plantes utilisées pourraient avoir un rôle dans la prévention et le traitement des maladies hépatiques en agissant sur plusieurs cibles pharmacologiques.

Aussi, deux espèces (*Cananga latifolia* et *Willughbeia edulis*[12]) ont été très citées dans l'enquête et peu d'études ont été réalisées pour évaluer leur activité pharmacologique, il serait donc intéressant d'entreprendre un examen approfondi de ces espèces.

[12] Cette espèce a déjà été reportée comme très citée dans les maladies du foie dans la revue bibliographique de l'introduction (voir §3.4). Cette troisième étude permet de confirmer l'importance de l'usage traditionnel de cette espèce dans les pathologies hépatiques, en particulier au Cambodge.

Finalement, les pratiques des médecins traditionnels semblent avoir largement évolué depuis ces dernières années, notamment grâce à la mise en place de cette formation spécifique dispensée au Centre National de Médecine Traditionnelle de Phnom Penh. Ainsi, leurs méthodes de diagnostic reposent désormais sur la lecture d'examens médicaux, leurs conditions d'exercice se rapprochent de celles des médecins modernes (cabinet privatif, formulation galénique élaborée, notice d'emploi détaillée), et ils utilisent des concepts médicaux empruntés à la biomédecine (notion de *psah* et de *mérok*).

Ce travail s'est traduit par la rédaction d'un article soumis le 2 Janvier 2017 à la revue scientifique internationale indexée *Journal of Ethnopharmacology*, publié dans cette même revue le 9 mars 2017 après révision, et intitulé :

Article 3. « Treatment and management of liver diseases by Khmer traditional healers practicing in Phnom Penh area, Cambodia »

François Chassagne, Eric Deharo, Hieng Punley, Geneviève Bourdy.

L'article est présenté ci-dessous *in extenso* dans sa forme originale.

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Treatment and management of liver diseases by Khmer traditional healers practicing in Phnom Penh area, Cambodia

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ABSTRACT

Ethnopharmacological relevance: Liver disorders are a major health problem in Cambodia, where some patients prefer to seek treatment from traditional healers. The aim of the study was to document the knowledge and practices of these healers in four Southern Cambodian provinces.

Materials and methods: An ethnopharmacological survey was carried out from September 2015 to January 2016 in Cambodian urban and rural areas. Thirty-three Khmer traditional healers (KTH) were interviewed using a semi-structured questionnaire including socio-demographic data, healer's formation and their professional practice conditions, perception of liver diseases (types and causes of liver disorders, diagnostic methods and symptoms of liver problems), dietary recommendations given to patients, and herbal remedies used to treat them. For each medicinal plant mentioned in herbal remedies, the local name, part of the plant, mode of preparation and administration, and their properties, according to the healers, were recorded. The plants mentioned by the traditional therapists were collected and later identified by specialists.

Results: Different types of liver disease are identified by the healers, and diagnosis was mostly based on reading medical records, and by observing the yellow discoloration of the skin and eyes. A total of 42 herbal remedies including 83 medicinal plants belonging to 40 families were mentioned for treating liver disorders. The most predominant families were Leguminosae and Poaceae. Among the plants reported, *Cananga latifolia*, *Andrographis paniculata*, *Smilax* aff. *glabra*, *Gomphrena celosioides*, *Passiflora foetida and Physalis minima* were the most cited species. A large part of the herbal remedies used were multi-ingredient recipes, and were prepared mainly by a decoction administered orally. Plants are combined in multi-ingredient recipes, and selected on the basis of their properties (*trocheak*, *psah*, *somrap mé rok*, *ktchol*) which originate from Khmer medical concepts. Most of the plants used by healers have a wide ethnobotanical use for liver disorders, and have been studied for their hepatoprotective activity and related activities on the liver.

Conclusion: In the diagnosis and treatment of liver diseases, KTH have incorporated biomedical concepts and new practices, which suggest that they could be defined as neotraditional healers. Medicinal plants constitute the core of traditional medicine practice by these healers, and these plants play a very important role in the health care of people with liver problems in Cambodia. Therefore, more attention should be paid to the integration of healers in national health care programs for the development of combined therapies. Furthermore, two plant species (i.e. *Cananga latifolia* and *Willughbeia edulis*) were found to be widely used for treating liver disorders in our survey, and should be studied for their pharmacological potential for liver problems.

1. Introduction

According to Beers et al. (2008), more than 100 different forms of liver diseases can affect men, women and children in an acute or chronic process. Chronic liver disorders, in particular, represent a major cause of mortality and morbidity worldwide; liver cirrhosis is one of the leading causes of death in Western countries (Stickel and Schuppan, 2007). Unfortunately, medical therapies developed for these diseases are often limited in efficacy and too costly which make them unaffordable for people living in developing countries (Seeff et al., 2001).

In these countries, chronic hepatitis B and chronic hepatitis C

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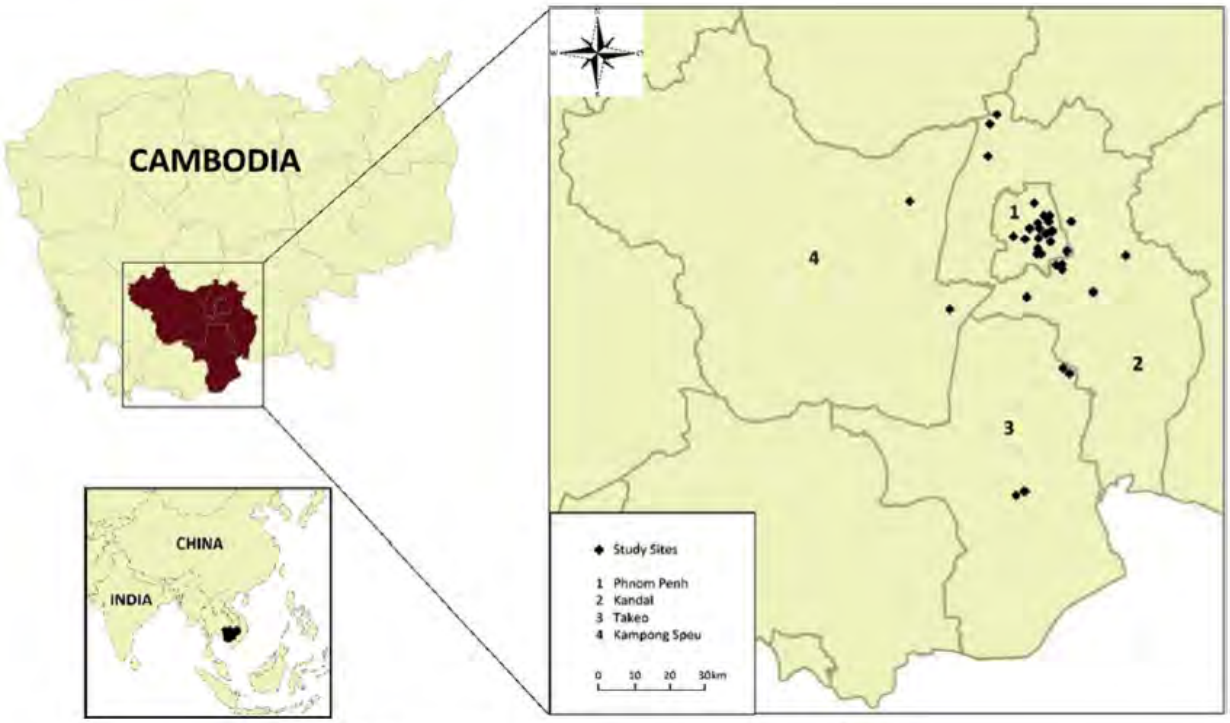

Fig. 1. Study area and locations of KTH (KTH = Khmer Traditional Healers).

infections are the two main risk factors responsible for the burden of liver diseases (Williams, 2006). These infections can lead to various liver cancers, among which hepatocellular carcinoma is the main type. While liver cancer is known to be the fifth most common cancer in the world, it represents the second most common cancer in Southeast Asia (Kao and Chen, 2005; Kimman et al., 2012). In Cambodia, no epidemiological data are available on the different types of liver disorders encountered in the area. However, hepatocellular carcinoma is estimated to rank first among all cancer types in men (Chassagne et al., 2016b).

Nowadays, it is estimated that 40–50% of people in Cambodia use traditional medicine in their everyday life (WHO, 2012). In the country, a broad range of traditional medicines are found, reflecting the cultural diversity of people living in the area. Indeed, Cambodian society is multi-ethnic and comprises a majority of Khmer people (90% of the total population in Cambodia), some ethnic minorities (Cham, Chinese, and Vietnamese) and different indigenous groups (e.g. Bunong, Tampuon, Jarai) (Moul and Seng, 2012).

Khmer traditional medicine can be considered as the main traditional medical system in Cambodia. It is known to have been employed for nearly a thousand years and it includes the use of various remedies (i.e. plants, animals, minerals) and practices (i.e. spiritual rites, offerings, dermabrasive treatments) (Finot, 1903; Richman et al., 2010). Khmer medicine has recently gained official status through recognition by the government. In 1997, the National Center of Traditional Medicine (NCTM) was created in the capital city, Phnom Penh. This public institution aims to promote traditional medical knowledge and to ensure the quality, safety and efficacy of TM products. From 2009–2012, it has also helped to train about 250 traditional healers coming from all over the country. The training course was designed to share and improve knowledge in traditional medicine (i.e. information on medicinal plants, pharmaceutical formulation, marketing, basic medical education), to promote safe and effective practices, and finally to register and license these practitioners.

According to Guillou (2001), different types of Khmer traditional healers (KTH) can be distinguished:

a) the ones called "*ruup*" (physical body), a medium who act as an intermediate between the spirit and physical worlds, and use guidance from the spirit for treating various ailments;
b) "*preah sang*" (monk), a religious man who lives in a pagoda, practices Buddhism and treat patients occasionally upon demand;
c) "*kruu* khmer" (khmer teacher), a layman who practices traditional medicine at home and treats patients occasionally upon demand. In this category of healers, Martin (1983) distinguished different practitioners classified according to their specialization: "*kruu meul tchumnguh*" (teachers who read the diseases), "*kruu mon akom*" (teachers who recite magic words), "*kruu thnam*" (teachers who use remedies), and based upon their care practices (liquid spraying, grinding of TM products, scarification, pinching). In addition to these traditional therapists, others "*kruu*", less studied, have been identified: the fortune teller (*kruu tiey*) and the sorcerer (*kruu thmop*) (Eisenbruch, 1992; Martin, 1983).

As already mentioned by Guillou (2001), Khmer traditional healers are undergoing a process of professional change, which suggests that this classification is evolving.

Although few data are available in Cambodia on the management of liver diseases, some studies have emphasized that the cost of treatment, the lack of medical equipment and specialized medical doctors, and geographical constraints are a major barrier to access to healthcare for Cambodian patients (Eav et al., 2012; Hardeman et al., 2004). Moreover, in a recent study on liver cancer in the country, it was reported that 84% of patients receive only palliative treatment (Chassagne et al., 2016b).

Therefore, because of the high prevalence of liver diseases in the country, the difficult access to biomedical healthcare for patients, the significant use of traditional medicine and the importance of KTH playing an important role for the population, we decided to undergo a study aiming to document how Khmer traditional healers care for patients affected with liver problems and treat them. The purpose of this survey was to give an overview of KTH knowledge on liver disorders, to understand the management of patients with liver diseases, and to document the treatment practices of KTH including medicinal plant uses.

2. Materials and methods

2.1. Study area

The study was conducted in four southern Cambodian provinces: Phnom Penh, Kandal, Takeo, and Kampong Speu (Fig. 1). Phnom Penh capital city is mostly an urban area, whereas the three other provinces are rural areas.

According to NIS (2009), Phnom Penh has the highest population of the four provinces with 1,327,615 inhabitants and a population density of 4516 pers./km^2, Kandal ranks second in terms of population size with 1,265,280 (355 pers./km^2), Takeo ranks third with a population size of 844,906 (237 pers./km^2), and Kampong Speu has the smallest population size and density, with respectively 716,944 inhabitants and 102 pers./km^2. Khmer people are the dominant ethnic population in the study area.

These provinces can be divided in two geographically distinct regions (ICEM, 2003):

- Mekong Delta region: an alluvial area dominated by rice cultivation and semi-natural wetlands. These lowlands lie below 100 m elevation. It includes Phnom Penh, Kandal and Takeo provinces.
- Plateau and Mountain region: an area comprising central Indochina dry forest and Cardamom mountains rain forest that can reach 1800 m high. It includes Kampong Speu province.

In this area, the mean temperature ranges between 26 °C (December) and 31 °C (April). The annual rainfall is 1500 mm, with more than 80% falling in the rainy season (May to October). The dry season is typically 5–6 months length (November to April) (Kubo, 2008).

2.2. Data collection

A survey was carried out from September 2015 to January 2016. First, a total of 115 traditional healers were selected based on the information given by the National Center of Traditional Medicine (NCTM) of Cambodia. All the informants were contacted by phone and 33 people agreed to be interviewed. Finally, a meeting was set up between them and our team according to their availability. All the interviews were held in their home with the help of a translator. Before each interview, the purpose of the study was explained to the participants.

Data were collected by interviewing traditional healers through a questionnaire (See supplementary data 1) following the guidelines on ethnobotanical study written by Alexiades (1996) and Martin (1995).

- First, sociodemographic data were recorded (gender, age, education, profession)
- Then, questions were oriented toward the healer's formation and their professional practices
- Third, questions related to KTH perception and description of liver diseases were asked, including (i) the different type of liver diseases known by the healers (open questions), (ii) the causes of liver diseases (open questions), (iii) diagnosis methods employed by healers (open questions) and the most common symptoms known for liver problems (open questions). The translation of Khmer names of liver problems to probable biomedical terms were realized with the help of medical practitioners specialized in hepatology.
- Finally, a set of questions on disease management was submitted, including dietary recommendations and treatments used by KTH for liver diseases. In addition, complete herbal recipes were recorded (local name and part of plants used, mode of preparation and administration). For each medicinal plant species included in these recipes, its properties or effects (according to KTH) were recorded by free listing.

Interviews were supplemented by a field trip with traditional healers. During these walks, plants used for treating liver diseases were collected for further identification.

2.3. Data mapping

Relationships between medicinal plants and their five most common pharmacological properties according to Khmer medicine were displayed as an interaction network using the software Gephi v. 0.9.1. (https://gephi.org) with the layout *ForceAtlas*.

2.4. Botanical identification

Plants were collected, and dried to prepare herbarium specimens. The taxonomic identification was realized by specialists using specialized literature such as "Flore du Cambodge, du Laos et du Vietnam" and "Flora of Thailand". Plants names have been checked and updated according to the standard database "The Plant List" (http://www.theplantlist.org/).

Voucher specimens were deposited at the National Herbarium of Cambodia, Department of Biology, Faculty of Science, Royal University of Phnom Penh (RUPP), Cambodia.

2.5. Ethical considerations

Ethical approval was obtained from the National Ethics Committee for Health Research under the guidance of the Ministry of Health of Cambodia in January 2015 (006NECHR2015). Before each interview, a prior informed consent was obtained from all respondents. Participants were also given the option to choose whether or not they wished to share their knowledge / be interviewed.

3. Results and discussion

3.1. Socio-demographic data, training and professional practices conditions of KTH

Table 1 shows the socio-demographic characteristics of all the healers who participated in this survey. A total of 33 healers from 32 to 73 years old were interviewed. Most of the informants were males (31, 93.9%), thus reflecting the overall sex ratio status of KTH in Cambodia. Indeed, Crochet (2000) already mentioned the predominance of men among KTH. According to Eisenbruch (1992), this gender difference in Cambodia is also seen in Thailand. However, this is not a common feature of all traditional healers in Southeast Asia across all ethnic groups. For example, in the case of Red Yao people living in Thailand, Vietnam and Laos, as well as in Ati culture from Philippines, ethnomedicinal knowledge is mainly a woman's prerogative (Long and Li, 2004; Ong and Kim, 2014).

Among the 33 healers interviewed, 31 were trained by the NCTM in the years 2009–2012 and thus received an official license from the Ministry of Health. Some of them belong to an association (CaTHA, Cambodian Traditional Healers Association), and two were affiliated to institutions (NCTM). One was "*ruup*" (a medium), 6 were "*preah sang*" (Buddhist monks), and the others (26) identified themselves as "*kruu khmer*".

According to the socio-demographic data and the characteristics of professional practice, two different types of "*kruu khmer*" can be distinguished: the first group (10 KTH) live in rural areas, work as farmers, and practice traditional medicine at home upon demand, whereas the second group (16 KTH) live mostly in urban areas, have a higher level of education, and practice traditional medicine in a "modern way" by receiving patients in a consultation room every day. Moreover, the latter group deliver elaborate galenic formulations (pills, ointments) and packaging with labels indicating the plant's name, the mode of preparation and administration of the remedy. The first group

Table 1
Socio-demographic characteristics and type of KTH.

Characteristics		Frequency	Percent (%)
Gender			
	Male	31	93.9
	Female	2	6.1
Age			
	30–45 years	9	27.3
	46–60 years	14	42.4
	61–75 years	10	30.3
Residence			
	Urban area	20	60.6
	Rural area	13	39.4
Education			
	Primary School	3	9.1
	Secondary School	12	36.4
	High School	8	24.2
	University	4	12.1
	Unknown	6	18.2
Type of Healers			
	ruup	1	3
	preah sang	6	18.2
	kruu khmer	26	78.8

delivers remedies which are mainly mixtures of crude plant parts placed in plastic bags without instructions on the package. In both groups, the remedies are prepared by the healers.

3.2. Khmer medical concepts

Nowadays, traditional Khmer medicine is a syncretic development which combines various cultural and religious traditions: Theravada Buddhism, animism, traditional Chinese medicine, Ayurveda and French pharmaceutical traditions (Burke et al., 2011; Chhem, 2002; Guillou, 2001; Sargent and Marcucci, 1984).

Different concepts serve as a theoretical basis of Khmer medicine, including humoral theories which represent one of the most significant medical concepts in Southeast Asia (Manderson, 1981; Van Esterik, 1988). According to humoral theory, the body is composed of four elements: earth, water, fire, air (or wind). Health is maintained through equilibrium, so a dysfunction of these elements can lead to illness. The hot (called *kedaeu* in Khmer) and cold (*trocheak*) qualities, sometimes associated with the fire and water elements, should be equilibrated within the body. An imbalance between these two qualities can lead to "hot" or "cold diseases", which should be restored by "cold" and "hot treatments" respectively. Moreover, wind element (*ktchol*) is directly related with the movement of body parts and fluids in the body (i.e. bile, faeces, urine). Dysfunction of the wind element is recognized as an important cause of illness (Chhem, 2002; Muecke, 1979).

Besides these main concepts stemming from the humoral theory, Khmer medicine has also incorporated one concept originating from the biomedical model (also called *tnam baraing*, French medicine): "*mé rok*", meaning "origin of the diseases". This term is associated with the germ theory of the diseases introduced by medical doctors during the French Protectorate of Cambodia (Guillou, 2001), and "*mé rok*" is also commonly used to designate microbes. This concept is now used in Khmer medicine to describe the pharmacological activity of some medicinal plants designated as "*somrap mé rok*" (against the origin of the diseases/microbes).

According to Martin (1983), the pharmacological activity of plants used in Khmer medicine is classified according to their taste (astringent, bitter, sweet, salty and sour), as well as in other Asian humoral medicinal systems (Ayurveda, Chinese, Tibetan medicine) (Gilca and Barbulescu, 2015; Richardson-Boedler, 1999).

3.3. Perception of liver diseases by traditional healers

3.3.1. Different types of liver diseases

When asked about the different types of liver diseases "*tchumnguh thlaeum*" known, KTH listed the following conditions:

"*Krenn thlaeum*" (tough liver), a condition related to cirrhosis (according to biomedical practitioners) was mentioned by 6 KTH, "*relik thlaeum*" (liver inflammation) identified as hepatitis was cited by 4 KTH, "*bok thlaeum*" (furuncle liver) or liver abscess by 2 healers. "*Moharik thlaeum*" (gigantic liver) or liver cancer, "*thlaeum krem*" (dysfunctional liver), "*ksaoy liver*" (weak liver) and "*thlaeum rik*" (enlarged liver) or hepatomegaly were cited by only one healer each. In addition, "*propet A*", "*propet B*", and "*propet C*" (type A, B or C) identified as viral hepatitis A, B or C were also reported by 6, 8 and 8 traditional healers respectively.

Six KTH also mentioned, without prompting, that liver diseases evolve in different stages. For 3 KTH, it was assumed that "*propet A*" (first stage of illness) can lead to "*propet B*" which can lead to "*propet C*" (the most severe one). On the contrary, one KTH said that "*propet C*" (first stage of illness) can lead to "*propet B*" which can lead to "*propet A*" (the worst one). Moreover, two KTH identified "*relik thlaeum*" (hepatitis) as the first step of liver diseases, then "*krenn thlaeum*" (cirrhosis) with "*tiyek teuk*" (ascites) was cited as the second step, and "*moharik thlaeum*" (liver cancer) as the final step.

In a study aiming to document the perception of liver diseases in Cambodian migrants in the United States, "*krenn thlaeum*" (cirrhosis), "*thlaeum krem*" (dysfunctional liver), "*tiyek teuk*" (ascites), "*kat luong*" (jaundice), "*propet A*", "*propet B*", "*propet C*" were also listed as different types of diseases affecting the liver (Burke et al., 2011). In this survey, Cambodian migrants also stated that "*propet A*", "*propet B*" and "*propet C*" represent different stages of liver diseases, thus are not 3 different illnesses.

3.3.2. Causes of liver diseases

Concerning the causes of liver diseases, KTH first mentioned contaminated food (30.3%), then alcohol (27.3%) and microbes (*mé rok*) (21.2%) (Table 2). During the interviews, KTH explained that food can be contaminated by toxins (such as pesticides), or by infected people when sharing food with them. The latter explanation was also cited by US Cambodian immigrants as the main causes of liver diseases (Burke et al., 2011). Interestingly, although 9 traditional healers cited one, two or three of the following type of liver diseases: "*propet A*", "*propet B*" and "*propet C*", only one KTH mentioned them as the cause of these diseases.

In our opinion, the importance placed upon contaminated food, and "*mé rok*" as the main causes of liver diseases, reflect the public health messages delivered in the country on the prevention of different types of viral hepatitis (A, B and C) reinterpreted by Cambodian people. Indeed, according to the biomedical system, the food sharing is only in cause for hepatitis A transmission, but it is perceived by KTH as a factor allowing transmission of all types of viral hepatitis, since they seem to consider that hepatitis A, B, and C is the same disease.

Table 2
Causes of liver diseases (causes cited only one time not included).

Causes	Frequency	Percent (%)
Contaminated food	10	30.3
Alcohol	9	27.3
Microbes	7	21.2
Tobacco	3	9.1
Sexual intercourse	3	9.1
Malaria	2	6.1
Heredity	2	6.1
Poor sanitation	2	6.1

3.3.3. Diagnostic methods and symptoms of liver diseases

Interestingly, most of the healers (25, 75.7%) said that they knew that the patient was suffering from a liver disorder by reading their medical report, and for three of them, it was the only diagnostic criteria used. The three KTH explained that people suffering from liver diseases are often asymptomatic. Medical report results were also presented as criteria for making treatment decisions. One KTH mentioned that he would not treat patients with a low level of hemoglobin (< 8 g/dl), and another healer said that the viral load results allow him to know the severity of the illness.

In addition to the medical records reading, other diagnostic methods were used by KTH. Twenty-two healers (66.7%) said that they make or complete their diagnosis by examining the patient's body (e.g. body and eyes coloration), and by asking them for their medical history.

Finally, three healers (9.1%) mentioned that they also perform palpation in order to detect any abnormality (i.e. liver enlargement, ascites).

Clinical symptoms cited by KTH for describing liver diseases are summarized in Table 3. The most common symptoms mentioned were yellow eyes (54.5%), followed by yellow skin (36.4%) and spots on the skin (36.4%).

Although knowledge is not homogenous between the different healers, answers seem to be made in accordance with biomedical concepts, and is more or less well understood. As an example, some healers (9) described the different types of liver problems under the neologism *propet* (A, B, C) and diagnosis was mostly based on medical report reading, completed with anamnesis and the search for secondary signs of jaundice and aggravation (liver enlargement, ascites). By comparison, in two other ethnobotanical studies on liver diseases in Togo and Rwanda, less than 20% of traditional healers based their diagnosis on medical records, and most of them identified liver diseases by observing the yellow discoloration of the skin and mucous membranes (Kpodar et al., 2016; Mukazayire et al., 2011). These differences could be explained by the fact that KTH have followed a training on basic medical knowledge provided by the NCTM.

3.4. Liver diseases management and treatment

3.4.1. Dietary recommendations

Before treating a patient with liver diseases, most of the healers give specific dietary recommendations, based mainly upon food avoidance (*thorm*, prohibition). In our study, KTH cited some foods strictly prohibited such as seafood, chicken eggs, duck eggs, fermented fish, bamboo shoots, liver, beef, chili, and alcohol. Moreover, some of them said they would recommend patients to do some physical activities, to stop cigarette smoking and to avoid hard work.

In Cambodia, as well as in others countries from Southeast-Asia, dietary adjustments are a part of medical treatments administered to the patients (Eisenbruch, 1992; Laderman and Van Esterik, 1988). Food avoidance is well documented in the country for pregnancy and postpartum (Montesanti, 2011). In case of liver problems, Burke et al. (2011) already mentioned that wine, oils, meats and fried foods should be avoided. Indeed, all these types of food are perceived as "hot" food, according the humoral theory, which make them detrimental to liver health conditions.

Table 3
Clinical symptoms related to liver diseases.

Symptoms	Frequency	Percent (%)
Yellow eyes	18	54.5
Yellow skin	12	36.4
Spots on the skin	12	36.4
Abdominal pain	9	27.3
Abdominal swelling	9	27.3
Dark skin	6	18.2
Itching	6	18.2
Asthenia	5	15.2
Insomnia	4	12.1
Pale appearance	4	12.1
Dark urine	4	12.1
Light eyes	3	9.1
Hard liver	2	6.1
Loss of appetite	2	6.1
Fever	2	6.1
Leg swelling	2	6.1
Others: dark eyes, weight loss, constipation, difficulty urinating, headache, nausea	1	3.0

3.4.2. Herbal remedies

3.4.2.1. Overview of medicinal plants used in recipes. Altogether, 27 KTH reported the use of 42 different herbal remedies for treating liver disorders comprising a total of 383 ingredients. Among these ingredients, 83 different plant species have been identified along with two components (sugar, baking powder). Six KTH did not mention any herbal remedy.

The eighty-three medicinal plants reported by KTH belong to 40 families (Table 4). The most cited plants were *Cananga latifolia* (Hook.f. & Thomson) Finet & Gagnep. (15 citations), *Andrographis paniculata* (Burm.f.) Nees (12 cit.), *Smilax* aff. *glabra* (10 cit.), *Gomphrena celosioides* Mart. (9 cit.), *Passiflora foetida* L. (9 cit.), *Physalis minima* L. (9 cit.), *Willughbeia edulis* Roxb. (8 cit.), *Phyllanthus amarus* Schumach. & Thonn. (7 cit)., and *Salacia chinensis* L. (6 cit).

The most predominant plant families were Leguminosae (8 sp.), Poaceae (7 sp.), and Compositae (5 sp.). Leguminosae was also reported to be the most cited family in other ethnobotanical studies in Cambodia, Thailand and Laos (Chassagne et al., 2016a; Junsongduang et al., 2014; Khuankaew et al., 2014; Libman et al., 2006). Indeed, it is considered to be the most important plant family in the region (Dy Phon and Rollet, 1999).

Of the overall species, 49 are wild and 34 are cultivated. 32 species are herbaceous plants (38.5%), 24 are trees (28.9%), 14 are shrubs (16.9%), 10 are lianas (12.0%) and 3 are palms or epiphytic plants (3.6%). In our study, herbs represent the most important type of plants used, which is also the case in two other ethnobotanical studies on liver disorders in Asia (Chhetri et al., 2008; Sharma et al., 2012). Moreover, a large percentage of medicinal plants used are cultivated species, which could be explained by the practices of KTH coming from urban area who prioritize cultivated species (growing in their homegardens) for their daily practices. As already reported by Thomas (2012), modernization and integration in the market economy of traditional healers can result in a higher use of cultivated species and market-based consumer goods compare to wild plants.

Concerning the geographical origin of plants used, 34 species are widespread in Asia, 33 are pantropical or originate from old world tropics (Africa, Asia, Australia), 14 are found in the Indochinese Peninsula (Cambodia, Laos, Myanmar, Thailand, Vietnam and Southern China), and 2 are endemic to Cambodia and Vietnam (*Calamus salicifolius* Becc., *Garcinia cochinchinensis* (Lour.) Choisy) (Ashwell and Walston, 2008; Leti et al., 2013).

To the best of our knowledge, new medicinal usage for liver problems in Cambodia has been reported for the first time for the 18 following plants: *Alangium salviifolium* (L.f.) Wangerin, *Amaranthus viridis* L., *Benincasa hispida* (Thunb.) Cogn., *Cayratia trifolia* (L.) Domin, *Coccinia grandis* (L.) Voigt, *Dillenia hookeri* Pierre, *Dillenia ovata* Wall. ex Hook.f. & Thomson, *Imperata cylindrica* (L.) Raeusch., *Jatropha curcas* L., *Limnophila aromatica* (Lam.) Merr., *Morinda citrifolia* L., *Moringa oleifera* Lam., *Morus alba* L., *Phyllanthus taxodiifolius* Beille, *Physalis minima* L., *Plumeria alba*

Table 4
Medicinal plants used by KTH for treating liver disorders.

No	Scientific name	Family	Voucher No	Khmer name	Parts used	Method of preparation[a]	Properties[b]	No of citations
1	*Abutilon indicum* (L.) Sweet	Malvaceae	FC299	Tebal Kenne	Stem	Decoction/Infusion/Pill	*trocheak*(1), *psah*(1), blood circulation(1)	3
2	*Acacia harmandiana* (Pierre) Gagnep.	Leguminosae	FC234	Thmea	Bark	Decoction	ND	1
3	*Alangium salviifolium* (L.f.) Wangerin	Cornaceae	FC359	Angkaol	Wood	Decoction	ND	2
4	*Allium sativum* L.	Amaryllidaceae	NO	Kteum Sor	Bulb	Crushed	ND	1
5	*Aloe vera* (L.) Burm.f.	Xanthorrhoeaceae	FC170	Kontoy Krepeu	Leaf	Fresh latex extracted from leaves	*trocheak*(1), *ktchol* [laxative](1)	1
6	*Amaranthus viridis* L.	Amaranthaceae	FC300	Slok Pti	Whole plant	Decoction	*trocheak*(1)	1
7	*Ampelocissus arachnoidea* (Hausskn.) Planch.	Vitaceae	FC187	Tompeaing Bay Tchou Prey	Stem	Decoction	*ktchol* [diuretic](2)	2
8	*Andrographis paniculata* (Burm.f.) Nees	Acanthaceae	FC324	Promat Meunouh	Whole plant	Decoction/Syrup/Pill	*trocheak*(8), *psah*(4), *levign*(4), *mé rok*(2)	12
9	*Antidesma ghaesembilla* Gaertn.	Phyllanthaceae	FC036	Dongkiep Kdam	Wood	Decoction/Infusion/Pill	*psah*(1)	3
10	*Areca catechu* L.	Arecaceae	FC262	Sla	Wood	Decoction	*mé rok*(1)	1
11	*Artocarpus heterophyllus* Lam.	Moraceae	FC366	Knao	Wood	Decoction	*psah*(2)	3
12	*Azadirachta indica* A.Juss.	Meliaceae	FC272	Sedaeu	Bark/Wood	Decoction	*trocheak*(3)	4
13	*Bambusa* spp.	Poaceae	FC099	Russey Srok/Russey Prey	Wood	Decoction	*psah*(2), *ktchol* [diuretic](1)	4
14	*Bauhinia bassacensis* Gagnep.	Leguminosae	FC190	Kleign Poa	Wood	Decoction	*trocheak*(1), liver detoxifier(1)	1
15	*Benincasa hispida* (Thunb.) Cogn.	Cucurbitaceae	FC303	Tror Lach	Fruit	Decoction	*trocheak*(1)	1
16	*Blumea balsamifera* (L.) DC.	Compositae	FC035	Bay Mat	Whole plant	Decoction	ND	1
17	*Boerhavia diffusa* L.	Nyctaginaceae	FC329	Tchan Kon Roméain	Whole plant	Decoction/Syrup	*psah*(2), *trocheak*(2), *ktchol* [diuretic](1), *levign*(1)	5
18	*Borassus flabellifer* L.	Arecaceae	FC363	Tnaot	Flower	Decoction	*ktchol* [diuretic](1)	1
19	*Calamus salicifolius* Becc.	Arecaceae	FC312	Rôpeak	Root/Stem	Decoction	*psah*(1)	2
20	*Cananga latifolia* (Hook.f. & Thomson) Finet & Gagnep.	Annonaceae	FC054	Chkae Sreng	Root/Wood	Decoction/Infusion/Pill	*psah*(14), *trocheak*(1)	15
21	*Cardiospermum halicacabum* L.	Sapindaceae	FC341	Pous Am Beng	Whole plant	Decoction	ND	1
22	*Cayratia trifolia* (L.) Domin	Vitaceae	FC295	Tror Dèt	Stem	Decoction	*trocheak*(1), *psah*(1)	3
23	*Ceiba pentandra* (L.) Gaertn.	Malvaceae	FC024	Ko	Bark	Decoction	*trocheak*(2)	2
24	*Cheilocostus speciosus* (J.Koenig) C.D.Specht	Costaceae	FC177	Tror Thok	Rhizome	Decoction/Infusion/Pill	*trocheak*(2)	2
25	*Coccinia grandis* (L.) Voigt	Cucurbitaceae	FC306	Vor Bass	Stem	Decoction	*trocheak*(1), *mé rok*(1)	1
26	*Combretum quadrangulare* Kurz	Combretaceae	FC050	Sangkae	Stem	Decoction	*mé rok*(1)	1
27	*Cyanthillium cinereum* (L.) H.Rob.	Compositae	FC346	Kbal Roy	Whole plant	Decoction/Syrup	*trocheak*(1)	1
28	*Cymbopogon citratus* (DC.) Stapf	Poaceae	FC089	Slok Krey	Whole plant	Decoction	*ktchol* [laxative](1), *psah*(1)	1
29	*Cyperus rotundus* L.	Cyperaceae	FC049	Krovagn Tchrouk	Whole plant	Decoction	*ktchol* [diuretic, laxative](1)	1
30	*Dillenia hookeri* Pierre	Dilleniaceae	FC068	Plou Bat	Root	Decoction	ND	1
31	*Dillenia ovata* Wall. ex Hook.f. & Thomson	Dilleniaceae	FC013	Plou Thom	Bark	Decoction	ND	1
32	*Eclipta prostrata* (L.) L.	Compositae	FC331	Kmagn	Whole plant	Decoction/Syrup	*psah*(3), *trocheak*(1), *mé rok*(1), *levign*(1)	5
33	*Elephantopus mollis* Kunth	Compositae	FC351	Prokrap Domrey	Whole plant	Decoction	*psah*(2), *trocheak*(2), energy(1), appetite stimulant(1), good sleep(1)	5
34	*Eleusine indica* (L.) Gaertn.	Poaceae	FC326	Tcheung Krass	Whole plant	Decoction	*ktchol* [diuretic](1), *mé rok*(1), *psah*(1)	4
35	*Eurycoma longifolia* Jack	Simaroubaceae	FC320	Antong Krohom	Root	Decoction	*trocheak*(1), *psah*(1), *mé rok*(1), *levign*(1)	2
36	*Flueggea virosa* (Roxb. ex Willd.) Royle	Phyllanthaceae	FC364	Lieach Ptous	Wood	Decoction	*psah*(2), *mé rok*(1), *ktchol* [laxative](1)	4
37	*Garcinia cochinchinensis* (Lour.) Choisy	Clusiaceae	FC343	Sondan	Fruit	Crushed	painkiller(1)	2
38	*Gmelina asiatica* L.	Lamiaceae	FC365	Ang Tchagn	Wood	Decoction	*trocheak*(1), *psah*(1)	1
40	*Gomphrena celosioides* Mart.	Amaranthaceae	FC305	Pkaa Toum Hou	Whole plant	Decoction	liver aid(5), *psah*(2), *trocheak*(2)	9
39	*Gomphrena globosa* L.	Amaranthaceae	FC356	Trochiek Toun Say	Whole plant	Decoction	ND	1
41	*Heliotropium indicum* L.	Boraginaceae	FC296	Pramoy Domrey	Whole plant	Decoction	*trocheak*(1)	3
42	*Hoya kerrii* Craib	Apocynaceae	FC002	Trochiek Domrey	Stem	Decoction	*trocheak*(2)	3
43	*Hydnophytum formicarum* Jack	Rubiaceae	FC109	Sout Domrey	Rhizome	Decoction	*psah*(2), *trocheak*(1), respiratory support(1)	3
44	*Imperata cylindrica* (L.) Raeusch.	Poaceae	FC361	Sböv/ Sböv Pléaing	Root	Decoction	*ktchol* [diuretic](2), *psah*(1)	4
45	*Jatropha curcas* L.	Euphorbiaceae	FC078	Lehong Kwong	Wood	Decoction	*psah*(1)	1
46	*Kyllinga nemoralis* (J.R.Forst. & G.Forst.) Dandy ex Hutch. & Dalziel	Cyperaceae	FC328	Kâk Kdam	Whole plant	Decoction	ND	1
47	*Leea rubra* Blume ex Spreng.	Vitaceae	FC182	Kedaing Bay	Root	Decoction/Syrup/Infusion/Pill	appetite stimulant(3), good sleep(1), *psah*(1), *trocheak*(1)	5

(continued on next page)

Table 4 *(continued)*

No	Scientific name	Family	Voucher No	Khmer name	Parts used	Method of preparation[a]	Properties[b]	No of citations
48	*Limnophila aromatica* (Lam.) Merr.	Plantaginaceae	FC148	Ma ôm	Whole plant	Decoction	ND	1
49	*Mimosa pudica* L.	Leguminosae	FC071	Preah *Kl* op	Whole plant	Decoction	*psah*(1)	1
50	*Morinda citrifolia* L.	Rubiaceae	FC039	Gnô Srok	Fruit/Root/ Wood	Macerated in salt and Decoction	*psah*(1)	2
51	*Moringa oleifera* Lam.	Moringaceae	FC284	Mrom	Leaf	Crushed	general health(2)	2
52	*Morus alba* L.	Moraceae	FC294	Mone	Wood	Decoction/Infusion/Pill	*trocheak*(1)	3
53	*Nauclea orientalis* (L.) L.	Rubiaceae	FC332	Kdol	Bark	Decoction	*mé rok*(1)	2
54	*Oroxylum indicum* (L.) Kurz	Bignoniaceae	FC142	Pika	Bark/Wood	Decoction/Infusion/Pill	*trocheak*(4), *psah*(2)	5
55	*Orthosiphon aristatus* (Blume) Miq.	Lamiaceae	FC337	Pouk Mouat Tchma	Whole plant	Decoction	*ktchol* [diuretic] (1)	2
56	*Oryza sativa* L.	Poaceae	FC278	Angkor Domneub Kmaeu	Seed	Soaked in water and grilled on fire. Decoction	*mé rok*(1), energy(1)	1
57	*Passiflora foetida* L.	Passifloraceae	FC067	Sao Mao Prey	Stem/Root	Decoction	*trocheak*(5), sedative(2)	9
58	*Phyllanthus amarus* Schumach. & Thonn.	Phyllanthaceae	FC297	Prok Plae	Whole plant	Decoction/Syrup/Pill	*trocheak*(4), *psah*(2), *ktchol* [diuretic](1)	7
59	*Phyllanthus taxodiifolius* Beille	Phyllanthaceae	FC310	Kro Phegn	Wood	Decoction	ND	1
60	*Physalis minima* L.	Solanaceae	FC330	Peng Poa Srom	Whole plant	Decoction	*trocheak*(7), liver aid(3), *levign*(2), *psah*(2), *ktchol* [diuretic](1)	9
61	*Piper nigrum* L.	Piperaceae	FC266	Marek	Seed	Crushed	ND	1
62	*Plumeria alba* L.	Apocynaceae	FC338	Champey Sô	Wood/Flower	Decoction	*trocheak*(2), *mé rok*(1), blood enhancer(1)	3
63	*Pouzolzia zeylanica* (L.) Benn.	Urticaceae	FC066	Mouk Tchineng	Whole plant	Decoction	*psah*(3), *trocheak*(2), antiparasite(1), *mé rok*(1)	5
64	*Prismatomeris tetrandra* (Roxb.) K.Schum.	Rubiaceae	FC318	Romdegn Meas	Wood	Decoction	energy(1)	1
65	*Rhinacanthus nasutus* (L.) Kurz	Acanthaceae	FC333	Tchong Ambok	Whole plant	Decoction	*psah*(1)	3
66	*Rhodamnia dumetorum* (DC.) Merr. & L.M.Perry	Myrtaceae	FC313	Pouïch	Wood	Decoction	energy(1), appetite stimulant(1)	1
67	*Saccharum officinarum* L.	Poaceae	FC277	Ampeu Kmao	Stem	Decoction	pus drainage(1)	1
68	*Salacia chinensis* L.	Celastraceae	FC314	Veay	Root/Wood	Decoction	*ktchol* [laxative](4), dizziness(1)	6
69	*Scoparia dulcis* L.	Plantaginaceae	FC048	Ey Sey Psom Srach	Whole plant	Decoction	ND	2
70	*Senna alata* (L.) Roxb.	Leguminosae	FC169	Dong Heut Thom	Stem	Decoction/Infusion/Pill	*ktchol* [laxative](4)	5
71	*Senna siamea* (Lam.) H.S.Irwin & Barneby	Leguminosae	FC335	Angkagn	Wood	Decoction	*mé rok*(1)	3
72	*Senna tora* (L.) Roxb.	Leguminosae	FC327	Dong Heut Tchineng	Seed	Decoction	*ktchol* [laxative](1)	2
73	*Smilax* aff. *glabra* L.	Smilacaceae	FC355	Meum Tam Chen	Root	Decoction	psah(8), energy(1)	10
74	*Streblus asper* Lour.	Moraceae	FC051	Snay	Wood	Decoction	*psah*(1)	1
75	*Streptocaulon juventas* (Lour.) Merr.	Apocynaceae	FC032	Vor Chuy	Root	Decoction	*psah*(2), *trocheak*(1)	3
76	*Suregada multiflora* (A.Juss.) Baill.	Euphorbiaceae	FC360	Tro Mong Sek	Wood	Decoction	*mé rok*(1), *ktchol* [bloating](1)	2
78	*Tamarindus indica* L.	Leguminosae	FC161	Ampeul	Bark	Decoction	*psah* (1)	1
77	*Tarlmounia elliptica* (DC.) "H.Rob., S.C.Keeley, Skvarla & R.Chan"	Compositae	FC336	Konleut Tchork	Whole plant	Decoction	*psah*(4), blood circulation(1), *trocheak*(1)	4
79	*Terminalia bialata* (Roxb.) Steud.	Combretaceae	FC110	Popiel Kaé	Bark	Decoction	ND	1
80	*Tinospora crispa* (L.) Hook. f. & Thomson	Menispermaceae	FC165	Vor Pich	Whole plant	Decoction/Pill	*trocheak*(2), *mé rok*(1), *levign*(1)	2
81	*Willughbeia edulis* Roxb.	Apocynaceae	FC316	Vor Koy	Stem	Decoction/Infusion/Pill	*psah*(5), *trocheak*(2), ktchol [laxative](2), blood circulation(1)	8
82	*Xylia xylocarpa* (Roxb.) Taub.	Leguminosae	FC031	Sokrom	Fruit	Decoction	blood purifier(1)	1
83	*Zea mays* L.	Poaceae	FC263	Pôte	Cornsilk	Decoction	*mé rok*(1)	1

ND = Not Determined

[a] All the herbal preparation are administered orally (drunk or swallowed).

[b] Number of KTH who cited the properties are indicated in the brackets

Fig. 2. Different types of formulations used by KTH. Legend (Fig. 2): A = Mix of dried plants. B = Plant powder. C = Pills [to prepare the pills: dry plant powder is mixed in a dragee drum machine with manioc flour and water]. D = Syrup [to prepare the syrup: mix 30 l of water and 4 kg of dried plants, then reduce to 4 l, then add alcohol (2 L) and sugar (850 g)].

L., *Saccharum officinarum* L., *Scoparia dulcis* L.

3.4.2.2. Method of preparation and administration of herbal remedies. Among the 42 herbal recipes used by the 27 KTH, 36 (85.7%) are multi-ingredient recipes and 6 (14.3%) are single-ingredient recipes. Multi-ingredient formulae can comprise from 2 to 27 different plant species depending on the KTH.

Of the 36 multi-herb formulae, 29 (80.6%) were presented in form of transparent plastic bags containing pieces of dried plants part already chopped (see Fig. 2). These formulation types are prepared by decoction (i.e. boiling dried plants parts for 10–15 min) and administered orally (water is filtered and drunk).

Four multi-herb remedies were prepared in form of a syrup and administered orally. Syrup was said to reduce the bitterness of some plants, especially *Andrographis paniculata* (Burm.f.) Nees.

Four other multi-ingredient formulae were presented in form of pills, and ingested orally.

Plants from three multi-herb recipes were ground finely and the powder was packed in transparent plastic bags. The latter was prepared by infusion (i.e. adding hot water [70–80 °C] to the plant powder) and administered orally.

Finally, one multi-herb recipe was prepared by mixing and crushing five different ingredients (*Garcinia cochinchinensis* (Lour.) Choisy, *Allium sativum* L., *Piper nigrum* L., baking powder and sugar), and was administered orally in the form of a paste.

Three KTH stated that they prepare their recipes in three different formulation types (i.e. mix of dried plants, plant powder or pills), each one with the same medicinal plants, so the patient can choose what is the most convenient for him.

Of the 6 single-herb formulas, three were presented in form of plant powder, prepared by infusion. One was a dried plant to be prepared by decoction. Another one was a pill made out of the whole plant of *Andrographis paniculata*. The last one was a fresh part of plant (*Aloe vera* gel).

In this survey, oral administration of plants was the only used way of administration, and the same was also reported from three others ethnobotanical surveys on liver diseases in Asia and Africa (Kpodar et al., 2016; Mukazayire et al., 2011; Sharma et al., 2012). However, it should be noted that other ways of administration are used by Khmer traditional healers depending upon the health problem treated; asthma is usually treated with fumigation of plants, and wounds are mainly cured with local application of plants (Crochet, 2000).

3.4.2.3. Herbal therapy protocols. Altogether, 27 healers reported to use from 1 to 5 recipes for treating liver disorders.

For 18 KTH, the treatment protocol is based on the use of one recipe alone, while seven KTH use an association of at least 2 different herbal recipes, administered simultaneously. Among the latter group, three healers reported to combine two different herbal remedies in order to have a complementary effect on the liver. For the other four healers, the two different herbal recipes they prescribe to the patient have different activities: one is specific for liver disorders, and the other

recipe is said to have general regulatory effects on stomach, intestines, blood circulation, or is presented as a panacea.

Besides, two KTH reported the use of different recipes depending on the stage of the disease. For both healers, three different stages can be distinguished. According to one healer, the first stage corresponds to a patient with liver problems only, the second stage of the disease is characterized by a loss of appetite and the third stage is characterized by extreme weakness, and inability to walk. In the first stage, the treatment is based on the use of a mix of 8 dried plants. In the second stage, a syrup combining 4 plant species (*Andrographis paniculata* (Burm.f.) Nees, *Boerhavia diffusa* L., *Eclipta prostrata* (L.) L., and *Phyllanthus amarus* Schumach. & Thonn.) is added to the previous preparation. In the third stage, the same recipes are prepared with freshly collected plants. For the other healer, the first stage, described as "*relik*" (inflammation) is treated by a mix of dried plants (25 plants species). The second stage called "*mé rok*" (origin of disease) is cured by the same previous mixture plus one more plant (*Areca catechu* L.) said to have "*somrap mé rok*" properties. The last stage corresponds to "*krenn thlaeum*" (cirrhosis) and "*tiyek teuk*" (ascites), and is treated by a mix of 6 dried plants.

Among the various recipes used by KTH, one was mentioned several times (4 citations). In this preparation, four plant species are combined together: *Andrographis paniculata* (Burm.f.) Nees (whole plant), *Boerhavia diffusa* L. (whole plant), *Eclipta prostrata* (L.) L. (whole plant), and *Phyllanthus amarus* Schumach. & Thonn (whole plant).

3.4.2.4. Activity of plants according to Khmer traditional healers. For each plant mentioned in the survey, Khmer traditional healers cited their different properties/effects. The most common properties reported by KTH are: "*ktchol*" (wind), "*trocheak*" (cold), "*somrap mé rok*" (against the origin of the diseases) and "*psah*" (heal, cicatrize) (Fig. 3).

In this survey, 18 plants have been said to have "*ktchol*" properties. Regarding "*ktchol*" effect, it was explained to us that a deficiency in "*ktchol*" results in constipation, bloating or liquid accumulation (ascites). Thus, "*ktchol*" plants will help to restore the movements of fluids blocked within the body and will help in the general well-being and recovery of the patient. In this group of plants, *Salacia chinensis* L. and *Senna alata* (L.) Roxb. are amongst the most popular, and are used for their laxative properties (4 citations each), together with *Ampelocissus arachnoidea* (Hausskn.) Planch. and *Imperata cylindrica* (L.) Raeusch. (2 citations each) said to be diuretic.

Thirty-three and thirty-four plants were said to have "*trocheak*" (cool) and "*psah*" (heal, cicatrize) effects respectively, and 19 plants were said to have both properties hence displaying a significant overlapping between these 2 properties.

Use of plants said to have "*trocheak*" effect are in accordance with the humoral theory, as cool plants are used to treat "hot conditions" such as liver diseases (Burke et al., 2011). Among the 33 medicinal plants mentioned to have "*trocheak*" property, *Andrographis paniculata* (Burm.f.) Nees, *Physalis minima* L., and *Passiflora foetida* L. were the most cited plants with 8, 7 and 5 citations respectively. For some plants of this category (*Andrographis paniculata* (Burm.f.) Nees, *Boerhavia diffusa* L., *Eclipta prostrata* (L.) L., *Eurycoma longifolia* Jack, *Physalis minima* L. and *Tinospora crispa* (L.) Hook. f. & Thomson), it was stated that their cooling effect was due to their bitterness.

Thirty-four plants have been noted to possess "*psah*" properties. The word "*psah*" is assigned to plants used to cure inflammations, infections, wounds, burns and repair tissue damage, whether internal or external, in a very efficient manner. From French colonial times until now, this term has also been used to describe the effects of antibiotics (*thnam psah, or psah* medicine) perceived to heal very fast. Plants with "*psah*" high consensus are *Cananga latifolia* (Hook.f. & Thomson) Finet & Gagnep. (14 citations), *Smilax glabra* L. (8 citations), and *Willughbeia edulis* Roxb. (5 citations).

Andrographis paniculata (Burm.f.) Nees, *Phyllanthus amarus* Schumach. & Thonn., *Physalis minima* L. and *Pouzolzia zeylanica* (L.) Benn. were highly cited for both "*psah*" and "*trocheak*" activity. According healers, combination of both "*trocheak*" and "*psah*" properties (e.g. *Gomphrena celoisioides* Mart. and *Physalis minima* L.) will help in the restoration of liver functions.

Finally, "*somrap mé rok*" (against the origin of disease) activity has been recorded 17 times, for 16 species: thus, only one species has been cited more than once for this activity (*Andrographis paniculata* (Burm.f.) Nees). This absence of consensus might be explained by the fact that "*mé rok*" concept is of recent origin, hence the difficulty for the healers to allocate it to a specific species.

Interestingly, four species are said to share the three above properties (*somrap mé rok, psah, trocheak*), which are *Pouzolzia zeylanica* (L.) Benn., *Andrographis paniculata* (Burm.f.) Nees, *Eclipta prostrata* (L.) L. and *Eurycoma longifolia* Jack, this last species being cited only once in each category. No species has been noted to have the 4 properties (*ktchol, trocheak, somrap mé rok, psah*).

During the free listing of the alleged pharmacological activity of plant species, healers also mentioned some symptomatic uses of the plants they use in their recipe. Most frequently cited uses were liver aid (*Gomphrena celosioides* Mart. and *Physalis minima* L.), appetite stimulant (*Leea rubra* Blume ex Spreng. and *Rhodamnia dumetorum* (DC.) Merr. & L.M.Perry), tonic effect (*Oryza sativa* L. and *Prismatomeris tetrandra* (Roxb.) K.Schum.), and for blood circulation (*Abutilon indicum* (L.) Sweet, *Tarlmounia elliptica* (DC.) "H.Rob., S.C.Keeley, Skvarla & R.Chan", and *Willughbeia edulis* Roxb.) with 8, 5, 5 and 3 citations respectively. Moreover, some plants were said to be general health improvers (*Moringa oleifera* Lam.), helping to reduce anxiety and to induce sleep (*Passiflora foetida* L.), to drain pus (*Saccharum officinarum* L.), to ease breathing (*Hydnophytum formicarum* Jack) and to relieve pain (*Garcinia cochinchinensis* (Lour.) Choisy). According to KTH, these plants might be selected depending on the health status of the patient, and thus the ingredients of the recipe can vary from a patient to another.

Altogether, these data are in accordance with the theoretical concepts of the Khmer medicine, and more broadly humoral medicine, in which cool plants helps to cure "hot diseases" and thus helps to reduce inflammation and heal damaged tissues. Also, the linking between the organoleptic properties (bitterness) of some plants with their pharmacological activity (*trocheak*) is characteristic of this type of medicine (Burke et al., 2011; Manderson, 1981; Van Esterik, 1988). In Traditional Chinese Medicine, bitterness is said to dissipate excessive heat in any part of the body (Bensky et al., 2015).

3.4.2.5. Pharmacological activities of the most cited plants. In this survey, 16 species were cited at least 5 times. Table 5 shows their relevant ethnopharmacological uses and pharmacological activities already reported by previous studies.

Of the 16 most cited plants, 15 (93.7%) have been already reported to be used for treating liver disorders in others ethnobotanical studies (in Asia, Africa and America), 12 species (75%) have shown to possess hepatoprotective activity on different animal models, and 6 plants (37.5%) have been proven to possess anti-hepatocarcinogenic property either on *in vitro* or *in vivo* models. Moreover, *Phyllanthus amarus* Schumach. & Thonn. has been studied for its anti-hepatitis B and anti-hepatitis C activities, *Eclipta prostrata* (L.) L.) has been shown to possess anti-hepatitis C properties, and *Andrographis paniculata* (Burm.f.) Nees has proven to exhibit anti-hepatitis B activity.

Since patients with liver diseases are particularly prone to develop bacterial infections (e.g. during liver abscess or ascites infections), and

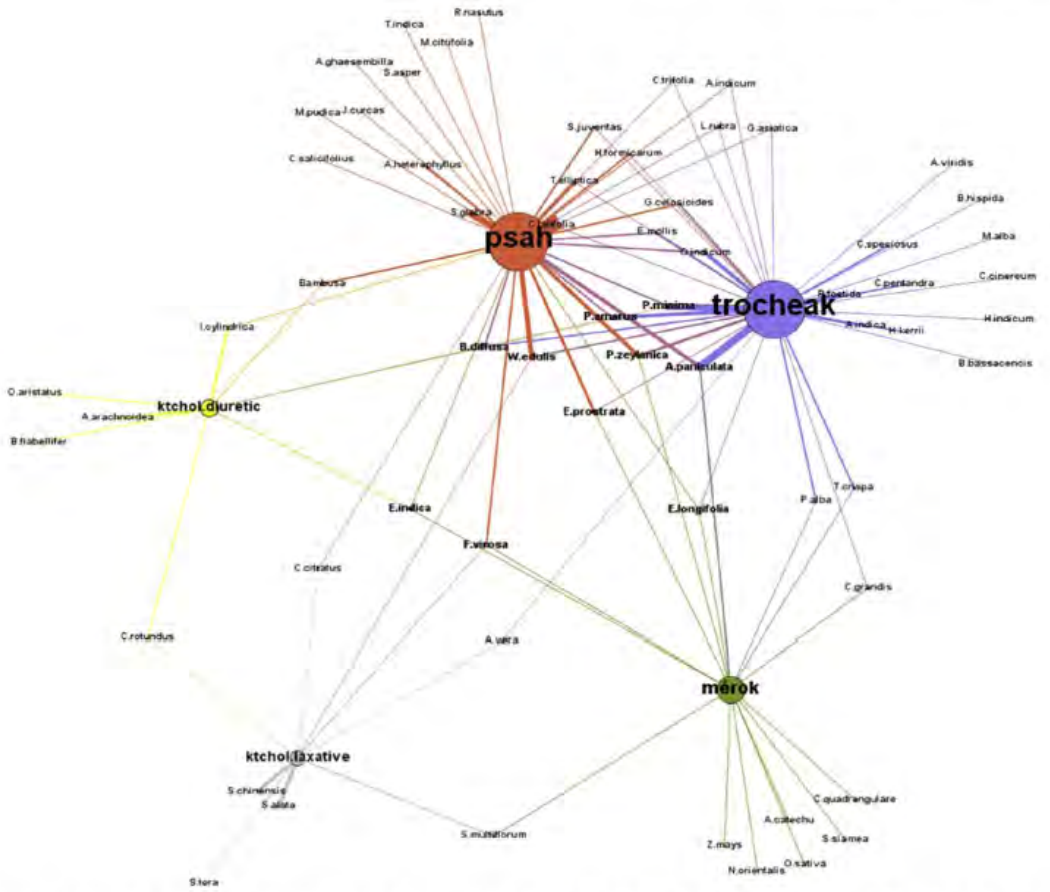

Fig. 3. Relationship between medicinal plants and the five most common Khmer properties (psah, trocheak, mérok, ktchol diuretic, ktchol laxative). Five colors are attributed to each properties and the most cited plants for each properties. The size of the circles and the lines are proportional to the frequency of citations. The closer is the plant to the node (property), the stronger is the relationship between the plant and the property. Only the medicinal plants mentioned for the five properties have been included. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the most common bacteria involved in these infections are: *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pneumonia* and *Staphylococcus aureus* (Borzio et al., 2001), we also focused on the antimicrobial activities of these plants. In total, 13 species have been shown to possess antimicrobial property and the most common bacteria inhibited were *Staphylococcus aureus* (11 plants) followed by *Escherichia coli* (9 plants).

Moreover, inflammation is a common process involved in various chronic and acute liver diseases (e.g. viral hepatitis, cirrhosis, liver cancer) (Coussens and Werb, 2002; Goodman, 2007). Thus, medicinal plants with anti-inflammatory activity might also be of interest in the treatment of liver diseases. In our survey, 12 of the 16 most cited species have been shown to possess anti-inflammatory activities on various animal models.

Finally, *Passiflora foetida* L., highly cited in this survey (9 citations), has been shown to possess activity in the liver and also to have sedative and antidepressant activity, thus confirming its use as a sedative and anxiolytic in our survey (Miyasaka et al., 2007).

Besides these directly related liver activities, 18 plants from the "*ktchol*" category were said to have diuretic (10 species) or laxative effect (8 species). Among the plants said to have "*ktchol*" activity with diuretic effect, nine of them (*Bambusa vulgaris* Schrad., *Boerhavia diffusa* L., *Borassus flabellifer* L., *Cyperus rotundus* L., *Eleusine indica* (L.) Gaertn., *Imperata cylindrica* (L.) Raeusch., *Orthosiphon aristatus* (Blume) Miq., *Phyllanthus amarus* Schumach. & Thonn and *Physalis minima* L.) have been reported in traditional use for this effect, and 5 of them have demonstrated diuretic activity in pharmacological studies (*Bambusa vulgaris* Schrad., *Boerhavia diffusa* L, *Imperata cylindrica* (L.) Raeusch., *Orthosiphon aristatus* (Blume) Miq., and *Phyllanthus amarus* Schumach. & Thonn) (Chothani and Vaghasiya, 2012; Du Dat et al., 1992; Iqbal and Gnanaraj, 2011; Mishra et al., 2014; Paschapur et al., 2009; Patel et al., 2011; Peerzada et al., 2015; Potdar et al., 2010).

Moreover, of the 8 species said to have "*ktchol*" activity with laxative effect, 5 plants (*Aloe vera* (L.) Burm.f., *Cyperus rotundus* L., *Salacia chinensis* L., *Senna alata* (L.) Roxb., and *Senna tora* (L.) Roxb.) have been already reported to treat constipation, and 2 plants species have been proven effective (*Aloe vera* (L.) Burm.f., and *Senna alata* (L.) Roxb.) (Chuakul et al., 2002; Hennebelle et al., 2009; Peerzada et al., 2015; Perry, 1980; Vogler and Ernst, 1999).

Among the multi-ingredients recipes reported in our survey, only the combination of *Andrographis paniculata* (Burm.f.) Nees, *Boerhavia diffusa* L., *Eclipta prostrata* (L.) L. and *Phyllanthus amarus* Schumach. & Thonn was mentioned several times. These four plants species are also commonly found in poly-herbal formulations such as Virgoliv® and Stimuliv® which are claimed to be Ayurvedic medicine (Ghosh et al., 2011; Ingawale et al., 2015). Indeed, these four plants are well known in Indian system medicine for having liver protection properties (Thyagarajan et al., 2002), and all of them have shown to possess hepatoprotective activities in pharmacological studies (Akbar, 2011; Mishra et al., 2014; Patel et al., 2011; Priya et al., 2010). Moreover, multi-herbal drug strategy is widely employed in traditional medicine, since combinations of herbal medicines can exhibit synergistic effects and can treat multicausal diseases (Wagner and Ulrich-Merzenich, 2009; Williamson, 2001). In this case, *Phyllanthus amarus* Schumach. & Thonn has been reported to have

Table 5
Ethnobotanical uses and pharmacological activities of plants cited more than five times as previously reported in the literature.

Scientific name	No of citations	Relevant ethnobotanical uses	Relevant pharmacological activities			
			Hepatoprotective	Anti-hepatocarcinogenic	Antimicrobial/ Antiviral	Anti-inflammatory
Cananga latifolia	15	Liver diseases in Cambodia (Chassagne et al., 2016a).	ND	ND	ND	ND
Andrographis paniculata	12	Jaundice in Thailand (Inta et al., 2013). Hepatitis, jaundice, liver problems in India (Hossain et al., 2014; Sharma et al., 2012; Singh et al., 2002). Hepatitis in China (Hossain et al., 2014).	The leaf extract of *A. paniculata* shows protective effect on thioacetamide induced liver cirrhosis in rats (Bardi et al., 2014). Its major diterpenoid (i.e. andrographolide, neoandrographolide, andrographiside) show protective effects on carbon tetrachloride induced liver damage in mice (Kapil et al., 1993).	Andrographolide possesses anti-proliferative activity against hepatocellular carcinoma cells (HepG2, Hep3G, SMMC–7721) (Ji et al., 2008; Li et al., 2007; Yang et al., 2009). Andrographolide enhances the effect of 5-FU (5-Fluorouracile) in hepatocellular carcinoma cells (Yang et al., 2009).	Dehydroandrographolide and andrographolide possess anti-hepatitis B virus activity (Chen et al., 2014). The extract water of *A. paniculata* has antibacterial activities against *Staphylococcus aureus* and Methicillin resistant *S. aureus* (Zaidan et al., 2005).	Intraperitoneal administration of *A. paniculata* extract produces complete inhibition on carrageenan induced inflammation models (Sheeja et al., 2006). The ethyl-acetate fraction of *A.paniculata* possesses also anti-inflammatory activities in vitro (Chao et al., 2010).
Smilax glabra	10	Liver complaints in Cambodia (Menaut, 1930). Liver disorders in Vietnam (Vo et al., 2014). Hepatitis in China (Au et al., 2008).	The flavonoid-rich fraction from rhizomes of *S. glabra* Roxb. displays protective effect on carbon tetrachloride induced hepatotoxicity in rats (Xia et al., 2013).	The root extract of *S. glabra* possesses anti-proliferative and apoptotic effect on human hepatoma cell lines (HepG2 and Hep3B) (Sa et al., 2008).	The ethanolic extract, ethyl acetate fraction and *n*-butanol fraction of *S.glabra* displays antibacterial activity against *S. aureus* (Xu et al., 2013).	The water extract of *S.glabra* inhibits the production of NO and TNF-α in LPS and IFN-γ activated mouse macrophages (Ravipati et al., 2012).
Gomphrena celosioides	9	Liver diseases in Togo (Kpodar et al., 2016). Viral hepatitis, jaundice, liver problems in Benin (Guinnin et al., 2015; Sangare et al., 2014).	The aqueous extract of *G.celosioides* shows hepatoprotective effect on carbon tetrachloride induced liver damage in rats (Sangare et al., 2012).	ND	The ethanolic extract and pure compounds of *G.celosioides* shows antibacterial effect against *S.aureus* (de Moura et al., 2004).	The aqueous leaf extract of *G.celosioides* produces antiinflammatory effect on carrageenan induced paw edema in rats (Oladele et al., 2009).
Passiflora foetida	9	Liver disorders and cooling medicine for liver in India (Lim, 2012). Liver diseases in Togo (Kpodar et al., 2016).	The ethanolic extract of fruits of *P. foetida* possesses hepatoprotective effect on carbon tetrachloride induced hepatic injury in rats (Lim, 2012).	ND	Leaves and stem extracts of *P.foetida* inhibit the growth of *Escherichia coli* (Bendini et al., 2006).	The ethanolic leaf extract of *P. foetida* produces anti-inflammatory effect on carrageenan induced paw edema in rats (Sasikala et al., 2011).
Physalis minima	9	Liver disorders and jaundice in India (Salave et al., 2011).	The leaf extract of *P. minima* shows hepatoprotective activity on rifampicin-isoniazid induced liver injury in rats (Tammu and Ramana, 2014).	ND	Chloroform, ethanol, ethyl acetate and methanol extracts of *P. minima* possess antibacterial activities against *E.coli*, *K.pneumoniae* and *S.aureus* (Chothani and Vaghasiya, 2012).	Crude extract and chloroform fraction of *P.minima* exhibit anti-inflammatory activity on carrageenan induced paw edema in rats (Khan et al., 2009).
Willughbeia edulis	8	Hepatitis in Cambodia (Perry, 1980). Jaundice in Malaysia (Wiart, 2006).	ND	ND	ND	ND
Phyllanthus amarus	7	Jaundice in Thailand (Inta et al., 2013). Hepatitis, jaundice and liver diseases in India (Patel et al., 2011). Liver diseases in Togo (Kpodar et al., 2016).	The ethanolic extract of *P. amarus* possesses hepatoprotective effect on aflatoxin-B1 induced hepatic damage in mice (Naaz et al., 2007).	The aqueous extract of *P. amarus* increases the life span of rats with hepatocellular carcinoma (Rajeshkumar and Kuttan, 2000).	The dried powder of the whole plant shows anti-hepatitis B virus effect on chronic carriers of hepatitis B virus (Thyagarajan et al., 1988). The methanolic extract of *P. amarus* inhibits Hepatitis C virus replication *in vitro* (Ravikumar et al., 2011). The aqueous and methanolic extracts of *P. amarus* is active against *E. coli* and *S. aureus* (Patel et al., 2011).	The hexan extract and the lignan-rich fraction of *P. amarus* exhibit anti- inflammatory activity on agents induced paw edema in rats (Kassuya et al., 2005). The aqueous, ethanol and hexane extracts of *P.amarus* inhibit the induction of COX–2, iNOS, TNF-α *in vitro* (Kiemer et al., 2003).
Salacia chinensis	6	Liver diseases in Cambodia (Schmitt, 2004). Liver cancer in Thailand (Poonthananiwatkul et al., 2015).	The methanolic extract from the leaves of *S. chinensis* shows a protective effect on α-galactosamine induced cytotoxicity in hepatocytes (Nakamura et al., 2011).	ND	The aqueous and ethanolic extracts of *S. chinensis* show no significant activities against *E. coli*, *K. pneumoniae* and *S. aureus* (Kannaiyan et al., 2012).	ND
Boerhavia diffusa	5	Hepatitis and jaundice in Cambodia (Cheng and Huon, 1996). Hepatic system and jaundice in India (Sharma et al., 2012). Hepatitis in Pakistan	The aqueous extract of roots of *B. diffusa* shows protective effect on thioacetamide induced liver damage in rats (Rawat et al., 1997).	ND	The aqueous and ethanolic extracts of *B. diffusa* exhibit antimicrobial activities against *E. coli* and *S. aureus* (Akinnibosun et al., 2009).	The alcoholic extract of *B. diffusa* produces anti-inflammatory activities on agents induced paw edema in rats (Mishra et al., 2014).

(continued on next page)

Table 5 *(continued)*

Scientific name	No of citations	Relevant ethnobotanical uses	Relevant pharmacological activities			
			Hepatoprotective	Anti-hepatocarcinogenic	Antimicrobial/ Antiviral	Anti-inflammatory
		(Ullah et al., 2014). Liver diseases in Togo (Kpodar et al., 2016).				
Eclipta prostrata	5	Hepatitis, jaundice and liver cirrhosis in China (Li and Xing, 2016). Jaundice, liver disorders and viral hepatitis in India (Sharma et al., 2012).	The crude extract of *E. prostrata* exhibits protective effect on carbon tetrachloride and acetaminophen induced hepatotoxicity in rats (Lin et al., 1996)	The hydroalcoholic extract of *E. prostrata* exhibits anti-proliferative effect on hepatocellular carcinoma cells (HepG2) (Chaudhary et al., 2011).	*E. prostrata* extract and its isolates exhibit anti-hepatitis C virus activity *in vitro* (Manvar et al., 2012). The ethyl acetate fraction of *E. prostrata* shows antimicrobial activity against *S. aureus* (Dalal et al., 2010).	The methanolic extract of leaves of *E. prostrata* shows anti-inflammatory activity on carrageenan induced paw edema in rats (Arunachalam et al., 2009).
Elephantopus mollis	5	Hepatitis in Taiwan (Lin and Kan, 1990). Jaundice in Brazil (Botsaris, 2007).	The ethanolic extract of *E. mollis* displays protective effect on alcohol induced liver damage in mice (Ho et al., 2012).	The ethyl acetate extract of *E. mollis* produces anti-proliferative effect on hepatoma cells (HepG2) (Ooi et al., 2014)	Triterpenoids from *E. mollis* exhibit antimicrobial activities against *E. coli* (Kabiru and Por, 2013).	A formulation containing *E. mollis* and two others plants have demonstrated its activity on carrageenan induced paw edema in rats (Tsai and Lin, 1998).
Leea rubra	5	Liver diseases in Cambodia (Chassagne et al., 2016a).	ND	ND	ND	ND
Oroxylum indicum	5	Jaundice in Cambodia, Laos and Vietnam (Audibert et al., 2015; de Boer et al., 2012; Dinda et al., 2015). Hepatoprotective, jaundice and viral hepatitis in India (Dinda et al., 2015).	The methanolic bark extract of *O. indicum* shows protective effect on carbon tetrachloride induced hepatotoxicity in rats (Tripathy et al., 2011).	Oroxylin A, a flavonoid isolated from the stem bark of *O. indicum*, induces apoptosis of human hepatocellular carcinoma cell line (HepG2) (Hu et al., 2006).	Dichloromethane of the stem bark and root of *O. indicum* possess antimicrobial activities against *E. coli* and *S. aureus* (Mat Ali et al., 1998)	Dichloromethane of the stem bark and root of *O. indicum* exhibit anti-inflammatory activity *in vitro* (Mat Ali et al., 1998).
Pouzolzia zeylanica	5	ND	ND	ND	The methanolic extract of *P. zeylanica* exhibits moderate antibacterial activity against *E. coli* and *S. aureus* (Hossain et al., 2017)	The methanolic extract of *P. zeylanica* possesses anti-inflammatory effect on xylene ear edema model (Hossain et al., 2017).
Senna alata	5	Liver disorders in Cambodia (Menaut, 1930). Hepatalgia and jaundice in Vietnam (Lim, 2014). Liver diseases in Togo (Kpodar et al., 2016).	The leaf extract of *S. alata* exhibits protective effect on paracetamol induced liver damage in rats (Lim, 2014).	ND	The water and ethanol extract of *S. alata* possess antibacterial activity against *E. coli* and *S. aureus* (Lim, 2014)	The alcoholic extract of the leaves of *S. alata* shows anti-inflammatory activity in mice (Palanichamy and Nagarajan, 1990)

ND = Not Documented.

anti-cancer activity on hepatocellular carcinoma models, while *Eclipta prostrata* (L.) L. has been shown to possess analgesic activity, *Andrographis paniculata* (Burm.f.) Nees has been studied for its hepatostimulating activities and *Boerhavia diffusa* L. has been proven to possess immunostimulating properties (Chua, 2014; Patil and Bhalsing, 2016; Rajeshkumar and Kuttan, 2000; Sawant et al., 2004).

4. Conclusion

The present study reports 42 herbal remedies and identified 83 medicinal plant species from 40 families commonly used by Khmer traditional healers to treat liver disorders.

A large number of these plants species have been already mentioned for the treatment of liver disorders in others countries, and have been proven to possess a protective role on liver damage. Thus, it suggests that most of the plants cited in this survey could be considered as an appropriate therapy for liver disorders. However, KTH should be aware that the use of some species might also present a risk, as they might present some toxicity. This is the case for *Heliotropium indicum* L., *Morinda citrifolia* L., *Senna siamea* (Lam.) H.S.Irwin & Barneby and *Tinospora crispa* (L.) Hook. f. & Thomson, reported to be hepatotoxic and thus not to be recommended for patients with liver impairments (Ahmad et al., 2016; Kamagaté et al., 2014; Roeder and Wiedenfeld, 2011; Yüce et al., 2006). Finally, two species are widely used in the treatment of liver diseases but have not being investigated for their hepatoprotective properties. Therefore, these plants should be recommended for further pharmacological investigations: *Cananga latifolia* (Hook.f. & Thomson) Finet & Gagnep. and *Willughbeia edulis* Roxb.

Nowadays, Khmer traditional healers are undergoing a process of professional change. Traditional healers' practices and knowledge evolves and takes the form of neotraditionalism. According to Pordié (2008), neotraditionalism is mainly characterized by: "the appropriation of ideologies and epistemologies, the use of modern rhetoric and practices that are, at least initially, foreign to (traditional) medicine. The clearest example is that of biomedical science". As shown in this survey, KTH use the pharmacological category "*somrap mé rok*", imported from the biomedical model to describe the activity of some species; most of them base their diagnosis on medical records; and they also describe different types of liver disease and causes using the occidental medicinal theoretical frame, more or less well understood. Moreover, it appears that these neotraditional healers are practicing medicine as a living, thus playing a growing role in the health care of the population. Because, in the case of liver diseases, treatments provided by KTH are often the only ones financially accessible to patients, further studies should be performed in order to understand how to include the neotraditional healers (and the medicinal plants) in the national program management of patients suffering from liver disorders.

Author contributions

Conceived and designed the survey: FC, GB, ED. Performed the survey: FC, HP. Identified the plants: FC, HP. Analyzed the data: FC, GB. Wrote the paper: FC, GB, ED.

Supplementary material 2

Vernacular names in Khmer characters of the 83 medicinal plants are given in Supplementary data 2.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jep.2017.03.002.

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CHAPITRE 4 : IDENTIFICATION DE COMPOSES BIOACTIFS SUR CELLULES HEPG2 A PARTIR DE PLANTES SELECTIONNEES

1. Contexte et objectifs

Dans les chapitres précédents, nous avons vu qu'une majorité de patients atteints de cancer du foie se tourne vers l'utilisation de plantes médicinales en complément de la médecine moderne. Au total, 86 plantes ont été indiquées par les médecins traditionnels (83 espèces) et par les patients (8 espèces, dont 5 communes aux médecins traditionnels) pour le traitement de diverses affections du foie et notamment le cancer du foie. Suite à une recherche bibliographique, nous avons calculé que 57 espèces (66,2%) ont déjà été étudiées pour leur activité hépatoprotective *in vivo*, justifiant ainsi leur rôle dans le traitement des maladies du foie. Cependant, 28 espèces (32,5%) seulement ont été étudiées pour leur activité sur cellules cancéreuses hépatiques, ce qui suggère l'intérêt de continuer les investigations pharmacologiques sur le pool total de plantes avec ce type de modèle d'étude *in vitro*.

Dans cette étude, notre démarche a été de sélectionner des espèces de plantes selon leur convergence d'usage dans les maladies du foie en Asie du Sud-Est, puis selon leur aptitude à inhiber la prolifération de cellules d'hépatocarcinome humain (HepG2), avec pour objectif final

d'identifier les composés susceptibles d'être impliqués dans l'activité antiproliférative par une méthode de déréplication[13].

2. Matériels et Méthodes

La sélection des espèces de plantes à étudier a été faite sur la base de plusieurs critères :

- Un score élevé (S) dans l'analyse bibliographique (cf. Introduction §3.4)
- Un nombre de citations important dans l'étude précédente (cf. chapitre 3)
- L'absence d'étude pharmacologique des extraits de plantes sur lignées cellulaires HepG2 *in vitro*
- L'accessibilité et l'abondance de la ressource dans l'aire de collecte prospectée
- La facilité d'identification botanique de l'espèce

Au final, dix espèces de plantes ont été retenues et collectées. Pour chaque espèce sélectionnée, une extraction éthanolique et une extraction aqueuse (proche de la préparation traditionnelle) de la partie de plante utilisé en MT ont été réalisées. Un test d'inhibition de prolifération de cellules d'hépatocarcinome humain (HepG2) a ensuite été effectué pour tous les extraits de plantes en utilisant la méthode colorimétrique avec le sel de tétrazolium MTT. Les extraits présentant un IC_{50} (concentration inhibitrice médiane) inférieur à 250 µg/ml ont été fractionnés par une méthode d'extraction en phase solide. Une analyse des métabolites présents dans chaque extrait et fraction actifs a été réalisée par chromatographie liquide haute performance couplée à un spectromètre de masse haute résolution (UHPLC-HRMS). Puis une analyse multivariée a été appliquée à ces profils UHPLC-HRMS afin de classer les métabolites en fonction de leur activité antiproliférative. Finalement, les composés susceptibles d'être responsables de l'activité antiproliférative ont été provisoirement identifiés en utilisant des bases de données spécifiques aux plantes et les données de la fragmentation MS/MS *in silico*.

[13] La déréplication consiste à identifier, par des méthodes de chimie analytique précise, la présence de composés déjà connus dans un mélange avant même leur isolement physique.

3. Principaux résultats

Les résultats du test indiquent que les extraits éthanoliques d'*Andrographis paniculata*, *Oroxylum indicum*, *Orthosiphon aristatus* et *Willughbeia edulis* présentent une meilleure activité antiproliférative (IC_{50} = 195.9, 64.1, 71.3, 66.7 µg/ml respectivement), comparé aux extraits aqueux et éthanoliques des six autres espèces (*Boerhavia diffusa*, *Cananga latifolia*, *Gomphrena celosioides*, *Melastoma saigonense*, *Salacia chinensis*, et *Senna alata*) (IC_{50} > 250 µg/ml). En fonction de ces résultats, les extraits éthanoliques ont été choisis pour l'analyse métabolomique. Au moyen d'un procédé de déréplication, les composés susceptibles d'être responsables de l'activité antiproliférative ont été classées par ordre d'activité antiproliférative puis identifiés pour *A. paniculata* (andrographolactone et dehydroandrographolide), *O. indicum* (baicalein, chrysin, oroxylin A et scutellarein) et *O. aristatus* (5-desmethylsinensetin). Dans le cas de *W. edulis*, le manque d'études phytochimiques sur cette espèce ne nous a pas permis d'identifier précisément les composés impliqués dans l'activité antiproliférative.

4. Discussion et conclusion

A partir d'une analyse bibliographique de 378 espèces de plantes utilisées traditionnellement dans les maladies du foie en Asie du Sud-Est, dix plantes ont été sélectionnés puis comparés pour leur aptitude, dose-dépendante, à inhiber la prolifération d'une lignée de cellules cancéreuses hépatiques. Parmi celles-ci, quatre ont été choisis puisqu'elles présentaient une meilleure activité antiproliférative. Les composés susceptibles d'être responsables de l'activité ont été classés par ordre d'activité antiproliférative puis identifiés pour trois d'entre elles (*Andrographis paniculata*, *Oroxylum indicum*, *Orthosiphon aristatus*). De nombreuses informations bibliographiques confirment l'activité pharmacologique de ces trois plantes dans le traitement des maladies du foie, cependant il existe peu de donnée disponible sur *Willughbeia edulis*. Cette espèce qui est parmi les plus citées à la fois par les médecins traditionnels et dans

la bibliographie apparaît donc comme une espèce très intéressante, pour laquelle des examens complémentaires devraient être réalisés. En particulier, la sélectivité de son activité antiproliférative devrait être évaluée, en identifiant précisément les composés responsables de cette activité, conjointement à d'autres activités pharmacologiques relatives aux maladies du foie.

Par ailleurs, le procédé de déréplication utilisé dans cette étude pourrait être appliqué à d'autres tests biologiques, notamment d'hépatoprotection, d'activité anti-oxydante ou d'anti-inflammatoire afin de mieux comprendre les composés susceptibles d'être responsables de l'activité des plantes dans le traitement des maladies du foie.

Ce travail s'est traduit par la rédaction d'un article à soumettre dans la revue scientifique internationale indexée *Journal of Ethnopharmacology*, et intitulé :

Article 4. « From an ethnobotanical literature review of Southeast-Asian plants used traditionally to treat liver disorders to a dereplication of antiproliferative compounds on HepG2 cells using a metabolomics approach »

François Chassagne, Mohamed Haddad, Aurélien Amiel, Chiobouaphong Pharkeovilay, Chanthanom Manithip, Geneviève Bourdy, Eric Deharo, Guillaume Marti.

L'article est présenté ci-dessous *in extenso* dans sa forme manuscrite.

Title:

From an ethnobotanical literature review of Southeast-Asian plants used traditionally to treat liver disorders to a dereplication of antiproliferative compounds on HepG2 cells using a metabolomics approach

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Statement conflict of interest:

The authors declare no conflict of interest.

Abstract:

Ethnopharmacological relevance:

Liver cancer is a major health burden in Southeast Asia, and most of patients turns towards the use of medicinal plants to alleviate their symptoms. Traditional plants were selected according to a point system from a comprehensive literature survey. The aim of this work was to apply a dereplication approach in order to identify compounds *in mixture* putatively responsible for the antiproliferative activity on human hepatoma cells of selected species.

Materials and Methods:

A comprehensive review focused on Southeast Asian literature was performed to determine plant species reported in the treatment of liver disorders. A point system allowed the selection of plants for further collection and extraction. Antiproliferative activities of extracts were assessed on HepG2 cell lines. Extracts with IC_{50} < 250 µg/ml were fractionated using solid phase extraction (SPE). Constituents of crude extracts and their respective fractions were characterized by ultra-high-performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS). Multivariate analysis of UHPLC-HRMS data allowed the ranking of metabolites according to their antiproliferative activity. Top ranked compounds were putatively identified using plant databases and *in silico* fragmentation pattern.

Results:

In the review process, 45 publications were analyzed providing a list of 378 plant species. Ten species were selected and tested on HepG2 model. Ethanolic extracts of *Andrographis paniculata*, *Oroxylum indicum*, *Orthosiphon aristatus* and *Willughbeia edulis* showed the highest antiproliferative effect (IC_{50} = 195.9, 64.1, 71.3, 66.7 µg/ml respectively) and were submitted to metabolomic analysis. A dereplication approach allowed the putative annotation of compounds responsible for the antiproliferative property in *A. paniculata* (andrographolactone and dehydroandrographolide), *O. indicum* (baicalein, chrysin, oroxylin A and scutellarein) and *O. aristatus* (5-desmethylsinensetin). For *W. edulis*, no compound belonging to the species was annotated in the top ranked features, thus underlining the value of the workflow depicted here to focus on further bioassay guided fractionation approach on this species.

Conclusion:

Starting from a literature review of 378 medicinal plants used traditionally for liver disorders in Southeast Asia, ten plant species were selected, among which, four were further investigated for their ability to inhibit proliferation of human hepatoma cell lines. Putative active compounds were ranked and identified for three plants. Although these three plants are well known for their hepatoprotective effect, no information is available for *W. edulis*. Thus, further investigations should be performed to determine its phytochemical constituents, to evaluate the selectivity of the antiproliferative effect and others pharmacological activities related to liver disorders.

Keywords: hepatocellular carcinoma; medicinal plants; HepG2; antiproliferative effect; Southeast Asia; metabolomics; dereplication

1. Introduction:

Liver cancer represent the second leading cause of cancer-related death worldwide and is therefore a major health public problem (Ferlay et al., 2015), hepatocellular carcinoma (HCC) being responsible of 80% of all liver cancers. The geographical distribution of HCC is heterogeneous with a high prevalence in developing regions of Eastern and South-eastern Asia (Pourhoseingholi et al., 2015). Curative treatments for HCC are mainly based on surgical resection, liver transplantation and local ablation. Although, these therapies can improve the prognosis of patients, there are indicated mainly for patients diagnosed at an early stage (Bruix and Sherman, 2011). Unfortunately, in developing countries, most of the patients are diagnosed at late stage and thus receive palliative treatments only (Chassagne et al., 2016b; Ruiz et al., 2016). Moreover, the high cost of treatment does not allow poor people to have access to good quality healthcare. Therefore, recent studies pointed out that liver cancer patients from low-income countries turns towards the use of traditional, complementary and alternative medicine (TCAM) and especially medicinal plants (Chassagne et al., *in press*; Rojas Rojas et al., 2016).

Hepatocarcinogenesis is a complex process involving a series of individual steps related to proliferation, cell cycle regulation, differentiation, apoptosis, angiogenesis, invasion and metastasis (Surh, 1999; Whittaker et al., 2010). Natural agents can exert therapeutic effects in one or more specific stage of this multistep process. As an example, milk thistle (*Silybum marianum* (L.) Gaertn.) which has been used for the treatment of liver disorders since the ancient Greeks is by far the most studied plant species for its hepatoprotective activity (Schuppan et al., 1999). A flavanolignan mixture, called silymarin, have been isolated from the seeds and fruits of the milk thistle, and its anticarcinogenic effect have been attributed to various actions: anti-inflammation, cell cycle regulation, apoptosis induction, growth inhibition, inhibition of angiogenesis, inhibition of invasion and metastasis (Feher and Lengyel, 2012; Ramasamy and Agarwal, 2008). Despite its limitation, *in vitro* antiproliferative assays on human hepatoma cells are widely used as a starting point for the evaluation of anti-hepatocarcinogenic effect (Carraz et al., 2015; Chaudhary et al., 2011; Mahavorasirikul et al., 2010). Coupled with a dereplication

approach and a multivariate analysis, this assay might help to rank and identify compounds responsible for the antiproliferative effect, providing a better understanding of the mechanism of action of medicinal plants (Verpoorte et al., 2005; Yuliana et al., 2013). Recently, Chervin et al. (2017) have described a new method for the dereplication of natural products in complex mixture which was employed for the identification of redox active compounds from *Viola* extracts.

Most of researches on medicinal plants used for the treatment of chronic liver diseases are based on the study of pharmacopeias of major traditional medicines (i.e. Traditional Chinese Medicine (TCM), Ayurvedic medicine (India), Kampo medicine (Japan) or Western herbal medicine) (Batey et al., 2005). However, in Southeast Asia, information on medicinal plants used for treating liver diseases is scattered and, to the best of our knowledge, only one publication has focused on medicinal plants used by Khmer traditional healers in Phnom Penh area (Cambodia) for liver pathologies (Chassagne et al., 2017). In this study, authors documented the use of 83 plants species, and found out that most of them have been shown to possess pharmacological properties (hepatoprotective, antimicrobial, anti-inflammatory, etc.) justifying their use with varying degrees of certainty. Still, for many of these plants species, further in-depth investigations are needed in order to recommend their use, and to highlight their mechanism of action.

Therefore, because in Southeast-Asia, traditional healers are frequently consulted by patients suffering from liver cancer, and also because they rely on medicinal plants for their cure, we decided to undergo the antiproliferative evaluation of selected extracts on HepG2 cells. Then, with the help of a dereplication approach, to provide some clues of putative active compounds present in the most active ones.

2. Materials and Methods

2.1. Ethnopharmacological selection of plants

A comprehensive review of the scientific literature was realized from May to July 2016 by focusing on Southeast Asian ethnobotanical documents. Books, PhD or master's thesis, and reports available in Lao, Cambodian and French libraries were reviewed. Scientific articles published in international journals were screened by consulting electronic databases (Google Scholar, PubMed, Web of Science); and by using specific keywords: "traditional medicine", "medicinal plants", combined with terms related to liver diseases: "liver", "jaundice", "icterus", "hepatitis"; and geographical names: "Southeast Asia", "Cambodia", "Laos", "Vietnam", Thailand", "China".

References in French, English, Cambodian and Lao were consulted, and translated if necessary. Botanical names were validated by referring to the Plant List database (http://www.theplantlist.org/).

As described in Elkington et al. (2014), a point system was employed to prioritize species recorded. Each plant species was given one point: for each citation in the overall literature; and for each Southeast Asian country where the plant was cited. The final score (S1) was obtained after the multiplication of the two values. All the plants recorded were entered in an excel spreadsheet (Microsoft 2013) (Suppl. Mat S1). Altogether 378 species were listed. From this list, we selected species with: (i) the highest score S1, (ii) the highest number of citations in Chassagne et al. (2017), (iii) no previous pharmacological evaluation on hepatocarcinoma cells (HepG2) for their crude extract, and (iv) a high frequency and abundance in the prospected area.

2.2. Plant materials

Plants were collected in July 2016 in Champasak province, Lao PDR with the assistance of traditional healers working in the Traditional Medicine Department, Champasak Regional Hospital. Authorization to collect the plant was obtained from relevant authorities. Ethical

approval was obtained from the National Ethics Committee for Health Research (NECHR) under the supervision of the Ministry of Health of Lao PDR in February 2016 (010NIOPH/NECHR).

Voucher specimens were deposited at the herbarium of the Department of Biology, Faculty of Sciences, National University of Laos (NUoL) in Vientiane. Botanical identification was realized by the first author, and confirmed by specialists.

2.3. Plant extraction and fractionation procedures

All the selected plant materials were shade-dried at room temperature for seven days and pulverized into fine powder. Then, the air-dried and powdered part of plants (15g) were exhaustively macerated with 80% EtOH (v/v, 2 x 150ml) at room temperature. The ethanolic solution was filtered, then concentrated under reduce pressure at 40°C to obtain the crude extracts. In parallel, aqueous extracts were obtained as follows: 15g of dried powdered plants were stirred in 150ml of deionized water, in a water bath at 90°C for 30 min, then kept at room temperature for 2 hours. The solution was filtered, stored in a freezer (-20°C) for one day, and freeze-dried for 3 days.

The ethanolic and aqueous dry extracts of each bioactive plants were fractionated using SPE (1 g Sep-Pak® C18 cartridge, Waters, USA). The SPE cartridges were primarily activated with 10 ml of methanol 100% (v/v), then equilibrated with 10 ml of methanol 5% (v/v). One hundred milligram of each extract were finally suspended in 1 ml of methanol 5% (ethanolic extracts) or 1 ml of water (aqueous extracts) and eluted successively with MeOH/H_2O: 5/95 (Fr1), 20/80 (Fr2), 40/60 (Fr3), 60/40 (Fr4), 80/20 (Fr5) and 100% MeOH (Fr6) to yield six fractions.

For each fraction and extract, stock solutions at a concentration of 10mg/ml in DMSO (Dimethyl sulfoxide) 100% (v/v) were prepared for antiproliferative assays on HepG2 model; and at a concentration of 2mg/ml in methanol 100% (v/v) for UHPLC-HRMS analysis.

2.4. Cell culture and antiproliferative assay

HepG2 (human hepatoma carcinoma) cell lines were purchased from the American Type Culture Collection (ATCC, USA). HepG2 cells were cultured at 37°C, 5% CO_2, in RPMI 1640 medium (Gibco®, Life Technologies, USA), supplemented with 10% fetal bovine serum, and penicillin/streptomycin (100U/ml). For antiproliferative assay, HepG2 were cultured in the same conditions without antibiotics. The medium was renewed twice a week.

HepG2 cells under exponential growth were seeded in 96-well plates at a density of 10 000 cells per well. After overnight growth, cells were treated with various concentrations of extracts/fractions for 72 h. The concentration tested range from 1.95 to 250 µg/ml. Then, the cells were washed with Phosphate-Buffered Saline (Gibco®, Life Technologies, USA), and incubated with MTT (Sigma Aldrich, USA) at a concentration of 0.5 mg/ml for 4 h. DMSO (Fisher Chemicals, UK) (100 µl/well) was then added into each well, and the plates were read at 570 nm using a microplate reader (EON, BioTek, USA). The inhibition rate was calculated and IC_{50} values were determined using Graph Pad Prism v.6 software (Graph Pad, USA). For each set of experiments, a positive control (Triton X-100 1%) was used to induce 100% cell death. All experiments were performed in triplicate.

2.5. UHPLC-HRMS profiling

Metabolite profiles were acquired using a UHPLC-DAD-LTQ Orbitrap XL instrument (Thermo Fisher Scientific, UK) equipped with an electrospray ionization source (ESI). The UHPLC system consisted of an Ultimate 3000 UHPLC (Thermo Fisher Scientific, UK) equipped with a Acquity BEH C_{18} column (100 × 2.1 mm i.d., 1.7 µm, Waters, USA). The mobile phase was composed of solvent A (0.1% formic acid-water) and solvent B (0.1% formic acid-acetonitrile) with a gradient elution (0-0.5 min, 95% A; 0.5-12 min, 95-5% A; 12-15 min, 5% A; 15-15.5min, 5-95% A; 15.5-19 min, 95%A). The flow rate of the mobile phase was 0.3 ml/min. The injection volume was 2 µL and the column temperature was maintained at 40°C. Electrospray ionization was applied in negative ion (NI) and positive ion (PI) mode under the following conditions:

capillary voltage at 3.0 kV and 4.2 kV for NI and PI respectively, and capillary temperature at 300°C. The UV detection was performed by a diode array detector (DAD) from 210 to 400 nm. Full mass spectra were recorded between 100 and 1500 Da. CID (Collision Induced Dissociation) mass spectra were obtained using the following parameters: 35% normalized collision energy, isolation width 2 Da, activation Q 0.250. External mass calibration was accomplished before starting the experiment.

2.6. Data processing and statistical analysis

Data processing and statistical analysis were performed as previously described by Chervin et al. (2017). Briefly, the UHPLC-HRMS raw data were processed with MS-Dial v.2.56 (Tsugawa et al., 2015) for mass signal extraction and peaks alignment. The resulting peak list was then exported into a comma-separated value (CSV) file format for proceeding to multivariate data analysis using SIMCA-P+ v. 14.0 (Umerics, Sweden). The OPLS (Orthogonal Projections to Latent Structures) regression was done with HepG2 IC_{50} values as Y input. For each model, a leave-one-subject-out cross-validation was performed to assess the model fit. The validity of the discriminant model was verified using permutation tests (Y-scrambling). Coefficient score were used to rank variables according to their correlation with the measured antiproliferative activity. Molecular formula prediction and compound annotation of significant features (*m/z*, RT pairs) were calculated with MS-FINDER 2.10 (Tsugawa et al., 2016). Only natural product databases focused on plants were selected (i.e. Universal Natural Products Database (UNPD), KNApSAc, PlantCyc, Dictionary of Natural Products (DNP, CRC press, v25:2) and CheBI). Database interrogation was performed following a three-step process. Already known compounds belonging to the plant species were first analyzed, then those belonging to the botanical family, and finally, molecules present in all plant databases were interrogated. Results were presented as a list of compounds sorted according to a score value for each match. This value encompassed uncertainty on accurate mass, the isotopic pattern score and the experimental MS/MS fragmentation mirrored to *in silico* fragmentation of the candidate structure.

3. Results

3.1. Plants selected

The bibliographical review process covered a total of 45 publications including 23 books, 18 scientific articles, 2 PhD thesis and 2 reports. A total of 378 plant species belonging to 112 families were identified from the literature. The three most cited plants in the literature were: *Melastoma saigonense* (Kuntze) Merr. (9 citations), *Willughbeia edulis* Roxb. (8 cit.), and *Phyllanthus urinaria* L. (8 cit.). Two plant species were reported from five and four countries respectively: *Curcuma longa* L. and *Oroxylum indicum* (L.) Kurz. The most predominant plant genus were *Phyllanthus* (13 cit., 4 species) and *Senna* (11 cit., 5 species). The most cited plant families in the literature were Leguminosae (57 cit., 39 plant species recorded), Rubiaceae (26 cit., 17 species), Apocynaceae (26 cit., 13 species) and Compositae (22 cit., 17 species).

The highest final score (S1) was obtained for: *Curcuma longa* L. (28), followed by *Oroxylum indicum* (L.) Kurz (24), *Melastoma sanguineum* Sims (18), *Orthosiphon aristatus* (Blume) Miq. (18), *Senna alata* (L.) Roxb. (18), *Phyllanthus urinaria* L. (16) and *Willughbeia edulis* Roxb. (16).

Based on the number of citations obtained in Chassagne et al. (2017), 10 species were selected for further analysis (Table 1).

Table 1: Plants cited for the treatment of liver diseases in the literature, and selected for antiproliferative assay on HepG2 model

Scientific name	Family	Part of plants used	Voucher specimen	Score	Number of citations in Chassagne et al. (2017)
Andrographis paniculata (Burm.f.) Nees	Acanthaceae	Aerial part	FC371	12	12
Boerhavia diffusa L.	Nyctaginaceae	Aerial part	FC369	4	5
Cananga latifolia (Hook.f. & Thomson) Finet & Gagnep.	Annonaceae	Stem bark	FC054	5	15
Gomphrena celosioides Mart.	Amaranthaceae	Aerial part	FC370	2	9
Melastoma saigonense (Kuntze) Merr.	Melastomataceae	Root	FC455	18	0
Oroxylum indicum (L.) Kurz	Bignoniaceae	Stem bark	FC376	24	5
Orthosiphon aristatus (Blume) Miq.	Lamiaceae	Aerial part	FC457	18	2
Salacia chinensis L.	Celastraceae	Stem bark	FC454	9	6
Senna alata (L.) Roxb.	Leguminosae	Leaf	FC453	18	5
Willughbeia edulis Roxb.	Apocynaceae	Stem bark	FC451	16	8

Score (S1) was calculated as follow: (number of citations in the literature) x (number of countries where the plant is used)

3.2. Antiproliferative assay

Of the 20 plant extracts tested on HepG2 model, only four ethanolic extracts (*A. paniculata*, *O. indicum*, *O. aristatus* and *W. edulis*) and one aqueous extract (*W. edulis*) have IC_{50} values inferior to 200 µg/ml. (Table 2).

Table 2: Antiproliferative activity of 10 plant species on HepG2 cell lines

Scientific name	Ethanolic extract IC_{50} (µg/ml)	Aqueous extract IC_{50} (µg/ml)
Andrographis paniculata	195.9 ± 13.86	>250
Boerhavia diffusa	>250	>250
Cananga latifolia	>250	>250
Gomphrena celosioides	>250	>250
Melastoma saigonense	>250	>250
Oroxylum indicum	64.1 ± 10.56	>250
Orthosiphon aristatus	71.3 ± 4.75	>250
Salacia chinensis	>250	>250
Senna alata	>250	>250
Willughbeia edulis	66.7 ± 8.37	127.2 ± 8.22

Data are presented as mean ± SD of three replicate experiments
IC50=half-maximal inhibitory concentration
In bold letters: Plants species with the best antiproliferative activity (IC_{50}<250µg/ml)

Regarding these preliminary results, the ethanolic extracts of *A. paniculata*, *O. indicum*, *O. aristatus* and *W. edulis* were selected for further fractionation using different concentrations of methanol. Each fraction obtained was then retested on HepG2 model (Table 3).

Of the 20 fractions evaluated, the highest antiproliferative activities were obtained for the fractions 5 of *O. aristatus* and *A. paniculata* (IC_{50} = 15.6 and 33.3 µg/ml respectively).

Table 3: Antiproliferative activity of the fractions of the four most antiproliferative plant species

Scientific name		Ethanolic extract IC_{50} (µg/ml)
Andrographis paniculata		
	Fr1	ND
	Fr2	ND
	Fr3	>100
	Fr4	66.2 ± 10.39
	Fr5	33.3 ± 7.79
	Fr6	>100
Oroxylum indicum		
	Fr1	>100
	Fr2	>100
	Fr3	>100
	Fr4	>100
	Fr5	84.4 ± 12.69
	Fr6	ND
Orthosiphon aristatus		
	Fr1	>100
	Fr2	>100
	Fr3	>100
	Fr4	>100
	Fr5	15.6 ± 0.51
	Fr6	90.8 ± 3.06
Willughbeia edulis		
	Fr1	>100
	Fr2	90.7 ± 2.26
	Fr3	69.3 ± 14.63
	Fr4	>100
	Fr5	>100
	Fr6	ND

Data are presented as mean ± SD of three replicate experiments

In bold letters: fractions with the best antiproliferative activity (IC_{50}<100µg/ml)

Fr = Fraction

ND = Not Determined

3.3. UHPLC-HRMS- based metabolomics approach

Metabolite profiling of all the 61 extracts (48 fractions, 8 crude extracts, 5 QC samples prepared by pooling an aliquot of all fractions) were acquired in positive and negative ionization mode.

When comparing the UHPLC-HRMS profiles obtained from the aqueous and the ethanolic extracts of each plant, it was clearly shown that ethanolic extracts exhibited a higher number of peaks than aqueous extracts (Figure 1). Thus, the ethanolic extract can be considered as richer in compounds than the aqueous extract, which corroborate with the antiproliferative activity (best activities for ethanolic extracts, see Table 2). In the case of *W. edulis*, the two profiles were similar in the number and intensity of peaks, which may be attributed to the presence of antiproliferative compounds in both extracts (Figure 2).

Figure 1: Total ion chromatograms in negative mode obtained from Orthosiphon aristatus ethanolic and aqueous extracts

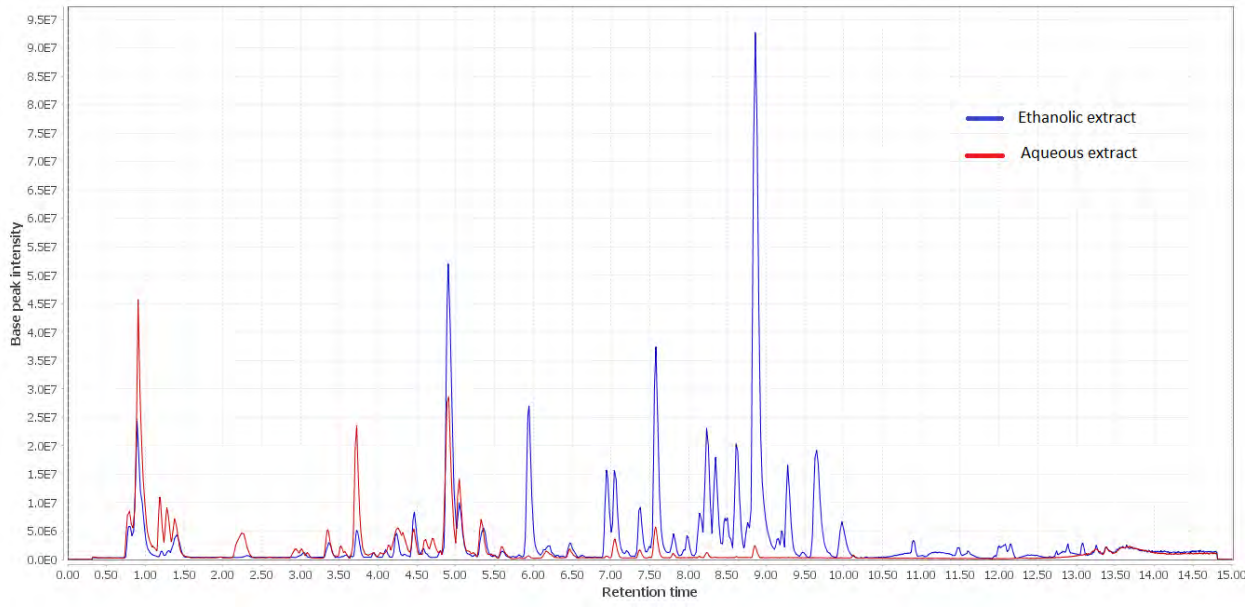
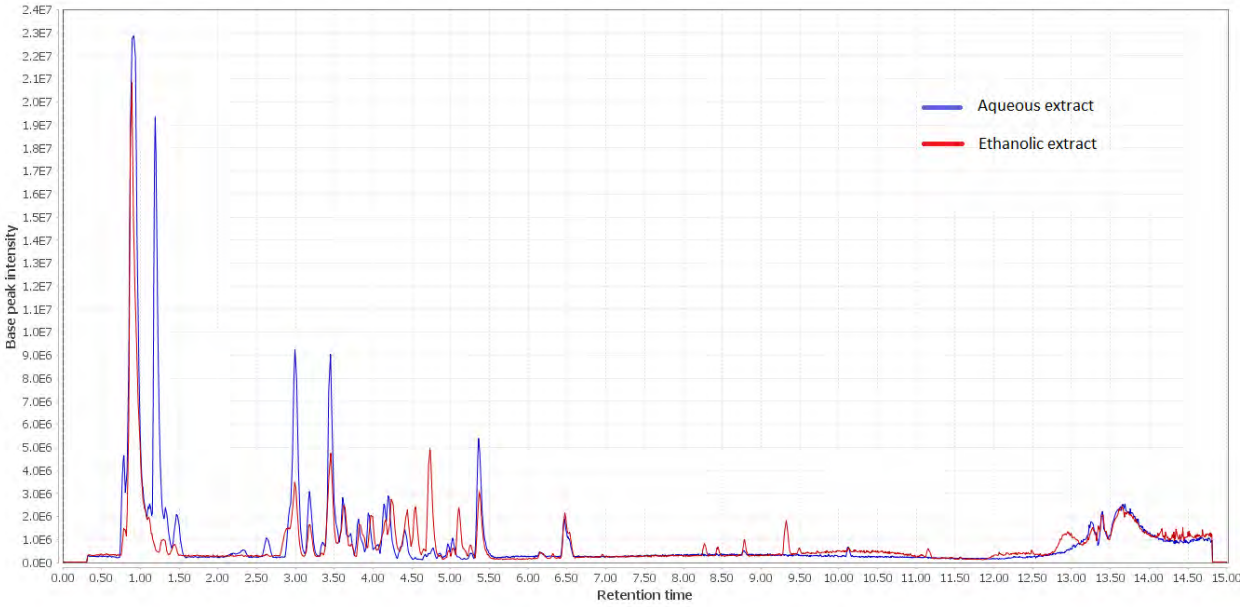


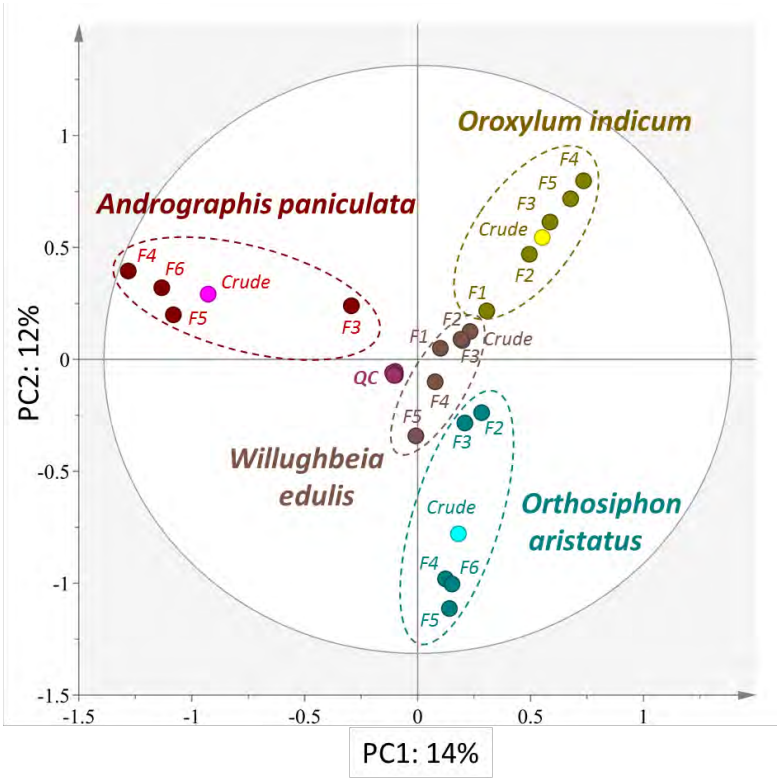
Figure 2: Total ion chromatograms in negative mode obtained from Willughbeia edulis ethanolic and aqueous extracts



According to antiproliferative assay results, ethanolic extracts were chosen for a deeper metabolomic analysis in order to dereplicate active compounds. The processing of UHPLC-HRMS profiles afforded a data table comprising each detected features (m/z, RT pairs) in all samples with their relative intensity. In total, 1451 and 1188 features were detected in positive and negative mode respectively for *A. paniculata*; 1339 and 1503 for *O. indicum*; 1526 and 787 for *O. aristatus*; 762 and 1186 for *W. edulis*.

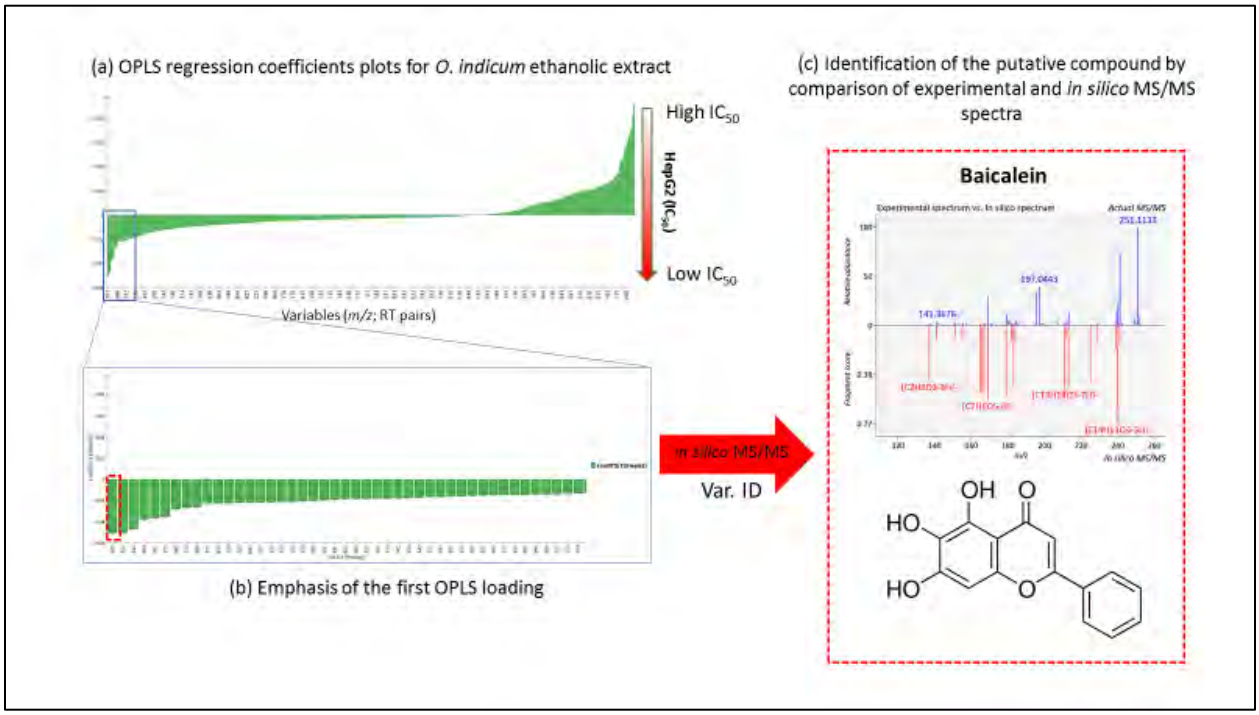
In order to better visualize the similarities and the differences among these complex data sets, PCA (Principal Component Analysis) was used to classify the fractions and the ethanolic extracts from each four plants species. In the PCA score plots, each spot represents a sample (Figure 3). As expected, PCA grouped QC (a pooled sample of all extracts) near the plot center. Moreover, PCA could well separate the four-plant species and their fractions, indicating the variability and the reproducibility of the dataset.

Figure 3: PCA score plots of UHPLC-HRMS profiles of ethanolic extracts from four plant species and their fractions (F) in negative mode



For each plant species, we performed OPLS regression analysis to obtain a classification of the metabolites regarding the HepG2 IC_{50} value (input Y). The ranking of the antiproliferative compounds was realized according to their regression coefficient values: negative coefficients were correlated to potential antiproliferative compounds and positive coefficients to less-antiproliferative compounds. Then, the compounds with the best rank were identified based on comparison between experimental MS/MS fragments and *in silico* spectra of structure candidates (Figure 4).

Figure 4: OPLS regression analysis and identification of putative compounds for *Oroxylum indicum* ethanolic extract



3.4. Identification of potential antiproliferative compounds

Using the OPLS regression analysis results, the top ranked compounds of the four plants species were tentatively identified by interrogating natural product databases.

For *A*. *paniculata*, two compounds (andrographolactone and dehydroandrographolide), one compound (19-Hydroxyjolkinolide E), and two compounds (Viteoside A, (1,8E,12E,14Z)-heptadecatetraene-4,6-diyne-3,11-diol) were putatively identified from the database related to the plant species, to the botanical family (Acanthaceae) and to all plant databases respectively. In this top five ranked compounds, four are diterpenoids molecules and two have been already evaluated for their antiproliferative activity (Table 4).

For *O. indicum*, of the top six compounds annotated, four (baicalein, scutellarein, oroxylin A and chrysin) and two compounds (ladanein, 3-Hydroxydehydroiso-alpha-lapachone-(2S,3S)-form) respectively were putatively determined from the databases related to the plant species and to the botanical family (Bignoniaceae) respectively. Among them, five are flavonoids molecules which have been already evaluated for their antiproliferative activity.

Of the top five compounds dereplicated for *O. aristatus*, only one compound (5-desmethylsinensetin) was putatively identified from the databases related to the plant species, and has been already evaluated for its antiproliferative activity. Besides, two compounds (6,8-Dihydroxy-13,14,15,16-tetranor-12-labdanoic acid-(6alpha,8alpha)-form-8-Ac, Prinoid B) and one compound (Brasiliensophyllic acid A) were putatively determined from the database related to the botanical family (Lamiaceae), and to all plant databases respectively.

For *W. edulis*, no previous phytochemical studies have been performed to isolate compounds present in the species. Thus, only molecules belonging to the Apocynaceae family (3716 compounds) and others plant families were analyzed. In the top five ranked molecules, one compound (Parabaroside C) and four compounds (procyanidin, hydnocarpin 7-O-[4-Hydroxy-E-cinnamoyl-(→4)-β-D-glucopyranoside], 3,8-Dihydroxy-3,10(14)-guaiadien-12,6-olid-13-oic acid, (1α,5α,6α,8α,11α)-form, 8-O-(3-Piperidinyl), 3-O-β-D-glucopyranoside, lysidicichin) were putatively determined from the databases related to the botanical family (Apocynaceae) and to

all plant databases respectively. Among them, two have been already evaluated for their antiproliferative activity, but these compounds are not present in plants from the Apocynaceae family, thus their annotation remain elusive.

Table 4: Summary of the top ranked dereplicated compounds for the four plant species

Rank[a]	RT (min)	[M-H]-/ [M-H]+[b]	Molecular formula	Mass error (mDa)	Type of database[c]	Percentage in aqueous extract[d]	Potential Id[e]	Chemical class	Biological source	Biological activity	References
Andrographis paniculata											
1	9.64	555.2831 (-)	C28H44O11	0.0021	A	7.9%	Viteoside A	Diterpene	*Vitex rotundifolia* (Vitaceae)	NR	
2	6.78	317.2099 (+)	C20H28O3	0.0010	F	15.2%	19-Hydroxyjolkinolide E	Diterpene	*Phlogacanthus* sp. (Acanthaceae)	NR	
3	6.83	297.1836 (+)	C20H24O2	0.0012	S	3.8%	Andrographolactone	Diterpene	*Andrographis paniculata*	Antiproliferative activity on LoVo (colon cancer) and NCI-H460 cells (lung cancer) [IC_{50}= 50 and 60µM respectively]	Wang et al., 2009
4	6.84	257.1526 (+)	C17H20O2	0.0009	A	4.5%	(1,8E,12E,14Z)-heptadecatetraene-4,6-diyne-3,11-diol	Polyacetylene	*Artemisia eriopoda* (Compositae)	NR	
5	6.83	333.2047 (+)	C20H28O4	0.0012	S	4.6%	Dehydroandrographolide	Diterpene	*Andrographis paniculata*	Antiproliferative activity on **HepG2** and T-47D models (breast cancer) [IC_{50}= 11.5 and 1.5µg/ml resp.]	Tan et al., 2005
Oroxylum indicum											
1	7.45	269.0449 (-)	C15H10O5	0.0006	S	0.6%	Baicalein	Flavonoid	*Oroxylum indicum*	Antiproliferative activity on **HepG2**, A549 (lung cancer) and BCG-823 (gastric cancer) cells [IC_{50}= 28.3, 13.0 and 19.1 µg/ml resp.]	Luo et al., 2014
2	6.69	313.0711 (-)	C17H14O6	0.0007	F	ND	Ladanein	Flavonoid	*Baccharis sp.* (Compositae), *Catalpa* sp. (Bignoniaceae), *Marrubium* sp. (Lamiaceae)	Antiproliferative activity on K562 and K562R cells (leukemia) [IC_{50}= 25.1 and 38.0 µg/ml]	Alkhatib et al., 2010
3	6.44	287.0539 (+)	C15H10O6	0.0012	S	0%	Scutellarein	Flavonoid	*Oroxylum indicum*	Antiproliferative activity on **HepG2**, DLA (lymphoma), A-549 (lung cancer), and MCF-7 (breast cancer) cells [IC_{50}= 8.4, 5.8, 6.8 and 13.7 µg/ml resp.]	Thirusangu et al., 2017
4	7.46	285.0749 (+)	C16H12O5	0.0009	S	0.6%	Oroxylin A	Flavonoid	*Oroxylum indicum*	Antiproliferative activity on **HepG2** and SMMC-7721 models (liver cancer) [IC_{50}= 59.5 and 104.1 µM]	Zou et al., 2012
5	7.44	255.0657 (-)	C15H12O4	0.0006	F	0%	3-Hydroxydehydroiso-alpha-lapachone-(2S,3S)-form	Naphtoquinone	*Catalpa* sp. (Bignoniaceae)	NR	
6	7.29	253.05 (-)	C15H10O4	0.0005	S	2.6%	Chrysin	Flavonoid	*Oroxylum indicum*	Antiproliferative activity on **HepG2** cell lines [IC_{50}= 5 µM]	Sun et al., 2011

Rank[a]	RT (min)	[M-H]-/[M-H]+[b]	Molecular formula	Mass error (mDa)	Type of database[c]	Percentage in aqueous extract[d]	Potential Id[e]	Chemical class	Biological source	Biological activity	References
Orthosiphon aristatus											
1	9.17	559.3112 (-)	C35H44O6	0.0048	A	0%	Brasiliensophyllic acid A	Chromanone acid	*Calophyllum inophyllum* (Clusiaceae)	No antiproliferative effect on Jukart T (leukemia) and myosarcoma cell lines	Cottiglia et al., 2004
2	8.18	737.28 (-)	C39H46O14	0.0017	A	4.1%	Febrinin A	Triterpene	*Soymida febrifuga* (Meliaceae)	NR	
3	7.47	325.2013 (-)	C18H30O5	0.0007	F	ND	6,8-Dihydroxy-13,14,15,16-tetranor-12-labdanoic acid-(6alpha,8alpha)-form-8-Ac	Diterpene	*Salvia yosgadensis* (Lamiaceae)	NR	
4	7.63	359.1107 (+)	C19H18O7	0.0014	S	0.9%	5-desmethylsinensetin	Flavonoid	*Orthosiphon aristatus*	Antiproliferative activity on MDA-MB-435 (breast cancer), MCF-7 (breast cancer), DU-145 (prostate cancer), HT-29 (colon cancer), DMS-114 (lung cancer), SK-MEL5 (melanoma cancer) [IC_{50}= 0.06, 0.03, 2.2, 5.0, 0.11, 1.1 μM resp.]	Manthey and Guthrie, 2002
5	8.92	311. 1626 (+)	C20H22O3	0.0016	F	0%	Prionoid B	Diterpene	*Salvia prionitis* (Lamiaceae)	No antiproliferative activity on A-549 (lung cancer) and P-388 (leukemia)	Chang et al., 2005
Willughbeia edulis											
1	3.91	613.1550 (-)	C30H30O14	0.0014	F	46.8%	Parabaroside C	Flavonoid	*Parabarium huaitingii* (Apocynaceae)	NR	
2	3.6	577.1338 (-)	C30H26O12	0.0015	A	63.3%	Procyanidin	Flavonoid	Widespread in plants	Antiproliferative effect of structured related procyanidin on various cancerous cell lines	Actis-Goretta et al., 2008
3	4.44	773.2059 (+)	C40H36O16	0.0017	A	13.9%	Hydnocarpin; 7-O-[4-Hydroxy-E-cinnamoyl-(→4)-β-D-glucopyranoside]	Flavonolignan	*Mallotus metcalfianus* (Euphorbiaceae)	Antiproliferative activity of hydnocarpin against various cancerous cell lines (nasopharynx, colon, osteosarcoma, lung, glioma)	Sharm and Hall, 1991
4	3.78	540.242 (+)	C26H37NO11	0.0019	A	146.9%	3,8-Dihydroxy-3,10(14)-guaiadien-12,6-olid-13-oic acid, (1α,5α,6α,8α,11α)-form, 8-O-(3-Piperidinyl), 3-O-β-D-glucopyranoside	Sesquiterpene alkaloid	*Youngia japonica* (Compositae)	NR	
5	3.46	469.1134 (-)	C24H22O10	0.0006	A	17.3%	Lysidicichin	Flavonoid	*Lysidice* sp. (Leguminosae)	NR	

[a] Based on OPLS coefficient regression values

[b] Molecular mass obtained in negative mode (-) or in positive mode (+)

[c] Three types of database are analyzed: species level (S), family level (F) and all plants (A)

[d] Each compound dereplicated from the ethanolic extract were searched in the aqueous extract, and its abundance compared to the ethanolic extract based on the relative peak intensity from extracted ion chromatogram (XIC)
[e]Top ranked hits determined by in silico MS/MS fragmentation with MS Finder

ND=Not Detected
NR=No references related to antiproliferative activity found in the literature

4. Discussion

A scoring bibliographic approach was set up for the selection of plants frequently used in the treatment of liver disorders in Southeast Asia. Starting from 378 plants, 10 plants were selected and their antiproliferative activity were tested on HepG2 cells.

Among the four plants species exhibiting the highest antiproliferative activity on HepG2 cell lines, three (*A. paniculata*, *O. indicum*, *O. aristatus*) are well known for the treatment of liver disorders in various countries and have been evaluated for different pharmacological activities related to liver impairments.

Andrographis paniculata (Acanthaceae) is an herbaceous plant native from Asia, which is cultivated in various countries including China and India. Due to its bitter taste, it is known as "the king of bitters", and it is employed as a bitter tonic in Ayurvedic medicine and is indicated for "hot" conditions in TCM (Thakur et al., 2015). According to our point system, *A. paniculata* is the tenth most employed plant species in Southeast Asia, where it has been reported to be used for jaundice, hepatitis and liver disorders (Chassagne et al., 2017; Hossain et al., 2014; Inta et al., 2013). The high value of this herb in the treatment of liver disorders can be attributed to its numerous pharmacological effects such as hepatoprotective effect, antiviral activity against hepatitis B virus, immunomodulatory, anti-inflammatory, analgesic and antipyretic properties (Chen et al., 2014; Hossain et al., 2014; Jayakumar et al., 2013). Major constituents of *A. paniculata* are diterpenes lactones (more than 50 compounds described, including andrographolide the major active principle), flavonoids, quinic acid derivatives and xanthones (Subramanian et al., 2011).

In our study, the ethanolic extract of *A. paniculata* and one of its fraction (fraction 5) showed an IC50 value of 195.9 µg/ml and 33.3 µg/ml respectively on HepG2 cell lines. Our dereplication approach allowed the annotation of two compounds already reported in the literature for this species, and thus putatively responsible for the antiproliferative effect (Tan et al., 2005; Wang et al., 2009). Dehydroandrographolide (also known as 14-Deoxy-11,12-didehydroandrographolide) is a diterpene molecule which has been shown to possess antiproliferative activity on various cancerous cell lines: T-47D (breast cancer), Hs-578T (breast

cancer), NCI-H23 (lung cancer) and HepG2 with IC_{50} of 1.5, 34.9, 41.8 and 11.5 µg/ml respectively. Compare to others major diterpenoid constituents of *A. paniculata*, dehydroandrographolide showed greater antiproliferative activity on HepG2 model, thus confirming its putative role in the antiproliferative effect of the ethanolic extract (Tan et al., 2005). Moreover, Tan et al. (2012) reported that this compound exerts its antiproliferative effect by regulating genes that are known to inhibit cell proliferation, induce growth arrest and suppress cell growth. Andrographolactone, is another diterpenoid molecule recently isolated from the ethanolic extract of *A. paniculata.* It has been reported to exhibit antiproliferative effect on two human cancerous cell lines (LoVo, colon cancer and NCI-H460, non-small cell lung cancer) (Wang et al., 2009). However, no studies have evaluated its antiproliferative effect on HepG2 model, thus further investigations are needed to confirm its role in the antiproliferative activity. Besides, the others top ranked compounds identified at the family level should be first isolated from *A. paniculata* and then evaluated for their antiproliferative effect on HepG2 model in order to confirm our results.

Oroxylum indicum (Bignoniaceae) is a medium-sized tree, native to the Indian sub-continent and extends into Southeast Asia and Southern China. It is traditionally used in many Asian countries including Indian Ayurvedic medicine and TCM (Dinda et al., 2015). From our literature review, this tree is the second most employed species in the region for treating liver disorders and jaundice. To justify this traditional use, numerous pharmacological activities have been reported such as hepatoprotective, antimicrobial, anti-oxidant, anti-inflammatory, immunostimulant and analgesic activities (Dev et al., 2010; Lim, 2012). Flavonoids are the most abundant constituents of this plant, among which baicalein, chrysin and oroxylin A are the major chemical constituents of the stem bark (Dinda et al., 2015).

The ethanolic extract of *O. indicum* showed the highest antiproliferative activity on HepG2 model (IC_{50} = 64.1 µg/ml) compare to other plants evaluated in our study. Based on the results of our dereplication approach, the antiproliferative effect might be attributed to the presence of four compounds belonging to the flavonoid chemical class and present in the plant species: baicalein, scutellarein, oroxylin A and chrysin. Indeed, these four molecules have been reported to possess antiproliferative activity on HepG2 model in previous studies with IC_{50} ranging from

1.27 to 28.3 µg/ml (Luo et al., 2014; Sun et al., 2011; Thirusangu et al., 2017; Zou et al., 2012). Although, another flavonoid compound (ladanein) found in some plants from the Bignoniaceae family was annotated and has demonstrated antiproliferative effect on leukemia cell lines (Alkhatib et al., 2010), further investigations are necessary to confirm the presence of this constituent in *O. indicum* and to evaluate its antiproliferative activity on HepG2 model.

Orthosiphon aristatus (Lamiaceae) is an herbaceous plant native from Southeast Asia, and widely distributed in temperate and tropical areas. The plant is registered at the European pharmacopeia for its diuretic properties. In our analysis, *O. aristatus* rank third and is used in Cambodia, Laos and Vietnam for treating hepatitis and jaundice (Cheng and Huon, 1996; Tezuka et al., 2000). Besides its diuretic activity, the plant species have been shown to possess anti-inflammatory, analgesic, antipyretic and hepatoprotective properties, thus supporting its role in the treatment of liver disorders (Ameer et al., 2012). Three types of phytochemicals have been isolated from this species including flavonoids, phenylpropanoids (caffeic acid derivatives) and terpenoids (mainly diterpenes and triterpenes).

O. aristatus ethanolic extract exhibited an IC_{50} value of 71.3 µg/ml, while one of its fraction (fraction 5) showed the highest antiproliferative activity (IC_{50} = 15.6 µg/ml) compare to other fractions obtained from other plants species. The results from our dereplication approach indicated that only one compound (5-desmethylsinensetin), already isolated from *O. aristatus*, was annotated in the top ranked features. This flavonoid has been already evaluated for its antiproliferative activity on six cancerous cell lines and displayed good antiproliferative activity with an IC_{50} ranging from 0.3 to 5.0 µM (Manthey and Guthrie, 2002). Further studies should be realized to determine the antiproliferative effect of this compound on HepG2 model, and thus to confirm its role in the antiproliferative activity. Besides, the others top ranked compounds identified at the family level should be isolated from the plant and also tested on HepG2 cell lines.

Willughbeia edulis is a liana endemic to Southeast-Asia, and its edible fruits are much prized. In our literature review, *W. edulis* rank fourth among the most employed species for treating liver disorders, and it has been cited mainly in Cambodia and Malaysia (Chassagne et al.,

2016a; Perry, 1980; Wiart, 2006). No previous pharmacological investigations were found in the literature to support traditional usage. In our study, the ethanolic and the aqueous extract of this species showed an IC_{50} value of 66.7 and 127.2 µg/ml respectively on HepG2 model.

Furthermore, no previous phytochemical studies have been performed to isolate compounds present in the species. Thus, no database was available for identifying molecules at the species level. Although some proposed compounds (procyanidins and hydnocarpin derivatives) have shown to possess antiproliferative activity on cancerous cell lines (Actis-Goretta et al., 2008; Sharma and Hall, 1991), further investigations are needed to confirm the presence of these compounds in the plant.

In our analysis, we also evaluate the presence of top ranked compounds in the aqueous extracts prepared following a similar method to the traditional formulation (decoction). UHPLC-HRMS analysis of aqueous extract of *W. edulis* reveal that top ranked compounds were detected in the range of 9.8 to 146.9% compare to the ethanolic extract. Due to the high quantity of putative antiproliferative compounds in this extract which aims to mimic the traditional formulation, further analysis should be performed to determine the selectivity of its antiproliferative activity on HepG2 model, and thus to make sure that these compounds do not display a general toxicity which could impair the safety of this plant species.

Altogether, our results suggest that the antiproliferative activity obtained *in vitro* for the ethanolic extract from three plants (*A. paniculata*, *O. indicum* and *O. aristatus*) can be attributed to the presence of specific compounds ranked according to OPLS regression model and further tentatively annotated based on accurate mass spectra and MS/MS patterns. Nevertheless, in the case of *W. edulis*, the lack of phytochemical investigations is a major barrier to the precise identification of compounds involved in the antiproliferative effect. Thus, our dereplication strategy pointed out some interesting features which could serve as a starting point for bioassays guided fractionation studies.

5. Conclusion

In this study, a rapid dereplication approach allowed the putative annotation of compounds responsible for this biological activity for three plants: *A. paniculata*, *O. indicum* and *O. aristatus*. Among dereplicated compounds, most of the top ranked molecules have been already evaluated for their antiproliferative activities on various cancerous cell lines including HepG2 cells, thus substantiating our approach.

In the case of *W. edulis*, the relative proportion of putatively active compounds was comparable in both aqueous and ethanolic extracts which corroborate their antiproliferative effect on HepG2 cells. Given the high value of *W. edulis* as a medicinal plant for liver diseases in Southeast Asia and the lack of pharmacological and phytochemical studies, further investigations should be performed to confirm its hepatoprotective activity, to determine the compound responsible for the biological activity, and to evaluate its toxicity.

Because of the multifactorial nature of liver related diseases, a multi-assays approach should give more detailed results on active components and intricate molecular mechanisms of these medicinal plants. For instance, our dereplicative approach could be applied to other models such as *in vivo* hepatoprotective, anti-inflammatory, antioxidant and antiproliferative assays (on others liver cancer cell lines and normal liver cells) in order to get a better picture of compounds involved in the biological activities related to liver disorders.

Overall, the workflow presented here combine an ethnopharmacological survey aiming to select most interesting TCAM species hyphenated with a rapid dereplicative approach for structural annotation. This bridge from field studies to bioactive compounds provide a competitive edge to be applied to other ethnopharmacological studies in order to rapidly decipher molecular basis of traditionally used medicinal plants.

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DISCUSSION GÉNÉRALE ET CONCLUSION

Ce projet de thèse s'est focalisé sur l'étude du cancer du foie au Cambodge. L'objectif de ce travail était multiple, puisqu'il s'agissait : (i) de décrire les caractéristiques épidémiologiques des patients atteints de cancer du foie à l'hôpital Calmette, (ii) de documenter les connaissances de ces patients vis-à-vis de leur maladie et de détailler les thérapeutiques utilisées, (iii) de comprendre les stratégies de prise en charge des patients souffrant de maladies hépatiques par les médecins traditionnels, (iv) d'évaluer quelques plantes médicinales, sélectionnées sur des critères bibliographiques et de terrain, sur un modèle *in vitro* de cellules cancéreuses hépatiques afin d'identifier les composés potentiellement responsables de cette activité antiproliférative.

Cette discussion générale dresse un bilan des principaux résultats, et fait le lien entre les différentes études réalisées, tout en mettant en perspective ces informations.

1. Cancer du foie au Cambodge : facteurs de risque et prévention

Dans la première étude (cf. chapitre 1), une analyse de 553 dossiers patients atteints de cancer du foie à l'hôpital Calmette (Phnom Penh, Cambodge) sur une période de 2003 à 2015 a permis d'identifier le carcinome hépatocellulaire comme type de cancer du foie prédominant (92% des cas). Parmi les patients atteints de CHC, près de 92% des cas étaient infectés chroniquement par le virus de l'hépatite B et/ou par le virus de l'hépatite C.

Exception faite de notre analyse, seule une étude épidémiologique a porté sur l'étude du cancer du foie dans le pays. (Narin et al. 2015) ont focalisé leur travail sur le deuxième hôpital spécialisé en cancérologie au Cambodge : l'hôpital de l'Amitié Khméro-Soviétique (AKS). Ils ont étudié 281 cas de carcinome hépatocellulaire admis dans l'établissement entre 2006 et 2011. Ce travail indique que trois-quarts des patients sont porteurs du VHB et/ou du VHC. Ces chiffres corroborent les valeurs obtenues dans notre étude, et donc confirment l'importance de ces deux facteurs de risque (VHB et VHC) au Cambodge.

Cependant, dans notre étude, il a été montré qu'une proportion équivalente de patients étaient infectés par le VHB (44,3%) ou par le VHC (43%). Ces chiffres ne sont mentionnées ni dans

les résultats obtenus par (Narin et al. 2015), ni dans les chiffres rapportés dans d'autres pays d'Asie du Sud-Est tel que le Vietnam, la Thaïlande ou la Malaisie, où la proportion d'infection au VHB par rapport au VHC chez les patients atteints de cancer du foie est de deux à vingt fois plus élevée (Norsa'adah and Nurhazalini-Zayani 2013; Raza, Clifford, and Franceschi 2007; Tangkijvanich, Suwangool, and Mahachai 2003).

D'une manière générale, les facteurs de risque les plus fortement associés à l'infection par le VHC sont les transfusions de sang et de produits sanguins, la réutilisation ou la mauvaise stérilisation du matériel médical, en particulier des seringues et des aiguilles, et la consommation de drogues injectables en partageant le matériel d'injection (Shepard, Finelli, and Alter 2005).

Au Cambodge, dans une étude portant sur les modes de transmission du VHC chez des patients atteints du virus du SIDA, (Goyet et al. 2014) suggère que l'infection au VHC pourrait être due à des facteurs iatrogènes tel que la mauvaise stérilisation des équipements médicaux utilisés lors de fibroscopie ou de chirurgie, ou la réutilisation de seringues usagées. (Vong et al. 2005) évaluant les pratiques d'injections au Cambodge, précisent qu'une grande majorité des injections (injections thérapeutiques, perfusion intraveineuse ou immunisation) sont réalisées par le secteur privé. Dans cette étude, il semblerait que les praticiens interrogés ont des pratiques d'injection sécurisées et ne réutilisent pas de seringues usagées. Cependant, (Saphonn et al. 2017) ont mis en exergue le rôle de praticiens privés dans la contamination par le virus du SIDA d'une partie de la population vivant à Roka, un village situé à l'Est du Cambodge. Selon les auteurs, cette épidémie est liée à l'utilisation de pratiques d'injections non sécurisées, et notamment à la réutilisation de seringues usagées. En effet, étant donné le nombre pléthorique de structures de soin privées existantes au Cambodge et le manque de cadre réglementaire permettant d'assurer la qualité des soins (Goyet et al. 2014), il semble raisonnable de penser que le risque de contamination dû à des mauvaises pratiques d'injections, même réalisées par des professionnels de santé n'est pas négligeable et que malgré les efforts déployés par le gouvernement pour inciter les praticiens à utiliser des seringues à usage unique, le risque de contaminations iatrogènes reste très élevé au Cambodge.

À ce risque d'être exposé à une infection au VHC par défaillance du personnel médical et des structures de santé, se rajoutent les attitudes des patients. Nous avons montré dans notre deuxième étude (cf. chapitre 2) qu'une forte proportion des patients atteints de cancer du foie interrogés avaient réalisé au moins une injection à visée thérapeutique depuis leur enfance. D'autres études mettent en avant l'idée partagée au sein de la population cambodgienne, que les injections ont une efficacité supérieure à celle des comprimés ou d'autres formes galéniques non injectables (Vong et al. 2005). De fait, le Cambodge n'est pas le seul pays où l'abus des injections est important. La Thaïlande, la Chine, l'Indonésie, l'Inde et le Pakistan sont d'autres pays dans lesquels ce genre de pratique est aussi très fréquente (Hadiyono et al. 1996; Janjua et al. 2006; Jiang et al. 2012). Pour (Simonsen et al. 1999), cette forte demande d'injections, de la part des patients, a pour effet la multiplication de consultation de praticiens peu ou pas qualifiés, et donc susceptibles de ne pas connaître ou ne pas respecter les bonnes pratiques d'injections.

En plus de cet engouement pour les injections, potentiellement contaminantes, les techniques de dermabrasion (*coining*) sont fréquemment utilisées au sein de la population cambodgienne pour traiter diverses affections (Richman et al. 2010). Dans notre étude, les patients atteints de cancer du foie ont également reporté cette pratique (cf. chapitre 2). Bien que le rôle du « *coining* » dans la contamination de pathogènes transmissibles par le sang n'est pas été clairement démontrée, (Hurie, Mast, and Davis 1992) suggèrent l'importance de cette pratique dans la transmission de l'hépatite B et C. Par ailleurs, il est à noter que d'autres pratiques conduisant à une effraction de la barrière cutanée tel que le tatouage et le piercing ont été statistiquement liés à la transmission de pathogènes comme le VHB et le VHC (Pérez-Cotapos, Cuyper, and Cossio 2009).

Un troisième facteur pouvant favoriser, indirectement, la contamination par ce virus est la méconnaissance, doublée d'une perception erronée des individus vis-à-vis des causes du cancer du foie et des modes de transmission des hépatites virales. Lors d'une étude portant sur la perception des pathologies hépatiques par les cambodgiens immigrés aux U.S.A., (Burke et al. 2011) confirment les résultats obtenus auprès des patients (cf. chapitre 2) et des médecins

traditionnels (cf. chapitre 3), à savoir que le partage de nourriture[14] est très fréquemment cité comme l'une des causes principales de transmission des pathologies hépatiques et que les trois formes d'hépatites (A, B et C) sont expliquées comme des stades évolutifs d'une même maladie. Il est donc possible de formuler l'hypothèse selon laquelle cette perception est à l'origine de la difficulté d'appréhender les différents modes de transmission propre à chaque hépatite par les patients. Il est également possible, dans ce contexte, que les patients et thérapeutes, mentionnant le partage de la nourriture comme cause de la pathologie, répètent certains messages hygiénistes relatifs à l'hépatite A. D'autres études font également état des méconnaissances des modes de transmission des maladies hépatiques et en particulier de l'hépatite virale B chez les cambodgiens. (Taylor et al. 2002) note que seulement un quart des 320 femmes d'origine cambodgienne vivant à Seattle interrogées mentionne que les patients asymptomatiques peuvent transmettre la maladie. Par ailleurs, dans une autre étude, (Taylor et al. 2009) note que seule la moitié des 111 cambodgiens vivant aux U.S.A. interviewés sait que le VHB peut se transmettre par voie sexuelle.

Il semblerait donc que plusieurs facteurs soient réunis, tous concourant à divers niveaux à favoriser et perpétuer la transmission du VHC : des pratiques d'injection ou de "*coining*" fortement valorisées d'un point de vue thérapeutique et très communément utilisées, couplées à un manque de connaissance ou une connaissance erronée empêchant une prise de conscience individuelle pouvant orienter ces pratiques dans le sens d'une plus grande sûreté d'usage.

Néanmoins, il est possible qu'il existe d'autres pratiques, favorisant la transmission du VHC, que nos enquêtes n'ont pu mettre en évidence. En particulier, le fait que les patients atteints de CHC et infectés par le VHC soient majoritairement des femmes et des habitants de Phnom Penh interpelle (cf. chapitre 1). On peut formuler l'hypothèse que les habitants de la capitale ont un meilleur accès aux soins de santé, et donc ont plus de contact avec des professionnels de santé. D'autre part, on pourrait aussi formuler l'hypothèse que les femmes, statistiquement plus infectées par le VHC dans notre étude, ont elle-aussi plus recours aux

[14] En effet, dans la culture khmère et asiatique plus généralement, les personnes partagent les plats principaux, et se servent à tour de rôle avec leurs couverts respectifs qu'ils portent ensuite à leur bouche.

injections lors de grossesses, d'accouchements, ou pour tout autre raison (campagnes d'immunisation mère-enfant, etc.), ce qui a aussi été suggéré dans une autre étude portant sur les étiologies associés au cancer du foie chez des patients d'Asie du Sud-Est (Lin et al. 2013). Mais ces hypothèses restent encore à démontrer, et des études complémentaires devraient être réalisées afin de mieux comprendre l'importance du virus de l'hépatite C dans les cas du cancer du foie au Cambodge.

Dans ce contexte, afin de diminuer l'incidence du cancer du foie, et en particulier la transmission de l'hépatite C, il conviendrait donc, dans un premier temps de réaliser des campagnes d'éducation à la santé auprès de la population afin de les sensibiliser aux risques liés aux injections et aux pratiques de « *coining* ». Si preuve était faite que cette pratique est réellement source de contamination, des alternatives devraient être proposées (généralisation de l'utilisation de jetons en plastiques jetables par exemple). Par ailleurs, un contrôle plus strict des praticiens exerçant dans le privé, et une meilleure formation de ceux-ci, concernant les règles de bonnes pratiques d'injections et de stérilisation du matériel médical, devraient être réalisé.

En ce qui concerne la transmission du virus de l'hépatite B, des campagnes de vaccination sont mises progressivement en place au Cambodge (Soeung et al. 2012), ce qui permet de penser qu'à l'instar d'autres pays ou ces campagnes ont été réalisées (Taiwan etc.), l'incidence de ces hépatites et donc du carcinome hépatocellulaire va diminuer fortement dans les années qui viennent (Ni et al. 2007).

Enfin, toutes ces informations devraient inciter les autorités locales à mettre en place des études épidémiologiques plus poussées afin de déterminer le rôle des autres facteurs de risques non étudiés dans ce travail, tel que les parasites de la famille Opisthorchidae, et les aflatoxines.

2. Type et rôle des thérapeutiques utilisées

Dans le chapitre 1, les informations relevées sur les dossiers médicaux nous ont permis de constater le manque de moyens matériels et techniques disponibles à l'hôpital Calmette. Plus

précisément, les options thérapeutiques disponibles pour les patients atteints de cancer du foie étaient très limitées. Ainsi, la majorité des patients ont reçu un traitement palliatif, et seules certaines chimiothérapies chères (sorafenib) ou inefficaces (tamoxifène) étaient administrées à quelques patients.

Dans un article détaillant la situation des services oncologiques au Cambodge, (Eav et al. 2012) notaient déjà l'absence de structures adéquates pour prendre en charge les patients cancéreux, le manque d'équipement de radiothérapie, le faible nombre de chirurgiens spécialisés en cancérologie, et le coût excessif des quelques traitements disponibles ne permettant pas à la majorité de la population d'y accéder.

Dans ce contexte, notre deuxième étude s'est intéressée aux pratiques thérapeutiques des patients atteints de cancer du foie afin de mieux comprendre comment ils pallient le manque de moyens de l'hôpital.

Les résultats de cette étude montrent que près de 83% des patients ont recours à la médecine traditionnelle en complément de la médecine moderne. Celle-ci inclut une variété de pratiques et de remèdes, qui sont, soit pris en automédication soit prescrits par un médecin traditionnel.

Parmi les substances utilisées par les patients, deux plantes médicinales (*Annona muricata* et *Moringa oleifera*) et un produit d'origine animale (nid d'hirondelle) ont été particulièrement cités dans notre étude.

A notre connaissance, une seule autre étude portant sur les médecines complémentaires et alternatives utilisées par les patients atteints de cancer du foie dans les pays à faible niveau de revenu a été réalisée. (Rojas Rojas et al. 2016) ont décrit les substances utilisées par 88 patients traités à l'Institut National du Cancer au Pérou. Au total, 56,8% des patients avaient pris des plantes pour traiter les symptômes associés à leur maladie du foie, incluant 46 espèces de plantes. Parmi celles-ci, la plante *Annona muricata* a également été très citée par les patients péruviens, indiquant une convergence d'usage pour cette espèce, originaire d'Amérique du Sud, dans le traitement du cancer du foie. L'importance de cette plante en tant que « anticancer naturel » semble être relayé par les réseaux sociaux et l'internet[15], ce qui est confirmé par (M'zoughi 2017)

[15] Il suffit de taper les mots-clés « anticancer » et « plantes » sur un moteur de recherche pour trouver le corossolier dans les premiers résultats.

dans son étude anthropologique sur l'alimentation des patients atteints de cancer au Cambodge.

M'zoughi (2017) reporte aussi l'usage de boissons à base de salive d'hirondelle et de remèdes à base de feuille de corossol (*A. muricata*) chez ces personnes. Il semblerait donc que la consommation de nid d'hirondelles, ainsi que l'usage du corossolier tel que relevé dans notre étude ne soit pas uniquement le fait de malades atteints du cancer du foie, mais plus largement de ceux atteint de cancer.

Les deux espèces de plantes citées dans notre étude ont montré des propriétés pharmacologiques pouvant confirmer leur rôle dans les pathologies hépatiques. *A. muricata* possède une activité antiproliférative *in vitro* sur plusieurs lignées cancéreuses et notamment sur des cellules cancéreuses hépatiques[16], ainsi qu'une activité hépatoprotectrice *in vivo* (Coria-Téllez et al., 2016; Moghadamtousi et al., 2015). *M. oleifera* possède une activité anti-inflammatoire, anti-oxydante, hépatoprotectrice *in vivo*, anti-VHB, et antiproliférative sur cellules cancéreuses hépatiques (Singh et al., 2014; Waiyaput et al., 2012).

Par ailleurs, l'usage populaire des nids d'hirondelles dans les cas de cancer peut s'expliquer en partie par ses activités biologiques. Ainsi, il a été prouvé que les nids d'hirondelles possèdent des activités anti-inflammatoires, anti-oxydantes et immunostimulantes, et qu'ils contiennent de nombreux composés bioactifs : acide sialique, glucosamine, lactoferrine, etc. (Ma and Liu, 2012; Yida et al., 2015). De plus, le nid d'hirondelle renferme de nombreuses substances nutritives tel que glycoprotéines, vitamines, acides aminés et minéraux (Marcone, 2005), ce qui peut justifier son usage comme complément alimentaire.

En ce qui concerne les plantes prescrites par les médecins traditionnels, notre troisième étude (cf. chapitre 3) a permis d'identifier un total de 83 espèces de plantes utilisées par les médecins traditionnels khmers dans le traitement des pathologies hépatiques.

[16] Certains sites internet grand public font état de plusieurs centaines d'études scientifiques sur cette plante. Et pourtant, en cherchant sur les moteurs de recherches spécialisés, elle ne semble pas être plus étudié que les autres plantes. 3100 résultats obtenus sur google scholar pour les mots-clés : « annona muricata » et « cancer », contre 7800 pour « moringa oleifera » et « cancer », ou 19 900 pour « panax ginseng » et « cancer ».

Parmi celles-ci, 72 ont déjà été reportées comme traditionnellement utilisées dans les maladies du foie dans d'autres pays du monde (Asie, Afrique, Europe et Amérique). Pour 54 espèces, une activité hépatoprotectrice *in vivo* a été mise en évidence, 57 espèces possèdent une activité antimicrobienne, 50 espèces ont une activité anti-inflammatoire et 25 ont été évaluées pour leur activité antiproliférative sur cellules cancéreuses hépatiques.

Dans la continuité de l'analyse ethnopharmacologique des plantes utilisées dans les maladies du foie, notre quatrième étude (cf. chapitre 4) s'est attachée à déterminer les composés susceptibles d'être responsables de l'effet antiprolifératif sur lignées cellulaires d'hépatocarcinome humain (HepG2), de dix plantes sélectionnées pour leur importance dans le traitement des pathologies du foie en Asie du Sud-Est.

Parmi ces dix plantes, les extraits éthanoliques de quatre espèces (*Andrographis paniculata*, *Oroxylum indicum* et *Orthosiphon aristatus*, *Willughbeia edulis*) ont présenté les activités antiprolifératives les plus élevées sur cellules HepG2. Grâce à la méthode de déréplication utilisée et à une analyse multivariée, les composés susceptibles d'être responsable de l'activité ont été classés selon leur activité antiproliférative, puis identifiés pour *A. paniculata* (andrographolactone et dehydroandrographolide), *O. indicum* (baicalein, chrysin, oroxylin A et scutellarein) et *O. aristatus* (5-desmethylsinensetin). De plus, ce procédé de déréplication nous a permis d'identifier une plante *W. edulis*, potentiellement source de nouvelles structures chimiques puisque peu de composés susceptibles d'être responsables de l'activité antiproliférative ont pu être dérépliqués à partir des bases de données de l'espèce et de sa famille botanique.

Ces résultats viennent s'ajouter aux nombreuses propriétés pharmacologiques liées à l'hépatoprotection de trois plantes déjà bien étudiées : *A. paniculata*, *O. indicum* et *O. aristatus*. Aussi, *W. edulis* plante endémique à l'Asie du Sud-Est et très connue pour la valeur nutritive de ses fruits, apparaît comme à la fois très utilisée dans le traitement des maladies hépatiques et également très peu étudiée d'un point de vue phytochimique et pharmacologique.

Ainsi, les substances prises en automédication par les patients ou prescrites par les médecins traditionnels présentent dans la majorité des cas une convergence d'usage et des propriétés pharmacologiques pouvant suggérer une action sur les maladies du foie. Par exemple, les propriétés anti-inflammatoires et antioxydantes de ces produits naturels pourraient contribuer à contrôler certains produits de l'inflammation tel que les cytokines, les facteurs de croissance et les facteurs de transcription impliqués dans l'expression de gènes suppresseurs de tumeurs et d'oncogènes (Hofseth and Wargovich, 2007), et également impliqués dans l'apparition de stéatose hépatique non-alcoolique, d'hépatite fulminante, de fibrose et de cirrhose (Chou et al., 2013; Plat et al., 2014). Un effet antiprolifératif sélectif sur cellules cancéreuses hépatiques couplé à un effet anti-angiogénique, immunomodulateur et inducteur de l'apoptose pourrait participer à limiter le développement de tumeur hépatique (Ramasamy and Agarwal, 2008). Des propriétés antimicrobiennes pourraient combattre certaines infections liées à la prolifération de bactéries induisant des abcès du foie. Ou encore des propriétés anti-VHB ou anti-VHC pourraient aidées à combattre la cause des hépatites virales B et C.

Malheureusement, peu d'études permettent de préciser les mécanismes d'action exactes de ces produits naturels. De plus, le manque d'études cliniques sur ces produits ne permet pas actuellement de confirmer l'efficacité et l'innocuité de ces substances. Ainsi, quatre plantes (*Heliotropium indicum*, *Morinda citrifolia*, *Senna siamea*, et *Tinospora crispa*) utilisées dans la troisième étude sont susceptibles de présenter un risque de toxicité, puisque des cas cliniques d'hépatotoxicité ont été décrits (Ahmad et al., 2016; Kamagaté et al., 2014; Roeder and Wiedenfeld, 2011; Yüce et al., 2006). Il est donc nécessaire de continuer les investigations phytochimiques, pharmacologiques et cliniques sur ces plantes afin de valider leur utilisation dans le traitement des pathologies hépatiques. Aussi, le procédé de réplication employé dans cette étude pourrait être employé en combinaison avec d'autres tests pharmacologiques (hépatoprotection *in vivo*, anti-inflammation, antimicrobien) afin de mieux identifier les composés impliqués dans l'action de ces plantes sur les maladies du foie.

Enfin, deux plantes mériteraient d'être étudiées d'une manière approfondie pour leur action sur les maladies du foie : *Cananga latifolia* et *Willughbeia edulis*.

3. Concepts médicaux associés aux pathologies hépatiques

Un autre résultat signifiant de notre étude est l'importance donnée à certains concepts médicaux propres au système nosologique khmer, liés aux maladies du foie et à ses traitements.

Dans la deuxième étude, la majorité des patients ont cité les aliments « chauds » comme cause de la maladie.

Burke et al. (2011), dans leur analyse de la perception des maladies du foie par les cambodgiens immigrés aux U.S.A., avaient déjà noté l'importance de veiller à réguler l'équilibre chaud/froid pour les personnes atteintes. En effet, les maladies du foie sont perçues comme des maladies « chaudes », dont le traitement passe par l'éviction des produits « chauds » (alcools, huiles, viandes, aliments frits) et la consommation de produits « froids » (légumes verts, brocoli, concombre amer, lait de soja).

L'utilisation de technique de dermabrasion est aussi reportée dans cette même étude dans le but « d'évacuer le vent ». En effet, les vents, parcourant le corps, sont les principes de fonctionnement du corps permettant la circulation des fluides plus ou moins subtils et des matières au sein de ce même corps. Un dérèglement (stagnation, accumulation, dispersion, orientation inversée, etc.) dans la circulation de ces vents entraine certaines manifestations symptomatiques. De plus, une forte altération du ou des vents est quelque fois reconnue comme la cause principale de certaines pathologies (ce sont souvent des désordres mentaux qui en résultent), mais le plus souvent on considère que toute pathologie est accompagnée et/ou aggravée par un dérèglement des vents, et donc améliorée si ces désordres sont corrigés. Dans notre étude deux pratiques visant à rétablir une circulation harmonieuse de ces vents ont été documentées ; le « *coining* » et l'utilisation de certaines plantes médicinales, laxatives et diurétique, afin de lever les blocages liés aux vents permettant l'évacuation de matières corporelles internes.

Dans l'étude anthropologique sur les pratiques alimentaires de patients atteints de cancer au Cambodge, M'zoughi (2017) développe les principes explicatifs des modes de consommation alimentaire qui renvoient à la fois à une recherche d'équilibre, de modération et de gain d'énergie, et qui s'inscrivent dans le cadre d'une médecine humorale où l'alimentation

joue un très grand rôle comme premier régulateur, utilisé préventivement ou curativement, de désordres de santé. Dans son analyse, certains breuvages sont décrits comme « tonique », et ont pour fonction de prodiguer de la force et d'améliorer l'état de santé des patients. C'est le cas des boissons à base de nids d'hirondelles consommés par une grande partie des patients interrogés, mais également des boissons énergétiques type Redbull®. Par ailleurs, l'utilisation du corossolier (*Annona muricata*) chez les patients cancéreux est décrite comme traitement curatif du cancer.

Dans la troisième étude, la classification des plantes médicinales, selon les critères ethnopharmacologiques des médecins traditionnels a été envisagée.

L'action des plantes se distribue entre quatre propriétés : « *trocheak* » (froid), « *ktchol* » (vent), « *somrap mé rok* » (contre l'origine de la maladie) et « *psah* » (cicatrisant). Aussi, certaines espèces cumulent deux voire trois activités, sachant que les propriétés « *psah* » et « *trocheak* » sont souvent associés. Ici, les plantes « *ktchol* » peuvent soit favoriser le transit intestinal, soit augmenter la diurèse.

La prépondérance d'utilisation de plantes aux propriétés « froides » dans notre étude est corroborée par M'zoughi (2017) dont les patients cancéreux disent : « le cancer est une maladie échauffante, il modifie les vents dans le corps ». Le cancer semble donc perçu comme une maladie « chaude », au même titre que les maladies du foie (Burke et al., 2011).

Aussi, la propriété « *psah* » est la deuxième la plus citée après l'activité « *trocheak* », et la moitié des plantes « *trocheak* » possédait également des effets « *psah* ». Ce dernier terme qualifie les traitements aux propriétés curatives et cicatrisantes, agissant à la fois sur des désordres internes ou externes, tel que les inflammations, les infections, les blessures et les brûlures. Dans son étude sur la popularité de la balance chaud/froid dans les différents systèmes médicaux à travers le monde, Anderson (1987) explique que « too much heat clearly cause damage : burns… » et que « fever and inflammation are obvious pathological signs ». Ce qui suggère l'importance à la fois de refroidir le corps (avec des plantes *trocheak*), et de soigner les inflammations associées à cet excès de chaleur (avec des plantes *psah*).

En ce qui concerne les propriétés « *somrap mé rok* », le terme « *mé rok* » est composé de « *mé* » (la mère, le chef, l'origine) et « *rok* » (la maladie), il renvoie à l'agent pathogène qui est à

« l'origine de la maladie », et qui est assimilé à des « microbes » (Guillou, 2001). Le terme « *somrap mé rok* » prit dans sa globalité désigne les traitements agissant contre l'origine de la maladie.

D'un point de vue pharmacologique, il serait intéressant d'essayer de mettre en perspective les propriétés des plantes, classifiées selon la nosologie khmère, avec leurs activités biologiques pour essayer de déterminer les grandes orientations d'activités des plantes qualifiées de « *psah* », « *trocheak* » ou « *somrap mé rok* ». Cette analyse pourrait permettre de discuter avec les médecins traditionnels khmers des similitudes retrouvées entre leur raisonnement thérapeutique et les stratégies de traitement adoptées dans le système médical occidental.

4. Conclusion générale

Nos recherches épidémiologique et ethnopharmacologique sur le cancer du foie au Cambodge ont mis en évidence l'importance du virus de l'hépatite C comme facteur de risque prédominant de la maladie à l'hôpital Calmette de Phnom Penh.

Nous avons suggéré que certaines pratiques employées par le personnel médical (mauvaise stérilisation des équipements médicaux et réutilisation de seringues usagées) pouvaient être responsables de la forte incidence des cas d'infection au VHC.

De plus certaines pratiques employées par les patients, tel que l'usage excessif des injections thérapeutiques et l'utilisation de technique de dermabrasion pourraient être aussi à l'origine de contaminations.

Par ailleurs, la méconnaissance des causes du cancer du foie et des modes de transmission des hépatites virales pourrait contribuer à l'augmentation du risque de contamination des patients.

Nous avons également suggéré la mise en place d'études épidémiologiques complémentaires afin de confirmer l'importance du VHC dans le développement de cancer du

foie au sein de la population générale cambodgienne. Ces études devraient être complétées par une campagne d'information et de dépistage pour une prise en charge précoce des patients.

Cela dit, pour être efficaces dans le contexte culturel cambodgien, toutes démarches préventives et thérapeutiques devraient impérativement tenir compte des perceptions culturelles locales afin d'éviter incompréhension et/ou rejet de la part des populations concernées.

C'est la raison pour laquelle nous nous sommes intéressés à l'étude des pratiques thérapeutiques dites traditionnelles, focalisées sur les pathologies de la sphère hépatique. Ainsi, nous avons mis en évidence que patients et tradipraticiens utilisaient un large éventail de plantes médicinales, de substances d'origine animale, de suppléments nutritionnels mais aussi de technique de dermabrasion.

Bien que la plupart de ces traitements n'aient pas été évalués au moyen d'essais cliniques contrôlés, la convergence d'usage et les propriétés pharmacologiques de ces traitements permettent de souligner leur intérêt dans le traitement des « maladies du foie ». Cependant, l'utilisation de tous ces éléments de la pharmacopée ne peut être dissociée du système nosologique khmer, et de ses concepts sur le fonctionnement symbolique du corps, auxquels se superposent et s'agrègent quelques termes empruntés à la biomédecine, plus ou moins bien compris et interprétés.

Aussi, d'autres investigations phytochimiques, pharmacologiques et cliniques devraient être poursuivies afin de déterminer les modes d'action de ces remèdes, et de savoir si leur utilisation pourrait être intégrée au programme national de santé pour le traitement du cancer du foie.

Enfin, le procédé de déréplication décrit dans cette étude pourrait être appliqué à d'autres modèles d'évaluation biologique et ainsi contribuer à la mise en évidence d'agents potentiellement thérapeutiques.

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ANNEXES

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Natural remedies used by Bunong people in Mondulkiri province (Northeast Cambodia) with special reference to the treatment of 11 most common ailments

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ABSTRACT

Ethnopharmacological relevance: In this paper we present a comprehensive ethnomedicinal study conducted in Mondulkiri province. Traditional knowledge about natural medicine (plants, animals, mushrooms) was investigated in Cambodia's largest indigenous community: the Bunong people. The survey aims to document the medicinal plant use of this ethnic, by focusing on the eleven most frequent diseases encountered in the area, in order to highlight species that could be recommended in public health programs.

Materials and methods: During the years 2013 and 2014, 202 villagers were interviewed in 28 villages from the five districts in Mondulkiri. Two types of methodology were employed: (1) an ethnobotanical field survey (walk-in-the-wood interviews) and (2) semi-structured household interviews with a special emphasis on the treatment of 11 most common ailments encountered in the area. Medicinal plants and mushrooms were collected and identified together with medicinal animals. The factor informant consensus (F_{IC}) and fidelity level (FL) were calculated.

Results: Bunong people use a total of 214 plants belonging to 72 families, 1 mushroom and 22 animal species in their traditional healthcare practices in order to treat 51 different ailments. Among the medicinal plants, Fabaceae was the most predominant family; *Chromolaena odorata* (L.) R.M. King and H. Rob. (Asteraceae), *Zingiber montanum* (J.Koenig) Link ex A.Dietr. (Zingiberaceae) and *Kalanchoe pinnata* (Lam.) Pers. (Crassulaceae) were the most cited medicinal plants; and four ailments (cold/fever, diarrhea, postpartum disorders and stomachache) were described as major ailments in the community. The root was the most important part of plants used, and decoction was the most cited method of preparation. During our survey, we also discovered a "new to science" plant species called *Ardisia mondulkiriensis* Hul and Chassagne, and we recorded for the second time the plant species recently described, *Solanum sakhanii* Hul.

Conclusion: Most of the species reported for the treatment of the 11 most frequent ailments have already been proven to be efficient and safe. Furthermore, 10 plant species are reported for the first time as medicinal and some of them are widely used in the community. Further pharmacological and phytochemical investigations should be undergone to assess the pharmaceutical potential of these species.

While undergoing considerable changes, Bunong people have maintained extensive traditional medicine knowledge. As this indigenous hill tribe depend mainly on natural remedies for their daily healthcare, environmental preservation is of high importance for the community.

1. Introduction

Southeast Asia is known to have rich biodiversity and overlaps with 4 of the 25 "biodiversity hotspots" (Myers et al., 2000). Covering three per cent of the earth's total surface, this region provides habitat for 18% of all known species (ASEAN, 2010). Compared to the other tropical regions, Southeast Asia contains the

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highest mean proportion of country-endemic bird (9%) and mammal species (11%) and the second-highest proportion of country-endemic vascular plant species (25%) (Sodhi et al., 2010).

Cambodia does not exceed 4% (181,035 km^2) of the total area of Southeast Asia, but possesses rich natural resources and a unique original ecosystem due to the Mekong River and Tonle Sap lake (ASEAN, 2010). In 2010, Cambodia was considered largely forested but since then, due to economic development, high deforestation rates have been registered (Kapos et al., 2010). It is estimated that the country contains a large number of bird (545), mammals (123) and fish species (874), and vascular plant species (over 3000) including around 214 endemic species (Anonymous, 2010).

Besides its natural diversity, Cambodia is characterized by a high cultural diversity. Cambodian society is multi-ethnic with a majority of Khmer people (90% of the total population), living near-by the so called "ethnic minorities" (Cham, Chinese and Vietnamese) and more than 20 indigenous groups (Moul and Seng, 2012). Historically, Khmer civilization has been strongly shaped for centuries by Indian and Chinese cultural traits through migration (Chhem, 2001), and indigenous groups, part of the "Proto-Indo-Chinese civilization" dwelling in the highlands, are said to be the most ancient inhabitants of the region (Condominas and Haudricourt, 1952).

Regarding traditional medicine practised in Cambodia, Khmer medicine, which is based on written scripts and is known to have been used for nearly a thousand years, is still widely used and plays an important role in everyday life (Cœdès, 1906; Chakravarti, 1979; Leti, et al., 2010). Medicinal plants especially are the core of this medicine and their uses have been well documented (Cheng and Huon, 1996; Dy Phon, 2000; Martin, 1971; Menaut, 1930; Petelot, 1952, 1953, 1954a, 1954b). Moreover, ethnopharmacological research has been conducted to evaluate some of the claimed activities (Bun et al., 2009; Chea et al., 2007a, 2007b; Hout et al., 2006; Jonville et al., 2010).

However, few studies focus on medicinal systems and natural remedies from Cambodian indigenous groups, other than the Mnong Gar and Brou hill tribe (Condominas and Haudricourt, 1952; Matras and Martin, 1972), and also some recent studies in the Bunong community (Pordié, 1998; Schmitt, 2004).

Bunong people (Môn-Khmer linguistic family), also called Phnong, is one of the largest indigenous groups from Cambodia with an estimated number of 33,000 people. They mainly dwell in Northeastern Cambodia and are spread over four provinces: Kratie, Mondulkiri, Stung Treng and Ratanakiri provinces. In Mondulkiri province, this ethnic group represents 52% of the total population (Moul and Seng, 2012; NIS, 2009).

This indigenous tribe used to live in remote areas, and in small settlements of 400–500 inhabitants. Their livelihood and culture is mainly based on a shifting cultivation system that revolves around planting upland rice and a large diversity of crops (such as banana and papaya) grown together in the fields, after the forest has been cut and burnt. Hunting, fishing and collecting non-timber forest products such as honey bee, oleoresin [*Dipterocarpus* spp., *Shorea* spp.], bamboo shoots [*Bambusa* spp.], rattans [*Calamus* spp.], and wild fruits [*Antidesma acidum* Retz., *Dillenia ovata* Wall., *Flacourtia indica* (Burm. f.) Merr., *Terminalia chebula* Retz., *Willughbeia edulis* Roxb., *Ziziphus* spp.] are also in daily use. Nowadays, Bunong people have introduced some plants with economic value, such as cassava [*Manihot esculenta* Crantz], cashew nut [*Anacardium occidentale* L.] and rubber plantations [*Hevea brasiliensis* (Willd. ex A. Juss.) Müll.Arg.] (Nikles, 2006; Schmitt, 2004).

Pordié (1998) was the first to study Bunong traditional medicine, focusing on medicinal plants used to treat malaria, reporting the use of 24 species. Then, Schmitt (2004) undertook ethnobotanical research with some Bunong traditional healers, and listed the use of 130 plant species. Finally, a case study aiming to highlight the contribution of wild medicinal plants towards poverty alleviation and health improvements was done by Laval et al. (2011), and a socio-economic profile of some communities living around the Mondulkiri protected forest was defined by Maling (2007).

These studies point out the importance of medicinal plant use within Bunong communities, and also list the 11 most common ailments found in the area: wounds, colds (associated with feverish conditions), cough, sprain, stomachache, headache, diarrhea, burns, backache, malaria, as well as the post-partum period, considered to be a period at risk during which special treatments should be administered to the mother.

Therefore, because of the importance of medicinal plant use and the lack of practical and contextualized information regarding the care of these most common ailments, we decided to implement a study. This aimed to report the management of these 11 most common health problems faced by Bunong people in their everyday life, using ethnopharmacological quantitative methods.

2. Materials and methods

2.1. Study area

With a total area of 14,288 km^2, Mondulkiri is the largest province in Cambodia. The region lies between latitude 12°4′ and 13°24′N and longitude 106°21′ and 107°36′E, and rises in elevation from 100 m to over 1078 m above sea level. Sen Monorom, in the south, is the main provincial town with more than 7500 inhabitants. According to Maurice (1993), three differents ecosystem co-exist in the province:

- In the Southeast, the Sen Monorom plateau, (600–900 m high) is described as a savanna with patches of evergreen forest, considered fertile land. With an average temperature between 15 and 20 °C during the cold season, and 25 °C to 30 °C from February to June, this part of Mondulkiri is one of the coolest areas of Cambodia.
- The foothills (300 and 600 m high) in the south, are characterized by high forest cover and comprises different types of forest: evergreen, semi-evergreen, deciduous dipterocarp forests and bamboo forest.
- Finally, the lowlands in the north (from 100 to 300 m) are very similar to the central Cambodian plains. Here, the climate is warmer with a minimum average temperature of 20 °C in the cold season, and 35–40 °C from April to May.

Mondulkiri's climate is dominated by monsoons, and the area receives an average annual rainfall of about 1500–2000 mm in the lowland, and over 2000 mm in the Sen Monorom plateau.

Administratively, the province is divided into 5 districts (Kao Seima, O Reang, Sen Monorom, Pechrada and Koh Neak), 21 communes and 98 villages. Mondulkiri has 61,107 inhabitants and possesses the lowest population density in Cambodia. Thirteen different ethnic groups dwell in the province; Bunong (52.3%) and Khmer (34.6%) are by far the most represented groups (NIS, 2009).

2.2. Data collection

The study was carried out between April 2013 and January 2014 in Mondulkiri province. Twenty-eight villages were visited (Fig. 1), including 9 in the Sen Monorom plateau, 5 in the lowlands and 14 in the foothills. The largest village was Pu Hiam with about 1500 inhabitants and 300 households. The smallest one was Sre Ampil with less than 300 inhabitants and 50 households. In these villages, more than 85% of the total population were Bunong, other

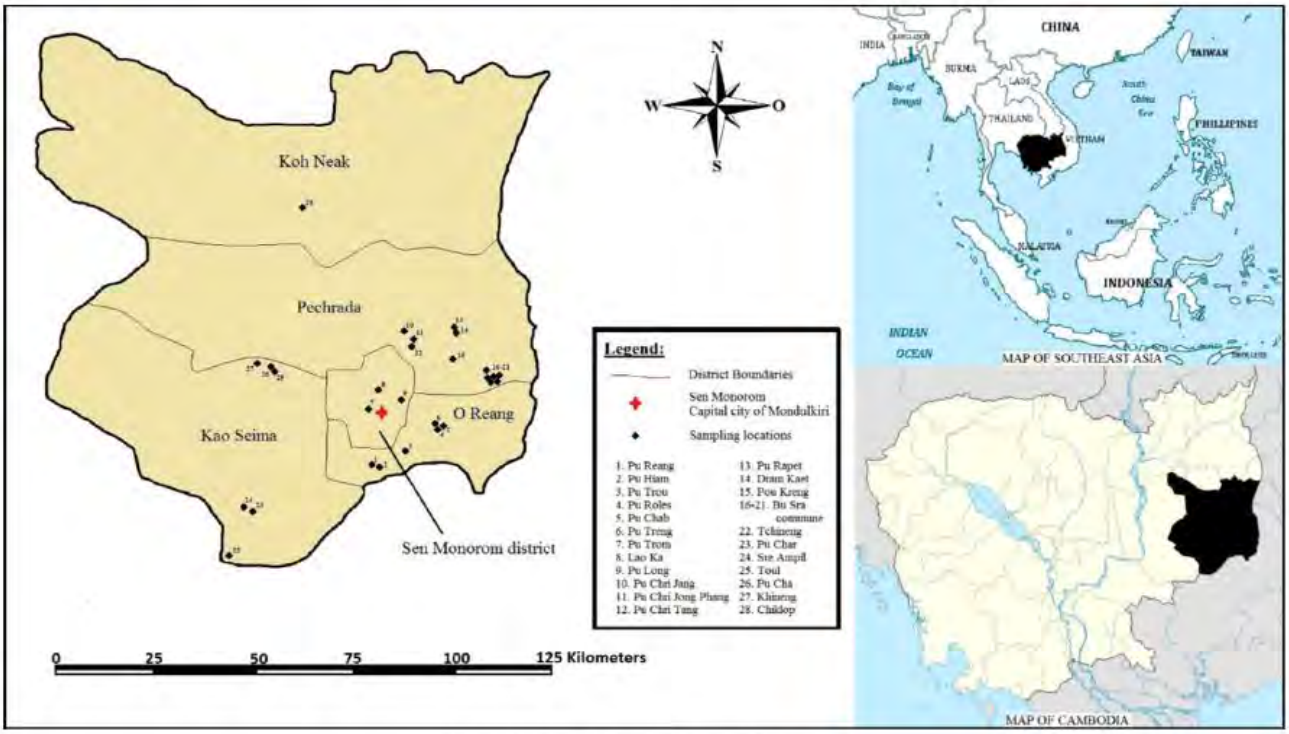

Fig. 1. Mondulkiri map and sampling locations.

than three (11, 12, 25) in which up to 40% of the total population were Khmer. All the villages had access to a health post (distance to the nearest health post < 15 km), except three villages (10, 27, 28), but most of these health centers were lacking in resources and personnel. First, 18 households were pre-selected as a traditional healer was living there, and then 184 households were randomly selected.

A total of 202 informants (including 18 traditional healers and 184 non-specialist informants), all over 18 years old were interviewed. Ethnobotanical data was recorded according to Martin (1995) and Alexiades (1996). Interviews were performed by the first author with the help of a translator. During the survey, two types of methodology were used:

a) A questionnaire was administered in each household. The questionnaire included four different parts.

- First, socio-demographic data were recorded (age, gender, occupation, religion, education level, length of stay in the place of interview).
- Second, for each of the 11 determined ailments, we asked if people manage this problem either by self-medication (based upon the use of local pharmacopoeia), with the help of a traditional healer, or by going to the nearest health center.
- Then, if the inhabitants mentioned that the problem could be treated by themselves (self-medication), they were asked to list the natural remedies that can be used to alleviate this symptom/ailment. Remedies were carefully noted and the same day or a few days after, plant species were collected with the help of the informant. For each plant cited, its vernacular names, part used, preparation method, posology, and way of administration were recorded. When the name of an animal part was mentioned, a book from the Wildlife Conservation Society with a local fauna photographer (Walston, 2008) was shown to people, in order to check identification.

a) Interviews in-situ during a walk-in-the-woods in different ecotopes were done with five traditional healers (herbalists) and one villager who were both identified as having a good plant knowledge through the first methodology and willing to participate in this sampling. Plants were collected, and information concerning the plant's local name, its uses, mode of preparation, administration and the posology were obtained, and a herbarium voucher specimen was collected for further determination.

2.3. Ethical concerns

This project was approved by the provincial authorities in accordance with the laws and regulations of the Kingdom of Cambodia, and all the participants signed a preliminary consent form before starting the survey.

2.4. Botanical identification of voucher specimens

All the collected plants from the study area were dried, numbered, registered and deposited at the National Herbarium of Cambodia, Department of Biology, Faculty of Science, Royal University of Phnom Penh (RUPP), Cambodia. Moreover, some vouchers were also deposited at the Museum National d'Histoire Naturelle (MNHN, Paris). The taxonomic identification was realized by specialists.

2.5. Data analysis

Factor informant consensus (F_{IC}) and Fidelity level (FL) were calculated.

- Factor informant consensus (F_{IC}) is used to determine the homogeneity of the data collected (Heinrich et al., 1998; Trotter and Logan, 1986). In other words, it indicates the level of agreement in the use of plants between the plant users for

one specific ailment. It was calculated as follows:

$$F_{IC} = (N_{ur} - N_t)/(N_{ur} - 1)$$

where "N_{ur}" refers to the number of use reports in each category of illness, and "N_t" refers to the number of taxa/species in each use category. F_{IC} values range from 0 to 1. A value near 1 indicate that relatively few taxa are used by a large proportion of informants for a specific ailment. F_{IC} was calculated for each of the 11 selected ailments.

- Fidelity level (FL) is the percentage of informants claiming the use of a certain plant for the same major purpose (Friedman et al., 1986). The indice was calculated with the following formula:

$$FL = (I_p/I_u) \times 100$$

where I_p is the number of informants who cited the species for a particular use, and I_u is the total number of informants that mentioned the plants for any use. High FL value indicates a high convergence in the use of a species. It was calculated for the first methodology most cited medicinal plants.

3. Results and discussion

3.1. Informant's socio-demographic characteristics

In total, we interviewed 92 men and 110 women (sex ratio=0.84) from 18 to 80 years old (see Table 1). Most of the informants subsist from their agricultural practices (90.1%), sometimes with another activity (such as weaver, school teacher, shop seller, village staff) in order to supplement their income. All traditional healers interviewed practice both agriculture and medicine, and treat patients occasionally upon demand. Among the 18 interviewed healers, we were able to work with 10 herbalists, 1 bonesetter, 3 shamans, and 4 midwifes. All healers were males, except the four midwives and one shaman.

Table 1
Socio-demographic data of the informants (n=202).

Socio-demographic data		Number	Percent (%)
Gender			
	Male	92	45.5
	Female	110	54.5
Age			
	18–30 years	50	24.8
	31–40 years	50	24.8
	41–50 years	41	20.3
	51–60 years	35	17.3
	61 and more	26	12.9
Education			
	Illiterate	124	61.4
	Elementary school	56	27.7
	Secondary school	20	9.9
	Higher education level	2	1.0
Occupation			
	Farmer	182	90.1
	Employed (village, school, health post,...)	22	10.9
	Shop seller	7	3.5
	Traditional healer	18	8.9
	Retired	7	3.5
Religion			
	Animism	173	85.6
	Christian	28	13.9
	Buddhism	1	0.5
Duration of residence			
	Born here	111	55.0
	> 15 years	59	29.2
	< 15 years	32	15.8
Number of people per household			
	1–4	48	23.8
	5–8	103	51.0
	More than 8	51	25.2

3.2. Bunong traditional medicine

Even if a small number of Bunong people converted to Christianity a few decades ago (Bourdier, 2006; Guérin et al., 2003), Bunong animistic beliefs still play a key role in their traditional medicine. People perceive themselves as part of an environment shared with spirits and ancestors. If not respected through special rituals and offerings or if some prohibitions are violated by human beings, these invisible entities are able to cause damage and may be the cause of misfortune, accidents, diseases, death, or bad harvests (Nikles, 2006). Contrarily, harmonious relations, and respectful behaviour towards entities will ensure protection and prosperity.

Bunong traditional medicine relies on the use of a rich pharmacopoeia, either used as self-medication or prescribed by traditional healers. According to Savajol et al. (2011), traditional healers can be classified in four categories: the herbalist (called *Bu Blao Nam Tchi*) who uses mainly medicinal plants to treat their patients; the bonesetter (*Bu Blao Chian Klet*) who can treat broken bones; the midwife (*Bu Blao Gnot Ndol*) who monitor and control pregnancy, delivery and postpartum; and the shaman (*Bu Blao Po Ohm*) who are able to remove agents of the disease from the body by "sucking the disease", and who can also perform spiritual rituals in case of serious diseases. Moreover, the fortune teller (*Bu Blao Pol*), is sometimes consulted for his ability to ascertain the cause of the illnesses.

All traditional healers, but mostly the shaman (*Bu Blao Po Ohm*), are said to have the capacity to communicate with spirits, through sacrificial rituals involving an animal (chicken, pig), offerings (incense, rice, jar rice alcohol, candles), prayers, use of special plants or stones, and dreams. Dreams play an important role as it is through them that spirits will ask people to become a traditional healer and teach them about medicinal plants (Nikles, 2006; Schmitt, 2004). Dreams can also help traditional healers to find the location of medicinal plants which can cure their patients.

Regarding the theoretical functioning of the body, Bunong traditional medicine states that health is the result of a balance between hot and cold elements. This hot-cold theoretical framework is supposed to originate from the humoral theories of Hippocrates and Galen, and was spread over Southeast Asia, through ancient cultural exchanges with Chinese, Indian and Arabic medical traditions (Laderman, 1981; Manderson, 1981). In this theory, it is thought that an excess of heat in the body (i.e. fever) has to be regulated with cold or fresh plants, and contrarily an excess of cold should be balanced with "warm" plants. This is why after giving birth and during the post-partum period, which is considered to be at risk for women because of heat loss, Bunong people use "warm" plants to restore this hot-cold imbalance.

As in Thai or Khmer humoral theories, wind or air (*Chial*) are also described as a major element in Bunong traditional medicine (Chhem, 2001; Muecke, 1979). This element is a vital force which

allows the movement of liquids (blood, bile, faeces, and urine) and body parts. Therefore, diarrhea can be perceived to be due to an excess of wind, and a constipation to a lack of this element. Moreover, "wind illness" is often described in the community, and refers to various signs and symptoms affecting the body (cramp, dizziness, muscle aches, indigestion, loss of consciousness, etc.). The most common treatment of "wind illness" consists of dermabrasive practices such as coining and massage (*Kos Chial*) (Schmitt, 2004).

Another characteristic of Bunong traditional medicine is the classification of the pharmacological activities of the medicinal plants according their organoleptic properties, an assumption also noted in Ayurveda, Chinese medicine and in Khmer medicine (Gilca and Barbulescu, 2015; Leonti et al., 2002; Menaut, 1930). For example, bitterness is thought to be an indication of the potential activity of plants against malaria, and astringent tastes can help to treat diarrhea.

3.3. Overview of Bunong pharmacopoeia

3.3.1. Medicinal plants

3.3.1.1. Repartition of plant family. A total of 214 medicinal plant species and 1 medicinal mushroom were collected (see Supplementary material). These 214 medicinal plants species belong to 178 genera from 72 families. Considering the types of methodology used, 196 species were mentioned in the first one, 98 in the second one, and 81 were mentioned in both methodologies. The most predominant plant families are Fabaceae (27 sp.), following by Malvaceae (12 sp.) and Poaceae (10 sp.) (see Fig. 2). Fabaceae was also reported to be the most important family in other ethnobotanical studies undertaken in Thailand and Laos (Junsongduang et al., 2014; Khuankaew et al., 2014; Libman et al., 2006) which could be explained by the predominance of the family in the region (Dy Phon and Rollet, 1999). The most cited plants (mentioned more than 10 times by the informants) are presented in Table 2.

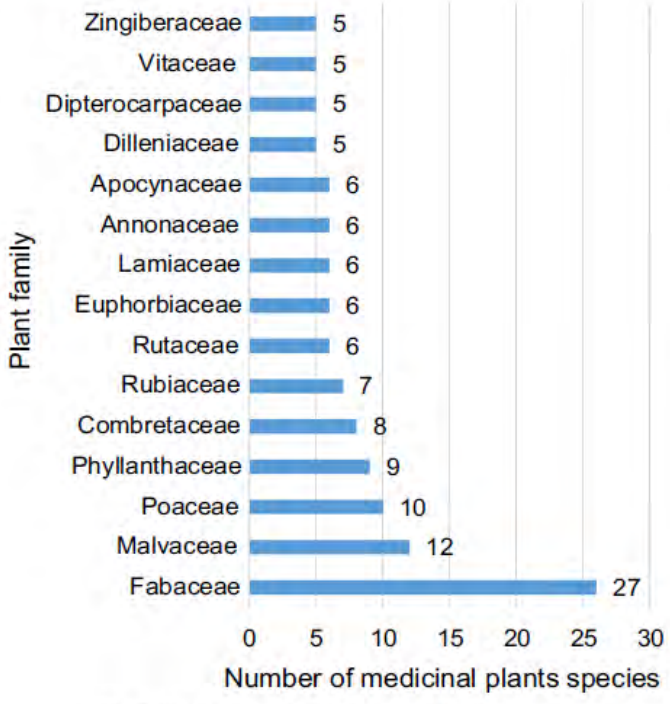

Fig. 2. Number of medicinal plants used by plant family.

3.3.1.2. Biological type of plants used. Of the 214 species, 161 species are wild and 53 are cultivated. 116 species (54.2%) are trees and shrubs, 66 (30.8%) are herbaceous plants, 27 (12.7%) are lianas, and 5 (2.3%) are palms or epiphytic species. The high number of wild plants used may suggest that Bunong people have a good knowledge of forest plants, and so still have access to this area. The frequent use of trees and shrubs species was also reported by Schmitt (2004).

3.3.1.3. Geographical origin of plants used. Among the 214 plants, 115 species are widespread in Asia, 41 are pantropical or originating from old world tropics (Africa, Asia, Australia), 41 are found in the Indochinese Peninsula (Cambodia, Laos, Vietnam, Myanmar, Thailand and southern China), 14 are endemic species from Cambodia, Laos and Vietnam, and three (*Ardisia mondulkiriensis* Hul & Chassagne, *Smilax cambodiana* Gagnep. and *Solanum sakhanii* Hul) are only reported from Cambodia (Ashwell and Walston, 2008; Chassagne and Hul, 2014; Leti et al., 2013, http://www.tropicos.org/).

3.3.1.4. Repartition of species according place of study. The sampling locations (28 villages) were classified into three groups, corresponding to the three ecosystems. Then, we analyzed the repartition of species between these groups (cultivated species were not included in the analysis). Among the 159 plants cited, 115 species were used by people living in lowland, 95 species were used by people from foothills and 89 by Bunong people living in the "Sen Monorom plateau". Our data indicate that plants used in Sen Monorom plateau are different from plants used in the lowland. Only a few species were cited with an equal number of citations in the three ecotypes: *Cananga latifolia* (Hook.f. & Thomson) Finet & Gagnep., *Careya arborea* Roxb., *Chromolaena odorata*, *Mimosa pudica* L., *Terminalia chebula* and *Willughbeia edulis*.

Twenty-five species are mostly (> 50% of all citations) recorded in the lowlands; 16 plants are mostly mentioned in the Sen Monorom plateau; and 10 plants are mostly cited in the foothills. These results suggest that there is a variation in Bunong medicinal practice according the place, linked with the different type of ecosystem, hence the availability of some species.

3.3.1.5. Part of plants used. As already noted by Schmitt (2004), underground parts (rhizome and root) are the most highly used plant part (Fig. 3).

The pharmacopeias of several countries in Asia relied on remedies from underground parts (Flores, 1999). For example, in a compilation of traditional Chinese materials, more than one-quarter of the over 400 preparations are derived from roots and/or rhizomes (Bensky and Gamble, 1986). Indeed, roots/rhizomes possess the ability to synthetize a remarkable diversity of secondary metabolites (Bais et al., 2001; Flores, 1999). Therefore, high concentration of bioactive compounds in the underground part of some species could explain the use of this part of plants (Kunwar et al., 2006; Srithi et al., 2009).

Besides these pharmacological considerations, Bunong people believe that roots are inhabited by spirits, which is why the underground part of plants are often used by traditional healers during spiritual healing rites. Most well known preparations based upon underground ingredients (mainly rhizomes from the Zingiberaceae family) used in healing sessions are called *Kun*. *Kun* display different functions: to cast a spell on somebody, to ward off the curse, to protect people from witchcraft and also helps in treating various illnesses. For example, *Kun Yang Paa* (from *Eleutherine bulbosa*) is cultivated in paddy fields in order to keep the "rice spirit", to ensure a good harvest sufficient to feed up all the family. Plants used to prepare *kun* are kept far from villages, and only specialists can collect these plants, after asking permission. In

Table 2
Medicinal plants and medicinal mushroom used by Bunong people (only the species mentioned more than 10 times are shown).

Scientific name/Voucher specimen	Family name	Bunong name (a)	Source (b)	Part used	Uses	Total citations	Recipe (s) (c)
Acacia harmandiana (Pierre) Gagnep. (FC234)	Fabaceae	Marr	W	Bark	Stomachache	36	Bark decocted until reducing the volume of water by one third. Drunk as often as possible
Ageratum conyzoides L. (FC084)	Asteraceae	Ak Kra	W	Whole plant	Cold/Fever	12	Boiled the whole plant with *Blumea balsamifera* (leaves), *Chromolaena odorata* (leaves and root), *Citrus aurantiifolia* (leaves), *Cymbopogon nardus* (leaves) and *Zingiber montanum* (rhizome). Bath at dawn, or steam bath above the pot
				Root	Diarrhea	3	Root decocted. Drunk
				Leaves	Wound	2	Leaves crushed and applied directly on the wound
Anacardium occidentale L. (FC080)	Anacardiaceae	Dio/Svay Chanti	C	Bark	Diarrhea	14	Bark grilled on fire and boiled. Drunk until getting better
				Bark	Stomachache	1	Bark decocted *Cananga latifolia* (bark/root), *Ceiba pentandra* (bark), *Chrysophyllum cainito* (bark), *Psidium guajava* (bark) and Tamarindus indica (bark). Drunk
Ananas comosus (L.) Merr. (FC230)	Bromeliaceae	Prit Ngo/Ngo Bone	C	Leaves	Cold/Fever	5	Leaves decocted with *Ageratum conyzoides* (leaves), *Blumea balsamifera* (leaves), *Chromolaena odorata* (leaves), *Spondias pinnata* (leaves). Bath with the water or steam bath with the vapor
				Leaves	Post-partum (headache, reduce pain)	4	Leaves decocted. Drunk, or applied on the body, or steam bath
				Leaves/Root	Malaria	3	Leaves and root decocted, and mix with *Chromolaena odorata* (root), *Imperata cylindrica* (root) and *Tinospora crispa* (wood). Drunk
				Leaves	Headache	2	Leaves decocted. Make a steam bath with the vapor above the pot
				Leaves	Itching	1	Leaves soaked in water with *Ceiba pentandra* (bark) and *Mangifera indica* (bark). Bathed
				Leaves	Sprain	1	Leaves decocted and applied on the affected limb
Antidesma ghaesembilla Gaertn. (FC036)	Phyllanthaceae	Ak Kraegn/Kraegn lot	W	Bark/Wood	Diarrhea / Diarrhea and vomiting	11	Bark with wood decocted. Drunk
				Bark/Root/Wood	Post-partum	8	Grilled the bark with the wood on fire, then boiled it. Drunk*
				Bark	Stomachache	2	Bark grilled on fire, sprinkled salt, then boiled. Drunk
				Bark/Wood	Hemorrhoids	2	Bark with wood decocted with mix 1. Drunk
				Leaves	Fever	1	Leaves rubbed on the body (for children)
				Bark	Cough	1	Grilled the bark on fire, soaked in water. Drunk
				Bark	Wound (cleaning)	1	Bark decocted. Applied the water on the wound
				Bark	Repellent (insect)	1	Bark grated and mixed with water. Rubbed the preparation on the body
ApoRosa villosa (Lindl.) Baill. (FC087)	Phyllanthaceae	Ngong	W	Leaves	Diarrhea	5	Leaves decocted. Drunk or applied on the stomach
				Leaves/Wood	Post-partum (strengthen the body)	5	Boiled the wood and the leaves in water. Drunk or applied on the body*
				Leaves	Stomachache	3	Leaves decocted or macerated in alcohol. Drunk
				Leaves	Cold//Fever	2	Leaves decocted. Bath with the water or steam bath with the vapor above the pot
				Leaves	Headache	1	Leaves decocted with *Ageratum conyzoides* (leaves), *Blumea balsamifera* (leaves), *Chromolaena odorata* (leaves), *Melicope pteleifolia* (leaves). Steam bath above the pot
				Leaves	Sprain	1	Leaves decocted. Applied the water on the affected limb
Azadirachta indica A. Juss. (FC272)	Meliaceae	Ndaing	C	Bark/Leaves/Wood	Malaria	8	Boiled the leaves, the bark and the wood in water. Drunk
				Wood	Diarrhea	2	Wood decocted. Drunk
				Wood	Cough	1	Wood decocted. Drunk
Bambusa bambos (L.) Voss (FC099)	Poaceae	Kless	W	Bark	Wound	29	Young bark grated and applied as a poultice
				Leaves	Cold/Fever	2	Leaves decocted. Bath with the water or steam bath with the vapor above the pot
Bauhinia malabarica Roxb. (FC139)	Fabaceae	Tchung Po	W	Leaves	Sprain	16	Leaves decocted. Applied the leaves on the affected limb

				Leaves	Post-partum (general health, reduce pain)	8	Leaves decocted. Applied it on the body, or took a bath with the water, or a steam bath with the vapor[a]
				Leaves/Root	Backache	5	Leaves decocted. Applied the leaves on the body, or steam bath with the vapor Root decocted, drunk the water
				Leaves	Cold/Fever	4	Leaves decocted with *Ceiba pentandra* (leaves), *Chromolaena odorata* (leaves), *Melicope pteleifolia* (leaves) and *Mangifera indica* (bark) and *Musa* spp. (trunk). Bathed with the water or steam bath with the vapor
				Bark/Leaves	Diarrhea	2	Boiled the bark and the leaves in water. Drunk
				Leaves	Stomachache	2	Leaves decocted or macerated in alcohol. Drunk or applied on the stomach
Blumea balsamifera (L.) DC. (FC035)	Asteraceae	Gum	C	Leaves	Cold/Fever	30	Leaves decocted or soaked in water. Bath 3 times/day for 3 days, or steam bath 2 times/day until getting better. Use alone or with *Ageratum conyzoides* (whole plant), *Chromolaena odorata* (leaves), *Melicope pteleifolia* (leaves), *Zingiber montanum* (rhizome).
				Leaves	Headache	7	Leaves decocted. Use alone or with *Ageratum conyzoides* (whole plant), *Chromolaena odorata* (leaves), *Melicope pteleifolia* (leaves), *Zingiber montanum* (rhizome). Steam bath
				Leaves	Cramp	3	Leaves decocted. Steam bath or bath
				Leaves	Cough (serious)	1	Leaves decocted with *Chromolaena odorata* (leaves and root). Drunk
Cananga latifolia Finet & Gagnep. (FC054)	Annonaceae	Rata / Chkae sreng	W	Bark/Root/Wood	Stomachache	22	Root decocted or a piece of bark with wood decocted. Reduced the volume of water by one third. Drunk as often as possible until getting better. Used alone or mix with *Acacia harmandiana* (bark), *Leea* spp. (root) and *Uraria crinita/lagopodiodes* (root).
				Bark/Root/Wood	Post-partum (recovery)	13	Root decocted or a piece of bark with wood decocted. Reduced the volume of water by one third. Drunk as often as possible until getting better. Or root, wood and bark macerated in alcohol for few days and drunk 3 times/day after eating foods[a].
				Root	Wound	3	Root soaked in water with *Ampelocissus arachnoidea* (root) and *Spondias pinnata* (bark). Applied on the affected limb
				Bark/Root/Wood	Diarrhea/Bloody diarrhea	2	Root, wood and bark decocted. Drunk until getting better
				Root	Cough	2	Root decocted with *Ampelocissus arachnoidea* (root) and *Cheilocostus speciosus* (root). Drunk
				Bark/Wood	Cold/Fever	1	Root and bark decocted with *Ceiba pentandra* (bark/leaves). Drunk
				Wood	Malaria	1	Wood decocted. Drunk
				Wood	Backache	1	Wood decocted. Drunk
				Bark/Wood	Leucorrhea	1	Pieces of bark and wood decocted with mix 2. Reduced the volume of water by one third. Drunk as often as possible
				Wood	Liver disease	1	Bark decocted with *Bombax ceiba* (bark), *Hoya kerri* (leaves), *Hydnophytum formicarum* (tuber), *Leea* spp. (root) and *Terminalia triptera* (bark). Drunk
				Root	Constipation	1	Root decocted with *Ampelocissus arachnoidea* (root) and *Cananga latifolia* (root). Drunk
Careya arborea Roxb. (FC015)	Lecythidaceae	Teuk	W	Bark	Diarrhea / Bloody diarrhea	28	Grilled the bark on fire, then boiled it. Drunk as often as possible, until getting better
				Bark/Sap	Wound (cleaning)	8	Bark decocted and water applied on the wound. Or applied the sap on it
				Bark/Root	Stomachache	5	Bark and root decocted. Drunk
				Bark/Sap	Burn	3	Bark decocted and water applied on the wound. Or applied the sap on it
				Bark	Hemorrhoids	1	Bark decocted with mix 1. Drunk
				Bark	Itching	1	Bark decocted with *Spondias pinnata* (bark). Applied on the body
				Bark	Toothache	1	Bark chewed and kept in mouth
Ceiba pentandra (L.) Gaertn. (FC024)	Malvaceae	Ko	C	Bark/Leaves	Cold/Fever (adult and baby)	52	Leaves and/or bark decocted or soaked in water. Bath at dawn or steam bath 3 times/day. Use alone or mix with *Blumea*

Table 2 (*continued*)

Scientific name/Voucher specimen	Family name	Bunong name (a)	Source (b)	Part used	Uses	Total citations	Recipe (s) (c)
							balsamifera (leaves), *Chromolaena odorata* (leaves), *Cymbopogon nardus* (leaves) and *Musa* spp. (stem).
				Leaves	Cough	4	Leaves decocted or soaked in water. Bathed. Or leaves crushed and rubbed on the throat
				Bark	Stomachache	3	Grilled the bark on fire, mixed it with salt and boiled with *Anacardium occidentale* (bark), *Cananga latifolia* (bark/root), *Psidium guajava* (bark) and *Tamarindus indica* (bark). Drunk
				Leaves/Sap	Burn	2	Sap mixed with ashes, and applied on the burn. Leaves crushed with water and applied on the burn.
				Bark	Itching	1	Bark soaked in water with *Ananas comosus* (leaves) and *Mangifera indica* (bark). Bathed
Chromolaena odorata (L.) R. M. King & H. Rob. (FC090)	Asteraceae	Mangleun/Mrep	W	Leaves	Wound	150	Leaves crushed and mixed with urine. Applied the paste on the wound to stop the bleeding
				Leaves/Root	Cold/Fever	77	Leaves and root decocted or soaked in water. Bath or steam bath. Used alone or mixed with *Ageratum conyzoides* (leaves), *Blumea balsamifera* (leaves), *Ceiba pentandra* (leaves), *Melicope pteleifolia* (leaves) and *Mangifera indica* (bark).
				Leaves/Root	Malaria	10	Leaves and root decocted with *Ananas comosus* (leaves), *Dendrolobium lanceolatum* (leaves/root/wood) and *Tinospora crispa* (wood). Drunk
				Leaves/Root	Diarrhea	10	Leaves crushed with sugar and water. Or root decocted. Drunk.
				Leaves	Cough	6	Leaves decocted or crushed with water. Drunk or bathed
				Leaves/Root/Wood	Headache	6	Leaves and root and wood decocted. Steam bath
				Leaves	Cramp	2	Leaves boiled with *Citrus aurantiifolia* (leaves), *Coleus amboinicus* (leaves) and *Melicope pteleifolia* (leaves). Bath or steam bath
				Root	Stomachache	2	Root decocted with *Acacia harmandiana* (bark), *Cananga latifolia* (root) and *Dillenia ovata* (bark). Drunk
				Root	Painful menstruation	1	Root decocted. Drunk
Citrus × *aurantiifolia* (Christm.) Swingle (FC091)	Rutaceae	Kroyk Chma	C	Leaves	Cold/Fever	33	Leaves decocted or soaked in water. Steam bath or bath. Use alone or mix with *Blumea balsamifera* (leaves), *Chromolaena odorata* (leaves), *Cymbopogon nardus* (leaves) and *Zingiber montanum* (rhizome).
				Fruit	Cough	12	Fresh juice mixed with sugar and hot water. Drunk
				Leaves	Cramp	2	Leaves boiled with *Chromolaena odorata* (leaves), *Coleus amboinicus* (leaves) and *Melicope pteleifolia* (leaves). Steam bath
				Leaves	Headache	2	Leaves decocted. Steam bath
Coleus amboinicus Lour. (FC076)	Lamiaceae	Torechor	C	Leaves	Cold/Fever	3	Leaves pressed in order to get the liquid. Let the liquid outside for one night. Drunk at dawn
				Leaves	Burn	2	Leaves crushed with salt. Applied on the burn
				Leaves	Cough	2	Leaves pressed in order to get the liquid. Let the liquid outside for one night. Drunk at dawn
				Leaves	Wound	2	Leaves crushed. Applied on the wound
				Leaves	Cramp	1	Leaves decocted with *Chromolaena odorata* (leaves), *Citrus aurantiifolia* (leaves) and *Melicope pteleifolia* (leaves). Steam bath
Curcuma aromatica Salisb. (FC104)	Zingiberaceae	Kun Bao	C	Rhizome	Post-partum (reduce pain, food intolerance)	11	Rhizome eaten, or decocted and drunk
Curcuma longa L. (FC096)	Zingiberaceae	Rameut/Ramat	C	Rhizome	Stomachache	6	Rhizome dried, crushed with honey, *Hystrix brachyura* (dried stomach) and *Nycticebus* sp. (dried animal). Eaten the paste
				Rhizome	Wound	3	Rhizome crushed. Applied on the wound
				Rhizome	Post-partum (tonic, appetence, sleepiness)	1	Rhizome crushed with alcohol. Drunk after meals
				Rhizome	Sprain	1	Rhizome crushed with alcohol. Applied on the affected limb
Cymbopogon citratus (DC.) Stapf	Poaceae	Mlaing	C	Whole plant	Sprain	18	Whole plant decocted. Applied on the affected limb

(FC089)				Leaves	Cold/Fever	7	Leaves decocted. Steam bath or bath. Use alone or mix with *Blumea balsamifera* (leaves), *Chromolaena odorata* (leaves), *Citrus aurantiifolia* (leaves) and *Zingiber montanum* (rhizome).
				Leaves	Cramp	2	Leaves decocted. Drunk the water or made a steam bath with the vapor above the pot
				Leaves	Backache	1	Leaves decocted. Applied on the back
				Leaves	Headache	1	Leaves decocted with *Chromolaena odorata* (whole plant), *Diospyros ehretioides* (leaves) and *Zingiber montanum* (rhizome). Steam bath
Cymbopogon nardus (L.) Rendle (FC093)	Poaceae	Mlong Ho	C	Leaves	Cold/Fever	40	Leaves decocted and soaked in water. Steam bath or bath. Use alone or mix with *Blumea balsamifera* (leaves), *Chromolaena odorata* (leaves), *Citrus aurantiifolia* (leaves) and *Zingiber montanum* (rhizome).
				Leaves	Headache	2	Leaves decocted. Steam bath
				Leaves	Cramp	1	Leaves decocted. Steam bath or bath
				Leaves	Cough	1	Leaves decocted. Used the water for bathing or applying on the throat
Dalbergia nigrescens Kurz (FC085)	Fabaceae	Rapet	W	Bark/Wood	Cough/Sore throat	11	Bark grilled on fire and soaked in water. Or Wood decocted. Drunk
				Leaves	Burn	8	Leaves crushed with water and honey. Applied on the affected limb
				Bark	Diarrhea	2	Grilled the bark on fire. Crushed with water before eaten
				Bark	Cold/Fever	1	Bark soaked in water. Drunk
				Leaves	Wound	1	Leaves crushed and applied on the wound
Dalbergia stipulacea Roxb. (FC157)	Fabaceae	Oundreng/Tchaeh Kon	W	Resin	Backache	13	Collected the resin made by insects on the bark. Boiled the resin in water. Drunk
				Resin	Sprain	4	Collected the resin made by insects on the bark. Boiled the resin in water. Drunk
				Resin	Rheumatism	3	Collected the resin made by insects on the bark. Boiled the resin in water. Drunk
				Resin	Contusion	1	Collected the resin made by insects on the bark. Boiled the resin in water. Drunk
				Resin	Diarrhea	1	Collected the resin made by insects on the bark. Boiled the resin in water. Drunk
				Resin	Insect bite	1	Collected the resin made by insects on the bark. Applied on the affected body's part
Dendrolobium lanceolatum (Dunn) Schnidl. (FC175)	Fabaceae	Rangay	W	Leaves/Root/Wood	Malaria	15	Leaves, root and wood decocted, or root decocted only. Drunk
				Root	Cold/Fever	4	Root decocted. Drunk, bathed or took a steam bath
				Whole plant	Loss of appetite	1	Whole plant decocted with *Gardenia obtusifolia* (bark/wood) and *Ochna integerrima* (wood). Drunk
Dialium cochinchinense Pierre (FC245)	Fabaceae	Kralan	W	Bark	Diarrhea / Bloody diarrhea	15	Bark decocted or macerated in alcohol. Drunk
				Bark/Wood	Post-partum (blood enhancer)	4	Pieces of bark with wood decocted or macerated in alcohol. Drunk[a]
				Bark	Stomachache	2	Bark decocted. Drunk
Dillenia hookeri Pierre (FC068)	Dilleniaceae	Mloo Roy/Mloo Ché	W	Root	Post-partum (galactagogue, tonic)	11	Root decocted or macerated in alcohol. Drunk[a]
				Root	Diarrhea	1	Root decocted with *Bridelia tomentosa* (root). Drunk
				Root	Hemorrhoids	1	Root decocted with mix 1. Drunk
				Root	Malaria	1	Root decocted with *Chromolaena odorata* (root), *Chrysopogon aciculatus* (root), *Dendrolobium lanceolatum* (root) and *Imperata cylindrica* (root). Drunk
Dillenia ovata Wall. (FC013)	Dilleniaceae	Mloo/Slotoap	W	Bark	Stomachache	5	Bark decocted with *Acacia harmandiana* (bark), *Cananga latifolia* (root), *Chromolaena odorata* (root) and *Willughbeia edulis* (wood). Drunk
				Bark	Diarrhea	3	Bark decocted. Drunk
				Bark	Cough	1	Bark decocted. Drunk
				Root/Bark	Hemorrhoids	1	Bark and root decocted with mix 1. Drunk
				Leaves/Root	Hypertension	1	Leaves and root decocted with *Phyllanthus emblica* (bark/fruit).

Table 2 (*continued*)

Scientific name/Voucher specimen	Family name	Bunong name (a)	Source (b)	Part used	Uses	Total citations	Recipe (s) (c)
							Drunk
				Bark	Wound	1	Bark decocted. Applied the water for cleaning the wound
Diospyros ehretioides Wall. ex A. DC. (FC064)	Ebenaceae	Paum	W	Bark/Root/Wood	Post-partum (warming effect, food supplement)	16	Bark, root and wood decocted or macerated in alcohol. Drunk*
				Leaves	Headache	1	Leaves decocted with *Chromolaena odorata* (whole plant), *Cymbopogon citratus* (leaves) and *Zingiber montanum* (rhizome). Steam bath
				Root	Malaria	1	Root decocted. Drunk (in second step, if the decoction of *Dendrolobium lanceolatum* don't treat the patient)
Dipterocarpus intricatus Dyer (FC098)	Dipterocarpaceae	Paing	W	Resin	Diarrhea / Diarrhea and vomiting	18	Resin drunk
				Resin	Ring worm	1	Applied on the skin
				Resin	Stomachache	1	Resin drunk
Dipterocarpus obtusifolius Teijsm. ex Miq. var *obtusifolius* (FC261)	Dipterocarpaceae	Paing	W	Resin	Diarrhea / Diarrhea and vomiting	18	Resin drunk
				Resin	Ring worm	1	Applied on the skin
				Resin	Stomachache	1	Resin drunk
Dunbaria bella Prain (FC209)	Fabaceae	Damrey Pram Do/ Neha Paeh	W	Root	Post-partum	11	Root decocted or macerated in alcohol. Drunk*
Euonymus cochinchinensis Pierre (FC196)	Celastraceae	Ko Mouy/Bof	W	Leaves/Root/ Wood	Post-partum (galactagogue, tonic, warming effect)	45	Leaves, root and wood decocted or macerated in alcohol. Drunk*
				Wood	Backache	1	Wood macerated in alcohol. Drunk
Flacourtia indica (Burm. f.) Merr. (FC004)	Salicaceae	Kop	W	Root/Wood	Post-partum (galactagogue, blood enhancer)	24	Root and wood decocted. Drunk*
				Bark/Wood	Tonic	2	Pieces of bark with wood decocted with *Mitragyna speciosa* (bark/wood), *Piper lolot* (leaves), *Salacia chinensis* (bark/Wood) and *Vitex peduncularis* (bark/wood). Drunk
				Root	Diarrhea / Diarrhea and vomiting	1	Root decocted with *Bombax ceiba* (root). Bathed
Helicteres angustifolia L. (FC016)	Malvaceae	Tan Yang/Keté Pray	W	Root	Diarrhea / Bloody diarrhea	38	Root decocted. Drunk
				Whole plant	Malaria	2	Whole plant decocted with *Ananas comosus* (leaves), *Chromolaena odorata* (leaves) and *Oroxylum indicum* (bark/leaves). Drunk
				Root	Constipation	1	Root decocted. Drunk
				Wood	Headache	1	Wood decocted with *Strychnos nux-blanda* (bark). Drunk
				Root	Intestine pain	1	Root decocted. Drunk
				Root	Stomachache	1	Root decocted. Drunk
				Root	Urinary retention	1	Root decocted. Drunk
Hiptage benghalensis (L.) Kurz (FC160)	Malpighiaceae	Rova Kong	W	Root/Wood	Post-partum (appetence, blood purifier)	33	Root and wood decocted or macerated in alcohol. Drunk*
				Bark/Root/Wood	Diarrhea	3	Bark, root and wood decocted. Drunk
				Root	Loss of appetite	1	Root decocted. Drunk
Hydnophytum formicarum Jack (FC109)	Rubiaceae	Pouk pou	W	Whole plant	Stomachache	9	Whole plant decocted. Drunk
				Whole plant	Post-partum	4	Whole plant decocted. Drunk*
				Whole plant	Cough	3	Whole plant decocted, drunk. Or whole plant soaked in water, bathed.
				Whole plant	Backache	1	Whole plant decocted or macerated in alcohol, with *Smilax cambodiana* (root). Drunk
				Whole plant	Cold/Fever	1	Whole plant soaked in water. Bathed
				Whole plant	Headache	1	Grilled the whole plant on fire. Crushed it and applied the pieces on the head
				Whole plant	Intestine pain	1	Whole plant decocted with *Phellinus rimosus* (whole mushroom) and honey. Drunk
				Whole plant	Liver disease	1	Leaves decocted with *Bombax ceiba* (bark), *Cananga latifolia* (wood), *Hoya kerrii* (leaves), *Leea* spp. (root) and *Terminalia*

							triptera (bark). Drunk
				Whole plant	Malaria	1	Whole plant soaked in water. Bathed
Imperata cylindrica (L.) P. Beauv. (FC145)	Poaceae	Jaa	W	Root	Malaria	4	Root decocted with *Ananas comosus* (leaves/root), *Chromolaena odorata* (leaves/root) and *Tinospora crispa* (wood). Drunk or bathed
				Root	Cold/Fever	2	Root decocted with *Chromolaena odorata* (root), *Mimosa pudica* (root) and *Sida acuta* subsp. *acuta* (root). Drunk
				Root	Post-partum (reduce pain)	2	Root decocted. Steam bath, bathed or drunk[a]
				Root	Urinary retention	2	Root decocted with *Cheilocostus speciosus* (root), *Saccharum officinarum* (bark) and *Zea mays* (cornsilk). Drunk
				Root	Cough	1	Root decocted. Drunk
				Root	Diarrhea	1	Root decocted with *Lagerstroemia cochinchinensis* (bark). Drunk
Irvingia malayana Oliver ex Bennett (FC154)	Irvingiaceae	Yarr/Niharr	W	Bark/Wood	Cough, sore throat	15	Grilled the bark on fire, then added salt and boiled in water. Or wood decocted. Drunk
				Wood	Headache	1	Wood decocted. Drunk
				Wood	Malaria	1	Wood decocted. Drunk
Jatropha curcas L. (FC078)	Euphorbiaceae	Tong	C	Bark/Resin	Burn	14	Grilled the bark on fire and crushed it. Applied on the burn. Or applied the resin directly on the burn
Kalanchoe pinnata (Lam.) Pers. (FC018)	Crassulaceae	Kun Klet	C	Leaves	Sprain	96	Leaves decocted or grilled on fire. Applied the leaves on the affected limb for few hours
				Leaves	Backache	10	Leaves decocted or grilled on fire. Applied the leaves on the back for few hours
Lagerstroemia cochinchinensis Pierre (FC100)	Lythraceae	Kouar	W	Bark	Diarrhea	28	Bark decocted, or grilled on fire and soaked in water, or bark soaked in water. Drunk
				Bark	Cough	2	Bark grated. Chewed
				Bark	Stomachache	2	Bark decocted. Drunk
				Bark	Wound	2	Bark grated. Applied on the wound
				Bark	Burn	1	Bark grated. Applied on the burn
				Wood	Post-partum (tonic)	1	Wood decocted. Drunk[a]
Leea aequata L. (FC183)	Vitaceae	Péang Kra/To Rokon/Dal lo	W	Root	Stomachache	15	Root decocted. Drunk. Used alone or mixed with *Acacia harmandiana* (bark), *Cananga latifolia* (bark/root/wood), *Hydnophytum formicarum* (whole plant), *Uraria* spp. (root) and *Zizyphus cambodiana* (fruit)
				Root	Backache	1	Root decocted. Drunk
				Root	Cough	1	Root decocted. Drunk
				Root	Leucorrhea	1	Root decocted with mix 2. Drunk
				Root	Liver disease	1	Root decocted with *Bombax ceiba* (bark), *Cananga latifolia* (wood), *Hoya kerrii* (leaves), *Hydnophytum formicarum* (whole plant) and *Terminalia triptera* (bark).
				Root	Hemorrhoids	1	Root decocted with mix 1. Drunk
Leea rubra Blume ex Spreng. (FC182)	Vitaceae	Péang Kra/To Rokon/Dal lo	W	Root	Stomachache	15	Root decocted. Drunk. Used alone or mixed with *Acacia harmandiana* (bark), *Cananga latifolia* (bark/root/wood), *Hydnophytum formicarum* (whole plant), *Uraria* spp. (root) and *Zizyphus cambodiana* (fruit)
				Root	Backache	1	Root decocted. Drunk
				Root	Cough	1	Root decocted. Drunk
				Root	Leucorrhea	1	Root decocted with mix 2. Drunk
				Root	Liver disease	1	Root decocted with *Bombax ceiba* (bark), *Cananga latifolia* (wood), *Hoya kerrii* (leaves), *Hydnophytum formicarum* (whole plant) and *Terminalia triptera* (bark).
				Root	Hemorrhoids	1	Root decocted with mix 1. Drunk
Leea thorelii Gagnep. (FC026)	Vitaceae	Péang Kra / To Rokon / Dal lo	W	Root	Stomachache	15	Root decocted. Drunk. Used alone or mixed with *Acacia harmandiana* (bark), *Cananga latifolia* (bark/root/wood), *Hydnophytum formicarum* (whole plant), *Uraria* spp. (root) and *Zizyphus cambodiana* (fruit)
				Root	Backache	1	Root decocted. Drunk
				Root	Cough	1	Root decocted. Drunk
				Root	Leucorrhea	1	Root decocted with mix 2. Drunk
				Root	Liver disease	1	Root decocted with *Bombax ceiba* (bark), *Cananga latifolia* (wood), *Hoya kerrii* (leaves), *Hydnophytum formicarum* (whole

Table 2 (*continued*)

Scientific name/Voucher specimen	Family name	Bunong name (a)	Source (b)	Part used	Uses	Total citations	Recipe (s) (c)
							plant) and *Terminalia triptera* (bark).
				Root	Hemorrhoids	1	Root decocted with mix 1. Drunk
Luffa aegyptiaca Mill. (FC140)	Cucurbitaceae	Tchan	C	Leaves	Burn	19	Leaves crushed with *Gossypium barbadense* (leaves). Applied on the burn
Mangifera indica L. (FC162)	Anacardiaceae	Svay	C	Bark	Cold/Fever	8	Bark decocted or soaked in water with *Bauhinia malabarica* (leaves), *Ceiba pentandra* (leaves), *Chromolaena odorata* (leaves), *Melicope pteleifolia* (leaves), *Mangifera indica* (bark) and *Musa* spp. (trunk). Bathed
				Bark	Diarrhea	2	Bark decocted. Drunk
				Bark	Itching	2	Bark soaked in water with *Ananas comosus* (leaves) and *Ceiba pentandra* (bark). Bathed
Melicope pteleifolia (Champ. ex Benth.) T. G. Hartley. (FC086)	Rutaceae	Nahing	W	Leaves	Cold/Fever	21	Leaves decocted or soaked in water. Drunk the remedy, bathed or steam bath. Or fresh leaves rubbed on the body. Used alone or mixed with *Blumea balsamifera* (leaves), *Chromolaena odorata* (leaves), *Cymbopogon nardus* (leaves), *Mimosa pudica* (whole plant).
				Leaves	Repellent (insect/leeches)	6	Fresh leaves rubbed on the skin.
				Leaves	Cough	4	Leaves decocted. Steam bath, bath or applied directly on the throat and the body
				Leaves	Cramp	2	Leaves decocted with *Chromolaena odorata* (leaves), *Coleus amboinicus* (leaves) and *Citrus aurantiifolia* (leaves). Steam bath or bath
				Leaves	Headache	2	Leaves decocted. Steam bath or bath
Mimosa pudica L. (FC071)	Fabaceae	Lok Krap/Lok Gnop/Lok Ambel	W	Whole plant	Post-partum (reduce pain, hemorrhage)	4	Whole plant decocted. Drunk, bathed or steam bath*
				Root	Cold/Fever	2	Root decocted with *Ageratum conyzoides* (whole plant), *Blumea balsamifera* (leaves), *Chromolaena odorata* (leaves), *Citrus aurantiifolia* (leaves), *Cymbopogon nardus* (leaves) and *Melicope pteleifolia* (leaves). Bath or steam bath
				Whole plant	Stomachache	2	Whole plant decocted with *Acacia harmandiana* (bark), *Cananga latifolia* (root), *Leea* spp. (root), *Pouzolzia zeylanica* (whole plant). Drunk
				Whole plant	Cough	1	Whole plant decocted. Drunk
				Whole plant	Headache	1	Whole plant decocted with *Bauhinia malabarica* (leaves), *Blumea balsamifera* (leaves), *Lygodium microphyllum* (whole plant), *Rubus annamensis* (leaves) and *Solanum sakhanii* (whole plant). Steam bath
Musa spp. L. (FC094)	Musaceae	Prit Kof/Prit Drone	C	Fruit (immature)	Stomachache	26	Fruit eaten. Or fruits decocted, drunk.
				Trunk	Cold/Fever	21	Trunk minced, then decocted or soaked in water with *Bauhinia malabarica* (leaves), *Ceiba pentandra* (leaves), *Chromolaena odorata* (leaves), *Melicope pteleifolia* (leaves) and *Mangifera indica* (bark). Steam bath or bath
				Resin	Burn	1	Resin applied directly on the burn
				Trunk	Cough	1	Trunk minced, then soaked in water with *Ceiba pentandra* (leaves). Bathed
				Flower	Diarrhea	1	Flower crushed with *Calamus palustris* (stem) and weaver ants. Eaten
				Trunk	Malaria	1	Trunk decocted with *Ananas comosus* (leaves). Steam bath or bath
Nicotiana tabacum L. (FC111)	Solanaceae	Mhat	C	Leaves	Wound (hemostatic)	58	Leaves dried (or used fresh), crushed with water. Applied on the wound
Ochna integerrima (Lour.) Merr. (FC038)	Ochnaceae	KonKie / Angkie	W	Bark/Root/Wood	Post-partum (tonic)	15	Bark, root and wood decocted or macerated in alcohol. Drunk for 2–3 months*
				Root/Wood	Tonic	2	Root and wood decocted. Drunk
				Wood	Loss of appetite	1	Wood decocted with *Dendrolobium lanceolatum* (whole plant)

							and *Gardenia obtusifolia* (bark/wood). Drunk
Oroxylum indicum (L.) Kurz (FC142)	Bignoniaceae	Pou Long	W	Bark	Burn	14	Bark grated with water. Applied on the burn
				Bark/Root	Cough	4	Bark and root decocted. Drunk
				Bark/Wood	Cold/Fever	2	Pieces of bark with wood decocted with *Dillenia pentagyna* (bark/wood). Drunk
				Bark/Leaves/Root	Malaria	2	Bark and leaves decocted, for a steam bath. Or root decocted, drunk.
				Root	Leucorrhea	1	Root decocted with mix 2. Drunk
				Fruit	Post-partum	1	Fruit decocted or macerated in alcohol. Drunk*
Phellinus rimosus (Berk.) Pilát (FC181)	Hymenochaetaceae	Tchet Rapeh/Tchet Marr	W	Whole mushroom	Backache	2	Whole mushroom decocted or macerated in alcohol. Drunk
				Whole mushroom	Post-partum (warming effect)	2	Whole mushroom grated in alcohol. Drunk
				Whole mushroom	Stomachache	2	Whole mushroom decocted with *Hydnophytum formicarum* (whole pant) and honey. Drunk
				Whole mushroom	Tonic	2	Whole mushroom decocted. Drunk
				Whole mushroom	Diarrhea	1	Whole mushroom decocted. Drunk
				Whole mushroom	Intestine pain	1	Whole mushroom decocted with *Hydnophytum formicarum* (whole pant) and honey. Drunk
				Whole mushroom	Wound	1	Whole mushroom chewed. Spit on the wound
Piper betle L. (FC141)	Piperaceae	Mlou	C	Leaves/Root	Sprain	8	Leaves and root decocted or soaked in water, with *Areca catechu* (seed) and *Scoparia dulcis* (whole plant). Applied on the affected limb
				Leaves	Burn	6	Leaves crushed with salt and water or buffalo mess. Applied on the burn
				Leaves	Backache	1	Leaves crushed with water and *Heliotropium indicum* (root). Applied on the back
				Leaves	Constipation	1	Leaves crushed with water. Eaten
Psidium guajava L. (FC017)	Myrtaceae	Trobaek	C	Bark/Leaves/Wood	Diarrhea	86	Pieces of bark with wood grilled on fire and decocted, drunk. Or leaves crushed with sugar and water, eaten.
				Bark/Leaves	Stomachache	4	Bark decocted, drunk. Or leaves crushed with salt, eaten
				Leaves	Post-partum (diarrhea prevention)	1	Leaves decocted with *ApoRosa villosa* (leaves), drunk and massage on the body for 1 or 2 months
				Leaves	Wound	1	Leaves crushed. Applied on the wound
Salacia chinensis L. (FC003)	Celastraceae	Pouk Rolan/Moy Roy Slotoap	W	Wood	Post-partum (hemorrhage)	8	Wood decocted or macerated in alcohol. Drunk*
				Wood	Stomachache	1	Wood decocted with *Cananga latifolia* (bark/root/wood), *Leea* spp. (root), *Pouzolzia hirta* (whole plant) and *Uraria crinita/lagopodioides* (root). Drunk
				Bark/Wood	Tonic	1	Pieces of bark with wood decocted with *Flacourtia indica* (bark/wood), *Mitragyna speciosa* (bark/wood), *Piper lolot* (leaves) and *Vitex peduncularis* (bark/wood). Drunk
Scoparia dulcis L. (FC048)	Plantaginaceae	Paupaegn ka/Pouk Ro Houay	W	Whole plant	Sprain	11	Whole plant decocted, drunk. Or whole plant decocted or soaked in water, with *Areca catechu* (seed) and *Piper betle* (leaves/root). Applied on the affected limb
				Root	Backache	1	Root chewed. Spit on the body and massage
				Whole plant	Stomachache	1	Whole plant decocted with *Acacia harmandiana* (bark), *Cananga latifolia* (root), *Cyperus rotundus* (root), *Mimosa pudica* (whole plant) and *Pouzolzia zeylanica* (whole plant). Drunk
				Whole plant	Swelling	1	Whole plant decocted. Applied the water on the affected limb
Shorea obtusa Wall. (FC237)	Dipterocarpaceae	Pot	W	Bark/Wood	Diarrhea	17	Pieces of bark with wood grilled on fire, then soaked in water. Or bark and wood decocted. Drunk
				Bark/Root	Stomachache	2	Bark and root decocted with *Careya arborea* (root) and *Xylia xylocarpa* (root). Drunk
Smilax cambodiana Gagnep. (FC136)	Smilacaceae	Dam Djang	W	Root	Post-partum (warming effect)	8	Root decocted or macerated in alcohol. Drunk*
				Root	Stomachache	3	Root decocted with *Cananga latifolia* (bark/root), or with *Leea*

Table 2 (*continued*)

Scientific name/Voucher specimen	Family name	Bunong name (a)	Source (b)	Part used	Uses	Total citations	Recipe (s) (c)
							spp. (root). Drunk
				Root	Backache	2	Root decocted or macerated in alcohol with *Hydnophytum formicarum* (whole plant). Drunk
				Root	Headache	2	Root decocted with *Blumea balsamifera* (leaves) and *Zingiber montanum* (rhizome). Steam bath
				Root	Diarrhea / Blood diarrhea	1	Root decocted. Drunk
Spondias pinnata (L. f.) Kurz (FC108)	Anacardiaceae	Ramouane	W	Bark	Wound (cleaning)	9	Bark decocted. Applied the water on the wound
				Leaves	Sprain	3	Leaves decocted. Applied the leaves on the affected limb and massage
				Leaves	Backache (painkiller)	2	Leaves decocted or grilled on fire. Applied the leaves on the affected limb and massage, or steam bath with the leaves decocted
				Leaves	Cold/Fever	2	Leaves decocted with *Ageratum conyzoides* (leaves), *Ananas comosus* (leaves), *Blumea balsamifera* (leaves), *Chromolaena odorata* (leaves). Steam bath or bath
				Bark	Diarrhea / Bloody diarrhea	2	Bark decocted. Drunk
				Leaves	Stomachache	2	Leaves decocted with *ApoRosa villosa* (leaves). Drunk
				Bark	Itching	1	Bark decocted with *Careya arborea* (bark). Applied on the body
				Leaves	Post-partum (reduce pain)	1	Leaves decocted with *Bauhinia malabarica* (leaves), *Imperata cylindrica* (root) and *Mimosa pudica* (whole plant). Bathed, drunk, or steam bath for 2 weeks
Strychnos nux-blanda A. W. Hill (FC158)	Loganiaceae	Rovak	W	Bark	Wound (cleaning)	9	Bark grated, applied pieces of bark on the wound. Or bark decocted, applied the water
				Bark	Headache	2	Bark decocted, drunk. Or bark soaked in water and applied on the forehead
				Wood	Cough	1	Wood decocted. Drunk
				Bark	Post-partum	1	Bark decocted. Drunk[a]
Taxillus chinensis (DC.) Danser	Loranthaceae	Totot/Kae Tot	W	Leaves/Wood	Headache	7	Wood and leaves decocted or crushed in water. Steam bath, bath or applied on the head
				Leaves/Wood	Cough	2	Wood and leaves decocted with *Ziziphus cambodiana* (Leaves/Wood). Drunk
				Whole plant	Stomachache	1	Whole plant decocted. Drunk
Terminalia bialata (Roxb.) Steud. (FC110)	Combretaceae	Riya	W	Bark	Diarrhea / Bloody diarrhea	17	Bark decocted. Drunk
				Bark	Cold/Fever	3	Bark decocted or soaked in water with *Ageratum conyzoides* (leaves), *Musa* spp. (trunk) and *Zingiber montanum* (rhizome). Drunk or bathed or steam bath
				Bark	Cough	1	Bark decocted. Drunk
				Bark	Jaundice	1	Bark decocted. Drunk
				Bark/Wood	Post-partum	1	Pieces of bark with wood decocted or macerated in alcohol. Drunk[a]
				Bark	Stomachache	1	Grilled the bark on fire, then boiled it. Drunk
Terminalia chebula Retz. (FC059)	Combretaceae	Leo	W	Bark/Leaves	Burn	3	Bark decocted, or leaves crushed in water. Applied the water on the burn
				Bark/Fruit/Leaves/Wood	Diarrhea	3	Bark or wood decocted, drunk. Or fruits eaten. Or young leaves eaten
				Bark	Wound	2	Bark decocted. Applied the water on the burn
				Fruit	Backache	1	Fruits crushed in alcohol. Drunk
				Fruit	Hemorrhoids	1	Fruits decocted with mix 1. Drunk
				Bark	Malaria	1	Bark decocted. Drunk
				Bark	Stomachache	1	Bark decocted. Drunk
Uraria crinita (L.) Desv. ex DC. (FC146)	Fabaceae	Tchiang Prok	W	Root	Stomachache	16	Root decocted. Drunk. Used alone or mixed with *Acacia harmandiana* (bark), *Cananga latifolia* (bark/root/wood), *Hydnophytum formicarum* (whole plant), *Leea* spp. and *Ziziphus cambodiana* (fruit)

Uraria lagopodiodes (L.) DC. (FC077)	Fabaceae	Tchiang Prok	W	Root	Stomachache	16	Root decocted. Drunk. Used alone or mixed with *Acacia harmandiana* (bark), *Cananga latifolia* (bark/root/wood), *Hydnophytum formicarum* (whole plant), *Leea* spp. and *Ziziphus cambodiana* (fruit)
Willughbeia edulis Roxb. (FC122)	Apocynaceae	Play Tchi/Couy	W	Wood	Post-partum (galactagogue, blood purifier)	13	Wood decocted. Drunk[a]
				Root	Stomachache	4	Root decocted with *Ampelocissus arachnoidea* (root), *Cananga latifolia* (root), *Cinnamomum iners* (bark) and *Leea* spp. (root). Drunk
				Bark/Wood	Diarrhea	1	Pieces of bark with wood decocted. Drunk
Xylia xylocarpa Taub. (FC031)	Fabaceae	Rapeh	W	Bark/Fruit/Wood	Diarrhea/Bloody diarrhea	16	Pieces of bark, or wood, or fruits decocted, or mixed together. Drunk
				Bark/Wood	Post-partum (blood purifier)	8	Pieces of bark with wood decocted. Drunk[a]
				Bark/Wood	Wound (cleaning)	4	Pieces of bark with wood decocted. Applied the liquid on the wound
				Bark/Root	Stomachache	2	Bark and root decocted with *Careya arborea* (root) and *Shorea obtusa* (root). Drunk
				Wood	Backache	1	Wood decocted with *Aganonerion polymorphum* (whole plant) and *Phellinus rimosus* (whole plant). Drunk
				Bark/Wood	Insect bite	1	Pieces of bark with wood decocted. Drunk
				Bark	Sprain	1	Bark decocted. Drunk or applied on the affected limb
Zingiber montanum (J. Koenig ex Retz.) Link ex A. Dietr. (FC034)	Zingiberaceae	Cha Rannay/Tiyoh	C	Rhizome	Cold, fever (adult)	43	Rhizome decocted (for example with) *Blumea balsamifera* (leaves), *Chromolaena odorata* (leaves), *Cymbopogon nardus* (leaves), *Melicope pteleifolia* (leaves). Steam bath or bath
				Rhizome	Sprain	41	Rhizome crushed in alcohol. Applied the liquid on the affected limb
				Rhizome	Backache	7	Rhizome crushed in alcohol, applied on the back. Or rhizome decocted, drunk
				Rhizome	Headache	7	Rhizome decocted. Steam bath
				Rhizome	Post-partum (warming effect)	4	Rhizome macerated in alcohol with *Nycticebus* spp. (whole animal) and honey, drunk. Or rhizome decocted alone and applied on the body
				Rhizome	Cough	3	Rhizome eaten
				Rhizome	Cold/Fever (baby)	2	Rhizome crushed in water. Applied on the head
				Rhizome	Wound	2	Rhizome crushed or decocted. Applied the liquid on the wound
				Rhizome	Body pain	1	Rhizome crushed in water. Applied on the body
				Rhizome	Contusion	1	Rhizome crushed in alcohol. Applied on the affected limb
				Rhizome	Stomachache	1	Rhizome macerated in alcohol (for 1–2 h) with *ApoRosa villosa* (leaves) and *Bauhinia malabarica* (leaves). Drunk
Zingiber officinale Roscoe (FC097)	Zingiberaceae	Tchaa	C	Rhizome	Sprain	16	Rhizome crushed and applied 2–3 times per day. Or rhizome decocted, applied the water
				Rhizome	Wound (cleaning)	6	Rhizome decocted. Applied the liquid on the wound. Or rhizome crushed and applied with bandage
				Rhizome	Rheumatism	4	Rhizome decocted or macerated in alcohol for 1–2 h. Drunk and massage
				Rhizome	Diarrhea	1	Rhizome crushed with salt, then decocted. Drunk
Ziziphus cambodiana Pierre (FC005)	Rhamnaceae	Rangong	W	Fruit	Stomachache	22	Fruits eaten. Or fruits decocted alone or mixed with *Acacia harmandiana* (bark), *Cananga latifolia* (bark/root/wood), *Hydnophytum forimcarum* (whole plant), *Leea* spp. (root) and *Uraria crinita/lagopodiodes* (root), drunk.
				Bark/Fruit/Root	Diarrhea / Bloody diarrhea	5	Bark or fruits or root decocted. Drunk
				Leaves/Wood	Cough	1	Leaves and wood decocted with *Taxillus chinensis* (leaves/wood). Drunk
				Bark/Wood	Leucorrhea	1	Pieces of bark with wood decocted with mix 2. Drunk
				Root	Post-partum (food intolerance)	1	Root decocted. Drunk[a]

(a) Here are listed the name of each plants without the prefix used to indicate the type of plants: Tarm for a tree, a shrub or a plant. Ratao for a liana
(b) W = Wild; C = Cultivated
(c) In general, when they decoct plants, they reduce by a third. And they drink as often as possible (when they are thirsty).

For bathing, they take it at dawn for cold/fever, 2 or 3 times a day for 4 days.
For steam bath, they do it above the boiling pot and cover themselves with a towel until getting entirely wet.
Mix 1 (for hemorrhoids)=*Albizia lebbekoides* (bark/wood), *Ampelocissus arachnoidea* (root), *Amphineurion marginatum* (leaves), *Antidesma ghaesembilla* (bark/wood), *Careya arborea* (bark), *Combretum quadrangulare* (root), *Dillenia hookeri* (root), *Dillenia ovata* (bark/root), *Euphorbia thymifolia* (whole plant), *Ficus hispida* (fruit), *Leea* spp. (root), *Schleicheria oleosa* (bark), *Sindora siamensis* (bark/wood), *Spatholobus parviflorus* (leaves/wood), *Terminalia chebula* (fruit).
Mix 2 (for leucorrhea)=*Amphineurion marginatum* (bark/wood), *Cananga latifolia* (bark/wood), *Harrisiona perforata* (bark/wood), *Hoya kerrii* (leaves/wood), *Leea* spp. (root), *Oroxylum indicum* (root), *Polyalthia cerasoides* (bark/wood), *Uvaria rufa* (bark/wood), *Ziziphus cambodiana* (bark/wood), *Ziziphus oenopolia* (bark/wood).
[a] Most of the plants for post-partum are combined together in order to enhance the efficacy of the remedy. The patient have to drink the remedy as often as possible for 2–6 months.

Laos, Vidal (1958) reported similar practices, also using Zingiberaceae rhizome. Moreover, the spiritual importance of roots has been also described in an ethnobotanical study in Mozambique (Bruschi et al., 2011).

In this study the most cited underground parts belong to the Fabaceae family (i.e: *Dendrolobium lanceolatum* (Dunn)) Schindl., *Uraria* spp., *Mimosa pudica*), together with Zingiberaceae (e.g.: *Zingiber montanum*, *Zingiber officinale* Roscoe, *Curcuma longa* L.).

3.3.1.6. Formulation, mode of preparation and mode of administration of remedies. Most of the preparations used by the Bunong community are multi-ingredient recipes. From our data we observed that even if some villagers might use few single ingredient recipe as well as more complex preparations, all traditional healers prefer to use multi-ingredient recipes, such as formulations containing ten or more medicinal plants. In our survey, 85 multi-ingredient recipes, made out of 145 species (corresponding to 68% of the total number of plants collected) were described thanks to the first methodology. As an example, urinary retention was reported to be treated by a mixture of 5 plant species, all of them with antipyretic and diuretic effect (Schmitt, 2004): *Cheilocostus speciosus* (J.Koenig) C.D.Specht, *Eleusine indica* (L.) Gaertn., *Imperata cylindrica* (L.) Raeusch., *Saccharum officinarum* L. and *Zea mays* L.

The multi-herbal drug strategy is not only the fact of Bunong people, and it is widely described in Traditional Chinese Medicine and Ayurvedic medicine (Wang et al., 2005; Wang et al., 2008). Indeed, many diseases have a multi-causal etiology and a complex pathophysiology. Combination of herbal medicine can treat diseases more effectively than single herbal medicine, by having synergistic effects (Wagner and Ulrich-Merzenich, 2009). The multi-target therapy has already been proven to be effective in various phytopreparations such as Iberogast® which is composed of nine plant extracts and is employed for the treatment of functional intestinal disorders (Wagner, 2006). Moreover, crude extracts from plants contain various compounds which can also display synergistic effect, and so can be more effective than isolated constituents (Rasoanaivo et al., 2011).

In our study, decoction was the most cited method of plant preparation (68% of the plants). Plant decoctions are administered orally and also in form of steam baths, inhalations, and baths.

To a lesser degree (11%), plants are crushed, with the help of some water (*Christia vespertilionis* (L.f.) Bakh.f., root/wood, fever), urine (*Chromolaena odorata*, leaves, wound), honey (*Curcuma longa*, rhizome, stomachache), salt (*Psidium guajava* L., leaves, stomachache), or alcohol (*Zingiber montanum*, root, sprain). Then the resulting paste is applied locally in case of injuries (wound, sprain) or eaten in case of gastrointestinal disorders.

Plant parts can also be macerated in alcohol (9%), soaked in cold water (7%), chewed (3%), pressed in order to get the juice (1%) or used fresh in their crude form (4%).

Most of the plants are administered orally (59% species), through local application (22%), in form of a wash (10%) and steam bath (9%). Topical application is mainly used in the case of eye problems, as this is the case for *Aloe vera* (L.) Burm.f. gel applied directly, or for toothache. In that case *Caesalpinia digyna* Rottler root is chewed. Mosquito repellent plants, such as *Melicope pteleifolia* (Champ. ex Benth.) T.G. Hartley fresh leaves, are rubbed on the skin, and plants used to relieve snake bites symptoms are also applied in form of a poultice. This was mentioned for a "new to science" plant species (Hul and Chassagne, in prep.), which belongs to the Primulaceae family, *Ardisia mondulkiriensis* Hul & Chassagne. According to one Bunong traditional healer from Dram Kaet village, the leaves and roots of this plant should be crushed together and the resulting paste applied locally for this purpose.

Steam bath and baths are very much in use in cases of colds and fever, headache, cramps, and during post-partum period. In

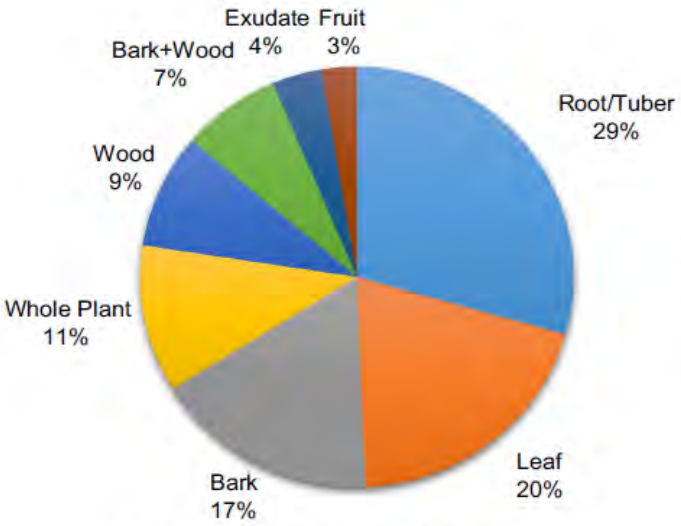

Fig. 3. Distribution percentage of plant parts used.

these circumstances, leaves are the most used plant part and several plant species are used together as this is the case for the leaves of *Chromolaena odorata*, *Coleus amboinicus* Lour., *Citrus* × *aurantiifolia* (Christm.) Swingle, and *Melicope pteleifolia* which are decocted together, then administered either as a bath or in form of a steam bath for relieving cramps symptoms. Essential oils contained in the leaves of these aromatic plants could explain the use of these species (Bakkali et al., 2008).

3.3.2. Zootherapy

3.3.2.1. Zootherapy ingredients. As summarized in Table 3 and as already highlighted in Khmer medicine (Ashwell and Walston, 2008), animal and animal by-products represent a significant part of the Bunong pharmacopeia. In this study, a total of 22 animal species were cited and 322 citations were recorded, making zootherapy an undeniably important part of the health system in Cambodia.

In our study, mammals (75%) were the most commonly used in traditional medicine, followed by insects (16%), birds (6%) and reptiles (3%). For more than half of the animals cited, only one specific organ or substance is said to be used in medicine, i.e. the stomach of the East Asian porcupine, or the fatty part of the Hog badger. In only a few cases, the whole animal is used (Slow/Pygmy loris, weaver ants).

Among the 22 animal species mentioned, the Slow Loris/Pygmy Loris (74 citations) was the most frequently used in medicine. The whole animal is used for post partum disorders, stomachache and burn, while its hairs are used to treat wounds. Due to a high market value, these two species are widely traded all over Cambodia for their medicinal qualities, and Mondulkiri province is one of the main sources of supply, since the region has the largest distribution area of pygmy lorises in Cambodia (Starr et al., 2011). With 51 citations, the East Asian Porcupine (stomach) was the second most common animal cited. Meat, together with faeces and quill from this animal were also reported to be used in Malaysia and in Nepal for treating various ailments such as asthma, stomach disorders and fever (Azliza et al., 2012; Joshi et al., 2011). Finally, honey from wild bees (40 citations) was the third most frequent animal by-product used in medicine, followed by tiger milk and Lesser Mousedeer bones (24 and 29 citations respectively).

During our survey, many people pointed out that tiger milk, used to treat burns, could in the past be found on the forest floor, but that unfortunately tigers have now disappeared. The same was noted for other animals, said to be more and more difficult to find. Nowadays, Bunong people are well aware of the threat to animals due to the wildlife trade, and this is why, when an animal is caught, its medicinal part is kept for many years.

3.3.2.2. Formulation, mode of preparation and mode of administration of remedies. Zootherapy ingredients can be used alone or in combination, depending on the type of disorders treated. For example, up to 3 animals can be mixed together in order to alleviate pain: *Centropus sinensis*, *Cynocephalus variegatus*, *Dinopium javanense* (all whole animals), whereas for male impotence only one ingredient is used (gall bladder from *Bos gaurus*). In seven preparations, plants and animal parts were reported to be combined together and the most popular recipe against stomachache includes *Curcuma longa* rhizome, honey from wild bees, East Asian porcupine dried stomach, and dried lorises combined together.

Previous to preparation and administration, whole animals or animal organs are placed over a fire in order to dry them. Only, honey, fresh eggs, breastmilk, and the fatty part of animals are used in their crude state, and do not undergo a specific preparation before their formulation and administration. For example, fresh eggs from a domesticated chicken and tiger milk are applied directly on the skin to treat burns.

The predominant method of preparation of animal ingredients recorded was alcoholic macerations (62%), which are drunk and mainly used during the post-partum period. These types of preparation might include up to 8 different animals or animal parts together. Then, administration of plain animal and animal by-products represent the second main method of preparation (30%), mostly for treating burns.

The most frequently used mode of administration was oral ingestion (78%) followed by topical uses (22%). In the case of weaver ants, used for diarrhea, the whole insect is crushed and added to banana flowers (*Musa* spp.) and rattan stem (*Calamus palustris* Griff.), ground together then eaten.

3.3.3. Other natural products

The first methodology revealed that in addition to medicinal plants, mushrooms and animals, other ingredients are found to be used in Bunong traditional medicine. As shown in Table 4, ingredients with high number of citations were found to be human urine (applied directly on the skin, in case of wounds or burn) (28 citations) and common salt (applied directly on the skin or ingested) (21 cit.). Also, cupping therapy (13 cit.) especially for headache, was widely practised. Cups are heated with a candle flame in order to create local suction and applied directly on the skin (head, neck, body). Also, to a lesser degree, mud and ashes are used (both in case of burns and wounds).

3.4. Natural remedies used for the 11 selected ailments

Laval et al. (2011), Maling (2007) and Schmitt (2004) listed the eleven main health problems faced by Bunong people in their everyday life. Wounds (*On Tchaa Tey* in Bunong language), injuries and skin encroachments are frequently encountered due to an arduous life, and agricultural practices that involve the constant use of knives. Burns (*Taow*) on the calf and on the ankle are generally due to contact with the exhaust pipe of the motorbike and occur frequently. Cold (*Tchi Kat*) is a common condition among this indigenous group, and the most frequent associated symptoms are runny noses, sneezing, fever and nasal congestion. Bunong people also complain about cough (*Ktchiak*), a condition that for some authors might be due to an infectious cause, either viral or bacterial, since there are also cases of tuberculosis in the province (Schmitt, 2004). When a person suffers from a persistent fever in combination with chills, headache and profuse sweating, malaria (*Krougn Tchagn*) is often suspected, and this pathology represents

Table 3
Animals used in medicine by Bunong people.

Family/English Name	Scientific name	Bunong name	Part used (a)	Uses	Total citations	Recipe (s)
Birds						
Greater coucal	*Centropus sinensis*	Yar eut	Wh	Pain	2	Grilled the bird on fire, then macerated in alcohol with *Cynocephalus variegatus*, *Dinopium javanense* and *Areca catechu* (root). Drunk 3 times/day
Common Flameback	*Dinopium javanense*	Ra Kleh	Wh	Pain	2	Grilled the bird on fire, then macerated in alcohol with *Centropus sinensis*, *Cynocephalus variegatus* and *Areca catechu* (root). Drunk 3 times/day
Domesticated chicken	*Gallus gallus domesticus*	Yarr	Eggs	Burn	16	Fresh eggs (white and yolk) applied directly on the burn
Insecta						
Wild Bee	*Apis dorsata*	Teak Tchot	Honey	Burn	25	Honey mixed with fresh eggs or *Ipomoea batatas* (leaves) and salt, or used alone. Applied on the burn
			Honey	Post-partum (food intolerance)	7	Honey macerated in alcohol (0.5 l) for 3 months with *Nycticebus bengalensis/pygmaeus* (dried animal) and *Zingiber purpureum* (rhizome grated). Drunk
			Honey	Stomachache	6	Honey crushed with *Curcuma longa* (rhizome), *Hystrix brachyuran* (dried stomach) and *Nycticebus bengalensis/pygmaeus* (dried animal). Eaten
			Honey	Wound	2	Honey crushed with *Chromolaena odorata* (leaves) and *Areca catechu* (seed). Applied on the wound
Asian Subterranean Termite	*Coptotermes* spp.	Yokatol (Njaa Rapoon)	Mub tubes	Burn	7	Mub tubes collected from a termite mound. Grilled it on fire, then applied with water on the burn
Weaver ant	*Oecophylla smaragdina*	Trek	Wh	Diarrhea	2	Whole ants crushed with *Calamus palustris* (stem) and *Musa spp.* (flower). Eaten
Mammalia						
Hog badger	*Arctonyx collaris*	Blourr	Fatty part	Burn	7	Oil extracted from the fatty part directly applied on the burn
			Fatty part	Sprain	5	Oil extracted from the fatty part directly applied on the affected limb
			Fatty part	Pain	3	Oil extracted from the fatty part directly applied on the pain
			Blood/Fatty part	Backache	3	Blood macerated in alcohol with oil extracted from the fatty part. Drunk
Gaur	*Bos gaurus*	Paille	Gall bladder	Male impotence	1	Gall bladder dried, then mixed with alcohol. Drunk
Water buffalo	*Bubalus bubalis*	Rapoh	Faeces	Burn	5	Faeces applied directly on the burn. Or mixed with *Luffa aegyptiaca* (leaves) and applied
Sunda Colugo	*Cynocephalus variegatus*	Big Parr	Wh	Post-partum (food intolerance)	4	Whole animal grilled on fire, then macerated in alcohol with mix 2. Drunk
			Wh	Pain	2	Whole animal grilled on fire, then macerated in alcohol with *Centropus sinensis*, *Dinopium javanense* and *Areca catechu* (root). Drunk 3 times/day
			Stomach	Stomachache	1	Stomach grilled on fire, and macerated in alcohol with *Nycticebus bengalensis/pygmaeus* (dried animal) and *Hystrix brachyura* (dried stomach). Drunk
East Asian Porcupine	*Hystrix brachyura*	Blo	Stomach	Post-partum (general health, food intolerance)	45	Whole animal grilled on fire, and macerated in alcohol with mix 1 or mix 2. Drunk
			Stomach	Stomachache	6	Stomach grilled on fire, then crushed with honey, *Ananas comosus* (fruit), *Curcuma longa* (root), and potentially *Nycticebus bengalensis/pygmaeus* (dried animal). Eaten
Otters	*Lutrogale perspicillata/Lutra sumatrana/Aonyx cinerea*	Ping	Skin	Post-partum (general health, food intolerance)	9	Skin grilled on fire, and macerated in alcohol with mix 2. Drunk
Red Muntjac	*Muntiacus muntjak*	Tchil/Njil	Leg/Jawbone	Post-partum (food intolerance)	5	Leg or jawbone grilled on fire, and macerated in alcohol with mix 2. Drunk
Southern Serow	*Naemorhedus sumatraensis*	Kess	Skin	Post-partum (general health, food intolerance)	9	Skin grilled on fire, and macerated in alcohol with mix 2. Drunk
			Horn	Backache, broken bones	5	Horn dried, then grated with alcohol. Drunk every day
			Bone	Malaria	1	Bone grated in alcohol with *Tragulus javanicus* (bone). Drunk
			Blood/Gall bladder/Horn	Tonic, recovery	1	Dried blood, dried gall bladder and dried horn macerated in alcohol. Drunk
Slow Loris/Pygmy Loris	*Nycticebus bengalensis/*	Tou Kless	Wh	Post-partum (general health,	33	Whole animal grilled on fire, and macerated in alcohol with mix 1 or mix

	Nycticebus pygmaeus			food intolerance)		2. Drunk
			Hair	Wound (hemostatic)	25	Hair collected from dried animal. Applied it on the wound
			Wh	Burn	9	Whole animal grilled on fire, crushed with honey. Applied directly on the burn
			Wh	Stomachache	7	Whole animal grilled on fire, then crushed with honey, *Ananas comosus* (fruit), *Curcuma longa* (rhizome) and *Hystrix brachyura* (dried stomach). Eaten
Tiger	*Panthera tigris*	Teak Tor Yao	Breastmilk	Burn	29	Applied directly on the burn*
Wild Pig	*Sus scrofa*	Natt	Gall bladder	Post-partum (food intolerance)	3	Gall bladder grilled on fire, and macerated in alcohol with mix 2. Drunk
			Tusk	Stomachache	1	Tusk decocted, or grated in alcohol. Drunk
			Tusk	Contusion (bruise)	1	Tusk grated in water with *Tragulus javanicus* (bone). Drunk
Lesser Mousedeer	*Tragulus javanicus*	Njouych	Bone/Jawbone	Post-partum (general health, food intolerance)	22	Bone or jawbone macerated in alcohol with mix 2. Drunk
			Bone	Contusion (bruise)	1	Bone grated in water with *Sus scrofa* (tusk). Drunk
			Bone	Malaria	1	Bone grated in alcohol with *Naemorhedus sumatraensis* (bone). Drunk
Reptilia						
(Asiatic) Reticulated python	*Python reticulatus*	Pae Klan	Fatty part	Burn	8	Oil extracted from the fatty part applied on the burn
			Fatty part	Sprain	1	Oil extracted from the fatty part applied on the affected limb

(a) Part Used: Wh=Whole animal. Mix 1=Honey+*Hystrix brachyuran* (dried stomach)+*Nycticebus bengalensis/pygmaeus* (dried animal)+*Zingiber purpureum* (root) Mix 2=*Cynocephalus variegatus* (dried animal)+*Hystrix brachyura* (dried stomach)+*Lutrogale perspicillata/Lutra sumatrana/ Aonyx cinerea* (dried skin)+*Muntiacus muntjak* (jawbone)+*Naemorhedus sumatraensis* (dried skin)+*Nycticebus bengalensis/pygmaeus* (dried animal)+*Sus scrofa* (gall bladder)+*Tragulus javanicus* (jawbone).

*The use of breastmilk was cited many times, but all the informants told us that they don't find tiger's milk anymore since few decades.

the first cause of illness in the community (Maling, 2007). Rheumatic pain and backache (*Tchi Peut*) which can be due to strong arduous physical work is also a widespread concern, together with sprains (*Klét*). Gastro-intestinal disorders such as diarrhea (*Iéro Ach*) and stomachache (*Tchi Kong*) are two main health problems in Cambodia, and diarrhea is the third major cause of mortality among children under 6 years old in the province (Maling, 2007). Finally, post-partum disorders prevention and treatment (*Con tchik*) is a general concern in many Asian traditional societies, and especially in Cambodia which also has the highest fertility rate in Southeast Asian countries, in tandem with a high maternal mortality rate (Lamxay et al., 2011; Lo, 2007; White, 2002).

3.4.1. *Overall use of plants*

During this survey, a total of 196 medicinal plant species and one medicinal mushroom were cited and collected. Altogether, the most frequently cited plants are: *Chromolaena odorata* (Total citations=261), *Zingiber montanum* (110), *Kalanchoe pinnata* (106), *Psidium guajava* (92), *Ceiba pentandra* (L.) Gaertn. (61), *Nicotiana tabacum* L. (58), *Musa* spp. (51), *Citrus × aurantiifolia* (47), *Euonymus cochinchinensis* Pierre (46), *Cananga latifolia* (45), *Careya arborea* (44) and *Helicteres angustifolia* (42). Most of these species are introduced plants or widespread in Asia.

The most cited plant *Chromolaena odorata* is an invasive species, a native of South America, which has already been reported as an important medicinal plant in several Southeast Asian ethnobotanical studies. *Zingiber montanum*, a plant widespread in Asia, is also widely used by indigenous people from the region (Inta et al., 2013; Junsongduang et al., 2014; Khuankaew et al., 2014; Ong and Kim, 2014; Tangjitman et al., 2013). The predominant use of introduced species in the Bunong pharmacopeia might be explained by their commonness (widespread species [*Chromolaena odorata*] or easily cultivated species [*Zingiber montanum*, *Kalanchoe pinnata*]), and by their value as a food (*Citrus × aurantiifolia*, *Musa* spp., *Psidium guajava*), ornamental (*Ceiba pentandra*) or recreational (*Nicotiana tabacum*) (Bennett and Prance, 2000). Moreover, it can be hypothetized that Bunong traditional medicine and medicinal plants uses have been shaped through exchanges with other ethnic groups (e.g. Khmer people), hence the introduction of these pantropical species and associated uses in local pharmacopeias.

In our survey we collected only two endemic Cambodian species: *Smilax cambodiana* and *Solanum sakhanii*. Before our survey, *Solanum sakhanii* was only known to be present in the south of Cambodia, and it was never reported as medicinal. Villagers from Dak Dam commune employed the plant for treating malaria and fever. The whole plant is boiled in water, then drunk for a few days until the person feels better (Chassagne and Hul, 2014).

Some species with high number of citations display a low level of fidelity (FL), such as *Antidesma ghaesembilla*, *Cananga latifolia*, *Hydnophytum formicarum* Jack, *Spondias pinnata* (L. f.) Kurz and *Xylia xylocarpa* (Roxb.) Taub. For most of them, they are combined with other plants in complex herbal preparations. One of the most popular plant is *Cananga latifolia* (bark, wood and root) which is part of recipes aiming to treat more than 7 different ailments (stomachache, postpartum, diarrhea, cough, cold/fever, malaria, wounds). This plant might appear as panacea since it is widely used by traditional healers, and it was one of the few species to be mentioned in each of the three ecotypes. *Cananga latifolia* is used as antipyretic, antituberculosis and anti-dizziness according to the Khmer pharmacopeia and is currently considered by Khmer traditional healers to be a very efficient medicinal plant with special therapeutic and healing properties (Dy Phon, 2000; Kham, 2004; Petelot, 1952). However, it would be interesting to undergo further pharmacological studies in order to better define its activities especially when combined with other species, to evidentiate

Table 4
Others natural remedies used by Bunong people.

English name	Uses	Total citations
Ashes	Take the ashes from the fireplace, add water and apply it on the burn	6
Salt	Mix salt and water or saliva and apply on the burn. For cough, eat and swallow the salt.	21
Urine	Use urine on a wound. Or crush the leaves of Chromolaena odorata and mix with urine for applying on the wound.	28
Cupping glass	Cups are warmed and placed on the forehead for treating headache.	13
River stone	Boil the stone and cover your head with a towel above the stone in order to take a steam bath. For headache and malaria.	7
Mud	Apply the mud directly on a wound or a burn	2

possible synergism. In this study, as well as in other previous studies from Schmitt (2004), *Cananga latifolia* appears thus as a major plant species in the Bunong's pharmacopeia.

Other medicinal plants with a high number of citations display a high FL, suggesting that these plants could be a specific treatment for the considered health problem. *Careya arborea*, *Helicteres angustifolia*, *Musa* spp., and *Psidium guajava* are mainly used for digestive disorders (stomachache and diarrhea), *Euonymus cochinchinensis* for the postpartum period, *Chromolaena odorata* and *Nicotiana tabacum* for wounds, *Ceiba pentandra* and *Citrus x aurantifolia* for cold/fever, *Kalanchoe pinnata* and *Zingiber montanum* for sprains.

3.4.2. Diseases and treatments

3.4.2.1. Cold/fever and malaria. Colds, fever (*Tchi Kat*) and malaria (*Krougn Tchagn*) have been mentioned as the two main illnesses in the community (Laval et al., 2011; Maling, 2007). *Tchi Kat* refers to a cold and/or a fever, but this term could also be used for malaria. In the latter case, villagers add other informations, such as *Tchi Kat Tchi Sregn* (fever and chills) or *Tchi Kat Tchi Pou* (fever and headache). Nowadays, health education programs have led to the introduction of a special term (*Krougn Tchagn*) borrowed from Khmer people, for malaria, largely used by the community.

3.4.2.1.1. Cold/fever. In our study, this ailment, well known by Bunong people, displayed a high number of species used (53) and has also a high number of citations (426) (Table 5). Altogether, 76% of interviewed people cited natural remedies as the first step to manage this disease, thus confirming Laval's observations (Laval et al., 2011). In our study, 59% of the plants mentioned are cultivated or widespread species.

Amongst the five most cited species, 4 are cultivated plants, and one displays a high fidelity level: *Cymbopogon nardus* (L.) Rendle (FL=0.98, see Table 6) also used as a flavouring herb in dishes. When used against this health problem, a leaf decoction is prepared, and administered in form of a steam bath or bath.

Bunong people also employed a combination of herbal plants, which can be composed of about 10 different medicinal plants and one of the favourite recipes is based upon the use of *Ageratum conyzoides* (L.) L. (leaves) with *Blumea balsamifera* (leaves), *Ceiba pentandra* (leaves), *Chromolaena odorata* (leaves), *Mangifera indica* L. (bark) and *Melicope pteleifolia* (leaves). The vapor of the decoction can be used to make a steam bath, or the plants can be soaked in water and a bath is administered.

Leaves seems to be the plant parts most in favour to treat cold/fever (Fig. 4), not only because they might contain essential oils, but also according to their "cold" properties. According Shiroyama et al. (2001), the borneol and camphor content of *Blumea balsamifera* leaves, reported in our survey to treat cold/fever might explain its classification as a "cold" plant, hence its use for treating colds and fever.

Among the most common plants used by Bunong people for treating colds, some have been shown to possess antimicrobial activity (*Blumea balsamifera*, *Chromolaena odorata*, *Citrus × aurantiifolia*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Mangifera indica* and *Ocimum tenuiflorum*), anti-inflammatory activity (*Ageratum conyzoides*, *Blumea balsamifera*, *Ceiba pentandra*, *Chromolaena odorata*, *Cymbopogon citratus*, *Mangifera indica*, *Melicope pteleifolia*, *Ocimum tenuiflorum* and *Zingiber montanum*), analgesic properties (*Chromolaena odorata*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Mangifera indica*, *Ocimum tenuiflorum* and *Zingiber montanum*), antipyretic effects (*Ageratum conyzoides* and *Chromolaena odorata*), and antiviral activities (*Cymbopogon nardus*), thus substantiating their traditional uses by Bunong people (Aini et al., 2006; Doughari and Manzara, 2008; Elumalai et al., 2012; Garrido et al., 2001; Kokate et al., 1971; Koontongkaew et al., 2013; Makri and Kintzios, 2008; Negrelle and Gomes, 2007; Okunade, 2002; Onawunmi et al., 1984; Owoyele et al., 2013; Pang et al., 2014; Pathan et al., 2012; Sakee et al., 2011; Vital and Rivera, 2009; Wei and Wee, 2013; Yoon et al., 2013). Moreover, toxicity studies on *Ageratum conyzoides*, *Ceiba pentandra* and *Citrus × aurantiifolia* showed neither acute nor chronic toxicity (Chunlaratthanaphorn et al., 2007; Diallo et al., 2010; Sarkiyayi et al., 2009).

3.4.2.1.2. Malaria. Medicinal plants are of relatively low importance in malaria treatment, as only 39% of interviewed people mentioned traditional medicine as the first step to manage the disease, and the majority said they would go to the health post to seek treatment. Indeed, over past years NGOs and governmental agencies have delivered health messages regarding the treatment of malaria encouraging people to consult a health post as soon as the first symptoms of malaria appear (Laval et al., 2011).

Concerning the treatment of malaria with plants, only 25 species have been mentioned, totalling 84 citations (F_{IC}=0.71).

Dendrolobium lanceolatum (15 citations, FL=0.75) a Southeast Asian endemic species (Ashwell and Walston, 2008) was the most cited species. This species has been shown to possess antiplasmodial activity, and some active compounds have been isolated (Kanokmedhakul et al., 2004).

Strychnos nux-vomica (9 cit., FL=1) and *Azadirachta indica* (8 cit., FL=0.8) display the highest FL, and these two species were already described as malaria treatments in the Khmer pharmacopeia (Cheng and Huon, 1996; Menaut, 1930; Petelot, 1952). Still, in the case of *Strychnos nux-vomica* L., seeds are known to be poisonous. Thus, traditional healers advise patients to swallow one quarter of a dried seed, and to take another quarter each day for 4 days, and it is strongly advised not to take more than half a seed per day. Use of poisonous nux vomica seeds have also been recorded within Khmer medicine, as an emetic or a stimulant according to the dose (Dy Phon, 2000), and also in Laos as a part of complex mixtures used as antimalarial preparations (Vidal, 1958). Unfortunately, despite toxicity warnings, strychnine poisoning still occurs among Cambodian population (Katz et al., 1996).

Roots are the preferred plant parts used to treat malaria, even if for few species (i.e. *Cananga latifolia*, *Irvingia malayana* (Oliv. ex A. W.Benn.) Tiegh., *Morinda tomentosa* B.Heyne, *Tinospora crispa*) pieces of stems, or wood are employed. One of the reasons for this large use of roots against malaria might be due to the fact that this disease is thought to be serious, and because underground parts

Table 5
Medicinal plants (196) and medicinal mushroom (1) used for 11 most common ailments.

Ailments	Number of citations	Number of plant species	F_{IC}	Plants species used (by number of citations)
Backache	57	23	0.61	*Dalbergia stipulacea* (13); *Kalanchoe pinnata* (10); *Zingiber montanum* (7); *Bauhinia malabarica* (5); *Phellinus rimosus* (2); *Smilax cambodiana* (2); *Spondias pinnata* (2); *Aganonerion polymorphum* (1); *Ancistrocladus wallichii* (1); *Cananga latifolia* (1); *Cayratia trifolia* (1); *Cymbopogon citratus* (1); *Eleutherine bulbosa* (1); *Euonymus cochinchinensis* (1); *Heliotropium indicum* (1); *Hydnophytum formicarum* (1); *Leea* spp. (1); *Melicope viticina* (1); *Piper betle* (1); *Poikilospermum suaveolens* (1); *Scoparia dulcis* (1); *Terminalia chebula* (1); *Xylia xylocarpa* (1)
Burn	106	26	0.76	*Luffa aegyptiaca* (19); *Jatropha curcas* (14); *Oroxylum indicum* (14); *Dalbergia nigrescens* (8); *Gnetum macrostachyum* (6); *Ipomoea batatas* (6); *Piper betle* (6); *Hopea odorata* (5); *Aloe vera* (4); *Careya arborea* (3); *Terminalia chebula* (3); *Ceiba pentandra* (2); *Clerodendrum serratum* (2); *Coleus amboinicus* (2); *Bombax ceiba* (1); *Cheilocostus speciosus* (1); *Cocos nucifera* (1); *Gossypium barbadense* (1); *Ipomoea aquatica* (1); *Lagerstroemia cochinchinensis* (1); *Mucuna pruriens* (1); *Musa* spp. (1); *Oryza sativa* (1); *Pterocarpus macrocarpus* (1); *Shorea siamensis* (1); *Sindora siamensis* (1)
Cold/Fever	426	53	0.88	*Chromolaena odorata* (77); *Ceiba pentandra* (52); *Zingiber montanum* (45); *Cymbopogon nardus* (40); *Citrus aurantiifolia* (33); *Blumea balsamifera* (30); *Melicope pteleifolia* (21); *Musa paradisiaca* (21); *Ageratum conyzoides* (12); *Mangifera indica* (8); *Cymbopogon citratus* (7); *Ocimum tenuiflorum* (7); *Ananas comosus* (5); *Bauhinia malabarica* (4); *Dendrolobium lanceolatum* (4); *Coleus amboinicus* (3); *Heliotropium indicum* (3); *Hibiscus rosa-sinensis* (3); *Kaempferia galanga* (3); *Terminalia bialata* (3); *Aporosa villosa* (2); *Bambusa bambos* (2); *Clausena excavata* (2); *Croton poilanei* (2); *Eleusine indica* (2); *Imperata cylindrica* (2); *Mallotus philippensis* (2); *Millettia pendulifformis* (2); *Mimosa pudica* (2); *Oroxylum indicum* (2); *Solanum sakhanii* (2); *Spondias pinnata* (2); *Agave americana* (1); *Bombax ceiba* (1); *Cananga latifolia* (1); *Capsicum frutescens* (1); *Dalbergia nigrescens* (1); *Dillenia pentagyna* (1); *Eurycoma longifolia* (1); *Grewia hirsuta* (1); *Harrisonia perforata* (1); *Hydnophytum formicarum* (1); *Limnophila chinensis* (1); *Lygodium microphyllum* (1); *Melicope viticina* (1); *Morinda tomentosa* (1); *Poikilospermum suaveolens* (1); *Polyalthia cerasoides* (1); *Rubus annamensis* (1); *Saccharum officinarum* (1); *Scleria terrestris* (1); *Sida acuta* (1); *Streblus asper* (1);
Cough	103	41	0.61	*Irvingia malayana* (15); *Citrus aurantiifolia* (12); *Dalbergia nigrescens* (11); *Chromolaena odorata* (6); *Millettia pendulifformis* (6); *Ceiba pentandra* (4); *Melicope pteleifolia* (4); *Oroxylum indicum* (4); *Hydnophytum formicarum* (3); *Zingiber montanum* (3); *Cananga latifolia* (2); *Coleus amboinicus* (2); *Lagerstroemia cochinchinensis* (2); *Passiflora foetida* (2); *Taxillus chinensis* (2); *Ampelocissus arachnoidea* (1); *Antidesma acidum* (1); *Antidesma ghaesembilla* (1); *Azadirachta indica* (1); *Blumea balsamifera* (1); *Cheilocostus speciosus* (1); *Cymbopogon nardus* (1); *Dillenia ovata* (1); *Dillenia pentagyna* (1); *Heliotropium indicum* (1); *Imperata cylindrica* (1); *Leea* spp. (1); *Mallotus philippensis* (1); *Mentha arvensis* (1); *Mimosa pudica* (1); *Morinda tomentosa* (1); *Musa* spp. (1); *Phyllanthus emblica* (1); *Pouzolzia zeylanica* (1); *Rubus annamensis* (1); *Saccharum officinarum* (1); *Smilax verticalis* (1); *Strychnos nux-blanda* (1); *Terminalia alata* (1); *Terminalia bialata* (1); *Ziziphus cambodiana* (1)
Diarrhea	433	71	0.84	*Psidium guajava* (86); *Helicteres angustifolia* (38); *Careya arborea* (28); *Lagerstroemia cochinchinensis* (28); *Dipterocarpus intricatus/obtusifolius* (18); *Shorea obtusa* (17); *Terminalia bialata* (17); *Xylia xylocarpa* (16); *Dialium cochinchinense* (15); *Anacardium occidentale* (14); *Antidesma ghaesembilla* (11); *Chromolaena odorata* (10); *Anogeissus acuminata* (9); *Terminalia triptera* (7); *Aporosa villosa* (5); *Bauhinia bassacencis* (5); *Poikilospermum suaveolens* (5); *Ziziphus cambodiana* (5); *Caesalpinia digyna* (4); *Rhodomyrtus tomentosa* (4); *Terminalia mucronata* (4); *Ageratum conyzoides* (3); *Calamus palustris* (3); *Combretum punctatum* (3); *Dillenia ovata* (3); *Hiptage benghalensis* (3); *Melastoma saigonense* (3); *Microcos tomentosa* (3); *Rubus annamensis* (3); *Shorea siamensis* (3); *Tamarindus indica* (3); *Terminalia chebula* (3); *Ziziphus rugosa* (3); *Aganonerion polymorphum* (2); *Ancistrocladus wallichii* (2); *Azadirachta indica* (2); *Bauhinia malabarica* (2); *Cananga latifolia* (2); *Crataxylum formosum* (2); *Dalbergia nigrescens* (2); *Helicteres isora* (2); *Mangifera indica* (2); *Oryza sativa* (2); *Schleicheria oleosa* (2); *Sesbania grandiflora* (2); *Spatholobus parviflorus* (2); *Spondias pinnata* (2); *Urena lobata* (2); *Allophyllus cobbe* (1); *Bombax ceiba* (1); *Bridelia tomentosa* (1); *Chrysophyllum cainito* (1); *Combretum quadrangulare* (1); *Dalbergia stipulacea* (1); *Dillenia hookeri* (1); *Ficus racemosa* (1); *Flacourtia indica* (1); *Imperata cylindrica* (1); *Manihot esculenta* (1); *Millettia pendulifformis* (1); *Musa* spp. (1); *Nauclea orientalis* (1); *Phellinus rimosus* (1); *Rourea minor* (1); *Smilax cambodiana* (1); *Streptocaulon juventas* (1); *Tinospora crispa* (1); *Willughbeia edulis* (1); *Zingiber officinale* (1); *Ziziphus mauritiana* (1)
Headache	56	25	0.56	*Blumea balsamifera* (7); *Taxillus chinensis* (7); *Zingiber montanum* (7); *Chromolaena odorata* (6); *Eurycoma longifolia* (3); *Ananas comosus* (2); *Citrus aurantiifolia* (2); *Cymbopogon nardus* (2); *Melicope pteleifolia* (2); *Smilax cambodiana* (2); *Strychnos nux-blanda* (2); *Aporosa villosa* (1); *Cymbopogon citratus* (1); *Diospyros ehretioides* (1); *Helicteres angustifolia* (1); *Hydnophytum formicarum* (1); *Irvingia malayana* (1); *Lygodium microphyllum* (1); *Mimosa pudica* (1); *Momordica charantia* (1); *Ocimum tenuiflorum* (1); *Oryza sativa* (1); *Osbeckia chinensis* (1); *Passiflora foetida* (1); *Scleria terrestris* (1)
Malaria	84	25	0.71	*Dendrolobium lanceolatum* (15); *Chromolaena odorata* (10); *Strychnos nux-vomica* (9); *Azadirachta indica* (8); *Tinospora crispa* (5); *Eurycoma longifolia* (4); *Imperata cylindrica* (4); *Morinda tomentosa* (4); *Ananas comosus* (3); *Coscinium fenestratum* (3); *Harrisonia perforata* (3); *Helicteres angustifolia* (2); *Oroxylum indicum* (2); *Aganonerion polymorphum* (1); *Cananga latifolia* (1); *Chrysopogon aciculatus* (1); *Clerodendrum paniculatum* (1); *Dillenia hookeri* (1); *Dioscorea glabra* (1); *Diospyros ehretioides* (1); *Hydnophytum formicarum* (1); *Irvingia malayana* (1); *Kaempferia galanga* (1); *Musa* spp. (1); *Terminalia chebula* (1)
Post-partum	349	75	0.79	*Euonymus cochinchinensis* (45); *Hiptage benghalensis* (33); *Flacourtia indica* (24); *Diospyros ehretioides* (16); *Ochna integerrima* (15); *Cananga latifolia* (13); *Willughbeia edulis* (13); *Curcuma aromatica* (11); *Dillenia hookeri* (11); *Dunbaria bella* (11); *Antidesma ghaesembilla* (8); *Bauhinia malabarica* (8); *Diospyros mollis/sylvatica* (8); *Salacia chinensis* (8); *Smilax cambodiana* (8); *Xylia xylocarpa* (8); *Polygonum odoratum* (7); *Aporosa villosa* (5); *Prismatomeris tetrandra* (5); *Ananas comosus* (4); *Dialium cochinchinense* (4); *Hydnophytum formicarum* (4); *Mimosa pudica* (4); *Zingiber montanum* (4); *Allium cepa* (3); *Cinnamomum iners* (3); *Gardenia obtusifolia* (3); *Piper nigrum* (3); *Agave americana* (2); *Annona squamosa* (2); *Dracaena elliptica* (2); *Eucalyptus camaldulensis* (2); *Euphorbia thymifolia* (2); *Ficus racemosa* (2); *Imperata cylindrica* (2); *Miliusa velutina* (2); *Peliosanthes teta* (2); *Phellinus rimosus* (2); *Tamarindus indica* (2); *Tetracera indica* (2); *Tetracera loureiri* (2); *Allophyllus cobbe* (1); *Amphineurion marginatum* (1); *Anacolosa griffithii* (1); *Calamus palustris* (1); *Cayratia trifolia* (1); *Curcuma longa* (1); *Eleusine indica* (1); *Eleutherine bulbosa* (1); *Euphorbia hirta* (1); *Harrisonia perforata* (1); *Helicteres lanata* (1); *Holarrhena pubescens* (1); *Hoya kerrii* (1); *Hyptis capitata* (1); *Lagerstroemia cochinchinensis* (1); *Microcos tomentosa* (1); *Morinda tomentosa* (1); *Nauclea orientalis* (1); *Oroxylum indicum* (1); *Pavetta indica* (1); *Phyllanthus emblica* (1); *Platycerium coronarium* (1); *Psidium guajava* (1); *Rourea minor* (1); *Saccharum*

Table 5 (continued)

Ailments	Number of citations	Number of plant species	F_{IC}	Plants species used (by number of citations)
				officinarum (1); *Spondias pinnata* (1); *Streblus asper* (1); *Streptocaulon juventas* (1); *Strychnos nux-blanda* (1); *Terminalia bialata* (1); *Ziziphus cambodiana* (1); *Ziziphus mauritiana* (1); *Ziziphus oenopolia* (1); *Ziziphus rugosa* (1)
Sprain	228	19	0.92	*Kalanchoe pinnata* (96); *Zingiber montanum* (41); *Cymbopogon citratus* (18); *Bauhinia malabarica* (16); *Zingiber officinale* (16); *Scoparia dulcis* (11); *Piper betle* (8); *Areca catechu* (4); *Dalbergia stipulacea* (4); *Phyllanthus taxodiifolius* (3); *Spondias pinnata* (3); *Ananas comosus* (1); *Aporosa villosa* (1); *Combretum quadrangulare* (1); *Curcuma longa* (1); *Cyperus rotundus* (1); *Lophopogon intermedium* (1); *Tamarindus indica* (1); *Xylia xylocarpa* (1)
Stomachache	243	60	0.76	*Acacia harmandiana* (36); *Musa* spp. (26); *Cananga lanifolia* (22); *Ziziphus cambodiana* (22); *Uraria crinita/lagopodioides* (16); *Leea* spp. (15); *Hydnophytum formicarum* (9); *Curcuma longa* (6); *Careya arborea* (5); *Dillenia ovata* (5); *Psidium guajava* (4); *Willughbeia edulis* (4); *Aporosa villosa* (3); *Celtis pentandra* (3); *Pouzolzia zeylanica* (3); *Smilax cambodiana* (3); *Ampelocissus arachnoidea* (2); *Antidesma ghaesembilla* (2); *Bauhinia malabarica* (2); *Bombax ceiba* (2); *Chromolaena odorata* (2); *Dialium cochinchinense* (2); *Ficus racemosa* (2); *Lagerstroemia cochinchinensis* (2); *Mimosa pudica* (2); *Oryza sativa* (2); *Passiflora foetida* (2); *Phellinus rimosus* (2); *Pouzolzia hirta* (2); *Schleichera oleosa* (2); *Shorea obtusa* (2); *Spondias pinnata* (2); *Tamarindus indica* (2); *Xylia xylocarpa* (2); *Aegle marmelos* (1); *Anacardium occidentale* (1); *Bauhinia bassacencis* (1); *Bridelia tomentosa* (1); *Chelocostus speciosus* (1); *Chrysophyllum cainito* (1); *Cinnamomum iners* (1); *Clerodendrum paniculatum* (1); *Cocos nucifera* (1); *Cyperus rotundus* (1); *Dipterocarpus intricatus/obtusifolius* (1); *Eurycoma longifolia* (1); *Ficus hirta* (1); *Helicteres angustifolia* (1); *Heliomopium indicum* (1); *Hoya kerrii* (1); *Physalis angulata* (1); *Salacia chinensis* (1); *Scoparia dulcis* (1); *Taxillus chinensis* (1); *Terminalia alata* (1); *Terminalia bialata* (1); *Terminalia chebula* (1); *Terracera indica* (1); *Zingiber montanum* (1); *Ziziphus oenopolia* (1)
Wound	316	39	0.94	*Chromolaena odorata* (150); *Nicotiana tabacum* (58); *Bambusa bambos* (29); *Spondias pinnata* (9); *Strychnos nux-blanda* (9); *Careya arborea* (8); *Zingiber officinale* (6); *Xylia xylocarpa* (4); *Cananga lanifolia* (3); *Crataxylum formosum* (3); *Curcuma longa* (3); *Ageratum conyzoides* (2); *Ampelocissus arachnoidea* (2); *Coleus amboinicus* (2); *Lagerstroemia cochinchinensis* (2); *Terminalia chebula* (2); *Zingiber montanum* (2); *Antidesma ghaesembilla* (1); *Areca catechu* (1); *Cassia fistula* (1); *Cayratia trifolia* (1); *Clerodendrum serratum* (1); *Colona auriculata* (1); *Dalbergia nigrescens* (1); *Dillenia ovata* (1); *Dracaena elliptica* (1); *Micrococos tomentosa* (1); *Oryza sativa* (1); *Peliosanthes teta* (1); *Phellinus rimosus* (1); *Phyllanthus emblica* (1); *Polyalthia cerasoides* (1); *Prismatomeris tetrandra* (1); *Psidium guajava* (1); *Pterocarpus macrocarpus* (1); *Seligmella helferi* (1); *Smilax verticalis* (1); *Tamarindus indica* (1); *Terminalia triptera* (1)

Table 6
Fidelity Level (FL) for the most cited medicinal plants.

Ailments	Medicinal plants	Fidelity Level (%)
Backache, rheumatism	*Dalbergia stipulacea*	94.7
Burn	*Jatropha curcas*	100
Burn	*Luffa aegyptiaca*	100
Burn	*Oroxylum indicum*	70
Cold/Fever	*Cymbopogon nardus*	97.6
Cold/Fever	*Citrus × aurantiifolia*	92.9
Cold/Fever	*Blumea balsamifera*	91.9
Cold/Fever	*Ocimum tenuiflorum*	87.5
Cold/Fever	*Ceiba pentandra*	86.7
Cold/Fever	*Melicope pteleifolia*	84
Cold/Fever	*Ageratum conyzoides*	70.6
Cough	*Irvingia malayana*	88.2
Diarrhea	*Anogeissus acuminata*	100
Diarrhea	*Psidium guajava*	97.7
Diarrhea	*Helicteres angustifolia*	95
Diarrhea	*Dipterocarpus intricatus/ obtusifolius*	94.7
Diarrhea	*Anacardium occidentale*	93.3
Diarrhea	*Terminalia triptera*	87.5
Diarrhea	*Shorea obtusa*	89.5
Diarrhea	*Lagerstroemia cochinchinensis*	82.4
Diarrhea	*Dialium cochinchinense*	78.9
Headache	*Taxillus chinensis*	77.8
Malaria	*Strychnos nux-vomica*	100
Malaria	*Azadirachta indica*	80
Malaria	*Dendrolobium lanceolatum*	75
Post-partum	*Curcuma aromatica*	100
Post-partum	*Diospyros ehretioides*	100
Post-partum	*Diospyros mollis/sylvatica*	100
Post-partum	*Dunbaria bella*	100
Post-partum	*Euonymus cochinchinensis*	100
Post-partum	*Hiptage benghalensis*	94.3
Post-partum	*Ochna integerrima*	93.8
Post-partum	*Flacourtia indica*	92.3
Post-partum	*Dillenia hookeri*	84.6
Post-partum	*Willughbeia edulis*	81.2
Post-partum	*Salacia chinensis*	80
Sprain	*Scoparia dulcis*	92.3
Sprain	*Kalanchoe pinnata*	90.6
Sprain	*Zingiber officinale*	82.6
Stomachache	*Acacia harmandiana*	100
Stomachache	*Uraria crinita/lagopodioides*	100
Stomachache	*Leea aequata/rubra/thorelii*	93.8
Stomachache	*Ziziphus cambodiana*	75.9
Wound	*Nicotiana tabacum*	100
Wound	*Bambusa bambos*	93.5
Wound	*Chromolaena odorata*	93.2

possess a special power, roots are more prone to be employed by traditional healers in that case.

Some species are used for both cold/fever and malaria. This is the case for *Eurycoma longifolia* (bark), *Harrisiona perforata* (Blanco) Merr. (root), *Imperata cylindrica* (root), *Kaempferia galanga* L. (rhizome), *Morinda tomentosa* (wood), *Oroxylum indicum* (L.) Kurz and *Solanum sakhanii* where the same part of plant is used in both cases. On the contrary, the part of plant differs for only one plant: roots and leaves of *Chromolaena odorata* are used against malaria while leaves of the same plants are used against cold/fever.

Whatever the plant part used, the mode of preparation of antimalarial recipes is always a decoction, which is drunk until feeling better.

In the scientific literature, 12 out of 25 of these plants have been evaluated for their antiplasmodial activity. The results show that 10 plants possess moderate or good antiplasmodial activity: *Azadirachta indica* (fruit and leaf), *Chromolaena odorata* (leaf), *Coscinium fenestratum* (stem), *Dendrolobium lanceolatum* (root), *Eurycoma longifolia* (bark and stem), *Harrisiona perforata* (leaf, root and stem), *Irvingia malayana* (leaf), *Kaempferia galanga* (leaf), *Terminalia chebula* (fruit) and *Tinospora crispa* (stem) (Chianese et al.,

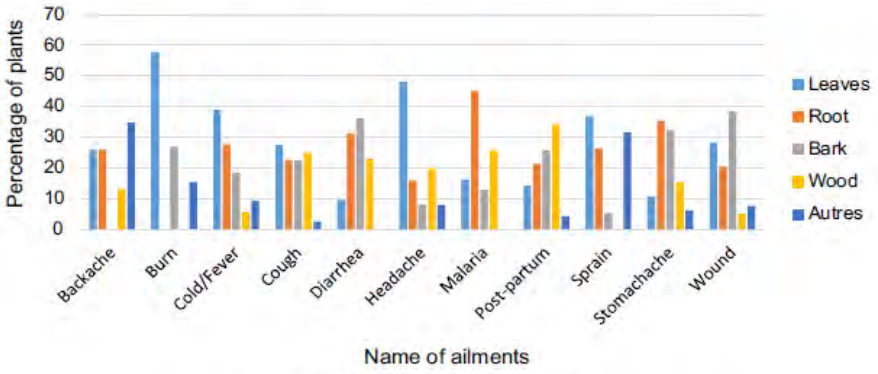

Fig. 4. Part of plants used by ailments categories.

2010; Ezenyi et al., 2014; Hout et al., 2006; Kanokmedhakul et al., 2004; Le Tran et al., 2003; Nguyen-Pouplin et al., 2007; Pinmai et al., 2010; Thiengsusuk et al., 2013). Two plants displayed weak or no activity: *Cananga latifolia* (bark, stem) and *Strychnos nux-vomica* (stem) (Hout et al., 2006; Philippe et al., 2005). Among the thirteen plants not yet evaluated for their antiplasmodial activity, the use of four plants for malaria treatment has been recorded in indigenous knowledge: *Ananas comosus*, *Clerodendrum paniculatum*, *Imperata cylindrica* and *Oroxylum indicum* (Adia et al., 2014; Asase et al., 2010; Nagendrappa et al., 2013; Iyamah and Idu, 2015).

3.4.2.2. Headache. The headache category displays the lowest factor informant consensus (0.56). Few plants are employed for this ailment (25 species), and there are none that are specific to this disease, with the notable exception of *Taxillus chinensis* (DC.) Danser. This could be explained by a lower level of knowledge in treating this ailment by traditional medicine, as most of the people interviewed (58.8%) mentioned modern medicine as the first step in the management of this ailment. For example, paracetamol (*Para*) was often mentioned as a cure for headache. Also, due to various health campaigns, headache is nowadays associated with malaria, a pathology for which modern medicine is much in use.

However, one species (*Taxillus chinensis*) was recorded 7 times for treating this ailment; this appeared to be quite a common practice in the community (Schmitt, 2004). Indeed, this parasitic plant, growing on a different tree species is said to be inhabited by a spirit. When people go under the tree without asking permission from this spirit, it is believed that the spiritual entities will cause a headache, and the only way to treat it is to employ the plant.

Headaches were mostly (45%) treated by the decoction of leaves, administered in the form of steam bath.

Previous pharmacological studies validate the traditional usage of the most cited plants: *Ananas comosus* (analgesic and anti-inflammatory), *Blumea balsamifera* (anti-inflammatory), *Chromolaena odorata* (analgesic and anti-inflammatory), *Cymbopogon nardus* (analgesic), *Eurycoma longifolia* (anti-inflammatory), *Melicope pteleifolia* (anti-inflammatory), *Taxillus chinensis* (anti-inflammatory), *Zingiber montanum* (analgesic and anti-inflammatory) (Kokate et al., 1971; Koontongkaew et al., 2013; Owoyele et al., 2013; Pang et al., 2014; Pavan et al., 2012; Varghese et al., 2013; Yoon et al., 2013; Zhang et al., 2011).

3.4.2.3. Cough. Forty-one plant species were cited for treating this ailment. Although, 68% of people mentioned traditional medicine as the first step to managing the ailment, no consensus about cough treatment seems to emerge from this survey ($F_{IC}=0.61$). Indeed, cough could refer to various diseases: pneumonia, asthma, tuberculosis, laryngitis, etc. Moreover, a large number of plants (22 species) are also employed for cold/fever treatment, and a few species are specific to this ailment. Amongst them, *Irvingia malayana*, a sacred tree, easy to find in the province, was mentioned 15 times and displays a high fidelity level (88.2). Its bark is grilled over a fire, then boiled in water with salt, and this remedy is drunk for few days. This tree was also reported as medicinal in Khmer traditional medicine and is known to possess tonic properties (Ashwell and Walston, 2008; Martin, 1971).

Another common practice only recorded to treat cough is to swallow lemon juice (*Citrus* × *aurantiifolia*) with sugar and hot water.

Finally, *Dalbergia nigrescens* and *Chromolaena odorata* use was also mentioned by Laval et al. (2011), so they represent two key plants in the management of this ailment.

Almost every part of the plant (leaves, root, bark and wood) were cited for treating these ailments, and most of the remedies used against cough were said to be administered orally (Fig. 5).

Irvingia malayana is used to treat cough in the Lao PDR, and it

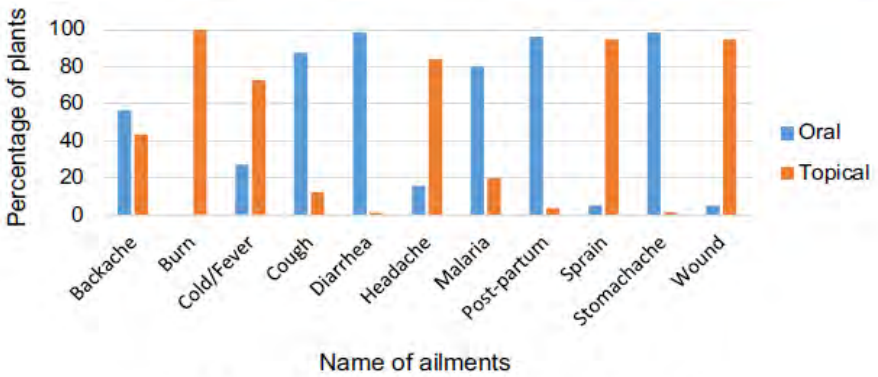

Fig. 5. Ways of administration employed by ailments categories.

has been evaluated for its activity against *Mycobacterium tuberculosis* (Elkington et al., 2009). *Citrus × aurantiifolia* is traditionally used for treating cough and sore throat, and it has been shown to possess antimicrobial activity against upper respiratory tract bacterial pathogens (Adeleye and Opiah, 2003; Apraj et al., 2011). *Dalbergia nigrescens* has been studied for its anti-inflammatory activity (Saha et al., 2013). The use of *Chromolaena odorata* as a cough remedy has already been recorded, and the plants showed analgesic activity, anti-inflammatory properties, and moderate antimycobacterial activity (Owoyele et al., 2013; Suksamrarn et al., 2004). *Ceiba pentandra*, *Hydnophytum formicarum*, *Melicope pteleifolia*, *Oroxylum indicum* and *Zingiber montanum* have also been shown to possess anti-inflammatory activities (Ali et al., 1998; Elumalai et al., 2012; Koontongkaew et al., 2013. Prachayasittikul et al., 2008; Yoon et al., 2013). Finally, to the best to our knowledge, it is the first time that *Millettia penduliformis* has been recorded as a traditional remedy. Further pharmacological investigations should be performed but its toxicity should be studied carefully as it is used by Bunong people as an herbal fish poison.

3.4.2.4. Stomachache and diarrhea. Digestive disorders such as stomachache (*Tchi Kong*) and diarrhea (*Ièro ach*) rank second in the common illnesses reported by Bunong people (Laval et al., 2011; Maling, 2007). As shown in Schmitt (2004), diarrhoeal disorders are named differently according associated symptoms. Simple diarrhea is called *Iéro ach*; diarrhea and vomiting: *Iéro ok*; and diarrhea with blood, also recorded as dysentery by Schmitt (2004) is named *Ach Moham*.

Altogether, 100 different plants species were mentioned. A high proportion of people (87% and 72% respectively) reported the use of traditional medicine as a first step for treating diarrhea and stomachache. Moreover, diarrhea and stomachache display high factor informant consensus (0.84 and 0.76 respectively). These data confirm that diarrhea and stomachache are two common health problems in the community. Secondly, it indicates that medicinal plants play an important role in the treatment of these ailments, as also reported by Laval et al. (2011).

Psidium guajava (86 citations, FL=0.98) is the most cited plants for treating simple diarrhea. It also displays a high fidelity level for this ailment, and quite a high number of different preparations were recorded with different part of plants. For example, a piece of bark can be grilled over a fire, then boiled in form of a decoction; or leaves can be eaten, or boiled and drunk. *Psidium guajava* is a pantropical species, introduced in South East-Asia, and also used in Khmer medicine. Since, the name of the plant (*Trobaek*) originated from Khmer people, and the uses of the tree are very similar to the Khmer medicine (Martin, 1971; Menaut, 1930), so we can suppose that Bunong people learned how to use this tree thanks to Khmer people.

Beside the significant use of guava for simple diarrhea, *Helicteres angustifolia* was one of the plants with the highest fidelity level (FL=0.95) regarding diarrhea with blood. It was mentioned 38 times, and rank second in the most cited species. A decoction of the root is prepared and administered orally.

To treat diarrhea, Bunong people use a "step by step" process. Recipes are made out of a sole ingredient, contrary to what can be observed for post-partum or cold/fever. The first plant to be used is the most common one (e.g. *Psidium guajava*), then in case of persistent diarrhea another species is employed, until getting better. In this case, the second plant species used can be *Anacardium occidentale* (bark), *Careya arborea* (bark), *Chromolaena odorata* (leaves or root), *Dipterocarpus* spp. (resin), *Lagerstroemia cochinchinensis* Pierre (bark), *Rhodomyrtus tomentosa* (Aiton) Hassk. (fruit) or *Terminalia bialata* (bark).

Anti-diarrhoeal plants which have been pharmacologically evaluated are: *Anacardium occidentale*, *Careya arborea*, *Chromolaena odorata*, *Dipterocarpus intricatus*, *Psidium guajava* and *Terminalia triptera* (Aba et al., 2015; Araújo et al., 2015; Gutiérrez et al., 2008; Rahman et al., 2003; Sireeratawong et al., 2012; Tradtrantip et al., 2014). Moreover, some species possess antibacterial activity against gastrointestinal pathogenic bacteria: *Anacardium occidentale*, *Antidesma ghaesembilla*, *Careya arborea*, *Helicteres angustifolia* and *Shorea obtusa* (Al Muqarrabun and Ahmat, 2015; Chea et al., 2007b; Sakunpak and Panichayupakaranant, 2012). Furthermore, usage of others plant species for diarrhea treatment in indigenous knowledge includes: *Anogeissus acuminata*, *Shorea obtusa* and *Xylia xylocarpa* (Fasola et al., 2014; Khuankaew et al., 2014; Raju and Reddy, 2005). Finally, we have recorded the use of *Lagerstroemia cochinchinensis* in traditional medicine for the first time. Another plant species from the same genus (*Lagerstroemia speciosa* (L.) Pers.) has already been shown to possess antidiarrhoeal activity, thus suggesting possible similar antidiarrhoeal compounds in both plants (Hussain et al., 2014).

Concerning the treatment of stomachache, a tree called *Tarm Marr* (*Acacia harmandiana*) (36 cit., FL=100) ranks first on the number of citations and displays the highest fidelity level. The bark is boiled until the volume of water is reduced by one third, and this preparation is drunk as often as possible.

More generally, the oral route of administration was used by Bunong people for stomachache in more than 98% of the total cases, with the prevailing use of roots and barks.

Acacia harmandiana is endemic to Southeast Asia and there have been few studies on its medicinal and biological properties; it could be interesting to study the bark of this species for its pharmacological activity. Besides, some *Musa* species have been shown to possess anti-ulcerogenic activity (Best et al., 1984; Vadivelan et al., 2006). *Cananga latifolia* is known for its uses as a medicinal plant in Thailand, but none of these studies mentioned its uses for stomach pain (Chuakul and Boonpleng, 2004; Chuakul et al., 2006). *Ziziphus cambodiana* was mentioned in another ethnomedicinal survey in Thailand for treating gastric ulcer (Tangjitman et al., 2015). *Uraria crinita* and *Uraria lagopodioides* are used as medicinal plants in Bangladesh, India and Thailand, but they were not reported for their uses in stomach disorders (Chuakul et al., 2002; Kumar and Bharati, 2014; Roy et al., 2008). *Leea rubra* is used for gastrointestinal disorders in Thailand (Chuakul et al., 2002; Ongsakul et al., 2009). *Hydnophytum formicarum* is a traditional remedy to cure stomach pain in Asian countries (Hanh and Thao, 2015). Finally, *Curcuma longa* has been well studied for the treatment of gastric ulcer and proven to be safe and efficient (Kim et al., 2005; Prucksunand et al., 2001; Rafatullah et al., 1990).

3.4.2.5. Wounds. This category of ailments displays the highest factor informant consensus (0.94). Indeed, only three out of thirty-nine plant species represent 75% of the total citations. *Chromolaena odorata* (150 citations, FL=0.93) is by far the most employed for wounds. Leaves are crushed with urine or water, and the paste is applied directly to the wound. *Nicotiana tabacum* (58 cit., FL=100) is the second most common plant used to treat wounds. Tobacco (*Mhat*) is widely cultivated in the Bunong community, since dried leaves are smoked in homemade cigarettes. For healing purposes, dried or fresh leaves are crushed, mixed with water and applied directly on the skin.

According to Bunong people, two differents kinds of plants are used to treat wounds. Some plants are said to be hemostatic and are used just after the cut (i.e. *Chromoalaena odorata* (leaves) or *Nicotiana tabacum* (leaves) in order to stop the blood), and other plants might be used later, in order to clean the skin and avoid infections: this is the case for *Spondias pinnata* (bark) and/ or *Xylia xylocarpa* (bark), prepared in form of a wash.

The use of *Chromolaena odorata* for wounds and cuts has

already been reported in other ethnobotanical surveys from Southeast Asia (Khuankaew et al., 2014; Ong and Kim, 2014; Tangjitman et al., 2013). Moreover, the plant was shown to have hemostatic activity in vivo and was extensively studied for its wound healing properties (Houghton et al., 2005; Pandith et al., 2012). *Nicotiana tabacum* is used to treat common skin problems (including wounds) in America, Ethiopia and India, and proven to have antimicrobial activity (Charlton, 2004; Kalayou et al., 2012; Upadhyay et al., 1998). *Bambusa bambos* is a traditional remedy for wounds and cuts in India (Ayyanar and Ignacimuthu, 2009; Subramanian et al., 2011). *Spondias pinnata* has been described in some ethnobotanical surveys for its common use for skin diseases, and was proven to have antimicrobial activity, especially against *Staphylococcus aureus* (Chea et al., 2007b; Gupta et al., 2010). *Strychnos nux-blanda* is used for treating wounds in Cambodia, and in traditional medicine for treating various ailments in Thailand (Chuakul et al., 2002; Inta et al., 2013; Schmitt, 2004). *Careya arborea* is used in traditional medicine for treating wounds in Bangladesh and in India, and has been shown to possess wound healing activity in rats (Bhat et al., 2012; Mia et al., 2009; Ramesh and Shenoy, 2013).

3.4.2.6. Sprains. In the case of sprain, only 19 differents plant species were recorded, and among them just 2 are mainly used. *Kalanchoe pinnata* is a very popular plant (96 citations, FL=0.92), primarily used for this purpose, and its local name (*Kun*=magic plant, *Klét*=sprain) suggests its use. Leaves are preferentially grilled over a fire for a few minutes, then applied directly on the affected limb. The second important plant reported is the rhizome of *Zingiber montanum*, also called *Cha Rannay*. In this case, the rhizome is crushed in alcohol, and the paste is applied directly on the affected part. *Kalanchoe pinnata* and *Zingiber montanum* were also the second and the third most frequently cited species in the treatment of backache, the last species being also cited in cases of cold/fever. These different uses might be explained by the analgesic and anti-inflammatory properties of these species, as reported by Afzal et al. (2012) and Bua-in and Paisooksantivatana (2009). Moreover, *Bauhinia malabarica*, *Cymbopogon citratus*, *Piper betle*, *Scoparia dulcis* and *Zingiber officinale* have analgesic and anti-inflammatory properties (Alam et al., 2013; Ali et al., 2008; Cechinel Filho, 2009; De Farias Freire et al., 1993; Negrelle and Gomes, 2007).

3.4.2.7. Burns. Two species display the highest fidelity level and are also the most cited plants for burn treatment. Leaves of *Luffa aegyptiaca* (19 citations, FL=1), a vegetable commonly cultivated in Cambodia are crushed and applied directly on the skin. In the case of *Jatropha curcas* (14 citations, FL=1), the bark can be grilled over a fire, then crushed and applied on the burn, or resin from the shrub can be directly applied locally.

Luffa aegyptiaca is used for treating skin diseases in Bangladesh and India, and has been proven to have wound healing and anti-inflammatory activities (Abirami et al., 2011; Darshan and Doreswamy, 2004; Mollik et al., 2010; Seliya and Patel, 2009). *Jatropha curcas* is used as a traditional remedy for burns and wounds in Peru and Colombia, and shown to have anti-inflammatory, anti-hemorrhagic, antibacterial and wound healing activities (Sabandar et al., 2013; Shetty et al., 2006; Villegas et al., 1997). *Oroxylum indicum* is used for skin disorders in India and Nepal, and possesses analgesic, anti-inflammatory, antimicrobial activity and wound healing activities (Dinda et al., 2015). *Dalbergia nigrescens* has been studied for its anti-inflammatory activity (Saha et al., 2013). *Gnetum macrostachyum* is used in traditional remedies for inflammation and pain relief in Thailand, and possesses anti-inflammatory activity (Kloypan et al., 2012; Zhang et al., 2015). *Ipomea batatas* is used for the treatment of burns in traditional medicine, and it has also been shown to possess analgesic, anti-inflammatory and antibacterial properties (Mohanraj and Sivasankar, 2014; Sathish et al., 2012).

3.4.2.8. Backache. Backache is one of the ailment categories with a low factor informant consensus (0.61), and only 23 species were mentioned for this ailment. This lack of consensus might be due to the fact that backache uncovers different types of etiology, i.e. musculoskeletal disorders, kidney infections or salpingitis. In the management of the disease, more than 69% of the people cited automedication with plants for primary care, but among them, 42.7% preferred to ask the help of a traditional healer.

Nevertheless, *Dalbergia stipulacea* displays the highest fidelity level (13 citations, FL=0.94). Resin produced by the tree in response to a mechanical damage induced by an undetermined species of insect is collected from the bark, then boiled and the resulting preparation is drunk. Although few studies have reported the use of *Dalbergia stipulacea* as a medicinal plant, the genus *Dalbergia* was already shown to possess antiarthritic activities (Kadir et al., 2012; Vasudeva et al., 2009). In this case it would be worthwhile to undergo pharmacological study in order to asset the anti-inflammatory activity of this resin.

As mentioned before, *Kalanchoe pinnata*, *Bauhinia malabarica* and *Zingiber montanum* possess analgesic and anti-inflammatory properties. *Kalanchoe pinnata* is frequently used in West African countries as an herbal remedy for arthritis and rheumatism, and has been evaluated for its muscle relaxant activities (Ojewole, 2005; Yemitan and Salahdeen, 2005). Moreover, acute and subacute toxicological assessments of the plant suggested that the aqueous leaf extract is safe (Ozolua et al., 2010). *Zingiber montanum* is used as a traditional remedy for rheumatism in Malaysia and for alleviating muscle pain in Thailand (Hamirah et al., 2010; Maneenoon et al., 2015). The same plant also possesses anti-arthritic activity (Siriarchavatana et al., 2009). *Bauhinia malabarica* is used for various ailments in Nepal and India, but no previous studies have reported its uses for back pain (Lingaraju et al., 2013; Manandhar, 1991). *Phellinus rimosus*, the only mushroom species reported by Bunong people in our study, possesses anti-inflammatory activity (Janardhanan et al., 2009).

3.4.2.9. Postpartum. In Southeast Asia, women commonly use medicinal plants after giving birth, during the post partum period (de Boer and Cotingting, 2014). The use of these plants aims to help in the recovery of the woman, to avoid complications as hemorrhages, puerperal fevers, to reduce abdominal pain and to increase lactation.

In Cambodia, as well as in other neighbouring countries such as Laos and Vietnam, it is said that women, just after giving birth, are in a "cold" state because they lost blood and energy while giving birth. This is why the body must be heated by drinking specific herbal remedies made out of "hot" plants, and also, stay close-by a small fire during few days, in order to warm up, thus restore the hot/cold balance (Lamxay et al., 2011; Lo, 2007).

Altogether, 75 plants have been mentioned for use in the postpartum period, which can last 2–6 months. This indicates a still vivid and extensive knowledge related to this period of women's life. This category also displays the highest number of plants (6) used solely for that purpose (FL=1): *Curcuma aromatica* Salisb., *Diospyros ehretioides* Wall. ex G.Don, *Diospyros mollis* Griff., *Diospyros sylvatica* Roxb., *Dunbaria bella* Prain and *Euonymus cochinchinensis*, hence making this moment very special, and different from the other ailments.

Most of the plants (85%) are mixed together, and some preparations are made out of over 20 different ingredients. One of the most widely used remedy is composed of a mix of *Euonymus cochinchinensis* (leaves/root/wood), *Ficus racemosa* L. (root/wood),

Flacourtia indica (root/wood), *Hiptage benghalensis* (L.) Kurz (root/wood), *Hydnophytum formicarum* (whole plant), *Salacia chinensis* (wood), *Smilax cambodiana* (rhizome) and *Willughbeia edulis* (wood).

Remedies are prepared in form of a decoction but sometimes also in the form of alcoholic maceration, believed to enhance the desired warming effect. In this survey, many plants used during the post-partum period were said to possess a warming effect, i.e. *Diospyros ehretioides*, *Euonymus cochinchinensis*, *Gardenia obtusifolia* Roxb. ex Hook.f. and *Prismatomeris tetrandra* (Roxb.) K.Schum.

Amongst the plants cited, some of them were specifically indicated to stop hemorrhage (*Salacia chinensis*), to treat abdominal pain (*Spondias pinnata*), to be useful in case of dizziness (*Polygonum odoratum* Lour.), headache (*Ananas comosus* (L.) Merr.), tiredness (*Ochna integerrima* (Lour.) Merr.) and loss of appetite (*Hiptage benghalensis*). All these symptoms are characteristic of a particular post-partum condition problem called "*Tech*". *Tech* is said to be due to non-observance of food restrictions by the mother, coupled with intense physical activities, exposition to rainy and windy environmental conditions and sexual intercourse. *Tech* treatment might be used either in a preventive or a curative form. *Tech*, as described by Bunong people seems to be similar to the post-partum condition called "Toas" in Khmer, mainly due to special food consumption (e.g. jackfruit, buffalo meat). To avoid relapse from food, specific prohibitions are given during the post-partum period (White, 2002).

Euonymus cochinchinensis, the most cited plant species for post-partum treatment in our survey, is used as an herbal remedy for bodily discomfort and genitourinary disorders in Thailand, but no previous pharmacological studies on this plant have been performed until now (Chuakul et al., 2002; Srithi et al., 2009). *Hiptage benghalensis* has been mentioned for its traditional uses in the treatment of various ailments (fever, cold, wound, ulcers, diabetes) in Bangladesh and India (Chenthurpandy et al., 2009; Hasan et al., 2010). It was also reported to have anti-inflammatory activity (Hsu et al., 2015). *Flacourtia indica* is widely used in traditional medicine as an appetizer, tonic, and in the treatment of headache or fever in India and Bangladesh (Hossain et al., 2010). It is also used in ethnoveterinary medicine for treating uterine prolapse and retention of the placenta (Upadhyay et al., 2011). Moreover, an ethnobotanical survey from Bangladesh mentioned its uses as an abortifacient (Haque et al., 2011). *Diospyros ehretioides* is used as a traditional remedy for leucorrhea and fever in Thailand (Chuakul et al., 2002). Some compounds extracted from dried fruits exhibited antimycobacterial, antifungal, antimalarial activities and cytotoxic activities (Prajoubklang et al., 2005). *Ochna integerrima* is used as a digestive tonic, antipyretic, antidysenteric in Thailand and Indonesia (Kaewamatawong et al., 2002), and has been shown to possess good antiviral and antiparasitic activity (Makhafola and Eloff, 2012). *Cananga latifolia* is used for the treatment of infectious diseases in early childhood, dizziness, nasal polyposis and fever in Thailand (Chuakul et al., 2002; Chuakul and Boonpleng, 2004; Chuakul et al., 2006; Phatchana et al., 2015), and has been studied for its antimicrobial and antiplasmodial activities (Chea et al., 2007b; Hout et al., 2006). *Willughbeia edulis* is used as a traditional remedy for oedema and yaws in Bangladesh and Thailand (Chuakul, 2005; Sarwar, 2015), and has been proven to possess antiviral activities (Lipipun et al., 2003). *Curcuma aromatica* is used for treating constipation, fever and gastrointestinal disorders in India and Thailand (Das et al., 2008; Rao et al., 2011). It has also been shown to possess anti-inflammatory and wound healing activities (Kumar et al., 2009).

Medicinal parts of animals are also employed during this period, since it is said that animals possess a vital power that humans can receive by ingesting them, as already recorded by Lamxay et al. (2011) in Laos. Various parts of animals were used such as the whole animal, skin, stomach, gall bladder, bones. During the post-partum period, the most popular preparation consists of a mixture of *Cynocephalus variegatus* (dried animal), *Hystrix brachyura* (dried stomach), Otters spp. (dried skin), *Muntiacus muntjak* (jawbone), *Naemorhedus sumatraensis* (dried skin), *Nycticebus* spp. (dried animal), *Sus scrofa* (gallbladder), and *Tragulus javanicus* (jawbone). If none of the eight previous animals are available, an alcoholic maceration of porcupine stomach is preferred.

3.5. Bunong traditional knowledge: loss or maintenance?

Nowadays, land and access to natural resources are threatened by different factors: migration of people from lowland provinces inside existing farms of Bunong inhabitants, economic land concession for agro-industrial development (especially rubber plantations) and climate change. This rapid change influences the biodiversity of the province and the livelihood of Bunong people (Bourdier, 2006; Guérin et al., 2003; Laval et al., 2011; Savajol et al., 2011). Furthermore, the remedies, especially medicinal plants, becomes less abundant, and people have more difficulty in finding them. According to Ashwell and Walston (2008), 50% of the native medicinal plants (14% of the Cambodian flora) could be threatened with extinction in the longer term. As a consequence of the loss of medicinal plants, Laval et al. (2011) demonstrate that 33% of the Bunong households would face an increase in their health expenditure.

Despite the change which occurs actually in the province, Bunong people have maintained a significant ethnobotanical knowledge and continue to use natural substances in medicine. Thus, the loss of knowledge among indigenous minorities is not an inevitable process leading to the complete disappearance of cultures. On the contrary, traditional knowledge is in a continuing process of evolution with the loss and gain of knowledge. As mentioned in some studies (Eyssartier et al., 2008; Gómez-Baggethun and Reyes-García, 2013), indigenous people, who face several changes, can maintain their traditional knowledge and continue to use medicinal plants. Likewise, Bunong culture is not a static tradition; they adapt to external and internal changes. They learn, incorporate new practices, modify ancestral skill, and generate hybrid knowledge (Ceuterick et al., 2011). Nevertheless, deforestation and the loss of biodiversity is a major threat to this process.

4. Conclusion

This study provides comprehensive information on the natural remedies used for 11 most frequent diseases encountered by the Bunong community. It appears that most of the plants reported have been already described as safe and efficient for the health problems cited. Moreover, some species that are widely used by the Bunong community have been recorded for the first time: *Dalbergia stipulacea* is mostly used for backache, *Lagerstroemia cochinchinensis* for diarrhea, *Millettia penduliformis* for cough and *Smilax cambodiana* for post-partum. Furthermore, *Cananga latifolia*, a well known medicinal plant in Thailand and Cambodia, was cited many times for treating various ailments. Therefore, further scientific researches should be undergone in order to assess the safety and the efficacy of these plants, and to evaluate their pharmacological potential.

Author contributions

Conceived and designed the survey: FC, GB. Performed the survey: FC. Identified the plants: FC, SH. Analyzed the data: FC. Wrote the paper: FC, GB, ED.

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Nota Bene: A documentary untitled "Forêts: Pharmacie du Monde", realized by Nadège Demanée and produced by AB international distribution in 2014, features the first and the second author during this ethnobotanical survey in Mondulkiri province.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2016.06.003.

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Annexe 2. Liste de références bibliographiques ayant trait à l'étude des substances naturelles utilisées en médecine traditionnelle au Cambodge (classée par date de parution)

Titre	Auteur	Année de parution	Langue	Type	Source	Lieu d'étude	Population d'étude	Pathologies étudiées	Type et nombre de substances citées	Références
Matière Médicale Cambodgienne	Menaut B. : médecin	1930	FR	Livre	Enquête	Cambodge	Khmer	Toutes	PLANTES [210 espèces médicinales], ANIMAUX [25], MINERAUX [13], CHAMPIGNONS [1]	(Menaut, 1930)
Les plantes médicinales du Cambodge, du Laos et du Vietnam (TOME I)	Petelot A.: botaniste	1952	FR	Livre	Bibliographie existante	Cambodge, Laos, Vietnam	Divers	Toutes	PLANTES [537 espèces médicinales]	(Petelot, 1952)
Les plantes médicinales du Cambodge, du Laos et du Vietnam (TOME II)	Petelot A.: botaniste	1953	FR	Livre	Bibliographie existante	Cambodge, Laos, Vietnam	Divers	Toutes	PLANTES [434 espèces médicinales]	(Petelot, 1953)

Annexe 2. Liste de références bibliographiques ayant trait à l'étude des substances naturelles utilisées en médecine traditionnelle au Cambodge (classée par date de parution)

Titre	Auteur	Année de parution	Langue	Type	Source	Lieu d'étude	Population d'étude	Pathologies étudiées	Type et nombre de substances citées	Références
Les plantes médicinales du Cambodge, du Laos et du Vietnam (TOME III)	Petelot A.: botaniste	1954	FR	Livre	Bibliographie existante	Cambodge, Laos, Vietnam	Divers	Toutes	PLANTES [503 espèces médicinales]	(Petelot, 1954a)
Les plantes médicinales du Cambodge, du Laos et du Vietnam (TOME IV)	Petelot A.: botaniste	1954	FR	Livre	Bibliographie existante	Cambodge, Laos, Vietnam	Divers	Toutes	INDEX des plantes citées dans les tomes I, II et III	(Petelot, 1954b)
Notes ethnobotaniques sur quelques plantes en usage au Cambodge	Vidal J.E. : botaniste, Martel G.: ethnographe, Lewitz S.: linguiste	1969	FR	Article	Enquête	Cambodge	ND	Toutes	PLANTES [160 espèces dont 40 médicinales]	(Vidal et al., 1969)
Introduction à l'ethnobotanique du Cambodge	Martin M.A.: ethnobotaniste	1971	FR	Livre	Enquête	Cambodge	ND	Toutes	PLANTES [752 espèces dont 239 médicinales]	(Martin, 1971)

Annexe 2. Liste de références bibliographiques ayant trait à l'étude des substances naturelles utilisées en médecine traditionnelle au Cambodge (classée par date de parution)

Titre	Auteur	Année de parution	Langue	Type	Source	Lieu d'étude	Population d'étude	Pathologies étudiées	Type et nombre de substances citées	Références
Contribution à l'ethnobotanique des Brou (Cambodge, Province de Ratanakiri) (Première partie)	Matras J. : anthropologue, Martin M.A.: ethnobotaniste	1972	FR	Article	Enquête	Province du Ratanakiri, Cambodge	Ethnie Brou	Toutes	PLANTES [154 espèces dont 14 médicinales]	(Matras and Martin, 1972a)
Contribution à l'ethnobotanique des Brou (Cambodge, Province de Ratanakiri) (Deuxième partie)	Matras J. : anthropologue, Martin M.A.: ethnobotaniste	1972	FR	Article	Enquête	Province du Ratanakiri, Cambodge	Ethnie Brou	Toutes	PLANTES [111 espèces dont 10 médicinales]	(Matras and Martin, 1972b)
Les Légumineuses-Papilionoidees du Cambodge: place systématique, noms khmers et usages éventuels	Dy Phon P. : botaniste. Vidal J.E. : botaniste	1984	FR	Article	Enquête	Cambodge	ND	Toutes	PLANTES [59 espèces dont 28 médicinales]	(Dy Phon and Vidal, 1984)

Annexe 2. Liste de références bibliographiques ayant trait à l'étude des substances naturelles utilisées en médecine traditionnelle au Cambodge (classée par date de parution)

Titre	Auteur	Année de parution	Langue	Type	Source	Lieu d'étude	Population d'étude	Pathologies étudiées	Type et nombre de substances citées	Références
Les plantes médicinales au Cambodge (partie 1)	Cheng S.K. : pharmacien, professeur des Universités, ex-directeur du CNMT. Huon C.: tradipraticien rattaché au CNMT	1996	KH	Livre	Bibliographie existante	Cambodge	NA	Toutes	PLANTES [115 espèces médicinales]	(Cheng and Huon, 1996a)
Les plantes médicinales au Cambodge (partie 2)	Cheng S.K. : pharmacien, professeur des Universités, ex-directeur du CNMT. Huon C.: tradipraticien rattaché au CNMT	1996	KH	Livre	Bibliographie existante	Cambodge	NA	Toutes	PLANTES [95 espèces médicinales]	(Cheng and Huon, 1996b)

Annexe 2. Liste de références bibliographiques ayant trait à l'étude des substances naturelles utilisées en médecine traditionnelle au Cambodge (classée par date de parution)

Titre	Auteur	Année de parution	Langue	Type	Source	Lieu d'étude	Population d'étude	Pathologies étudiées	Type et nombre de substances citées	Références
Contribution à l'étude des plantes médicinales utilisées dans le traitement du paludisme et des fièvres symptomatiques	Pordié L.: pharmacien, anthropologue	1998	FR	Rapport de recherche	Enquête	Province du Mondulkiri, Cambodge	Ethnie Bunong	Paludisme/fièvre	PLANTES [18 espèces médicinales]	(Pordié, 1998)
Dictionnaire des plantes utilisées au Cambodge	Dy Phon P.: botaniste	2000	FR; KH; ANG	Livre	Bibliographie existante	Cambodge	NA	Toutes	PLANTES [1254 espèces dont 576 médicinales]	(Dy Phon, 2000)
L'invisible guérison. Notes d'ethnomédecine en milieu rural au Cambodge	Crochet S.: anthropologue	2000	FR	Article	Enquête	Cambodge	Femme non-spécialiste	Toutes	PLANTES [21 espèces médicinales], ANIMAUX [5], MINERAUX [5]	(Crochet, 2000)
Medicinal Plants of Cambodia	Kham L.: scientifique	2004	ANG	Livre	Bibliographie existante	Cambodge	NA	Toutes	PLANTES [515 espèces médicinales]	(Kham, 2004)

Annexe 2. Liste de références bibliographiques ayant trait à l'étude des substances naturelles utilisées en médecine traditionnelle au Cambodge (classée par date de parution)

Titre	Auteur	Année de parution	Langue	Type	Source	Lieu d'étude	Population d'étude	Pathologies étudiées	Type et nombre de substances citées	Références
Etude ethnobotanique auprès des minorités ethniques de la province de Mondulkiri, Royaume du Cambodge	Schmitt A.: ethnobotaniste	2004	FR	Mémoire de Master	Enquête	Province du Mondulkiri, Cambodge	Ethnie Bunong	Paludisme/fièvre, désordres digestifs, fatigue	PLANTES [130 espèces médicinales]	(Schmitt, 2004)
Screening of selected indigenous plants of Cambodia for antiplasmodial activity	Hout et al.: pharmacognoste et parasitologue	2006	ANG	Article	Enquête	9 provinces, Cambodge	Khmer	Paludisme/fièvre	PLANTES [28 espèces médicinales]	(Hout et al., 2006)
Contribution à l'étude du paludisme chez les minorités Phnong du Cambodge	Schmitt A.: ethnobotaniste. Nicolas J.P.: ethnobotaniste	2007	FR	Article	Enquête	Province du Mondulkiri, Cambodge	Ethnie Bunong	Paludisme/fièvre	PLANTES [55 espèces médicinales]	(Schmitt and Nicolas, 2007)

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Titre	Auteur	Année de parution	Langue	Type	Source	Lieu d'étude	Population d'étude	Pathologies étudiées	Type et nombre de substances citées	Références
In vitro antimicrobial activity of plants used in Cambodian traditional medicine	Chea et al.: pharmacognoste, microbiologiste	2007	ANG	Article	Enquête	9 provinces, Cambodge	Khmer	Maladies infectieuses	PLANTES [27 espèces médicinales]	(Chea et al., 2007)
An overview of the use and trade of plants and animals in traditional medicine systems in Cambodia	Aswell D. : scientifique. Walston N. : scientifique	2008	ANG	Rapport	Enquête	Cambodge	ND	Toutes	PLANTES [824 espèces médicinales], ANIMAUX [43 espèces médicinales]	(Ashwell and Walston, 2008)
Traditional therapeutic knowledge of the Bunong people in North-eastern Cambodia	Savajol N. : ingénieur agronome, ex-chef de projet à Nomad RSI. Toun V.: intervenant local. Sam J.: intervenant local	2011	ANG	Livre	Bibliographie existante	Province du Mondulkiri, Cambodge	Ethnie Bunong	Toutes	PLANTES [27 espèces médicinales]	(Savajol et al., 2011)

Annexe 2. Liste de références bibliographiques ayant trait à l'étude des substances naturelles utilisées en médecine traditionnelle au Cambodge (classée par date de parution)

Titre	Auteur	Année de parution	Langue	Type	Source	Lieu d'étude	Population d'étude	Pathologies étudiées	Type et nombre de substances citées	Références
Flore photographique du Cambodge	Leti M. : phytochimiste. Hul S. : botaniste. Fouché J.G. : scientifique. Cheng S.K. : pharmacien, professeur des universités, ex-directeur du CNMT. David B.: pharmacien, phytochimiste	2013	FR	Livre	Enquête	Cambodge	ND	Toutes	PLANTES [524 espèces dont 301 médicinales]	(Leti et al., 2013)
Forest Healers. A guide to the medicinal plants of Cambodia's northeastern forests	Audibert V. : chef de projet Poh Kao. Behra O. : chef de projet Man and Nature. Hieng P. : médecin, ex-directeur du CNMT. Ky B.H. : tradipraticen	2015	ANG	Livre	Enquête	Province du Ratanakiri, Cambodge	Ethnie Kavet et Lao	Toutes	PLANTES [85 espèces médicinales]	(Audibert et al., 2015)

ព្រះរាជាណាចក្រកម្ពុជា
KINGDOM OF CAMBODIA
ជាតិ សាសនា ព្រះមហាក្សត្រ
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National Ethics Committee for Health Research

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រាជធានីភ្នំពេញ, ថ្ងៃទី..02..ខែ..01.....ឆ្នាំ2015....

Mr. François Chassage

Project: Epidemiological study: liber Cancer in Cambodia : Knowledge, Attitudes and Practices Survey. Version N° 1, dated 25 November, 2014.

Reference: - Your letter on 25 November , 2014
- Summary report of NECHR's secretaries on 29th December, 2014

Dear Mr. François Chassage,

I am pleased to notify you that your study protocol entitled "Epidemiological study: liber Cancer in Cambodia : Knowledge, Attitudes and Practices Survey. Version N° 1, dated 25 November, 2014" has been approved by National Ethic Committee for Health Research (NECHR). This approval is valid for twelve months after the approval date.

The Principal Investigator of the project shall submit following document to the committee's secretariat at the National Institute of Public Health at #2 Kim Il Sung Blvd, Khan Tuol Kok, Phnom Penh. (Tel: 855-23-880345, Fax: 855-23-881949):

- Annual progress report
- Final scientific report
- Patient/participant feedback (if any)
- Analyzing serious adverse events report (if applicable)

The Principal Investigator should be aware that there might be site monitoring visits at any time from NECHR team during the project implementation and should provide full cooperation to the team.

Regards,

Chairman

Prof. ENG HUOT

KINGDOM OF CAMBODIA
NATION RELIGION KING

MINISTRY OF HEALTH

HOPITAL Calmette

N°.3221. HC/2014

H.E. Prof. CHHEANG Ra
Director General
Calmette Hospital
3 Monivong Blvd.,
Phnom Penh, Cambodia

November 13, 2014

François CHASSAGNE,
PhD student/Doctor of Pharmacy
Paul Sabatier University – Toulouse III
Faculty of Pharmaceutical Sciences
F-31062 Toulouse Cedex 9

Objective: Letter of support for a study on Liver Cancer in Cambodia: Knowledge, Attitudes and Practices Surveys"

Dear Mr. François CHASSAGNE,

I am writing to inform you that Calmette Hospital has received and reviewed the study proposal entitled "Liver Cancer in Cambodia: Knowledge, Attitudes and Practices Surveys".

This study involves retrospective study of patient with hepatocarcinoma by lifting demographic clinical and biological character of patient's records.

Calmette Hospital, therefore, agrees to cooperate with you in conducting this study at Calmette Hospital upon approval by the National Ethics Committee for Health Research (NECHR) of Cambodia.

Sincerely yours,

H.E. Prof. CHHEANG Ra

No 3, Monivong Blvd, Phnom Penh, Cambodia, Tel: 023 426 948, Fax: 023 724 892, E-mail: hospital@calmette.gov.kh, www.calmette.gov.kh, P.B. 807.

Lao People's Democratic Republic
Peace Independence Democracy Unity Prosperity
===== 000 =====

Ministry of Health
National Institute of Public Health
National Ethics Committee
For Health Research (NECHR)

No. 010 NIOPH/NECHR

Approval Notice

Dr François Chassagne
Email : francois.chassagne@ird.fr
Phone: +33781820499

RE: " Traditional medicine used for liver diseases in Lao PDR: local knowledge and biological evaluation "

Dear Dr François Chassagne,
Members of the Ethics Committee of the Lao People's Democratic Republic (PDR) have reviewed and approved your research.

Please note the following information about your approved research protocol:

Approval period: March 2016 – March 2017
Approved Subject Enrollment: 180
Sponsor: FONDATION POUR LA RECHERCHE MEDICALE, Université de Toulouse, Institut de Recherche pour le Développement

Implementing Panel/Project Investigator: Dr François Chassagne

Please note that the Ethics Committee reserves the right to ask for further questions, seek additional or monitor the conduct of your research and consent process.

Vientiane Capital.. 26 FEB 2016
Director General
National Institute of Public Health

รองศาสตราจารย์ ดร กองแก้ว อักขะวิง
Assoc Prof Dr Kongsap AKKHAVONG

Communications orales et poster

Congrès internationaux

- **Chassagne, F.**, Rojas Rojas, T., Eav, S., Bertani, S., Pineau, P., & Deharo, E. (2016). A twelve-year retrospective study of liver cancer at Calmette Hospital in Cambodia: contribution of viral hepatitis. IARC 50th Anniversary Conference, 7-10 Juin 2016, Lyon, France. (poster)

- **Chassagne, F.**, Hul, S., Deharo, E., & Bourdy, G. (2015). Are ethnobotanical studies a good way to discover new plants species? A case study from Mondulkiri province, Cambodia. International Congress, Botanical Research in Tropical Asia, 6-10 Décembre 2015, Vientiane, Laos. (présentation orale)

- **Chassagne, F.**, Hul, S., Deharo, E., & Bourdy, G. (2014). Traditional remedies in Northeast Cambodia: a survey in the Bunong community. Biodiversity and Health Symposium, 17-18 Novembre 2014, Phnom Penh, Cambodia. (présentation orale)

- **Chassagne, F.**, Hul, S., Deharo, E., & Bourdy, G. (2014). Traditional remedies in Northeast Cambodia: a survey in the Bunong community. 8th National Health Research Forum, 16-17 Octobre 2014, Vientiane, Laos. (poster)

Congrès nationaux

- **Chassagne, F.**, Deharo, E., Hieng, P., & Bourdy, G. (2017). Ethnopharmacological study of plants used to treat liver cancer in Cambodia. 5ème Journées Internationales de l'AFERP, 17-19 Juillet 2017, Angers, France. (poster)

Conférence Grand Public

- **Chassagne, F.** (2015). Plantes médicinales : une efficacité prouvée ? Institut Français du Cambodge (IFC), 8 Septembre 2015, Phnom Penh, Cambodge. (Conférence de 1h)

Auteur : François Chassagne

Titre : Cancer du foie au Cambodge : état des lieux épidémiologiques, description des médecines traditionnelles utilisées et évaluation d'espèces médicinales utilisées

Directeurs de thèse : Eric Deharo et Geneviève Bourdy

Lieu et date de soutenance : Faculté de Pharmacie de Toulouse, le mardi 17 octobre 2017

Résumé en français :

Le cancer du foie est le 6ème cancer le plus fréquent et le 2ème plus meurtrier dans le monde. Au Cambodge, en raison du contexte historique et économique, les données précises concernant cette pathologie manquent. A l'aide d'outils épidémiologiques, nous avons décrit les caractéristiques de 553 patients atteints de cancer du foie à l'hôpital Calmette à Phnom Penh, et ainsi mis en évidence l'importance de l'infection par les virus des hépatites B et C chez les sujets étudiés. Puis, nous avons documenté les connaissances de 42 de ces patients vis-à-vis de leur maladie. Nous avons détaillé leurs itinéraires thérapeutiques, mis en évidences des pratiques à risques (forte utilisation d'injections thérapeutiques et de techniques de dermabrasion), et le recours fréquent à des médecines dites traditionnelles. Nous avons ensuite tenté de comprendre les stratégies de prise en charge des patients souffrant de maladies hépatiques par les médecins traditionnels, et mis en évidence la variété des remèdes utilisés et l'importance de la perception khmère des propriétés des plantes. Enfin, à l'aide d'un modèle *in vitro* de culture de cellules cancéreuses hépatiques couplé à des outils d'analyse métabolomique, nous avons évalué 10 espèces médicinales, sélectionnées sur des critères bibliographiques et de terrain, et tenté d'identifier les composés potentiellement responsables de l'activité antiproliférative observée.

Résumé en anglais :

Liver cancer is the 6th most common and 2nd most lethal cancer in the world. In Cambodia, due to the historical and economic context, there is a lack of accurate data on this pathology. Using epidemiological tools, we described the characteristics of 553 patients with liver cancer at the Calmette Hospital in Phnom Penh, and thus highlighted the importance of infection with hepatitis B and C viruses in the subjects studied. Then we documented the knowledge of 42 of these patients about their disease. We have detailed their therapeutic itineraries, highlighted risky practices (high use of therapeutic injections and dermabrasion techniques) and the use of traditional medicines. We then attempted to understand strategies for the management of patients with liver diseases by traditional healers, and highlighted the variety of remedies used and the importance of Khmer perception of plant properties. Finally, using an *in vitro* model of liver cancer cell culture coupled with metabolic analysis tools, we evaluated 10 medicinal species, selected on the basis of bibliographic and field criteria, and attempted to identify the compounds potentially responsible for the antiproliferative activity observed.

Mots-clés : activité antiproliférative ; Cambodge ; carcinome hépatocellulaire ; connaissance, attitude, pratique ; dossiers médicaux ; ethnopharmacologie ; hepG2 ; médecine traditionnelle ; métabolomique ; phytochimie ; plantes médicinales ; tradipraticiens.

Discipline administrative : Biologie, Santé, Biotechnologie

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