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Efficacy and safety of "Yahom" as a traditional Thai herbal therapy: A systematic review

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Abstract

Ethnopharmacological relevance: Yahom is a traditional Thai medicine used to treat syncope and abdominal discomfort.

Aim of the study: This study aimed to systematically review all available evidence which purports to support these claims.

Material and methods: The systematic review accorded with the Cochrane Collaboration framework and PRISMA reporting. Databases including MEDLINE, Excerpta Medica Database (EMBASE), Cochrane library database, and Google Scholar were searched by keywords, Yahom and Ya-hom. Pharmacological and toxicity data from non-animal and animal studies were included.

Results: Twenty-four articles: 2 on *in vitro* cell lines or bacteria, 3 *in vitro* cell-free, 5 *in vitro* animal, 13 *in vivo* and 1 human mainly reported (A) Cardiovascular effects (i) transient hypotension (0.2-0.8 g/kg, intravenous injection (i.v.)), increased cerebral blood flow (2 g/kg, single oral) and vascular dilatation/relaxation (ii) elevated blood pressure (BP) (0.2-0.8 g/kg, i.v. or 2-4 g/kg oral) and vasocontraction. Single Yahom doses (3 g) given to healthy volunteers had no effect on cutaneous blood flow, ECG or systolic BP although marginally increased diastolic BP was claimed. (B) Yahom (2-4 g/kg) completely inhibited gastric acid secretion evoked by gastric secretagogues. (C) Toxicity: Chronic oral doses of selected Yahoms to rodents (0.001-1 g/kg) supports its status as generally regarded as safe.

Conclusions: Most studies supported declared objectives relating to perceived Yahom actions, but lacked background demonstrating clinical efficacy, and mechanistic data that would validate conclusions. Our study suggests that research into traditional medicinal herbs needs underpinning by appropriate clinical interventions and pharmacovigilance, thereby optimising efficacy and minimizing toxicity by combining traditional wisdom and modern testing.

Key words: Thai traditional herbs, Yahom, Ya-hom, systematic review, folk medicines, herbal medicines

Running title: Yahom and its pharmacology

1. Introduction

Many folk and traditional medicines are founded on complex mixtures of natural ingredients and continue to be used widely throughout the world (World Health Organization, 2013). One of these is Yahom (meaning nice-smelling drug in Thai) which has a long history as a remedy for fainting, dizziness, flatulence, and abdominal discomfort (Department for development of Thai traditional and alternative medicine: Ministry of Public Health, 2011; Ministry of Public Health, 1999). Yahom is a generic term for preparations sold under numerous brand names all having different compositions and formulations and ~100 of these Yahom preparations are registered as traditional medicines with the Thai Food and Drug Administration that oversees their manufacture and sale. Only five (Tip Osot, Tepajit, Navagot, Kae Lom Wing Wien, and Intarachak or Intajak) are registered on the Thai National List of Herbal Medicinal Products which considers ethnopharmacological evidence and efficacy. Each Yahom is formulated from 30-60 components of desiccated medicinal plant parts ground into powders or tablets (Department for development of Thai traditional and alternative medicine: Ministry of Public Health, 2011). Ingredients and amounts are variable but the following plants are common to Yahom preparations e.g Pierre ex Lecomte, *Mesua ferrea* L., *Mimusops elengi* L., *Cinnamomum loureirii* Nees., *Saussurea lappa* C.B. Clarke, *Angelica dahurica, Ligusticum chuanxiong* Hort (Department for development of Thai traditional and alternative medicine: Ministry of Public Health, 2011). They are administered as tablets, or suspensions after soaking in hot water. Product labelling usually contains some information about product origin, recommended dosages and frequency, and symptoms appropriate to the medication. For others, recommendations are scant.

Yahom continues to enjoy widespread use, mainly among older Thais. It is usually used for acute conditions. Thus, a better understanding of their efficacy, interactions and adverse actions is needed to promote and protect public health and safety. Thus far, most scientific data about Yahom is derived from animal and *in vitro* studies (Jariyapongskul et al., 2006; Suvitayavat et al., 2004; Suvitayavat et al., 2005a). To date, no systematic review which integrates and assesses the reliability of this evidence on Yahom has been undertaken. Although systematic reviews normally correlate clinical studies (Higgins and Green, 2011), this approach is also a valuable strategy to correlate preclinical data thus helping to rationally direct future clinical work (Andersen et al., 2014; Liao et al., 2014; Ranasinghe et al., 2012). Therefore, the current study aims to systematically evaluate the evidence for Yahom efficacy and safety using *in vitro*, *in vivo* preclinical and clinical studies. In this, we identify several fundamental deficiencies in the studies and because of its widespread use, these need to be addressed.

2. Materials and methods

This systematic review was conducted according to the Cochrane Collaboration framework and reporting followed the PRISMA Statement (Higgins and Green, 2011; Moher et al., 2009).

2.1 Literature search

The following bibliographic databases were systematically searched since their inception dates to March 2016: MEDLINE, Excerpta Medica Database (EMBASE), Cochrane library database, and Google Scholar. The search terms: Yahom and Ya-hom were used. A manual search was performed using the references and asking experts on Yahom to seek additional data. Government reports and dissertation were also used as sources of data. Authors of the reports and papers were contacted where necessary to clarify missing or incomplete data, although some were unable or unwilling to provide extra data. We identified all relevant studies regardless of language.

2.2 Inclusion criteria

The inclusion criteria were studies that investigated the pharmacological effects and toxicities of Yahom in non-animal, animal, or human experiments. Review articles, abstracts, letters to the editor, comments, case reports and duplicated study populations were excluded.

2.3 Data extraction and analysis

Two reviewers (KC, CNS) independently reviewed and extracted the data using a common data extraction procedure. Data extraction for preclinical studies included animal & study models, Yahom brand and preparation, concentration/dose, route of administration and duration of treatment, protocols and outcome parameters measured and techniques used, and basic pharmacological data. The details of the extracted preclinical data of Yahom are summarised in Tables 1-3. A human study was extracted by study design, subject characteristics, Yahom preparation, intervention, outcome parameters, and results.

2.4 Quality assessment

Quality assessment was performed in animal and human studies. Animal studies were assessed for quality based on an approach proposed by Krauth et al. (Krauth et al., 2013), which included domains about sequence generation, allocation concealment, observer blinding to allocation (personnel collecting or analysing data were unaware of which subjects belonged to intervention or control groups), inclusion and exclusion criteria, sample size, animal welfare regulations, a conflict of interest statement, statistical tests, animal selection based on comorbidity, baseline data for the animals, dose-response model, withdrawal of animals from the study, time to the outcome assessment, and adequate

controls. We also added enough protocol and method information to repeat the study, and also 4 domains on herbal medicines which are supplementary to ARRIVE and CONSORT statements (Kilkenny et al., 2012; Gagnier et al., 2006). Use of positive controls and taxonomic validation of medicinal plant composition was also assessed. Risk of bias assessment and the CONSORT statement were used for the human study (Higgins and Green, 2011; Moher et al., 2010).

3. Results

3.1 Search results

A total of 35 articles were identified from the database searches. Nine additional articles were identified through manual searches of the references, bibliographies and consultations with experts. This yielded 24 articles which met the inclusion criteria including 2 *in vitro* cell lines or bacteria (Sripanidkulchai et al., 2007; Tepsuwan et al., 2011), 3 *in vitro* cell-free (Channarong et al., 2012; Nalinratana et al., 2014; Tuekaew et al., 2014), 5 *in vitro* animal tissue studies (Chantharangsikul et al., 2009; Nonthasawadsri et al., 2015; Nusuetrong et al., 2012; Pataloong and Sawasdimongkol, 1995; Suvitayavat et al., 2005b), 13 *in vivo* animal studies (Chavalittumrong et al., 2009; Intayoong, 2006; Jariyapongskul et al., 2006; Kengkoom and Ampawong, 2015; Kengkoom et al., 2012; Kengkoom et al., 2015; Matangkasombat, 1974; Nernpermpisooth et al., 2015; Sirisangtragul and Sripanidkulchai, 2013; Sirisangtrakul and Sripanidkulchai, 2011; Suvitayavat et al., 2004; Suvitayavat et al., 2005a; Thongpraditchote et al., 1999) and 1 human study (Suvitayavat et al., 2005) (Fig 1).

3.2 Study characteristics

The *in vivo* or *in vitro* experimental studies using either normal rats or mice (n = 3-16 per group for *in vivo* and n = 5-10 per group for *in vitro* studies), were performed to determine pharmacological actions of Yahom. In all studies, animals were allocated equally to each arm (control groups, receiving vehicle only and one or several different Yahom doses dissolved in vehicle). The cardiovascular system was the main focus in preclinical studies (Table 1). Mean arterial blood pressure (MAP) was measured in two *in vivo* studies on normal anesthetized rats after either intravenous or oral administration of Yahom (Jariyapongskul et al., 2006; Suvitayavat et al., 2004), and two *in vitro* studies measured contraction of rat isolated aorta or atria (Nusuetrong et al., 2012; Suvitayavat et al., 2005b). One study included cerebral blood flow (Jariyapongskul et al., 2006). Actions on the gastrointestinal system, another target possibly relevant to Yahom ethnopharmacology, were assessed as: (i) gastric acid secretion evoked by histamine or muscarinic agonists using either normal anesthetized rat (Suvitayavat et al., 2004) or isolated mouse whole stomach

(Chantharangsikul et al., 2009); or (ii) protection against ulceration in rats (Intayoong, 2006) (Table 2). Acute (14 days) and chronic (6 months) toxicity was tested in rats and mice (Chavalittumrong et al., 2009) and P450 enzyme function was measured in orally dosed mice for 4 weeks (Sirisangtragul and Sripanidkulchai, 2013; Sirisangtrakul and Sripanidkulchai, 2011).

The human study recruited 15 healthy females (age 20-23 yr) into an acute cross-over trial. *Five Pagodas brand* was administered as a single oral dose as an aqueous suspension (3 g) or a lyophilized aqueous extract (Suvitayavat et al., 2005) (Table 4). Electrocardiogram (ECG), blood pressure and forearm cutaneous blood flow were measured every 5 min for 60 min.

3.3 Nature of Yahom tested

All studies used different formulae and preparations of Yahom. Between the eight animal studies (Chavalittumrong et al., 2009; Kengkoom and Ampawong, 2015; Kengkoom et al., 2012; Kengkoom et al., 2015; Sirisangtragul and Sripanidkulchai, 2013; Sirisangtrakul and Sripanidkulchai, 2011; Suvitayavat et al., 2004; Suvitayavat et al., 2005a), four Yahom formulas were tested in all (Five Pagodas, Intajak, Navagot, Ampanthong, and Tultavai). Another study evaluated Prasarthong (Sripanidkulchai et al., 2007) while in 9 studies (Channarong et al., 2012; Intayoong, 2006; Jariyapongskul et al., 2006; Matangkasombat, 1974; Nernpermpisooth et al., 2015; Pataloong and Sawasdimongkol, 1995; Suvitayavat et al., 2005b; Tepsuwan et al., 2011; Thongpraditchote et al., 1999), the source and formulation were unreported, although 2 studies (Intayoong, 2006; Sirisangtrakul and Sripanidkulchai, 2011) listed the qualitative ingredients.

All studies used extracts rather than the raw commercial preparations that are consumed by users, except in one arm of the clinical study (Suvitayavat et al., 2005). The extracts were 5-19% of the original product by dry weights and the solvent was either water (Chantharangsikul et al., 2009; Intayoong, 2006; Suvitayavat et al., 2004; Suvitayavat et al., 2005; Suvitayavat et al., 2005a; Suvitayavat et al., 2005b), EtOH (Nusuetrong et al., 2012), or 80% EtOH, and 2 studies used $MeCl_2$, MeOH or water (Sirisangtragul and Sripanidkulchai, 2013; Sirisangtrakul and Sripanidkulchai, 2011). The extraction temperature, solvent volume, period, and mechanical treatment also varied.

Many studies quoted doses as a raw powder equivalent (Chantharangsikul et al., 2009; Nernpermpisooth et al., 2015; Pataloong and Sawasdimongkol, 1995; Sirisangtragul and Sripanidkulchai, 2013; Sirisangtrakul and Sripanidkulchai, 2011; Suvitayavat et al., 2005; Suvitayavat et al., 2005a; Suvitayavat et al., 2005b), otherwise all studies quoted the actual doses/concentrations of extract. The human study used the native powder, in one

study arm (Suvitayavat et al., 2005). The rationales for using extracts or doses were not stated.

3.4 Quality assessment

Thirteen *in vivo* animal studies (Chavalittumrong et al., 2009; Intayoong, 2006; Jariyapongskul et al., 2006; Kengkoom and Ampawong, 2015; Kengkoom et al., 2012; Kengkoom et al., 2015; Matangkasombat, 1974; Nernpermpisooth et al., 2015; Sirisangtragul and Sripanidkulchai, 2013; Sirisangtrakul and Sripanidkulchai, 2011; Suvitayavat et al., 2004; Suvitayavat et al., 2005a; Thongpraditchote et al., 1999) were assessed for their quality (Table 5). In all studies, sample size was clearly given and investigation time was sufficient for outcome assessment, but the allocation sequence, concealment, and blinding were unclear. None of the studies stated inclusion and exclusion criteria and only two of them detailed when animals were removed from study (Sirisangtragul and Sripanidkulchai, 2013; Sirisangtrakul and Sripanidkulchai, 2011). Complying with animal welfare regulations and declaring conflicts of interest were stated in 6 studies (Chantharangsikul et al., 2009; Chavalittumrong et al., 2009; Intayoong, 2006; Nusuetrong et al., 2012; Sirisangtragul and Sripanidkulchai, 2013; Sirisangtrakul and Sripanidkulchai, 2011) and 2 studies (Chantharangsikul et al., 2009; Nusuetrong et al., 2012), respectively. Seven studies performed dose-response effects of Yahom (Chantharangsikul et al., 2009; Chavalittumrong et al., 2009; Jariyapongskul et al., 2006; Nusuetrong et al., 2012; Suvitayavat et al., 2004; Suvitayavat et al., 2005a; Suvitayavat et al., 2005b), but only 5 studies used appropriate statistical tests (Chantharangsikul et al., 2009; Chavalittumrong et al., 2009; Nusuetrong et al., 2012; Suvitayavat et al., 2004; Suvitayavat et al., 2005b). None of the studies used any pathological animal model, so the appropriate co-morbidity domain was not determined. Quality of reporting was generally acceptable except for Matankasombat (Matangkasombat, 1974) where poor reporting made most of the data useless (Table 1). Some studies were underpowered especially Kengkoom with only 2 rats for ischemia/reperfusion against which to compare drug action (Kengkoom et al., 2015). In most studies validation of plant compositions of Yahom were not carried out (Table 5).

However, four studies on tissue pathologies (Chavalittumrong et al., 2009; Kengkoom and Ampawong, 2015; Kengkoom et al., 2012; Thongpraditchote et al., 1999) had no mention of who made the histological assessments and lacked validation. For the herbal domains, many studies stated Yahom herbal contents, some had HPLC profiles but did not compare powder and extract, and none provided all the information required by CONSORT and ARRIVE (Gagnier et al., 2006).

For the only human study (Suvitayavat et al., 2005), the risk of bias was unclear in 5 domains including sequence generation, allocation concealment, blinding, outcome data addressed, and other sources of bias, while the risk of bias was low for selective outcome reporting but high for selective data analysis (compared with baseline rather than control group).

CONSORT and ARRIVE also require reporting about the herbal treatments used. While several authors illustrated HPLC chromatograms of their extract, only 3 studies (Nalinratana et al., 2014; Sirisangtragul and Sripanidkulchai, 2013; Sirisangtrakul and Sripanidkulchai, 2011) compared different extraction methods and concluded that methylene chloride yielded most peaks with $MeCl_2$ extraction, MeOH showed some similarities, while aqueous extraction showed markedly fewer peaks (Nalinratana et al., 2014). Only one study identified peaks (Nalinratana et al., 2014). Three other studies (Suvitayavat et al., 2004; Suvitayavat et al., 2005a; Suvitayavat et al., 2005b) provided HPLC chromatographs but the nature of individual peaks were not identified and the rationale for showing them unclear.

3.5 Pharmacological effects of Yahom

Intravenous Yahom transiently reduced MAP (~30 mmHg, ~30 s) followed by a longer pressor action, ~10 mmHg (Suvitayavat et al., 2005b) while a similar elevation followed single oral gavages (Jariyapongskul et al., 2006; Matangkasombat, 1974) (Table 1). A single oral dose increased regional cerebral blood flow in rats, which correlated with cerebral arteriolar vasodilatation (Jariyapongskul et al., 2006). In rat isolated aorta, bath application of Yahom produced either a vasoconstriction (Suvitayavat et al., 2005a) or a vasodilatation (Nusuetrong et al., 2012), while in rat isolated atria Yahom increased force but reduced rate of contraction (Suvitayavat et al., 2005a).

Yahom in anaesthetised rats (Suvitayavat et al., 2004) or mice (Chantharangsikul et al., 2009) (Table 2), either applied serosally or duodenally almost completely inhibited the gastric secretion elicited by histamine or muscarinic agonists.

Cell-free studies rated Yahoms as moderately high phenolic content as judged by as high reducing capacities (Channarong et al., 2012; Nalinratana et al., 2014; Tuekaew et al., 2014) (Table 3). But how these translate to the *in vivo* environment is unclear.

The human study on 15 healthy female volunteers showed that single oral doses (3 g) of either Yahom powder in water or the same amount as lyophilized aqueous extract showed no convincing effects on blood pressure (MAP, systolic, or diastolic pressure) nor were ECG or cutaneous blood flow affected (Suvitayavat et al., 2005). For the native powder only, MAP and diastolic pressure reportedly increased by ~2 mmHg at several time

points when compared to '0' time. When each time point is compared to the control corresponding control value, there were no differences (Table 4).

In vitro, effective extract concentrations were highly variable: the most potent effects were aortic relaxation (0.001-0.1 mg/ml) (Nusuetrong et al., 2012), 1.7-17 mg/ml for aortic and atrial contraction (Suvitayavat et al., 2005b), and 2.5-20 mg/ml to inhibit gastric secretion (Chantharangsikul et al., 2009). However, Nusuetrong et al., (Nusuetrong et al., 2012) used a prolonged EtOH extraction which might explain the different effect to that of Suvitayavat et al study (Suvitayavat et al., 2005b).

Two studies used cell-free assays to measure phenolic/anti-oxidant potential and scavenging of specific oxidizing agents (Channarong et al., 2012; Nalinratana et al., 2014), but no evidence was cited that demonstrated these effects operated *in vivo* at the concentrations used, i.e., their biological contexts were unclear.

In general, the non-human pharmacological studies used much higher Yahom concentrations or doses than those likely to be relevant to plasma of humans taking Yahom orally.

3.6 Toxicity of Yahom

There were 6 studies where rats (0.01-1 g/kg/day) or mice (2-16 g/kg/day) were fed with Yahom extracts using various solvents. The most common maximum dose was 1 g/kg/day of either native powder or extract which translated from rats to be equivalent in humans of 0.130 g/kg/day or ~10 g/day using an allometric conversion (Sharma and McNeill, 2009). For up to 4 g/kg/day (mice, acute) and up to 1 g/kg/day for 12 months (rats), there were no consistent changes in body weight, appetite, spontaneous behaviour, general macroscopic health, blood chemistry, haematology, and histology of liver (Chavalittumrong et al., 2009; Kengkoom and Ampawong, 2015). For haematology, counts rose for leucocyte (males) and for platelets (females), both reversibly, in one study (Chavalittumrong et al., 2009) but showed no changes in other studies (Kengkoom and Ampawong, 2015; Kengkoom et al., 2012). The latter studies (Kengkoom and Ampawong, 2015; Kengkoom et al., 2012) also showed no changes blood chemistry, histology of liver, heart and lungs, nor other haematological parameters. However, at the highest Yahom dosage rate, glomerular mesangiopathy was evident (but renal function appeared normal as judged by BUN and plasma creatinine) (Kengkoom et al., 2012).

For LD_{50} determinations, 8 g/kg/day killed 1/10 mice while 7/10 died consuming 16 g/kg/day suggesting an LD_{50} of around 11 g/kg/day of extract (Chavalittumrong et al., 2009). This scales to ~60 g/day in humans. In two studies, mice fed two different Yahom

preparations for 4 weeks at low doses (1-5 mg/kg/day) showed reduced activities of hepatic drug metabolism enzymes including CYP1A1, CYP1A2 and CYP2E1 whereas CYP2B was increased by Yahom depending on the extraction solvent (Sirisangtragul and Sripanidkulchai, 2013; Sirisangtrakul and Sripanidkulchai, 2011). Pentobarbital 'sleeping' times in rats were shortened by up to 75% also suggesting liver enzyme induction.

Assessment of carcinogenicity and teratogenicity has relied on cell assays which showed no effect (Nernpermpisooth et al., 2015) but the concentrations tested were lower than some concentrations used to demonstrate cardiovascular effects. One endothelial cell culture study found concentration-dependent toxicity > 200 μg/ml extract (Nalinratana et al., 2014).

4. Discussion

To the best of our knowledge, this is the first study to systematically summarise and review the scientific evidence derived from investigations on Yahom in both preclinical and human studies. In preclinical studies, the cardiovascular effects were: (i) Vasorelaxation at the lowest concentrations (0.1 μg/ml) of the Yahoms tested, explaining in one *in vivo* study, the transient hypotension, the increased cerebral blood flow and accordant pial dilatation. (ii) Aortic contractions and increased atrial rate and force of contraction explains the pressor effects of Yahom, i.e., increased MAP, SBP and HR, but at concentrations (~1-17 mg/ml) higher than those showing endothelial toxicity (500 μg/ml). The main effect of Yahom (~20 mg/ml or ~4 g/kg) on gastro-intestinal tract from preclinical studies was its inhibitory action on the hormonally stimulated gastric acid and other gastric secretions. In most of these preclinical studies, it was suggested that the results support the traditional use of Yahom. But this is unlikely for anything needing in vitro concentrations > 1 mg/ml and in vivo doses > 300 mg/kg are unlikely to be relevant to their medicinal actions. More fundamentally, different Yahom formulations were used, or were undisclosed, especially in the cardiovascular studies. Thus, these actions may not be comparable with each other.

However, the relevance of all these actions in rodents to those underlying some therapeutic effects of Yahom in humans remains unclear. The use of Yahom may be of value in the treatment of syncope, because it increases blood pressure and cerebral blood-flow, however syncope arises from many causes, and an anti-syncope action is difficult to test in rodents. Yahom is a herbal remedy widely used among older Thais but ~50% of Thais aged over 50 yr are hypertensive, which contra-indicates any medicine raising BP *per se*. Its inhibition of gastric secretion was suggested to support the traditional use of Yahom to treat abdominal discomfort, but gastro-intestinal discomfort is multifactorial and focusing

on gastric secretions is presumptive. Thus, this pharmacological data sheds little light on the clinical actions of Yahom.

The discovered studies suggest an acceptable safety profile, at least for Navagot, at the oral dosages recommended by the National Drug List (1-6 g/day). Although Yahoms may share common therapeutic effects, their differing compositions does not necessarily mean that they have similar toxicity profiles. Furthermore, there is no human toxicity data. The low doses (1-5 mg/kg) that disrupted P450 enzymes is worrying because many of the consumers are elderly and likely to be prescribed other medications where potency changes could influence therapeutic or adverse outcomes (Sirisangtragul and Sripanidkulchai, 2013; Sirisangtrakul and Sripanidkulchai, 2011). One adverse effect report was where an infant with high fever died of renal/liver failure. In this case, the parents had over-dosed Five Pagodas, believing it would reverse her fever, although no causality was evident (WHO collaborating center for international drug monitoring, 1991). Consumers of Yahom point to its use over hundreds of years and that this is sufficient claim safety. But like all medicinal herbs, it should be integrated into pharmacovigilance programs. Therefore, toxicity data in the discovered articles should be seen as supporting the ethnopharmacological safety information.

No study reviewed carcinogenicity *in vivo* although potential renal toxicity was noted (Kengkoom and Ampawong, 2015). However, one ingredient (*Aristolochia pierei*) is now banned in Thailand because aristolochic acid in *Aristolochia* species is nephrotoxic and causes urothelial malignancy in humans. This ingredient was only found in the Navagot formula. It was classified as a human (class I) carcinogen (National Drug System Development Commission, 2011; World Health Organization International Agency for Research on Cancer, 2002).

While non-human studies produced clear, unambiguous effects, they all tested different extracts rather than the native formulation sold as a medicine, and from different products, although some authors quoted doses as powder-equivalents. The method of application also varied (oral, intraduodenal, intravenous, or bath addition for *in vitro* studies). Thus, the variation of effects recorded may reflect different spectra of constituents arising from product and extraction variations, as well as differences arising from limited bioavailability and metabolic degradation or activation. Because the active ingredients are unknown, and no metabolism nor pharmacokinetic data available, selecting concentrations for relevant *in vitro* or intraparentral *in vivo* study is guesswork. Above all, these studies cannot use the most appropriate animal models because the therapeutic actions and pathophysiological targets of Yahom are undefined. Thus this lack design cohesion makes it

difficult to answer the key questions: in humans, does Yahom work, how does it work, can its efficacies be established, if so, can it be used safely?

There are other limitations in our review that need to be highlighted. (i) The search terms were limited to Yahom/Ya-hom. After thorough database searches and consultations with experts in herbal medicine, alternative terms for Yahom were not found. However, additional papers (Kengkoom and Ampawong, 2015; Kengkoom et al., 2015; Nalinratana et al., 2014; Nusuetrong et al., 2012) were discovered that used the alternative term 'a Yahom'. Three abstracts that never materialized into papers or other detailed scientific output were also found. Similar products which might be sold elsewhere under different names were also not searched in this study. (ii) Some experts suggested that reports might be hidden under confidentiality agreements. (iii) There were some quality issues in the work covered in this study, where our analysis found numerous design, protocol defects, and lack of blinding which risks bias. Nevertheless, these studies were mostly conducted to the standards prevailing at their inception.

While most previous research focused on animal studies, human clinical trials using clearly defined symptomology to test the therapeutic value of Yahom are needed. The findings of this study suggest that a fundamental rethink is needed in clinical Yahom research by: (i) Quantifying real objectively assessable metrics and placebo effects. (ii) Recruiting participants who use a Yahom preparation to treat a well-defined symptom using a rigorously designed placebo-controlled randomized clinical trial with amelioration of the symptom as the primary endpoint. Dizziness in post-menopausal women might be a start, where the appropriate end-point might be postural hypotension. (iii) Characterise this symptomatic relief in physiological terms using appropriate non-invasive testing methods (e.g., hypovolemia, hypoglycemia, autonomic dysfunction, vaso-vagal activation, sinus dysfunction, and well-being). The latter cannot be ignored because of Thai traditional medicine's holistic approach to treatment which may contribute to clinical outcomes. (iv) Using HPLC to detect plasma compounds after consuming the medication and LC-MS to identify the likely chemical structures. (v) Using animals is appropriate only when a good model for the disease is available and is treated with the native Yahom formulation as used by human patients. (vi) Quality control of Yahom preparation is required and all species of plant material used need to be validated taxonomically, or traceable. Above all, there needs good coordination between studies thus permitting data pooling.

Although we focus here on experimental studies, the most valuable toxicity data comes from pharmacovigilance. Therefore, adverse events of herbal medicines should be entered into the national reporting systems like any other medicine and the simplest way of

effecting this would be through mandatory product-labelled and verbal instruction, and made a pre-requisite of registration.

Thus, the proposals which have emerged from this review might provide a model for redefining research into traditional medicines. Finally, although this present work studied a product sold in one country, the discordances identified here have wider implications and are equally applicable to other traditional medicines.

5. Conclusions

This systematic review identifies limitations of studies into the pharmacological characterization of Yahom. The included studies have some scientific rigor, yet are frustrated by their lack of fundamental therapeutic, pharmacological and pharmacokinetic clinical data relevant to Yahom as a herbal medicine to holistically treat specific symptoms. Instead, there needs a fundamental research re-alignment by:

(i) Running, rigorously designed clinical trials where studies on participants experiencing relevant symptoms/pathologies
(ii) Institute rigorous pharmacovigilance programs
(iii) Underpin with later 'preclinical' and pharmacokinetic studies to improve product efficacy and safety.
(iv) Identify the orally active ingredients in humans and weed out the non-essential components

The model that emerged here provides a template for future rational research strategies in optimizing traditional medicines in general.

Abbreviations

2-AA: 2-aminoanthracene, Ach: Acetylcholine, AF2: 2-aminofluorene, Aq: Aqueous, BMI: Body mass index, BP: Blood pressure, BUN: Blood urea nitrogen, CYPs: Cytochromes P450, DBP: Diastolic blood pressure, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ECG: Electrocardiogram FRAP assay: Ferric reducing antioxidant power assay, HPLC: High Performance Liquid Chromatography, HR: Heart rate, IC_{50}: Inhibitory concentration that cause 50% inhibition, ID_{50}: Inhibitory dose that cause 50% inhibition, IP: Intraperitoneal injection, LCMS: Liquid chromatography mass spectrometry, LDL: Low density lipoprotein, LD_{50}: Lethal dose required to kill 50% of the animal, MAP: Mean arterial blood pressure, NA: noradrenaline, NOAEL: No observed adverse effect level, 4-NQO: 4-nitroguinolene–1-oxide,

rCBF: Regional cerebral blood flow, SBS: Systolic blood pressure, SGOT: serum glutamic oxaloacetic transaminase , SGPT: serum glutamic pyruvic transaminase, TRABS: Thiobarbituric acid reactive substances, Wt: weight.

Authors' contributions

NC, AF, and KC designed the study. NS provided information of Yahom ethnopharmacology and sources of Yahom research. AF and KC made substantial contribution to data review, extraction, assessment and interpretation as well as drafting and revising manuscript. NC reviewed and interpreted the data, drafted and revised manuscript. CNS extracted the data and critically revised manuscript. All authors approved the final manuscript.

Competing interests

All authors declare that they have no conflicts of interest to disclose.

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Appendix A. Supplementary materials

Ingredients of commonly used Yahoms

Most of Yahom formulae include some of the following plants i.e., *Angelica dahurica* Benth., *Atractylodes lancea* (Thung.) DC., *Ligusticum chuanxiong* Hort**.**, *Angelica sinensis* (Oliv.) Diels, *Artemisia annua* L., *Saussurea lappa* C.B.Clarke, *Picrorrhiza kurroa* Royle. ex Benth., *Terminalia chebula* Retz., *Nardostachys jatamansi* **(**D**.** Don**)** DC., *Nigella sativa* L.*, Cuminum cyminum* L., *Lepidium sativum* L., *Foeniculum vulgare* Mill. var. *dulce* Alef, *Trachyspermum ammi* (L.) Sprague, *Anethum graveolens* L., *Pimpinella anisum* L., *Plantago ovata* Forssk, *Carum carvi* L.. In addition fragrant plants are also common to all Yahom preparations e.g. *Aquilaria crassna* Pierre ex Lecomte, *Mesua ferrea* L., *Mimusops elengi* L., *Cinnamomum loureirii* Nees

Five pagodas: 100 g contains

Agastache rugosa (Fisch. et Mey) O. Kuntze (whole plant, Lamiaceae) 7.1 g, *Acorus gramineus* Sol. ex Aiton (rhizomes, Araceae) 3.5 g, *Lysimachia foenum-graecum* Hance (whole plant, Primulaceae) 3.3 g, *Citrus nobilis* Lour. (outer yellow rind of the ripe fruit, Rutaceae) 7.1 g, *Magnolia officinalis* Rehd. et Wils. (bark of stem, Magnoliaceae) 11.8g, *Cinnamomum cassia* Presl (Chinese cinnamon, bark, Lauraceae) 7.1 g, *Mentha arvensis* L. (Japanese mint, whole plant, Lamiaceae) 3.5 g, *Asarum sieboldii* Miq. (whole plant, Aristolochiaceae) 2.3 g, *Ligusticum wallichii* Franch. (rhizomes, Apiaceae) 9.3 g, *Glycyrrhiza glabra* L. (licorice, rhizomes, Fabaceae) 4.8 g, *Eugenia caryophyllata* Thunb. (clove, flower-bud, Myrtaceae) 7.1 g, *Saussuria lappa* Clark (rhizomes, Asteraceae) 7.1 g, *Aquilaria agallocha* Roxb (wood, Thymelaeaceae) 7.1 g, *Atractylis ovata* Thunb. (rhizomes, Asteraceae) 9.3 g, menthol 4.7 g, *Borneo camphor* 1.4 g, *Angelica anomala Lallem* (rhizomes, Apiaceae) 3.5 g.

Navagot (54 plants):

Amomum testaceum Ridl., *Aquailaria crassna* Pierre ex Lecomte, *Gymnopetalum chinense* (Lour.) Merr., *Euphorbia antiquorum* L., *Syzygium aromaticum* (L.) Merr. et L.M. Perry, *Saussurea lappa* C.B.Clarke, *Anacyclus pyrethrum* (L.) Lagasca, *Picrorhiza kurroa* Royle ex Benth., *Atractylodes lancea* (Thung.) DC., *Astemisia annua* L., *Angelica sinensis* (Oliv.) Diels, *Terminalia chebula* Retz., *Angelica dahurica* Benth., *Ligusticum sinense* Oliv. cv. Chuanxiong, *Mimusops elengi* L., *Zingiber officinale* Roscoe, *Aristolochia pierrei* Lecomte, *Dracaena loureiri* Gagnep, *Myristica fragrans* Houtt, *Santalum album* L., *Plumbago indica* L., *Alyxia reinwardtii* Blume, *Glycyrrhiza glabra* L., *Piper sarmentosum* Roxb, *Piper chaba* Hunt, *Plantago ovata* Forssk, *Cuminum cyminum* L., *Foeniculum vulgare* Mill. var *dulce* Alef, *Nigella sativa* L., *Lepidium sativum* L., *Anethum graveolens* L., *Trachyspermum ammi* (L.) Sprague, *Pimpinella anisum* L., *Tinospora crispa* (L.) Miers ex Hook. f. & Thomson, *Nelumbo nucifera* Gaertn, *Mesua ferrea* L., *Kaempferia galangal* L., *Coriandrum sativum* L., *Vetiveria zizanioides* (L.) Nash ex Small, *Mimusops elengi* L., *Phyllanthus emblica* L.,*Jasminum sambac* (L.) Aiton, *Brucea javanica* (L.) Merr, *Pinus* sp.,*Terminalia bellirica* (Gaertn.) Roxb., *Cinnamomum bejolghota* (Buch.-Ham.) Sweet, *Piper ribesoides* Wall, *Dalbergia parviflora* Roxb, *Sophora tomentosa* L., *Mammea siamensis* Kosterm, *Mollugo pentaphylla* L., *Cyperus rotundus* L., *Cinnamomum loureirii* Nees.

Phikud Navagot: main herbs from Navagot in equal amounts (each 11%):

"Kot Soa" (root of *Angelica dahurica* (Fisch). Benth & Hook f., family Apiaceae), "Kot Khamao" (rhizome of *Atractylodes lancea* (Thunb.) DC., family Asteraceae), "Kot Hua Bua" (rhizome of *Ligusticum chuanxiong* Hort., family Apiaceae), "Kot Chiang" (root of *Angelica sinensis* (Oliv.) Diels, family Apiaceae), "Kot Chulalumpa" (aerial part of *Artemisia vulgaris* L., family Asteraceae), "Kot Kradook" (rhizome of *Saussurea costus* (Falc.) Lipsch.,

family Asteraceae), “Kot Kan-Prao” (rhizome of *Picrorhiza kurrooa* Royle ex Benth., family Scrophulariaceae), “Kot Pung Pla” (gall of *Terminalia chebula* Retz., family Combretaceae) and “Kot Jatamansi” (root and rhizome of *Nardostachys jatamansi* (D. Don) DC., family Valerianaceae).

Amanthong: Main ingredients as labelled*:*

Conioselinum univilatum (selinum) (7.45%), *Syzygium aromaticum* (clove) (7.45%), *Cinnamomum verum* (cinnamon) (7.45%), *Mesua ferrea* (Bun-nak) (7.45%), *Nelumbo nucifera* (lotus) (7.45%), *Glycyrrhiza glabra* (licorice) (7.45%), *Myristica fragrans* (mace) (0.74%), *Pogostemon cablin* (phim-sen) (1.49%), *Viverricula indica* (0.09%)

Intajak:

Alyxia reinwardtii Blume*, Amomum testaceum* Ridl.*, Anacyclus pyrethrum* (L.) Lagasca*, Angelica dahurica* Benth.*, Angelica sinensis* (Oliv.) Diels*, Aquilaria gallocha* Roxb.*, Aristolochia pierrei* Lecomte*, Artemisia annua* L.*, Atractylodes lancea* (Thung.) DC.*, Bixa orellana* L.*, Caesalpinia sappan* L.*, Cananga odorata* (Lam.) Hook. f & Thomson var. *odorata, Cinnamomum bejolghota* (Buch.-Ham.) Sweet*, Cinnamomum verum* J. Presl*, Coriandrum sativum* L.*, Cuminum cyminum* L.*, Dracaena loureiri* Gagnep.*, Enhalus acoroides* (L.f.) Royle*, Euphorbia antiquorum* L.*, Foeniculum vulgare* Mill. var *dulce* Alef*, Gymnopetalum chinense* (Lour.) Merr.*, Jasminum sambac* (L.) Aiton*, Lepidium sativum* L.*, Mammea siamensis (*Miq.*)* T. Anderson*, Mesua ferrea* L.*, Michelia champaca* L.*, Mimusops elengi* L.

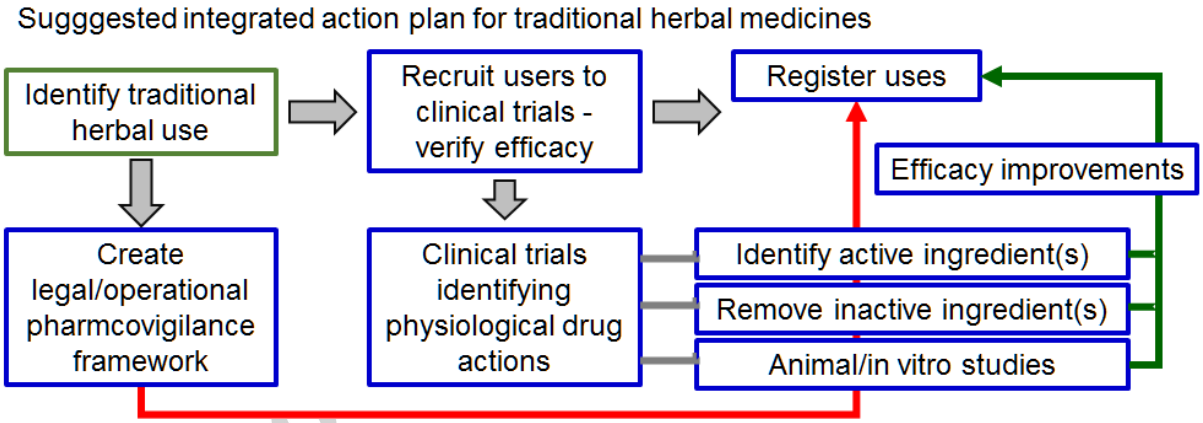

Table 1 Effects of Yahom on the Cardiovascular System

References	**Animal & Study Model**	**Yahom brand & Preparation***	**Concentration/Dose, Route of administration and Duration of treatment**	**Protocols & Outcome parameter measured and Technique used**	**Basic pharmacological data**
***In vitro* studies**					
Pataloong & Sawasdimongkol, 1995	Male Wistar rats, n=8-12/group *In vitro* study	Four un-named, brands, contents	Yahom extract solution 0.25-1 mg/ml (5 min) alone or with acetylcholine (Ach) or	Force and rate of atrial contraction to Yahom extract solution using	Two brands at concentration 1 mg/ml - slow onset with

References	Animal & Study Model	Yahom brand & Preparation*	Concentration/Dose, Route of administration and Duration of treatment	Protocols & Outcome parameter measured and Technique used	Basic pharmacological data
	using isolated atria	stated. Hot water extract solution	isoproterenol	organ bath technique	positive ionotropic action and antagonised Ach effect One brand - no effect. Another brand - negative chronotropism
Suvitayavat et al., 2005b	Male Wistar rats, n=8-10/group *In vitro* study using isolated aorta and atria	Un-named brand, contents stated. Lyophilised water extract (then dissolved in water)	Yahom extract solution 0.83, 1.67, 8.33, 16.67 and 83.33 mg/ml	Contraction of isolated aortic ring to Yahom extract solution using organ bath technique	Vasoconstriction of aortae via alpha receptors with maximal response at concentration 1.7-17 mg/ml.
				Force and rate of atrial contraction to Yahom extract solution using organ bath technique	Increased force but reduced rate of atrial contraction at concentration > 0.83 mg/ml
Nusuetrong et al., 2012	Male Sprague Dawley rats, n=5-6/group *In vitro* study using isolated aorta	Phikud Navagot, Ethanolic extract (then dissolved in DMSO)	Yahom solution 0.1-300 μg/ml, direct action or pretreatment (30 min)	Vasorelaxation of isolated aortic ring to Yahom solution and its pretreatment (100 μg/ml, 30 min) on carbachol, sodium nitroprusside or methoxamine responses using organ bath technique	Vasorelaxation with pEC_{50} ~ 4 μg/ml, max response ~ 70% (unaffected by pretreatment with N^G-nitro L-arginine methyl ester (LNAME) or indomethacin). Yahom pre-treatment decreased endothelium-dependent carbachol vasorelaxations
***In vivo* studies**					
Matankasombat, 1974	Rats and rabbits *In vivo* study	Two un-named brands Raw powder	Oral 500 mg/kg/day (12-21 days)	Blood pressure (BP) and heart rate (HR) measured via cannulation of femoral artery	Positive chronotropic action in rats but not rabbits. No effect on BP. No morphological

References	Animal & Study Model	Yahom brand & Preparation*	Concentration/Dose, Route of administration and Duration of treatment	Protocols & Outcome parameter measured and Technique used	Basic pharmacological data
				Gross anatomy and size of liver, spleen, and heart were also determined	changes (undefined).
				Acute cardiac studies *in vivo* Isolated atria to measure force and rate of contraction induced by noradrenaline (NA), adrenaline, isoproterenol, Ach	Doses and reporting confused, underpowered, no data analysis. Could not make conclusions.
Suvitayavat et al., 2005a	Male Wistar rats, n=8-12/group *In vivo* study using normal anesthetised rats	Five Pagodas, Lyophilised water extract (then dissolved in water)	Intravenous injection (IV) 0.2-0.8 g/kg	Mean arterial blood pressure (MAP) measured via cannulation of femoral artery	Transient decreased in MAP (max effect at 0.4 g/kg, duration 18-36 s) followed by long lasting increased in MAP at dose > 0.6 g/kg (duration 1-30 min depending on dose)
Jariyapongskul et al., 2006	Male Wistar rats, n=5-8/group *In vivo* study using normal anesthetised rats	Un-named brand, extract and formulation not stated Prepared by dissolving it in 5% Tween solution for oral administration and in 5% Tween plus artificial cerebrospinal fluid for superfusion on the pial cerebral cortex	Single oral doses 2 or 4 g/kg.bw	MAP measured via cannulation of femoral artery and regional cerebral blood flow (rCBF) measured using laser Doppler flow meter after 0, 5, 15, 30, 45, 60, 90, & 120 min.	Increased MAP with max response at 45 min (8% increase for 2 g/kg.bw and 16% increase for 4 g/kg.bw) and still 5% increase at 120 min Increased rCBF with max response at 15-30 min (21% increase for 2 g/kg.bw and 32% increase for 4 g/kg.bw) then back to baseline at 120 min
			Dose 4 g/kg.bw was dissolved in 10 ml 5% Tween solution, and then diluted in ACSF (ACSF; compositions: NaCl 118.0, KCl 4.0, $MgSO_4$ 1.2, $CaCl_2$	Videometric cerebral arteriole diameter after NA preconstriction, topical Yahom application to pial surface	Vasodilatation in pial microvessels (56% increase in arteriolar dilation compared to NA preconstriction)

References	Animal & Study Model	Yahom brand & Preparation*	Concentration/Dose, Route of administration and Duration of treatment	Protocols & Outcome parameter measured and Technique used	Basic pharmacological data
			1.5, NaH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 5.0 in mM) with a concentration of 1:100	(concentration unclear)	
Kengkoom et al, 2015	Male Sprague Dawley rats, n=2-5/group *In vivo* study, cardio-protection after ischemia/ reperfusion (I/R)	Phikud Navakot, Ethanolic extract, Dried + filler	Yahom extract 0, 10, 50,100, 200, 400 mg/kg/day orally (7 days)	Yahom extract give orally 30 min left coronary occlusion, then released for 24 hr then measured: Infarct size (not quantified), plasma troponin1, lactate dehydrogenase, myocardial eNOS, HO1, ERK1/2, AKT, BAX/BCL2, blood biochemistry	Controls too small (n=2 for IR alone) to make conclusions. Limited data suggest Yahom might promote myocardial damage. Blood biochemistry stable except all animals (inc shams) very hyperglycaemic.
Nernpermpisooth et al., 2015	Male Wistar rats, n=10-15/group *In vivo* chronic study	Un-named brand, contents stated. Lyophilised water extract (then dissolved in water)	Yahom extract 0, 1, 2.5, 5.0 g/kg/day orally (8 weeks)	Systolic blood pressure (SBP) and HR measured in conscious rats using tail-cuff plethysmography	Acute increase of SBP and HR (~3%, at 15-45 min) after the first dose of 2.5, 5 g/kg/day At week 8, SBP increased (~3%) in all groups compared to baseline and no change in HR. No difference between control and treatment group. (Note, base-line BP high in all groups (~138-143 mmHg)).
				Peripheral blood flow of hind paw skin measured in anesthetised rats using laser Doppler flowmetry	Acute increase of blood flow (~10% for 2.5 g/kg/d at 30 min and ~30% for 5 g/kg/d at 15-90 min)
			Yahom extract solution 1-50 mg/ml in organ bath	Contraction of aortic rings isolated from control and 5 g/kg/day group: measured contractile	NA sensitivity of aorta increased in Yahom chronic treatment group. Yahom (1-50 mg/ml) induced aortic ring

References	Animal & Study Model	Yahom brand & Preparation*	Concentration/Dose, Route of administration and Duration of treatment	Protocols & Outcome parameter measured and Technique used	Basic pharmacological data
				responses to Yahom and NA using organ bath technique	contraction (higher in chronic Yahom treated group compared to control) Yahom (10 mg/ml) reduced NA-induced contraction (~10% in both groups).

*See supplement material for available details of Yahom ingredients as it was stated in each reference

Table 2 Effects of Yahom on Gastrointestinal System

References	Animal & Study Model	Yahom brand & Preparation *	Concentration/Dose, Route of administration and Duration of treatment	Protocols & Outcome parameter measured and Technique used	Basic pharmacological data
***In vitro* study**					
Chantharangsikul et al., 2009	Male mice, n=6-10/group *In vitro* study using isolated whole stomach	Five Pagodas Lyophilised water extract	Extract 2.5, 5, 10, 20 mg/ml. (Concentration referred as powder) applied to serosal side (120 min)	Extract solution applied to serosal side then measured gastric acid secretion induced by histamine or bethanechol	Acid secretion dose dependently inhibited by Yahom (ID_{50} ~3 mg/ml). Atropine 50% or ranitidine 100% lower secretion.
***In vivo* studies**					
Intayoong, 2006	Male Wistar rats, n=6/group *In vivo* study	Ya-Hom powder, un-named but contents listed. Boiling water extract.	Yahom 1, 2, 4 g/kg (Dose referred as powder) Acute (4-6 hr) or Chronic (4 days)	Acute effect: Oral administration of – water, vehicle, Yahom, or cimetidine (100 mg/kg). +30 min gastric ulcer induction by: HCl (0.6N), aspirin (200 mg/kg), or stress induction by cold water-immersion. Rats were terminated at 4-6 hr, then measured lesion area and visible mucus secretion	All Yahom doses and cimetidine reduced all lesions, strongly for 2, 4 g/kg Yahom, and cimetidine. Yahom (4 g/kg) attenuated mucus secretion.
				Chronic effect: water-stress, then oral administration of 4 g/kg/day Yahom, cimetidine, or sucralfate for 4 days	Day 1 & 4, only Yahom protected in water-stress (gastric ulcers decreased by 60%).
Suvitayavat et	Male Wistar rats,	Five Pagodas,	Intraduodenal injection of Yahom	Intraduodenal injection of	Yahom increased visible mucus

al., 2004	n=10/group *In vivo* study using normal anesthetised rats	Lyophilised Water extract	0.5-4 g/kg	Yahom on secretion rates of gastric: blood flow, pepsin, acid, protein and soluble and visible mucus via gastric fistulae after histamine- or carbachol-induction	secretion, but decreased all other parameters dose-dependently

*See supplement material for more detail of Yahom ingredients

Table 3 Toxicity and drug metabolism study of Yahom

References	**Animal & Study Model**	**Yahom brand & Preparation***	**Concentration/Dose, Route of administration and Duration of treatment**	**Protocols & Outcome parameter measured and Technique used**	**Basic pharmacological data**
***In vitro* studies**					
Sripanidkulchai et al., 2007	*Salmonella typhimurium* TA98 and TA100	Navagot, Intarajuk, Prasarthong, Koklan and Sahasthara, Hexane pre-extraction, 95% ethanol and/or water extract	Yahom extract solution 1-10 mg/ml (Dissolved in DMSO) (48 h incubation)	Mutagenicity using *Salmonella*/microsome mutagenicity Test (Ames test) and antimutagenicity Tested native extract, extract after rat liver enzyme or S-9 digestion.	No mutagenic activity. All extracts showed antimutagenisis at ~1 mg/ml against mutagens, 2-aminoanthracene (2-AA), 2-aminofluorene (AF2), and 4-nitroguinolene-1-oxide (4-NQO).
Tepsuwaan et al., 2011	*Salmonella typhimurium* Human hepatoma cell-line (HepG2)	Fifty Thai traditional medicines - 11 were Yahoms. MeOH extraction	5-20 mg (Dissolved in DMSO) (48 h incubation)	Mutagenicity using *Salmonella*/microsome mutagenicity Test (Ames test)	One Yahom sample showed mutagenic activity.
			0.2-1 μg/ml (Dissolved in DMSO) (24 h incubation)	Genotoxicity on HepG2 by Comet and binucleation	Two Yahoms were genotoxic.
Channarong et al., 2012	Cell-free study	Five un-named brands i.e., A, B, C, D, E Water extract	1 g (Dried weight)	Analysis of total phenolic compounds of Yahom powder and antioxidant capacity of Yahom using FRAP assays	Fairly high amounts of total phenolic compounds and high FRAP values Brand E ~3-fold higher contents than A-D
Nalinratana et al., 2014	Cell-free	Phikud Navagot, 50% EtOH or Water extracts	200 mg/ml (Stock solution)	Intracellular ROS scavenging & specific reactive species	Herbal mixture more effective than sum of individual constituents
	Endothelial cell line		200 mg/ml (Stock solution)	Cytotoxicity Genotoxicity	IC_{50} ~500 μg/ml No gentoxicity up to 600 μg/ml
Tuekaew et al., 2014	Human blood Cell-free	Intajak and 47 individual components, 80% Ethanol extract: subfractions	20-100 μg/μl (For DPPH assay) 400-800 μg/ml (For FRAP assay)	Antioxidant effect study using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity and ferric reducing	Moderate DPPH scavenging activity: IC_{50} ~100 μg/ml and a FRAP equivalent of 1.12 mmol

References	Animal & Study Model	Yahom brand & Preparation*	Concentration/Dose, Route of administration and Duration of treatment	Protocols & Outcome parameter measured and Technique used	Basic pharmacological data
		into n-hexane or dichloromethane		antioxidant power (FRAP) assays	FeSO4/g. Not clear how this translates into treatments.
			0.08-1.25 μg/μl	Inhibition of human LDL peroxidation by thiobarbituric acid reactive substances (TBARS) assay	Dichloromethane fraction had highest inhibitory effect on LDL peroxidation. Not clear how this relates to *in vivo* actions in humans
***In vivo* studies**					
Thongpraditchote et al., 1999	Male & female Wistar rats and mice, n=6/group *In vivo* study	5 Un-named brands, Compositions stated Dissolved in distilled water	Oral single dose at 5 g/kg or intraperitoneal injection (IP) single dose at 1, 2, 5 g/kg Oral doses 0.5, 1, 2 g/kg/day for 5 weeks	Acute toxicity via oral or IP injection single dose, termination after 2 weeks Subacute toxicity oral doses for 5 weeks	LD_{50} > 5 g/kg No change in body weight and %haematocrit compared to control Some Yahom formulas reduced visceral organ weight. No change in body weight, %haematocrit, SGOT, SGPT, BUN, and histology of visceral organs compared to control
Chavalittumrong et al., 2009	Male/ female mice, n=10/group *In vivo* acute toxicity	Navagot, Hexane and 95% alcohol extract, then boiled in water 30 min then filtered and dissolved with water before use	Oral Yahom 2, 4, 8 or 16 g/kg/day (14 days)	Acute toxicity of oral Yahom	2 or 4 g/kg/day had no toxicity, 8 or 16 g/kg/day produced 10% or 70% mortality.
	Male/female Wistar rats, n=24/group *In vivo* chronic toxicity		Oral Yahom 10, 100, 500, 1000 or 1000 mg/kg/day (6 months)	Chronic toxicity of oral Yahom	No effect on body weight, food consumption, behaviour, general health and clinical chemistry values.

References	Animal & Study Model	Yahom brand & Preparation*	Concentration/Dose, Route of administration and Duration of treatment	Protocols & Outcome parameter measured and Technique used	Basic pharmacological data
	study				1000 mg/kg: increase in total leukocytes and platelet counts in male and female rats, respectively. Tended to recover 2 weeks after Yahom discontinuation.
Sirisangtrakul & Sripanidkulchai, 2011	Male mice, n=6-16/group *In vivo* study using normal rats	Ampanthong, CH_2Cl_2, MeOH or H_2O extracts	Oral each Yahom extract 1.2, 3, 5 mg/kg/day (4 weeks)	Effect of oral Yahom extract on hepatic CYP1A1, CYP1A2, CYP3A4, CYP2B and CYP2E1	All extracts inhibited CYP1A1, CYP1A2 and CYP2E1. CH_2Cl_2 & MeOH extracts enhanced CYP2B activity.
			IP Yahom extract 2 g/kg	Effect of IP Yahom i.e., CH_2Cl_2 extract on pentobarbital-induced sleeping time	Decreased pentobarbital-induced sleeping time.
Kengkoom et al, 2012	Male/female rats, n=6/group *In vivo* chronic toxicity testing	Phikud Navagot (9 herbs) 80% EtOH extract. Yield ~22% of dried powder	Oral Yahom extract 0, 10, 100, 1000 mg/kg/day (90 days)	Yahom extract for 90 days, +/-14 day recovery. Monitored daily. Body weight Histology major organs, full blood chemistry, haematology	Histology, blood chemistry - no changes with all doses of Yahom. Small, sporadic reductions in food intake and weight reductions. Increased uric acid (also in ref 39). Some hyperglycaemia but not in ref 39.
	Female rats, n=3/group Acute toxicity testing		Oral Yahom extract 2 g/kg (24 hr or 14 days)	2 g/kg 24hr acute, observed every 2 hr then daily for 14 days	Weight gain similar to chronic controls No changes up to 14 days
Sirisangtragul & Sripanidkulchai, 2013	Male mice, n=3-14/group *In vivo* study	Tultavai, CH_2Cl_2, MeOH or H_2O extracts	Oral each Yahom extract 1.2, 3, 5 mg/kg	Effect on: hepatic CYP1A1, CYP1A2, CYP3A4, CYP2B and CYP2E1	All extracts inhibited CYP1A1, CYP1A2 and CYP2E1. CH_2Cl_2 extract

References	Animal & Study Model	Yahom brand & Preparation*	Concentration/Dose, Route of administration and Duration of treatment	Protocols & Outcome parameter measured and Technique used	Basic pharmacological data
	using normal rats				enhanced CYP2B activity.
			IP Yahom extract 2 g/kg	CH_2Cl_2 extract on pentobarbital sleeping time, compared with ethyl-p-methoxycinnamate	Decreased pentobarbital-induced sleeping time by 75%
Kengkoom & Ampawong, 2015	Sprague Dawley rats, n=10/group Animal specification not stated *In vivo* study	Phikud Navagot (9 herbs) 80% EtOH extract	Yahom extract 10, 100, 1000 mg/kg/day (12 months)	Effect of Yahom extract on histology major organs, full blood chemistry, haematology and renal histochemistry.	No differences between any of the groups for all measures, except marked bilateral mesangiolysis at 1000 mg/kg/day with lowered aquaporin 1 expression and increased heat shock protein. But renal excretion maintained. Estimated NOAEL=100 mg/kg

*See supplement material for more details of Yahom ingredients

Table 4 Effect of Yahom in Human

References	Study Design	Participants	Yahom brand/ preparation	Interventions	Outcome Parameters	Basic pharmacological data
Suvitayavat et al., 2005	3-arm cross-over	15 healthy females Age 20-23y BMI 18-23 kg/m^2	Five Pagodas, powder, or Resuspended lyophilised water extract	1.Water only 2.Single dose of Yahom powder (3 g) suspended in 50 ml 35^{o}C water 3.Single dose of lyophilized water extract Eq to 3g powder of Yahom powder Measured every 5 min for 60 min	ECG	Neither Yahom preparation affected ECG or heart rate
					BP	**Within-group (compared to time=0)** *Ya-hom Powder:* Increased DBP by ~2mmHg at 10, 20, 30, 50 and 60 min compared to time 0 ($p < 0.05$) Increased MAP at 10, 30 and 50 min from 0 min ($p < 0.05$) Decreased pulse pressure at 60 min from 0 min ($p < 0.05$) *Lyophilised water extract of Ya-hom:* Increased MAP at 60 min compared with 0 min ($p < 0.05$) **Yahom preparations compared with control** No differences for any BP parameter
					Forearm cutaneous blood flow	No change in forearm cutaneous blood flow

	Adverse reactions	No comments

ECG = Electrocardiogram; BMI = body mass index; BP = blood pressure; DBP=diastolic blood pressure; MAP = mean arterial blood pressure

Table 5 Quality assessments of *in vivo* animal studies on Yahom action.

Domain	*Matankasombat, 1974*	*Thongpraditchot et al., 1999*	*Suvitayavat et al., 2004*	*Suvitayavat et al., 2005a*	*Jariyapongskul et al., 2006*	*Intayoong, 2006*	*Chavalittumrong et al., 2009*	*Sirisangtrakul and Sripanidkulcha, 2011, 2013*	*Kengkoom et al., 2012*	*Nernpermpisooth et al., 2015*	*Kengkoom and Ampawong, 2015*	*Kengkoom et al., 2015*
Allocation sequence adequately generated?	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Was allocation adequately concealed?	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Blinding: allocated intervention concealed during study?	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Inclusion/exclusion criteria stated?	No	No	No	No	No	No	No	No	No	No	No	No
Calculation or justification for sample size provided?	No	No	No	No	No	No	No	No	No	No	No	No
Complied with animal ethics/welfare regulations?	NR	NR	Yes	NR	NR	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Conflict of interest statement & funding source given?	Yes/No	Yes	No	No	No	No	No	No	Yes	Yes	Yes	Yes
Protocols/methods enough to repeat experiments	No	Yes	Yes	Yes	Yes	Yes/No	Yes	Yes	Yes	Yes	Yes	Yes
Statistical tests appropriate for study design?	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Animals had appropriate comorbidity? (a)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Test animal characteristics stated?	No	Yes	Yes	Yes	Yes	Yes	NA	Yes	Yes	Yes	Yes	Yes
Statement about dose-response model?	No	No	No	No	No	No	Yes	No	No	No	No	No
Details about why animals were removed from study?	No	No	NA	No	No	Yes	No	Yes	Yes	No	No	No
Intervention duration enough to assess outcome?	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Product name, supplier, batch, etc.	No	No	Yes/No	Yes/No	No	No	Yes	Yes/No	Yes	No	Yes/No	Yes/No

Herbal composition adequately characterized & tested	No	Yes	Yes	Yes	No	Yes	No	Yes	No	Yes/No	No	No
Taxonomic validation of medicinal plant composition	No	No	No	No	No	No	Yes	No	No	No	No	No
Yahom source/brand stated	No	No	Yes	No	Yes	No	No	Yes	Yes	No	No	No
Dosage range, method of administration	Yes/ No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Suitable placebo/negative control	No	No	No	No	No	No	No	No	No	No	No	No
Positive control included	Yes	NA	No	Yes	No	Yes	NA	No	NA	No	NA	No

NR = no report; NA = not applicable; These were marked as “NA” because Yahom actions in humans is unclear or study was toxicological.

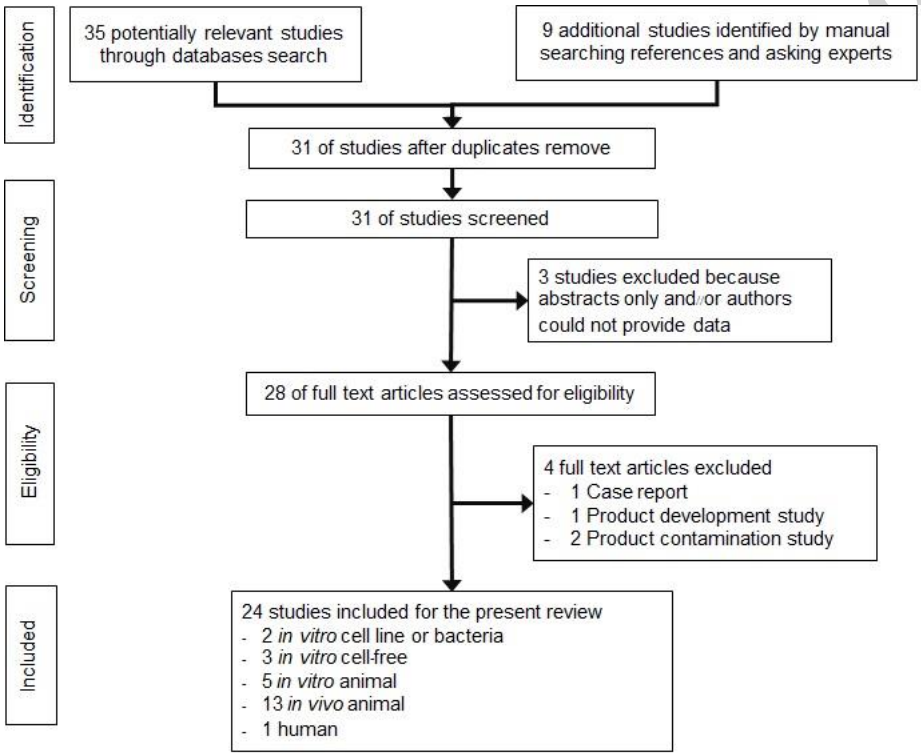

Figure 1. The flow diagram for study identification, inclusion and exclusion

Sugggested integrated action plan for traditional herbal medicines