

DOCUMENTATION AND EVALUATION OF MEDICINAL PLANTS WITH
POTENTIAL FOR BREAST CANCER THERAPY AS USED BY JAKUN
COMMUNITY OF KAMPUNG PETA, MERSING, JOHOR

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ABSTRACT

Breast cancer (BC) is the most commonly diagnosed cancer and the leading cause of cancer-related death among women globally. Traditional knowledge (TK) could be a means for the development of new BC treatment; however, TK is perishing due to socialisation and lack of natural resources conservation. The research aimed to document the TK of medicinal plants used for the treatment of breast cancer by Jakun community of Kampung Peta, Mersing Johor; to examine the secondary metabolites, the antioxidant and cytotoxic effect of the selected plants extract. Documentation of TK was analysed qualitatively from semi-structured interviews. The six species (*Artocarpus. altilis*, *Dioscorea bulbifera*, *Morinda citrifolia*, *Pinanga limosa*, *Ipomoea aquatica* and *Dianella ensifolia*) from six families and six genera were documented. They were evaluated for their antioxidant properties using FRAP, ABTS and DPPH assays and the effects of the plant's extracts on the proliferation of MCF-7 and MDA-MB-231 cell lines were evaluated using MTT assay, cell cycle arrest and apoptosis. GC-MS was used to identify active the compounds. Findings showed that *A. altilis* have the highest antioxidant capacity and *M. citrifolia*, the least. *A. altilis* methanol extract (AAM), *D. bulbifera* methanol extract (DBM) and *P. limosa* methanol extract (PIM) were found to induce cytotoxicity against MCF-7 and MDA-MB-231 with the IC₅₀ value range of 3.27 - 41.70µg/ml. Cell cycle analysis showed that the plant's extracts induced significant arrest at Sub G₁ and G₂/M at 24 hrs of treatment. Afterwards, 48 and 72 hrs of treatment, the proportion of cells in other phases had reduced significantly as compared to their control. Apoptosis occurred during the first 24 hrs and increased dramatically after 48 and 72 hrs of treatment. The GC-MS analysis revealed the presence of phytol, acetic acid and hexadecanoic acid among the major metabolites that might contribute to the anti-proliferative effect. The studies scientifically justified the use of the selected medicinal plant species by Jakun people. Further studies are necessary for detailed chemical characterisation and broader biological evaluation of the most active ingredients.

ABSTRAK

Kanser payu dara (KPD) ialah kanser paling umum didiagnosis dan sedunia merupakan penyebab kematian berkaitan kanser di kalangan wanita. Pengetahuan tradisional (PT) mungkin boleh menjadi salah satu cara untuk mengembangkan rawatan KPD yang baru; sayangnya PT kini mula berkurangan disebabkan sosialisasi serta berkurangnya pemuliharaan sumber asli. Kajian ini bertujuan untuk mendokumenkan PT tumbuhan ubatan yang digunakan oleh masyarakat Jakun dari Kampung Peta, Mersing, Johor dalam perawatan KPD. Di samping itu kajian juga memeriksa metabolit sekunder, antioksidan dan kesan sitotoksik daripada ekstrak tumbuh-tumbuhan terpilih. Pengdokumentasian PT telah dianalisis secara kuantitatif daripada temubual. Enam spesies tumbuhan (*A. altilis*, *D. bulbifera*, *M. citrifolia*, *P. limosa*, *I. aquatica* dan *D. ensifolia*) mewakili enam famili dan enam genus telah didokumenkan. Tumbuh-tumbuhan ini telah dinilai sifat-sifat antioksidannya menggunakan esei FRAP, ABTS dan DPPH. Kesan ekstrak tumbuhan terhadap proliferasi MCF-7 dan garisan sel MDA-MB-231 telah dinilai menggunakan esei MTT, perencatan kitaran sel dan apoptosis. GC-MS telah digunakan untuk mengenalpasti kompaun-kompaun aktif. Dapatan menunjukkan *A. altilis* mempunyai keupayaan antioksidan tertinggi sedang *M. citrifolia* terendah. Ekstrak methanol *A. altilis* (AAM), ekstrak methanol *D. bulbifera* (DBM) dan *P. limosa* (PIM) didapati menginduksi sitotoksik terhadap garisan sel dengan nilai IC_{50} dalam julat 3.27–41.70 $\mu\text{g/ml}$. Analisis kitaran sel menunjukkan bahawa ekstrak tumbuhan mengindus perencatan yang bererti di Sub G_1 dan G_2/M pada 24j rawatan. Selepas itu pada 48 dan 72j rawatan, nisbah sel-sel dalam fasa-fasa lain telah berkurang secara bererti apabila dibanding dengan kawalan masing-masing. Apoptosis berlaku dalam tempoh 24j dan meningkat secara mendadak selepas 48 dan 72j rawatan. Analisis GC-MS menunjukkan kehadiran fitol, asid asetik, dan asid heksadekanoik sebagai metabolit utama yang mungkin menyumbang kepada kesan antiproliferatif. Secara saintifik kajian telah menjustifikasikan penggunaan tumbuhan ubatan terpilih oleh masyarakat jakun. Kajian lanjutan diperlukan bagi pencirian kimia yang terperinci dan penilaian yang lebih luas bagi ramuan yang paling aktif.

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LIST OF SYMBOLS AND ABBREVIATIONS

<	-	less than
%	-	percentage
°C	-	Degree Celsius
>	-	greater than
≤	-	less than or equal
≥	-	greater than or equal
µg	-	microgram
µl	-	microliters
µm	-	micrometre
mg	-	milligram
min	-	minute
ml	-	Millilitre
mm	-	millimetre
nm	-	nanometres
AA	-	Antioxidant Activity
ABS	-	Access and Benefit Sharing
ABTS	-	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid
APG	-	Angiosperm Phylogeny Group
AAM	-	<i>Artocarpus altilis</i> methanol
AAE	-	<i>Artocarpus altilis</i> ethyl acetate
AIDS	-	Acquired immune deficiency syndrome
Bca	-	Breast Cancer cell line
BCS	-	Breast cancer sensitivity
BHT	-	Butylated Hydroxytoluene
CAT	-	Catalase
CGM	-	Complete Growth Media
CO ₂	-	Carbon dioxide

CNS	-	Central Nervous System
DEM	-	<i>Dianella ensifolia</i> ethyl acetate
DBM	-	<i>Dioscorea bulbifera</i> methanol
DMSO	-	Dimethyl sulphoxide
DNA	-	Deoxyribonucleic acid
DPPH	-	2,2-Diphenyl-1-picrylhydrazyl.
DOX	-	Doxorubicin
DRs	-	Death Receptors
ELISA	-	Enzymes Linked Immunosorbent Assay
FBS	-	Fetal Bovine Serum
FRAP	-	Ferric Reducing Antioxidant Power
GCMS	-	Gas Chromatography–Mass Spectrometry
GC	-	Gas Chromatography
HPLC	-	High-performance liquid chromatography
IAM	-	<i>Ipomoea aquatica</i> methanol
JAKOA	-	Pejabat Ketua Pengarah Jabatan Kemajuan Orang Asli
JNPC	-	Johor National Park Cooperation
KEP	-	Kepong Natural Herbarium
MCF-7	-	Michigan Cancer Foundation
MDA-MB-231	-	Human Breast Adenocarcinoma Cell Line
MTT	-	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCI	-	National Cancer Institute
NCR	-	National Cancer Registry
NIST	-	National Institute Standard and Technology
OD	-	Optical density
ORAC	-	Oxygen radical absorbance capacity
PBS	-	Phosphate Buffered Saline
PI	-	Propidium Iodide
PtNPs	-	Platinum nanoparticles
PdNPs	-	Palladium
Pt–PdNPs	-	Platinum–Palladium Bimetallic Nanoparticles
PCD	-	Programmed cell death
PIC	-	Prior Informed Consent
RNA	-	Ribonucleic acid
rpm	-	Revolution per minute

RT	-	Retention time
ROS	-	Reactive Oxygen Species
RMPI	-	Roswell Park Memorial Institute
SOD	-	Superoxide dismutase
TAC	-	Total Antioxidant Capacity
TNJER	-	Taman Negara Johor Endau Rompin
TK	-	Traditional Knowledge
TLC	-	Thin-Layer Chromatography
T & CM	-	Traditional and Complementary Medicine
TPTZ	-	2,4,6-Tri(2-pyridyl)-s-triazine
TNF	-	Tumour Necrosis Factor
UTHM	-	Universiti Tun Hussein Onn Malaysia
VCEAC	-	Vitamin C equivalent antioxidant capacity
XO	-	Xanthine Oxidase
WHO	-	World Health Organisation
WWF	-	World Wide Fund for Nature



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LIST OF PUBLICATIONS

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- (i) **Mainasara, Muhammad Murtala**, Bakar, Mohd Fadzelly Abu and Linatoc, Alona C (2018), Malaysian Medicinal Plants' potential for Breast Cancer Therapy. *Asian Journal of Pharmaceutical and Clinical Resources*. Vol 11, No. 6. Pp. 101-117
- (ii) **Mainasara, M. M.**, Bakar, M. F. A., Mohamed, M., Linatoc, A. C., & Sanusi, S. B. (2017). Ethnomedical Knowledge of Plants Used for the Treatment of Breast cancer by Jakun community in Kampung Peta Endau Rompin Johor, Malaysia. *Journal of Science and Technology*, 9(1) p. 42-49.
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- (v) **Muhammad Murtala Mainasara**, Mohd Fadzelly Abu Bakar and Alona C. Linatoc "Phytochemical Constituents, Antioxidant and Antiproliferative Properties of *Artocarpus altilis* (Sukun) from Endau Rompin, Johor, Malaysia. Submitted to "Kuwait Journal of Sciences" Scopus/ISI- **Under review**
Muhammad Murtala Mainasara and Mohd Fadzelly Abu Bakar "Phytochemical Constituents, Antioxidant and Antiproliferative Properties of *Dioscorea bulbifera* from Endau Rompin, Johor, Malaysia. Submitted to "Oriental Pharmacy and Experimental Medicine" Scopus/ISI- **Under review**

Book chapter

- (i) **Mainasara, M. M.,** Bakar, M. F. A., Mohamed, M., Linatoc, A. C., & Sabran, F. (2018). Sugar Apple—*Annona squamosa* Linn. In Exotic Fruits (pp. 397-402). Elsevier

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- (ii) International Conference on Biodiversity (ICB 2018) “Diversity beyond borders organised by Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (UTHM) 11 -13 November 2018, UTHM (Pagoh Campus), KM 1, Jalan Panchor Lama, 84000 Muar, Johor Darul Takzim, Malaysia



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CHAPTER 1

INTRODUCTION

1.1 Introduction

This study highlighted the process of traditional knowledge (TK) documentation of the Jakun community in Kampung Peta, Mersing Johor. The documented knowledge was based on medicinal plants (MP) that have the potential for the treatment of breast cancer. The study also describes the efforts to justify claims made by Jakun people on the potential of these plants scientifically. These were carried out using standardised bioactive assays to assess their cytotoxicity in human breast cancer cell lines. Hopeful discoveries were successfully proven, which demonstrate the ability of the TK approach in searching for new medications in the current age.

1.2 Background of the study

Various communities hold TK passed down to them from preceding generations through verbal tradition and cultural rituals and has been the basis for agriculture, food preparation, health care, education, conservation and the full range of other activities that sustain societies in many parts of the world. This TK is the foundation of inherent cultural uniqueness and subsistence as one entity. Some aspects of TK are common and shared throughout some communities. Other elements are more restricted and precise to specific communities, families and even individuals (Pearce *et al.*, 2015)

Moreover, TK is not just traditional it is a system based on the shared practices, customs, morals, ethnicities, lifestyles, social interactions, conceptual

orientations and spiritual beliefs specific to indigenous communities. These are forever evolving as new knowledge is obtained or generated (Gibson, 2016). TK is more than the sum of its parts. These parts formulate or unite to form unique, vibrant and evolving systems of local knowledge. The richness and intricacy of TK systems originated principally from the fact that they incorporate, and are often the resolution of two very different world views. A researcher cannot separate any one aspect or component of local knowledge (e.g. traditional ecological knowledge) to the exclusion of any other without misinterpreting it as indigenous see and comprehend it. This is why the native want control over how their experience is collected, interpreted and used (Spradley, 2016).

TK includes knowledge of ecosystem relations and a code of ethics governing seemly use of the environment. This code comprises rules and conventions promoting desirable ecosystem relations, human-animal interactions and even social relationships since the latter continue to be established and reaffirmed through hunting and other activities on the land. TK enunciates with non-traditional knowledge to form a rich and distinct understanding of life and the world (Gordon, 2017).

Malaysia is among the countries which allow the use of herbal medicines in primary health care. Because of the vast diversity of plant species, reported to be the twelfth largest in the world, Malaysia has a massive resource of potential medicinal plants (Azmir *et al.*, 2013). Every tribe and race in Malaysia has its method or ways of curing the affliction of disease, it depends very much on practice, belief and knowledge each one possesses. Beside chanting, prayers and other practices, the human beings also utilise the various resources especially plants and animals around them. Based on their experience and observation through the generations, they are also able to identify the effectiveness of specific practices (Zakaria & Mohd, 2010). Malaysia is one of the countries with vast areas of tropical rainforest. According to the World Wide Fund for Nature (WWF, 2017), 59.5% of total land in Malaysia is covered with vegetation even though deforestation has been increasing immensely over the years. The vast areas of the forest in Malaysia made the country to be rich in natural resources, with over 6000 species of tropical plants all over the country (Saad *et al.*, 2014).

Malaysia was placed as the twelfth mega-diverse nation on the planet because of its richness of various indigenous vegetation. Peninsular Malaysia has been assessed to have more than 2,000 types of healing plants, and 200 species being utilised by several ethnic communities all around the country. Jamal, Barkat & Amid (2010), Nadzirah, Rusop & Noriham (2014); Sabran, Mohamed & Abu Bakar (2015) stated that about 12,000 flowering plants might have potential health-promoting properties.

However, only 1,300 species have been identified for medicinal purposes. Despite the establishment of modern medicine, traditional medicine (TM) is still one of the most famous healing methods that are sought by many Malay people. Malay TM has significantly been influenced since ancient times by traders from Indonesia, India, the Middle East and China. Before the introduction of Islam in Malaysia. Malay TM was influenced mainly by the beliefs and practice existing then, which included chanting and the use of supernatural powers. Traditional healers use various methods of curing or treating patients who suffer from multiple illnesses. TM is one of the bases in conventional medicine. According to statistics from the Ministry of Health, there are about 7,282 traditional and Chinese medicine practitioners in Malaysia. There are no figures for foreign practitioners in the country, but statistics show that the leading complementary treatments these practitioners use include Thai massage, reflexology and acupuncture. Moreover, the state has about 26 training centres (Pillay, 2006).

The government is committed to seeing traditional and complementary medicine (T&CM) integrated into the health system. It has proposed the T&CM Act that will force undocumented or unqualified practitioners out of business. Furthermore, the Health Ministry has agreed to include Islamic medicine practices in T&CM Bill 2012, and Islamic medicine practitioners have to be registered through a council before it's established. According to Siti *et al.*, 2009, a survey was conducted in 2004 in all states of Malaysia in which various traditional and complementary alternative medicine modalities used by the Malaysian population were captured. The results show that, (88.9%) of the population used biological based therapy which includes herbal treatment for health problems and health maintenance is (87.3%), under this category herb based application/herb based beauty product/herb based

hygiene product group was most commonly used for health issues 23.6% while pure herbs were the ones most widely used for health maintenance (29.6%).

In Malaysia, the use of herbal and MP for many applications either for prevention, curing and treatment of certain diseases have been in use as an alternative medications alongside modern medicines. These traditional medications have come together under the T&CM body which has been formed by the Ministry of Health, Malaysia. The Ministry of Health offers T&CM in some of the public hospitals as an alternative medication to the patients, including treatments such as acupuncture and massage. World Health Organisation (WHO) also has recognised the importance of T&CM. Nowadays, herbals use in Malaysia are not only restricted for healing and treatment purposes but also extended on a worldwide basis to cosmetics and nutraceutical. In Malaysia herbal products have generated substantial income in the years 2000-2005 the annual sales for traditional medicines increased from US\$ 385 million (RM 1 billion) to US\$ 1.29billion (RM4.5billion) (Bernama, 2010; Rezai *et al.*, 2013).

Documentation is a process of collecting, recording, and keeping data systematically until the available information can be used for future references (WIPO, 2013). While TK documentation is primarily a process in which TK is identified, collected, organised, registered or recorded in some way, as a means to dynamically maintain, manage, use, disseminate and protect TK according to specific goals (WIPO, 2017a). TK documentation can take many shapes and forms – through written registries and files, video, images and audio; in a traditional indigenous language or other languages; and using modern or more classical technologies (digital versus written filing). Some ethnics from the early civilised world such as Chinese and the Indians, have established how systematic and proper records of TK benefits future needs. The written version of their traditional medicine was documented in the form of medical pharmacopoeia, and the systems are appropriately called as Traditional Chinese Medicine (TCM) (Siow *et al.*, 2005) and Ayurveda (Patwardhan *et al.*, 2005). When compared with indigenous Malaysian ethnics, the knowledge of traditional used of MP remains uncertain since they only preserve and transfer the knowledge verbally and informally adapting it as their way of life. Recently, government agencies of Malaysia initiate continuous efforts to document the knowledge through publications such as Malaysian Herbal Monograph

and Compendium of Medicinal Plants used in Malaysia (Sabran, 2016). Also, there is also an effort to create an integral database such as the Global Information Hub on Integrated Medicine (GLOBNMED) and Traditional Knowledge Digital Library (TKDL). These act as a starting point to encourage advance researchers that could provide evidence derived from the documented TK.

Worldwide, BC is the most common invasive cancer among women; It affects about 12% of women worldwide (McGuire *et al.*, 2015). Breast cancer comprises 22.9% of invasive cancers in women (Boyle & Levin, 2008; Ozmen, 2008) and 16% of all female cancers in 2012, it comprised 25.2% of cancers diagnosed in women, making it the most common female cancer (Ayanian *et al.*, 1993; DeSantis *et al.*, 2011).

In the year 2000 death as a result of breast cancer was recorded around 375,000 as reported by (Bray, McCarron & Parkin, 2004). Nearly 1.15 million new cases were diagnosed in 2002, which is more than the combined incidence of colorectal and cervical cancer, the second and third most common cancers in women, respectively (Hortobagyi *et al.*, 2005). About 411,000 deaths cases which are approximately 15% of all cancer-related deaths in 2002 as reported by (Ozmen, 2008). Bray *et al.* (2013) reported that in 2008, BC caused 458,503 deaths worldwide (13.7% of cancer deaths in women and 6.0% of all cancer deaths for men and women together). Since the 2008 estimates, breast cancer incidence has increased by more than 20%, while mortality has increased by 14%. Breast cancer is also the most common cause of cancer death among women (522 000 deaths in 2012) and the most frequently diagnosed cancer among women in 140 of 184 countries worldwide. It now represents one in four of all cancers in women. Lung cancer, the second most fundamental reason for cancer-related death in females, caused 12.8% of tumour death in women (18.2% of all malignancy death for people together).

Breast cancer is the most prevalent cancer in Malaysia with the age-standardised rate for females of 47.4 per 100,000 (Taib *et al.*, 2011). The incidence was higher in Chinese women (ASR 59.9 per 100,000 women) compared to Indian (ASR 54.2) and Malay (ASR 34.9) (Taib *et al.*, 2011). According to estimates, there are about 90-100,000 people in Malaysia living with cancer at any one time. The National Cancer Registry of Malaysia (NCR) records 21,773 Malaysians being diagnosed with cancer but estimates that almost 10,000 cases are unregistered every

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