PHYTOCHEMICAL ANALYSIS ON VARIOUS PARTS OF *PHALERIA MACROCARPA* METHANOL EXTRACTS AND THEIR LARVICIDAL AND ANTIMICROBIAL ACTIVITIES

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FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH
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DEGREE : **MASTER OF SCIENCE (ENVIRONMENTAL SCIENCE)**

DATE OF VIVA: **13th AUGUST 2018**

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Noorehan Rastaon
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ABSTRACT

Phaleria macrocarpa is a herbal plant that originates from Indonesia and can be abundantly found in tropical areas. This plant had been reported with antioxidant, antimicrobial, and anticancer attributes. However, the larvicidal effect of this plant against mosquito larvae has not been widely inspected. Over a long period of time, the use of synthetic larvicide to control vector mosquitoes has not been very effective, as it has aroused physiological resistance as well as adverse environmental effects. Therefore, in this study, the potentials of *P. macrocarpa* as mosquito larvicide against *Ae. aegypti* larvae and adult mosquito, and as antimicrobial agent against human pathogens were investigated. Larvicidal activity of various parts of *P. macrocarpa* methanol crude extracts were studied in the laboratory assay against third and fourth instar larvae of *Ae. aegypti*. Seed extract was showed to be more toxic against *Ae. aegypti* larvae that caused 100% mortality after 24 hours of treatment compared to fruit (20%), leaf (40%), and stalk (60%) extracts. The LC_{50} and LC_{90} values for seed methanol extract of *P. macrocarpa* were 182 $mgml^{-1}$ and 302 $mgml^{-1}$ respectively. *P. macrocarpa* seed methanol extract also demonstrated 100% mortality against adult *Ae. aegypti* at a minimum inhibitory concentration of 211 $mgml^{-1}$. When tested against human pathogens, *P. macrocarpa* crude methanol extracts showed weak antimicrobial activity against *Candida tropicalis* and *Bacillus cereus* strains. The Kirby Bauer sensitivity test showed that all parts of *P. macrocarpa* extracts were ineffective against *C. tropicalis* with inhibition zones <10mm. Seed extract treatment exhibited the highest inhibition zone (8.75±0.4mm), followed by stalk (7.45±0.7mm), fruit (6.5±0mm), and leaf (6.45±0.1mm) extracts. Meanwhile, antibacterial activity of *P. macrocarpa* crude methanol extracts against *B. cereus* showed that stalk exhibited the highest inhibition zone (9.5±0.7mm), followed by seed (8.65±0.5mm), leaf (8.5±0.7mm), and fruit (7.35±0.2mm). The larvicidal and antimicrobial activities recorded indicated the presence of active compounds such as saponin and flavonoid in *P. macrocarpa*. Saponin content was highly found in the *P. macrocarpa* seed (66.93±1.56mg/g) compared to other parts which were stalk (42.75±1.02mg/g), fruit (27.4±2.86mg/g), and leaf (7.11±3.01mg/g). Meanwhile, *P. macrocarpa* fruit contained the highest amount of quercetin (19.66±1.27mg/g), followed by stalk (13.16±2.56mg/g), leaf (1.78±1.03mg/g) and seed (0.62±1.98mg/g) extracts. Therefore, this present results showed the high potential of *P. macrocarpa* seed to be included as an alternative control of vector-borne diseases.

ABSTRAK

ANALISIS FITOKIMIA PADA PELBAGAI BAHAGIAN EKSTRAK METANOL PHALERIA MACROCARPA DAN AKTIVITI LARVICIDAL DAN ANTIMIKROBNYA

Phaleria macrocarpa adalah tumbuhan herba yang berasal dari Indonesia dan boleh didapati dengan banyaknya di kawasan tropika. Tumbuhan ini telah dikaji mempunyai sifat antioxidant, antimikrob, dan anticancer. Walau bagaimanapun, kesan larvicidal dari tumbuhan ini terhadap larva nyamuk tidak dilaporkan secara meluas. Dalam jangka masa panjang, penggunaan larvicide sintetik untuk mengawal nyamuk vektor tidak begitu berkesan, kerana ia telah menimbulkan ketahanan fisiologi serta kesan buruk terhadap alam sekitar. Oleh itu, dalam kajian ini, potensi P. macrocarpa sebagai larvicide nyamuk terhadap larva Ae. aegypti dan nyamuk dewasa, dan juga sebagai agen antimikrobial terhadap patogen manusia disiasat. Aktiviti larvicidal dari pelbagai bahagian ekstrak methanol P. macrocarpa telah dikaji di assay makmal terhadap larva instar ketiga dan keempat Ae. aegypti. Ekstrak methanol benih lebih menunjukkan kesan toksik terhadap larva Ae. aegypti yang menyebabkan kematian 100% selepas 24 jam rawatan berbanding dengan ekstrak buah (20%), daun (40%), dan batang (60%). Nilai LC_{50} dan LC_{90} untuk ekstrak metanol benih P. macrocarpa masing-masing adalah 182 $mgml^{-1}$ dan 302 $mgml^{-1}$. Ekstrak metanol benih P. macrocarpa juga menunjukkan kematian 100% terhadap nyamuk dewasa Ae. aegypti pada kepekatan penghalang minimum 211 $mgml^{-1}$. Apabila diuji terhadap patogen manusia, ekstrak methanol P. macrocarpa menunjukkan aktiviti antimikrob yang lemah terhadap strain Candida tropicalis dan Bacillus cereus. Ujian kepekaan Kirby Bauer menunjukkan bahawa semua bahagian ekstrak P. macrocarpa tidak berkesan terhadap C. tropicalis dengan zon perencatan <10mm. Rawatan ekstrak methanol benih P. macrocarpa menunjukkan zon hambatan tertinggi (8.75±0.4mm), diikuti oleh ekstrak batang (7.45±0.7mm), buah (6.5±0mm), dan daun (6.45±0.1mm). Sementara itu, aktiviti antibakteria ekstrak methanol P. macrocarpa terhadap B. cereus menunjukkan bahawa ekstrak tangkai menunjukkan zon hambatan tertinggi (9.5±0.7mm), diikuti oleh ekstrak benih (8.65±0.5mm), daun (8.5±0.7mm), dan buah (7.35±0.2mm). Aktiviti larvicidal dan antimikrobik menunjukkan adanya sebatian aktif seperti saponin dan flavonoid di P. macrocarpa. Kandungan saponin didapati sangat baik dalam benih P. macrocarpa (66.93±1.56mg/g) berbanding bahagian lain iaitu tangkai (42.75±1.02mg/g), buah (27.4±2.86mg/g) dan daun (7.11±3.01mg/g). Sementara itu, buah P. macrocarpa mengandungi jumlah quercetin tertinggi (19.66±1.27mg/g), diikuti dengan ekstrak tangkai (13.16±2.56mg/g), daun (1.78±1.03mg/g) dan benih (0.62±1.98mg/g). Oleh itu, hasil kajian ini menunjukkan adanya potensi benih P. macrocarpa untuk dimasukkan sebagai kawalan alternatif terhadap penyakit bawaan vektor.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial Resistant
AR	Antibiotic Resistant
ARGs	Antibiotic Resistant Genes
Bti	*Bacillus thuringiensis israelensis*
DHF	Dengue Haemorrhagic Fever
DIZ	Diameter of Inhibitory Zone
EEE	Eastern Equine Encephalitis
EO	Essential Oils
HBL	Hemolysin BL
HPLC	High Performance Liquid Chromatography
IGR	Insect Growth Regulators
IPM	Integrated Pest Management
JE	Japanese Encephalitis
MIC	Minimum Inhibitory Concentration
NHE	Nonhemolytic Enterotoxin
VRE	Vancomycin Resistant Enterococci
WHO	World Health Organization

LIST OF SYMBOLS

%	percent
°C	Degree Celsius
<	less than
>	more than
g	gram
ml	milliliter
mm	millimeter
ppm	parts per million
v/v	volume per volume
µg	microgram
µg/g	microgram per gram
$\mu g ml^{-1}$	microgram per milliliter
µl	microliter

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CHAPTER 1

INTRODUCTION

1.1 Introduction

In the recent years, research on plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of herbal plants used in various field. The herbal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protection from various diseases.

Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds. These compounds are also known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property (Saxena *et al*., 2013).

Substantial antibacterial, antifungal and moderate insecticidal, sporicidal and cytotoxic activities were observed with the hexane extract of the stem bark of *Amona glabra* (Sher, 2009). Chromatographic fractionation of the stem led to the isolation of kaur-16-en, 19-oic acid, which was found to be largely responsible for its biological activities (Padmaja *et al*., 1995). Previously, studies have been conducted to test the larvicidal potential of the *Azadirachta indica* extracts against fourth instar larvae of *Culex quinquefasciatus*, and it was reported positive (Nour *et al*., 2012).

One of the most concerned environmental problems that the world is currently facing is mosquito-borne disease. Mosquitoes belong to the family Culicidae within the order Diptera. There are approximately 3400 species and 42 genera throughout the world. Mosquitoes are vectors to many different human diseases, causing million of deaths every year (Kamaraj *et al*., 2011).

Dengue fever is among the most widespread vector-borne infectious disease. At present, dengue is endemic in 112 countries in the world (Pinheiro and Corber, 1997). Around 2.5 to 3 billion people, living mainly in urban areas of tropical and subtropical regions are estimated to be at risk of acquiring dengue viral infections (WHO, 1999).

In Malaysia, dengue is predominantly an urban disease due to the abundance of the principle vector *Aedes aegypti* which is at a close proximity to high densities of susceptible hosts. The states of Selangor, Wilayah Persekutuan Kuala Lumpur and Johor are the areas that have been largely affected by the disease and are reporting high numbers of cases.

Mosquitoes belonging to the genus *Aedes* (*Aedes aegypti*, *Aedes albopictus*, and *Aedes polynesiensis*) play an important role in transmission of dengue. However, the primary and most important vector is *Ae. agypti*. *Ae. agypti*, a container breeding, day biting mosquito is usually found in tropical and subtropical areas.

They can breed in polluted or small collection of water such as flower vases, coconut shells, and also abandoned rubber tires. Their eggs can survive for long periods, as they are capable of withstanding desiccation. *Ae. agypti* normally rest indoors, mainly in living rooms and bedrooms. This maximizes man-vector contact and minimizes contact with insecticides, hence contributing to difficulty in controlling this vector (Perich *et al*., 2000).

To prevent proliferation of this mosquito-borne disease and also to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation that has been used widely is the application of synthetic insecticides such as organochlorine and organophosphate compounds. However, this method has not been very successful nowadays due to human, technical, operational, ecological, and economic factors.

The adverse effects of chemical insecticides for the control of mosquito vectors have received wide public apprehension because of several problems that arise, such as insecticide resistance, resurgence of pest species, environmental pollution, toxic hazards to humans, and non-target organisms. Although insecticides such as malathion 4%, fenitrothion 1%, or pirimiphos-methyl have proved to be very effective in many control programmes, mosquito vectors develop different patterns of resistance to them (Gratz, 1999; Huong, 1999).

These problems have initiated the need to explore and develop alternative strategies using eco-friendly, environmentally safe, biodegradable plant products, which are non-toxic to non-target organisms too. One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a sustainable method of mosquito control.

Plant extracts are safer for non-target organisms including human. Therefore, plant based formulations would be more feasible environmental products, with proven potential as insecticide or repellent. It can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level (Imam *et al*., 2014).

Thus, in this research study, the potential of herbal plant *Phaleria macrocarpa* (Scheff.) Boerl as mosquito larvicide against *Ae. aegypti* larvae and adult mosquito was investigated. Its extracts are non-toxic, easily available at affordable prices, biodegradable and reported to have active compounds such as saponins, flavonoid, and alkaloid that carry insecticidal value.

Besides that, the recent failure of antibiotics due to the dramatic emergence of multidrug resistant pathogens and the rapid spread of the new infections, urge the health organizations and pharmaceutical industries all over the world to change their strategy and stop going on the slow growing production of more synthetic antibiotics against the fast growing antibiotics-resistant microorganisms. There are considerable alternative sources of natural antimicrobials from plants with different mode of actions. Leaves and seeds of *P. macrocarpa* are reported to have profound antibacterial activity (Altaf *et al*., 2013). Therefore, this research also aimed to determine the antimicrobial activity of *P. macrocarpa* extracts against human pathogens *Candida tropicalis* and *Bacillus cereus*.

1.2 Objectives of Study

The aim of this research study was to evaluate the larvicidal and antimicrobial activity possessed by *Phaleria macrocarpa* (Scheff.) Boerl. The specific objectives of the study were as listed below:

(i) To determine the amount of flavonoid and saponin present in various parts of *P. macrocarpa* that was its leaf, stalk, fruit, and seed.

(ii) To investigate and compare the larvicidal activity of various parts *P. macrocarpa* methanol extracts against *Ae. aegypti* larvae and adult *Ae. aegypti* mosquitoes.

(iii) To examine the antimicrobial property of various parts of *P. macrocarpa* methanol extracts against human pathogens: *Candida tropicalis* RETL-Cr1 and *Bacillus cereus* NMeHI-Cr2.

CHAPTER 2

LITERATURE REVIEW

2.1 Phytochemicals and Biological Activities of Plants

The secondary compounds of plants are huge repository of compounds with a wide range of biological activities (Duke, 1990). Unlike compounds synthesized in the laboratory, secondary compounds from plants are virtually guaranteed to have biological activity and that activity is highly likely to function in protecting the producing plant from a pathogen, herbivore, or competitor.

Plants are rich in a wide variety of secondary metabolites or phytochemicals, such as tannins, terpenoids, alkaloids, and flavonoids, which have been reported to have many biological properties (Table 2.1). For example, the essential oil isolated from the leaves of *Aegle marmelos* exhibited significant antifungal activity against different fungal isolates and 100% inhibition of spore germination of all the tested fungi when evaluated using the spore germination assay (Rana *et al*., 1997).

Since decades ago, it is believed that a good source of anti-infective agents actually comes from plants. Moreover, scientists nowadays from divergent field are investigating plants with an intention to discover as much valuable phytochemicals as possible. Laboratories all over the world have found literally thousands of phytochemicals, which have inhibitory effects on all types of microorganisms *in vitro* (Cowan, 1999).

Table 2.1 Phytochemicals and their biological activities of various species of plant

Species	Phytochemicals	Biological Activities	Reference
Amona glabra	Kaur16-en, 19-oic acid	Antibacterial, antifungal and moderate insecticidal, sporicidal, and cytotoxic activities	Padmaja *et al.*, 1995
Clausena anisata	Clausenol and clasenine-two carbazole alkaloids	Antibacterial and antifungal activities	Chakraborty *et al.*, 1995
Garcinia mangostana	Xanthones	Antifungal activity	Gopalakrishnan *et al.*, 1997
Terminalia belerica	Termilignan, thannilignan, 7 hydroxy 3,4 (methylenedioxy) flavones, and anolignan B	Anti HIV-1, antimalarial and antifungal activities	Valsaraj *et al.*, 1997
Capparis spinosa	Caffeic acid, vanillic acid, quercetin	Anticancer, antiinflammatory, antioxidant and antiatherogenic	Proestos *et al.*, 2006
Castanea vulgaris	Gallic acid, apigenin, rutin	Antioxidant, antimicrobial, antifungal, and anti-allergic agent	Proestos *et al.*, 2006
Helichrysum patulum	Arbutin, β-caryophyllene, terpineol, limonene	Antiinflammatory, antioxidant, and antitumor activities	Swartz, 2006
Solanum nigrum	Alkaloids, reducing sugar, tannins, flavonoids, phlobatannins, steroids	Antioxidant, antiulcer, antidiabetic, anticancer, antihyperlipidaemic, antinflammatory, antimicrobial, antispermatogenic activities	Venkatesan *et al.*, 2009
Acacia nilotica	3-picoline-2-nitro, 1-acetyl beta carboline, hydroxyl citronellal	Antibacterial, antimalarial, antifungal, antibiotic, anti-diarrhea, molluscidal, and anti hypertensive activities	Hemamalini and Nirmala, 2013

Table 2.1 Phytochemicals and their biological activities of various species of plant

(cont.)

Species	Phytochemicals	Biological Activities	Reference
Aegle marmelos	Alkaloids, flavonoids, phenols, cardiac glycosides, saponins, terpenoids, steroids, and tannins	Antioxidant, antiulcer, antidiabetic, anticancer, antihyperlipidaemic, anti-inflammatory, antimicrobial, antispermatogenic	Mujeeb *et al.*, 2014
Ziziphus jujube	Triterpenic acids, flavonoids, cerebrosides, phenolic acids, α-tocopherol, β-carotene, and polysaccharides	Antioxidant, anticancer, anti-inflammatory, antimicrobial, and hepatoprotective	Tahergorabi *et al.*, 2015
Syzygium cumini	Phenols, flavonoids, tannins, terpenes, anthocynins	Antihyperglycemic, antidiabetic, antiinflammatory, antibacterial, cardioprotective, and antioxidant	Chagas *et al.*, 2015
Cymbopogon nardus	Geraniol, trans-citral, cis-citral, geranyl acetate, citronellal (6-octenal, 3, 7-dimethyl) and citronellol	Antifungal, antimicrobial, and mosquito repellent	Singh *et al.*, 2015
Panax ginseng	Triterpene saponins, ginsenoside, acetylenic compounds, kaempferol, trifolin, panaxynol, lechitin, amino acid	Anti-tumor, anti-inflammatory, anti-pruritic, antiallergic, hepatoprotective, hypoglycemic hypolipidemic, and immunomodulatory activity	Ru *et al.*, 2015
Acorus calamus	Phenols, flavonoids, phenolic acids, 4-terpineol, lysidine, epieudesmin, spathulenol, furylethyl ketone	Insecticidal, larvicidal, antibacterial, mutagenic, cytotoxic, hepatoprotective, anticonvulsant, neuroleptic,	Parki *et al.*, 2017

Table 2.1 Phytochemicals and their biological activities of various species of plant

(cont.)

Species	Phytochemicals	Biological Activities	Reference
Azadirachta indica	Alkaloids, triterpenoids, and their glycosides, limonoids, flavonoids, fatty acids, and steroids	Anticarcinogenic, anti-inflammatory, antiulcer, antioxidant, immunomodulatory, antifungal, antibacterial, antiviral, antimalarial, antimutagenic, and antihyperglycemic	Maan *et al.*, 2017
Gymnema Sylvestre	Alkaloids, flavonoids, saponins, phenol, tannins, cardiac glycosides	Antiviral, anti allergic, diuretic, hypoglycemic, hypolipidemic, for the treatment of obesity and dental cares	Kumar and Patra, 2017
Balanites aegyptiaca	Quercetin 3-rutinoside, diosgenin glucoside, saponin, phytosterol, sterol esters and tocopherols	Anticancer, antivirus, antimicrobial, antidiabetic and antioxidant agents	Al-Thobaiti and Zeid, 2018

The term "medicinal plant" includes various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses. Medicinal plants are considered as a rich resources of ingredients which can be used in drug development either pharmacopoeial, non-pharmacopoeial or synthetic drugs (Table 2.2) for anticancer, antimicrobial, antifungal, and pesticidal activities. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values. Some of these plants include ginger, green tea, walnuts, aloe, pepper and turmeric. Some plants and their derivatives are considered as important source for active ingredients.