PHYTOCHEMICAL PROPERTIES AND ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF Macaranga triloba (Thunb.) Müll.Arg. AND Macaranga gigantea (Rchb.f. & Zoll.) Müll.Arg.

KEW KOK YONG

FH 2019 7

PHYTOCHEMICAL PROPERTIES AND ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *Macaranga triloba* (Thunb.) Müll.Arg. AND *Macaranga gigantea* (Rchb.f. & Zoll.) Müll.Arg.

KEW KOK YONG

FACULTY OF FORESTRY

UNIVERSITI PUTRA MALAYSIA

2019

PHYTOCHEMICAL PROPERTIES AND ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *Macaranga triloba* (Thunb.) Müll.Arg. AND *Macaranga gigantea* (Rchb.f. & Zoll.) Müll.Arg.

By

KEW KOK YONG

A Project Report Submitted in Partial Fulfilment of the Requirements for the Degree of Bachelor of Forestry Science in the

Faculty of Forestry

University Putra Malaysia

2019

DEDICATION

Specially dedicated to my:

My Family

Prof. Dr. Rozi Mohamed

Lab members in Biotechnology Lab

And All My Friends

Thank you for your supervision and supports.

Deeply appreciation for all the supports along the way.

ABSTRACT

Macaranga triloba (Thunb.) Müll.Arg. and *Macaranga gigantea* (Rchb.f. & Zoll.) Müll.Arg. are medicinal plants found in Peninsular Malaysia. However, there are limited scientific evidence to validate the medicinal properties of both species. This study is to identify the phytochemical materials and the antimicrobial and antioxidant activities of the leaves of *M. triloba* and *M. gigantea*. In this study, *M. triloba* and *M. gigantea* specimends were collected at Ayer Hitam Forest Reserve. Identification of the species was made through morphological characteristcs and DNA identification. Phytochemical screening of the leaf extracts of *M. triloba* and *M. gigantea* using methanol and hexane was conducted. Antimicrobial activities were determined using the agar disk-diffusion and well-diffusion methods while antioxidant activities were determined using the DPPH assay. For phytochemical screening, flavanoids, alkaloids and terpenoids were found in both species. Antimicrobial activity against *Bacillus subtilis* was detected in the methanol extract of *M. gigantea* while against *Staphylococcus aureus* was detected in the hexane extract of *M. triloba*. Comparing to the ascorbic acid as positive control, hexane extract of both species showed 2 times higher antioxidant activities than the methanol extract. The presence of phytochemicals and antimicrobial and antioxidant activities in *M. triloba* and *M. gigantea* indicated the potential medicinal properties of both species.

ABSTRAK

Macaranga triloba (Thunb.) Müll.Arg. and *Macaranga gigantea* (Rchb.f. & Zoll.) Müll.Arg. merupakan sejenis tumbuhan ubatan yang digunakan oleh penduduk tempatan di Malaysia. Namun begitu, hanya bukti saintifik yang terhad telah dikemukakan untuk menunjuk kegunaan *M.triloba* dan *M. gigantea* sebagai ubatan. Penyelidikan ini adalah untuk mendapatkan fitokimia dan aktiviti antimikrob dan aktiviti antioksida dalam ekstrak bahagian daun *M.triloba* dan *M. gigantea*. Spesimens *M. triloba* dan *M. gigantea* telah dikumpul di Ayer Hitam Forest Reserve. Pengenalan spesies telah dijalankan melalui pengenalan ciri-ciri morfologi dan penganalan DNA. Fitokimia dalam ekstrak bahagian daun *M. triloba* dan *M. gigantea* dengan menggunakan metanol dan heksana telah dikenalpastikan dalam kajian ini. Aktiviti antimikrob oleh ekstrak daun M. triloba dan M. gigantea telah dikenalpasti dengan kaedah kajian perepasan cakera dan kajian penyebaran cakera manakala antioksida dekenalpasti dengan kaedah DPPH assay. Ekstrak telah didapati mengandungi fitokimia seperti alkaloid, fenol dan terpenoid dalam kedua-dua spesies. Aktiviti antimikrob tehadap *Bacillus subtilis* hanya dapat dikesan dalam ekstrak metanol dari *M. gigantea* manakala aktivity antimikrob terhadap *Staphylococcus aureus* dapat dikesan dalam ekstrak heksana dari *M. triloba*. Berbanding dengan asid askorbik sebagai kawalan positif, ekstrak heksana menunjukkan aktiviti antioksida yang dua kali lebih tinggi berbanding dengan ekstrak metanol. Kegunaan *M. triloba* dan *M. gigantea* sebagai tumbuhan ubatan telah dibuktikan dengan kandungan fitokimia dan aktiviti antimikrob dan antioksida di bahagian daun.

ACKNOWLEDGEMENTS

First and foremost, I have to thank my research supervisor, Prof. Dr. Rozi Mohamed. Without her assistance and dedicated involvement in every step throughout the process, this thesis would have never been accomplished. I would like to thank you very much for your support and understanding over these past three years.

I would also like to show gratitude to lab members in the Forest Biotechnology Lab including Dr. Lee Shiou Yih, Siti Rahimah Jumaat, Tuan Noraida Hamzah and Muhammad Syahmi. I am extremely thankful to them for sharing their experiences and providing valuable guidance to me throughout this research and thesis writing.

Last but not least, I would like to express my appreciation to my family and my friends for sharing their knowledge and accompany me throughout this research. Thank you once again to my friends for the continuous guidance and advice for my research. I would like to extend my thankfulness to my parents as well for their encouragement and support over the years.

APPROVAL SHEET

I certify that this research project report entitled "PHYTOCHEMICAL PROPERTIES OF *Macaranga triloba* (Thunb.) Müll.Arg. AND *Macaranga gigantea* (Rchb.f. & Zoll.) Müll.Arg. AND THEIR ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES" by Kew Kok Yong has been examined and approved as a partial fulfillment of the requirements for the Degree of Bachelor of Forestry Science in the Faculty of Forestry, Universiti Putra Malaysia.

Approved by:

_____

Prof. Dr. Rozi Mohamed

Faculty of Forestry

Universiti Putra Malaysia

(Supervisor)

_____

Prof. Dr. Mohamed Zakaria bin Hussin

Dean

Faculty of Forestry

Universiti Putra Malaysia

Date: 3 January 2019

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

T&CM	Traditional and Complementary Medicine
^{o}C	Degree Celsius
mL	Milliliter
µL	Microliter
mg	Milligram
NCBI	National Center for Biotechnologt Information
EtBr	Ethidium bromide
BLAST	Basic Local Alignment Search Tool
WHO	World Health Organization
UPM	Univerisiti Putra Malaysia
AHFR	Ayer Hitam Forest Reserve
FAO	Food and Agriculture Organization
DPPH	2,2-Diphenyl-1-picrylhydrazyl
CH_3OH	Methanol
nm	nanometer
UV	ultraviolet
mm	millimeter
TAE	Tris base, acetic acid, Ethylenediaminetetraacetic acid
bp	base pair
Ab value	Absorbance value
µg/mL	Microgram per millimeter
mg/mL	Milligram per milliliter
IC_{50}	Half maximal inhibitory concentration

CHAPTER ONE

INTRODUCTION

1.1 General background

1.1.1 Traditional plant medicine

Traditional medicine comprises medical aspects of traditional knowledge that developed over generation within various societies before the era of modern medicine. The World Health Organization (WHO) defines traditional medicine as the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness.

A large proportion of the population in a number of developing countries still relies on traditional practitioners, including traditional birth attendants, herbalists and bone-setters and on local medicinal plants to satisfy their primary health care needs. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes.

It is frequently stated in the scientific literature (Stekelenburg et al., 2005; Tilburt & Kaptchuk, 2008; Gude, 2013), official fact sheets and reports (WHO, 2008) and the press (BBC News, 2014) that 80% of people in

Asian and African countries use traditional medicine to meet their primary health care needs. In low and middle income countries where the number of practitioners of modern medicine may not be enough to meet the health care needs of the country, Traditional medicine and its practitioners are considered an important resource for population health. Compared to modern medicine, traditional medicine is perceived to be more affordable, accessible and acceptable to the communities in which it operates (Sato, 2012).

Plant extracts and compounds derived from plants are in use as drug from the ancient times (John & Koperuncholan, 2012). Numerous studies have identified compounds within herbal plants that are effective antibiotics (Basile et al., 2000). Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics (Okpekon et al., 2004). Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities (Rice-Evans et al., 1995; Sala et al., 2002). To promote the proper use and to determine their potential as sources for new drugs, it is essential to study the medicinal plants and their derivatives. Therefore, researchers are increasingly turning their attention to folk medicine to develop better drugs against many diseases (Parekh & Chanda, 2007).

1.1.2 Present and future prospect of traditional and complementary medicine in Malaysia

Thousands of years ago, Malaysia had an extensive variety of plant species and traditional medical systems. More than 1300 medicinal plant species

have been recorded in Peninsular Malaysia alone (Burkill, 1935). The Malaysian system has been affected by Indonesian, Chinese, Indian, and Orang Asli traditional practices (Zakaria, 1994). In the Malaysian medicine system, herbal products form an important component. Approximately 7,411 plant species excluding bryophyta, algae and fungi have been identified in Sabah and about 80% of the indigenous plants were used by local communities. In addition, about 1,200 were used statewide for medicinal purposes (Kulip, 2010).

Malaysian plants are widely valued for their aromas and tastes and many of these plants are medicinal and used to treat various human illnesses. Studies have shown that several plants in Malaysia are poisonous to animals and humans because of the presence of specific components. Screening botanical extracts for potential toxins is a significant step in assessing their appropriateness for the market (Qader, 2011).

Recently, interest has grown in discovering antioxidants derived from plant sources to replace artificial antioxidants. Natural antioxidants are seen as being safer and more desirable than their synthetic counterparts because they occur in plant foods, data from scientific reports and laboratory studies indicate that plants contain a large variety of phytochemicals that have antioxidant activities (Chanwitheesuk, 2005).

1.2 Problem Statement

The genus *Macaranga* have a long history of use in traditional medicine to treat cuts, swellings, boils, bruises and sores. In folk medicine, traditional healers use fresh or dried leaves of some *Macaranga* species to treat swellings, cuts, sores, boils and bruises (Nick et al., 1995). An increasing number of phytochemical studies are being carried out on plants belonging to the genus *Macaranga* due to their various traditional uses.

This is a resourceful area of research as many species of *Macaranga* are use in traditional medicine as well as exhibits various pharmacological properties; while their chemistry indicates varied chemical structures. However, not much studies of *Macaranga gigantea* and *Macaranga triloba* have been established in Malaysia. Thus it is important for us to evaluate the phytochemical properties and chemistry contents of *Macaranga* species for future research on plants in this genus and their potential for development as herbal drugs.

1.3 Objectives

I) To identify the phytochemical materials present in the leaves of *Macaranga triloba* and *Macaranga gigantea.*

II) To investigate the antimicrobial and antioxidant activities of the leaves of *M. triloba* and *M. gigantea*.

REFERENCES

Ahmed, J. S. & Koperuncholan, M. (2012). Antibacterial activities of various solvent extracts from *Impatiens balsamina*. *International Journal of Pharma and Biosciences,* 3*,* 401-406.

Alothman, M., Rajeev, B. A. & Karim, A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry,* 115(3), 785-788.

Anbu Jeba Sunilson J. S., Anandarajagopal, K., Diniesh, K. C., Rejitha, G., Suraj, R., Azman, H., Vignesh, M. & Proom, P. (2010). Ethnomedical survey of plants used by the Orang Asli in Kampung Bawong, Perak, West Malaysia. *Journal of Ethnobiology and Ethnomedicine* 6:5.

Andrews, R. E., Parks, L. W. & Spence, K. D. (1980). Some effects of Douglas fir terpenes on certain microorganisms. *Applied Environmental Microbiology*, 40, 301-304.

Andri, C. K., Masitah, H. & Harcharan, S. (2009). Effects of solvent properties on the Soxhlet extraction of diterpenoid lactones from Andrographis paniculata leaves. *ScienceAsia,* 35, 306-309.

Basile, A., Sorbo, S., Giordano, S., Ricciardi, L., Ferrara, S., Montesano, D., Castaldo, C. R., Vuotto, M. L. & Ferrara, L. (2000). Antibacterial and allelopathic activity of extract from Castanea sativa leaves. *Fitoterapia,* 71, 110-116.

Betancur-Galvis, L. A., Morales, G. E., Forero, J. E. & Roldan, J. (2002). Cytotoxic and Antiviral Activities of Colombian Medicinal Plant Extracts of the Euphorbia genus. *Memórias do Instituto Oswaldo Cruz*, 97(4), 541-546.

Brigitte, F., Armin, J., Ulrich, M. & Eduard, L. (1998). Diversity, evolutionary specialization and geographic distribution of a mutualistic ant-plant complex: Macaranga and Crematogaster in South East Asia. *Biological Joumal of the Linnean Society,* 66, 305-331.

Brown, D. F. & Kothari, D. (1975). Comparison of antibiotic discs from different sources. *Journal of Clinical Pathology,* 28, 779-783.

Burkill, I. H. (2002). *Dictionary of the economic products of the Malay Peninsula.* Oxford: Vol. 1.

Cai, Y.Z., Sun, M. & Corke, H., (2003). Antioxidant activity of betalains from plants of the Amaranthaceae. *Journal of Agricultural and Food Chemistry* 51 (8), 2288-2294.

CDC.gov. (2016). *"Staphylococcal Food Poisoning".* Retrieved from https://www.cdc.gov/foodsafety/diseases/staphylococcal.html.

Chanwitheesuk, A., Teerawutgulrag, A. & Rakariyatham, N. (2005). Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chemistry,* 92(3), 491-497.

Cheng, T., Xu, C., Lei, L., Li, C., Zhang, Y. & Zhou, S. (2016). Barcoding the kingdom Plantae: new PCR primers for *ITS* regions of plants with improved universality and specificity. *Molecular Ecology Resources, 16*(1), 138-149.

Donovan, L. A., Maherali, H., Caruso, C. M., Huber, H. & Kroon, H. (2001). The evolution of the worldwide leaf economics spectrum. *Trends in ecology & evolution (Personal edition)*. 26(2), 88-95.

Edberg, S. C. (1991). *US EPA human health assessment: Bacillus subtilis*. Unpublished, Washington, D.C.: U.S. Environmental Protection Agency.

El Akrem, H., Manaf, A., Marielle, B. & Moktar, H. (2007). The effect of solvents and extraction method on the phenolic content and biological activites in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. *Food Chemistry,* 105(3), 1126-1134.

Evans, W. (1996). *Trease and evans pharmacognosy*. London: W. B. Saunders Company Ltd.

Fereidoon, S. & Ying, Z. (2015). Measurement of antioxidant activity. J*ournal of Functional Foods*, 18(B), 757-781.

Fiala, B., Linsenmair, K. E. & Maschwitz, U. (1994). Diversity of ant-plant interactions: protective efficacy in *Macaranga* species with different degrees of ant association. *Oecologia*, 97, 186-192.

Fiala, B., Maschwitz, U., Tho, Y. P. & Helbig, A. J. (1989). Studies of a South East Asian ant-plant interaction: protection of Macaranga trees by *Crematogaster borneensis*. *Oecologia*, 79, 463-470.

Firn, R. (2010). *Nature's Chemicals*. Oxford University Press, Oxford. 74-75.

Gardener, S., Sidisunthorn, P. & Anusarnsunthorn, V. (2000). *A Field Guide to Forest Trees of Northern Thailand*. Kobfari Publishing Project, Bangkok.

Gilbert, R. J., Turnbull, P. C. B., Parry, J. M. & Kramer, J. M. (1981). *Bacillus cereus and other Bacillus species: their part in food poisoning and other clinical infections.* Academic Press, London, pp 297-314.

Grosvenor, P. W., Gothard, P. K., McWilliam, N. C., Supriono, A. & Gray, D. O. (1995). Medicinal plants from Riau Province, Sumatra, Indonesia. *Journal of Ethnopharmacology*, 45, 75-95.

Gude, D. (2013). Indigenous medicines: a wake-up slap. *Indian Journal of Public Health,* 57, 183-184.

Harborne, J. B. (1973). *Phytochemical Methods*. London: Chapman & Hall.

Heatley, N. G. (1944). A method for the assay of penicillin. *Biochemical Journal*, 3, 861-865.

Hervert-Hernández, D., García, O. P., Rosado, J. L. & Goñi, I. (2011). The contribution of fruits and vegetables to dietary intake of polyphenols and antioxidant capacity in a Mexican rural diet: Importance of fruit and vegetable variety. *Food Research International.* 44, 1182-1189.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A. & Deemer, E. K. (2002). Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated beta-cyclodextrin as the solubility enhancer. *Journal of Agricultural and Food Chemistry.* 50, 1815-1821.

Hyde, M. A., Wursten, B. T., Ballings, P. & Coates Palgrave, M. (2018). *Flora of Zimbabwe: Genus page: Macaranga.* Retrived from https://www.zimbabweflora.co.zw/.

Ibrahim, R. K. & Towers, G. H. N. (1960). The Identification of Plant Phenolics by Paper Chromatography. *Academic Press*, *New York*, 133-149.

Ingold, K. U. (1961). Inhibition of the autoxidation of organic substances in the liquid-phase. *Chemical Review*, 61, 563-589.

Iqbal, M. Z. (1985).Cuticular and anatomical studies of white clover leaves from clean and air-polluted areas. *Pollution Research*, 4, 59-61.

Jaish, B. M. (2015). Medicinal Plants Wealth of the Family Euphorbiaceae in Azamgarh District. *Indian Journal of Science Research*, 11(1), 149-152.

Joseph, J. M. (2014). *Phytochemistry and pharmacology of the genus Macaranga: A review.* vol 8(12), pp. 489-503.

Kittakoop, P., Mahidol, C. & Ruchirawat, S. (2014). Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation. *Current Topics in Medicinal Chemistry,* 14(2), 239-252.

Klepacka, J., Gujska, E. & Michalak, J. (2011). Phenolic Compounds as Cultivar- and Variety-distinguishing Factors in Some Plant Products. *Plant Foods for Human Nutrition,* 66(1), 64-69.

Kochummen, K. M. (1966). Natural Plant Succession after Farming at Sg Kroh. *Malayan Forester 29*, 170-181.

Kovacic, S. & Nikolic, T. (2005). Relations between Betula pendula Roth. (Betulaceae) leaf morphology and environmental factors in five regions of Croatia. *Acta Biologica Cracoviensia.* 47, 7-13.

Kulip, J. (2003). An ethnobotanical survey of medicinal and other useful plants of Muruts in Sabah, Malaysia. *Telopea*, 10(1), 81-98.

Lewis, J. K. (1969). Range management viewed in the ecosystem framework: The ecosystem concept in natural resource management. *Academic Press, New York,* 97-188.

Luque de Castro, M. D. & Priego-Capote, F. (2010). Soxhlet extraction: Past and present panacea, *Journal of Chromatography A*, 1217(16), 2383-2389.

Magaldi, S., Mata-Essayag, C., Hartung de Capriles, C., Perez, C., Colella, M. T., Olaizola, C. & Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Disease*, 8, 39-45.

Manske, R. H. F. (1965). *The Alkaloids. Chemistry and Physiology*. Volume VIII. New York: Academic Press, 673.

Marian, N. & Fereidoon, S. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis,* 41(5), 1523-1542.

Markstaedter, C., Federle, W., Jette, R., Riederer M. & Hoelldobler, B. (2000). Chemical composition of the slippery epicuticular wax blooms on *Macaranga* (Euphorbiaceae) ant-plants. *Chemoecology,* 10, 33-40.

Michael, S. (2009). A Life of Its Own*. Synthetic biology and evolution: The New Yorker,* 1-16. Retrived from https://www.newyorker.com/magazine/a-life-of-its-own.

Naczk, M. & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis,* 41, 1523-1542.

Nihal, T., Ferda, S. Y. & Sedat, V. (2006). Effect of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. *Food Chemistry*, 99(4), 835-841.

Ninoval, D. J., Dushkova, P. I. & Kovacheva, G. V. (1983). Anatomical, Morphological studies of Platanusacerifolia at various degrees of air pollution. *Ekologiya (Sofia)* 6, 35-47.

Norman, S. R. (1981). Extraction of tissue lipids with a solvent of low toxidity. *Method in Enzymology*, 72, 5-7.

Okpekon, T., Yolou, S., Gleye, C., Roblot, F., Loiseau, P., Bories, C., Grellier, F., Frappier, F., Laurens, A. & Hocquemiller, R. (2004). Antiparasitic activities of medicinal plants used in Ivory Coast. *Journal of Ethnopharmacology,* 90, 91-97.

Ovando, C., Hernandez, D., Hernandez, E., Rodriguez, A. & Galan-Vidal, A. (2009). Chemical studies of anthocyanins: a review. *Food Chemistry*, 113, 859-871.

Owen, R. W., Giacosa, A., Hull, W. E., Haubner, R., Spiegelhalder, B. & Bartsch, H. (2000). The antioxidant/anticancer potential of phenolic

compounds isolated from olive oil. *European Journal of Cancer,* 36 (10), 1235-1247.

Parekh, J. & Chanda, S. (2007). *In vitro* antibacterial activity of the crude methanol extract of *Woodfordia fruticosa* kurz. Flower (Lythraceae). *Brazilian Journal of Microbiology*, 38, 204-207.

Prabuseenivasan, S., Jayakumar, M. & Ignacimuthu, S. (2006). *In vitro* antibacterial activity of some plant essential oils. *BMC Complementary and Alternative Medicine*, 6, 39.

Prior, R. L., Wu, X. & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53, 4290-4302.

Priyadi, H., Takao, G., Rahmawati, I., Supriyanto, B., Ikbal Nursal, W. & Rahman, I. (2010). *Five hundred plant species in Gunung Halimum Salak National Park, West Java: a checklist including Sundanese names, distribution and use*. Bogor: Center for International Forestry Research.

Pumza, F. (2014). *Witnessing a South African healer at work*. BBC News. Retrieved from https://www.bbc.com/news/world-africa-22306869.

Qader, S. W., Abdulla, M. A., Chua, L. S., Najim, N., Zain, M. M. & Hamdan, S. (2011). Antioxidant, total phenolic content and cytotoxicity evaluation of selected Malaysian plants. *Molecules,* 16(4), 3433-3443.

Qiu, S., Sun, H., Zhang, A. H., Xu, H. Y., Yan, G. L., Han, Y. & Wang, X. J. (2014). Natural alkaloids: basic aspects, biological roles, and future perspectives. *Chinese Journal of Natural Medicine*, 12(6), 401-406.

Rahmad, Z., Nik, F., Nik, R., Mashhor, M. & Mohd Yunus, Z. (2008). The distribution of Macaranga in Penang Island, Peninsular Malaysia. *Journal of Bioscience*, 19(2), 91-99.

Raymond, S. S., Jonathan, S. J., Michael, J. & Watkins, P. (2010). *The Essence of Analgesia and Analgesics*. Cambridge University Press. 82-90.

Rice-Evans, C. A., Miller, N. J., Bolwell, P. G., Bramley, P. M. & Pridham, J. B. (1995). The relative activities of Plant-derived polyphenolic flavonoid. *Free Radical Research,* 22, 375-383.

Russo, P., Frustaci, A., Del Bufalo, A., Fini, M. & Cesario, A. (2013). Multitarget drugs of plants origin acting on Alzheimer's disease. *Current Medicinal Chemistry*. 20(13), 1686-1693.

Sala, A., Recio, M. D., Giner, R. M., Manez, S., Tournier, H., Schinella, G. & Rios, J. L. (2002). Anti-inflammatory and antioxidant properties of Helichrysum italicum. *Journal of Pharmacy and Pharmacology,* 54 (3), 365-371.

Sato, A. (2012). Revealing the popularity of traditional medicine in light of multiple recourses and outcome measurements from a user's perspective in Ghana. *Health Policy and Planning,* 27, 625-637.

Stekelenburg, J., Jager, B. E. & Kolk, P. R. (2005). Health care seeking behaviour and utilisation of traditional healers in Kalabo, Zambia. *Health Policy*, 71, 67-81.

Sun, J., Chu, Y. F., Wu, X. Z. & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural and Food Chemistry,* 50(25), 7449-7454.

Tilburt, J. C. & Kaptchuk, T. J. (2008). Herbal medicine research and global health: an ethical analysis. *Bulletin of the World Health Organization*, 86, 594-599.

Ting, S. & Chi, T. H. (2005). Antioxidant activities of buckwheat extracts. *Food Chemistry*, 90(4), 743-749.

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L. & Fowler, V. G. (2015). "Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management". *Clinical Microbiology Reviews*. 28 (3): 603-61.

United States Environmental Protection Agency (1997). *Final Risk Assessment of Bacillus subtilis.*

Uphof, J. C. (1959). *Dictionary of Economic Plants*. Weinheim.

US Department of Agriculture. (2016). *Why is it important to eat vegetables?* USDA Center for Nutrition Policy & Promotion.

Ushnie, T. P., Cushnie, B. & Lamb, A. J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents*, 44(5), 377-386.

Valko, M., Rhodes, C. J., Moncol, J. & Izakovic, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*, 160, 1-40.

Whitmore, T. C. (1969). First thoughts on the species evolution in Malayan Macaranga. *Biological Journal of the Linnean Society*, 1, 223-231.

Whitmore, T.C. (2008). *The genus Macaranga, a Prodromus*. Royal Botanic Gardens, Kew.

William, L. H. (2008). *Euphorbiaceae*. The Editors of Encyclopaedia Britannica.

World Health Organization. (2016). *Traditional Medicine*. Fact Sheet No 134.

World Health Organization. (2008). *Traditional Medicine: Definitions*.

Yang, C. S., Landau, J. M., Huang, M. T. & Newmark, H. L. (2001). Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annual Review Nutrition,* 21, 381-406.

Yao, L. H., Jiang, Y. M., Shi, J., Tomas-Barberan, F. A., Datta, N., Singanusong, R. & Chen, S. S. (2004). Flavonoids in food and their health benefits. *Plant Foods for Human Nutrition*, 59(3), 113-122.

Zakaria, M. & Mohd, M. A. (1994). *Traditional Malay Medicinal Plants*. Kuala Lumpur: Penerbit Fajar Bakti, Sdn. Bhd.

Zhang, Y. J., Gan, R. Y., Li, S., Zhou, Y., Li, A. N., Xu, D. P. & Li, H. B. (2015). Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. *Molecules*, 20(12), 21138-21156.

Zheng, W., Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry,* 49 (11), 5165-5170.