CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF TWO VARIETIES OF *ETLINGERA ELATIOR* FLOWERS

NURUL IRYANI BINTI MUHAMAD AZHAR

UNIVERSITI TEKNOLOGI MALAYSIA

CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF TWO VARIETIES OF *ETLINGERA ELATIOR* FLOWERS

NURUL IRYANI BINTI MUHAMAD AZHAR

A report submitted in partial fulfilment of the
requirements for the award of the degree of
Master of Science (Chemistry)

Faculty of Science
Universiti Teknologi Malaysia

2013

Special dedication to my beloved mother and father,
Muhamad Azhar Baba
Rosna Hj. Ismail

My siblings, my teacher, my supervisor, my beloved friends and all lab members.

For all your love, care support, and believe in me.
Thank you so much.

ACKNOWLEDGEMENT

Praise to God for His help and guidance that I am able to complete my research as one of my requirement to complete my master study. I would like to express my deepest gratitude to all the parties involved in this research. First and foremost, I wish to express my sincere thanks to my supervisor Prof. Dr. Hasnah Mohd Sirat for her untiring cum outstanding supervision, guidance and support to me in order to complete this work.

Secondly, I would like to thanks the lecturers at the Department of Chemistry especially, Assoc. Prof. Dr. Farediah Ahmad, Dr. Shajarahtunnur Jamil and Dr. Norazah Basar for their encouragement and helpful suggestions throughout the course of this research. I also would like to forward my appreciation to the technical and laboratory staffs of the Department of Chemistry, in particular Mr. Azmi Md. Rais and Mr. Rasyidi Abd. Mubin for their assistance and guidance in the NMR analysis of the compounds isolated. Mr. Hamzah Basinon and Ms. Fariza for their help with GC and GC-MS analyses.

To all my friends and lab mates, thank you for believing in me and helping me to go through the difficult time. And lastly, I owe my special gratitude to my family members who always care and support me throughout my studies in Universiti Teknologi Malaysia (UTM), without their encouragement and understanding it would have been impossible for me to finish this work.

ABSTRACT

Etlingera elatior is synonymous with *Phaeomeria speciosa, Alpinia elatior, Elettaria speciosa, Nicolaia elatior* and *Nicolaia speciosa.* The species is native to Peninsular Malaysia and Indonesia. In Malaysia, it is called *kantan*. The essential oil and phytochemicals of two *Etlingera elatior* flowers varieties (pink and red flower variety) have been studied. The essential oil of the fresh *E. elatior* flowers was extracted using the hydrodistillation technique and analysed by Kovats Indices and Gas Chromatography-Mass Spectrometry (GC-MS). Hydrodistillation of *E. elatior* (pink flower variety) flowers gave 70.63% of essential oil classified as monoterpenes, sesquiterpenes, and oxygenated of monoterpenes and sesquiterpenes derivatives. The major constituent identified in the essential oil is cyclodecane (38.07%). Extraction of the dried flowers was done by soxhlet and cold extraction methods using chloroform and dichloromethane, respectively. Fractionation and purification on the crude extracts using Vacuum Liquid Chromatography (VLC) and Column Chromatography (CC) was successfully isolated β-sitosterol and a mixture of stigmasterol and β-sitosterol. Their structures were elucidated by GC, GC-MS, IR, ^{1}H NMR and ^{13}C NMR spectroscopies. The antimicrobial activity of essential oil, crude extracts and β-sitosterol was carried out using disc diffusion method, Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assay with eight strains of bacteria, *Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* (Gram positive), *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Pseudomonas putida* (Gram negative). Antimicrobial screening showed that the essential oil gave moderate to strong inhibition to most of the bacteria with minimum inhibition concentration between 225-900 μg/mL.

ABSTRAK

Etlingera elatior adalah sinonim dengan *Phaeomeria speciosa, Alpinia elatior, Elettaria speciosa, Nicolaia elatior* dan *Nicolaia speciosa.* Spesies ini berasal dari Semenanjung Malaysia dan Indonesia. Di Malaysia ia dikenali sebagai kantan. Kandungan minyak pati dan fitokimia daripada dua jenis bunga *E. elatior* (jenis bunga merah jambu dan merah) telah dikaji. Minyak pati daripada bunga segar *E. elatior* diekstrak dengan kaedah penyulingan hidro dan seterusnya dianalisis menggunakan Indeks Kovats dan kromatografi gas-spektrometri jisim (KG-SJ). Penyulingan hidro ke atas bunga *E. elatior* (jenis bunga merah jambu) telah memberikan 70.63% minyak pati, yang diklasifikasikan sebagai monoterpena, sesquiterpena, dan terbitan teroksigen monoterpena dan sesquiterpena. Siklodekana (38.07%) dikenalpasti sebagai konstituen utama di dalam kandungan minyak pati tersebut. Pengekstrakan kandungan fitokimia daripada bunga kering dilakukan secara kaedah pengekstrakan 'soxhlet' menggunakan kloroform dan pengekstrakan sejuk menggunakan diklorometana. Pemeringkatan dan penulenan ke atas ekstrak mentah menggunakan kromatografi cecair vakum dan kromatografi turus telah berjaya mengasingkan β-sitosterol dan campuran stigmasterol dan β-sitosterol. Struktur sebatian tersebut telah dikenalpasti melalui teknik spektroskopi iaitu KG. KG-SJ, IM, RMN ^{1}H dan RMN ^{13}C. Ujian antimikrob ke atas minyak pati, ekstrak mentah dan β-sitosterol telah dijalankan menggunakan teknik pembauran cakera, kaedah Kepekatan Minimum Perencatan (KMP) dan Kepekatan Minimum Bakteria (KMB) dengan lapan jenis bakteria iaitu, *Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* (Gram positif), *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* dan *Pseudomonas putida* (Gram negatif). Penyaringan antimikrob menunjukkan minyak pati mempunyai aktiviti perencatan bakteria yang sederhana-kuat dengan nilai kepekatan minimum perencatan di antara 225-900 µg/mL.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	-	Alpha
$BaCl_2$	-	Barium chloride
β	-	Beta
QCA	-	Caeffeoylquinic acid
^{13}C	-	Carbon
cm	-	Centimeter
δ	-	Chemical shifts
$CHCl_3$	-	Chloroform
CGA	-	Chlorogenic acid
CC	-	Column Chromatography
J	-	Coupling constant
$CDCl_3$	-	Deuterated chloroform
CH_2Cl_2	-	Dichloromethane
Et_2O	-	Diethyl ether
DMSO	-	Dimethyl sulfoxide
DEPT	-	Distortionless Enhancement by Polarization Transfer
d	-	Doublet
dd	-	Doublet of doublet
EBV	-	Eipstein-Barr Virus
EtOAc	-	Ethyl acetate
FTC	-	Ferric thiocyanate
GC	-	Gas Chromatography
GC-MS	-	Gas Chromatography-Mass Spectrometry
g	-	Gram
Hz	-	Hertz
IR	-	Infrared
i.d.	-	Internal diameter

kg	-	Kilogram
L	-	Liter
lit.	-	Literature
MeOH		Methanol
$MgSO_4$	-	Magnesium sulphate
m/z	-	Mass to charge ion
MHz	-	Megahertz
m.p	-	Melting point
m	-	Meter
µg	-	Microgram
µL	-	Microliter
mg	-	Milligram
mL	-	Milliliter
mm	-	Millimeter
MBC	-	Minimum Bactericidal Concentration
MIC	-	Minimum Inhibition Concentration
min	-	Minute
M^+	-	Molecular ion
m	-	Multiplet
nm	-	Nanometer
NMR	-	Nuclear Magnectic Resonance
NA	-	Nutrient Agar
NB	-	Nutrient Broth
ppm	-	Parts per million
cm^{-1}	-	Per centimeter
Prep-TLC	-	Preparative Thin Layer Chromatography
^1H	-	Proton
R_f	-	Retention factor
s	-	Singlet
H_2SO_4	-	Sulfuric acid
t	-	Triplet
UV	-	Ultraviolet
VLC	-	Vacuum Liquid Chromatography

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

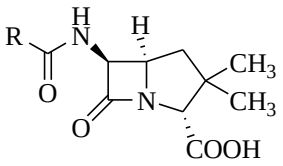
1.1 General Introduction

Nature always stands as golden mark to exemplify the outstanding phenomena of symbiosis [1]. Natural products produced by plant, animal and minerals have been isolated as biologically active pharmacophores and have been used as the basis of treatment of human diseases [1-3]. Natural products are typically secondary metabolites produced by the organisms in response to external stimuli such as nutritional changes, infection and competition [3].

It is estimated today that about 80% of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal uses are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine [1].

History of medicine started practically due to the existence of human civilization. The current accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies [2]. In pre-industrialized society and agrarian societies, plant-derived natural products were used by indigenous populations as therapies for many diseases raging from infections to emphysema. A seminal point in the use of natural products

as single, pharmaceutical entities was the well-known discovery of penicillin **(1)** (1928) [3].



R = the variable group

(1)

Malaysia has been classified as the world's 12 mega biodiversity-rich countries in term of number of plant species with an abundance of variety of medicinal plants [4]. Among the famous plant species used in traditional medicinal purpose are ginger, nutmeg, cloves, turmeric, black pepper and cardamom. Various studies have been carried out on the phytochemicals and bioactivities of these plants because of the wide usage of these plants in traditional medicines.

Ginger is one of the most useful and well-known spice in the world. Most gingers are used not only as spice but also for drugs purposes. Many gingers have been used in traditional medicine which appeared in prescriptions of Chinese medicine, Ayurveda in India and Jamu in Indonesia. Gingers are medicinal plants belonging to Zingiberaceae family that consists of about 47 genera and 1400 species [5]. Ginger is native to Southeast Asia and has been cultivated in countries such as India and China for over 3,000 years [5].

In Chinese traditional medicine, the fresh rhizome of common ginger, *Zingiber officinale* is prescribed as antiemetic, a cough and cold remedies, an antitoxic and digestive stimulant. The dried rhizome of *Z. officinale* is regarded as a good remedy for stomach-ache. Ginger also had been used for the remedies of arthritis, rheumatism, sprains, muscular aches, sore throats, cramps, constipation, vomiting, hypertension, dementia, infectious disease and helminthiasis [5, 6]. The rhizomes of some members of the Zingiberaceae family such as galangal (*Alpinia galangal*), ginger (*Z. officinale*), turmeric (*Curcuma longa*) and krachai

(*Boesenbergia pandurata*) have been extensively used as condiment for flavouring and local medicines for relief from stomach-ache, carminative and treating diarrhoea. They are known to contain various antimicrobial agents [7-9].

The recent progress of science has brought out developments in the studies of the chemistry, pharmacology and biochemistry of natural products such as ginger [5]. Many plants of the Zingiberaceae family traditionally used as spices and drugs possess a variety of important biological properties, the chemical constituents of the plant of the family Zingiberaceae and the biological activities of these constituents must be studied to determine the medicinal and pharmaceutical values of the plant species. Further investigations are required for the prevention of disease and improvement of human health by these plants and their bioactive compounds.

1.2 Statement of Problem

Etlingera is one of the genera in Zingiberaceae family. Several species of *Etlingera* are widely used for many different purposes; as condiment, food flavouring, and as medicine to treat headaches, stomach ache, earache and for cleaning wounds. There have not been many phytochemicals and bioactivity studies conducted on the species of *Etlingera*, however several species have been repoted on *Etlingera elatior*. Previous research conducted on *E. elatior* mostly done on the rhizomes and leaves, and there are only a few reports on the flowers. Thus, the isolation and evaluation of chemical compounds in the flowers of *E. elatior* is essential to be carried out to determine the chemical compounds. It is also important to study the bioactivities of the chemical compounds to determine the medicinal value of the plant.

1.3 Objectives of Study

The objectives of this research are to investigate the chemical composition of the essential oil and the phytochemicals present in the two varieties of *E. elatior* flowers. The first part is to identify the essential oil constituents from the fresh samples. The second part is to isolate and elucidate the structure of the phytochemicals from the dried samples. The final objective is to evaluate the antimicrobial activities of the essential oil, crude extracts and isolated compounds.

1.4 Scope of Study

This research is divided into three parts. The first part is focused on the extraction of the essential oil constituents from the fresh flowers of two varieties of *E. elatior* using hydrodistillation technique. The essential oil constituents will be analysed using GC-MS and Kovats Indices. The second part is to extract the compounds from dried flowers using soxhlet and cold extraction methods. The crude extracts will be fractionated by using vacuum liquid chromatography (VLC), followed by purification of the fractions using gravity column chromatography (CC), and preparative thin layer chromatography (Prep-TLC) to obtain the compounds. The structure of the isolated compounds will be characterized using spectroscopic methods including GC, GC-MS, IR, NMR. The third part is to evaluate the antimicrobial activities on the essential oil, the crude extracts and isolated compounds from two varieties of *E. elatior* flowers by using disc diffusion method.

REFERENCES

1. Sheetal, V., and Singh, S. P. (2008). Current future status of herbal medicines. *Veterinary World.* **1 (11)**, 347-350.
2. Silvia, R. (2007). Natural Products in Drug Discovery. *CHIMIA.* ***61 (6)****, 312.*
3. William, R. S. (2000). The role of natural products in a modern drug discovery program. *Drug Discovery Today.* **5 (2)**, 39-41.
4. Compendium of Medicinal Plants used in Malaysia. (2002). Institute for Medicinal Research. Kuala Lumpur.
5. Mazza, G. and Oomah, B. D. (1998). Herbs, Botanicals and Teas. New York: CRC Press.
6. Ali, B. H., Blunden, G., Tanira, M. O. and Nemmar, A. (2008). Some Phytochemical, Pharmacological and Toxicological Properties of Ginger (*Zingiber officinale* Roscoe): A Review of Recent Research. *Food Chemistry Toxicol*. **46**, 409-420.
7. Oonmetta-aree, J., Suzuku, T., Gasaluck, P. and Eumkeb, G. (2006). Antimicrobial Properties and Action of Galangal (*Alpinia galangal* Linn.) on *Staphylococcus aureus. LWT*. **39**, 1214-1220.
8. Ramirez-Ahumada, M. D. C., Timmermann, B. N. and Gang, D. R. (2006). Biosynthesis of Curcuminoids and Gingerols in Tumeric (*Curcuma longa*) and Ginger (*Zingiber officinale)*; Identification of Curcuminoid Synthease and Hydroxycinnamoyl-CoA Thioesterases. *Phytochemistry*. **67**, 2017-2029.
9. Sabula, B. Dan, M., John, A. J., Kurup, R., Pradeep, N. S., Valsammaa, R. K. and George, V. (2006). Caryophyllene-rich Rhizome Oil of *Zingiber nimmonii* from South India: Chemical Characterization and Antimicrobial Activity. *Phytochemistry*. **67**, 2469-2473.

10. Kress, W. J., Prince, L. M. and Williams, K. J. (2002). The Phylogeny and New Classification of the Gingers (Zingiberaceae): Evidence from Molecular Data. *American Journal of Botany.* **89**, 1682-1696.

11. Larsen, K., Ibrahim, H., Khaw, S. H. and Saw, L. G. (1999). Gingers of Peninsular Malaysia and Singapore. Kota Kinabalu: Natural History Publication. 135.

12. Chan, E. W. C. (2009). Bioactivities and Chemical Constituents of Leaves of some *Etlingera* Species (Zingiberaceae) in Peninsular Malaysia. Monash University, Sunway Campus, Malaysia:PhD Thesis.

13. Sirirugsa, P. (1999). Thai zingiberaceae: Species diversity and their uses. <http: iupac.org/symposia/proceedings/phuket97/sirirugsa.html>

14. Yasodha, S. (2008). Phytochemical Investigation on some Species from the Genera *Elettariopsis* and *Etlingera*. Universiti Sains Malaysia: PhD Thesis.

15. Ibrahim, H. (1992). Malaysian Zingiberaceae: Ecological, Morphological and Economic Aspect. *Bulletin of Heliconia Society International*. **6**, 4-8.

16. Abbas, F., Lajis, N. H., Shaari, K., Israf, D. A., Stanslas, J., Yusuf, U. K. and Raof, S. M. (2005). Alabdane diterpene glucoside from the rhizomes of *Curcuma manga*. *Journal of Natural Products.* **68**, 1090-1093.

17. Lim, C. K. (2003). Taxanomic notes on *Elettariopsis* Baker and new taxa from Peninsular Malaysia and Thailand. *Folia Malaysiana.* **4**, 205-226.

18. Noweg, T., Abdullah, A. R. and Nidang, D. (2003). Forest plants as vegetables for communities bordering the Crocker Rangr National Park. *ASEAN Review of Biodiversity and Environmental Conservation ARBEC.* January-March 2003, 1-18.

19. Ong, H. C. and Norazlina, J. (1999). Malay herbal medicine in Gemencheh, Negeri Sembilan, Malaysia. *Fitoterapia*. **70**, 10-14.

20. Ibrahim, H. and Setyowati, F. M. (1999). *Etlingera*. In: De Guzman, C. C. and Siemonsma, J. S.(Eds) Plant Resources of South-east Asia, Vol: 13, Backhuys Publisher, Leiden, Netherlands. 123-126.

21. Rozanida, A. R., Nurul Izza, N., Mohd Helme, M. H. and Zanariah, H. (2006). Xanzwhite^{TM} – A Cosmeceutical product from species in the family Zingiberaceae. In: Mazura, M. P. *et. al.* (Eds) Harnessing Cures from Nature: trends and Prospects, Publication of Forest Research Institute

Malaysia.

22. Khaw, S. H. (2001). The genus *Etlingera* (Zingiberaceae) in Peninsular Malaysia including a new species. Gardens' Bulletin of Singapore. **53**, 191-239.
23. Poulsen, A. D. (2006). *Etlingera* of Borneo. Kota Kinabalu, Sabah: Natural History Publication (Borneo). 1-174.
24. Lim, C. K. (2000). Taxonomic notes on *Etlingera* Giseke (Zingiberaceae) in Peninsular Malaysia: the '*Nicolaia'* taxa. *Folia Malaysiana*. **1**, 1-12.
25. Lim, C. K. (2001a). Taxanomic notes on *Etlingera* Giseke (Zingiberaceae) in Peninsular Malaysia: the '*Achasma'* taxa and supplementary notes on the '*Nicolaia'* taxa. *Folia Malaysiana.* **2**, 141-178.
26. Boyce, P. (2006). The Gingers of Sarawak I – The Giants. The HSPR Newsletters. **11** (1).
27. Keng, H., Chin, S. C. and Tan, H. T. W. (1998). The Concise Flora of Singapore, Vol II: Monocotyledons. Singapore University Press, Singapore.
28. Chan, E. W. C., Lim, y. Y. and Mohd Ali, N. S. (2010). Composition and Antibacterial Activity of essential oil from leaves of *Etlingera* (Zingiberacea). *International Journal for The Advancement of Science and Arts.* **1** (2), 1-12.
29. Wong, K. C., Sivasothyl, Y, Boey, P. L. and Osman, H. (2010). Essential Oils of *Etlingera elatior* (Jack) R. M. Smith and *Etlingera littoralis* (Koenig) Giseke. *Journal of Essential Oil Research. **22**, 461-466.*
30. Yahya, M. A. A., Yaacob, W. A., Din, L. E. and Nazlina, I. (2010). Analysis of Essential Oils of *Etlingera sphaerocephala* Var. *grandiflora* by two-dimensional gas chromatography with time-of-flight mass spectrometry. *The Malaysian Journal of Analytical Science.* **4** (1), 32-40.
31. Abdelwahab, S. I, Qamaruz, Z. F., Mariod, A. A., Yaacob, M., Abdel Imageed, A. H. A. and Khamis, S. (2010). Chemical composition, antioxidant and antibacterial properties of the essential oils of *Etlingera elatior* and *Cinnamomum pubescens* kochummen. *Journal of the Science of Food and Agriculture.* **90**, 2668-2682.
32. Tadtong, S. Wannakhot, P., Poolswat, W., Athikomkulchai, S.,

Ruangrungsi, N. (2009). Antimicrobial activities of essential oil from *Etlingera puniceae* rhizome. *Journal of Health Research.* **23** (2), 77-79.

33. Jaafar, F. M., Osman, C. P., Ismail, N. H. and Awang, K. (2007). Analysis of Essential Oils of the Leaves, Stems, Flowers and Rhizomes of *Etlingera elatior* (Jack) R. M. Smith. *Malaysian Journal of Analytical Science* **11**, 269-273.

34. Zoghbi, M. D. B.G. and Andrade, E. H. A. (2005). Volatiles of the *Etlingera elatior* (Jack) R. M. Smith and *Zingiber spectabile* Griff.: Two Zingiberaceae cultivated in the Amazon. *Journal of Essential Oil Research.* **17**, 209-211.

35. Wong K. C., Yap, Y. F. and Ham, L. K. (1993). The Esential Oil of Young Shoots of *Phaemoria speciosa* Koord. *Journal of Essential Oil. Research.* **5**, 135-138.

36. Lechat-Vahirua, I., Francois, P., Menut, C., Lamaty, G.and Bessiere, J. M. (1993). Aromatic plants of French Polynesia I. Constituents of three Zingiberacea: *Zingiber zerumbet* Smith, *Hedychium coronarium* Koeing and *Etlingera cevuga* Smith. *Journal of Essential Oil Research.* **5**, 55-59.

37. Sirat, H. M., Masri, D. and Rahman, A. A. (1994). The distribution of labdane diterpenes in the Zingiberaceae of Malaysia. *Phytochemistry*. **36**, 699-701.

38. Mohammad, H., Lajis, N. H. Abas, F., Ali, A. M., Sukari, M. A., Kikuzaki, H. and Nakatani, N. (2005). Antioxidative constituents of *Etlingera elatior*. *Journal of Natural Product.* **68**, 285-288.

39. Mohammad, H., Lajis, N. H. Abas, F., Ali, A. M., Sukari, M. A., Kikuzaki, H. and Nakatani, N. (2005). Antitumor-promoting and Cytotoxic constituents of *Etlingera elatior. Malaysian Journal of Medical Science.* **12**, 6-12.

40. Williams, C. A. and Harborne, J. B. (1977). The Leaf Flavonoids of Zingiberals. *Biochemical Systematics and Ecology.* **5**, 221-229.

41. Chan, E. W. C., Lim, V. Y., Ling, S. K., Tan, S. P., Lim, K. K. and Khoo, M. G H. (2009). Caeffeoylquinic Acids from Leaves of *Etlingera* Species (Zingiberaceae). *LWT-Food Science and Technology.* **42**, 1026-1030.

42. Arjun, P., Jha, S., Murthy, P. N., Manik, Sharone, A. (2010). Isolation and

characterization of stigmast-5-en-3β-ol (β-sitosterol) from the leaves of *Hygrophila spinosa* T. Andres. *International Journal of Pharma Sciences and Research.* **1** (2), 95-100.

43. Pateh, U. U., haruna, A. K., Garba, M., Ilya, I., Sue, I. M., Abubakar, M. S. and Ambi, A.A. (2009). Isolation of stigmasterol, β-sitosterol and 2-hydroxyhexadecanoic acid methyl ester from the rhozomes of *Stylochiton lancifolius* Pyer and Kotchy (Areceae). *Nigerian Journal of Pharmceutical Science.* **8** (1), 19-25.

44. Mackken, M. M., Ali, a. M., El-Sharkawy, S. H., Manap, M. X., Salleh, K. M., Lajis, N. H. and Kawazu, K. (1997). Antimicrobial and Cytotoxic Properties of some Malaysian Traditional Vegetables. *International Journal of Pharmacognosy.* **35**, 174-178.

45. Zavala, M. A., Perez, G. S. and Perez, G. R. M. (1997). Antimicrobial Screening of some Medicinal Plants. *Phytotherapy. Research.* **11**, 368-371.

46. Arias, M. E., Gomez, J. D. Cudmani, N. M. and Isla, M. I. (2004). Antibacterial Activity of Athanolic and Aqueous Extracts of *Acacia aroma* Gill. Ex Hook et Arn. *Life Sciences.***75**, 191-202.

47. Vahirua-Lechat, I. and Mitermite, Y. (2010). Aromatic Plants of French Polynesia. IV. Composition and Chemical Variations of the Essential Oils of Leaves of *Etlingera cevuga* (Seeman) R.E. Smith. *Journal of Essential Oil Research*. **22**, 407-409.

48. Abdelmageed, A. H. A., Faridah, Q. Z. Nur Amalina, A. and Yaacob, M. (2011). The Influence of Organ and Post-harvest Drying Period on Yield and Chemical Composition of Essential Oils of *Etlingera elatior* (Zingiberaceae). *Journal of Medicinal Plants Research*, **5** (15), 3432-3439.

49. Joseph, R. (1998). Karyomorphometrical Analysis and Exploration of Major Essential Oil Constituents in Zingiberaceae. Mahatma Gandhi University, Kottayam: PhD Thesis.

50. Adams, R. P. (2001). Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Corporation, Carol Stream, IL.

51. Greca, M. D., Monaca, P. and Previtera, L. J. (1990). Stigmasterols from

Typha latifolia. *Journal of Natural Product.* **53**, 1430-1435.

52. Anjoo, K. and Kumar, S. (2011). Isolation of stigmasterol and β-sitosterol from petroleum ether extract of aerial part of *Ageratum conyzoides* (Asteraceae). *International Journal of Pharmacy and Pharmaceutical Science.* **3** (1), 94-96.

53. Au, T. S., Yusof, M. Y., Wiart, C., Hassan, H., Hanifah, Y. A. and Kamaruddin, M. Y. (2003). Antibacterial Activity of *Annona squamosal* Linnaeus (Annonaceae). *Investing in Innovation.* **3**, 7-10.

54. Nascimento, G. G. F., Locatelli, J., Freitas, P. C. and Silva, G. L. (2000). Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic-Resistance Bacteria. *Brazillian Journal of Microbialogy*. **31**, 247-256.

55. Al-Reza, S. M., Rahman, A., Lee, J. and Kang, S. C. (2010). Potential Roles of Essential Oils and Organic Extracts of *Zizyphus jujube* in Inhibiting Food-borne Pathogens. *Food Chemistry.* **119**, 981-986.

56. Singh, R., Chandra, R., Bose, M. and Luthra. P. M. (2000). Antibacterial Activity of *Curcuma longa* Rhizome Extract on Pathigenic Bacteria. *Current Science.* **83**, 737-740.

57. Subramanion, J. T. L., Sreenivasan, S., Vello, S. and Zakaria, Z. (2010). Pharmacological activity, phytochemical analysis and toxicity of methanol extract of *Etlingera elatior* (torch ginger) flowers. *Asian Pacific Journal of Tropical Medicine*. 769-774.