PHYTOCHEMICALS AND ANTIBACTERIAL ACTIVITY OF *PIPER RETROFRACTUM* VAHL. AND *PIPER ARBORESCENS* ROXB.

NADYA PUTRI FABARANI

UNIVERSITI TEKNOLOGI MALAYSIA

PHYTOCHEMICALS AND ANTIBACTERIAL ACTIVITY OF *PIPER RETROFRACTUM* VAHL. AND *PIPER ARBORESCENS* ROXB.

NADYA PUTRI FABARANI

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

Faculty of Science
Universiti Teknologi Malaysia

MARCH 2012

For my beloved father, mother, sister and grandmother

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my supervisor, Assoc. Prof. Dr. Farediah Ahmad for her guidance, patience, motivation, support and advices during my research.

I am grateful to the Ministry of Science, Technology and Innovation (MOSTI) Malaysia for financial support *via* vote number 79074. I also thank the Department of Chemistry for the access of using the UV, IR and NMR instruments. Sincerely thanks to all lab assistants who helped me through these years.

Special thanks to my lab mates in the organic chemistry research laboratory, Della, Safina, Noraini, Saidah, Raihan, Athirah, Nuzul, Tarmizi, Syafiqah, Encik Samshul, Kak Shariha and Taysir, for their support and sharing to make the lab works meaningful. I also thank the seniors, Pak Emrizal, Yau, Mala, Azlin, Syamil, Fariz and Salihin for their help and cooperation.

I highly express my appreciation for all my Indonesian friends, especially for the July 2008 intake and the KTF members, thanks for being so nice, friendly and made me have a family here.

Last but not least, special thanks to my father, mother and sister for their love, caring, spirits and encouragement in every single step I make. I also thank to my beloved grandmother for every prayer she made for me. All of you made everything possible for me. Thank you.

ABSTRACT

The phytochemical investigations of the fruits of *Piper retrofractum* Vahl. and the stems and leaves of *Piper arborescens* Roxb. have been carried out. The fruits of *Piper retrofractum* Vahl. were bought from a spice shop at Pandan, Johor Bahru and the methanolic crude extracts of stems and leaves of *Piper arborescens* Roxb. were obtained from UiTM Sarawak, Malaysia. Samples were extracted with Soxhlet extractor using methanol as the solvent to give the crude extracts. The crude extracts were fractionated using vacuum-liquid chromatography and then purified using several chromatographic techniques. The isolated compounds were identified by analysis of various spectral data using infrared, 1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, and HMBC) nuclear magnetic resonance spectroscopies and mass spectrometry. Seven compounds were isolated from these plants. The purification of methanol crude extract of the fruits of *Piper retrofractum* Vahl. yielded four compounds identified as piperine, oleic acid, *N*-isobutyl-2*E*,4*E*,14*Z*-eicosatrienamide and methyl piperate. Another three compounds, characterized as sesartemin, diayangambin and 3-(3,4-dimethoxybenzyl)-4-(3',4',5'-trimethoxybenzyl)-tetrahydrofuran-2-ol had been isolated from the methanol crude extract of the stems of *Piper arborescens* Roxb. The hydrolysis of piperine was done using potassium hydroxide and ethanol to yield piperic acid. The antibacterial test against Gram positive bacteria (*Bascillus subtilis* and *Staphylococcus aureus*) and Gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) were performed on the crude extracts and isolated compounds. The crude extracts and piperine were found to show the strongest inhibition against both Gram positive bacteria with MIC and MBC values of 225 µg/mL.

ABSTRAK

Kajian fitokimia ke atas buah *Piper retrofractum* Vahl., batang dan daun *Piper arborescens* Roxb. telah dijalankan. Buah *Piper retrofractum* Vahl. dibeli daripada kedai rempah di Pandan, Johor Bahru dan ekstrak mentah metanol daripada batang dan daun *Piper arborescens* Roxb. diekstrak di UiTM Sarawak, Malaysia. Sampel telah diekstrak menggunakan Soxhlet dengan metanol sebagai pelarut untuk mendapatkan ekstrak mentah. Ekstrak mentah diperingkatkan menggunakan kromatografi cecair vakum dan kemudian ditulenkan dengan menggunakan beberapa teknik kromatografi. Sebatian tulen dikenalpasti dengan analisis data pelbagai spektrum iaitu spektroskopi inframerah, 1D (^{1}H, ^{13}C dan DEPT) dan 2D (COSY, dan HMBC) resonans magnet nukleus dan spektrometri jisim. Tujuh sebatian telah dipisahkan daripada tumbuhan kajian ini. Penulenan ekstrak mentah metanol buah *Piper retrofractum* Vahl. menghasilkan empat sebatian tulen yang dikenalpasti sebagai piperina, asid oleik, *N*-isobutil-2*E*,4*E*,14*Z*-eikosatrienamida dan metil piperat. Tiga sebatian yang dicirikan sebagai sesartemin, diayangambin dan 3-(3,4-dimetoksihidroksibenzil)-4-(3',4',5'-trimetoksibenzil)-tetrahidrofuran-2-ol telah dipisahkan daripada ekstrak mentah metanol bahagian batang *Piper arborescens* Roxb. Hidrolisis piperina dilakukan dengan menggunakan kalium hidroksida dan etanol untuk menghasilkan asid piperik. Ujian antibakteria terhadap bakteria Gram positif (*Bascillus subtilis* dan *Staphylococcus aureus*) dan bakteria Gram negatif (*Pseudomonas aeruginosa* dan *Escherichia coli*) telah dilakukan ke atas ekstrak mentah dan sebatian tulen. Ekstrak mentah dan piperina didapati menunjukkan perencatan terkuat terhadap kedua-dua bakteria Gram positif dengan nilai MIC dan MBC 225 µg/mL.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	Alpha
β	Beta
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
br	Broad
^{13}C	Carbon-13
CC	Column Chromatography
COSY	Correlation Spectroscopy
COX	cyclooxygenase enzyme
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
cm^{-1}	Per centimeter
δ	Chemical shift
d	Doublet
1D	1 Dimension
2D	2 Dimension
dd	Doublet of doublets
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulfoxide
E	Entgegen
EtOAc	Ethyl acetate
EtOH	Ethanol
EIMS	Electron Impact Mass Spectrometry
^{1}H	Proton
HCl	Hydrochloric acid
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence

Hz	Hertz
IR	Infrared
IC_{50}	Inhibition Concentration at 50%
J	Coupling constant
KBr	Potassium bromide
KOH	Potassium hydroxide
L	Liter
lit.	Literature
LOX	lypoxygenase enzyme
µg	Microgram
M	Molar
M^+	Molecular ion
max	Maximum
MIC	Minimum Inhibition Concentration
min	Minimum
MBC	Minimum Bactericidal Concentration
mg	Milligram
mL	Milliliter
MS	Mass Spectrometry
mM	Millimolar
m/z	Mass to charge ion
MeOH	Methanol
mp	Melting point
MHz	Megahertz
m	Multiplet
NA	Nutrient Agar
NB	Nutrient Broth
Na_2CO_3	Sodium Carbonate
nm	Nanometer
NMR	Nuclear Magnetic Resonance
PE	Petroleum ether
ppm	Parts per million
R*f*	Retention factor
s	Singlet

SD	Standard Deviation
SiO_2	Silica gel
t	Triplet
TLC	Thin Layer Chromatography
TNF	Tumor Necrosis Factor
UV	Ultraviolet
VLC	Vacuum Liquid Chromatography
Z	Zusammen

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background

Today, although science and technology are very advanced, many diseases still trouble us and even threaten our lives. During the process of understanding and treating diseases, humans have discovered a variety of plants with therapeutic value. Many of these medicinal plants have been used for thousand of years by significant fraction of population and are still applied to health care, either alone or in combination with modern medicines. Indeed, it is estimated that about 25 percent of the drugs prescribed worldwide come from plants and 60 percent of antitumor/anti-infectious drugs already on the market or under clinical trial are of natural origin [1]. For people from developing countries, medicinal plants are popular because their products are safe and widely available in low cost [2]. Medicinal properties of plants can be utilized for blood refining, alleviate blood pressure, heart diseases, diarrhea, cough and fever [3].

The family Piperaceae consists of 10 genera and about 2000 species of tropical plants of which about 30 species are medicinal plants and used in Asia-Pasific region. Some of the genera are *Piper, Arctottonia, Micropiper, Peperomia* and *Manekia*. The best known genus is *Piper*. Piperaceae can be recognized by three main features: articulate stems, asymmetrical or cordate leaves and axillary spikes of little round berry-like fruits [4].

Plants belonging to the family of Piperaceae are reputed in the Indian Ayurvedic system of medicine for their medicinal properties and in folklore medicine of Latin America and West Indies. In particular they are useful to cure asthma, bronchitis, fever, pain in abdomen, as stimulant and in haemorrhoidal afflictions [5]. Plants from the family of Piperaceae have many promising phytochemicals with insecticidal activity, among of them are from *Piper nigrum*, *Piper guineense* and *Piper tuberculatum* [6]. The genus *Piper* has been an important source of secondary metabolites which are used for medicinal purposes in various manners [7].

Several *Piper* species from India, Southeast Asia and Africa are of economic importance since they are used as spices and traditional medicines [8]. The antibacterial and fever-reducing activities of *Piper* extracts are well known from ancient Asian medicinal practices in South Asia as well as in other parts of the world [9].

The most famous species of Piperaceae is *Piper nigrum*. The fruit of *Piper nigrum* (black pepper) is widely used in many physiological activities i.e., stimulation of the central nervous system, analgesic, and antipyretic activities [10]. The leaves and fruits are also used as a cough remedy and the seeds for treating stomach-aches [11]. Various uses which include abortifactants, antibiotic, arrow or fish poisons, diuretic, toothache remedy, tobacco snuff substitute and insect repellant, and treatment of anxiety and epilepsy were reported from this plants [12]. As the spice, black pepper has been traded world-wide for many centuries and represents a highly important cash crop for many tropical countries including India, Indonesia, Vietnam, Malaysia and Brazil [8] and so it is called the “King of spices”. It is an important spice, appreciated for both its aroma and its pungency. It is one of the oldest spices used for both culinary and medicinal purposes [13, 14].

Some species such as *Piper clusii*, *Piper longifolium*, *Piper longum*, and *Piper aduncum* have been used in several countries on a small scale as substitutes for pepper. *Piper methysticum* and *Piper medium* are widely used in the Pasific and tropical

America respectively as ceremonial and social non-intoxicant drinks. The leaves of *Piper betle* have been traditionally used for chewing puposes throughout the Southeast Asian region. In peninsular Malaysia some species such as *Piper nigrum* is used traditionally as ingredients in dart-poison owing to their sufficiently strong irritant properties [15]. Several *Piper* species are taken internally as tonics, indigestion mixtures and postnatal medications [16].

1.2 Problem Statement

It is reported that only 10% of the Asian tropic *Piper* species have been investigated for their phytochemicals and biological activities. Based on this statement, this research is focused on the chemical investigation and antibacterial activity of phytochemicals from *Piper arborescens* Roxb. which has not been investigated thoroughly by other researchers and reinvestigation of the phytochemicals of *Piper retrofractum* Vahl.

1.3 Research Objectives

The objectives of this research are to isolate and characterize the phytochemicals from *Piper retrofractum* Vahl. and *Piper arborescens* Roxb. In addition, the crude extracts and characterized phytochemicals are screened for antibacterial activity.

1.4 Scope of Study

The phytochemicals from *Piper arborescens* Roxb. and *Piper retrofractum* Vahl. will be extracted by Soxhlet apparatus using methanol as the solvent. The crude extracts

will be fractionated into several fractions based on the solvent polarity by petroleum ether, chloroform, ethyl acetate, acetone and methanol using vacuum liquid chromatography technique and further purified by multiple column chromatography, preparative thin layer chromatography, and centrifugal preparative thin layer chromatography using chromatotron to get pure phytochemicals. The structures of the pure phytochemicals will be characterized spectroscopically by using IR, NMR (1D and 2D) and MS. The crude extracts and pure phytochemicals will be screened for antimicrobial activity by using disc diffusion method with *Bascillus subtilis* and *Staphylococcus aureus* as Gram positive bacteria and *Escherichia coli* and *Psedomonas aeruginosa* as Gram negative bacteria.

REFERENCES

1. Yaniv, Z. and Bachrach, U. (2005). *Handbook of Medicinal Plants*. (pp. 214). New York : Food Product Press.

2. Sawangjaroen, N., Sawangjaroen, K. and Poonpanang, P. (2004). Effects of *Piper longum* fruit, *Piper sarmentosum* root and *Quercus infectoria* nut gall on caecal amoebiasis in mice. *Journal of Ethnopharmacology*. 91, 357-360.

3. Walton, N.J., Nicholas, J. (1999). *Chemicals from Plants*. (pp. 215). London : World Scientific Publishing.

4. Wiart, C. (2006). *Medicinal Plants of Asia and The Pasific*. (pp. 23). Taylor&Francis Group.

5. Parmar, V., Jain, S.C., Bisht, K.S., Jain, R., Taneja, P., Jha, A. Tyagi, O.D., Prashad, A.K., Wengel, J., Olsen, C.E. and Boll, P. (1997). Phytochemistry of the genus *Piper*. *Phytochemistry*. 46. (4). 597-673

6. Arnason, JT., Durst, T., Philogène, BJR. (2002). Prospection d'insecticides phytochimiques de plantes tempérées et tropicales communes ou rares**.** Biopesticides d'origine végétale. Editions TEC and DOC, Paris. 37-51.

7. Bristow, S.(2004). *The Herb Handbook, Discovering, Using and Enjoying Herbs*. England : D&S Books Ltd.

8. Simpson, BB. and Orgozaly, MO. (1995). *Economic Botany : Plants in our World*. (2nd Ed). (pp. 742). New York : McGraw-Hill.

9. Evans, PH., Bowers, WS. and Funk, EJ. (1984). Identification of fungicidal and nematocidal components in the leaves of *Piper betle* (Piperaceae). *J. Agric. Food Chem*. 32, 1254-1256

10. Wei, K., Li, W., Koike, K., Pei Y., Chen, Y. and Nikaido, T., (2004). New Amide Alkaloids from the Roots of *Piper nigrum*. *J.Nat.Prod.* 67, 1005-1009

11. Shultes, RE. and Raffauf, RF. (1990). *The Healing Forest. Medicinal and Toxic Plants of the Northwest Amazonia. Historical, Ethno- and Economic Botany Series*. (2nd vol). (pp. 484). Portland, OR : Dioscorides Press.

12. Bourbonnais-Spear, N., Awad, R., Maquin, P., Cal V., Sánchez-Vindaz, P., Poveda L. and Arnason, JT. (2005). Plant use by the Q'Eqchi' Maya of Belize in Ethnopsychiatry and Neurological Pathology. *Economic Botany.* 59, 326-336.

13. Madsen, H.L and Bertelsen, G. (1995). Spices as antioxidants. *Trends Food Sci Tech*. 6, 271-277.

14. Ravindran, P.N. (2000). *Black pepper : Piper nigrum*; Medicinal and Aromatic Plants – Industrial Profiles. Centre for Medicinal Plants Research: Kerala, India. 1-526.

15. Jantan, I.B., Ahmad, A.R., Ahmad, A.S. and Ali, N.A.M. (1994). A Comparative Study of the Essential Oils of Five *Piper* Species from Peninsular Malaysia. *Flavour and fragrance journal*. 9, 339-342.

16. Burkill, I.H. (1935). A Dictionary of Economic Products of the Malay Peninsula. (pp. 1736). London : Crown Agents for the Colonies.

17. Burger, W. (1971). Flora Costaricensis. Fieldiana Botany

18. de Morais, S.M., Facundo, V.A., Bertini, L.M., Cavalcanti, E.S.B., dos Anjos Jr, J.F., Ferreira, S.A., de Brito, E.S. and de Souza Neto, M.A. (2007). Chemical Composition and Larvicidal Activity of Essential Oils from *Piper* species. *Biochemical Systematic and Ecology*. 35, 670-675.

19. Ahn, J., Ahn, M., Zee, O., Kim, E., Lee, S., Kim, H and Kubo, I. (1992). Piperidine alkaloids from *Piper retrofractum* fruits. *Phytochemistry*.31.(10), 3609 – 3612.

20. Kikuzaki, H., Kawabata, M., Ishida, E., Akazawa, Y., Takei, Y., Nakatani, N. (1993). LC-MS Analysis and Structural Determination of New Amides from Javanese Long Pepper (*Piper retrofractum*). *Bioscience Biotechnology and Biochemistry*, 57(8), 1329-1333.

21. Duh, C.Y., Wu, Y.C., and Wang, S.K., (1990). Cytotoxic Pyridone Alkaloids from *Piper arborescens*. *Phytochemistry*. 29 (8), 2689-2691

22. Duh, C.Y. and Wu, Y.C. (1990). Cytotoxic Pyridone Alkaloids from the leaves of *Piper arborescens*. *J.Nat.Prod*. 53 (6), 1575-1577.

23. Reshmi, S.K., Sathya, E. and Devi, P.S. (2010). Isolation of piperdine from *Piper nigrum* and its antiproliferative activity. *Journal of Medicinal Plants Research*. 4, 1535-1546.

24. Siddiqui, B.S., Begum, S., Gulzar, T. and Noor F. (1997). An amide from fruits of *Piper nigrum*. *Phytochemistry*. 45, 1617-1619.

25. Banerji, A., Sarkar, M., Datta, R., Sangupta, P. and Abraham, K. (2002). Amides from *Piper brachystachyum* and *Piper retrofracum*. *Phytochemistry*. 59, 897-901.

26. Araujo-Jr, J.X., Da-Cunha, E.V.L., De O-Chaves, M.C., and Gray, A.I. (1997). Piperdardine, A Piperidine Alkaloid from *Piper tuberculatum*. *Phytochemistry*. 44(3), 559-561.

27. Reddy, S.V., Srivinas, P.V., Praveen, B., Kishore, K.H., Raju, B.C., Murthy, U.S. and Rao, J.M. (2004). Antibacterial constituent from the berries of *Piper nigrum*. *Phytomedicine*. 11, 697-700.

28. Tsai, I.L., Lee, F.P., Wu, C.C., Duh, C.Y., Ishikawa, T., Chen, J.J., Chen, Y.C., Seki, H. and Chen, I.S. (2005). New cytotoxic cyclobutanoid amides, a new furanoid lignin and anti-platelet aggregation constituents from *Piper arborescens*. *Planta Medica*. 71 (6), 535-542.

29. Rukachaisirikul, T., Sirriwattanakit, P., Sukcharoenphol, K., Wongvein, C., Ruttanaweang, P, Wongwattanavuch, P. and Suksamrarn, A. (2004). Chemical constituent and bioactivity of *Piper sarmentosum*. *Journal of Ethnopharmacology*. 93, 173 – 176.

30. Tabopda, T.K., Ngoupayo, J., Liu, J., Mitaine-Offer, A., Tanoli, S., Khan, S., Ali, M., Ngadjui, B., Tsamo, E., Lacaille-Dubois, M. and Luu, B. (2008).

Bioactive aristolactams from *Piper umbellatum*. *Phytochemistry*. 69, 1726 – 1731.

31. Li, C.Y., Tsai, W.J., Damu, A.G., Lee, E.J., Wu, T.S., Dung, N.X., Thang, T.D. and Thanh, L. (2007). Isolation and identification of antiplatelet aggregatory principles from the leaves of *Piper lolot*. *J. Agric. Food Chem*. 55, 9436-9442

32. Tawan, C.S., Ipor, I.B., Fashihuddin, B.A., & Sani, H. (2002). A brief account on the wild *Piper* (Piperaceae) of the Crocker range, Sabah. ASEAN Review of Biodiversity and Environmental Conservation (ARBEC), (July–September), 1–11.

33. Rajudin, E., Ahmad, F., Sirat, H.M., Arbain, D. and Aboul-Enein, H.Y. (2010). Chemical constituents from tiger's betel, *Piper prophyrophyllum* N.E.Br. (Fam. Piperaceae). *Natural Product Research*. 24 (4), 387-390.

34. Masuoka, C., Ono, M., Ito, Y. and Nohara, T. (2003). Antioxidative, antihyaluronidase and antityrosinase activities of some constituents from the aerial part of *Piper elongatum* Vahl. *Food Science Technology Research*. 9 (2), 197-201.

35. da S. Alves, H., de Souza, Maria de F.V. and de O. Chaves, Maria C. (2011). Three new compounds from *Piper montealegreanum* Yuncker (Piperaceae). *Journal of Brazilian Chemical Society*. 1-6.

36. Duan, S., Zhang, P. and Yu, P. (2010). Neolignans and lignin from *Piper wallichii*. *Zhonggo zhongyao zazhi*. 35 (2), 180-182.

37. Lin, L-C., Shen, C-C., Shen, Y-C. and Tsai, T-H. (2006). Anti-inflammatory neolignans from *Piper kadsura*. *J. Nat. Prod*. 69, 842-844.

38. Elfahmi, Ruslan, K., Batterman, S., Bos, R., Kayser, O., Woerdenbag, H.J. and Quax, W.J. (2007). Lignan profile of *Piper cubeba*, an Indonesian medicinal plant. *Biochemical Systematics and Ecology*. 35, 397-402.

39. Solis, P.N., Olmedo, D., Nakamura, N., Calderón, A.I., Hattori, M. and Gupta, M.P. (2005). A new larvicidal lignan from *Piper fimbriulatum*. *Pharmaceutical Biology*. 43 (4), 378-381.

40. Kanaki, N., Dave, M., Padh, H. And Rajani, M. (2008). A rapid method for isolation of piperine from the fruits of *Piper nigrum* Linn. *Journal of Natural Medicine*. 62, 281-283.

41. Dahiya, J. S., Woods, D. L., and Tewari, J. P. Piperine from an *Ulocladium sp*. *Phytochemistry*, 1988. 27(7), 2366.

42. Mohrig, J.R. (1979). *Laboratory Experiments in Organic Chemistry*. (3rd ed.). (pp. 528). New York : D. Van Nostrand Company.

43. Gunston, F. D. (1996). *Fatty Acid and Lipid Chemistry*. London, Glasgow, New York: Blacky Academic & Professional.

44. Akita, C., Tatsuya, K., Kaneko, F. and Yamamoto, H. (2004). Solid-state ^{13}C NMR study on Order → Disorder Phase Transition in Oleic Acid. *Journal of Physical Chemistry B*.(108), 4862-4868.

45. Wu, N., Fu, L., Su, M., Aslam, M., Wong, K.C. and Dravid, V.P. (2004). Interaction of Fatty Acid Monolayers with Cobalt Nanoparticles. Nano Letter. *American Chemical Society*. 4.(2), 383 – 386.

46. Ternes, W., Krause, E. (2002). Characterization and Determination of Piperine and Piperin isomers in Eggs. *Analytical Bioanalytical Chemistry*.(374), 155-160

47. Kijjoa, A., Pinto, M.M.M., Tantisewie, B. and Herz, W. (1989). A new Linalool Derivative and Other Constituents from *Piper ribesoides*. *Planta Medica*. 55, 193.

48. Christov, R., Bankova, V., Tsvetkova, I., Kujumgiev, A., and Tejera A.D. (1999). Antibacterial furofuran lignans from Canary Islands propolis. *Fitoterapia*. 70, 89 – 92.

49. Russel, G.B. and Fenemore, P. G. (1973). New lignans from leaves of *Micropiper excelsum*. *Phytochemistry*. 12, 1799 – 1803.

50. Ohashi, H., Kawai, S., Sakurai, Y. And Yasue, M. (1992). Norlignan from The Knot Resin of *Araucaria angustifolia*. *Phytochemistry*. 31 (4), 1371 – 1373.

51. Singleton, P. (2004). *Bacteria, in Biology, Biotechnology and Medicine*. (pp. 1). England : John Wiley & Sons Ltd.

52. Ravage, B., and Brunelle, L. (2004). *Bacteria*. (pp. 10). USA : Gareth Stevens Publishing.

53. Wearing, J. (2010). *Bacteria Staph, Step, Clostridium and Other Bacteria*. (pp. 7). USA : Crabtree Publishing Company.

54. Escudero, M.R., Escudero, D.F.R., Remsberg, C.M., Takemoto, J.K., Davies, N.M. and Yáñez, J.A. (2008). Identification of Polyphenols and Anti-Oxidant Capacity of *Piper anduncum* L. *The Open Bioactive Compounds Journal*. 1, 18 – 21.

55. Mbaveng, A.T., Ngameni, B., Kuete, V., Simo, I.K., Ambassa, P., Roy, R., Bezabih, M., Etoa, F., Ngadjui, B., Abegaz, B., Meyer, J.J.M., Lall, N. and Beng, V.P. (2008). Antimicrobial activity of the crude extract and five flavonoids from the twigs of *Dorstenia barteri* (Moraceae). *Journal of Ethnopharmacology*. 116, 483-489.