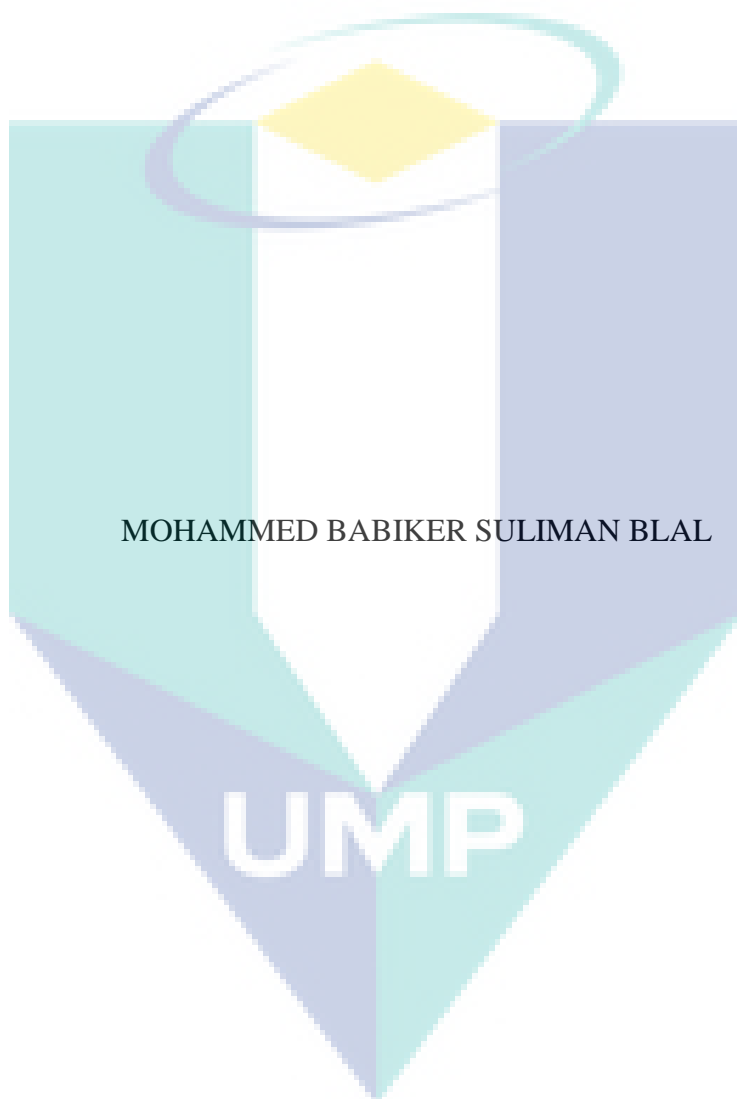


INVESTIGATION OF ANTIMICROBIAL ACTIVITY, BIOASSAY-GUIDED
ISOLATION AND IDENTIFICATION OF ANTIMICROBIAL COMPOUNDS
FROM *SWIETENIA MACROPHYLLA* KING



DOCTOR OF PHILOSOPHY (INDUSTRIAL CHEMISTRY)

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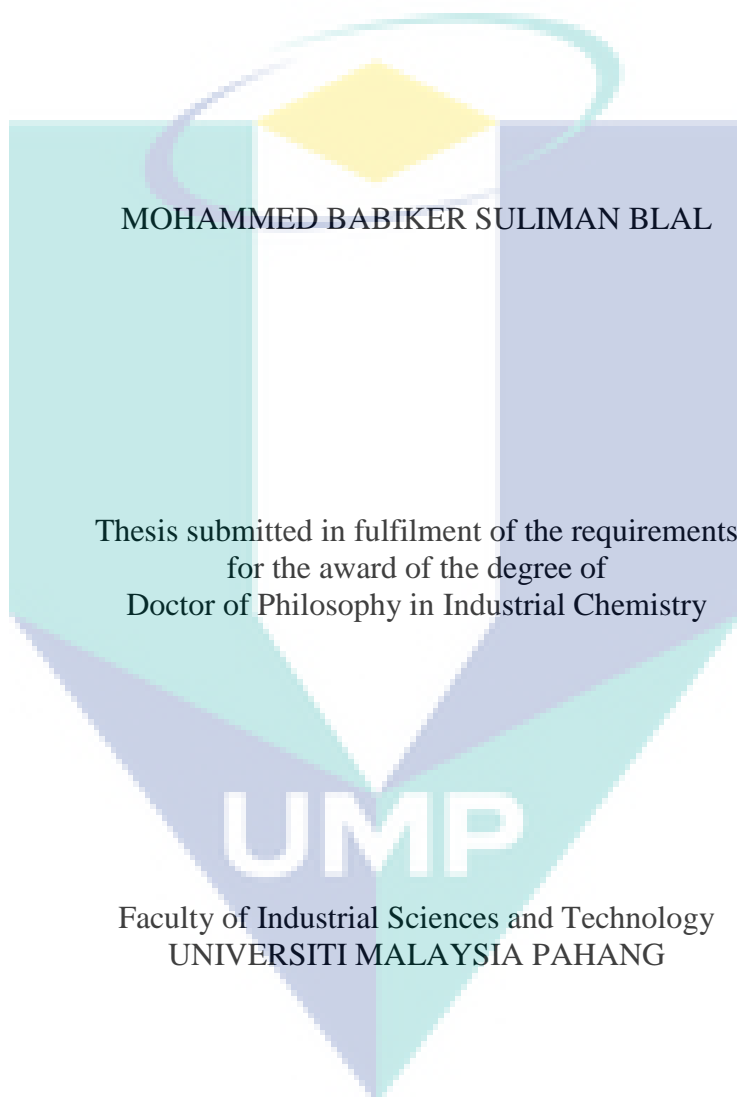
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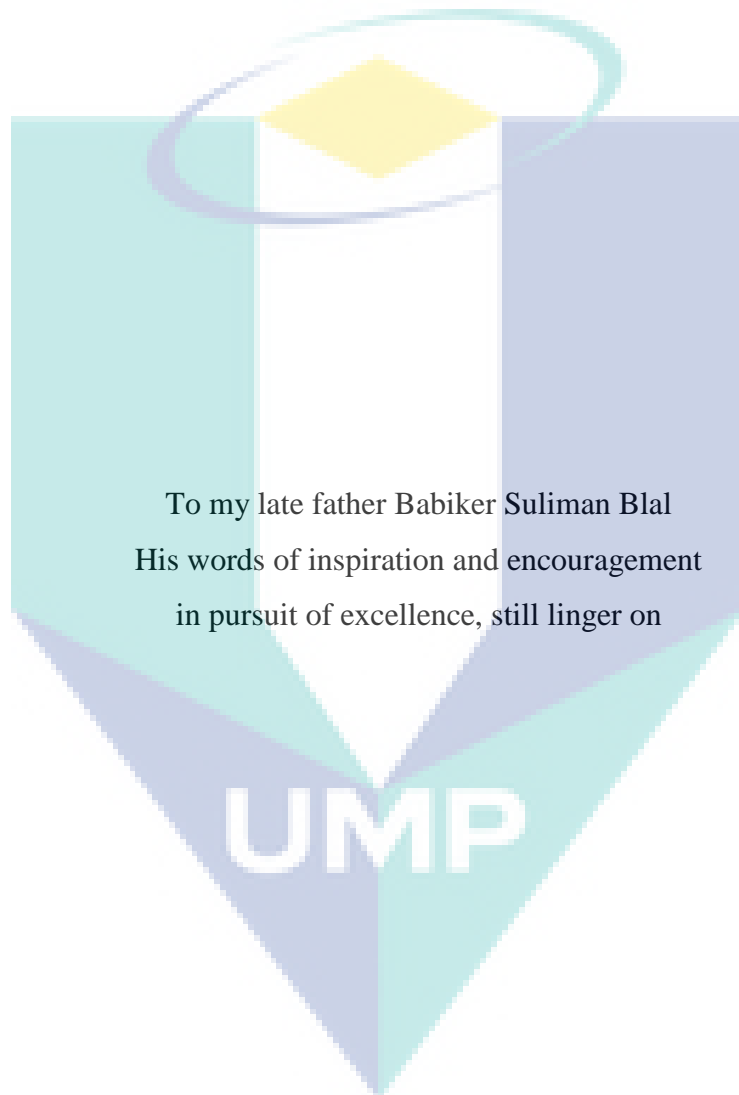
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JULY 2016

DEDICATION

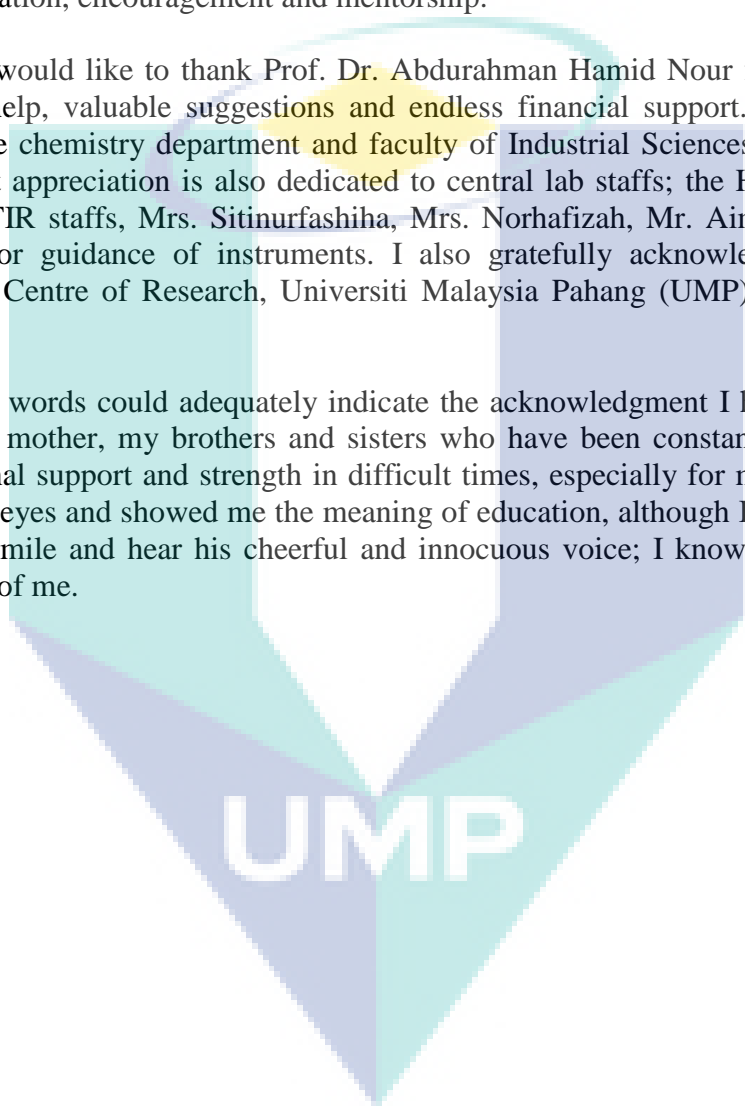


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ABSTRACT

The plant genus *Swietenia* of which *Swietenia macrophylla* (Family: Meliaceae) belongs to, is a large *mahogany* tree growing in the rainforest of Malaysia and widely used in traditional medicine to treat various diseases. In this study, the physicochemical properties of the lipid, phytochemical and antimicrobial activity of extracts and fractions from *Swietenia macrophylla* were investigated. Then the compounds from the most active fraction were isolated and identified. The Minimum Inhibitory Concentrations (MIC) of these compounds were also identified. The dried plant parts (seeds, leaves, stems and roots) were subjected to maceration and later the most active crude extract (seeds extract) was fractionated into different classes according to the polarity with various solvents. The seed oil was extracted by solvent semi-continuous extraction method (Soxhlet) with hexane for six hours. The volatile compounds were identified in the extracts by GC-MS analysis, and the physicochemical properties of the seed oil were determined according to the standard methods. In the antimicrobial test, all the crude, fractions, seed oil and isolated compounds were investigated against nine microorganisms (all were lab strains) by using agar diffusion method. The microbes were: six bacteria, namely; *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Escherichia coli*; and three fungi namely; *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*. The isolation and purification of active compounds has been carried out using chromatographic techniques (analytical HPLC and preparative-LC), and the structure elucidation of the isolated compounds based on spectroscopic techniques including, MS, IR and NMR. The GC-MS results showed three, five, eight, and ten compounds in the roots, stems, leaves, and seeds, respectively. The predominant compound existed in the all extracts was palmitic acid, with relative abundances of 2.03% (roots), 5.79% (seeds), 6.40% (stems), and 14.15% (leaves). The major compound in each part as: 1-tetradecene (18.08%); phytol (19.68%); linoleic acid (39.76%) and stearic acid (52.0%) for the stems, leaves, seeds and roots respectively. The physicochemical properties of the seeds oil were refractive index (1.46), specific gravity (0.954), viscosity (412.7), iodine value (71.5 mL/g), saponification value (211.75. mg KOH/g) and peroxide value (3.25%) are an attribute of the oil to be used for industrial purposes, such as in manufacturing of paint, varnish, and ink industries. The antimicrobial activity among the extracts was extremely broad against all tested organisms. Overall, among the crude extracts, the seeds extract showed more potent as antimicrobial activity than other parts against most tested microbes, whereas within fractionated seed fractions; fraction 3 (ethyl acetate fraction) is more potent. Compounds isolation and structural elucidation of the most bioactive fraction yielded four limonoids, namely swietenolide (**1**), proceranolide (**2**), 3-*O*-tigloyl-6-*O*-acetylswietenolide (**3**) and swietenine acetate (**4**). Among the isolated limonoids, swietenolide (**1**) showed highest activity against all of the tested organisms. The MIC values of the compounds ranged from 4 to 256 µg/mL. Conclusively, these results suggested that limonoids present in *S. macrophylla* were associated with antimicrobial activity. This provides the scientific evidence for the possible use of limonoids derived from *S. macrophylla* as a source of herbal antimicrobial agent(s).

ABSTRAK

Kumpulan tumbuhan *Swietenia* yang berasal dari *Swietenia macrophylla* (Keluarga: Meliaceae) ialah sebuah pokok *mahogany* besar yang tumbuh dalam hutan Malaysia dan digunakan secara meluas dalam perubatan tradisional bagi merawat pelbagai penyakit. Dalam kajian ini, sifat fizikokimia lipid, fitokimia dan aktiviti antimikrobial ekstrak dan pecahan dari *Swietenia macrophylla* telah disiasat. Kemudian sebatian daripada pecahan yang paling aktif telah diasingkan dan dikenal pasti. The Kepekatan Perencatan Minimum (MIC) sebatian ini juga telah dikenal pasti. Kemudian sebatian daripada bahagian yang paling aktif telah diasingkan dan dikenal pasti. Bahagian tumbuhan yang dikeringkan (benih, dedaun, tangkai dan akar) adalah tertakluk kepada pameratan dan kemudiannya, ekstrak mentah yang paling aktif (ekstrak biji) dipecahkan kepada kelas berlainan mengikut kekutuban dengan pelbagai bahan pelarut. Minyak dari benih diekstrak menggunakan kaedah pengekstrakan pelarut separa-selanjur (Soxhlet) dengan heksana selama enam jam. Sebatian meruap dalam ekstrak dikenalpasti melalui analisa GC-MS dan sifat fizikokimia minyak benih ini ditentukan mengikut kaedah umum. Dalam ujian antimikrobial, segala bahan mentah, pecahan, minyak benih dan sebatian terasing telah dikaji terhadap sembilan mikroorganisma (segalanya adalah strain makmal) dengan menggunakan kaedah resapan. Mikrob terlibat adalah enam bakteria, iaitu: *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, dan *Escherichia coli*; serta tiga kulat, iaitu: *Candida albicans*, *Aspergillus flavus*, dan *Aspergillus niger*. Pengasingan dan pemurnian sebatian aktif telah dijalankan menggunakan teknik kromatografi (analisa dan sediaan HPLC) dan struktur penjelasan sebatian terasing berdasarkan teknik spektroskopik adalah termasuk MS, IR dan NMR. Keputusan GC-MS menunjukkan tiga, lima, lapan dan sepuluh dalam akar, tangkai, dedaun dan benih. Sebatian pradominan yang wujud dalam kesemua ekstrak adalah asid palmitic, dengan limpahan relatifnya adalah 2.03% (akar), 5.79% (benih), 6.4% (tangkai), dan 14.15% (dedaun). Sebatian utama dalam setiap bahagian adalah 1-tetradecene (18.08%); fitol (19.68%) asid linoleik (39.76%) dan steric asid (52.0%) bagi tangkai, dedaun, benih dan tangkai. Ciri-ciri fizikokimia minyak benih adalah indeks biasan (1.46), graviti tentu (0.954), kelikatan (412.7), nilai iodin (71.5 mL/g), nilai saponifikasi (211.75. mg KOH/g) dan nilai peroksida (3.25%) yang merupakan ciri-ciri minyak yang digunakan dalam industri seperti penghasilan cat, varnis dan industri dakwat. Aktiviti mikrobial antara sebatian adalah berkesan terhadap setiap organisma yang diuji. Secara keseluruhannya antara ekstrak mentah, ekstrak benih adalah lebih berkesan untuk aktiviti mikrobial berbanding bahagian lain yang diuji terhadap mikrob yang kerap diuji sementara di antara pecahan benih; pecahan 3 (pecahan etil asetat) adalah lebih sesuai. Pengasingan sebatian dan penjelasan struktur pecahan bioaktif yang paling banyak menghasilkan empat limonoid iaitu swietenolide (1), proceranolide (2), 3-O-tigloyl-6-O-acetylswietenolide (3) dan swietenine acetate (4). Antara limonoid yang terasing, swietenolide (1) menunjukkan aktiviti tertinggi terhadap semua organisma yang diuji. Nilai MIC dalam sebatian adalah di paras 4 hingga 256 µg/mL. Secara kesimpulannya, keputusan ini mencadangkan bahawa limonoid yang wujud dalam *S. macrophylla* berhubungkait dengan aktiviti mikrobial. Ini memberikan bukti saintifik untuk kebarangkalian penggunaan limonoid yang terhasil dari *S. macrophylla* sebagai sumber ejen antimikrobial herba yang selamat.

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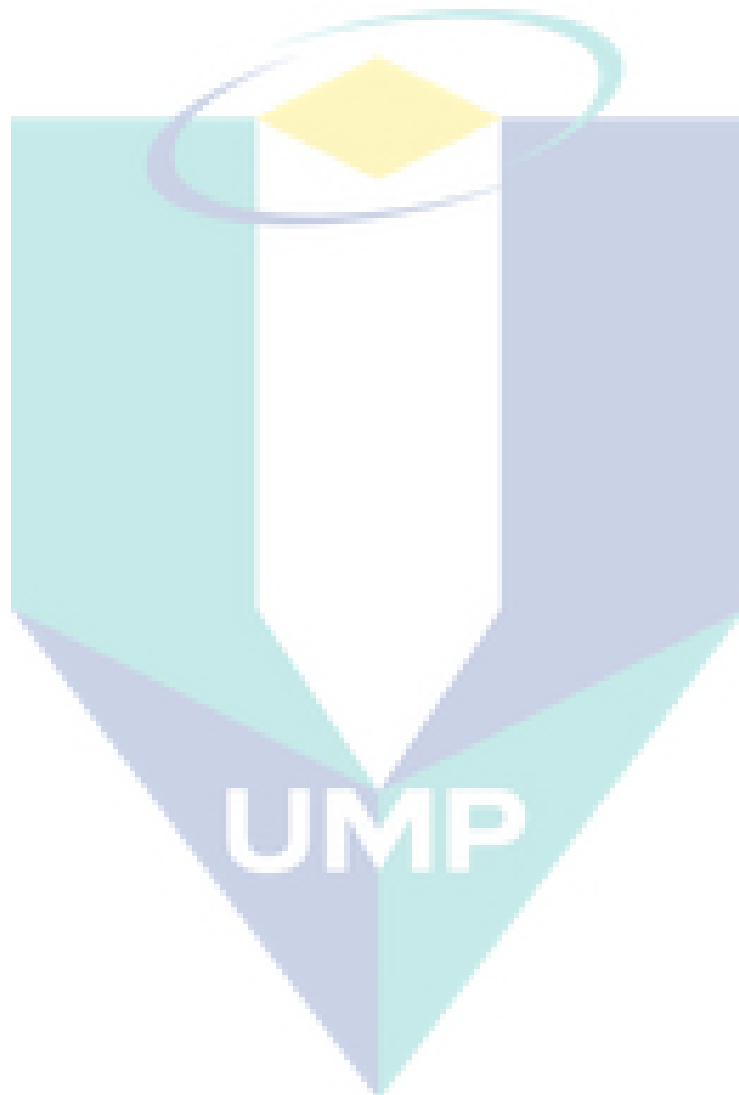
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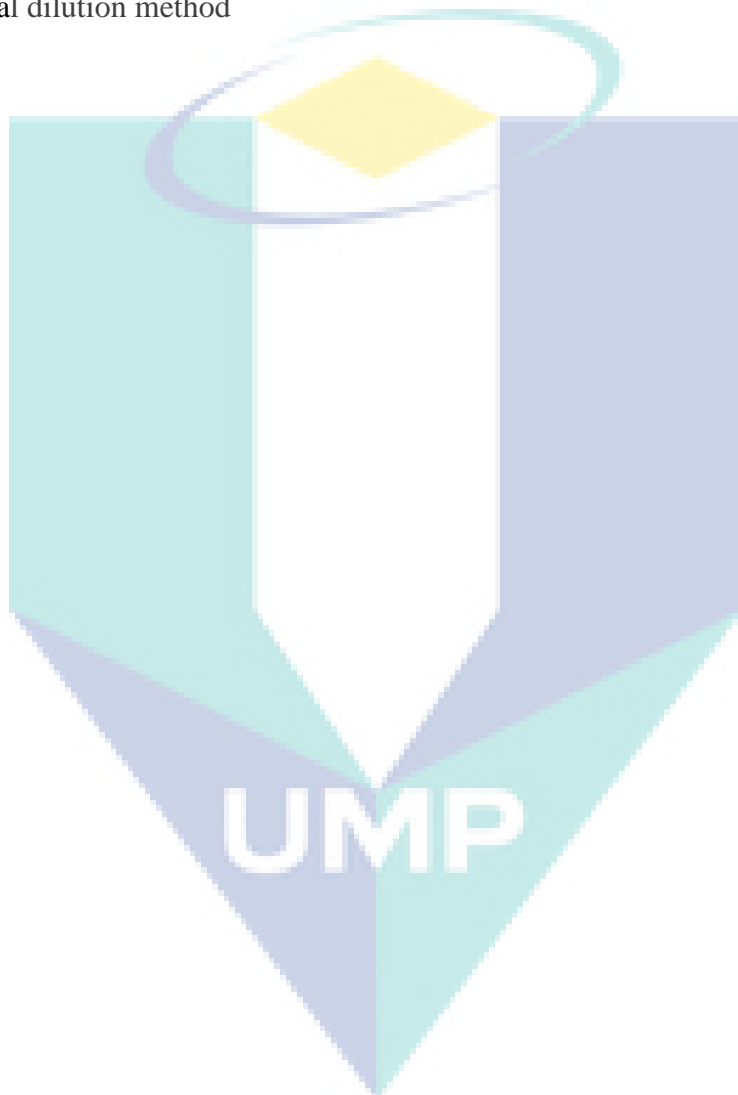
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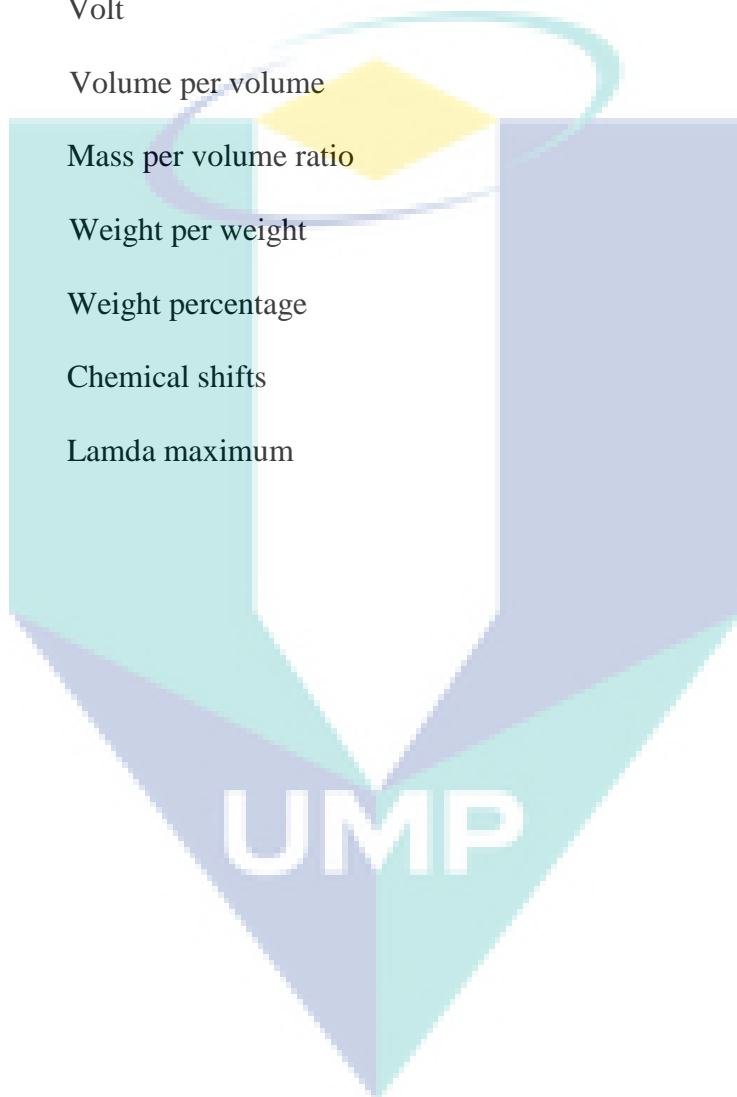
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LIST OF SYMBOLS

%	Percentage
=	Equals
° C	Degree Celsius
° C/min	Degree Celsius per minute
µg	Micro gram
µg/mL	Micrograms per milliliter
cm	Centimeter
cm ⁻¹	Reciprocal centimeter
eV	Electron volt
g	Gram
w/w	Weight per weight
g/L	Gram per liter
g/mol	Gram/mol
GHz	Gigahertz
h	Hour
Hz	Hertz
L	Liter
m	Meter
m/z	Mass-to-charge ratio
Meq/kg	Milliequivalents per kilogram
mg	Milligram
mg/g	Milligram per gram
mg/kg	Milligram per kilogram
mg/ml	Milligram per milliliter

min	Minutes
mL	Milliliter
mL/min	Milliliter per minute
N	Normality
s	Seconds
V	Volt
v/v	Volume per volume
w/v	Mass per volume ratio
w/w	Weight per weight
Wt%	Weight percentage
δ	Chemical shifts
λ_{\max}	Lamda maximum



LIST OF ABBREVIATIONS

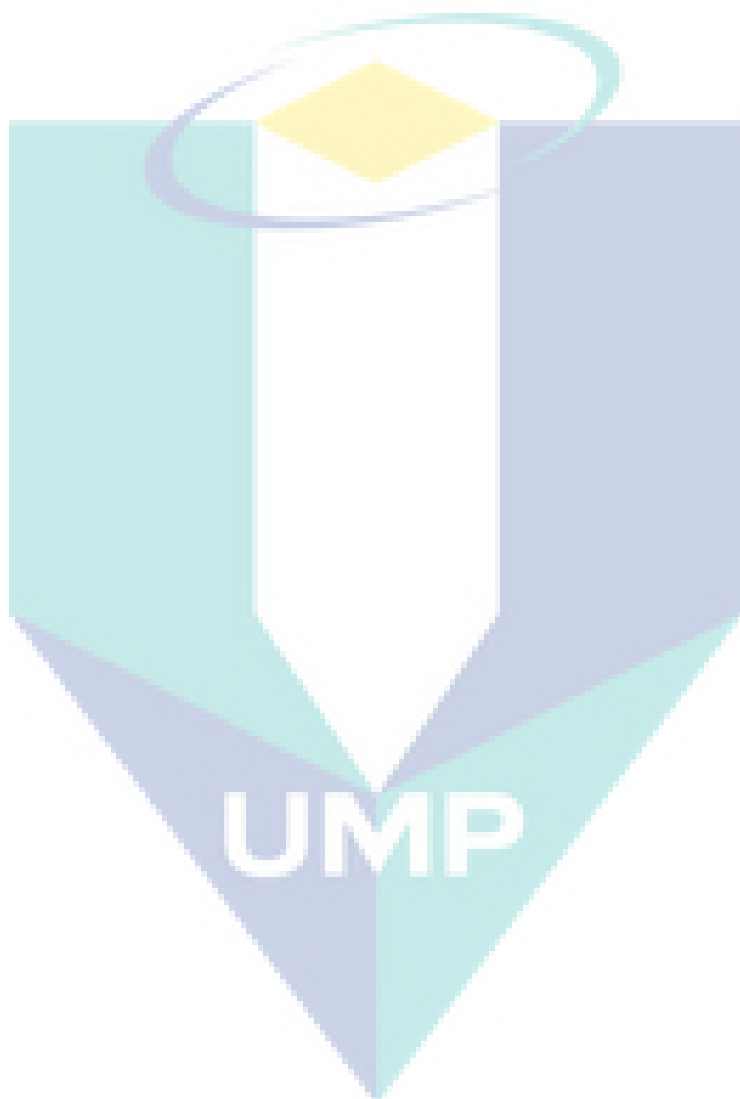
SF	Seed fraction
¹³ C-NMR	Carbon nuclear magnetic resonance
1D-NMR	One dimensional carbon nuclear magnetic resonance
¹ H-NMR	Proton nuclear magnetic resonance
2D-NMR	Two dimensional carbon nuclear magnetic resonance
ANOVA	One way analysis of variance
br	Broad resonance
br s	Broad singlet
C	Carbon
COSY	Correlated spectroscopy
d	Doublet
DAD	Diode array detector
dd	Doublet of doublets
DEPT	Distortionless enhancement by polarization transfer
DMSO	Dimethyl sulfoxide
dt	Doublet of triplets
<i>E. coli</i>	<i>Escherichia coli</i>
EI-MS	Electron ionization-mass spectrometry
et al.	And others
FFA	Free fatty acid
FID	Flame ionization detector
FTIR	Fourier transformed infrared Spectrometer
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry

H	Hydrogen
HETCOR	Hetronuclear correlation spectroscopy
HMBC	Hetronuclear multiple bond correlation
HMBQ	Hetronuclear multiple quantum correlation
HPLC	High performance liquid chromatography
IR	Infrared
LC-MS	Liquid chromatography-mass spectrometry
m	Multiplet
MS	Mass spectrometry
NA	Nutrient agar
NMR	Nuclear magnetic resonance
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
q	Quartet
s	Singlet
<i>S. aureus</i>	<i>Staphyococcus aureus</i>
<i>B. subtilis</i>	<i>Bacillus subtilis</i>
<i>S. macrophylla</i>	<i>Swietenia macrophylla</i>
<i>E. faecalis</i>	<i>Enterococcus faecalis</i>
<i>A. flavus</i>	<i>Aspergillus flavus</i>
<i>S. typhi</i>	<i>Salmonella typhi</i>
Soxhelt	Solvent semi-continuous extraction
SPSS	Statistical package for the social sciences
t	Triplet
TLC	Thin layer chromatography
USA	United States of America

USDA United State Department of Agriculture

UV Ultraviolet spectrometry

API American Petroleum Institute



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CHAPTER 1

INTRODUCTION

1.1 Introduction

The aim of presenting this chapter is to present the motivation and problem statement, objectives and scopes of the research together with the significance and contribution of the research. All these aspects will be the foundation to further discover the research.

1.2 Research Background and Problem Statement

Nowadays, diseases transmitted by microbes such as fungi and bacteria are one of the health problems in many countries worldwide. *Gram*-positive bacteria such *Staphylococcus aureus* causes several serious problems such as food poisoning, post-operative, endocarditis, osteomyelitis and wound infections (Benayache et al., 2001). *Gram*-negative bacteria such as *Pseudomonas aeruginosa* is mainly responsible for urinary tract infections, ventilator-associated pneumonia, surgical site infection, respiratory infections, ocular infections and burn sepsis (Brooks et al., 2007). In the past 60 years, effective antibiotic agents to treat most of the infections and diseases caused by bacteria are commercially available (Brooks et al., 2007). However, the indiscriminate use of available antimicrobial drugs, the wide and long use of antimicrobial drugs, the specific nature of the relationship of bacteria to antimicrobial drugs and environment factor led some bacteria to develop resistance to commercially available antimicrobial agents.

REFERENCES

- Abdel Rahman, S., Abdel-Ellatif, S., Deraz, S. and Kalil, A. 2011. Antibacterial activity of some wild medicinal plants collected from western mediterranean coast, Egypt: Natural alternatives for infectious disease treatment. *African Journal of Biotechnology*. 10(52): 10733-10743.
- Abdelgaleil, S.A.M., Doe, M., Morimoto, Y. and Nakatani, M. 2005. Ring B,D-Seco limonoids from the leaves of *Swietenia mahogany*. *Phytochemistry*. 67: 452-458.
- Abubakar, E.M. 2010. Antibacterial potential of crude leaf extracts of *Eucalyptus camaldulensis* against some pathogenic bacteria. *African Journal of Plant Science*. 4(6): 202-209.
- Adwan, G. and Mhanna, M. 2008. Synergistic effects of plant extracts and antibiotics on *Staphylococcus* strains isolated from clinical specimens. *Middle-East Journal of Scientific Research*. 3(3): 134-139.
- Ahmed, A., Alkarkhi, A.F.M., Hena, S. and Khim, L.H. 2009. Extraction, separation and identification of chemical ingredients of *Elephantopus scaberl* using factorial design of experiment. *International Journal of Chemistry*. 1(1):1 36-49.
- Akpan, U.G., Jimoh, A. and Mohammed, A.D. 2006. Extraction, characterization and modification of *Castor* seed oil. *Leonardo Journal of Sciences*. 8: 43-52.
- Akpuaka, A., Ekwen, M.M., Dashak, D.A. and Dildar, A. 2013. Biological activities of characterized isolates of *n*-hexane extract of *Azadirachta indica* A. juss (*Neem*) leaves. *Nature and Science*. 11(15): 141-147.
- Alekhya, V., Deepan, T., Shaktiprasanna, S. and Dhanaraju, M.D. 2013. Preliminary phytochemical screening and evaluation of *in vitro* antioxidant activity of *Anthocephalous cadamba* by using solvent extracts. *European Journal of Biological Sciences*. 5(1): 34-37.
- Ali, M.A., Sayeed, M.A., Islam, M.S., Yeasmin, M.S., Khan, M.A. and Muhamad Ida, I. 2011. Physicochemical and antimicrobial properties of *Trichosanthes anguina* and *Swietenia mahogani* seeds. *Bull. Bulletin of the chemical Society of Ethiopia*. 25(3): 427-436.
- Alves T.M., Silva, A.F., Brandao, M., Grandi, T.S., Samania, E.F., Junior, A.S. and Zani, C.L. 2000. Biological screening of Brazilian medicinal plants. *Memórias do Instituto Oswaldo Cruz*. 95(3): 367-373.
- Anderdw, E.H.A., Santos, A.S., Zoghbi, M.G.B. and Maia, J.G.S. 1998. Volatile constituents of fruits of *astrocarium vulgare mart.* and *bactris gasipaes H.B.K.* (arecaceae). *Flavour and Fragrance Journal*. 13(3): 151-153.

- Ansorena, D., Astiasaran, I. and Bel, O.J. 2000. Influence of the simultaneous addition of the protease flavourzyme and the lipase novozyme 677BG on dry fermented sausage compounds extracted by SDE and analyzed by GC-MS. *Journal of Agricultural and Food Chemistry*. 48(6): 2395-2400
- Anup, M., Subhash, C.M. and Saikat, D. 2007. *In vivo* evaluation of antidiarrhoeal activity of the seed of *Swietenia macrophylla* king (*meliaceae*). *Tropical Journal of Pharmaceutical Research*. 6(2): 711-716.
- AOAC, 1990. Official methods of analysis (15th ed.) Association of Official Analytical Chemists, Washington D.C. USA.
- AOAC, 1999. Official methods of analysis (16th ed.) Association of Official Analytical Chemists, Gaithersburg, M.D. USA.
- AOAC, 2002. Official methods of analysis (17th ed.) Association of Official Analytical Chemists, Gaithersburg, M.D. USA.
- AOCS, 2009. Official methods and recommended practices of the AOCS free fatty acids (6th ed). Press Champaign I.L. USA.
- Arora, S., Dhillon, S., Rani, G. and Nagpal, A. 2004. The *in vitro* antibacterial/synergistic activities of *Withania somnifera* extracts. *Fitoterapia*. 75: 385-388.
- Arumugasamy, K., Latha, K.V. and Kumar, N.H.S. 2004. Studies on some pharmacognostic profile of *Swietenia macrophylla* king. *Ancient Science of Life*, 24(2): 97-102.
- Asiedu, J.J. 1989. *Processing Tropical Crops. A Technological Approach*. MacMillan Publishers. London.
- Ayepola, O. and Adeniyi, B. 2008. The Antibacterial activity of leaf extracts of *Eucalyptus camaldulensis* (Myrtaceae). *Journal of Applied Sciences Research*. 4(11): 1410-1413.
- Aziz, R., Yaakob, H., Anuar, R.K., Samsulbahrin, J. and Abdul Hamid, M. 2004. Herbal industry potential in Malaysia: Issues and challenges to be a market player. University of Technology, Malaysia.
- Beaulieu, J.C. and Grimm, C.C. 2001. Identification of volatile compounds in cantaloupe at various developmental stages using solid phase microextraction. *Journal of Agricultural and Food Chemistry*. 49(3): 1345-1352.
- Beens, J., Tijssen, R. and Blomberg, J. 1998. *Prediction of comprehensive two-dimensional gas chromatographic separations*. A theoretical and practical exercise. *Journal of Chromatography A*. 822(2): 233-251.

- Benayache, S., Benayache, F. and Benyahia, S. 2001. Leaf oils of some *Eucalyptus* species growing in Algeria. *Journal of Essential Oil Research*. 13: 210-213.
- Bizuneh, A. 2015. Minerals and fatty acid composition analysis of *trichilia emetica* seed oil and the possibility of its use in cosmetic preparation. *World Journal of Pharmaceutical Science*. 3(11): 2185-2191.
- Boatright, W.L. and Crum, A.D. 1997. Nonpolar-volatile lipids from soy protein isolates and hexane-defatted flakes. *Journal of American Chemistry Oil Society*. 74(4): 461-467.
- Boekennoogen, H.A. 1964. *Analysis and characterization of oils, fats and product*. Interscience Publishers, London.
- Bracher, E. 1994. Polycyclic aromatic alkaloids 10. Annonaceous alkaloids with activity. *Arch Pharm (Weinheim)*. 327(6): 371-375.
- Brooks, G., Carroll, K., Butel, J., Morse, S. and Mietzner, T. 2007. Jawetz, melnick, and adelberg's medical microbiology, 24th ed. The McGraw-Hill Companies, USA.
- Chang, L.P., Sheng, L.S., Yang, M.Z. and An, D.K. 1989. Retention index of essential oil in temperature-programmed capillary column gas chromatography. *Acta Pharmacologica Sinica*. 24(11): 847-852
- Chen, J.J., Huang, S.S., Chang, H.L., Dau, C.W., Ping, J.S., Tai, C.W. and Ming J.C. 2010. A new phragmalin-type limonoid and anti-inflammatory constituents from the fruits of *Swietenia macrophylla*. *Food chemistry*. 120: 379-384.
- Chen, J. and Ho, C-T. 1999. Comparison of volatile generation in serine/threonine/glutamine-ribose/glucose/fructose model systems. *Journal of Agricultural and Food Chemistry*. 47(2): 643-647.
- Chen, Y.Y., Wang, X.N., Fan, C.Q., Yin, S. and Yue, J.M. 2007. Swiemahogins A and B, two novel limonoids from *swietenia mahogany*. *Tetrahedron Letter*. 48(42): 7480-7484.
- Cocks, L.V. and Rede Van, C. 1966. *Laboratory handbook for oil and fat analysis*. Academic press Inc. London and New York.
- Das, A., Sunilson, J.J., Gopinath, R., Radhamani, S. and Nilugal. 2009. Anti-nociceptive activity of fruits of *Swietenia macrophylla* king. *Journal of Pharmacy research*. 2(9)1367-1369.
- Demetrio, L.V. J., Jeannie, I.A., Juliana, J.M., Puzon, 1., Esperanza, C.C. and Windell, L. 2015. Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. *Asian Pacific Journal of Tropical Biomedicine*. 5(7): 532-540.

- Diana, M., Mariana-Atena, P. and Ioan, G. 2012. Quality characteristics and oxidative stability of coconut oil during storage. *Journal of Agroalimentary Processes and Technologies*. 18 (4): 272-276.
- Dewanjee, S., Maiti, A., Das, A.K., Mandal, S.C. and Dey, S.P. 2009. Swietenine: A potential oral hypoglycemic from *Swietenia macrophylla* seed. *Fitoterapia*. 80: 249-251.
- Duke, J.A. 2007. Phytochemical and ethnobotanical databases. <http://www.ars-grin.gov/duke/chem-activities.html>.
- Eid, A.M.M., ELMarzugi, N. and EL-Enshasy. 2013. A review on the pharmacological of *Swietenia macrophylla*. *International Journal of Pharmaceutical Sciences*. 3(5): 47-53.
- Ekimoto, H., Irie, Y., Araki, Y., Han, GQ., Kadota, S. and Kikuchi, T. 1991. Platelet aggregation inhibitors from the seeds of *Swietenia mahagoni*: inhibition of *in vitro* and *in vivo* platelet-activating factor-induced effects of tetranortriterpenoids related to swietenine and swietenolide. *Planta Medica*. 57(1): 56-58.
- El-Shazily, A.M., Hafez, S.S. and Wink, M. 2004. Comparative study of the essential oils and extracts of *achillea fragrantissima (forssk) sch. bip.* and *achillea santolina L.* (asteraceae) from Egypt. *Pharmazie*. 59: 226-230.
- Falah, S., Safithri, M., Katayama, T. and Suzuki, T. 2010. Hypoglycemic effect of *mahogany (Swietenia macrophylla king)* bark extracts in alloxan-induced diabetic rats. *Wood Research Journal*. 1(2): 89-94.
- Fan, X.H., Cheng, Y.Y., Ye, Z.L., Lin, R.C. and Qian, Z.Z. 2006. Multiple chromatographic fingerprinting and its application to the quality control of herbal medicines. *Analytica Chimica Acta*. 555: 217-224.
- Farnsworth, N.R., Akerele, O., Bingel, A. S., Soejarto, D.D. and Guo, Z. 1991. *Global importance of medicinal plants*. In *The Conservation of Medicinal Plants*. Cambridge University Press.
- Ganjewala, D., Sam, S. and Khan, K. 2009. Biochemical compositions and antibacterial activities of *Lantana camara* plants with yellow, lavender, red and white flowers. *EurAsian Journal of BioSciences*. 3: 69-77.
- Ghisberti, E.L. 2000. *Lantana camara L.(Verbenaceae)*. *Fitoterapia*. 71(5): 467-486.
- Goh, B.H. and Kadir, H. 2011. *In vitro* cytotoxic potential of *Swietenia macrophylla* King seeds against human Carcinoma cell lines. *Journal of Medicinal Plant Research*. 5(8): 1395-1404.
- Goun, E., Cunningham, G., Chu, D., Nguyen, C. Miles, D. 2003. Antibacterial and antifungal activity of Indonesian ethnomedical plants. *Fitoterapia*. 74(6): 592-596.

- Govindachari, T.R., Suresh, G., Banumathy, B., Masilamani, S., Gopalakrishnan, G. and Kumari, G.N.K. 1999. Antifungal activity of some B, D-seco limonoids from two meliaceous plants. *Journal of Chemical Ecology*. 25(4): 923-933.
- Grayer, R.J. and Harborne, J.B. 1994. A survey of antifungal compounds from higher plant 1982-1993. *Phytochemistry*. 37: 19-42.
- Guo, Z. and Xu, X. 2005. New opportunity for enzymatic modification of fats and oils with industrial potentials. *Organic and Biomolecular Chemistry*. 3(14): 2615-2619.
- Gunstone, F.D., John, L., Harwood. and Albert, J.D. 2007. *The Lipid Handbook with Cd-Rom*. 3rd ed. Boca Raton: CRC Press.
- Haefner, B. 2003. Drugs from the deep: marine natural products as drug candidates. *Drug Discovery Today*. 8(12): 536-544.
- Halabalaki, M., Vougianniopoulou, K., Mikros, E. and Skaltsounis, A.L. 2014. Recent advances and new strategies in the NMR-based identification of natural products. *Current Opinion in Biotechnology*. 25: 1-7.
- Harborne, J.B. 1998. *Phytochemical methods, a guide to modern techniques of plant analysis*. 3rd ed. Chapman and Hall. New York.
- Hashim, M.A., Yam, M.F., Hor, S.Y., Lim, C.P., Asmawi, M.Z. and Sadikun. A. 2013. Anti-hyperglycaemic activity of *Swietenia macrophylla* king (meliaceae) seed extracts in normoglycaemic rats undergoing glucose tolerance tests. *Chinese Medicine*. 8(1): 11-19.
- Heinzen, V.E.F., Soares, M.F. and Yunes, R.A. 1999. *Semi-empirical topological method for the prediction of the chromatographic retention of cis- and trans-alkene isomers and alkanes*. *Journal of Chromatography A*. 849(2): 495-506.
- Hussain, M. and Gorski, M. 2004. Antimicrobial activity of *Nerium oleander* Linn. *Asian Journal of Plant Sciences*. 3(2): 177-180.
- Inoue, Y., Hada, T., Shiraishi, A., Hirose, K., Hamashima, H. and Kobayashi, S. 2005. Biphasic effects of geranylgeraniol, teprenone, and phytol on the growth of *staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*. 49(5): 1770-1774.
- Issaka, S. 2007. *Stability and demulsification of water-in-crude oil emulsion via microwave heating*. M. Eng. Thesis. University Technology of Malaysia.
- Jimenez, A., Villarreal, C., Toscano, R.A., Cook, M., Arnason, J.T., Bye, R., and Mata, R. 1998. Limonoids from *swietenia humilis* and *guarea grandiflora* (Meliaceae). *Phytochemistry* 49(7):1981-1988.
- Kadota, S., Marpaung, L., Kikuchi, T. and Ekimoto, H. 1990. Constituents of the seed of *Swietenia mahagoni* Jacq. I. Isolation, structures and ¹H- and ¹³C-Nuclear

- Magnetic Resonance signal assignments of new tetranortriterpenoids related to swietenine and swietenolide. *Chemical and Pharmaceutical Bulletin*. 38: 639-651.
- Kamisah, Y., Othman, F., Qodriyah, S.H. and Jaarin, K. 2013. *Parkia speciosa* Hassk: A Potential Phytomedicine. *Evidence-Based Complementary and Alternative Medicine*. 1-9.
- Khan, A., Usman, R., Wang, M., Rauf, A., Muhammad, N., Aman, A. and Tahir, T.H. 2013 *In vitro* biological screening of the stem of *Desmodium elegans*. *Asian Pacific Journal of Tropical Biomedicine*. 3(9): 711-715.
- Kirk, R.S. and Sawyer, R. 1991. *Pearson's composition and analysis of foods*. 9th ed. Addison Wesley, Longman.
- Kleiman, R. and Payne-Wahl, L. 1984. Fatty acid composition of seed oils of the meliaceae including one genus rich in cis-vaccenic acid. *Journal of the American Oil Chemists Society*. 61(12): 1836-1838.
- Kowalski, R. 2005. Analysis of lipophylic fraction from leaves inflorescences and rhizomes of *siphium perfoliatum* L. *Acta Societatis Botanicorum Poloniae*. 74(1): 5-10.
- Kumar, V., Mahdi, F., Chander, R., Singh, R., Mahdi, A.A. and Khanna. 2010. Hypolipidemic and antioxidant activity of *Anthocephalus indicus* (kadam) root extract. *Indian Journal of Biochemistry and Biophysics* 47:104-109.
- kumari, S., Moorthi, S. and Kalpans, S. 2014. Comparative analysis of antimicrobial activity of petroleum ether extract with other different extracts of *Syzygium aromaticum* (Linn.) against food borne pathogens. *Journal of Antimicrobials*. 129: 288-293.
- Lahlou, M. 2007. Screening of natural products for drug discovery. *Expert Opinion on Drug Discovery*. 2(5): 697-705.
- Leffingwell, J.C. and Alford. E.D. 2005. Volatile constituents of perique tobacco. *Electronic Journal of Environmental, Agricultural and Food Chemistry*. 4(2): 899-915.
- Linda, 2009. *Different solvents on the extraction of soya bean oil*. M. Sc. Thesis. University Science and Technology, Tamale. <http://dspace.knust.edu.gh:8080/jspui/bitstream/123456789/715/1/DARI%20LINDA.pdf>.
- Lin, B.D., Yuan, T., Zhang, C.R., Dong, L., Zhang, B., Wu, Y. and Yue, J.M. 2009. Structurally diverse limonoids from the fruits of *swietenia mahagoni*. *Natural Products*. 72(12): 2084-2090.

- Lubeck, A.J. and Sutton, DL. 1983. *Kovats retention indices of selected hydrocarbons through C10 on bonded phase fused silica capillaries. Journal of High Resolution. Chromatography.* 6(6): 328-332.
- Lucie, A.T., Dogo, S., Lakouetene, D.B., Florent, B.O., Talla, G.M. and Anna, T. 2013. Chemical characterization and insecticidal activity of ethyl acetate and dichloromethane extracts of *Drypetes gossweileri* against *sitophilus zeamais*, *tribolium castaneum* and *rhyzopertha dominica*. *Journal of Life Sciences.* 7(10): 1030-1040.
- Madhuri, S. and Pandey, G. 2009. Some anticancer medicinal plants of foreign origin. *Current Science.* 96(6): 779-783.
- Maiti, A., Dewanjee, S., Mandal, S.C. and Annadurai, S. 2007. Exploration of antimicrobial potential of methanol and water extract of seeds of *Swietenia macrophylla* (family: meliaceae), to substantiate folklore claim. *Iranian Journal of Pharmacology and Therapeutics.* 6: 99-102.
- Majid, M.A., Rahman, I.M.M., Shipar, M.A.H., Uddin, M.H. and Chowdhury, R. 2004. Physico-chemical characterization, antimicrobial activity and toxicity analysis of *Swietenia mahogany* seed oil. *International Journal of Agriculture and Biology.* 6: 350-354.
- Mallik, J. and Banik, R.K. 2012. *In vitro* studies on antimicrobial and thrombolytic activity of *Swietenia macrophylla* King. *Journal of Pharmaceutical Research and Opinion.* 2(5): 45-48.
- Manivachagam, C., Krishnan, K. and Venugopalan, V. 2008. Antimicrobial activity of fatty acid methyl esters of some members of *Chenopodiaceae*. *Zeitschrift für Naturforschung C.* 63: 331-336
- Maria, J.R., Kannan, P.S.M. and Kumaravel, S. 2001. GC-MS Analysis of *Lantana camara* L. leaves. *International Journal of Pharmaceutical research and development.* 2(11): 63-66.
- Marpaung, H. 2003. The analysis of fatty acid compositions in the seeds of *Swietenia mahogany* JAQ. *Jurnal Sains Kimia.*,7(1): 26-27.
- Massada, Y. 1996. *Analysis of essential oils by gas chromatography and mass spectrometry.* JohnWiley and Sons, New York, NY, USA.
- Mehta, S.D. 2005. *Making and breaking of water in crude oil emulsion.* M. Sc. Thesis. Texas University, USA
- Mendes, M.D., Trndade, H., Figueiredo, A.C., Pedro, L.G. and Barroso, L.G. 2009. *Chaerophyllum azoricun trel.* grown in the Azores archipelago, Portugal: evaluation of the genetic diversity using molecular markers and comparison with volatile oils profiles. *Flavour and Fragrance Journal.* 24(5): 259-265.

- Miguel, A.A., Evangelina, A., Raymundo, S.G., Jose, A.C., E., Jose, B.V. 2009. Extracts of *swietenia humilis* zucc. Seed with antifungal activity in *rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. *Revista Mexicana de Fitopatologia*. 27(2): 84-92.
- Mingarro, I., Lukovic, D., Vilar, M. and Perez-Gil J. 2008. Synthetic pulmonary surfactant preparations: New developments and future trends. *Current Medicinal Chemistry*. 15(4): 393-403.
- Modupe, O., Wessley, O., Morufu, A. and Osiboteii, E. 2010. Analysis of essential oil from the stem of *Chansmanthera dependens*. *Journal of natural product* .3: 47-53.
- Moghadamtousi, S.Z., Goh, B.H., Chan, C.K., Sabah, T. and Kadir, H.A. 2013. Biological activities and phytochemical of *Swietenia macrophylla* King. *Molecules*. 18(9): 10465-10483.
- Mohagheghzadeh, A., Schmidt, T.J. and Alfermann, A.W. 2002. Arylnaphthalene lignans from in vitro cultures of *linum austriacum*. *Journal of Natural Products*. 65: 69-71.
- Mohamed, A., El-Sayed, M., Hegazy, M., Helaly, S., Esmail, A. and Mohamed, N. 2010. Chemical constituents and biological activities of *Artemisia herba-alba*. *Records of natural products*. 4(1): 1-25.
- Mootoo, B.S., Ali, A., Motilal, R., Pingal, R., Ramlal, A., Khan, A., Reynolds, W.F. and McLean, S. 1999. Limonoids from *Swietenia macrophylla* and *S. subrevilleana*. *Journal of Natural Products*. 62: 1514-1517.
- Mostafa, M., Jahan, A.I., Riaz, M., Hossain, H., Nimmi, I., Miah, A. and Chowdhury, U.J. 2011. Comprehensive analysis of the composition of seed cake and its fatty oil from *S. mahogany* Jacq. Growing in Bangladesh. *Journal of Pharmaceutical Sciences*. 10(1): 49-52.
- Muhammad, H.Tari., Bilal, G., Tauqeer, A., Abbas, S., Muhammad, I. Muhammad, J.F. 2015. Phytochemical and microbiological evaluation of different chemical extract of papaya seeds on clinical isolates of (FGSH hospital) islamabad. *International journal of pharmacy*. 5(1): 122-126.
- Mulyono, N., Bibiana, W.L., Ocktreya, L. and Rahayu, S. 2013. Antidiarrheal activity of *Apus bamboo* (*gigantochloa apus*) leaf extract and its bioactive compounds. *American Journal of Microbiology*. 4(1): 1-8.
- Nascimento, G., Locatelli, P., Freitas, C. and Silva, G. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. *Brazilian Journal of Microbiology*. 31(4):247-256.
- Nielsen, S.S. 1994. *Introduction to the chemical analysis of foods*. Chapman and Hall, New York.

- Norman, J.H. 2001. *Nontechnical guide to petroleum geology, exploration, drilling and production*. 2nd ed. Pennwell nontechnical series. Penn Well Publisher.
- Nour, A.H., Elhussein, S.A., Osman, N.A. and Nour, Ab.H. 2009. Characterization and chemical composition of the fixed oil of fourteen basil (*ocimum basilicum L*) accession grown in Sudan. *International journal of chemical technology*. 1(2): 52-58.
- Nour, A.H., Nour, Ab.H., Sandanasamy, J.A/P. and Yusoff, M.M. 2012. Antibacterial Activity of Different Extracts of *Swietenia macrophylla* King. Proceedings of the 13th Seminar on Medicinal and Aromatic Plants, pp. 17-18.
- Nouros, P.G., Georgiou, C.A. and Polissiou, M.G. 1999. Direct parallel flow injection multichannel spectrophotometric determination of olive oil peroxide value. *Analytica Chimica Acta* 389: 239-245.
- Obeidat, M., Shatnawi, M., Al-alawi, M., Al-Zu`bi, E., Al-Dmoor, H., Al-Qudah, M., El-Qudah, J. and Otri, I. 2012. Antimicrobial activity of crude extracts of some plant leaves. *Research Journal of Microbiology*. 7(1): 59-67.
- Omar, J.M.A. 2002. Effects of feeding different levels of sesame oil cake on performance and digestibility of Awassi lambs. *Small Ruminant Research*. 46: 187-190.
- Ovalle-Magallane, B., Medina-Campos, O.N., Pedraza-Chaverri, J. and Mata, R. 2015. Hypoglycemic and antihyperglycemic effects of phytopreparations and limonoids from *Swietenia humilis*. *Phytochemistry*. 110: 111-119.
- Owais, M., Sharad, K. and Saleemuddin, M. 2005. Antibacterial efficacy of *Withania somnifera* (ashwagandha) an indigenous medicinal plant against experimental murine salmonellosis. *Phytomedicine*. 12(3): 229-235.
- O'Neill, C., Gill, M.S., Hobbs, D., Morgan, C. and Bancroft, I. 2003. Natural variation for seed oil composition in arabidopsis thaliana. *Photochemistry*. 64: 1077-1090.
- Perez-Rubio, V., Heredia, J.B., Chaidez-Quiroz, C., Valdez-Torres, J.B., Salazar-Villa, E., Allenda-Molar, R. and Anqulo-Escalante, M.A. 2012. Physicochemical characterization and fatty acid content of 'Venadillo' (*Swietenia humilis zucc.*) seed oil. *African Journal of Biotechnology*. 11(22): 6138-6142.
- Ping, C.L., Ibrahim, H.N. and Yusof, M.H. 2012. Effect of pretreatments on chemical and antioxidant properties of sky fruit (*Swietenia macrophylla*) seed oil. *Jurnal of Teknolgi dan Industri Pangan*. 23(2): 205-209.
- Prashant, T., Bimlesh, K., Mandeep, K., Gurpreet, K. Harleen, K. 2011. Phytochemical screening and extraction: A review. *International Pharmaceutical Sciencia*. 1(1): 98-106.

- Praveen, k.P., Kumaravel, S. and Lalitha, C. 2010. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. *African. Journal of Biochemistry Research* 4(7): 191-195.
- Radulovic, N.S. and Dordevic, N.D. 2011. Steroids from poison hemlock (*conium maculatum* L.): a GC-MS analysis. *Serbian Chemical Society*. 76 (11): 1471-1483.
- Ramalakshmi. S. and Muthuchelian. K. 2001. Analysis of bioactive constituents from the ethanolic leaf extract of *Tabebuia rosea* (Berrol.) DC by gas chromatography-mass spectrometry. *International Journal of ChemTech Research*. 3(3): 1054-1059.
- Rastogi, N., Moreau, B., Capmau, M.L., Goh, K.S. and David, H.L. 1998. Antibacterial action of amphiphatic derivatives of isoniazid against the mycobacterium avium complex. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene*. 4: 456-462.
- Reazazadeh, S., Hamedani, M.P., Dowlatabdi, R., Yazdani, D. Shafiee, A. 2006. Chemical composition of the essential oils of *stachys schtschegleevii* sosn. and *stachys balansae boiss kotschy* from Iran. *Flavour and Fragrance Journal*. 21(2): 290-293.
- Roy, A. and Saraf, S. 2006. Limonoids: overview of significant bioactive triterpenes distributed in plants kingdom. *Biological and Pharmaceutical Bulletin*. 29(2): 191-201.
- Rudel, L.L., Kelly, K., Sawyer, J.K., Shah, R. and Wilso, M.D. 1998. Dietary monounsaturated fatty acids promote aorti atherosclerosis in LDL receptor-null ApoB100-overexpressing transgenic mice. *Arteriosclerosis Thrombosis and Vascular Biology*. 18: 1818–1827.
- Rudnick, L.R. and Erhan, S.Z. 2006. “*Natural oils as lubricants*”, in *synthetics, mineral oils, and bio-based lubricants*, Boca Raton, Florida.
- Sahgal, G.S., Ramanathan, S., Sasidharan, M.N., Mordi, S., Ismail. and Mansor, S.M. 2009. Phytochemical and antimicrobial activity of *Swietenia mahagoni* crude methanolic seed extract. *Tropical biomedicine*. 26(3): 274-279.
- Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M and, Yoga Latha, L. 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative medicines*. 8(1): 1-10
- Schefer, A.B., Braumann, U., Tseng, L.H., Spraul, M., Soares, M.G., Fernandes, J.B., da Silva, M.F., Vieira, P.C. and Ferreira, A.G. 2006. Application of high-performance liquid chromatography nuclearmagnetic resonance coupling to the identification of limonoids from *mahogany* tree (*Swietenia macrophylla*, Meliaceae) by stopped-flow 1D and 2D NMR spectroscopy. *Journal of Chromatography A*. 1128: 152-163.

- Seided, V. 2012. Initial and bulk extraction of natural products isolation. *Methods in Molecular in Biology*. 864: 27-41.
- Shahidur Rahman, A.K.M., Azad Chowdhury, A.K., Ali, H., Raihan, S.Z., Ali, M.S., Nahar, L. and Sarker, S.D. 2009. Antibacterial activity of two limonoids from *Swietenia mahogani* against multiple-drug-resistant (MDR) bacterial strains. *Journal of Natural Medicines*. 63: 41-45.
- Shailendra Kumar, M.B., Bharath, A.C., Nandini, K.C., Prashith Kekuda, T.R., Raghavendra, H.L., Rakesh Kumar, M.C., Rakshitha, M.N. and Vinod Kumar, H.R. 2010. Screening of selected biological activities of *Artocarpus lakoocha roxb* (*Moraceae*) fruit pericarp. *Journal of Basic and Clinical Pharmacy*. 1(4): 239-245.
- Shakeri, A., Hazeri, N., Vlizabeth, J., Ghasemi, A. and Tavallaei, F. 2012. Photochemical screening, antimicrobial and antioxidant activity of *Anabasis aphylla* L. extracts. *Kragujevac Journal of Science*. 34: 71-78.
- Shobana, S., Vidhya, V.G. and Ramya, M. 2009. Antibacterial activity of *Garlic* varieties (*ophioscordon* and *Sativum*) on enteric pathogens. *Current Research Journal of Biological Sciences*. 1(3): 123-126.
- Skaltsa, H.D., Demetzos, C., Lazari, D. And Sokovic, M. 2003. Essential oil analysis and antimicrobial activity of eight *Stachys* species from Greece. *Phytochemistry*. 64(3): 743-752.
- Sridharan., Sriram.,Vaidyanathan., Meena., Venkatesh., Kavitha., Nayagam. and Agnel, A J. 2001. GC-MS study and phytochemical profiling of *Mimosa pudica* Linn. *Journal of Pharmacy. Research*. 4(3): 741-742.
- Stojanovic, I.Z., RAdulovic, N.S., Mitrovic, Y.L.J., Stamenkovic, S.M. and Stojanovic, G.S. 2011. Volatile constituents of selected Parmeliaceae lichens. *Journal of the Serbian Chemical Society*. 76(7): 987-994.
- Subashri, B. and Pillai, Y.J.K. 2014. A comparative study of antioxidant activity of *Baccopa monnieri* (L.) pennell using various solvent extracts and its GC-MS analysis. *International Journal of Pharmacy and Pharmaceutical Sciences*. 6(2): 494-8.
- Sudha, P., Saikat, H., Fayaj, M., Smita, Z., Hirekodathakallu, T. and Ameeta, R. 2015. Gedunin and azadiradione: Human pancreatic alpha-amylase inhibiting limonoids from *neem* (*azadirachta indica*) as anti-Diabetic agents. *PLoS One*. 10(10): 1-19.
- Suffredini, I.B., Paciencia, L.B., Nepomuceno, D.C., Younes, R.N. and Varella, A.D. 2006. Antibacterial and cytotoxic activity of Brazilian plant extracts-clusiaceae. *Memórias do Instituto Oswaldo Cruz*. 101(3): 287-290.

- Sumitra, R., Sudheer K.S., Christian, L., Carlos, R.S. and Ashok, P. 2007. Oil cakes and their biotechnological applications - A review. *Bioresource Technology*. 98: 2000-2009.
- Takahashi, H., Hirata, S., Minami, H. and Fukuyama, Y. 2001 Triterpene and flavanone glycoside from *rhododendron simsii*. *Phytochemistry*. 56: 875-879.
- Tan, S., Osman, H., Wong, K. and Boey, P. 2009. Antimicrobial and antitoxic activities of *Swietenia macrophylla* leaf extract. *Asian Journal of Food and Agro-Industry*. 2(20): 181-188.
- Tan, S., 2009. *Isolation and identification of limonoids from Swietenia macrophylla and their antioxidant and antimicrobial activities*. Ph. D. Thesis. University of Science, Malaysia.
- Ting, W.T.E. and Deibel, K.E. 1991. Sensitivity of *Listeria monocytogenes* to spices at two temperatures. *Journal of Food Safety*. 12(2): 129-137.
- Trabalon, M., Niogret, J. and Legrand-Frossi, C. 2005. Effect of 20-hydroxyecdysone on cannibalism, sexual behavior, and contact sex pheromone in the solitary female spider, *Tegenaria atrica*. *General Comparative Endocrinology*. 144(1): 60-66.
- Tret'yakov, K.V. 2007. Retention Data. NIST Mass Spectrometry Data Center. NIST Mass Spectrometry Data Center.
- Ugbogu, O.C. and Akukwe, A.R. 2009. The antimicrobial effect of oils from *Pentaclethra macrophylla* Bent, *Ghrysophyllum albidum* G. don and *Persea gratissima* gaerth F on some local clinical bacteria isolates. *African Journal of Biotechnology*. 8(2): 285-287.
- Vasudevan, P., Sharma, S. and Kumar, A. 2005. Liquid fuel from biomass: An overview. *Journal of Scientific and Industrial Research*. 64: 822-831.
- Warra, A.A., Wawata, I.G., Gunu, S.Y. and Aujara, K.M. 2011. Extraction and physicochemical analysis of some selected Northern Nigerian industrial oils. *Archives of Applied Science Research*. 3 (4):536-541.
- WHO, 2002. Traditional medicine strategy launched. World Health Organization. 80, 610.
- Wu, S., Lin, G., Chuang, Y., Chang, F., Tseng, C., Wu, Y. and Lee, C. 2011. Anti-hepatitis C virus of 3--hydroxy caruillignan C from *Swietenia macrophylla* stems. *Journal of Viral Hepat*. 19(5): 364-370.
- Xu, X., Tang, Z. and Liang, Y. 2010. Comparative analysis of plant essential oils by GC-MS coupled with integrated chemometric resolution methods. *Analytical Methods*. 2(4): 359-367.

- Yelaware, P.N., Gunashekar, D.R., Faiyaz, A. and Asna, U. 2014. Pharmacological effects and active phytoconstituents of *swietenia mahagoni*: a review. *Journal of Integrative Medicine Editorial office*. 12(2): 86-93.
- Zaha, A.E., Rajashri, R.Naik., Ashok, K.S. and Sanaa, K.B. 2016. Fatty acids analysis, antioxidant and biological activity of fixed oil of *Annona muricata L.* seeds. *Journal of Chemistry*. 2016: 2-6.
- Zaki, N.N. 1996. Surfactant stablized crude oil-in-water emulsion for pipeline transcation of viscous crude oil. *Collides and surfaces A. Physicochemical and Engineering Aspects*. 125: 19-25.
- Zulkania, A. 2004. *Formation and stability study of some Malaysian crude oil emulsions*. M. Eng. Thesis. University Technology of Malaysia.

