ANTIBACTERIAL ACTIVITY OF *Persicaria minor* (Huds.) LEAF-EXTRACTS AGAINST BACTERIAL PATHOGENS

MUSA AHMED ABUBAKAR

UNIVERSITI TEKNOLOGI MALAYSIA

ANTIBACTERIAL ACTIVITY OF *Persicaria minor* (Huds.) LEAF-EXTRACTS AGAINST BACTERIAL PATHOGENS

MUSA AHMED ABUBAKAR

A dissertation submitted in partial fulfillment of the
Requirements for the award of
Master of Science (Biotechnology)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

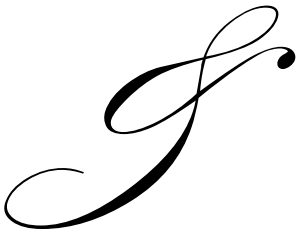
JANUARY 2015

DEDICATION

To

AR-RAZAQ

The provider of assets and all Biotechnogists and Microbiologists who work assiduously towards ensuring the Nutritional values and Antimicrobial actions of naturally occurring plants



HIS EXCELLENCY ENGR. DR. RABIU MUSA KWANKWASO

for providing the scholarship and may the blessings of Allah continue to follow him throughout his future endeavour- Amen.

ACKNOWLEDGEMENT

A research dissertation such as this, usually involves the efforts of many. I would like to start by expressing my profound gratitude to god Almighty Allah, the creator of plants, animals and tiny giants such as microbes and to whom all our praise is due, for making this journey up to the conclusion of my Masters degree, a relatively smooth and successful one. I also wish to express my sincere appreciation to my versatile supervisor, Dr. Razauden Bin Mohamed Zulkifli, for his encouragement, guidance, criticism and friendship without whose support, this research wouldn't have been as presented here.

I also admire and thank my respected parents, Alh. Modu Bukar and Haj. Rashidah Abubakar; without whom, I would not have the chance to understand the beauty of our universe and the tue meaning of love and patience. To this extent, I owe all the nice and valuable moments of my life to them.

Leaving no stone unturned, I am so grateful to my darling wife, Hafsat Ahmad Maigida, for her kindness and never-ending motivations and encouragement; without her understanding and patience, I would't have been able to dedicate my time towards the path of greater success in absolute tranquility. May god Almighty continue to bless her- Amen.

May I also use this opportunity to recognize and appreciate the support of fellow postgraduate students at Nutritional Biochemistry Laboratory who in one way or the other assisted in the successful accomplishment of my research work and finally to all my family members especially Ismael Ibrahim and Fatima Moh'd Abubakar, for their moral and encouragement throughout my study in Malaysia.

ABSTRACT

Persicaria minor (Huds.) Opiz known as Small water-pepper and well recognized locally in Malaysia as "daun kesum" is an edible vegetable with nutritional and medicinal benefits utilized generally by South-east Asians. The present study was conducted to evaluate the antibacterial activity of standardized aqueous-ethanolic and water extracts of *P. minor* leaves. The leaves of the plant undergone extraction based on Malaysian Standard Guideline which is 30% aqueous-ethanol and absolute water as normally used in traditional medicine to produce the respective extract concentrates. Both extracts were evaluated for total protein and polysaccharide contents in which aqueous-ethanolic extract was found to possess high contents of proteins (1713.67 µg/ml) while contents of polysaccharides were high in absolute water extract (17.6 µg/ml). These measurements were used as a standard for different batch extract. The extracts were then tested against four standard strains of bacteria which are *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442 at different concentrations using disc-diffusion (qualitative) and microplate dilution (quantitative) assays. For positive and negative control, penicillin and dimethylsulfoxide were used as controls, respectively. Both extracts showed antibacterial activity with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values in the range of 50 to 100 mg/mL against *S. aureus*, *E. faecalis*, and *E. coli,* respectively with aqueous-ethanolic extract being more potent. However, none of the extracts were active against *P. aeruginosa.* Results from this study truly illustrated high potential of *P. minor* leaves as natural antibacterial agent for the elimination of various bacterial disease and infections.

ABSTRAK

Sejumlah besar herba telah digunakan sebagai sumber pemakanan dan perubatan berasaskan tumbuhan dan dianggap berperanan dalam meningkatkan taraf kesihatan . *Daun kesum* dikenali sebagai Kecil waater-lada dan juga dikenali di Malaysia sebagai adalah sayuran yang membekalkan manfaat nutrisi dan perubatan yang diambil oleh kebanyakan penduduk Asia Tenggara. Kajian ini dijalankan bagi menganalisa aktiviti antibakteria daripada ekstrak daun *P. minor* iaitu ekstrak akues-etanol dan ekstrak. Untuk penghasilan ekstrak, daun diesktrak mengikut Garis Panduan Piawai Malaysia di mana 30% akeus-etanol digunakan dan juga teknik ekstrak air yang sering digunakan dalam perubatan tradisional. kandungan jumlah protin dan dan didapati ekstrak ekues-ethanol mempunyai kandungan protein yang tinggi (1713,67 μg / ml) manakala kandungan polisakarida yang tinggi dalam ekstrak air (17.6 μg / ml). Sukatan ini telah digunakan sebagai piawai untuk kumpulan ekstrak yang berbeza. Ekstrak kemudiannya diuji terhadap empat jenis bacteria piawai iaitu yang *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442 pada kepekatan yang berbeza menggunakan kaedah kualitatif (resapan cakera) dan kaedah kuantitatif (pencairan papan kecil) dengan penisilin digunakan sebagai kawalan positif dan dimethylsulfoxide (pembawa) sebagai kawalan negatif. Kedua-dua ekstrak menunjukkan aktiviti antibakteria dengan nilai kepekatan perencatan minimum (MIC) dan kepekatan bakteria minimum (MBC) masing-masing dalam lingkungan 50 hingga 100 mg/mL terhadap *S. aureus, E. faecalis, dan E. Coli dengan* ekstrak akueus-etanol menunjukkan kesan yang lebih ketara. Walau bagaimanapun, tiada ekstrak aktif terhadap *P. aeruginosa.* Oleh itu, keputusan yang diperolehi dalam kajian ini telah menunjukkan nilai-nilai nutrisi potensi tinggi daun P. minor untuk digunakan sebagai agen antibakteria semulajadi bagi menghapuskan kebanyakan penyakit dan jangkitan bawaan bakteria.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

(A)	-	*Staphylococcus aureus*
ATCC	-	American Type Culture Collection
(B)	-	*Escherichia coli*
$BaCl_2$	-	Barium chloride
B-caryoph	-	Beta-caryophyllene
BSA	-	Bovine Serum Albumin
(C)	-	*Enterococcus faecalis*
C10	-	Position 10 carbon
C12	-	Position 12 carbon
(D)	-	*Pseudomonas aeruginosa*
DDT	-	Disc diffusion test
DMSO	-	Dimethylsulfoxide
E. coli	-	*Escherichia coli*
E. faecalis	-	*Enterococcus faecalis*
e.t.c	-	Et cetera
FRIM	-	Forest Research Institute of Malaysia
H_2O	-	Water molecule
H_2SO_4	-	Sulphuric acid
M	-	McFarland standard solution
MBC	-	Minimum Bactericidal Concentration
mg	-	Milligram
MIC	-	Minimum Inhibitory Concentration
mL	-	Milliliter
mm	-	Millimeter
MRSA	-	Methicilin resistant *Staphylococcus aureus*
N	-	No activity

NA	-	Nutrient agar
NB	-	Nutrient broth
NHCP	-	National Health Care Programmes
nm	-	Nanometer
No	-	Number
P. minor	-	*Persicaria mionr*
P. odorata	-	*Persicaria odorata*
R	-	Resistant
rpm	-	Revolution per minute
R^2	-	Fitness value
SD	-	Standard deviation
Sample-A	-	30% aqueous-ethanolic extract
Sample-B	-	100% aqueous extract
S. aureus	-	*Staphylococcus aureus*
S. *epidermidis*	-	*Streptococcus epidermidis*
S. *pneumonia*	-	*Streptococcus pneumonia*
S. *pyogenes*	-	*Streptococcus pyogenes*
TSI	-	triple Sugar Iron
TSS	-	Toxic Shock Syndrome
TSSE	-	Toxic Shock Skin Exfoliation
UTCTAD	-	United Nations Conference on Trade Development
USSR	-	Union of Soviet Socialist Republics
UTI	-	Urinary tract infection
UTIs	-	Urinary tract infections
UTM	-	Universiti Teknologi Malaysia
nm	-	Nanometer
UV	-	Ultra violent
VRSA	-	Vancomycin resistant *Staphylococcus aureus*
WHO	-	World Health Organization
X	-	Concentration of the unknown
Y	-	Absorbance
%	-	Percentage
µg	-	Microgram
µL	-	Microliter

+	-	Addition
±	-	Plus or minus
>	-	Greater than
<	-	Less than

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

The utilization of medicinal plants as raw materials in the development of drugs is again getting to be well known in the world. Since the beginning of time, plants have been the backbone of medicinal remedies of which is a vital part of every society where the Food and Drug Administration and control have approved many plant herbs for therapeutic purposes (Basu, 2004).

Medicinal plants are said to be considered as vital element in various traditional systems of medications such as Traditional Chinese and Ayuverdic. China utilized about 50% of total consumption of medicine by its people through traditional herbal medication. However, in Africa, almost 60% of its countries such as Nigeria, Mali and Zambia utilize herbal medicine traditionally at home to treat some children infectious diseases which includes malaria, typhoid fever and many more (Krainsitu, 2003).

The potential plant herb for the present study is *Persicaria minor (*Huds.) Opiz which is previously known as *Polygonum minus* is a species in the family *Polygonocaea* collectively referred to as smart-weeds (Thomson *et al.,* 2013). *Persicaria minor* has many common names depending on the country. Its English

name includes Small water pepper, Pygmy smart weeds, Tear-thumb, Slender persicaria (Christophe, 2003 and Hamidun *et al.*, 2011). Nevertheless, based on the reports of Vimala *et al.*, 2011, small water pepper is recognised as "kesom" in Malaysia, Vietnam and Singapore. The plant has a cosmopolitan distribution in Southeast Asia and some wild-weather areas of Canada, Europe and USA.

Table 1.0 *Persicaria minor* Description (Syarul *et al.*, 2010; Thomson *et al.*, 2013 and Vimala *et al.*, 1999)

Name	**Criteria**			
	Condition	**Distribution**	**Traditional uses**	**Types of active ingredients**
P. minor	Flourishes well in wet and damp environments. It grows best at temperatures between 15 to 35^{O}C.	Tropical, sub-tropical, Canada, Europe and USA.	**1.** Mainly used as culinary herb. **2.** Used for stomach indigestion, dandroff, diarrhea, taken after chil-birth. **3.** For sexual desire suppression	Aldehydes (decanals, do-decanal), alcohols, terpenes, flavonoids, phenolic mixes, oxalic acids and proteins.

The species of *Persicaria,* is made up to about 150 different species with cosmopolitan distribution in Southeast Asia. The decoction or boiling down the crushed leaves or the plant itself of many species of *Persicaria*, including *P. barbata* and, *P. odorata* and *P. chinensis*, are used for the treatment of skin diseases such as scabies, ring-worms, boils, and ulcers (Wilson, 1990); and also used for the treatment of fresh wounds, snake bites, dog bites and insect bites due to the disinfection property of the plants (Nguyen, 1993). *Persicaria chinensis* alone, is therefore considered, traditionally for the treatment of eye infection, cholera, dysentery and

headache (Do, 2001). *Persicaria minor* is used as vegetable for cooking or mixed into salads. It has a pungent taste and therefore used as a spice , but also reported to have some medicinal importance especially as antioxidant agent (Vimala *et al.,* 2011). Some populace of the regions utilize its leaves to be used in folk medicine to treat various ailments; the leaves have generally been controlled to treat the following : indigestion, stomach associated wounds and fungal infections. Its volatile aromatic components are utilized as flavor and fragnance agents (Vimala *et al.,* 2011) and hence, its leaves are used worldwide in medicine, cuisines, pharmacy and cosmetics.

Generally, the medicinal value of a drug plant is due to the presence of some bioactive chemical substances that produce a definitive physiological action on the body. The most important of these substances include aldehydes, alcohols, alkaloids, compounds of carbon, hydrogen, nitrogen, and many more. Some of these substances are poisonous so that the preparation and administration of the drug are left in the hands of skilful pharmacists and physicians (Geissman, 1963).

1.2 Statement of the Research Problem

Some microorganisms of bacterial species have beneficial role in nature such as *Lactic-acid bacteria, Enterobacter* and some species of *Clostridium,* in which they contribute immensely to the production of many useful biological end-products in the fermentation industries. However, some species of bacteria are etiological agents of human diseases. *Persicaria minor (*Huds.) has long been used traditionally by most of the people in Southeast Asia especially in Malaysia for the treatment of dandroffs, fugal infections, diarrhoea and other digestive tract disorders (Thomson *et al.,* 2013 and Wilson,1990). However, to date, little is known about its effect on various pathogenic bacteria. Therefore, the antibacterial activities of *Persicaria minor* leaf-extracts against some pathogenic bacterial isolates need to be re-investigated to explore its antimicrobial properties and scientifically support its traditional claims for preventive health care.

1.3 Significance of the Study

To help in creating awareness on the therapeutic potentials of *Persicaria minor* plant other than its vegetable uses. Likewise to generate standard information about the plant on which different studies can be based upon that can be valuable for exploration and pharmaceutical industries.

1.4 Specific Objective of the Study

i. To extract *Persicaria minor* leaves using aqueous-ethanol and absolute water.
ii. To standardize *Pesicaria minor* leaf-extracts by quantitative analysis.
iii. To evaluate the antibacterial activity of *P. minor* extracts against some pathogenic bacterial strains using disc diffusion method.

1.5 Scope of the Study

The present study focused only on plant **extraction, standardization** and **antibacterial screening**. The dry leaves of *Persicaria minor* herb were crushed and extracted (by 30% aqueous-ethanol and absolute water). After the extraction process, standardization of the extracts were demonstrated using calorimetric analysis for the quantitative identification of total protein and polysaccharide contents present in the plant leaves. Bioassay procedure using disc-diffusion method for each of the extracts was conducted for antibacterial sensitivity testing against four strains of bacteria namely; *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Finally, concentration of minmum inhibition (MIC) and that of minimum bactericidal (MBC) of both extracts were also demonstrated. All data designed for the experiment were measured and presented statistically.

REFERENCES

Adamu, H.M., Blar, A, Odise, M, Metchawe, C. (2000). Antimicrobial Activity and Phytochemical Screening of some Selected Plants in Bauchi (Nigeria). *Journal of Ecology*. Taxo. Bot: 2, 123-127.

Ahmad, I., Mehmood, Z., Mohd, F. (1998). Screening of some Indian Medicine Plants for their Antimicrobial Properties. *Journal Ethenopharmocol.* Vol. 62: 183-193.

Akpulu, I.N., J.D., Odama, E.L., and Galadima H. (1994). " Antimicrobial Activity of Aqueous Extracts of some Savanna Medicinal Plants of Nigeria". *Nigerian Journal of Botany.* 7: 46-51.

Amita Pandey, Shalini Tripathi (2014). Concept of Standardization, Extraction and Pre-phytochemical Screening Strategies for Herbal Drug. *Journal of Pharmacognosy and Phytochemistry.* 2 (5): 115-119.

Ampofo, O., (1999). The Practice of Physiotherapy in Ghana in Africa Medicinal Plants (ed. Sofowora, E.A). University of Ife, Nigeria. 67-70.

Annie Corapi (2011). "The 10 healthiest ethnic cuisines". *CNN Health*. Retrieved 3 December 2011.

Annie George, sasikala Chinnappan, Yogendra Choudhary, Praveen Bommu, Murthy Sridhar (2014). Immunomodulary activity of an aqueous extract of polygonum minus Huds on Swiss albino mice using carbon clearance assay. *Asian Pacific Journal of Tropical Disease;* dio:10.1016/S2222-1808(4)60594-6.

Apata, L., (1979). Practice of Herbalism in Nigeria. University of Ife Press.

Baharum SN, Bunawan H, Ghani MA, *et al.*, (2010). Analysis of the chemical composition of the essential oil of Polygonum minus Huds. using two-dimensional gas chromatography-time-of-flight mass spectrometry (GC-TOF MS). Molecules; 15(10):7006-7015.

Bala, S.A., (2006). Some Ethnomedicinal Plants of the Savanna Regions of West Africa: *Description and Phytochemicals.* Triumph Publishers, Kano, Nigeria. Vol. 2: 3-5.

Balcht, Aldona; Smith, Raymond (1994). Pseudomonas aeruginosa*: Infections and Treatment*. Informa Health Care. pp. 83–84. ISBN 0-8247-9210-6.

Basu P. (2004). Trading on Traditional Medicines (N. Biotechnology, Trans.); Beijing China March.

Benitez DE, Cadwallader KR & Suriyaphan O. (2001) Aroma-active Components of Vietnamese Coriander (*Polygonum odoratum*). In IFT Annual Meeting. Louisiana, New Orlands.

Bentley R., Meganathan R (1982)."Biosynthesis of Vitamin K (menaquinone) in bacteria".*Microbiol. Rev.* **46** (3): 241–80. PMC 281544.PMID 6127606.

Biopharma, AVI (2007). "Antisense Antibacterial Method and Compound". World Intellectual Property Organization. Retrieved 2008.

Brooks, G.E, Janet, S.B. and Stephens, A.M., (2001). Medical Microbiology. Second Edition. 99, 198-201.

Cheesborough M., (2012). Summary of the Clinical and Laboratory Features of Microorganisms. Cheesborough M. (ed.). Cambrdge Edition. 157-233.

Christophe Wiar (2003). Medicinal Plants of Asia and Facific. Bocha Raton: CRC Press,. 49.

Do, N.T (2001). *Persicaria Miller* [Internet] Record from Proseabase. van Valkenburg, J.L.C.H. and Bunyapraphatsara, N. (Editors).

Duraipandiyan, V., M, Anyyanar and S. Ignacimuthu, (2006). Antimicrobial Activity of some Ethnomeedicinal Plants used Paliyar tribe from Tamil Nadu, India. BMC Complement. Altern. Med., 6: 35-35.

Eastwood, M.A.; Nyhlin, H. (1995). "Beeturia and Colonic Oxalic Acid". *QJM : monthly journal of the Association of Physicians* 88 (10): 711–7.

Evans C.E., Banso A. and Samuel, O.A. (2002). Efficacy of some Nine Medicinal Plants against *Salmonella typhi:* an *In-vitro* study. *Journal of Ethnopharmacology.* Vol. 80: 21-25.

Faujan, N.H., N. Abdullah, N. Abdullah Sani and A.S. Babji, (2007). Antioxidative Activities of Water Extracts of some Malaysian Herbs. *ASEAN Food Journal,* 14: 61-68.

Fotadar U, Zaveloff P, Terracio L (2005). "Growth of *Escherichia coli* at Elevated Temperatures". *Journal of Basic Microbiol.* **45** (5): 403-4.

Francois P. and Schrenzel J. (2008). "Rapid Diagnosis and Typing of *Staphylococcus aureus*". *Staphylococcus: Molecular Genetics*. Caister Academic Press. ISBN 978-1-904455-29-5.

Freeman, C.C. and Hinds; Reveal. J.L. *Polygonaceae.* In *Flora of North America*; Oxford University Press: New York, NY, USA, 2005; Volume 5, pp. 216–601.

Giessman, T. A. (1963). " Flavonoids Compounds, Tannins, Lignins, and Related Compounds". P. 256. *In* M. Florkin and E. H. Totz (ed.*) Pyrrole Pigments, Isoprenoid Compounds and Phenolic Plant Constituents*, Vol. 9 Elsevier, New York. NY.

Hachem RY, Chemaly RF, Ahmar CA, Jiang Y, Boktour MR, Rjaili GA, Bodey GP, Raad II (2007). "Colistin is Effective in Treatment of Infections caused by Multidrug-resistant *Pseudomonas aeruginosa* in cancer Patients".*Antimicrobial Agents Chemother.* **51** (6): 1905–11. 011.

Hamidun B, Noraini T, Noor M. (2011). Foliar Anatomy and Micromorphology of *Polygonum minus* Huds. and their Taxonomic Implications. Aust J. Crop Sci; 5(2):123-127.

Handa SS, Khanuja SPS, Longo G, Rakesh DD. (2008). Extraction Technologies for Medicinal and Aromatic Plants. *International centre for science and high technology, Trieste;* 21-25.

Hudault S, Guignot J, Servin AL (2001)."*Escherichia coli* strains Colonizing the Gastrointestinal Tract Protect Germ-free Mice against *Salmonella typhimurium* Infection". *Gut* **49** (1): 47–55.

Hunter M. (1996). Australian Kesom Oil- a new ssential oil for the flavour and fragrance industry. Agro-Food-Industry Hi-Tech Vol. 7, pp.26-28.

Hussain, K., Ismail, Z., Sadikun, A., & Ibrahim, P. (2008). Analysis of Proteins, Polysaccharides, Glycosaponins contents of *Piper sarmentosum* Roxb. and anti-TB evaluation for Bioenhancing/Interaction Effects of Leaf-extracts with Isoniazid (INH). *Natural Product Radiance, 7*(5), 402-408.

Jeller AH, Silva DHS, Liao LM, Bolzani VS, Furlan M. (2004). Antioxidant and Phenolic Triterpenes from Quinomethide *Cheiloclinium cognatum.* Phytochemistry; 65:1977-1982.

Julio G. Urones, Isidro Sanchez Marcos, Bel~~N G~Mez Perez and Pilar Basabe Barcala (1990). FLAVONOIDS FROM POL YGONUM *MINUS. Phytochemistry*, Vol. 29, No. 11, pp. 3687-36891.

Karakoca Kubra, Meltem Asan Ozusaglam, Yavuz Selim Cakmak, Seher Karaman Erkul (2013). EXCLI. 12, 150-167.

Kluytmans J, van Belkum A, Verbrugh H., (1997)."Nasal Carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks". *Clin. Microbiol. Rev.* **10** (3): 505–20. PMC 172932.PMID 9227864.

Kraisintu, K. (2003). The Status of Medicinal and Aromatic Plants in Cambodia, Laos, The Philippines, Thailand and Vietnam Medicinal plants and their Utilization (pp. 3-54); Italy.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein Measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry* **193,** 265–275.

Mabberley, D.J. (2008). Mabberley's Plant-Book: a Portable Dictionary of Plants, their Classification and Uses.*Cambridge University Press: Cambridge.*

Madigan MT, Martinko JM (2006). *Brock Biology of Microorganisms* (11th ed.). Pearson. ISBN 0-13-196893-9.

Magina, M. D., Dalmarco, E. M., Wisniewski Jr, A., Simionatto, E. L., Dalmarco, J. B., Pizzolatti, M. G., & Brighente, I. M. (2009). Chemical Composition and Antibacterial Activity of Essential Oils of *Eugenia species. Journal of natural medicines*, *63*(3), 345-350.

Matasyoh, J.C., Maiyo, Z.C., Ngure, R.M. and Chepkorir, R., (2008). Chemical Composition and Antimicrobial Activity of The Essential Oil of *Coriandrum sativum, Journal of Food Chemistry,* 113(2): 526-529.

Michael Tierra, L.AC. O.M.D. (1999). **"**Why Standardized Herbal Extracts?". https://www.planetherbs.com/.../why-standardized-herbal-extracts.html.

Misharina TA (2001). Effect of conditions and Duration of Storage on Composition of Essential Oil from Coriander seeds. Prikladnaia Biokhimiia i Mikrobiologiia Vol. 37, pp.726-732.

M.M. Mackeen, A.M. Ali, S.H. El-Sharkawy, M.Y. Manap, K.M. Salleh, N.H. Lajis, K. Kawazu (1997). Antimicrobial and Cytotoxic Properties of Some Malaysian Traditional Vegetables (Ulam). *Pharmaceutical Biology,* Vol. 35, No. 3 : Pages 174-178.

Moerman, D.E. (1996). An Analysis of the Food Plants and Drugs Plants of Native North America. *A Journal of Ethnopharmacology.* Vol. 52: 171-175.

Murray, BE. (1990). "The life and times of the *Enterococcus*." *Clinical Microbiology Rev* 3 (1): 46–65.PMC 358140. PMID 2404568.

Nguyen Van Duong (1993). Medicinal plants of Vietnam, Cambodia and Laos. Mekong Printing, Santa Ana, California, United States. Pp. 341-342.

Ogbe Raphael John, Adenkola Adeshina Yahaya, Anefu Emmanuel (2012). Aqueous Ethanolic Extract of *Mangifera indica* Stem Bark Effect on the Biochemical and Haematological Parameters of Albino Rats. Archives of Applied Science Research; 4 (4):1618-1622

Ogko, N.T. (2002). Antimicrobial Activity of some Selected Medicinal Plants against Respiratory Tract Pathogens. *Published M. Sc. Thesis, Bayero University Kano, Nigeia.*

Olaleye, O. and Merry, T. (2007). Cytotoxicity and Antibacterial Activity of Methanolic Extracts of *Hibiscus sabdariffa. Journal of Mediciinal Plants Research.* Vol. 1: 9-14.

Oliver, B. (1960). Nigerian Medicinal Plants. Nigerian College of Arts, Science and Technology, Ibadan. 138-139.

Onawunmi, Grace O., and Ogunlana, E.O. (1986). A Study of the Antibiotic Activity of Essential oil of Lemongrass. *Journal crude Drug resistance*, Vol. 24: 64-98.

Othman R., M. Hana-Marlin, L.Y. Chang (2010). Proteomics Analysis of *Polygonum minus* Leaves. *Special Abstracts / Journal of Biotechnology* 150S (2010) S1–S576; doi:10.1016/j.jbiotec.2010.09.738.

Philpson, J. D., (2001). Phytochemistry and Medicinal Plants. Phytochemistry: 56, 265-243.

Prescott, H.K., (2006). General Microbiology Text-book. Sixth Edition. Pp 314-338.

Qader, S.W., *et al.*, (2011). Antioxidant, Total Phenolic Content and Cytotoxicity Evaluation of Selected Malaysian Plants. *Molecules;* 16(4): 3433-3443.

Reanmongkol W, Subhadhirasakul S, Panichayupakaranant P, Kim KM (2003). Anti-allergic and Antioxidative Activities of some Compounds from Thai Medicinal Plants. *Pharm. Biol,* 41:592-597.

Ridzuan P.M, H. Hairul Aini, M. H. Norazian2 A. Shah, Roesnita and K. S. Aminah (2013). Antibacterial and Antifungal Properties of *persicaria odorata* Leaf Against Pathogenic Bacteria and Fungi. *The Open Conference Proceedings.* (Suppl-2, M17). 71-74.

Roia, F.C and Smith, R.A. (1977). The Antibacterial Screening of some Common Ornamental Plants. *Journal of Economic Botany.* Vol. 31: 28-27.

Ryan, KJ; Ray, CG, eds. (2004). *Sherris Medical Microbiology* (4th ed.). McGraw Hill. ISBN 0-8385-8529-9.

Saad, R., Khan, J., Krishnanmurthi, V., Asmani, F., & Yusuf, E. (2014). Effect of Different Extraction Techniques of *Persicaria odorata* Extracts Utilizing Anti-bacterial Bioassay. *British Journal of Pharmaceutical Research*, *4*(18).

Saad, R., Murugiah, G., Abdulhamid, J., Yusuf, E., & Fadli, M. (2014). Comparative Study between Percolation and Ultrasonication for the Extraction of Hibiscus and Jasmine Flowers Utilizing Antibacterial Bioassay.

Sandberg, F. And Bruhn, J. G. (1979). Screening of Plants for Bioactive Substances in Africa Medicinal Plants (ed., Sofowora, E.A). *The University Press, Ife, Nigeria.* Pp 119.

Sasongko, P.; Laohankunjit, N.; Kerdchoechuen, O. (2011). Antibacterial Activity of the Essential Oil from *Persicaria odorata* Leaves. *J. Agr. Sci., 42*(2), 105-108.

Schultes, R.E. (1978). "*The Kindom of Plants." In* W.A.R Theomson (ed.) Medicinal from the Earth. McGraw-Hill Book Co, New York, N. Pp 208.

Shanmugam, B., Shanmugam, K. R., Ravi, S., Subbaiah, G. V., Mallikarjuna, K., & Reddy, K. S. (2014). Antibacterial Activity and Phytochemical Screening of *Phyllanthus niruri* in Ethanolic, Methanolic and Aqueous Extracts. *International Journal of Pharmaceutical Sciences Review & Research*, *27*(2).

Shavandi, Mohammad Amin, Zahra Haddadian, and Mohd Halim Shah Ismail (2014). "*Eryngium Foetidum L. Coriandrum Sativum* and *Persicaria Odorata* L.: A Review." *Journal of Asian Scientific Research* 2.8: 410-426.

Sofowora, E. A (1993). *"Medicinal Plants and Traditional Medicine in Africa*." Spectrum Books L.t.d. Second edition.

Stuart, Charles H., DDS, Scott A. Schwartz, DDS, Thomas J. Beeson, DDS, and Christopher B. Owatz, DMD (2006). *"Enterococcus faecalis:* Its Role in Root Canal Treatment Failure and Current Concepts in Retreatment." *Journal of endodontics* 32.2: 93-98.

Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. *Molecules*, *14*(6), 2167-2180Sofowora, E. A (1993). *"Medicinal Plants and Traditional Medicine in Africa*." Spectrum Books L.t.d.

Sumazian Y, Ahmad S, Mansor H, *et al.,* (2010). Antioxidant Activities, Flavonoids, Ascorbic acid and Phenolic content of Malaysian Vegetables. *Journal of Medicinal Plants Res;* 4(10):881-890.

Syarul Nataqain Baharum 1, Hamidun Bunawan, Ma'aruf Abd. Ghani, Wan Aida Wan Mustapha, and Normah Mohd Noor (2010). Analysis of the Chemical Composition of the Essential Oil of *Polygonum minus* Huds. using Two-Dimensional Gas Chromatography-Time-of-Flight Mass Spectrometry (GC-TOF MS). *Molecules: 15*, 7006-7015.

Teh CP. (1996). Studies on the Relationships Between Wood Anatomical Structures of Local species and its Potential Uses. PhD Thesis. Universiti Kebangsaan Malaysia, Bangi, Malaysia.

Thomson, I. S. I. (2013). Norliza Muhammad, Elliza Mansor, Yap Chuan Sang, Nor Syahira Shariffudin, Amri Dahdi, Ahmad Fadhil Alias, Norazlina Mohamed, Ahmad Nazrun Shuid, Abdul Salam Babji and Ima Nirwana Soelaiman. "Acute and Subacute Toxicity of Persicari minor in Wistar Rats". *Asian Journal of Animal Sciences*, *7*(2), 47-55.

Todar, K. (2007). "Pathogenic *E. coli*". *Online Textbook of Bacteriology*. University of Wisconsin–Madison Department of Bacteriology.

Todar's Online Textbook of Bacteriology (2004). Textbook of bacteriology.net. Retrieved:

T Tolosa, H. Wagaye, F. Regassa (2009). A Study On *In-vitro* Antimicrobial Effects of some Selected Plants on *Staphylococcus aureus* Isolated from Bovine Clinical Mastitis. *The Internet Journal of Veterinary Medicine.* Vol. 8 (1).

UNCTAD (1994). Market for Selected Medicinal Plants and their Derivatives. United Nations Conference on Trade Development, Geneva.

Uyub, A.M., *et al.*, (2010). *In-vitro* antibacterial Activity and Cytotoxicity of Selected Medicinal Plant Extracts from Penang Island Malaysia on Metronidazole-resistant-Helicobacter pylori and some pathogenic bacteria.

Vimala, S., and M.I. Adenan (1999). Malaysian Tropical Forest Medicinal Plants: A source of natural antioxidants. *Journal Trop. For. Prod.*, 5: 32-38.

Vimala S., Ilham, M.A., Rashih A.A. & Rohana S., (2003). Nature's Choice To Wellness: Antioxidant Vegetables/Ulam. SiriAlam & Rimba 7. *Forest Research Institute Malaysia (FRIM).* Pp 131.

Vimala S., Rohana S., Rashih A.A. & Juliza M., (2011). Antioxidant Evaluation in Malaysian Medicinal Plant: *Persicaria minor* (Huds.) Leaf. *Science Journal of Medicine & Clinical Trials.* ISSN: 2276-7487.

Wilson K.L., (1990). Some Widespread Species of *Persicaria* (Polygonaceae) and their Allies. Kew Bulletin 45(4): 621-636.

Yaacob, K.B., (1987). Kesom oil: A atural source of aliphatic aldehydes. *Perfumer Flavorist*, *12*, 27-30.

Yentema, O., Alioune, O. and Dorosso, S.A., (2007). Chemical Composition and Physical Characteristic of the Essential oil of *Cymbopogon schoenanthus* (L.) Spreng of Burkina Faso, *Journal of Applied Sciences.* 7(4): 503-506.