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ANTIMICROBIAL PROPERTIES OF DIFFERENT FRACTIONS OF *SWERTIA CHIRATA*

Nik Aiman Afiq Nik Ab Rahman, Isma Syahril Ismail and Lwin Lwin Nyein
University Teknologi MARA, Malaysia

ABSTRACT

Complementary and alternative medicine (CAM) is becoming increasingly popular and experienced a high growth all over the world. A medicinal plant, Swertia chirata, has been widely use as herbal medicine in Asian countries and some parts of the world. The aim of this study was to evaluate the antibacterial properties of Swertia chirata and to assess the cytotoxicity using brine shrimp lethality test. Plants were extracted using ethanol. Disc diffusion technique was used to determine antimicrobial activity. Crude extraction of the whole plant showed significant antimicrobial activities against some clinical bacterial isolates. Plant extracts at a concentration of 100 µg disc^{-1} were used to evaluate the antimicrobial activity and 30 µg disc^{-1} concentration of Chloramphenicol was used as a positive control. Zone of inhibition against Staphylococcus aureus, Bacillus cereus, Escherichia coli and Salmonella arizonae were 10 mm, 6 mm, 9 mm and 7 mm respectively. Minimum Inhibitory Concentration (MIC) of the crude extract was determined by serial dilution technique which showed 128 µg/ml, 64 µg/ml, 128 µg/ml and 128 µg/ml respectively. Plant showed no significant cytotoxitcity with LC_{50} >5000 µg.The results obtained revealed inhibition against some gram positive and gram negative bacteria and low cytotoxicity value indicates its effective use as traditional medicine plant. Thus, Swertia chirata which possesses antimicrobial activity, with low cytotoxicity, will be of use in complementing a standard antimicrobial drug and will contribute to the current status of the public health where emergence of drug resistance is a major problem.

Keywords: Swertia chirata; antibacterial, complementary and alternative Medicine (CAM), minimum inhibitory concentration (MIC), LC_{50}

INTRODUCTION

Traditional systems of medicine are fast emerging as an alternative to modern medical and health science. In recent years the emphasis on herbal plants as a source for drug discovery and development has been realized globally. The concept of scientific validation of the basis of traditional uses of herbal medicines has given birth to a new concept of reverse pharmacology, and interactions between traditional and modern systems of medicine are being increasingly encouraged. Recent Trends in Herbal Drug Research and Therapy showcases some of these crucial and emerging issues relating to herbal drugs (Arunabha *et al.*, 2010). The use of higher plants and preparations made from them to treat infections is an old-age practice in a large part of the world population, especially in developing countries, where there is dependence on traditional medicine for variety of diseases (Ahmad *et al.*, 1998, Alam *et al.*, 2009). The economic crisis, high cost of industrialized medicines, inefficient public access to medicinal and pharmaceutical care, in addition to the side effects caused by synthetic drugs are of some of the factors contributing to the central role of medicinal plants in health care (Johann *et al.*, 2007, Alam *et al.*, 2009)

Swertia chirata, is a robust annual herb which grows up to about 1.5 meters in height. It has leaves in opposite pair about 10 cms long, without stalks, pointed at the tip. The plant has numerous flowers, pale green in colour, tinged with purple, with long white or pink hairs and minute sharp pointed fruits. The whole plant, collected in its flowering stage and dried, constitutes the drug. It is found in the Himalayan ranges of India from Kashmir to Bhutan at an altitude of 1,200-3,000 m. It is also found in the Khasi Hills of Meghalaya at an altitude of 1,200-1,500 m. (Joshi & Dhawan 2005, Anwar *et al.,* 2011). It has long been used by the ayurvedic physicians as a bitter tonic. The plant contains a bitter glycoside chiratin, which yields on hydrolysis, two bitter principle, ophelic acid and chiratin. The ophelic acid is a brown hydroscopic substance which is soluble in water and alcohol. It also contains resin, tannin and 4 to 8 per cent of ash (Joshi & Dhawan 2005). Chirata is an effective drug for reducing fevers (Sampath Kumar *et al.*, 2010). It has been widely use as herbal medicine in Asian countries particularly in India, Nepal, Myanmar, Arab and

some parts of the European countries. Reported studies showed extracts of this plant has attributable properties as hypoglycemic, antipyretic (Bhargava *et al*., 2009), anti-inflammatory (Banerjee *et al.,* 2000), antibacterial (Ali *et al*.2009), antiviral (Verma *et al*), antimalarial, antihepatotoxic (Joshi & Dhawan 2005) and wound healing activity (Rafatullah *et al.,* 1993). In 2009, it was announced in Annual Professional Conference of Diabetes UK, proven to have an anti-diabetic effect (Health News 2011). In this study, ethanolic extract of different fractions of *Swertia chirata* was evaluated to assess its antimicrobial property and brine shrimp lethality test was done to evaluate the cytotoxicity of the plant.

MATERIAL AND METHODS

Identification of samples

Dried plants of *S. chirata* were obtained from registered traditional herbal medicine center, Myanmar. Locally known as *Seykhagyi,* identification and confirmation was performed by licensed traditional herbal medicine expert.

Preparation of plant materials

Parts of the plants were separated into stem and leaves and cut into smaller pieces and placed in an autoclave at 40^0C 24 hours for further drying. Then it was weighed about 100g for each stem and leaves and ground into coarse powder with grinding machine at the Institute of Medical Molecular Biotechnology (IMMB), Sungai Buloh, Malaysia.

Extraction, evaporation and isolation of compounds

100 g of plant samples were taken to dissolved with 80% ethanol in a 500 ml conical flask and placed on a shaker for 7 days. It was decanted and filtered using fresh cotton wool. This procedure was repeated three times to the same filtrate to achieve as much active compounds as possible. Thus, total of 1500 ml of filtrates was lastly obtained in a beaker. The solvent was then placed in rotatory evaporator until semisolid gummy mass was obtained. It was then preserved at 4^0C until further analysis. 100 mg of crude extract of leaf and stem powder of *S. chirata* were then dissolved in 10 ml of 80% ethanol and ready to be tested with different microbial agents.

Tested microbial agents

Pure cultures of *Staphylococcus aureus* and *Bacillus cereus* for gram positive bacteria, *Escherichia coli* and *Salmonella arizonae* for gram negative bacteria were used as the experimental microorganisms isolated from Center for Pathology & Diagnostic Research Laboratories (CPDRL), Universiti Teknologi MARA, Sungai Buloh, Malaysia.

Antimicrobial study

Disc diffusion method was performed according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2012) and Minimal Inhibitory Concentration (MIC) was carried out using the reference broth microdilution method as described by the National Committee for Clinical Laboratory Standards. Standard Chloramphenicol disc (30µg $disc^{-1}$) was use as a positive control. Test materials impregnated with respective solvents (different strength 100%, 75%, 50% & 25%) were soaked into 100µg $disc^{-1}$ (Whatman filter paper disc) and antimicrobial potency was measured.

Cytotoxicity bioassay

100mg of crude extract of leaf and stem powder of *S. chirata* were then dissolved in 10ml of 80% ethanol and ready to be tested for the brine shrimp bioassay test. Commercially available cysts of brine shrimps were obtained from Ocean star international (O.S.I.) Inc. made in U.S.A. Artificial seawater (ASW) was used as culture medium. Two unequal compartments were used; one darkened and smaller one was illuminated with lump (25^0C) and was connected with small holes. Brine shrimp eggs were placed in larger compartment filled with artificial seawater (ASW). After 48 h the eggs were hatched and phototropic nauplii were collected by pipette from the lighted side which had passed through the small holes from the darker compartment. Bioassay was done with the vials to which a drop of dimetyl sulfoxide (DMSO) solvent was added. Then, ten nauplii were placed using Pasteur pipette, and 4.5 ml ASW was added. To this vials 0.5ml of plant extract

was added with total volume of 5ml and maintained at room temperature. Different concentration of plant extract was used to detect LC_{50} (concentration of 50% mortality of the nauplii). After 24 hours, nauplii were counted against a lighted background. Experiments were conducted along with podophyllotoxin as positive control and different concentrations (1-5000 µg/ml) of the test substances.

RESULTS AND DISCUSSION

The study showed that different fraction of both leaves and stems showed antimicrobial property against some gram positive and gram negative microorganisms. Zone of inhibition against *S. aureus*, *B. cereus*, *E. coli* and *S. arizonae* were 10mm, 6 mm, 9 mm and 7 mm respectively. Results were summarized in Table I and Table II.

Table I: Antibacterial activity of the crude extract of *S. chirata* leaf

Tested Microbial agents	Concentrations of tested crude extracts of *S. chrata* (µg disc^{-1})				Standard Chloramphenicol disc (30µg disc^{-1})
	100%	75%	50%	25%	
Staphylococcus aureus	10*	6	-	-	33
Bacillus cereus	6	-	-	-	33
Escherichia coli	9	4	-	-	34
Salmonella arizonae	7	-	-	-	33

Table II: Antibacterial activity of the crude extract of *S. chirata* stem

Tested Microbial agents	Concentrations of tested crude extracts of *S. chrata* (µg disc^{-1})				Standard Chloremphenicol disc (30µg disc^{-1})
	100%	75%	50%	25%	
Staphylococcus aureus	9*	7	-	-	33
Bacillus cereus	6	-	-	-	33
Escherichia coli	9	4	-	-	34
Salmonella arizonae	7	-	-	-	33

*Values indicate zone of inhibition (diameter in mm)

Antibacterial property of ethanolic crude extract of both *S. chirata* leaves and stem showed both possessed inhibition against test organisms without much difference in inhibition zones. For gram positive bacteria, zone of inhibition for *S. aureus* was 10 mm and 9 mm whereas for *B. cereus* 6 mm and 6 mm respectively. For gram negative bacteria, *E.coli* showed inhibition zone of 9 mm and 9 mm and *S. arizonae* showed 7 mm and 7 mm respectively for full strength. In concentration of 75%, only *S. aureus* and *E.coli* showed inhibition zone a little less than full strength, others revealed no significant inhibition. The results support previous studies (Jesmin *et al.*, 2007, *Alam et al.,* 2009) and showed that antimicrobial activity was best at full concentration.

Minimum inhibitory concentration (MIC) of the test sample

Minimum Inhibitory Concentration (MIC) of ethanolic crude extract mixture (stem and leaves) was determined by serial dilution technique, which showed 128 µg/ml, 64 µg/ml, 128 µg/ml and 128 µg/ml for *S. aureus, B. cereus, E.coli and S. arizonae* respectively. Results for *S. aureus, B. cereus* differ from results

obtained by Sultana *et al.*, which showed 64 µg/ml for *S.aureus* and 128 µg/ml for *B. cereus* whereas for gram negative bacteria showed no significant difference. Results were summarized in Table III.

Table III: Minimum Inhibitory Concentration (µg/ml) values of ethanolic extract of *S. chirata*

Tested Bacteria strain	MIC (µg/ml)
Gram positive	
Staphylococcus aureus	128 µg/ml
Bacillus cereus	64 µg/ml
Gram negative	
Escherichia coli	128 µg/ml
Salmonella arizonae	128 µg/ml

Cytotoxicity bioassay

The brine shrimp lethality assay is an easy, rapid, inexpensive bioassay for testing plant extracts bioactivity properties of the plants (Alluri *et al.,* 2006). In this study, the LC_{50} value of *S. chirata* extracts were determined using brine shrimp lethality assay which showed >5000 µg. This result showed no significant effect compared with the control.The results obtained in this study is in consistant with the experiment done by Alluri *et al.,*but did not support the study done by Sultana et al., which was 60.25 µg that indicates high cytotoxic effect of the plant.

CONCLUSION

The ethanolic extract of the medicinal plant *S. chirata* both stem and leaves demonstrate appreciable antibacterial property. From the preliminary testing, this plant has no significant cytotoxic effect with LC_{50} of >5000mg which support the safe traditional use as medicinal plant (Alam *et al.,* 2009). Although the results obtained revealed inhibition against some gram positive and gram negative bacteria, further study need to be carried out to detect the active compounds of this highly potent plant. Further work will be continued for the identification of the isolated pure compound as well as its biological activity against medically important microorganisms. Hence, *S. chirata*, a herbal medicine with potent antimicrobial activity, will not only complement a standard antimicrobial drug but will solve the issues of high cost of industrialized medicines and will contribute to CAM especially in developing countries where there is difficulty in public access to medicinal and pharmaceutical care.

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REFERENCES

Ahmad, I., Z. Mehamood and F. Mohammad (1998). "Screening of some Indian medicinal plants for their antimicrobial properties," *J. Ethnopharmacol.*, 62: 183-193.

Alam K.D., Ali M.S., Parvin S., Mahjabeen S., Akbar M.A. and Ahamed R.(2009). "*In vitro* Antimicrobial activities of different fractions of *Swertia chirata* ethanolic extract," *Pakistan J. Biol Sci.* 12(19): 1334-1337.

Ali MS, Parvin S, Akbar MA., Alam K.D., Mahjabeen S., Ahmed R. (2009). "*In vitro* Antimicrobial activities of different fractions of *Swertia chirata* ethanolic extract," *Pakistan Journal of Biological Sciences* 12 (19): 1334-1337.

Alluri V. Krishnaraju, Tayi V. N. R., Dodda S., Mulabagal V., Hsin-Sheng T. and Gottumukkala V. S. (2006). "Biological Screening of Medicinal Plants Collected from Eastern Ghats of India Using *Artemia salina* (Brine Shrimp Test)," *Int. J.Appl. Sci. Eng.,* 4, 2: 115-125.

Alluri V. Krishnaraju, Tayi V. N. R., Dodda S., Mulabagal V., Hsin-Sheng T., and Gottumukkala V. S. (2006). "Assessment of Bioactivity of Indian Medicinal Plants Using Brine Shrimp (*Artemia salina*)," *Int. J. Appl. Sci. Eng.,* 3, 2: 125-134.

Anwar K., Amir W., Malik S., Uzair-Ur-Rehman, Sonia K. A. Seth, Mhd Hassham H.B. A., Fatima R., Mhd Khurram, Waqas and Ghulam M.(2011). " Antibacterial activity analysis of extracts of various plants against gram -positive and -negative bacteria," *African Journal of Pharmacy and Pharmacology* Vol. 5(7): 887-893.

Arunabha Ray and Kavita Gulati (2010). Recent Advances in Herbal Drug Research and Therapy, I.K. International, 480.

Banerjee S, Kumar Sur T, Mandal S, Das PC, Sikdar S (2000)."Assessment of the anti- inflammatory effects of *Swertia chirata* in acute and chronic experimental models in male albino rats," *Indian Journal of Pharmacology* 32: 21-24.

Bhargava S., Rao P. S., Bhargava P., Shukla S., (2009). "Antipyretic potential of *Swertia chirata* Buch Ham. Root extract," *Sci Pharm* 77; 617-623.

Clinical and Laboratory Standards Institute guidelines, CLSI (2012) - Antimicrobial Susceptibility Testing Standards. January 13, 2012.

Health News : Alternative Medicine News Posted on March 12, 2011.

Johann, S., M.G, Pizzolatti, C.L., Donnici, M.A., de-Resende and S. Paulo (2007). "Antifungal properties of plants used in Brazilian traditional medicine against clinically relevant fungal pathogens," *Braz. J. Microbiol,* 38: 11-14.

Joshi P. and Dhawan V., (2005). "*Swertia chirayita* – an overview," *Current Science*, 89:(4): 25.

Mst. Jesmin S, Md. Molla T. H., Alam M.T., FShaki. R., Ahmad (2007). "Investigation on antimicrobial activities of the plant *Swertia chirata* Ham," *J. Life Earth Sci.,* 2(2): 31-34

Rafatullah S., Tariq M., Mossa J.S., Al-Yahya M.A., Al-Said M.S., Ageel A.M. (1993). "Protective effect of *Swertia chirata* against indomethacin and other ulcerogenic agent-induced gastric ulcers," *Drugs Exptl. Clin.Res.* XIX (2): 69-73.

Sampath Kumar K. P., Debjit B., Chiranjib, Biswajit, Margret C., (2010). "*Swertia chirata*: A traditional herb and its medicinal uses," *J. Chem. Pharm*. Res.: 34(2): 262-266.

Verma H., Patil P.R., Kolhapure R.M., Gopalkrishna V. (2008). "Antiviral activity of the Indian medicinal plant extract, *Swertia Chirata* against herpes simplex viruses," *Indian Journal of Medical Microbiology*, 26(4): 322-6.