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Analysis of Different Fractions of *Swertia Chirata* Against Gram Positive and Gram Negative Bacteria

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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 property of *Swertia chirata.* Plants were extracted using ethanol. Disc diffusion technique was used to determine antimicrobial activity. Crude extraction of whole plant showed significant antimicrobial activities against some gram positive and gram negative bacteria. Test materials 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 positive control. Zone of inhibition against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Salmonella arizonae* were 10mm, 6mm, 9mm and 7mm respectively. Minimum Inhibitory Concentration 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 . Although the results obtained revealed inhibition against some gram positive and gram negative bacteria, a further study need to be carried out to detect the active compounds of this highly potent plant. Moreover, problems of drug resistance leading to recurrent infections highlight a need to search for new compounds for treatment of microbial infections. Hence, *Swertia chirata* which possesses antimicrobial activity, hope will complement if not replace a standard antimicrobial drug that will be of benefits to the health of the mankind.

Keywords: *Swertia chirata*, antibacterial, Complementary & Alternative Medicine (CAM).

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 [1]. 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 [1]. 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 [2, 3]. 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 [3, 4]. Hence, in this study different fraction of *Swertia chirata*, leaves and stems were studied for its antimicrobial property.

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Biological Name: *Swertia chirata*, Family- *Gentianaceae* Indian Name: *Chirayata*, 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. [5, 6]. 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 latter is soluble in water. 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 [5]. Chirata is an effective drug for reducing fever [7]. 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 [8], anti-inflammatory [9], antibacterial [10], antiviral [11], antimalarial, antihepatotoxic [5] and wound healing activity [12]. Moreover, it was also announced in Annual Professional Conference of Diabetes UK, 2009, proven to have an anti-diabetic effect [13]. In this study,

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Table 1. Antibacterial Activity of the Crude Extract of *S. chirata* Leaf

Bacteria / Percentage	100%	75%	50%	25%	Standard Chloramphenicol Disc (30µg disc^{-1})
Staphylococcus aureus	10	6	-	-	33
Bacillus cereus	6	-	-	-	33
Escherichia coli	9	4	-	-	34
Salmonella arizonae	7	-	-	-	33

Table 2. Antibacterial Activity of the Crude Extract of *S. chirata* Stem

Bacteria / Percentage	100%	75%	50%	25%	Standard Chlorempheniol Disc (30µg disc^{-1})
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)

ethanolic extract of different fraction of *Swertia chirata* was evaluated to assess its antimicrobial property.

MATERIAL AND METHODS

Collection of plants: 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: Plants were separated into stem and leaves and cut into smaller pieces and placed in an autoclave at 40°C 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, Sungai Buloh, Malaysia.

Extraction, evaporation and isolation of compounds: 100g of plant sample were taken to dissolved with 80% ethanol in a 500ml conical flask and placed on a shaker for 7 days. It was decanted and filtered using fresh cotton wool. This procedure was repeated 3 times to the same filtrate to achieve as much active compounds as possible. Thus, total of 1500ml 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.

Crude extract of leaf and stem powder of *S. chirata* were then dissolved in 80% ethanol and ready to be tested with different microbial agents.

Tested microbial agents: *Staphylococcus aureus* and *Bacillus cereus* for gram positive bacteria, *Escherichia coli* and *Salmonella Arizonae* for gram negative bacteria were used as an experimental microorganisms isolated as pure culture 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 [14] 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} (Whattman filter paper disc) and antimicrobial potency was measured.

RESULTS AND DISCUSSION

The study revealed that different fraction of both leaves and stems of *S. chirata* showed antimicrobial property against some gram positive and gram negative microorganisms. Results were summarized in tables and figures. Table **1** showed antibacterial activity of the crude extract of *S. chirata* leaf and Table **2** showed antibacterial activity of the crude extract of *S. chirata* stem. Fig. (**1**) and Fig. (**2**) showed antibacterial activity of *S. charata* stem and leaves against *S. aureus, B. cereus, E. coli, S. arizonae* at different concentrations of 100%, 75%, 50% and 25 % respectively. Table **3** showed the MIC (µg/ml) values of ethonolic extract of *S. chirata*.

Antibacterial property of ethanolic crude extract of both *S. chirata* leaves and stem showed both possessed inhibition against test organisms. For gram positive bacteria, zone of inhibition for *S. aureus* was 10mm and 9mm whereas for *B. cereus* 6mm and 6mm respectively. For gram negative bacteria, *E.coli* showed inhibition zone of 9mm and 9mm and *S. Arizonae* showed 7mm and 7mm respectively for full strength. In concentration of 75%, only *S. aureus* and *E.coli*

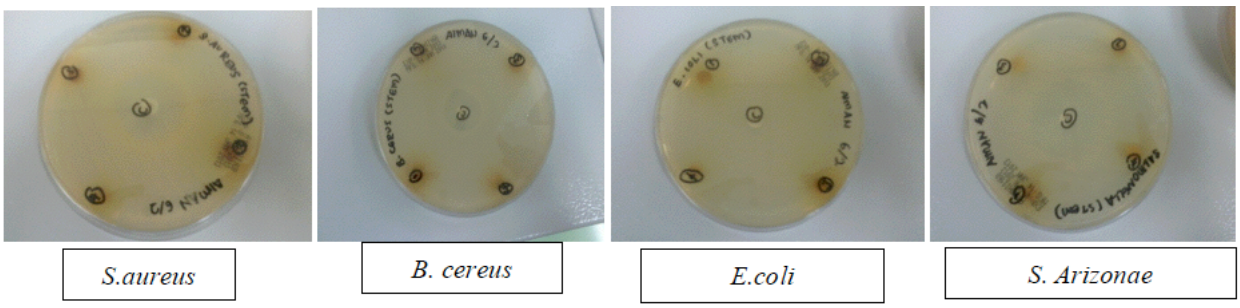

Fig. (1). Antibacterial activity of *S. charata* stem against *S. aureus, B. cereus, E. coli, S. arizonae* at different concentrations of 100%, 75%, 50% & 25 %. Standard Chloramphenicol disc (30 µg $disc^{-1}$) placed at the center of the plate.

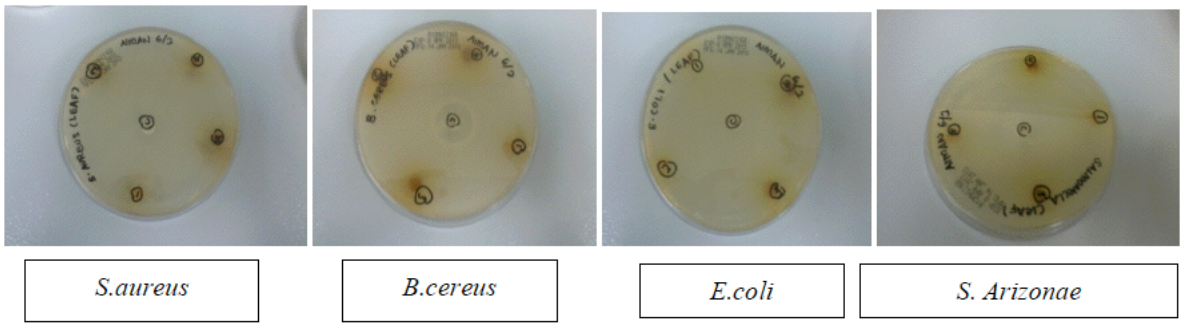

Fig (2). Antibacterial activity of *S. charata* leaves against *S. aureus, B. cereus, E. coli, S. Arizonae* at different concentrations of 100%, 75%, 50% & 25 %. Standard Chloramphenicol disc (30 µg $disc^{-1}$) placed at the center of the plate.

showed inhibition zone a little less than full strength, others revealed no significant inhibition. The results support previous studies [15, 3] 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 shown in Table **3**.

Table 3. Minimum Inhibitory Concentration (µg/ml) Values of ethonolic extract of *S. chirata*

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

Minimal inhibition Concentration revealed 128 µg/ml for S. *aureus*, 64 µg/ml *B. cereus,* 128 µg/ml for both *E.coli* and *S. Arizonae* respectively as shown in Table **3**.

CONCLUSION

The ethanolic extract of the medicinal plant *S. chirata* both stem and leaves demonstrate appreciable antibacterial property. Although the results obtained revealed inhibition against some gram positive & gram negative bacteria, a further study need to be carried out to detect the active compounds of this highly potent plant. Moreover, problems of drug resistance leading to recurrent infections highlight a need to search for new compounds for treatment of microbial infections. Hence, *S. chirata* which possesses antimicrobial activity will complement if not replace a standard antimicrobial drug that will partly solved the problem of emergence of drug resistance. Further work will be continued for the identification of the isolated pure compound as well as its biological activity against microorganisms.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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