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Antibacterial Activity of *Swertia chirata* Buch.-Hams. A Highly Valuable Medicinal Herb

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In the present study we have carried out to evaluate the antibacterial activity of crude extracts from the whole plant of *Swertia chirata* Buch.-Hams. Hexane, ethyl acetate and methanol extracts were obtained by cold percolation method and their activity was carried out with gram positive and gram negative bacterial strains. Preliminary phytochemical screening was performed for all the extracts. In the present results ethyl acetate extract inhibited many bacteria with MIC values ranging from 0.312 to > 1 mg/ml. Phytochemical screening revealed the presence of terpenoids, steroids, flavonoids, alkaloids and carbohydrates. This study showed that *S. chirata* can be one of the potential sources of new antibacterial agents for the tested bacterial strains.

Key words: Secondary metabolites, antibacterial activity.

Microbial infections pose a health problem throughout the world with the alarming increase in the rate of infection by antibiotic resistant microorganisms[1]. The increasing resistance of most synthetically derived antimicrobial agents is of utmost concern. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject for very intense pharmacological studies. In recent years, there has been renewed interest in the treatment against different diseases as herbal drugs are generally known to be non-toxic[2]. The WHO has also recommended the evaluation of the effectiveness of plants in conditions where we lack safe modern drugs[3]. Evaluation of plant products for pharmacological and medicinal effects is of growing interest as they contain many bioactive substances which have therapeutic potential. Evaluation of antibacterial medicinal plants is essential because phytotheraphy is cheaper and locally available.

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Swertia chirata belongs to the family Gentianaceae; it is a native of temperate Himalayas, found at an altitude of 1200-3000m, from Kashmir to Bhutan. The entire plant is used in traditional medicine; however root is documented as the most powerful part for the pharmacological effect[4]. Phytochemical studies have revealed the presence of secondary metabolites such as xanthones, triterpenoids, glycosides, triterpenoid and alkaloid[5-6]. Most of the terpenoids, flavones and sterols were isolated from the whole plant of *S.chirata*. Biological activities like anti-inflammatory, antimalarial and hepatoprotective activity of this plant have also been reported[5]. A concoction of *S. chirata* with cardamom, turmeric and Kutti is given to treat gastro intestinal infections and skin problems. In our present study the entire plant was used to evaluate different solvent crude extracts of *S. chirata* on antibacterial activity *in vitro*.

MATERIAL AND METHODS

Collection of plant material

Healthy, disease free plants of *S. chirata* (whole plant) were collected from Darjeeling (West Bengal, India). The species was identified and authenticated by Dr. D. Narasimhan, Taxonomist, Department of Botany, Madras Christian College, Chennai and the voucher specimen (MPC-165) was deposited at the department herbarium, Loyola College, Chennai. Freshly collected plant material was thoroughly washed, shade dried and grounded into powder.

Crude extract preparation

The plant powder (3 kg) was soaked sequentially in hexane, ethyl acetate and methanol for 72 h respectively with intermittent shaking. After 72h the solution was filtered and the filtrate was concentrated under reduced pressure using rotary vacuum evaporator. The filtrate was air dried to yield 28gms of hexane extract, 38gms of ethyl acetate extract and 57 g of methanol extract and stored at 4°C in air tight containers until assay.

Preparation of test organism

Three strains of gram-positive bacteria *Staphylococcus epidermidis* (ATCC 3615), *Staphylococcus aureus* (ATCC 25983), *Bacillus subtilis* (MTCC 441) and six strains of gram-negative *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (MTCC 1771), *Enterobacter aerognes* (ATCC 111) *Klebsiella pneumonia* (ATCC 15380), *Salmonella typhi* (ATCC 43579) were obtained from department of Microbiology, Christian Medical College, Vellore, India. All bacterial strains were maintained in MHA slants; stocks were stored at 4°C throughout the study and used as stock cultures

MIC assay

Minimum Inhibitory concentrations were determined by the broth dilution method recommended by the National Committee for

Table 1. Minimum Inhibitory Concentration (MIC) of ethyl acetate extract of *S. chirata* (mg/ml)

Bacteria	Hexane	Ethyl acetate	Methanol
S. epidermidis	-	> 1.0	-
S. aureus	0.625	0.625	-
B. subtilis	0.625	0.312	0.312
P. aueroginosa	-	0.625	> 1.0
E.coli	> 1.0	> 1.0	> 1.0
P. vulgaris	-	> 1.0	-
E. aerogens	> 1.0	0.625	> 1.0
K. pneumonia	-	-	-
S. typhi	-	> 1.0	> 1.0

Table 2. Preliminary phytochemical screening of *S. chirata*

Chemical constituents	Hexane	Ethyl acetate	Methanol
Terpenoids	+	+	+
Steroids	+	+	+
Flavonoids	+	-	+
Alkaloids	+	+	-
Carbohydrates	+	+	+
Tannin	-	-	+
Soponin	-	-	-

Clinical laboratory Standards[7]. A series of two fold dilution of each solvent crude extract (hexane, ethyl acetate & methanol) ranging from 0.2 to 5 mg/ml was prepared in sterilized and cooled Mueller Hinton broth. Plates were dried at room temperature for 30 min prior to spot inoculation with 3 µl aliquots of culture containing ~ 10^5 CFU/ml of each organism. Inoculated plates were incubated at 37°C for 18 h and the MIC was determined. Experiments were carried out in triplicate. Inhibition of bacterial growth in the plates containing *S. chirata* fractions was judged by comparison with growth in blank control plates. The MICs were determined as the lowest concentration of crude extract inhibiting visible growth of colonies of each organism on the agar plate[8]. Three independent trials were conducted.

Phytochemical screening

Phytochemical analyses of all the evaporated solvent extracts were conducted [9]. By this analysis the presence of several phytochemicals listed in table (2) were tested.

RESULTS AND DISCUSSION

The antibacterial activities of *S. chirata* against tested bacteria are summarized in table 1. It can be observed that ethyl acetate extract was able to inhibit the majority of the bacteria tested with MIC values ranging from 0.312 to > 1 mg/ml. Hexane and methanol extracts were moderately active. There was no inhibitory activity against *K. pneumonia*. The ethyl acetate extract of *S. chirata* was highly active against *B. subitils* (0.312 mg/ml). *S. corymbosa* (aqueous extract) showed maximum inhibitory activity against *S. aureus*[10]. Several reports also suggest that Xanthones derivaties of *S. chirata* show potent anti-platelet, anticancer and antimalarial effect[11]. Antibacterial activity in our study may be due to the presence of secondary metabolites. Preliminary phytochemical screening revealed the presence of terpenoids, steroids, flavonoids, alkaloids and Carbohydrates. In previous findings flavonoids are found to be effective antimicrobial substances against a wide range of microorganisms, probably due to their ability to complex with extra cellular and soluble protein and to complex with bacterial cell wall[12]. Secondary metabolites of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens. In conclusion, all of these findings raise some interesting expectations about antibacterial activity of this plant extract, and it is possible that identification and elucidation of the active constituents in this plant may provide useful lead to the development of new and effective drugs.

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REFERENCES

1. Davies J. Inactivation of antibiotics and the dissemination of resistance genes. *Science.* 1994; **264**: 375-382.
2. Rao BK, Sudharshan PR, Rajasekhar M.D, Nagaraju N and Rao CA. Antidiabetic activity of *Terminalia pallida* fruit in alloxan induced diabetic rats. *J Ethnopharmacol.* 2003; **85**: 169-172.
3. Pari L and Amarnathsathesh A. Antidiabetic activity of *Boerhaavia diffusa* L. effect on key enzymes in experimental diabetes. *J Ethnopharmacol.* 2004; **91**:109-113.
4. Kirtikar KR, and Basu B.D. *Indian Medicinal Plants*. Alahabad 1984; **3**: 1664-1666.
5. Joshi P, and Dhawan V. *Swertia chirayita* – an overview. *Current Science* 2005; **89**:635-640.
6. Jensen S.R, and Schripsema J. Gentainaceae systematics and natural History (eds Struwe, L. and Albert, V.), Cambridge University Press. London. 2002; 573-632.
7. Prudent D, Perineau F, bessiere JM, Michel GM, Baccou J.C. Analysis of the essential oil of wild oregano from Martinique (*Coleus aromaticus* Benth.)- evaluation of its bacteriostatic and fungistatic properties. *J Essen oil Res.* 1995; **7**:165-173.
8. Delaquis P.J, Stanich K, Girard B, Mazza G. Antimicrobial activity of individual and mixex fractions of dill, cilantro, coriander and eucalyptus essential oils. *Inter J Food Microbiol*. 2002; **74**:10-109.
9. Kokate C.K, Purohith A.P and Gokhale S.B.

In: Pharmacognosy, Nirali Prakashan. Pune, 1990, 120.

10. Ramesh N, Viswanathan M,B, Saraswathy A, Balakrishna K, Brindha P, Lakshmana perumalsamy P. Antimicrobial and phytochemical studies of *Swertia corymbosa*. *Fitoterapia.* 2002; **73**: 160-164.
11. Rodriguez S, Wolfender J.L, Hakizamungu E, Hostelttmann K. An antifungal napthoquinone, Xanthones and Secoiridoids from *Swertia calcina*. *Planta Med.,* 1995; **61:** 362-364.
12. Tsuchiya H, Sato M, Miya Zaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T and Dinuma M. *J Ethnopharmacol*. 1996; **50**:27.