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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (19): 1334-1337, 2009
ISSN 1028-8880

In vitro Antimicrobial Activities of Different Fractions of *Swertia chirata* Ethanolic Extract

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Abstract: The aim of the present study was to evaluate the *in vitro* antimicrobial activity of pet-ether, dichloromethane and methanol fractions of *Swertia chirata* (Family: Gentianaceae) ethanolic extract. Disc diffusion technique and food poison method were used for antibacterial and antifungal activity, respectively. Dichloromethane fraction from both leaf and stem showed significant antimicrobial activities against some Gram-positive and Gram-negative bacteria and mild to moderate activity against some fungi. A large zone of inhibition was observed (19 mm) against *Staphylococcus aureus.* Test materials at a concentration of 400 μg $disc^{-1}$ were used to evaluate the antimicrobial activity while Kanamycin at a concentration of 30 μg $disc^{-1}$ was used as positive control in this study. Among different fractions, dichloromethane fraction showed significant antimicrobial activity against Gram-positive, Gramnegative and fungi. The most significant antimicrobial activity was seen against *Staphylococcus aureus* which reflects it potentiality to be used in skin infections.

Key words: Antimicrobial activity, *Swertia chirata*, Gentianaceae, disc diffusion technique, food poison method

INTRODUCTION

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 a variety of diseases (Ahmad *et al*., 1998). In recent time, interest with herbal medicine for antimicrobial activities has been increased significantly. The economic crisis, high cost of industrialized medicines, inefficient public access to medical and pharmaceutical care, in addition to the side effects caused by synthetic drugs are some of the factors contributing to the central role of medicinal plants in health care (Johann *et al*., 2007). Hence, the present study was undertaken to evaluate the antimicrobial activity of *Swertia chirata* (Family: Gentianaceae).

The plant is a native of temperate Himalayas, found at an altitude of 1200-3000 m (4000 to 10,000 ft), from Kashmir to Bhutan and in the Khasi hills at 1200-1500 m (4000 to 5000 ft) (Kirtikar and Basu, 1984; Clarke, 1885). It can be grown in sub-temperate regions between 1500 and 2100 m altitudes (Bentley and Trimen, 1880). The plant has about 2-3 feet long stem, the middle portion is round, while the upper is four-angled. The stems are orange brown (Anonymous, 1982) or purplish in color (Bentley and Trimen, 1880) and contain large continuous yellowish pith. The root is simple, tapering and stout, short, almost 7 cm long and usually half an inch thick (Clarke, 1885; Bentley and Trimen, 1880). Flowers are small, stalked, green-yellow, tinged with purple colour, rotate and tetramerous (Kirtikar and Basu, 1984; Bentley and Trimen, 1880). The plant is gathered during the late stages of flowering, commonly tied up in flattish bundles about 3 ft long and 1.5 to 2 lbs in weight (Bentley and Trimen, 1880) and is sold in the market as dried brownish stems with root and leaves intact. The plant extract have been reported to possess antipyretic (Bhargava *et al*., 2009), anti-viral (Verma *et al*., 2008), anthelmintic (Iqbal *et al*., 2006), anticarcinogenic (Saha *et al*., 2004), hepatoprotective (Mukherjee *et al*., 1997) hypoglycemic (Bajpai *et al*., 1991; Saxena *et al*., 1993) activities. Early studies documented the presence of flavonoids, xanthones, terpenoids, iridoid and secoiridoid glycosides in the *S. chirata* plant (Pant *et al*., 2002). In the present study, the antimicrobial properties of pet-ether, dichloromethane and methanol fractions of leaf and stem of *Swertia chirata* Ethanolic extract were investigated by disc diffusion technique and food poison method.

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MATERIALS AND METHODS

Collection and preparation of plant materials: Fresh plant sample of *Swertia chirata* was collected from Chawk bazar, Dhaka in November 2007 and taxonomically identified by the National Herbarium of Bangladesh, Mirpur, Dhaka having the identification accession number -34333. Leaf, stem and root were separated from the plant and dried in shade. The dried leaf and stem were then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Faculty of Pharmacy, University of Dhaka and preserved in an air tight containers.

Extraction and fractionation: Leaf and stem powder of *Swertia chirata* were extracted with ethanol by cold extraction procedure. The extracts were filtered and concentrated at 40°C with a Heidolph rotary evaporator until solid/semisolid mass were produced. The crude extract of leaf and stem were then dissolved in 10% water in methanol (100 mL) and partitioned between pet-ether, dichloromethane and methanol fractions.

Test organism: Both Gram-positive (*Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina lutea*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Salmonella typhi*, *Shigella boydii*, *Shigella dysenteriae*, *Vibrio mimicus*, *Vibrio parahemolyticus*), as well as fungal strains (*Candida albicans, Aspergillus niger*, *Sacharomyces cerevacae*) used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh.

Experimental procedure: The antimicrobial study of the test sample was carried out by Disc diffusion technique (Monica, 2000) for bacteria and Poison food technique (Dhingra and Sinclair, 1995) for fungi. Standard Kanamycin disc (30 µg disc^{-1}) and disc containing the test materials (400 µg disc^{-1}) impregnated with the respective solvents were used as positive and negative controls, respectively. The antimicrobial potency of the test samples was measured by determined the diameter of the zone of inhibition in millimeter.

RESULT

The study showed that among different fractions, dichloromethane fraction of both leaf and stem have significant antimicrobial activities at a dose of 400 µg disc^{-1} against some Gram-positive and Gram-negative bacteria and moderate activity against some fungi, whereas other fractions showed mild to moderate antimicrobial activities (7-11 mm). The results are summarized in the Table 1.

Results of antibacterial screening: Among Gram-positive bacteria, the most notable effect was seen against *Staphylococcus aureus* (19 mm with dichloromethane fraction of both leaf and stem) in comparison to others. Dichloromethane fraction of leaf and stem also showed

Table 1: Antimicrobial activity of different fractions of *Swertia chirata*

	Diameter of zone of inhibition (mm)						
Test microorganisms	PL	DCML	ML	PS	DCMS	MS	Kanamycin
Gram-positive bacteria							
Bacillus cereus	10	16	9	-	17	-	32
Bacillus megaterium	7	8	8	10	9	7	33
Bacillus subtilis	9	14	8	8	13	7	34
Staphylococcus aureus	9	19	7	-	19	7	30
Sarcina lutea	8	10	8	9	9	7	34
Gram-negative bacteria							
Escherichia coli	-	15	7	7	17	8	34
Pseudomonas aeruginosa	-	13	7	7	12	9	33
Salmonella paratyphi	9	10	-	7	11	7	30
Salmonella typhi	10	11	9	-	12	8	35
Shigella boydii	-	12	8	8	13	8	34
Shigella dysenteriae	10	10		7	12	7	34
Vibrio mimicus	9	12	8	7	11	-	30
Vibrio parahemolyticus	8	10	8	-	10	10	32
Fungi							
Candida albicans	8	12	-	10	12	8	37
Aspergillus niger	9	12	8	11	13	8	38
Sacharomyces cerevacae	8	12	10	10	13	-	33

PL: Pet-ether fraction of leaf, DCML: Dichloromethane fraction of leaf, ML: Methanol fraction of leaf, PS: Pet-ether fraction of stem, DCMS: Dichloromethane fraction of Stem, MS: Methanol fraction of stem, MS: Methanol fraction of stem, Dose -400 µg disc^{-1} (Sample) and 30 µg disc^{-1} (Kanamycin). -: Zone of inhibitions less than 6 mm are not included in the table marked antimicrobial activities against *Bacillus cereus* (16 mm for dichloromethane fraction of leaf and 17 mm for dichloromethane fraction of stem) and *Bacillus subtilis* (14 mm for dichloromethane fraction of leaf and 13 mm for dichloromethane fraction of stem).

Against Gram-negative bacteria, dichloromethane fraction of leaf and stem showed highest activity against *Escherichia coli* in comparison to others. The zone of inhibition against *Escherichia coli* was found to be 15 mm for Dichloromethane fraction of leaf and 17 mm for stem fraction. Dichloromethane fraction of stem and leaf also showed moderate antimicrobial activities (10-13 mm) against Gram-negative bacteria except *Escherichia coli*.

Result of antifungal screening: Against different fungi, dichloromethane fraction also showed moderate antimicrobial activity (12 mm for dichloromethane fraction of leaf and 12-13 mm for dichloromethane fraction of stem).

DISCUSSION

Herbal plants are an important source of new chemical substances with potential therapeutic uses. Approximately 119 pure chemical substances extracted from higher plants are used in medicine throughout the world (Farnsworth *et al*., 1985). The increased interest on plant medicines in today's world is from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects (Nair and Chanda, 2006).

In the present investigation we have studied the antimicrobial activity of *Swertia chirata*. As the result, dichloromethane fraction of *Swertia chirata* was most active against *Staphylococcus aureus* in comparison to other microorganisms tested. Among Gram-negative bacteria *E. coli* also showed significant susceptibility to dichloromethane fraction. On contrary, microorganisms were less susceptible to pet-ether and methanol fraction in relative to dichloromethane fraction. This may be due to good solubility of active chemical substance in dichloromethane fraction. The highest activity of dichloromethane fraction against *Staphylococcus aureus* reflects its potentiality to treat skin infections. Traditionally the plant is used for curing various skin diseases in Indian subcontinent where the plant is indigenous (Joshi and Dhawan, 2005). From the present investigation, the results obtained confirmed the therapeutic potency of *Swertia chirata* used in traditional medicine. The present study also set an important basis for further phytochemical and pharmacological investigation on *Swertia chirata*.

The present study suggests that the plant extract certainly possess some chemical constituents with antimicrobial properties and this finding is very important in discovering new drugs for the therapy of infectious diseases. However, further studies can be subjected to isolate and characterize the active constituents responsible for the antimicrobial property of *Swertia chirata*.

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