

Short Communication

Antimicrobial studies of rhizome of *Swertia petiolata*

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ABSTRACT

Swertia petiolata is used as folkore medicine in the treatment of skin diseases and mental disorders, ulcers, liver disorder and as bitter tonic, febrifuge, anthelmintic, antimalarial and anti-diarrheal. Extracts of *Swertia petiolata* were prepared by soxhlet method. Dilutions were made in DMSO and subjected to antimicrobial activity by using agar well diffusion method, plate count agar (PCA) plates were inoculated with 100 μ l of each pathogenic microorganism adjusted to standardized inoculum (1.5×10^8 CFU/ml) in triplicates and spread with sterile swabs. After incubation for 24 hrs at 37 °C, the plates were observed. The zone of inhibition was measured and expressed in millimeters. Whereas the standard antibiotics; cefutaxim and amoxicillin showed antimicrobial activity with zones of inhibition ranging from > 15 mm to 10-15 mm. The hydro methanol extract showed excellent activity against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis* = 10-15mm), Gram-negative bacteria (*Escherichia coli* = 10-15 mm). However chloroform and petroleum ether extracts did not show significant antibacterial activity reflected by >4mm (gram +ve) and 5-10 mm (Gram-ve) and >4 mm (Petroleum ether extract). Phytochemical analysis of the extract showed the presence of steroids, tannins, phenolics, saponins, alkaloids, flavonoids and glycosides. From the study, it can be concluded that *Swertia petiolata* possesses significant antimicrobial activity which might be due to the presence of any/all these active constituents.

INTRODUCTION

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Chashoo et al., 2012). Natural products have been the source of most of the active ingredients of medicines. This is widely accepted to be true when applied to drug discovery in 'olden times' before the advent of high-throughput screening and the post-genomic era: more than 80% of drug substances were natural products or inspired by a natural compound (Harvey, 2008). The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Medicinal plants represent a rich source of antimicrobial agents.

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated (Mahesh and Satish, 2008). Resistance to antimicrobial agents has become an increasingly important and pressing global problem. Of the 2 million people who acquire bacterial infections in US hospitals each year, 70% of cases now involve strains that are resistant to at least one drug. A major cause for concern in the UK is methicillin-resistant *Staphylococcus aureus* (MRSA), which was at low levels a decade ago but now accounts for ca. 50% of all *S. aureus* isolates. Substantial investment and research in the field of anti-infectives are now desperately needed if a public health crisis is to be averted (Cushnie and Lamb, 2005). Medicinal plants represent a rich source of antimicrobial agents. Enteric bacteria are major causes of food-borne illnesses and gastrointestinal problems in the developing countries and human beings around the world. (Stainer et al., 1987). Plant extracts are emerging alternatives to conventional natural preservatives for the control of microorganisms. Kashmir region of Himalaya is a rich source of diversified herbs and shrubs of medicinal importance.

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Among various species of plants of medicinal importance, the family *Gentianaceae* holds a distinct place, as various genera of this family are medicinally important and have been used over years in various parts of the world to treat different ailments. There are around 1000 species of this family. Among the plants often used in traditional medicine, *Swertia* species are quite important and have been used as crude drugs in Indian Pharmacopoeia. There are about 250 species of *Swertia*, distributed worldwide, out of which near about 32 species occur in India with 15 species in north west Himalaya. About 9 species of *Swertia* have been reported from Jammu and Kashmir.

These grow in grasslands, slopes or alpine bugyal. *Swertia* species are used as tonic and febrifuge. These have been used as bitter tonic, febrifuge, anthelmintic, antimalarial and anti-diarrheal, (Kirtikar and Basu, 1933). The present study was undertaken to explore the medicinal potential of *Swertia petiolata* found in Kashmir. These species have been used traditionally and regionally for various ailments by local healers.

The entire plant of *Swertia petiolata* is used in Tibetan medicine for its cooling potency (Tsewang, 1994), anti-inflammatory activity, as febrifuge, and as a liver tonic (Komatsu & Tomimori, 1966). Therefore, this study was undertaken to assess the antimicrobial activity.

MATERIALS AND METHODS

Plant material

Rhizomes of *Swertia petiolata* collected from Gulmarg and Banamarg areas (2600-3100 m). These herbs were collected, identified and authenticated by the expert taxonomist (Dr. AR Naqshi) of Kashmir University and the herbarium specimens were deposited in University of Kashmir, Srinagar.

Drying and size reduction of plant

The rhizomes of *Swertia petiolata* was subjected to shade drying for about two week. The dried material was further crushed to powder and the powder was passed through sieve mesh 40 and stored in air tight container at room temperature for further analysis.

Preparation of Extracts

Rhizomes of *Swertia petiolata* was shade dried, and powdered. The powdered herb was first soxhlet extracted with petroleum ether (60:80°C) for 24 hours and subsequently by chloroform and hydro-methanol (20:80). The extracts were dried under reduced pressure using a rotary flash evaporator (Heidolph, model-4011, USA). The percent yield was 5.8, 3.5 and 15.3% for *Swertia petiolata*. The extracts were preserved in a refrigerator at 4 °C.

Phytochemical study

Phytochemical screening for the presence of various chemical constituents was done on successive extracts (Kumar et al., 2011).

Test microorganisms and drugs

A total of four test bacteria namely *Staphylococcus aureus* MTCC-7443, *Bacillus subtilis* (Gram-positive bacteria), *Escherichia coli*, *K pneumonia* (Gram-negative bacterium) and *P chrysogenum* (fungus) were obtained from IMTECH, Chandigarh. The bacteria were maintained on nutrient broth (NB) at 37°C. Drugs like cephalaxin and ketoconazole disk were purchased from scientific distributor- Srinagar, Jammu and Kashmir, India. All extracts dilutions were made in DMSO solvent and the DMSO solvent was used as control.

Agar well diffusion method for antimicrobial activity

In agar well diffusion method, plate count agar (PCA) plates were inoculated with 100µl of each pathogenic microorganism adjusted to standardized inoculum (1.5×10^8 CFU/ml) in triplicates and spread with sterile swabs. Wells or cups of 8 mm size were made with sterile cork borer into agar plates containing the microbial (bacterial and yeast) inoculum and the lower portion was sealed with a little molten agar medium. Different extracts (5mg/50µl) of *Angelica archangelica* were poured into a well of inoculated plates. Cephalaxin (antibacterial) and ketoconazole (antifungal drug) were used as positive control which was introduced into a well instead of an extract. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extract into the agar. After incubation for 24 hrs at 37 °C, the plates were observed. Antimicrobial activity was indicated by an inhibition zone expressed in millimeters. Antimicrobial activity was recorded if the zone of inhibition was greater than 8 mm (Kumar et al., 2011).

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screenings of *Swertia petiolata* are shown in Table 1.

Table. 1: Phytochemical screening of *Swertia petiolata*.

Alkaloids	Present
Flavonoids	Present
Proteins	Present
Saponins	Present
Sterols	Present
Tannins	Present

Antimicrobial activity

The extracts of *Swertia petiolata* were evaluated against four human pathogenic bacteria and one fungus by agar well diffusion method. Results were depicted in Table 2. *Swertia petiolata* may be used to control the growth of Gram-positive and negative bacteria and fungus. The test antibiotic; cefutaxim and amoxicillin showed antimicrobial activity with zones of inhibition ranging from +++++ mm to +++ mm. The hydromethanol extract showed excellent activity against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis* = +++),

Table 2: Antimicrobial activity of different extracts of *Swertia petiolata* against human pathogenic.

Microorganism	Petroleum ether (3mg/ml)	Chloroform (3mg/ml)	Hydro-methanol (3mg/ml)	Amoxicillin (10µg/ml)	Cefutaxime (10µg/ml)
Bacillus subtilis (Gram +ve)	+	+	+++	++++	++++
Staphylococcus aureus (Gram +ve)	+	+	+++	++++	++++
Pseudomonas aeruginosa (Gram +ve)	+	++	+++	+++	++++
Escherichia Coli (Gram -ve)	+	++	+++	+++	++++
DMSO	#	#	#	#	#

The experiments were performed in triplicate, and the diameter of the zone of inhibition was measured in mm; Diameter >4 mm = +, 5-10 mm = ++, 10 – 15 mm = +++ , > 15 mm = +++++, # = 1-2 mm

*DMSO = Dimethylsulphoxide, NA = No activity

Gram-negative bacteria (*Escherichia coli* = +++), chloroform showed activity against Gram-positive bacteria only single + while in Gram-negative bacteria it showed double ++. Petroleum ether extract showed only single positive activity against all strains. DMSO (Control) does not show any kind of inhibition. Phytochemical analysis of the extracts showed the presence of steroids, tannins, phenolics, saponins, flavonoids, carbohydrates, proteins, amino acids, cardiac glycosides, glycosides. Earlier, most of the secondary metabolites were reported for antimicrobial activity (Soetan et al., 2006; Odebiyi, 1995). Therefore, the antimicrobial effect shown by *S. petiolata* might be due to the presence of these constituents. It can also be concluded from the present study that might be used as an alternative to the antibiotic used in pharmaceuticals on the basis of inhibition of pathogenic microorganisms. Further studies are needed to screen out the pure constituents, which help to kill the human pathogen and act as broad spectrum antimicrobial agent.

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