



Phytochemical analysis and in-vitro antimicrobial activity of *Swertia chirata* in different solvents

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Abstract : The present study was undertaken with an aim to analyse the distribution of the phytochemicals in the different parts of *Swertia chirata* in methanol, acetone, petroleum ether and chloroform extracts. The phytochemical analysis revealed that the maximum bioactive compounds were present in the methanol extract. The methanol and acetone extracts of *Swertia chirata* were evaluated for antimicrobial activity against Gram-positive cocci (*Staphylococcus aureus* and *Streptococcus pyogenes*), Gram-negative bacilli (*Escherichia coli* and *Salmonella typhi*) and fungi (*Aspergillus flavus* and *Candida albicans*) by agar diffusion method and further

confirmed by disc-diffusion method. The leaf and stem portion of the methanol extract showed the most significant antimicrobial activity against Gram-positive, Gram-negative and fungal strains. The most significant antimicrobial activity was seen against *Staphylococcus aureus* which reflects its potentiality to be used in skin infections.

Keywords: *Swertia chirata*, phytochemicals, antimicrobial, agar diffusion method, disc-diffusion method.

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Introduction:

The use of medicinal plants play an important role to cover the basic health needs of human beings. *Swertia chirata* an annual herb is reported for an array of therapeutic uses. 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 from Kashmir to Bhutan and in the Khashi hills at 1200-1500m (Clarke, 1885). It can be grown in subtemperate region between 1500 and 2100 altitudes. The plant extract have been reported to

possess antipyretic (Bhargava et. al., 2009), antiviral, antihelmintic, anticarcinogenic. (Saha et. al., 2004), hepatoprotective (Mukherjee et. al., 1997), hypoglycemic (Bajpai et. al., 1991) activities. In the present study, the methanol, acetone, chloroform and petroleum ether extracts of *Swertia chirata* (root, stem and leaves) were evaluated for phytochemical analysis and antimicrobial activity by agar diffusion method and disc diffusion method.

Materials and Methods :

Collection and preparation of plant materials: The plants were collected from a nursery in Patna in August 2012 and taxonomically identified. Leaf, stem and root were separated from the plant and dried in shade and were mechanically ground into coarse powder. The powder was then preserved in air tight containers.

Extraction of plant material: About 5g of the powdered material of each root, stem and leaf were dissolved in methanol, acetone, petroleum ether, and chloroform and left for 24 hrs in dark. These were then filtered and evaporated in hot air oven to remove the solvents and the extracts were ready for use.

Phytochemical analysis: Preliminary phytochemical analysis of all the extracts were conducted by the standard process (Manjunath et. al., 2006).

Detection of tannins : The extract (2 ml) was dissolved in 2 ml of distilled water. To this, few drops of neutral ferric chloride (5%) were added. A dark green colour indicated the presence of tannins.

Detection of flavonoids : To 2 ml of the extract few drops of concentrated sulphuric acid were added. An orange colour indicated the presence of flavonoids.

Detection of alkaloids : To 2 ml of the extract, a drop or two of Mayer's reagent were added by the side of the test tube. A white or creamy precipitate indicated the test as positive.

Detection of terpenoids/steroids : To 2 ml of the extract, 2 ml of chloroform was added followed by 3 ml of concentrated sulphuric acid. A reddish brown colour indicated the presence of terpenoids. To this reddish-brown colouration, 2 ml of acetic acid was added. A green coloured lower layer indicated the presence of steroids.

Antimicrobial assay :

Test microorganisms: Two Gram-positive cocci (*Staphylococcus aureus* and *Streptococcus pyogenes*), two Gram-negative bacilli (*Escherichia coli* and *Salmonella typhi*) and two fungi (*Aspergillus jlavus* and *Candida albicans*) used for the experiment were collected as pure cultures from from Bihar Veterinary College, Patna.

Experimental procedure :

The antimicrobial activity (Kweera et. al., 2011) of the extracts was determined by agar diffusion technique and disc diffusion method. The discs were impregnated with the plant extracts and were loaded on the MHA plates and SDA plates inoculated with the isolate's suspensions. These plates were then examined after 16-18 hrs of incubation. The antimicrobial potency of the test samples were measured by determining the diameter of the zone of inhibition in millimeter.

Result and Discussion :

Phytochemical analysis: Phytochemical analysis of all the four extracts revealed that the methanol extract contained the maximum bioactive compounds followed by the acetone extract. The results are depicted in Table 1.

Table 1. Phytochemical analysis of *Swertia chirata*

Solvents and Plant Parts	Tannin	Flavonoid	Alkaloid	Terpenoid	Steroid
1. Methanol					
Root	-	+	-	-	-
Stem	-	+	-	+	+
Leaf	+	+	+	+	+
2. Acetone					
Root	-	-	-	+	+
Stem	-	+	-	-	-
Leaf	-	+	-	+	+
3. Petroleum Ether					
Root	-	-	-	-	-
Stem	-	-	-	-	-
Leaf	-	-	+	+	+
4. Chloroform					
Root	-	-	-	-	-
Stem	-	-	+	-	-
Leaf	-	-	+	+	+

(+ Present ; - Absent)

Antimicrobial screening: The antimicrobial screening of the methanol and acetone extract revealed that the leaf and the stem portion of the methanol extract showed the highest antimicrobial property as depicted in Table 2.

Table No. 2. Antimicrobial assay of *Swertia chirata*

Plants Solvent Candida Parts	Test Microorganisms (zone diameters in mm)					
	Staphylo- coccus	Strepto- coccus	Escheri- chia	Salmo- nella	Asper- gillus	
albicans	aureus	pyogenes	coli	typhi	flavus	
Leaf Methanol	12	10	8	9	8	9
Leaf Acetone	11	9	7	8	7	8
Stem Methanol	8	6	7	8	10	7
Stem Acetone	9	9	8	7	7	6
Root Methanol	7	-	6	-	-	7
Root Acetone	8	7	6	-	-	-
*C (Control)	19	17	21	20		

**FU (Control)

18 20

Herbal plants are an important source of new chemical substance with potential therapeutic uses. (Kirtikar et. al., 1984) Approximately 119 pure chemical substances extracted from higher plants have been used in medicine throughout the world. The increased interest in plant medicine in today's world is safe and dependable compared

with costly synthetic drugs that have adverse effects. In the present investigation we have studied the antimicrobial activity of *Swertia chirata*. In a similar study the petroleum ether, dichloromethane and methanol fractions of *Swertia chirata* have also showed antimicrobial activity against some Grampositive and Gram-negative bacteria as well as some fungi. A large zone of inhibition has been observed (19 mm) against *Staphylococcus aureus* (Alam et. al., 2009). In the present study also the methanol fraction of *Swertia chirata* was most active against *Staphylococcus aureus* where the highest zone of inhibition (12 mm) was recorded. Among Gram-negative bacteria, *Salmonella typhi* also showed significant susceptibility to methanol fraction. On the contrary, microorganisms were less susceptible to acetone fraction III comparison to methanol fraction. The leaf and the stem fraction of methanol and acetone showed better activity against test microorganisms than their root fraction. This may be due to good solubility of active chemical substance in methanol fraction or due to polarity. The most active extract i.e. methanol extract contained tannins, flavonoids, alkaloids, glycosides. Similar results were obtained for the phytochemical analysis in a previous work done on the same plant with little variations (Kweera et. al., 2011). Tannins and flavonoids are known to possess antimicrobial potential against bacteria and fungi. The antimicrobial potential of the plant extract may be due to the presence of these phytochemicals. The highest activity of methanol fraction against *Staphylococcus aureus* reflects its potentiality to treat skin infections. Traditionally the plant is used for curing various skin diseases in India From the present investigation, the result obtained confirmed therapeutic potency of *Swertia chirata* used in traditional medicine.

Conclusion :

Swertia chirata is not a very common plant of Bihar. Moreover, this plant is not given as much importance as the other medicinal plants like neem, tulsi, etc. Hence we have tried to bring out the importance of this plant through this work, but there is still a wide scope for exploring different aspects of *S. chirata* .. A strong need is felt to screen the different chemo-types of *chirata* growing at different phyto-geographical locations. Similarly, biodiversity studies at morphological, biochemical and genetic levels will enable the research community to realize the extent of variability within the existing germplasm of *S.chirata* (Sampath et. al., 2010) and hence help in conservation of the plant.

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