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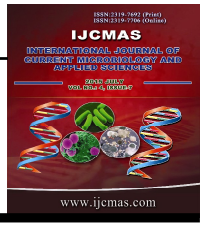

Original Research Article

Antibacterial activity of different extracts of medicinal plant *Swertia chirata*

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A B S T R A C T

A medicinal pant *Swertia chirata* has been widely used as herbal medicine in Asian countries and some parts of world. The aim of present study was to evaluate the antibacterial activity of the plant "*Swertia chirata*". The ethanol and methanol extract of leaves and stem of the plant were used for this purpose. The test organisms used were two Gram Positive (*Staphylococcus aureus* and *Bacillus* sp.) and three Gram Negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) bacteria. The antibacterial activity was determined by agar well diffusion method and MIC (Minimum Inhibitory Concentration) was also performed. MIC was performed by broth dilution method. Among all the strains, *Bacillus* sp. showed significant zone of inhibition against all extracts. As control, ethanol and methanol were used. It was found that among both the extracts, ethanol extract of both leaves and stem was more effective compared to methanol extract against the test microorganisms.

Keywords

Medicinal plant, *Swertia chirata*, antibacterial activity, MIC

Introduction

Nature has long been an important source of medicinal agents. An impressive number of modern drugs have been isolated or derived from natural source, based on their use in traditional medicine. The number of multidrug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the North East but it is doubtless an art as old as mankind. Various parts of plants such as root, stem, fruit, flower, twigs are used as a source of medicine in the form of extract. The crude extracts of plant parts and phytochemicals of known antimicrobial properties is of great significance in therapeutic treatment.

Swertia chirata belonging to family Gentianaceae is an annual, biennial or perennial herb of seasonal growth. It has an elongated stem, the size of which ranges from 60 to 150 cm. The stem is cylindrical at base and quadrangular upward. The colour of stem is greenish- brown when the plant is young and changes from light brown to light violet as the plant attains maturity. The root are generally small, 5-10 cm in length, light brown, somewhat twisted and

gradually tapering. It has leaves in opposite pair about 10 cm long, without stalks, pointed at the tip. Flowers are greenish yellow, tinged with purple and the seeds are small light brown to dark brown in colour. It is usually found at an altitude of 1200-3000 m.

The plant *Swertia chirata* is a therapeutic plant. It is known to show antimicrobial activities on various bacteria. The antibacterial property of *Swertia chirata* is attributed to the presence of the biologically active constituent present in it like amarogentin, swerchirin, triterpenoids, xanthones, opelic acid and gentiopicric (Abdul *et al*, 2011). It is useful in treatment of several diseases like bronchial asthma, liver disorder, fever, anaemia, diarrhoea and stomachic. It also exhibit several biological activities such as antibacterial, antioxidant, anticancer, hepatoprotective, antihelminthic, antiglycemic, antihepatotoxic and hypotensive.

In this study, the effect of ethanol and methanol extracts of *Swertia chirata* against various pathogens is examined. MIC also studied against these test microorganisms.

Materials and methods

Collection of plant material

The plant was collected and identified at Vasco, Goa.

Processing of sample

The leaves and stem of the plant sample were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. After washing, both leaves and stem samples were shade dried and then ground into fine powder. The powdered samples were stored in clean, dry and sterile container for further use.

Extract preparation

Dried and powdered stem and leaves were extracted using methanol and ethanol in soxhlet apparatus at 60°C for 48 hrs. The extracts were then cooled at room temperature, filtered through Whatmann No. 1 filter paper and the filtrate was evaporated to complete dryness in a flash evaporator. The dried extracts were then scraped and stored in clean sterile container for further use.

Test microorganisms

Both Gram Positive (*Staphylococcus aureus* and *Bacillus* sp.) and Gram Negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) organisms were obtained from the Department of Microbiology, Brindavan College, Bangalore.

Antibacterial activity by agar well diffusion method

The effect of different concentrations of ethanol and methanol extracts of leaves and stem of the plant under study on several bacterial strains was assayed by Agar Well Diffusion method. The MIC (minimum inhibitory concentration) of the different extracts was also performed to determine the minimum concentration of the extracts that inhibited the growth of micro-organisms.

20 ml of sterile Mueller Hinton Agar was added to sterile petriplates and allowed to solidify and swabbed with the overnight broth culture of the test organisms using sterile cotton swab. Wells were made using sterile cork borer and 20 µl of different concentrations of the plant extract (ethanol and methanol extracts of leaf and stem) ranging from 100-5000µ g/ml were added

into the well. The plates were then incubated at 37ºC for 24 hrs. The antibacterial activity was assayed by measuring the zone of inhibition. The results obtained from the ethanol and methanol extract of leaf and stem were compared to know the effectiveness of leaves and stem of plant under study.

MIC (Minimum Inhibitory Concentration)

The Minimum Inhibitory Concentration (MIC) was determined by adding various concentrations (100-5000µg/ml) of plant extract to 5 ml of sterile nutrient broth in test tubes. Then 100 µl of overnight broth culture of several test organisms were added into it. The test tubes were then incubated at 37ºC for 18-24 hrs for each microbial culture. The lowest concentration of the extract that inhibited the growth of micro-organism was considered as MIC.

Results and Discussion

The results of antibacterial sensitivity of both ethanol and methanol extracts of leaves and stem of the plant under study against the five different bacterial strains are interpreted in table I-IV, Plates I-IV and Graph I-IV.

The ethanol extract of leaves showed significant activity against *Bacillus* sp. and *Pseudomonas aeruginosa* with zone of inhibition ranging from 14-19 mm for *Bacillus* sp. and 10-18 mm for *Pseudomonas aeruginosa*. *S. aureus*, *E. coli* and *K. pneumoniae* were resistant at lower concentration and zone of inhibition was seen only at higher concentration.

The ethanol extract of stem was effective against all the bacteria with maximum zone of inhibition for *S. aureus* (20mm at 5000µg/ml). *P. aeruginosa*, *Bacillus* sp. and *K. pneumoniae* also showed significant zone of inhibition. *E. coli* was resistant at lower concentration but was sensitive at higher concentration.

Methanol extract of leaves showed moderate activity against *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *Bacillus* sp. *S. aureus* was completely resistant to the extract at all concentrations.

Methanol extract of stem showed moderate activity against *Bacillus* sp. with zone of inhibition ranging from 10-12 mm (100-5000 µg/ml) and *S. aureus* 9-10 mm (2500-5000 µg/ml). All other organisms were found to be resistant against the extract.

SCE: *Swertia chirata* ethanol extract
SCM: *Swertia chirata* methanol extract
- : No activity

The results of MIC (Minimum Inhibitory Concentration) were related to the results of antibacterial assay and are interpreted in table V.

In the present study, both ethanol and methanol extracts were effective against some bacterial strains. Ethanol extract was more effective against the bacteria compared to methanol extract. Also both the extracts were effective against *Bacillus* sp. From the above study, it can be concluded that the activity of the plant extract may be due to the secondary metabolites or broad spectrum antibiotic compounds present in it. The polarity of the solvent seems to play an important role in exhibiting potential antibacterial activity (Ahirwal *et al*, 2011).

In the present research, ethanol extract of leaf tested against various bacterial species, showed significant activity against *Bacillus* sp. and *Pseudomonas aeruginosa* with increasing concentration from 100-5000 µg/ml.

Table.1 Antibacterial activity of ethanol extract of leaf of Swertia chirata against various bacterial species

	Dilution (µg/ml)	Zone of inhibition (mm)				
		S. aureus	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*Bacillus* sp.
SCE (leaf)	100	-	-	-	-	14
	250	-	10	-	-	14
	500	-	11	-	-	14
	1000	-	11	-	-	14
	2000	-	12	-	-	15
	2500	10	12	10	09	15
	3000	11	14	11	09	15
	3500	12	15	13	09	16
	4000	13	15	15	10	18
	5000	14	18	15	12	19

Table.2 Antibacterial activity of ethanol extract of stem of *Swertia chirata* against various bacterial species

Plant extract	Dilution (µg/ml)	Zone of inhibition (mm)				
		S. aureus	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*Bacillus* sp.
SCE (stem)	100	-	-	-	-	12
	250	-	09	-	09	12
	500	-	09	-	09	12
	1000	12	10	-	09	12
	2000	12	10	-	09	12
	2500	15	11	12	13	13
	3000	15	12	13	16	13
	3500	16	16	14	16	14
	4000	17	17	14	17	15
	5000	20	18	15	17	17

Table.3 Antibacterial activity of methanol extract of leaf of *Swertia chirata* against various bacterial species

Plant extract	Dilution (µg/ml)	Zone of inhibition (mm)				
		S. aureus	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*Bacillus* sp.
SCM (stem)	100	-	-	09	-	-
	250	-	-	09	-	-
	500	-	-	10	09	-
	1000	-	-	10	10	-
	2000	-	-	10	10	10
	2500	-	09	11	10	11
	3000	-	10	11	10	11
	3500	-	10	11	11	11
	4000	-	10	11	11	12
	5000	-	10	11	11	12

Table.4 Antibacterial activity of methanol extract of stem of *Swertia chirata* against various bacterial species

Plant extract	Dilution (µg/ml)	Zone of inhibition (mm)				
		S. aureus	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*Bacillus* sp.
SCM (stem)	100	-	-	-	-	10
	250	-	-	-	-	10
	500	-	-	-	-	10
	1000	-	-	-	-	11
	2000	-	-	-	-	11
	2500	09	-	-	-	11
	3000	09	-	-	-	12
	3500	09	-	-	-	12
	4000	10	-	-	-	12
	5000	10	-	-	-	12

Table.5 MIC (Minimum Inhibitory Concentration) of different extract of leaves and stem of *Swertia chirata*

Plant extracts	MIC(µg/ml)				
	Bacillus sp.	*S. aureus*	*K. pneumoniae*	*P. aeruginosa*	*E .coli*
SCE (leaf)	100	3000	4000	3000	4000
SCE(stem)	4000	3500	4000	3500	5000
SCM (leaf)	4000	-	2000	3000	3500
SCM (stem)	1000	3500	-	-	-

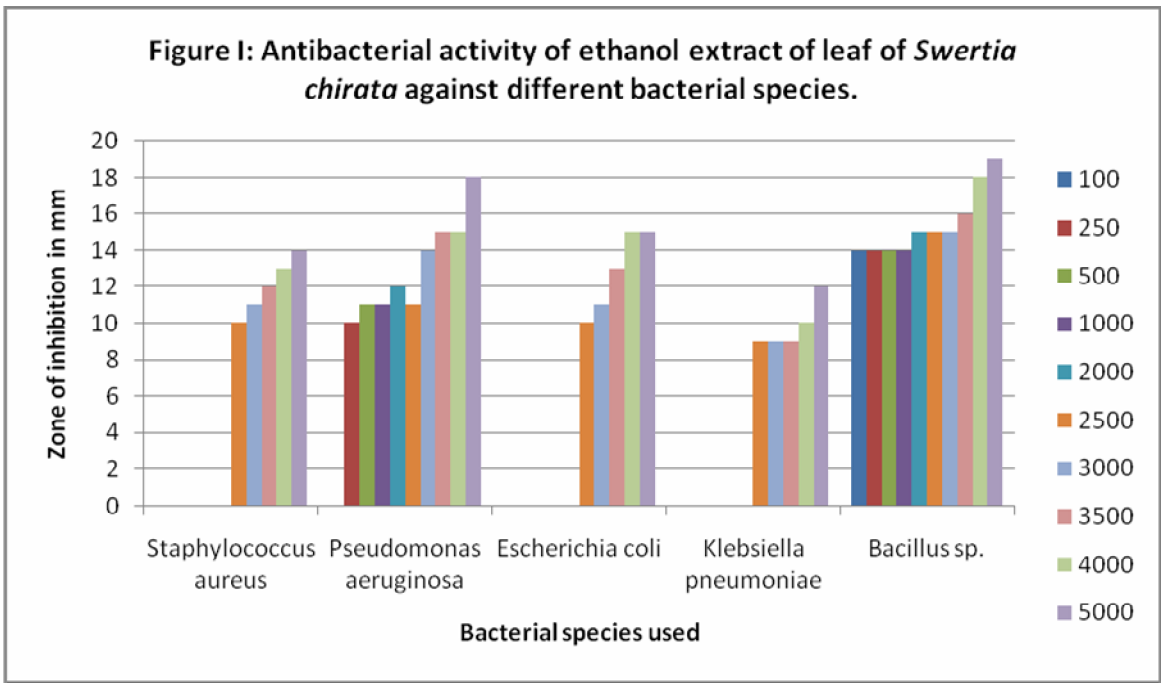


Figure I: Antibacterial activity of ethanol extract of leaf of *Swertia chirata* against different bacterial species.
Zone of inhibition in mm
20
18
16
14
12
10
8
6
4
2
0
Staphylococcus aureus
Pseudomonas aeruginosa
Escherichia coli
Klebsiella pneumoniae
Bacillus sp.
Bacterial species used
100
250
500
1000
2000
2500
3000
3500
4000
5000

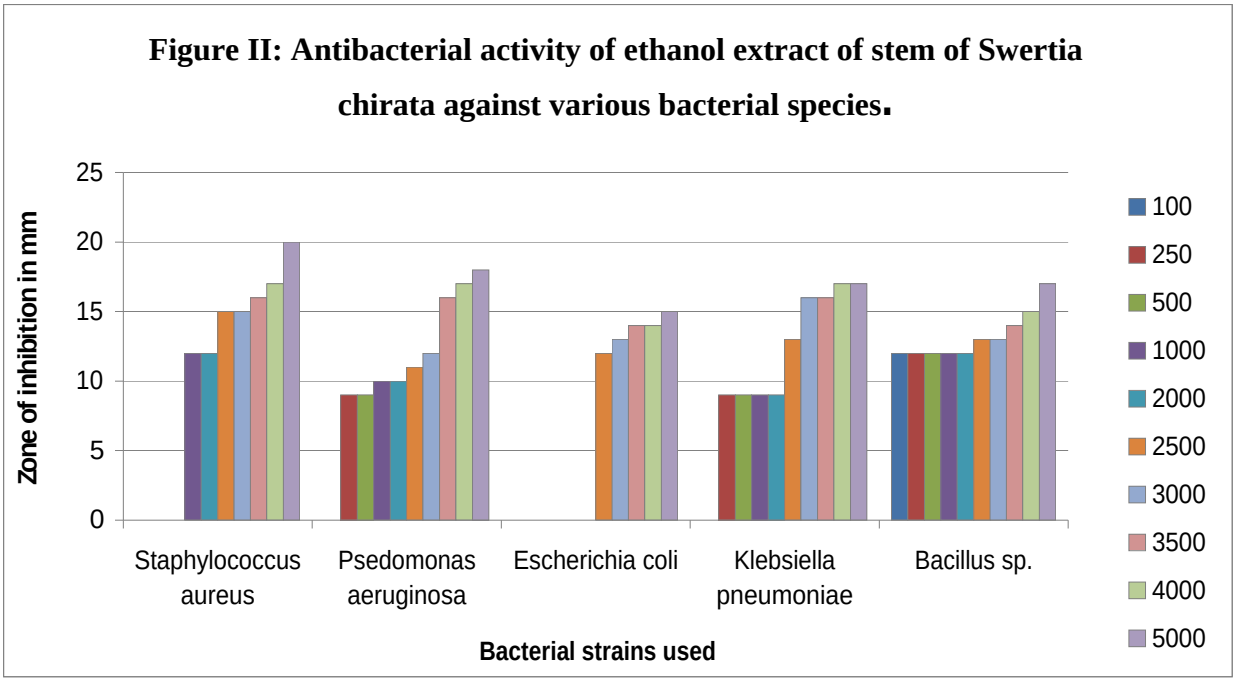


Figure II: Antibacterial activity of ethanol extract of stem of Swertia chirata against various bacterial species.
Zone of inhibition in mm
25
20
15
10
5
0
Staphylococcus aureus
Psedomonas aeruginosa
Escherichia coli
Klebsiella pneumoniae
Bacillus sp.
Bacterial strains used
100
250
500
1000
2000
2500
3000
3500
4000
5000

Figure III: Antibacterial activity of methanol extract of leaf of *Swertia chirata* against various bacterial species.

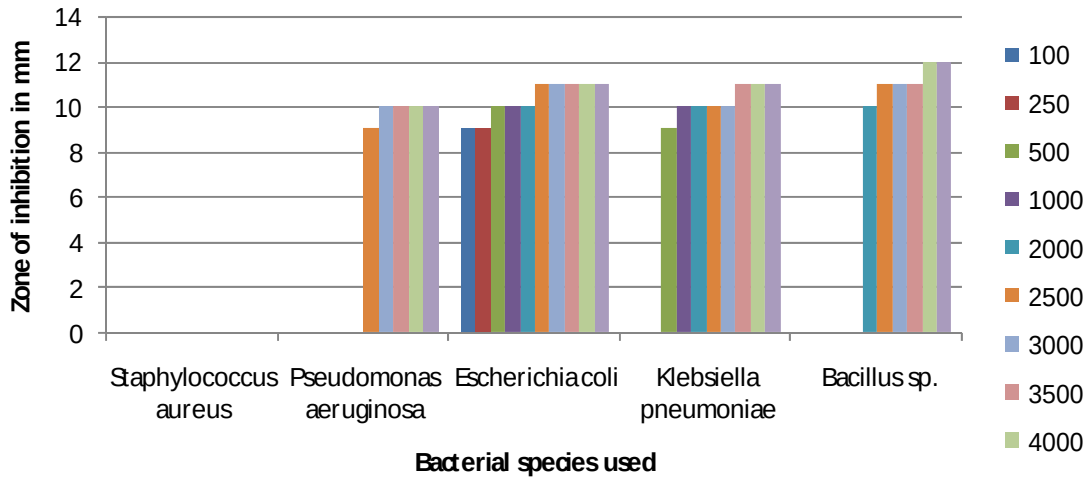
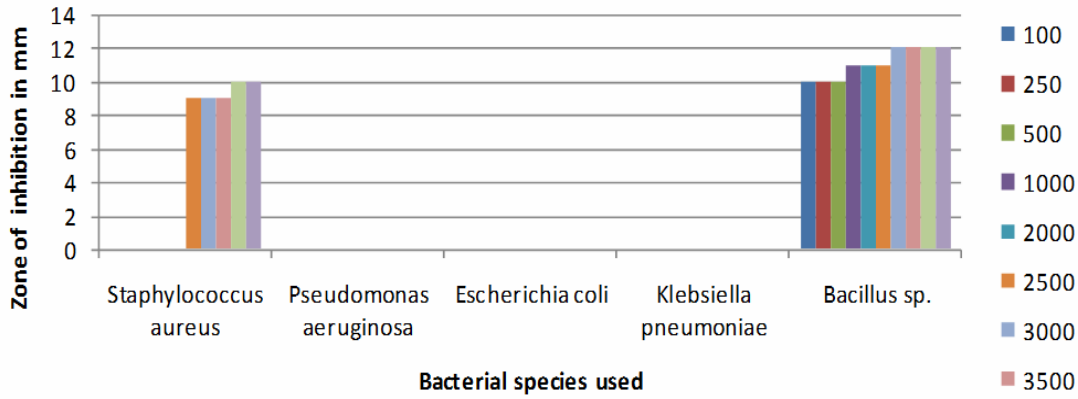


Figure IV: Antibacterial activity of methanol extract of stem of *Swertia chirata* against various bacterial species.



This result is in agreement with Nik *et al*, 2013 and Lwin *et al*, 2013 who also reported the similar fact *Bacillus* sp. whereas in the study conducted by Syed *et al*, 2013, no significant activity was observed against *Bacillus* sp. at concentration of 0-50 µg/ml. In our study, we observed moderate activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. Ahirwal *et al*, 2011 obtained moderate activity for *Staphylococcus aureus*, *Escherichia coli* and *Peudomonas aeruginosa* but using aqueous extract.

In ethanol extract of stem, our results were similar to Nik *et al*, 2013 where *E.coli* and

Staphylococcus aureus were sensitive at higher concentrations and Jesmin *et al*, 2007 and Lwin *et al*, 2013 where *Saphylococcus aureus*, *Bacillus* sp. and *E. coli* were sensitive.

In case of methanol extract of leaf, we found that *Staphylococcus aureus* was resistant but *Bacillus* sp. was sensitive from 2000 µg/ml. This was in accordance with Ahirwal *et al*, 2011 who also obtained resistant for *Staphylococcus aureus* and *Bacillus* sp. was sensitive (800 µg/ml).

In conclusion, as the search for new drugs are in demand, plant extracts may provide an attractive alternative source against various infections and chronic disease. Also due to MDR microorganisms and side effects of the synthetic drugs, these studies can be helpful in discovering new therapeutic agents with less or no side effects.

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