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Research Article

ANTIMICROBIAL SCREENING OF METHANOL AND AQUEOUS EXTRACTS OF *SWERTIA CHIRATA*

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ABSTRACT

Methanol and aqueous extracts of *Swertia chirata* were evaluated for antimicrobial activity against 10 bacteria and 3 fungi using agar diffusion method (well method). Gentamycin and Amphotericin were used as standard drug for antibacterial and antifungal activity respectively. The antimicrobial activity was determined by measuring the diameter of the zone of inhibition in term of millimeter (mm). The results showed that both the extracts showed activity but the methanol extract possess better activity than aqueous extract against test organisms. Both the extracts showed concentration dependent activity. The phytochemical analysis showed the presence of tannins and glycosides in both the extracts whereas, flavonoids and alkaloids were present only in methanol extract. It is concluded that the antimicrobial activity showed by the plant is due to the presence of these phytochemicals.

Keywords: Antimicrobial, *Swertia chirata*, Methanol extract, Aqueous extract.

INTRODUCTION

Today, the increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microorganisms has been a major problem to mankind and this leads to the screening of several medicinal plants for their potential for antimicrobial activity[1,2,3]. Beside this, plant originated antimicrobial drugs do not have much side effect and have an enormous therapeutic potential to heal many infectious diseases[4]. Medicinal plants represent a rich source of antimicrobial agents. In recent years, screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents[5,6,7].

Swertia chirata belongs to family Gentianaceae. It is an erect annual or perennial herb found in Himalaya and Meghalaya at an altitude of 1200–1300 meters. The plant has been reported to possess hypoglycemic activity[8], anti-inflammatory activity[9], hepatoprotective activity[10], wound healing activity[11] as well as antibacterial activity[12]. On the basis of these wide ranges of therapeutic uses, this plant is evaluated for its antimicrobial activity.

MATERIALS AND METHODS

Plant Material

Aerial parts of *Swertia chirata* were purchased from Jeevan herbs Agro, Sagar (M.P.) and authenticated at Department of Botany, Dr. H. S. Gour University, Sagar.

Extract Preparation

Dried and powdered plant material was defatted with petroleum ether and then successively extracted with methanol and water using soxhlet apparatus. The extracts were cooled at room temperature, filtered and evaporated to complete dryness.

Phytochemical Analysis

Both the extracts were subjected to various chemical tests to detect the presence of various compounds such as tannins, flavonoids, alkaloids, terpenoids, steroids etc. using standard procedure[13,14].

Test microorganisms

Bacteria:

Bacillus subtilis (MTCC 736), *Bacillus polymyxa, Staphylococcus aureus* (MTCC 3160), *Escherichia coli* (MTCC 723), *Salmonella typhi* (MTCC 3216), *Vibrio cholerae* (MTCC 3906), *Streptococcus pyogenes* (MTCC 1927), *Proteus mirabilis* (MTCC 1429), *Providentia alkalifaciens,* and *Pseudomonas aeruginosa* (MTCC 7837)

Fungi:

Aspergillus niger (MTCC 1881), *Aspergillus flavus* (MTCC 1883), *Cladosporium oxysporum* (MTCC 1777).

Chemicals and media

Gentamycin, Amphotericin, Muller Hilton's Agar media, Potato Dextrose Agar media, 10% Tween 80.

Antimicrobial Assay

For antibacterial assay[15], initially the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 h. The agar plates of the Muller Hilton's Agar media were prepared. Each plate was inoculated with an aliquot (0.1 ml) of the bacterial suspension (10^5-10^6 colony forming unit "CFU"/ml), which was spread evenly on the plate. After 20 min, wells were made with the help of cork borer in the plates and filled with test samples of different concentrations. The positive and negative control wells were filled with Gentamycin (Standard drug) and 10% Tween 80 respectively. All the plates were incubated at 37°C for 24 h and then the diameter of zone of inhibition was noted.

Similarly for the antifungal activity[16], the stock cultures of fungi were revived by inoculating in broth media and grown at 27°C for 72 h. The agar plates of the Potato Dextrose Agar media were prepared. Each plate was inoculated with an aliquot (0.1 ml) of the fungal suspension (10^3 spores/ml), which was spread evenly on the plate. After 20 min, wells were made and filled with test samples of different concentrations. The positive and negative control plates with Amphotericin (standard drug) and 10% Tween 80 were also prepared. All the plates were incubated at 27°C for 72 h and then the diameter of zone of inhibition was noted.

RESULTS

In the present study, the phytochemical tests of the extracts showed the presence of tannins, alkaloids, glycosides and flavonoids in methanol extract and only tannins and glycosides were present in aqueous extract (Table 1). For antimicrobial screening, methanol and aqueous extracts of *S. chirata* were tested against 10 bacterial and 3 fungal strains. The observations were compared with that of standard antibiotic drugs in terms of diameter of zone of inhibition.

Table 1: Phytochemical analysis of methanol and aqueous extracts of *S. chirata*

Phytochemical	Methanol extract (SCM)	Aqueous extract (SCA)
Steroids	-	-
Terpenoids	-	-
Tannins	+	+
Alkaloids	+	-
Glycosides	+	+
Flavonoids	+	-
Saponins	-	-

+ : Present; - : Absent

In antibacterial activity, methanol extract showed most significant activity against *B. subtilis* (5 mm at 800 µg/ml) and moderate activity against *E. coli* and *S. typhi* (4 mm at 800 µg/ml), very less activity against *P. mirabilis* and *B. polymyxa* (1mm at 800 µg/ml) and no activity against *S. aureus, V. cholerae, S. pyogenes, P. alkalifaciens,* and *P. aeruginosa.* Aqueous extract showed moderate activity against *E. coli, P. alkalifaciens, S. typhi,* and *V. cholerae* (2mm at 800 µg/ml) and was inactive against *S. aureus, B. subtilis, S. pyogenes, P. mirabilis, B. polymyxa* and *P. aeruginosa*. Both the extracts showed concentration dependent activity (Table 2 and Table 3; Fig. 1 and Fig. 2).

Table 2: Antibacterial activity of methanol extract of *Swertia chirata*

Organism	Extract/Drug (µg/ml)/ Zone of Inhibition (mm)												10%Tween 80
	SCM						Gentamycin						
	25	50	100	200	400	800	25	50	100	200	400	800	
E. coli	-	-	-	1	2	4	18	20	23	26	28	30	-
S. aureus	-	-	-	-	-	-	13	18	21	25	27	34	-
B. subtilis	-	-	1	2	4	5	8	10	15	19	22	25	-
S. typhi	-	-	-	-	2	4	2	13	16	21	25	27	-
V. cholerae	-	-	-	-	-	-	13	15	18	21	23	25	-
S. pyogenes	-	-	-	-	-	-	19	22	25	29	30	33	-
P. mirabilis	-	-	-	-	-	1	9	13	18	21	25	27	-
P. alkalifaciens	-	-	-	-	-	-	20	23	26	31	35	38	-
B. polymyxa	-	-	-	-	-	1	15	22	25	28	30	33	-
P. aeruginosa	-	-	-	-	-	-	-	-	1	3	8	14	-

SCM*: Swertia chirata* methanol extract

- : No activity

Table 3: Antibacterial activity of aqueous extract of *Swertia chirata*

Organism	Extract/Drug (µg/ml)/ Zone of Inhibition (mm)												10% Tween 80
	SCA						Gentamycin						
	25	50	100	200	400	800	25	50	100	200	400	800	
E. coli	-	-	-	-	-	2	18	20	23	26	28	30	-
S. aureus	-	-	-	-	-	-	13	18	21	25	27	34	-
B. subtilis	-	-	-	-	1	2	8	10	15	19	22	25	-
S. typhi	-	-	-	-	-	2	2	13	16	21	25	27	-
V. cholerae	-	-	-	-	1	2	13	15	18	21	23	25	-
S. pyogenes	-	-	-	-	-	-	19	22	25	29	30	33	-
P. mirabilis	-	-	-	-	-	-	9	13	18	21	25	27	-
P. alkalifaciens	-	-	-	-	-	2	20	23	26	31	35	38	-
B. polymyxa	-	-	-	-	-	-	15	22	25	28	30	33	-
P. aeruginosa	-	-	-	-	-	-	-	-	1	3	8	14	-

SCA: *Swertia chirata* aqueous extract

- : No activity

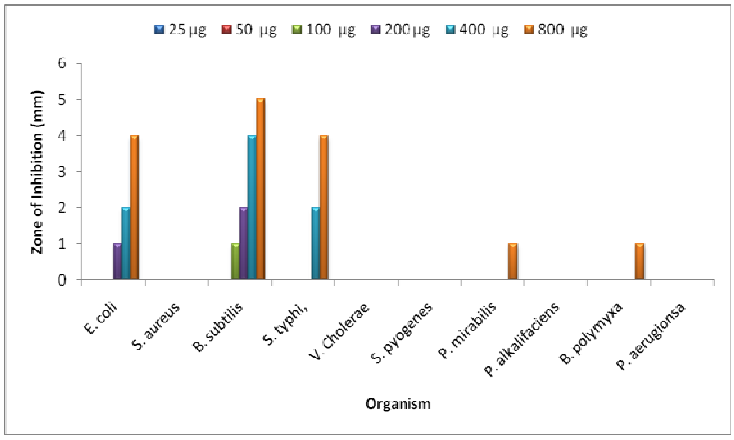

Fig. 1: Antibacterial activity of methanol extract of *Swertia chirata*

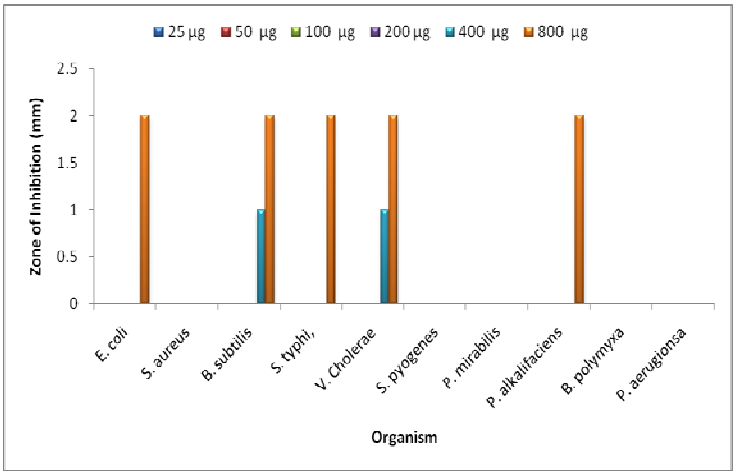

Fig. 2: Antibacterial activity of aqueous extract of *Swertia chirata*

Table 4: Minimum Inhibitory Concentration* (MIC) of methanol and aqueous extracts of *Swertia chirata*

Extract	*E. coli*	*S. aureus*	*B. subtilis*	*S. typhi*	*V. cholerae*	*S. pyogenes*	*P. mirabilis*	*P. alkalifaciens*	*B. polymyxa*	*P. aeruginosa*
SCM	400	800	100	800	800	800	800	800	800	800
SCA	800	800	400	800	400	800	800	800	800	800
Gentamycin	25	25	25	25	25	25	25	25	25	100

*Concentration in µg

SCM: *Swertia chirata* methanol extract

SCA: *Swertia chirata* aqueous extract

In antifungal activity, methanolic extract showed significant activity against *Cladosporium oxysporum* (9mm at 800 µg/ml), moderate activity against *Aspergillus niger* (2 mm at 800 µg/ml) and no activity against *Aspergillus flavus*. Aqueous extract showed significant activity against *Cladosporium oxysporum* (5 mm at 800 µg/ml) and *Aspergillus flavus* (4 mm at 800 µg/ml), while against *Aspergillus niger* it showed nil activity. Both the extracts showed concentration dependent activity (Table 5). The minimum inhibitory concentration of the extracts varied between 50 µg/ml to ≤ 800 µg/ml (Table 4 and Table 6).

Table 5: Antifungal activity of methanol and aqueous extracts of *Swertia chirata*

Test sample	Conc. (µg)	Zone of Inhibition (mm) Organism		
		A. niger	*C. oxysporum*	*A. flavus*
SCM	25	-	-	-
	50	-	1	-
	100	-	2	-
	200	-	2	-
	400	-	5	-
	800	2	9	-
SCA	25	-	-	-
	50	-	-	-
	100	-	-	-
	200	-	2	-
	400	-	4	2
	800	-	5	4
10% Tween 80 (Control)		-	-	-
Amphotericin (Standard drug)	25	-	-	-
	50	-	2	-
	100	2	7	-
	200	3	9	-
	400	5	13	7
	800	7	15	10

SCM: *Swertia chirata* methanol extract

SCA: *Swertia chirata* aqueous extract

- : No activity

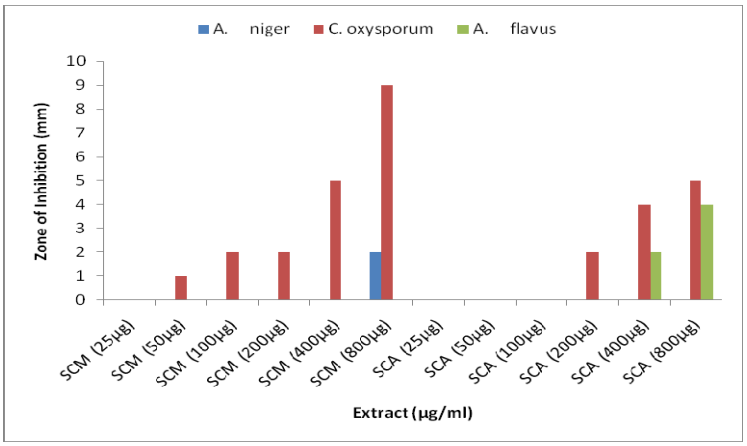

Fig. 3: Antifungal activity of methanol and aqueous extracts of *Swertia chirata*

Table 6: Minimum Inhibitory Concentration (MIC) of methanol and aqueous extracts of *Swertia chirata*

Extract	*A. niger*	*C. oxysporum*	*A. flavus*
SCM	800	50	?800
SCA	?800	200	?800
Amphotericin	100	50	400

*Concentration in µg

SCM: *Swertia chirata* methanol extract

SCA: *Swertia chirata* aqueous extract

DISCUSSION

In the present investigation, both the extracts showed activity against some bacteria and fungi, but methanol extract showed better activity than aqueous extract. The activity may be the indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins[17]. The polarity of the solvent seems to play an important role in exhibiting potential antibacterial activity[18]. The most active extract i.e. methanol extract contained tannins, flavonoids, alkaloids and glycosides (Table 1) and as tannins and flavonoids were known to possess antimicrobial potential against bacteria and fungi[19,20,21],the antimicrobial potential of these plant extracts may be due to the presence of these phytochemicals.

Antimicrobial agents can damage pathogens in several ways. The major modes of actions are interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, and inhibition of a metabolic pathway[22]. The most selective antimicrobial agents are those that interfere with the synthesis of bacterial cell walls e.g. penicillin, cephalosporin etc. these drugs have high therapeutic index because bacterial cell walls possess a unique structure, not found in eukaryotic cells[23]. Both the extracts may possess the similar mechanisms of action against the microbes.

As the search for new antimicrobial agents is in demand, plant extracts may provide attractive alternate sources of antimicrobial drug against various microbial diseases. The present study provides the evidence of antimicrobial property of *S. chirata* against bacteria and fungi.

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