



Phytochemical screening and evaluation of *Berginia ligulata* root extract for antimicrobial activity

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Abstract

Berginia ligulata popularly known as *paashaanbhed* belongs to family *Saxifragaceae*. The present study was designed to screen out various phytoconstituents present in the roots of *Berginia ligulata* and further its antimicrobial activity against human pathogenic bacteria. For this roots of the plant were shade dried, powdered and extracted using different solvents in increasing order of polarity i.e. petroleum ether, diethyl ether, acetone, ethanol and methanol respectively. Preliminary phytochemical screening of the extracts revealed the presence of steroids, terpenoids, alkaloids, tannins, flavanoids, glycosides and carbohydrates. The presence of these bioactive constituents is associated with the antimicrobial activity of the plant. Antimicrobial activity of extracts was performed by disc diffusion method at a concentration of 100% and 50% (w/v) against five bacterial strains. The maximum antimicrobial activity was found in ethanol extract followed by acetone, methanol, diethyl ether and least in petroleum ether extract respectively. Ethanol extract revealed broad spectrum of inhibition against *S. aureus* and *P. aeruginosa*.

Keyword: *Berginia ligulata*, *paashaanbhed*, antimicrobial activity, phytochemical screening

Introduction

Though the traditional Indian system of medicine has a long history of use, they lacked adequate scientific documentation, particularly in light modern scientific knowledge (WHO, 1998). *Berginia ligulata* Wall. (Syn. *Saxifraga ligulata*), family *Saxifragaceae*, is a perennial herb with thick rootstock, short stem, white, pink or purple flowers and found in South and East Asia. In India, it grows at high altitudes in the Himalayas usually in rocky areas and in the Kashmir valley where it is popularly known as *Paashaanbhed*. It is found in the Himalayas between the altitudes of 2,000 and 2,500 meters. The roots contain tannic acid, gallic acid, starch, mineral salt, albumin, glucose, mucilaginous matter, wax and aromatic substances. The roots are used as an antidiabetic, diuretic, astringent, cardiogenic, wound healer, expectorant, anti-haemorrhoidal, tonic in fever, diarrhea and also as an antiscorbutic. (Kritikar and Basu, 1983 & Gyanendra, 1988). Chemical investigations have shown the presence of β -sitosterol, β -sitosterol-D-glucoside, bergenin (Bahl *et al.*, 1974) and

paashaanolactone (Umashankar *et al.*, 1998). Bergenin and β -sitosterol are well known for pharmacological actions. This plant is explored a very little for pharmacological activity. Thus we have studied this plant for its antimicrobial activity so that its traditional use can be claimed. The present study explores the preliminary phytochemical and anti-microbial screening of roots of *Berginia ligulata* and establishes a correlation between the phytoconstituents and their antimicrobial activity.

Material and methods

Plant material

Fresh roots of *Berginia ligulata* were collected from Aushdhiya vanaspati van sansthan, Dehradun, Uttarakhand and authentication was done by Dr. Santosh Kumar Agarwal (Head Department of Botany, D.B.S. (P.G.) College Dehradun. The roots were shade dried, grind into powder and stored in polythene bags before use.

Extraction of plant material

104.367 g of powdered roots of *Berginia ligulata* was extracted in a soxhlet extractor sequentially with 700 ml of petroleum ether (40-60° C), diethyl ether, acetone, ethanol and methanol in increasing

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order of polarity. The obtained extracts were concentrated at reduced pressure in a rotary vacuum evaporator.

Preliminary phytochemical investigation

The major secondary metabolites like, alkaloids, flavonoids, saponins, phenols, terpenoids, anthraquinones, carbohydrates and glycosides were assessed according to the standard procedure described by Harborne (Harborne, 1984).

Microorganism used

A total of five out of which four gram negative and one gram positive human pathogenic bacterial strains were used in the present study. The bacterial species used were *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Enterobacter*

Culture media and inoculum

Muller Hinton Agar and Nutrient broth (Hi-Media Pvt. Ltd., Bombay, India) were used as culture for the test microorganism. Microbial cultures, freshly grown at 37°C were appropriately diluted in sterile normal saline solution and the turbidity of the suspension is adjusted equivalent to a 0.5 McFarland standard, so as to obtain the cell suspension between 10⁵ to 10⁸ CFU/ml (McFarland, 1907).

Preparation of test extract

Solvent free extracts obtained were dissolved in sterilized and filtered DMSO (filtered with whatman filter of pore size 0.45 micron) to prepare a test solution of extract of 50% and 100% (w/v) concentration.

Antimicrobial Activity

The antimicrobial activity of the extracts was performed by standard protocol of Kirby- Bauer disc diffusion susceptibility method (Bauer *et al.*, 1966). In this method, 6 mm sterilized filter paper discs were saturated with sterilized extracts of the test solution. The discs absorb approximately a 10 µl of the test solution. The discs were stored and dried in the cold. The seeded discs are then placed on to the surface of MHA pre-inoculated with test bacteria. The disc devoid of extract and presence of DMSO served as control. Ofloxacin (5 µg/ disc) and Ciprofloxacin (10 µg/ disc) were used as standard. The plates were kept at 4 °C for 1 hour for diffusion of extract, thereafter the plates were incubated at 37 °C for 24 hours. After incubation, zone of inhibition was measured in mm (millimeter). All the test processes were performed in a triplicate in laminar chamber.

Results and Discussion

Extractive Yield

The extractive yield (w/w) of *Berginia ligulata* root extracts is shown in Table 1. The yield was maximum in ethanol extract (10.124) followed by acetone (4.591), methanol (3.778), diethyl ether (1.184) and least in petroleum ether (0.777) extract respectively.

Preliminary phytochemical investigation

The result of phytochemical screening (Table 2) revealed that *Berginia ligulata* root contain a number of secondary metabolites like alkaloids, carbohydrates, glycosides, steroids, terpenoids, tannin, flavonoid and saponnin. Ethanol and acetone extract contains majority of phytoconstituents in comparison to others,

Table 1:- Extractive yield of different extracts of *Berginai ligulata* root extract

Extracts	Appearance	Consistency	% Yield (w/w)
Petroleum ether	Pale yellow	Fluidy and sticky	0.777
Diethyl ether	Pale brown	Waxy	1.184
Acetone	Dark Brown	Semisolid	4.591
Ethanol	Dark Brown	Semisolid	10.124
Methanol	Brown	Semisolid	3.778



extractive yield is also suggestive for it. The presence of various phytoconstituents implies that the roots of this plant might be of medicinal importance to pharmaceutical industries as the phytoconstituents of the plants account for their medicinal value.

Antimicrobial Activity

All the extracts screened, showed significant reduction in bacterial growth in terms of zone of inhibition, indicating that this plant exhibits antimicrobial activity against the microorganisms; the zone of inhibition was recorded and tabulated (Table 3).

Table 2:- Phytochemical evaluation of *Berginia ligulata* root extract

Phytoconstituents	Extracts				
	Petroleum ether	Diethyl ether	Acetone	Ethanol	Methanol
Steroid	+	+	+	+	-
Terpenoid	+	+	+	-	-
Alkaloid	+	+	+	+	-
Tannin	-	-	+	+	-
Flavonoid	-	-	+	+	-
Glycoside	-	-	+	+	+
Saponnin	-	-	-	+	+
Carbohydrate	-	-	-	+	+

Table 3:- Antimicrobial activity of *Berginia ligulata* root extracts compared with standard drug Ofloxacin and Ciprofloxacin

Bacterial strain (Gram stain)	Conc % (w/v)	Zone of Inhibition in mm*					Ofloxacin (5 µg/disc)	Ciprofloxacin (10 µg/disc)
		Petroleum Ether	Diethyl ether	Acetone	Ethanol	Methanol		
S. aureus (+)	100	14.100 ± 1.65	14.980 ± 2.30	17.565 ± 2.08	21.350 ± 2.96	14.960 ± 1.70	24.330 ± 2.08	28.600 ± 0.78
	50	12.000 ± 0.78	13.125 ± 1.25	14.563 ± 1.10	16.250 ± 1.59	13.990 ± 1.78		
E. coli (-)	100	10.220 ± 0.65	12.330 ± 0.56	13.660 ± 1.52	15.657 ± 1.05	12.085 ± 1.15	21.200 ± 1.17	28.000 ± 0.50
	50	8.020 ± 0.25	11.580 ± 1.32	11.220 ± 1.46	13.120 ± 2.08	10.090 ± 1.90		
K. pneumonia (-)	100	12.010 ± 1.32	14.211 ± 1.86	14.325 ± 4.16	16.694 ± 1.68	14.969 ± 2.33	20.000 ± 1.36	30.100 ± 1.89
	50	11.500 ± 0.36	12.860 ± 0.92	13.462 ± 2.19	14.085 ± 0.98	12.450 ± 2.98		
P. aeruginosa (-)	100	13.060 ± 1.87	15.830 ± 0.85	17.238 ± 0.57	20.696 ± 2.50	16.086 ± 1.05	16.500 ± 1.15	36.20 ± 1.10
	50	11.500 ± 1.23	13.056 ± 1.12	15.055 ± 1.85	18.114 ± 2.12	13.600 ± 1.45		
Enterobacter (-)	100	13.660 ± 2.08	13.000 ± 2.64	15.000 ± 2.08	18.326 ± 1.52	13.262 ± 1.52	19.500 ± 1.73	24.000 ± 0.79
	50	11.850 ± 1.42	10.400 ± 3.69	13.850 ± 1.14	14.780 ± 2.17	11.476 ± 2.40		

(-): No zone of inhibition, *Results are expressed as mean of 3 values ± s.d.



The extracts seem to be active against gram positive and gram negative bacteria. Ethanol extract shows maximum potential of inhibition against all bacterial strains followed by acetone and methanol extract. Maximum inhibition (21.350) was shown by *S. aureus* against ethanol extract. For *P. aeruginosa* ethanol extract is even inhibitorier at both concentrations when compared with standard ofloxacin. Similarly the zone of inhibition of ofloxacin and ethanol extract is nearly equal for *Enterobacter*. The standard ciprofloxacin reveals much larger inhibition zone than ofloxacin and extracts against all the strains. Generally, the use of synthetic antibiotics gives a more potent antimicrobial activity when compared to a crude extract. But one thing that always should be remembered that synthetic antimicrobial have undesirable side effects as compared to the natural antimicrobials of plant origin (Iwu *et al.*, 1999). The antimicrobial activity of extracts is possibly due to the presence of various phytoconstituents, however the tremendous activity of ethanol extract perhaps due to the presence of majority of these phytoconstituents in that single extract.

So the above study clearly illustrates the pharmacological use of *Berginia ligulata* roots as antimicrobial agents, due to which it can be used for preparing in formulation of various pharmaceutical preparations against highly expensive synthetic antimicrobial agent. Further its traditional use as antimicrobial agent is also well demonstrated by this study.

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