Innovare Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 7, Issue 4, 2015

Original Article

ANTIMICROBIAL ACTIVITY OF BARLERIA PRIONITIS ON PATHOGENIC STRAINS

PRIYANKA PANCHAL[a], KAMAL SINGH[b]

[a]IBMER, Mangalayatan University, Aligarh, India, [b]Vallabhbhai Patel Chest Institute, Delhi University, Delhi, India
Email: drpriyanka.biotech@gmail.com

Received: 24 Jul 2015, Revised and Accepted: 15 Sep 2015

ABSTRACT

Objectives: The present study was planned to investigate the antimicrobial activity of different part of the plant drug. Therefore, the preliminary successive solvent extraction and chemical test revealed the presence of secondary metabolites in various extracts, and provide us clue for further investigation.

Methods: Different solvents i.e., Chloroform, Acetonitrile, Ethanol, was used for extraction of active secondary metabolites by the Soxhlet method. Percentage of yield[4], and antimicrobial activity by gradient plate technique [13]. Statistical analysis has done by standard error by mean (SEM), in which n=3.

Results: The significant antimicrobial activity of leaf, stem and root along with 1.2±0.0136 mg/ml to 4±0.0128 mg/ml on bacterial strain and 9.4±0.0128 mg/ml to 14.5±0.0149 mg/ml MIC value with SEM. The highest percentage of yield was observed in Ethanol, chloroform and Acetonitrile i. e from 3.7% to 5.36%. Various numbers of secondary metabolites were extracted from 1 to 4 in *Barleria prionitis* Linn.

Conclusion: Ethanol has been shown as a best solvent to yield, extracted components and antimicrobial effects prospects while acetonitrile and chloroform were also good solvents to extract secondary metabolite components.

Keywords: *Barleria prionitis*, Secondary metabolites, Antimicrobial activity, Solvents.

INTRODUCTION

Medicinal plants are the important source of medicine, which is being used in the Ayurveda, Unani, Siddha, Chinese and Tibetan medicine worldwide since time immemorial and approximately 60-80 % of the world's population still depends on traditional medicines [1-4]. Several medicinal compounds have been successfully isolated from plants and presently being used for the treatment and management of different diseases. The discovery of new drugs through natural products is the single most successful strategy which led the invention of several modern drugs. Though the use of the plants, human beings have taken advantage of the medicinal compound known as secondary metabolites present in the leaves, stems, roots and sap of the plants. The secondary metabolites such as saponins, alkaloids, flavonoids, glycosides, anthraquinone, steroids and tannins from plants has also been used in the modern system of medicines for their extensive therapeutic values [5].

Barleria prionitis Linn. (Acanthaceae) commonly found in the tropical Asia, including Malaysia, India, Pakistan, Philippines, Sri Lanka and in tropical Africa and Yemen [7], is well known for its medicinal values and used by tribal's of malghat region for medicinal purpose [6]. The leaf, stem and bark of this plant has already been used as diuretic, hepato protective, anti-inflammatory agent and the juice of leaves was found useful in fungal infections, whooping cough, bleeding teeth, toothache and joint pain [6-8]. The root of this plant posse's anti-fertility activity and also used for controlling the fever; glandular swelling. The flowers have been used for the treatment of migraine, internal abscesses, edema, haemoptysis, urethral discharges, seminal disorders and to reduce the obesity [9] The crude extract of this plant along with oil helps in arresting the hair graying, arthritis and gout [10] This plant is widely used for the management of various neurological disorders like paraplegia, sciatica, also in leprosy and other skin diseases [9]. Through the therapeutic value of this plant has been investigated, but the detailed phytochemical studies in relation to its antimicrobial activity have not been investigated. Therefore, keeping the above facts in view, the present study has been planned to investigate the antimicrobial activity of leaf, stem, and root of *Barleria prionitis.*

MATERIALS AND METHODS

Collection of plant

The whole plant was collected from Govt. Nursery of Modinagar, Dist. Ghaziabad, U. P, India, and has been authenticated by NBRI, Lucknow, India (Voucher/Specimen no. is NBRI-SOP-202) a voucher specimen was deposited in the department.

Extraction

The plant's part (stems, leaves, roots) were separated from each other and washed carefully under running tap water followed by distilled water. These were shade dried under room temperature for one week and pulverized to a fine powder, using a sterilized mixer grinder. The 300 gm power of each plant part extracted by the Soxhlet method using Chloroform, Acetonitrile, Ethanol, as solvents on the basis of their polarity of 4.1,5.8 and19.4 respectively.

Yield

The percentage yields were calculated using following formula: [11]

$$\text{Yield in \%} = \frac{\text{Quantity of extraction in gm}}{\text{Quantity of dry powder of plant in gm}} \times 100$$

Qualitative phytochemical investigations of extract

A qualitative chemical test was conducted with the aim to check the presence of various phytochemical constituents in different parts of plant extracts. The phytochemical analysis was conducted using the test developed by Kolkata *et al.* (2013) and the presence of different phytochemicals in the extract was listed in the table which as further used for qualitative investigation.

Antimicrobial activity

Collection of strain

The strain of Bacterial pathogens *Corynebacterium diphtheriae*: MTCC 116, *Pseudomonas aeruginosa*: MTCC 10462, *Bacillus thuringiensis*: MTCC 10484, *Bacillus anthracis:* MTCC 10095, *Salmonella typhi*: MTCC 3231, *C. pneumoniae*: MTCC 7162 and Fungal pathogens like *A fumigatus*: MTCC 4163, *C. neoformans*: MTCC 6333, *C. albicans*: MTCC 7253, was collected from innovative life sciences, lucknow and *C. vaginitis:* Clinical isolate, *B dermatitidis* clinical isolate. Gaurang Homeo Clinic, Aliganj, Sector-1, Lucknow 226010, Uttar Pradesh.

Preparation of bacterial/fungal strains and culture conditions

The obtained cultures of *S. typhi, C. pneumoniae, B. thurengenesis, B. anthracis, P. aeruginosa* were maintained on nutrient agar and *A.*

fumigates, C. neoformans, C. albicans, C. vaginitis, B. dermatitidis on Potato Dextrose agar medium by making slants and the stock cultures of were transferred at monthly intervals. A single colony was transferred in sterile 100 ml of nutrient broth/potato dextrose media and incubated at 37 °C/25 °C in a shaker at 140 rpm for 14 h. Culture of bacteria and fungi was recovered by centrifugation and were suspended in sterile distilled water; the concentration of the pathogen was optimized by maintaining OD to 0.1 at 600 nm [12].

S. No.	Name of the Sec. metabolite	Method-1	Method-2
1	Alkaloid	Hager's method	Tannic acid method
2	Saponin	Foam test	
3	Steroids/Terpenoids	Salkowaski reaction	
4	Flavonoids	Shindona test	Sodium hydroxide test
5	Tannins	Lead Acetate test	
6	Card. Glycosides	Kellar Killani test	Beljet's test
7	Anthroquinone	Confirmation test given in kokate. (Pharmacology Hand Book)	

Screening of bioactive compounds against various microbes

The method used to screen plant extracts before determination of MIC, was agar disc diffusion method, in which 10 ml of nutrient agar media poured in a sterile Petri dish, 100 µl of test organisms were spread on the surface of media, wells were prepared with help of sterile borer and wells were aseptically filled by 50 µl of plant extract along with positive (antibacterial compound tetracycline and antifungal compound floconazole at 50 µg/ml and 100 µg/ml repectively) and negative control (autoclaved distilled water). Plates were incubated aerobically at 37 °C/25 °C for 72 h. The diameter of zones of inhibition was measured. The initial concentration of MIC was 10 mg/ml for bacterial strains and 20 mg/ml for fungal strains.

Minimum inhibitory concentration (MIC) determination

MIC is carried out by double agar double diffusion method. 10 ml molten agar medium (Nutrient agar for bacteria and Potato dextrose for Fungal strains) were poured into the plate without antibiotic and was allowed to harden. After the hardening of agar (2-5 min), the plate was set flat on the desk and 10 ml medium containing the antibiotic (10 mg/ml) was added. It was allowed to harden for 15 to 20 min. Using 100 µl fresh cultures (grow overnight) of concern microbe and spread with the help of inoculation spreader, microbes were spread out over the surface of the medium, taking care not to tear the agar. Later it was incubated for approximately 72 h. The plate was observed in the pattern of microbial growth. Tetracycline (Microxpress) was taken for bacterial strains as positive control and Fluconazole (Microxpress) was used as standard compound as positive control for fungal strains.

Analysis of data

Size of petri plate was 100 mm. Concentration of antibiotic/extracted drug-10 mg/ml, and total medium was poured on a single plate was 10 ml. 100 mg drugs have been poured into the 100 mm plate as a higher to lower concentration. Every 1 mm of plate surface area has been considered as 1 mg/ml concentration of the drug for inhibition for bacterial strains and 2 mg/ml= 1 mm was considered for fungal strains, the distance was measured from the top end and the concentration of the compound was calculated as MIC.

RESULTS AND DISCUSSION

In the present study, chloroform, acetonitrile, ethanol, extracts of stem, leaf, roots of *Barleria prionitis* were evaluated for the presence of phyto chemical constituents as well as their antimicrobial activity was tested.

Successive solvent extraction yield % values in various organic solvent were observed as ethanol, with all parts of the plant (table 1).

Table 1: Yield of *Barleria Prionitis* with different solvents

S. No.	Parts	Solvent	Quantity (mg)	Yield (%)	Appearance
1	Leaves	Eth	2680	5.36	Light brown
2	Stems	Act	1930	3.86	Light brown
3		Chl	1880	3.76	Light cream
4		Eth	2670	5.34	Light brown
5	Roots	Act	1860	3.72	Cream
6		Eth	1850	3.7	Cream

The results of the phyto chemical analysis were carried out in extracts of *Barleria prionitis* medicinal plant. The experiment showed the presence of secondary metabolites such as alkaloid, glycosides, flavonoids, saponins, tannins, steroids and anthraquinone. Hence the phyto chemical screening reveals that ethanol extracts show high no. of secondary metabolites. Thus, the preliminary screening analysis is helpful in the direction of bioactive components in discovery and development of new drug (table 2).

Table 2: Phytochemical study on *Barleria Prionitis*

S. No.	Code	Alk	Sap	Std	Flv	Tan	cd. gly	Antq	No. of S. M
1	LEt	-+	-+	--	++	++	--	--	4
2	SAcN	--	--	++	--	--	++	++	3
3	SCl	--	--	++	--	--	--	--	1
4	SEt	-+	-+	++	--	--	++	--	4
5	RAcN	--	--	--	--	--	--	++	1
6	REt	+-	++	++	++	--	--	--	4

Antimicrobial activity study showed that *Barleria* leaf extract and *Barleria* stem extract with ethanol gives best inhibitory effect against all experimental pathogens, while *Barleria* root extract with ethanol given good result rather than others. If we discussed about *barleria* leaf ethanol extract inhibitory effects, representing excellent results against multidrug resistant strain *Pseudomonas aeruginosa,*

Aspergillus fumigates and C. vaginitis. On the other hand it is showing average effects *against S. typhi, B. thruengenesis, B. anthracis, C. pneumonia* (bacterial cell), *C. neoformans, C. albicans* and *B. dermatidis* (fungal cell).

Barleria stem ethanol extract's inhibitory effects, representing excellent results against *B. thruengenesis, B. anthracis.* On the other hand, it is showing average effects *against S. typhi, Pseudomonas aeruginosa, C. vaginitis., C. pneumonia* (bacterial cell), *C. neoformans, A. fumigates, C. albicans* and *B. dermatidis* (fungal cell). *Barleria* root ethanol extract's inhibitory effects, representing excellent results candidal fungal species i.e. *C. vaginitis and C. albicans.* On the other hand it is showing average effects *against S. typhi, Pseudomonas aeruginosa, B. thruengenesis, B. anthracis, C. pneumonia* (bacterial cell), *Aspergillus fumigates, C. neoformans,* and *B. dermatidis* (fungal cell).

Table 3: MIC of *Barleria Prionitis* through gradient plate technique on pathogenic microbial strains with SEM

		Mg/ml MIC value with mean±SEM (n=5)							
S. N.	**Bacterial Strains**	**BLE**	**BSC**	**BSE**	**BSA**	**BRE**	**BRA**	**+ve control (µg/ml)**	**-ve control**
1	*P. aeruginosa*	1.2±0.0621**	6.8±0.0244	1.2±0.0241*	9.2±0.0446	7.2±0.2100	8.6±0.2233	36±3.31	-
2	*S. typhi*	3.2±0.0130*	8.2±0.0153	2.9±0.0143*	9.8±0.0151	2.4±0.0156*	8.6±0.0094	25±3.53	-
3	*B. thruengenesis*	2.9±0.0130*	4.6±0.0120	1.1±0.0130**	9.5±0.0221	1.2±0.0136**	7.2±0.0109	27.5±6.12	-
4	*B. anthracis*	3.8±0.0115*	7.8±0.0089	1.7±0.0092**	8.9±0.0128	1.8±0.0096**	9±0.0250	21±4.30	-
5	*C. pneumoniae*	4±0.0128*	5.7±0.0092	2.2±0.0136*	9.6±0.0092	2.3±0.0115*	9.7±0.0115	26±2.44	-
	Fungal Strains								
1	*A. fumigatus*	10.2±0.00927**	17.5±0.0142	14.5±0.0149*	19.2±0.0130	14.3±0.0169*	19.4±0.01303	65±5.00	-
2	*C. neoformans*	13.4±0.0153*	18.4±0.0124	11.7±0.0124*	19.9±0.0142	12.6±0.0128*	18.6±0.0128	72±4.63	-
3	*C. albicans*	11.9±0.0143*	19.3±0.0142	12.2±0.0216*	18.6±0.0135	10.9±0.01503**	18.9±0.0158	36±5.09	-
4	*C. vaginitis*	9.4±0.0128**	16.3±0.0115	13.7±0.0120*	18.9±0.0102	9.4±0.0149***	19.1±0.0172	46±4.30	-
5	*B. dermatidis*	13.6±0.0114*	17.2±0.0177	11±0.0092*	19.1±0.0149	10.2±0.1631*	19.4±0.0136	60±2.50	-

*=good, **=very good, SEM=±standard error with mean, -ve control= Double distilled water, +ve control= Tetracycline, (-) = No inhibition concentration.

CONCLUSION

In the present study, Ethanol has been shown as a best solvent to the highest yield for extracted components and antimicrobial effects while Acetonitrile and Chloroform were also good solvents to extract secondary metabolite components.

ACKNOWLEDGEMENT

The authors are thankful to Emeritus Professor Dr. A. K Srivastava (Department of Botany, Meerut University, India) We are thankful to Institute of Biomedical Education & Research, Mangalayatan University, Aligarh, for providing the laboratory facilities and other necessary support.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Menghai EA, Pareek RS, Negi RS, Ojha CK. Search antimicrobial potential from certain Indian medicinal plants. Res J Med Plant 2011;5:295-301.
2. Ansari JA, Inamdar NN. The promise of traditional medicines. Int J Pharmacol 2010;6:808-12.
3. Aneja KR, Joshi R, Sharma C. Potency of *Barleria prionitis* L. Bark extracts against oral disease causing strains of bacteria and fungi of clinical origin. New York Sci J 2010;3:5-12.
4. Kumar T, Chandershaker KS. Bauhinia purpurea L. a review of its ethanobotany, phytochemical and pharmacological profile. Res J Med Plant 2011;5:420-31.
5. Gantait A, Maji A, Barman T, Banerji P, Venkatesh P, Mukherjee PK. Estimation of capsaicin through scaning densitometry and evaluation of different varieties of capsicum in India. Nat Prod Res 2011;26:216-22.
6. Diwan PD, Gadhikar YA. Assessment of phytochemical composition and antibacterial activity of different extracts of *Barleria prionitis* leaves against oral microflora to improve dental hygiene. Asian J Pharm Sci Res 2012;5:182-4.
7. Dhar R, Bhatnagar P. A study of the antidiabetic activity of *Barleria prionitis* Linn. Indian J Pharmacol 2010;42:70-3.
8. Paul S, Salva D. Comparative study of the efficacy of *Barleria prionitis* leaf extracts against Bacteria. Asian J Pharm Clin Res 2012;2:107-10.
9. Khare CP. Indian Herbal Remedies: Rational Western Therapy, Ayurvedic and Other Traditional Usage, Botany. 1st Edn. Springer, New York; 2004. p. 93-4.
10. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. 1st Edn. Springer Science, New York; 2007. p. 82-3.
11. Pande M, Ingale S, Gupta S. The pharmacognostic and phytochemical studies on the leaves of *Murraya koenigll* (L) Spreng. Int J Sci Technol 2009;2:53-4.
12. Mishra RP. Effect of metal ions and drugs on antibacterial activities of *Nigella sativa* (L.) seeds. Eur J Exp Biol 2011;1:41-6.