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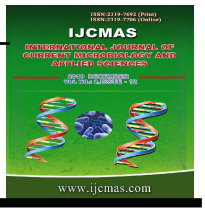

Original Research Article

Exploration of Antioxidant and Antibacterial Activity of *Barleria prionitis* Linn

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Keywords

Barleria prionitis; Antibacterial; Resazurin 96 Well assay; Antioxidant Activity.

A B S T R A C T

This investigation is mapped to evaluate the antioxidant activities of *Barleria prionitis Linn* (bark and leaf) in two extracts methanol and petroleum ether and antibacterial against six bacteria *(B. cereus* (MTTT 430), *B. licheniformis* (MTCC 1483), *S. aureus, E. coli, S. typhi, A. faecalis* **).** Methanol extract of leaf showed highest antibacterial activity against *B. cereus (*22.66 mm in diameter) followed by pet. ether leaf extract against *E. coli* (21.66 mm in diameter). Various extracts of *B. prionitis* were comparable to control antibacterial agent Ampicillin, Tetracycline. Maximum inhibition shown by tetracycline against *S. aureus* (28.20) followed by Ampicillin against *B. cereus* (28.40).Resazurin 96 well assay was used to assess the minimum inhibition concentration (MIC) **;** petroleum ether of leaf demonstrated the least MIC value against *B. cereus* (0.05 mg/ml) and *E.coli (0.2* mg/ml), while the methanol extract of bark and leaf demonstrated 0.2 mg/ml against B. cereus. In contrast, the methanolic leaf extract exhibited significantly higher antioxidant activity (61.73) in 6000 ppm concentration.

Introduction

In present scenario, there is failure of obtainable antimicrobials to treat infectious diseases, so many researchers have focused on the investigation of natural products as source of new bioactive molecules (Recio.; Rios, et al 1989 and Silver.; Bostian et al., 1993). The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility towards antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Sieradzki *et al*., 1999).

Oxidative stress, the consequence of an imbalance of prooxidants and antioxidants in the organisms, is gaining recognition as a key phenomenon in chronic illness like

inflammatory and heart diseases, hypertension, and some forms of cancer. (Oh et al, 2001 and zeynep et al., 2007) Plant based, antioxidant-rich foods traditionally formed the major part of the human diet, and plant based dietary antioxidants are hypothesized to have an important role in maintaining human health (Benzie, 2013).

Barleria prionitis Linn (Acanthaceae) is mostly distributed throughout Africa, India, Sri Lanka and tropical Asia. The crude extract of this plant is commonly used as folk medicine to treat whooping cough. The plant extract has also shown its potential applications as diaphoretic and expectorant alongwith its hepatoprotective activity in experimental animals (Sing et al., 2005). The plant has also shown anti-respiratory syncytial virus (Chen et al., 1998). Not only in this plant but there are several reports of antirespiratory, anti-arthritic, anti-inflammatory and anti-oxidant activities in other plants (Harish et al., 2006 and kikuzaki et al., 1993).In other experimental animals like male albino rats antifertility studies was conducted with its root extracts (Gupta et al.,2000). Hence more studies pertaining to use of *Barleria prionitis* parts as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The main investigation of this work is to screen and evaluate antibacterial activity and radical scavenging properties of *Barleria prionitis* parts (leaf and bark) in two different crude extracts methanol and petroleum ether.

Materials and Methods

Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl), BHT (butylated hydroxytoluene).

Plant materials and extract preparation

The required amount of healthy and fresh matured leaves of the *B. prionitis* was collected from Herbal Garden Cum Germ plasm Conservatory Non- Wood Forest Products Division of the FRI (Forest Research Institute, Dehradun) during the month of March, 2012. The collected samples of *B. prionitis* were cleaned, chopped and shade dried in the blotting paper at room temperature. The drying place was maintained dark in order to prevent the degradation of bioactive components of *B. prionitis* by interfering through light. After drying the plant parts, leaf and bark were grinded into fine powder separately.The 15gm of grind leaf sample of *B. prionitis* was taken and extracted by Soxhlet extractor. This cycle may be allowed to repeat many times, in the petroleum ether solvent 25 cycles was taken and in the methanol solvent 15 cycles was taken with 10g of grind bark sample of *B. prionitis* and extracted by Soxhlet extractor and vice- versa.

Removal of solvent

The separation of solvent from extracted plant sample was undertaken in chemistry laboratory of FRI, Dehradun. The petroleum ether solvent was removed with the help of rotary evaporator at 40ºC temperature and methanol solvent was removed by water-bath after that it was weighed in order to know the amount of extract of plant.

Bacterial strains

For the testing antibacterial activity, the microbial strains employed in the biological assay were Gram positive bacteria: *Bacillus cereus* (MTTT 430), *Bacillus licheniformis* (MTCC 1483),

Staphylococcus aureus; Gram negative bacteria: *Escherichia coli, salmonella typhi, Alcaligenes faecalis*, were obtained from Microbiology Laboratory of Dolphin (PG) Institute Of Biomedical & Natural Science, Dehradun, India. Original culture were further stored at low temperature in the refrigerator to maintain stock culture. Fresh cultures were used for testing antibacterial activity using disc diffusion assays method

Screening of the Antibacterial activity

Disc diffusion assay

The antibacterial activity of *Barleria prionitis* leaf and bark extract (petroleum ether and methanol) was tested by Disc diffusion (Bauer et al.,1966) against six pathogenic bacteria, three Gram negative (*E.coli, Salmonella typhi and Alcaligenes faecalis)* and three Gram positive *(Bacillus cereus, Bacillus licheniformis, Staphylococcus aureus***).** In this method, freshly prepared agar media is dispensed into the sterilized Petri-dish. The agar is allowed to solidify and 100µl of bacterial suspension poured over the agar media and spread by a spreader or a rod. Streptomycin, Ampicillin and Tetracycline (30µg/dish Himedia) were used as standards. In the each culture medium Petri-dish four discs were used, one disk of antibiotics, two discs separately for (petroleum ether and methanol) extract of *Barleria prionitis*, one disk used as a control (Sterile water. The plates were sealed and incubated overnight at 37ºC in the incubator. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The diameter of zone of inhibition (mean of three replicates SD) as indicated by clear area which was devoid of growth of microbes was measured to determine the antibacterial activity. The experiment was replicated three times to confirm the reproducible.

Determination of MIC via Resazurin assay

Resazurin assay was performed in 96 well titration plates (Karuppusamy and Rajasekaran, 2009). In complete nutrient broth two fold dilutions of plant extracts and antibiotics were prepared in the test wells. The final concentration would be 20µ of each bacterial suspension was added to 180 ml of antibiotics and plant extracts (30-0.02mg/ml in sequence) contained in culture medium as well as the antibiotics concentration would be 0.06mg/l streptomycin and tetracycline0.12mg/l. For comparative study control plates were prepared only with culture medium and bacterial suspension. The plates were sealed and incubated for 12 hours at 37◦C for additional 5 hour. At the intervals of 1 hour plates were observed for colour change blue to pink and pink to colorless in live bacterial strains containing wells. Preliminary micro titre plate assay revealed that the fast decolonization of resazurin extract doesn't possessed antibacterial potential. The bioactivity of extracts were screened which shows that the extracts inhibit the dye reduction.

Antioxidant assay

The free radical scavenging activity of the extracts was measured in term of hydrogen donating or radical scavenging activity of the stable radical 1, 1- diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Braca *et al.,* (2001). Test extract and DPPH was dissolved in methanol and kept in dark for 30 minutes. Plant extract

(10,100,1000,2000 and 6000ppm)) was added to 3ml of a 0.004% methanol solution of DPPH and absorbance at 517nm was determined after 30 min. Blank reading was taken by methanol and control was calculated by measuring the absorbance of DPPH solution through spectrometer. The antioxidant activity was compared with BHT.

The percentage inhibition activity was calculated by following formula

% scavenging DPPH free radical=100 x (1 – AE / AD)

Where AE is absorbance of the solution, when extract has been added at a particular level and AD is the absorbance of the DPPH solution with nothing added, without extract (control).

Statistical evaluation

In the disc diffusion assays, the average size of the zone of inhibition (N = 3) is reported for those extracts that resulted in a consistent and noticeable kill zone compared to the diameter of the filter paper disc (6 mm).The collected data were statistically analyzed using software STATISTICA 10.0. Data were subjected to analyses of variance and treatment means were compared through Bonferroni test (P= 0.05).

Results and Discussion

In the primary screening, Antibacterial activities of *Barleria prionitis* seeds extract were obtained by the disc diffusion display (Table 1). Both extracts revealed different degrees of antibacterial activity against test. Maximum inhibition was shown by methanol leaf extract against *B. cereus (*22.66 mm in diameter) followed by pet ether leaf extract against *E. coli* (21.66 mm in diameter).Effect of both the above extracts against *B. cereus* and *E. coli* are significantly same. Minimum inhibition was shown by pet ether leaf extract against *A. faecalis* (4.66 mm in diameter) followed by methanol bark extract against *A. faecalis* (5.33). Activities of the various extracts of *B. prionitis* were comparable to standard antibacterial agent Ampicillin, Tetracycline and streptomycin (Table 2).

Maximum inhibition was shown by tetracycline against *S. aureus* (28.20) followed by Ampicillin against *B. cereus* (28.40). Both the antibiotic has significantly same effect against both *S. aureus* and *B. cereus.* This shows that we can use any of the antibiotics to control both the organism. Minimum inhibition was shown by streptomycin against *S. typhi* (13.66) followed by Ampicillin against *B. licheniformis (15.66*).

Resazurin assay (MIC determination)

Resazurin is a blue non-fluorescent and non-toxic dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells. Resorufin is further reduced to hydroresorufin (uncolored and nonfluorescent). A resazurin reduction test has also been used for decades to demonstrate bacterial and yeast contamination of milk (Mc Nicholl et al., 2006). We were choosing Resazurin assay to assess the MIC value (Table 3) of extracts against test bacteria. According to the Table 3 petroleum ether of leaf illustrated the slightest MIC value against *B. cereus* (0.05 mg/ml) and *E.coli (0.2* mg/ml), while the methanol extract of bark and leaf illustrated 0.2 mg/ml against *B. cereus.*

Table.1 Antibacterial activity of *Barleria prionitis* (Zone of inhibition in mm diameter)

Bacteria/Solvent	Bark		Leaf	
	Pet ether	**Methanol**	**Pet ether**	**Methanol**
E. coli	18.33±2.51 hij	10.33±1.15 a-e	21.66±2.51j	13.66±1.52 c-h
A. faecalis	7.66±1.52 abc	5.33±1.52 ab	4.66±1.52 a	8.33±2.51 a-d
S. typhi	10.33±1.15 a-e	10.66±1.15 a-h	15.33±2.51 e-i	14.33±2.08 d-i
S. aureus	10.33±1.52 a-e	10.33±1.52 a-e	12.66±1.52 c-h	8.66±1.15 a-d
B. cereus	16.66±1.52 g-j	20.33±2.51ij	17.33±2.51h-j	22.66±2.08 j
B. licheniformis	11.66±1.52 c-g	9.33±1.52 a-e	11.33±2.08 b-g	8.33±1.52 a-d
	Organism	**Plant part X Solvent**	**Plant Part X Solvent X Organism**	
LSD (0.1%)	1.517	1.239	3.035	

Mean±S.D

Table.2 Antibacterial activity of *standard antibiotics against test bacteria* (Zone of inhibition in mm diameter)

Bacteria	Antibiotic		
	Streptomycin	**Ampicillin**	**Tetracycline**
E. coli	25.33±2.51 efg	24.33±2.51 efg	21.33±2.08 b-f
A. faecalis	16.66±1.52 abc	16.66±2.51abc	17.20±2.30 a-d
S. typhi	13.66±1.52 a	16.33±2.08 abc	22.83±1.89 c-g
S. aureus	20.33±1.52 a-f	24.33±2.51 efg	28.20±2.30 g
B. cereus	25.00±2.00 efg	28.40±2.62 g	26.73±1.85 fg
B. licheniformis	19.33±1.52 a-e	15.66±1.52 ab	23.66±2.08 d-g
	Bacteria (B)	**Antibiotic (A)**	**Interaction (B * A)**
LSD (0.1%)	1.997	1.412	3.459

Mean±S.D

Table.3 Resazurin assay for MIC (mg/ml)

Extracts(µg/ml)	Bark		Leaf	
	Methanol	***Pt. ether***	***Methanol***	***Pt. ether***
E. coli	0.9	0.4	1.8	0.2
A. faecalis	-	-	-	-
S. typhi	7.5	7.5	3.7	1.8
S. aureus	7.5	7.5	15	3.7
B. cereus	0.2	0.9	0.2	0.05
B. licheniformis	-	-	15	7.5

Table.4 In vitro *DPPH* Free Radicals scavenging activities of *B. prionitis* extract and standard BHT

Conc. (ppm)/Solvent	Bark		Leaf		BHT
	Pet ether	Methanol	Pet ether	Methanol	
10	13.06±1.00 bc	6.40±0.52 a	10.36±1.48 abc	8.16±0.20 ab	20.03±0.95b
100	29.80±1.92 ef	22.66±0.66 d	25.50±1.32 de	15.56±0.72 c	33.66±1.52c
1000	37.70±0.60 gh	29.86±0.32 ef	30.40±1.47 ef	32.10±0.90 f	41.00±1.00a
2000	47.36±1.18 jk	34.86±1.02 fgh	33.06±1.79 fg	40.06±1.05 hi	46.13±5.42a
4000	51.30±1.41kl	39.90±1.01hi	44.06±2.53 ij	46.00±1.00 jk	61.70±1.12d
6000	59.11±1.05 m	53.36±5.82 l	51.40±0.52 kl	61.73±1.41 m	71.36±0.55 e
	Conc.	**Plant part X Solvent**	**Plant Part X Solvent X Organism**		
LSD (0.1%)	1.381	1.128	2.762		4.311

Mean±S.D

Antioxidant activity

Antioxidant activity of the extracts was estimated by DPPH free radical scavenging, using butylated hydroxytoluene (BHT) as references or positive controls was shown (Table 4). Maximum inhibition was shown by methanol leaf extract (61.73) in 6000 ppm concentration followed by petroleum ether bark extract (59.11) again at 6000 ppm and methanol bark extract (53.36) in 6000 ppm. Methanol leaf extract and pet ether bark extract are showing significantly same effect at 6000 ppm concentration. As the conc. of all the extracts increases inhibition also increases. Minimum inhibition was shown by methanol bark extract (6.40) at 10 ppm concentration.

Methanol and petroleum ether extracts of *Barleria prionitis* (leaf and bark) were studied for its Antibacterial activity against Six human bacterial pathogens. According to the results of the present analysis, it can be concluded that *Barleria prionitis* leaf and bark has antibacterial effect which support to the established use of this plant for the treatment of related diseases.

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