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Studies on endophytes and antibacterial activity of *Bryophyllum pinnatum* Lam.

Anand Sagar, Nisha Devi and Ved Prakash

Abstract
Present study was conducted on a local plant of *Bryophyllum pinnatum* Lam. of distt Hamirpur (H.P.) for the presence of endophytes and antibacterial activity against three tested pathogens (*Staphylococcus aureus, E. coli* and *Yersinia pestis*). The whole plant or the plant parts (leaf, bark, stem, and root) are utilised for medicinal purposes. In the present study, different plant parts (bark, leaf, stem and root) are analysed for the presence of endophytes. 18 species of endophytic fungi were isolated from these plant parts in different seasons. A comparision of seasonal distribution revealed that maximum number of fungi were recorded in rainy season (15 spp.) followed by summer season (8 spp.) and winter season (5 spp.). Furthermore antibacterial screening of extracts (methanol, ethanol and acetone) of different plant parts (viz. bark, leaf, stem and root) of *B. pinnatum* against *S. aureus, E. coli* and *Y. pestis* bacteria was done using Agar-well diffusion method. The results revealed that *B. pinnatum* showed maximum antibacterial activity against *S. aureus* at all concentrations as compared to *E. coli* and *Y. pestis.*

Keywords: Endophytes, pathogens, medicinal plants, plant extracts, antibacterial activity, agar-well diffusion method

Introduction
There is vast microflora inhabiting the earth. Microorganisms are present everywhere on the earth in all types of soils, sands, snow covered soils, on rocks, deserts and crevices. The dominating groups of microorganisms are fungi, bacteria, actinomycetes, protozoa and soil nematodes [1].

Fungi are the second largest group after insects and are the key component of tropical ecosystems throughout the world. They are ubiquitous with diverse habitats ranging from psychrophilic to thermophilic and remarkably play a vital role in every ecosystem. Being heterotrophic, they are usually saprophytes and parasites. During evolution when plants colonized the land successfully, fungi developed different types of relationship with them. The group 'endophytes' form one of these associations and their existence have been traced in the fossil records suggesting that endophyte-host association may have evolved from the time of emergence of first higher plants on earth [2].

Fungi are diverse group of organisms comprising of single celled or multicellular filamentous forms. Some fungi live within the plants and are called Endophytes, enabling the plant to grow under harsh conditions which they could not do otherwise [3].

Endophytes have been shown to be present in leaves and stems of healthy plants ranging from conifers to grasses [4-6]. Endophytes play diverse indispensable functions such as plant growth, development, stress tolerance, and adaptation [7]. In the present study endophytes of a medicinal plant *Bryophyllum pinnatum* have been isolated.

The plants are very important to us. The plant kingdom is full of potential drugs and in the recent years the awareness about the importance of medicinal plants is increasing day by day. The drugs obtained from the plants are easily available, less expensive, safe, and efficient and have no side effects. The plant products can be derived from barks, leaves, flowers, roots, fruits and seeds [8].

The scientists have estimated over 250,000 species of angiosperms present on earth, Most of the plants for their medicinal properties have yet to be explored. Humans have been using medicinal plants form centuries. The knowledge and personal experience passed down for generations, people have learned which plant species may help to, induce labor, cure malaria, and toothaches. Now a day, there has been an explosion of interest regarding medicinal plants [9].

India is one of the 12 biodiversity centers with the presence of over 45000 different plant spp. Out **o**f the 2, 50,000 higher plant spp. in the world, more than 80,000 plant spp. are medicinal. There are16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes in India. Out of these only 15000-20000 plants have good medicinal value. Only 7000-7500 medicinal plant spp. are used by traditional peoples. The herbal drugs have been used since ancient times such as Unani and Ayurveda. During the Ayurveda systems about 700 spp. are used as medicines, Unani used 700 spp., Siddha and Amchi used 600 spp., and modern medicine around 30 plant spp. The drugs were derived from the whole plant or from different organs, like leaf, stem, bark, root, flower, and seed, etc [10].

Bryophyllum pinnatum Lam. belongs to family Crassulaceae, it is a perennial herb and grows 3-5 feet tall, with opposite, glabrous, and fleshy dark green leaves that are distinctively scalloped and trimmed in red, and bell like pendulous flowers. It is a widely used traditional medicine for the treatment of various ailments like hepatoprotective, antinociceptive, anti-inflammatory anthelmintic, immunosuppressive, and antidiabetic, nephroprotective, antioxidant activity, antimicrobial activity, analgesic, anticonvulsant, neuropharmacological and antipyretic. It has both haemostatic and wound healing properties. The whole plant or the plant parts (leaf, bark, stem, and root) are utilised for medicinal purposes [11].

Present investigation deals with the fungal endophytes and evaluation of the plant extracts (leaf, bark, stem, and root) of *B. pinnatum* against *S. aureus, E. coli* and *Y. pestis* with the following objectives:

- To isolate and identify different fungal endophytes from leaf, stem, bark, and root of *B. pinnatum.*
- To study antibacterial activities of medicinal plant *B. pinnatum.*

Materials and Methods

Study Area

Sampling Area

Village Cigar of District Hamirpur in Himachal Pradesh was selected for the collection of study material (leaf, stem, bark, and root) of medicinal plant *Bryophyllum pinnatum* Lam. The collections were made during summer season, rainy season and winter season.

Materials

The material used for present study of endophytic isolation and antibacterial activities of plants were root, stem, bark, and leaf of *B. pinnatum.*

Bryophyllum pinnatum Lam.

Bryophyllum pinnatum Lam. is commonly called as Patharchatta and Patharchur, which belongs to the family Crassulaceae. In India, it is found in Himalayas, Kashmir and Khasi Hills of Assam. Also found in Pakistan, Nepal, Egypt, Brazil, Arabia, Myanmar, Africa, Thailand, India, Central and East Asia. Nowadays the plant is cultivated in Andhra Pradesh, Karnataka, Tamil Nadu and Kerala in large scale and sold to pharmaceutical companies. It grows well in slopes of the dry hill, and in Indian plains. It is distinctive for the profusion of miniature plantlets that form on the margins of its phylloclades. The leaves of the species are actually leaf-stem combinations called phylloclades. It forms a cymose panicle. *B. pinnatum* have been found to contain bufadienolide cardiac glycosides. These can cause cardiac poisoning, particularly in grazing animals. *B. pinnatum* include bryophyllin A which showed strong anti-tumor promoting activity in vitro and bersaldegenin-3-acetate and bryophillin C which were less active, and show insecticidal properties.

Methods

Sampling technique

Sampling of plants parts

To acquire endophytes, *B. pinnatum* plant parts (viz. leaf, stem, bark and root) were collected from their natural habitat from the area of village Cigar of district Hamirpur (H.P.). The samples were brought to the laboratory. The collected samples were first washed thoroughly in running tap water. The plant material from different tissues of (i.e. leaf, stem, bark and root) *B. pinnatum* were cut into small pieces (5mm approx.) and screened for the presence of fungal endophytes.

Methodology for isolation of endophytes

(a) Hot water treatment

The endophytes were isolated from small pieces of leaf, stem, bark and root. These were washed with hot water (60 °C) for 15 min in a test tube. Then three pieces of each sample were inoculated on Petri plates containing PDA medium supplemented with penicillin or streptomycin (150 mg^{-1}). These Petri plates were incubated at 25±2 °C in an incubator for one week. After the fungal growth, sub-culturing was done on PDA slants and slants were preserved in refrigerator.

(b) Three step method

The samples were washed with sterilized distilled water. They were surface sterilized with 25% methanol for 5 minutes, followed by 50% methanol for 3 min, again followed by 75% methanol for 2 min. Finally these samples were washed in sterilized water for 5 minutes. Then three pieces of each sample were inoculated on Petri plates containing PDA medium supplemented with penicillin or streptomycin (150 mg^{-1}). The Petri plates were incubated at 25±2 °C for few days. The growing fungal colonies were then transferred on PDA slants.

Qualitative Analysis of Endophytic Fungi

Slide preparation

For identification, temporary mounts of fungi were made in 0.1 % cotton blue and lactophenol. An adequate high power microscope was used for observing the slides. Fungi were identified following Nagmani *et al.* (2005) [12].

Maintenance and Preservation of culture

Pure culture of different fungal genera was maintained on PDA which was preserved in refrigerator. Sub culturing was done at regular intervals in order to maintain culture. Each fungal species were transferred from parent source to a fresh slant in order to maintain and preserve the culture.

Methodology for Antibacterial Activity

Procurement of Bacteria

Different strains of bacteria (*Staphylococcus aureus, Escherichia coli, Yersinia pestis*) have been procured from IGMC Shimla, IMTECH Chandigarh and Department of Biotechnology, HPU Shimla for screening antibacterial properties of plant extracts.

Revival of Pathogens

The collected pathogens were revived in nutrient broth and stored in nutrient agar slants at 4 °C.

Extract Preparation

Extracts (acetone, ethanol, methanol and water) of plant have been prepared to check antimicrobial activity. 5 gm dried plant material was taken in separate Erlenmeyer flasks to which 50 mL of required solvents (i.e., methanol, acetone and ethanol) were added. The flasks were covered with aluminium foil and allowed to stand for 3-5 days for extraction. These extracts were filtered through Whatman filter paper no. 1 and evaporated at 40 °C using rotary evaporator. The extracts were collected and weighed. Finally, stock solution of concentration 50 mg/mL was prepared.

Screening Antibacterial Activity

Agar-well Diffusion Method

Screening of plant extracts root, stem, leaf, and bark (methanol, acetone, ethanol and water) of *B. pinnatum* Lam. was done using agar-well diffusion method. Nutrient agar medium (Beef extract 1 g, Yeast extract 2 g, Sodium Chloride 1 g, Peptone 5 g, Agar 20 g, Distilled Water 1000 mL) was used throughout the investigation. The medium was autoclaved at 121.6 °C for 30 minutes and poured into Petri plates. Bacteria were grown in nutrient broth for 24 hours. A 100 µL of bacterial suspension was spread on each nutrient agar plate. Agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plate were loaded with 25%, 50%, 75% and 100% concentration of prepared plant extracts. The Petri plate kept as a control contained pure solvent only. The plates were incubated at 37 ± 2^0C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all the three replicates and the average values were tabulated. Percentage inhibition of bacterial species was calculated after subtracting control from the values of inhibition diameter using control as standard [13].

Percentage of growth inhibition = (Control-Test/Control) x100

Control = average diameter of bacterial colony in control.

Test = average diameter of bacterial colony in treatment sets [14].

Results

Bryophyllum pinnatum Lam. (Crassulaceae) is a plant of great economic and medicinal importance and present worldwide. *B. pinnatum* has many properties like, antibacterial, antimicrobial, antifungal, antioxidant, analgesic and antipyretic. The present studies were conducted on the endophytes and antibacterial activities of *B. pinnatum* and observations are given below:

Isolation and identification of endophytic fungi from various plant parts (viz. bark, leaf, root and stem) of *B. pinnatum*.

The various parts of plant *B. pinnatum* (viz. Bark, leaf, root and stem) were collected from the sampling area (District Hamirpur) during different seasons (viz. summer, rainy and winter) and analysed for the presence of endophytic fungi by hot water treatment and three steps method.

Qualitative assessment of endophytic fungi isolated from various plant parts of *B. pinnatum*

In the present investigation, 18 species of fungi were isolated from plant parts (i.e. bark, leaf, root and stem) in different seasons which fall into 12 genera as shown in Table-1. These genera were *Alternaria, Aspergillus, Chaetomium, Epicoccum, Fusarium, Gliocladium, Mucor, Penicillium, Phoma, Rhizopus, Trematostroma* and *Trichoderma.*

A comparision of seasonal distribution of these isolates revealed that maximum number of fungi were recorded in rainy season (15spp.) followed by summer season (8spp.) and winter season (5spp.) as given in Table- 2. Most predominant genera reported were *Aspergillus, Fusarium, Penicillium, Rhizopus* and *Trichoderma* (2 sp. each), followed by *Alternaria, Chaetomium, Epicoccum, Gliocladium, Mucor, Phoma, Trematostroma* (1 sp. each)

These fungal isolates from plant parts (viz. bark, leaf, root and stem) of *Bryophyllum pinnatum* Lam. were further grouped into Zygomycota (i.e. *Mucor* and *Rhizopus*) and Ascomycota (i.e. *Alternaria, Aspergillus, Chaetomium, Epicoccum, Gliocladium, Penicillium Phoma* and *Trematostroma)* and Deuteromycota (*Fusarium Trichoderma*). The maximum representatives isolated were of Ascomycota as shown in Table-3.

Table 1: List of Endophytic Fungi Isolated from various plant parts viz. Stem, Bark, Leaf and Root, of *B. pinnatum*:

Sr. No.	Isolated Endophytic Fungi
1.	*Alternaria alternata*
2.	*Aspergillus niger*
3.	*Aspergillus ustus*
4.	*Chaetomium* sp.
5.	*Epicoccum nigrum*
6.	*Fusarium moniliforme*
7.	*Fusarium solani*
8.	*Gliocladium catenulatum*
9.	*Mucor plumbeus*
10.	*Penicillium chrysogenum*
11.	*Penicillium citrinum*
12.	*Penicillium notatum*
13.	*Phoma* sp.
14.	*Rhizopus nigricans*
15.	*Rhizopus oryzae*
16.	*Trematostroma* sp.
17.	*Trichoderma harzianum*
18.	*Trichoderma viride*

Table 2: Comparison of occurrence of endophytic fungi isolated from various plant parts (viz. stem, bark, leaf and root) of *B. pinnatum* during different seasons

Sr. No.	Isolated Endophytic Fungi	Summer	Rainy	Winter
1.	*Alternaria alternata*	-	+	-
2.	*Aspergillus niger*	+	+	+
3.	*Aspergillus ustus*	+	+	-
4.	*Chaetomium* sp.	-	+	+
5.	*Epicoccum nigrum*	-	+	-
6.	*Fusarium moniliforme*	+	-	-
7.	*Fusarium solani*	-	+	+
8.	*Gliocladium catenulatum*	-	+	-
9.	*Mucor plumbeus*	+	+	+
10.	*Penicillium chrysogenum*	-	+	-
11.	*Penicillium citrinum*	-	+	-
12.	*Penicillium notatum*	+	-	-
13.	*Phoma* sp.	-	+	-
14.	*Rhizopus nigricans*	+	+	+
15.	*Rhizopus oryzae*	-	+	-
16.	*Trematostroma* sp.	+	-	-
17.	*Trichoderma harzianum*	-	+	-
18.	*Trichoderma viride*	+	+	-

(+) - Present
(-) - Absent

Table 3: Categorization of endophytic fungi isolated from various plant parts (viz. stem, bark, leaf and root) of *B. pinnatum* into different fungal divisions

Sr. No.	Division	Genus
1.	Zygomycota	*Rhizopus, Mucor*
2.	Ascomycota	*Alternaria, Aspergillus, Chaetomium, Epicoccum, Penicillium, Phoma, Gliocladium, Trematostroma*
3.	Deuteromycota	*Fusarium, Trichoderma*

Antibacterial screening of extracts (methanol, ethanol and acetone) of different plant parts (viz. bark, leaf, stem and root) of *B. pinnatum* against *S. aureus, E. coli* and *Y. pestis* bacteria.

In the present study, the plant parts, such as bark, leaf, stem and root of *B. pinnatum* were tested for their antibacterial properties against selected human pathogens. Results obtained revealed that the tested plant extracts possess considerable potential antibacterial activity against *E. coli, Y. pestis* and *S. aureus.*

Antibacterial screening of leaf extracts (methanol, ethanol and acetone) of *B. pinnatum* against *S. aureus, E. coli* and *Y. pestis* bacteria.

The results are presented in Table-1 and plates 1, 2, 3.

Table 1: Percent Inhibition of growth of pathogenic bacterial spp. at different concentrations of methanol, acetone and ethanol leaf extracts of *B. pinnatum*

Extract	Concentrations (In %)	Inhibition zone diameter In mm (± S.D.)		
		S. aureus	***E. coli.***	***Y. pestis***
Methanol extract	Control	0.00 ±0.00	0.00±0.00	0.00 ±0.00
	25	12.77±0.17	19.17±0.42	15.14±0.24
	50	14.75±0.12	20.88±0.12	17.10±0.26
	75	17.62±0.32	21.81±0.18	18.82±0.15
	100	21.25±0.25	24.85±0.16	20.58±0.21
Acetone extract	Control	0.00 ±0.00	0.00±0.00	0.00 ±0.00
	25	10.51±0.27	9.64±0.32	11.10±0.5
	50	14.12±0.18	11.12±0.31	12.70±0.36
	75	16.16±0.14	11.84±0.33	14.65±0.35
	100	18.56±0.31	12.98±0.11	16.62±0.31
Ethanol extract	Control	0.00 ±0.00	0.00±0.00	0.00 ±0.00
	25	17.62±0.61	14.86±0.22	15.5±0.28
	50	20.09±0.14	17.64±0.25	17.16±0.16
	75	22.59±0.31	18.96±0.18	19.03±0.04
	100	24.83±0.22	21.00±0.05	21.2±0.46

±

It is evident from Table-1 that methanol leaf extracts showed maximum inhibition against *E. coli* as compared to *S. aureus* and *Y. pestis* whereas acetone and ethanol extracts showed maximum inhibition against *S. aureus* as compared to *E. coli* and *Y. pestis* at all concentrations.

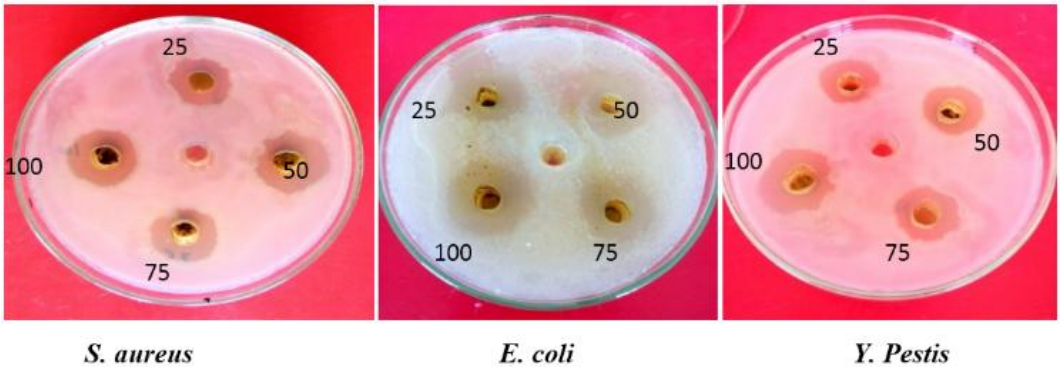
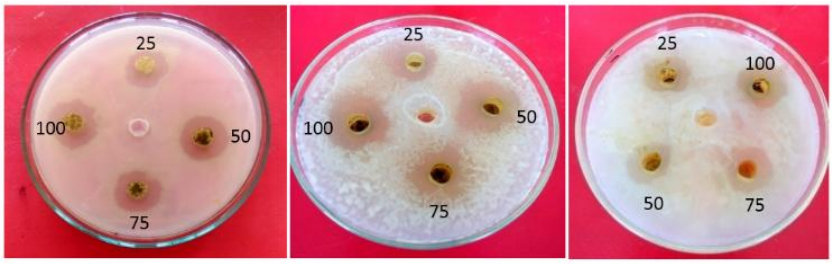
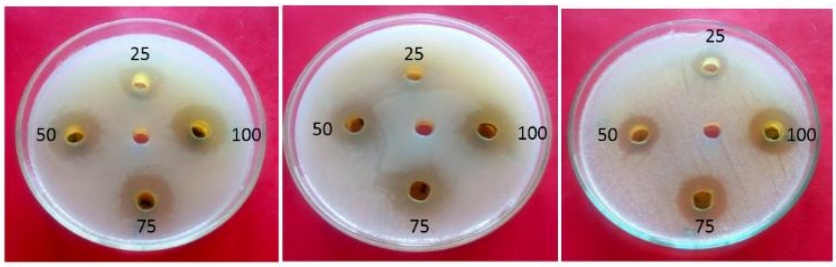


Plate 1: Percentage inhibition of growth of three bacteria by *B. pinnatum* methanol leaf extract *at* different concentrations.



S. aureus *E. coli* *Y. Pestis*

Plate 2: Percentage inhibition of growth of three bacteria by *B. pinnatum* acetone leaf extract *at* different concentrations.



S. aureus *E. coli* *Y. Pestis*

Plate 3: Percentage inhibition of growth of three bacteria by *B. pinnatum* ethanol leaf extract *at* different concentrations.

Antibacterial screening of root extracts (methanol, ethanol and acetone) of *B. pinnatum* against *S. aureus, E. coli* and *Y. pestis* bacteria.

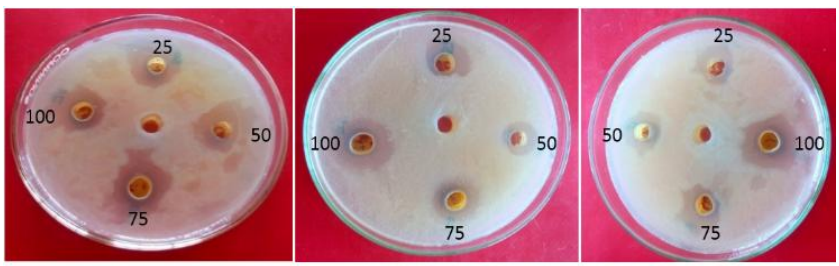
The results are presented in Table-2 and plates 4, 5, 6.

By comparing the three extracts of Table-2 the methanol and ethanol root extracts showed maximum inhibition against *S. aureus* as compared to *E. coli* and *Y. pestis* at all concentrations. Whereas acetone extract showed maximum inhibition against *E. coli* as compared to *S. aureus* and *Y. pestis.*

Table 2: Percent Inhibition of Growth of *pathogenic bacterial* spp. at different concentrations of methanol, acetone and ethanol root extracts of *B. pinnatum*:

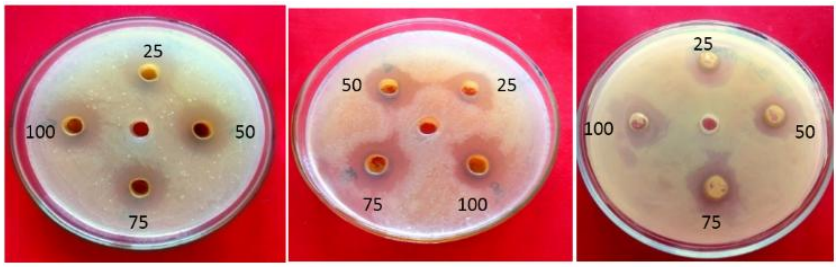
Extract	Concentrations (In %)	Inhibition zone diameter In mm (± S.D.)		
		S. aureus	***E. coli.***	***Y. pestis***
Methanol Extract	Control	0.00 ±0.00	0.00±0.00	0.00 ±0.00
	25	15.98±0.18	14.48±0.34	16.70±0.28
	50	17.45±0.34	16.64±0.29	19.67±0.19
	75	20.26±0.32	17.75±0.35	20.61±0.30
	100	21.93±0.10	19.54±0.32	21.91±0.19
Acetone Extract	Control	0.00 ±0.00	0.00±0.00	0.00 ±0.00
	25	10.13±0.45	11.23±0.39	10.32 ±0.39
	50	11.62±0.26	13.37±0.32	11.86±0.14
	75	13.67±0.22	15.65±0.20	13.86±0.28
	100	15.47±0.35	16.08±0.14	15.24±0.13
Ethanol Extract	Control	0.00 ±0.00	0.00±0.00	0.00 ±0.00
	25	15.45±0.45	10.66±0.30	15.67±0.54
	50	17.80±0.42	11.54±0.27	17.83±0.16
	75	19.38±0.30	12.60±0.21	19.51±0.27
	100	20.99 ±0.07	14.37±0.34	20.90±0.43

±



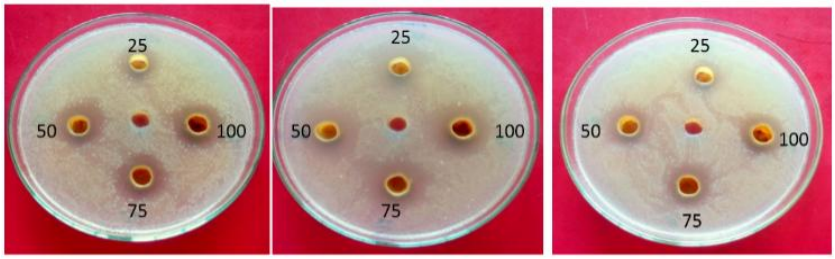
S. aureus *E. coli* *Y. Pestis*

Plate 4: Percentage inhibition of growth of three bacteria by *B. pinnatum* methanol root extract *at* different concentrations.



S. aureus *E. coli* *Y. Pestis*

Plate 5: Percentage inhibition of growth of three bacteria by *B. pinnatum* acetone root extract *at* different concentrations.



S. aureus *E. coli* *Y. Pestis*

Plate 6: Percentage inhibition of growth of three bacteria by *B. pinnatum* ethanol root extract *at* different concentrations.

Antibacterial screening of bark extracts (methanol, ethanol and acetone) of *B. pinnatum* against *S. aureus, E. coli* and *Y. pestis* bacteria.

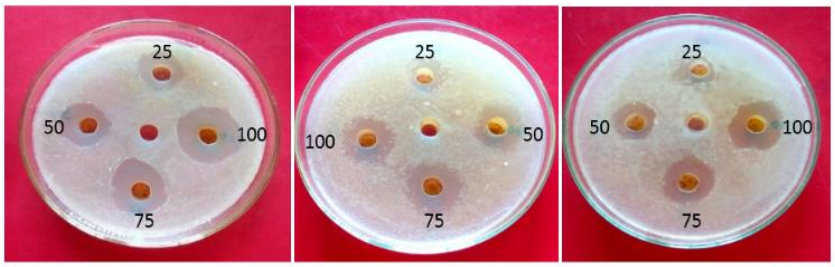
The results are presented in Table-3 and plates 7, 8, 9.

The comparison of the three extracts of Table-3 showed that the methanol root extracts showed maximum inhibition against *S. aureus* as compared to *E. coli* and *Y. pestis* at all concentrations. Whereas acetone and ethanol extracts showed maximum inhibition against *Y. pestis* as compared to *S. aureus* and *E. coli.*

Table 3: Percent inhibition of growth of pathogenic bacterial spp. at different concentrations of methanol, acetone and ethanol bark extracts of *B. pinnatum*

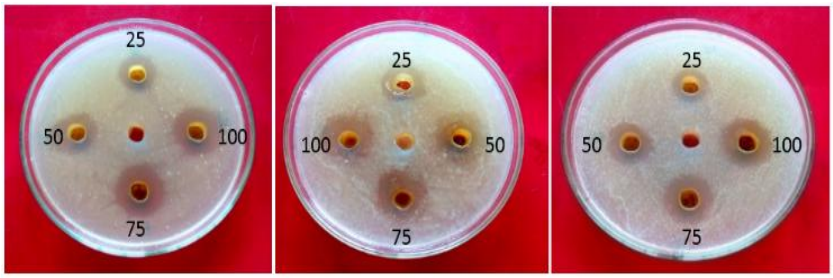
Extract	Concentrations (In %)	Inhibition zone diameter In mm (± S.D.)		
		S. aureus	***E. coli.***	***Y. pestis***
Methanol extract	Control	0.00 ±0.00	0.00±0.00	0.00 ±0.00
	25	16.03±0.26	13.70±0.20	14.80±0.17
	50	18.14±0.57	16.00±0.21	16.90±0.14
	75	20.41±0.29	18.91±0.14	19.01±0.01
	100	24.05±0.08	20.87±0.25	21.72±0.17
Acetone Extract	Control	0.00 ±0.00	0.00±0.00	0.00 ±0.00
	25	14.63±0.41	12.93±0.60	14.89±0.11
	50	16.55±0.40	15.46±0.25	18.39±0.17
	75	18.89±0.39	17.35±0.22	20.02±0.14
	100	21.03±0.08	18.73±0.14	23.45±0.35
Ethanol Extract	Control	0.00 ±0.00	0.00±0.00	0.00 ±0.00
	25	16.09±0.26	14.40 ±0.39	19.50±0.29
	50	17.66±0.16	16.18±0.49	22.11±0.53
	75	19.60±0.34	18.58±0.33	23.80±0.22
	100	21.66±0.44	19.85±0.42	25.75±0.14

±



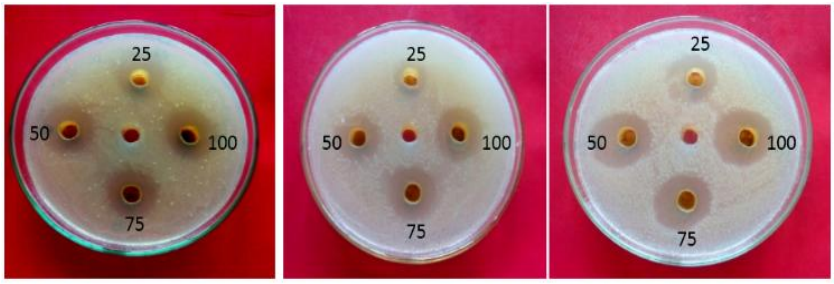
S. aureus *E. coli* *Y. Pestis*

Plate 7: Percentage inhibition of growth of three bacteria by *B. pinnatum* methanol bark extract *at* different concentrations.



S. aureus *E. coli* *Y. Pestis*

Plate 8: Percentage inhibitions of growth of three bacteria by *B. pinnatum* acetone bark extract *at* different concentrations.



S. aureus *E. coli* *Y. Pestis*

Plate 9: Percentage inhibitions of growth of three bacteria by *B. pinnatum* ethanol bark extract *at* different concentrations.

Antibacterial screening of stem extracts (methanol, ethanol and acetone) of *B. pinnatum* against *S. aureus, E. coli* and *Y. pestis* bacteria.

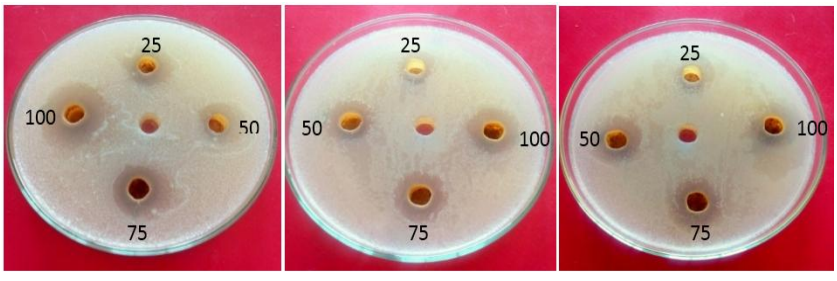
The results are presented in Table-4 and plates 10, 11, 12.

It is evident from Table-4 that methanol, ethanol and acetone extracts of stem of *B. pinnatum* showed maximum inhibition against *S. aureus* as compared to *E. coli* and *Y. pestis* at all concentrations.

Table 4: Percent inhibition of growth of pathogenic bacterial spp. at different concentrations of methanol, acetone and ethanol stem extracts of *B. pinnatum*:

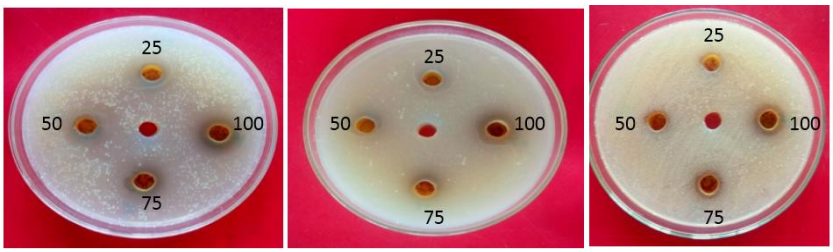
Extract	Concentrations (In %)	Inhibition zone diameter In mm (± S.D.)		
		S. aureus	***E. coli.***	***Y. pestis***
Methanol extract	Control	0.00 ±0.00	0.00±0.00	0.00 ±0.00
	25	11.56±0.64	10.61 ±0.30	12.29±0.41
	50	14.81±0.58	14.54±0.36	13.85±0.65
	75	19.06±0.27	15.70±0.28	15.75±0.19
	100	21.23±0.23	17.48±0.31	17.76 ±0.15
Acetone extract	Control	0.00 ±0.00	0.00±0.00	0.00 ±0.00
	25	18.56±0.30	11.44 ±0.34	15.92±0.22
	50	20.02±0.20	13.87±0.16	18.60±0.33
	75	21.47±0.33	14.47±0.27	20.80±0.30
	100	23.98±0.11	16.81±0.16	22.69±0.11
Ethanol extract	Control	0.00 ±0.00	0.00±0.00	0.00 ±0.00
	25	19.52 ±0.32	14.40 ±0.31	16.59 ±0.32
	50	23.01±0.30	16.08±0.22	18.97±0.09
	75	24.70±0.27	17.69±0.27	22.08±0.13
	100	25.99±0.09	19.11±0.21	24.80±0.18

±



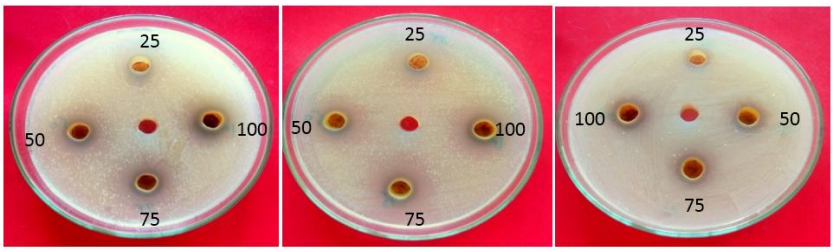
S. aureus *E. coli* *Y. Pestis*

Plate 10: Percentage inhibitions of growth of three bacteria by *B. pinnatum* methanol stem extract *at* different concentrations.



S. aureus *E. coli* *Y. Pestis*

Plate 11: Percentage inhibitions of growth of three bacteria by *B. pinnatum* acetone stem extract *at* different concentrations.



S. aureus *E. coli* *Y. Pestis*

Plate 12: Percentage inhibitions of growth of three bacteria by *B. pinnatum* ethanol stem extract *at* different concentrations.

Discussions

Bryophyllum pinnatum Lam. is a multipurpose plant having both economic and medicinal value. In the present investigation, work has been conducted on the endophytes and antibacterial activity of the *B. pinnatum* and the results are discussed in the following pages:

Isolation and identification of endophytes of *B. pinnatum*

Fungal endophytes colonize living tissues of plants without causing any apparent symptoms and negative effects [15-16]. Endophytes have useful abilities for the whole living world. Most of endophytic fungal cultures have yielded a wide range of bioactive compounds with antioxidants and immunosuppressant properties [17].

Different plant parts (bark, leaf, stem and root) were analysed for the presence of endophytes. 18 species of endophytic fungi were isolated from these plant parts in different seasons. A comparision of seasonal distribution revealed that maximum number of fungi were recorded in rainy season (15 spp.) followed by summer season (8 spp.) and winter season (5 spp.).

Suman (2007) [18] isolated *Fusarium solani* as fungal endophyte of *Azadirachta indica* and *Acacia catechu.* Shukla *et al.* (2014) [19] isolated fungal endophytes *Aspergillus, Penicillium, Alternaria, and Fusarium* from some medicinal plants. Sharma (2015) [20] isolated 12 species of endophytic fungi from *Taxus baccata.* Naik *et al.* [21] screened leaf segments of seven medicinal herbs during summer, rainy and winter seasons. They observed *Aspergillus niger, Chaetomium sp., Penicillium, Fusarium, Trichoderma viride* and in the present study they were also endophytes of *B. pinnatum.*

Results of the present study are in agreement with the work of earlier workers.

Antibacterial activity of *B. pinnatum*

The leaves and leaf juice of *B. pinnatum* were used traditionally as antiviral, antipyretic, antimicrobial, anti-inflammatory, antitumor, hypocholesterolemic, antioxidant, diuretic, antiulcer, styptic, antidiabetic, astringent, antiseptic, antilithic and as cough suppressant [22-23]. The plant shows various pharmacological activities like Urolithic, Diuretic, Anti-Diabetic, Wound healing property [24]. Its antimicrobial activity may be due to bioactive compounds [25].

Considering these, as a first step in the present investigation, leaf, bark, stem and root extracts of one of the local medicinal plant *B. pinnatum* was screened *in-vitro* for antibacterial activity against three pathogenic bacteria (*E. coli, S. aureus* and *Y. pestis*). Results obtained revealed that the tested plant extracts possess considerable potential antibacterial activity. The antibacterial activity of different leaf extracts showed that the methanol leaf extracts had maximum inhibition against *E. coli* (24.85mm at 100%) as compared to *S. aureus* (21.25mm at 100%) and *Y. pestis* (20.58mm at 100%) whereas acetone and ethanol leaf extracts showed maximum inhibition against *S. aureus* (18.56mm at 100%) (24.83mm at 100%) as compared to *E.coli* (12.98mm at 100%) (21.00mm at 100%) and *Y. pestis* (16.62mm at 100%) (21.20mm at 100%).

The antibacterial activity of different bark extracts revealed that the methanol and ethanol root extracts showed maximum inhibition against *S. aureus* (21.93mm at 100%) (20.99mm at 100%) as compared to *E.coli* (19.54mm at 100%) (14.37mm at 100%) and *Y. pestis* (21.91mm at 100%) (20.90mm at 100%). Whereas acetone extract showed maximum inhibition against *E. coli* (16.08mm at 100%) as compared to *S. aureus* (15.47mm at 100%) and *Y. Pestis* (15.24mm at 100%).

The antibacterial activity of different root extracts showed that the methanol root extracts displayed maximum inhibition against *S. aureus* (24.05mm at 100%) as compared to *E.coli* (20.87mm at 100%) and *Y. pestis* (21.72mm at 100%). Whereas acetone and ethanol extracts showed maximum inhibition against *Y. pestis* (23.45mm at 100%) (25.75mm at 100%) as compared to *S. aureus* (21.03mm at 100%) (21.66mm at 100%) and *E.coli* (18.73mm at 100%) (19.85mm at 100%).

The antibacterial activity of different stem extracts revealed that methanol, ethanol and acetone extracts showed

maximum inhibition against *S. aureus* (21.23mm at 100%), (25.99mm at 100%) and (23.98mm at 100%) as compared to *E. coli* (17.48mm at 100%), (19.11mm at 100%) and (16.81mm at 100%) and *Y. pestis* (17.76mm at 100%), (24.80mm at 100%) and (22.69mm at 100%) at all concentrations.

Nwadinigwe and Ogochukwu (2011) [26], Odunayo *et al*. (2007) [27] and Mudi *et al.* (2008) [28] have investigated antimicrobial activity on *B. pinnatum* and comparative account provided by them fits well to our results also. There is a need for further investigations on the bioactive compounds present in different extracts of *B. pinnatum*.

It was concluded from the above experimental observations that *B. pinnatum* was more effective against *S. aureus* at all concentrations as compared to *E. coli* and *Y. pestis.*

Findings of the present work confirm that the plant extracts of *B. pinnatum* can be used as potential antimicrobial agents against pathogenic bacteria. Extracts of these plants require further research formulation to control human diseases.

Although the present investigations are of the preliminary type yet they have established a base for further extension of this work on the lines of isolation and purification of bioactive compounds present in this medicinal plant. Time has come to explore the huge untapped potential of traditional medicinal herbs for the welfare of mankind.

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