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ANTIBACTERIAL POTENTIAL OF DIFFERENT EXTRACT OF ALEURITOPTERIS BICOLOR IN DOON VALLEY, UTTARAKHAND

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ABSTRACT:

Pteridophytes possess an important role in folklore medicine although neglected in modern days. These plants have been successfully used in different systems of medicines like Ayurvedic, Unani, Homeopathic and other systems of medicines. In view of literature review on these plants, the present study was focused on the antibacterial activity of *Aleuritopteris bicolor*, family Sinopteridaceae, in Doon valley, situated in foot hills of the Himalayas. Plant material was collected, dried and grinded out a powdered form which was then subjected to diffusion extraction using seven different solvent in the increasing order of their polarity. Different extract of the plants revealed its antibacterial activity against the studied pathogenic bacterial strains. Out of the seven extracts assayed the ethyl acetate extract was most active against four gram positive and one gram negative bacteria. Therefore, minimum inhibitory concentration (MIC) of the extract was determined against the selected bacteria showing zones of inhibition ≥ 10 mm. The MIC for different strains ranges between 125mg/ml to 62.50mg/ml. So, it can be concluded that ethyl acetate extract of *Aleuritopteris bicolor* possess good antibacterial activity against bacterial strains that cause infection. *Aleuritopteris bicolor* can be recommended in future for various biological activities such as antibacterial, antioxidant, anti-inflammatory.

Index terms: *Aleuritopteris bicolor*, antimicrobial activity, Pteridophyte

1. INTRODUCTION

Pteridophytes are vascular cryptogams which constitute ferns and fern allies and forms a conspicuous element of vegetation all over the earth's surface. These are very ancient group of plants; early fern fossils predate the beginning of the Mesozoic era, 360 million years ago. They are older than land animals and much older than the dinosaurs. They were thriving on earth for two hundred million years before the flowering plant involved but many of the current families and species did not appear until roughly 145 million years ago in the late Cretaceous (after flowering plants came to dominate many environments)(Benniaminet.al.,2008).

The world flora consists of roughly 12,000 species of Pteridophytes of which around 1000 species distributed in 70 families and 192 genera are likely to occur in India (Dixit, 2000). Most of the pteridophytes diversity is observed within the Himalayas, Eastern and Western Ghats. Though the pteridophytes occur in abundance in the tropical, sub-tropical and moist deciduous forests of India, large scale destruction of forests has drastically affected the diversity of Pteridophytes species. The life-cycle of the ferns and fern-allies depends upon the existence of forests, but thanks to habitat destruction many species are reduced and therefore the rare ferns are being extinct or are on the verge of extinction (Dixit, 2000). Many rare and endangered ferns and fern-allies like skeleton fork fern, *Tectaria zeylanica*, *Lindsaea malabarica*, *Cheilanthes rufa*,

Cyathea nilgiriensis, etc. have been recorded from the Western Ghats by different researchers and they need urgent attention for conservation.

The rich diversity of Indian medicinal ferns has been screened extensively for their antimicrobial potential worldwide. Ferns show various economic values towards food and fodder indicators, biofertilizers, insect repellents, medicine and folk medicines. The tree fern *Cyathea nilgiriensis*, which is endemic to South India, has analgesic and antidiabetic activities. The related species *Cyathea gigantea* (Maridass) has free radical scavenging activity, anti-inflammatory and hepato-protective effects (Patric Raja *et al.* 2012). In the present scenario, the utilization of herbs and herbal medicine is at its peak and majority of researchers are screening higher plants for an equivalent but, very few researchers are considering the lower plants for his or her antimicrobial potential. Since, these pteridophytic plants are considered to be the disease free plants and are getting used ethanobotanically by various tribal communities.

Recently, some phytoremediation techniques gained attention as an alternative low cost and affordable technology to remove contaminants from soil and water (Khan *et. al.*, 2000), among them, phytoextraction makes use of plants to remove contaminants from the soil and concentrate them in the aerial, harvestable biomass (McGrath and Zhao, 2003). Plants generally contain secondary metabolites like phenolics, flavonoids, glycosides, coumarins, saponins, terpenoids, alkaloids etc. which reveal their specific characteristic properties and attribute to their pharmacological properties (Okigbo and Anuagasi, 2009). These phytochemicals are qualitatively and quantitatively estimated by different spectrophotometric and chromatographic techniques. HPTLC being one of the powerful analytical tools can be utilized for linking the chemical constituents of the plant with high proficiency which in turn provides unique profiling of that particular plant (Bobby *et al.*, 2012). Pteridophytes are the second largest group of vascular plants out of which 61 medicinally important pteridophyte species are found in India (Benjamin and Manickam, 2006).

In view of demand of present scenario, the aims of the present study on antimicrobial potential of different extracts of *Aleuritopteris bicolor* in Doon Valley, Uttarakhand are an endeavour to find a good natural antimicrobial drug for treatment of the manifestation caused by microorganisms. Different extract of plant *Aleuritopteris bicolor* were tested for their Antimicrobial potential against various pathogenic bacterial strains.

II. MATERIAL AND METHODOLOGY

2.1. Location of the experiment and climate condition:

The present investigation was carried out at Microbiology Laboratory, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand. Located amongst Shivalik ranges on the Foothills of the Himalayas, the Doon Valley is nestled between two of India's mightiest rivers the Ganga on the east and Yamuna on the west. Dehradun is picturesque city with mild climate. It is the capital of Uttarakhand and is located between the latitude the climate of Dehradun is generally temperate, although it varies from tropical, to several cold, depending upon the season, and the altitude of area the nearby hill regions often get snowfalls during winter but the temperature in Dehradun does not go under 0⁰. During summer the temperature here is usually between 27-40⁰C where as during winter it is between 2-24⁰C. During monsoon there often constant and heavy rain falls. The main synclinal through receive an average of 210 cm rainfall annually. The weather is considered to be good during winter in the hilly regions but it is often hot in the Doon valley. The agriculture is good here due to the fertile alluvial soil and the adequate water drainage and rainfall.

2.2. MATERIALS

The material for the present study comprised of whole plant of fern, *Aleuritopteris bicolor* belongs to the family Sinopteridaceae. It grows on undisturbed moist and shady areas in Dehradun.



Figure2.1. Study plant: *Aleuritopteris bicolor*

2.3. EXPERIMENTAL METHODOLOGY

2.3.1. Collection of plant:

The fresh whole plant was collected from different places of Uttarakhand. The plant samples were dried in shade at 25°C to 35 °C for 15-20 days in the laboratory and then crushed to coarse powder using grinder. The dried plant materials were stored in paper bags.

2.3.2. Extraction:

The dried plant material powder was subjected to successive diffusion extraction with different solvents in increasing order of polarity (i.e. Petroleum ether < Benzene < Chloroform < Ethyl acetate < Acetone < Ethyl Alcohol < Distilled water). In the method of diffusion extraction, dried extract are dipped in solvent for 24 hour. About 50 gm accurately weighed dry plant sample powder was taken in thimble and about 250 ml of solvent taken in a beaker and it was covered with foil paper and left for extraction for 48 hrs. On completion of extraction of the plant sample was taken out of beaker and dried in shed. Then the residue was extraction with other solvents successively in the same manner. The aqueous extract of the plant left was obtained by infusion method that is by soaking drug in 250 ml of distilled water for 24 hrs. The extraction drug was then taken in a 100 ml beaker and the solvent was evaporated on water bath and it was finally reduced to dryness to get dry extract. The extract was then transferred to previously weigh air tight container, (weighed on an electronic balanced) and stored in refrigerator until they were screened for the antibacterial activity.

2.3.3. Calculation of percentage yield:

Percentage yield of the crude extract were calculated with the formula
 Percentage yield (%) = Weight of extract x 100/ Weight of powdered drug taken

2.4. Antibacterial assay:

2.4.1. Source of bacterial strains:

The antibacterial assay of different extracts was performed. All bacterial strains were isolated from different places (Hospital, Milk & Skin) in laboratory department of Microbiology, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Dehradun.

2.4.2. Evaluation of the antibacterial potential of plant extract:

A total of five bacterial strains i.e. four gram positive and one gram negative bacteria, were taken to evaluate anti bacterial potential of the different extracts of *Aleuritopteris bicolor*. The bacterial strains are isolated from different places and characterized (table 2.1). All the crude extracts were first screened for preliminary test with the concentration of 1000mg/ml to know whether they were active against the particular bacteria or not. The sensitivities against standard drug amikacin (30mcg) were also observed. Only extracts with good activity were then assayed further at different concentration for MIC test.

Table.no.2.1. Detail characteristics of bacterial strains used for present studies

S.N.	Bacterial	Arrangement	Characteristics	Isolated
Strain-1	<i>Streptobacilli</i> sps. (Sw-5)	Bacilli in chain,	Gram-positive	Hospital
Strain-2	<i>Lactobacilli</i> sps. (LB)	Bacilli	Gram-positive	Milk
Strain-3	<i>Streptobacilli</i> sps (2A)	Bacilli in chain	Gram- positive	Hospital
Strain-4	<i>Staphylococci</i> sps (2VJ)	Cocci in bunch	Gram-positive	Skin
Strain-5	<i>Streptobacilli</i> sps (SB)	Bacilli in chain	Gram-negative	Hospital

2.4.3. Antibacterial assay procedure:

The Nutrient Agar media for antimicrobial assay was prepared by dissolving beef extract (10 gm), peptone (10 gm), sodium chloride (5 gm) in 100 ml distilled water. The media was dissolved on hotplate by continuous stirring. The media was then autoclaved at 15 lbs (121°C) for 15 minutes. It was poured quickly in to sterile Petri dishes while hot to give a depth of 3-4mm under aseptic condition and allowed to cool and solidify. The activated bacterial culture (100µl) was introduced to solid surface of agar media with the help of micropipette it was then spread across the surface of solid agar media by means of a sterile spreader and kept at room temperature for 15 min. or absorption to occur. The pre sterilized discs dipped in different extract were then placed on the surface of the agar media. The Petri dish was then incubated in BOD incubator for 24 hrs at temperature 37°C. After incubation the degree of sensitivity was determined by measuring the zone of inhibition around the disc in mm used a ruler.

2.4.4. Minimum Inhibitory Concentration (MIC) analysis:

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent (drug) that will inhibit the visible growth of microorganisms after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agent. A lower MIC is an indication of a better antimicrobial agent. An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism.

Here the MIC value of the extract was determined only against those bacterial strains which showed high sensitivity during the preliminary antibacterial testing, MIC analysis performed by the serial dilution of the active concentrated extract in the pure DMSO to achieve a decreasing concentration range of 1000mg/ml to 31.25mg/ml. By using different concentration of the active extract i.e. The growth around the disc with lowest concentration to which the organism is susceptible would be determined as MIC of the extract against the particular organism.

III. RESULT AND DISCUSSION

The world flora consists of roughly 12,000 species of Pteridophytes of which around 1000 species distributed in 70 families and 192 genera are likely to occur in India (Dixit, 2000). Most of the Pteridophytes diversity is observed in the Himalayas, Eastern and Western Ghats (Manickam, 1984). Plants generally contain secondary metabolites like phenolics, flavonoids, glycosides, coumarins, saponins, terpenoids, alkaloids etc. which reveal their specific characteristic properties and attribute to their pharmacological properties (Okigbo and Anuagasi, 2009). Pteridophytes are not infected by the microbial pathogens, which may be one of the important factors for the evolutionary success of pteridophytes and the fact that they survived for more than 350 million years (Mandal and Mondal, 2008; Singh *et al.*, 2013).

Aleuritopteris bicolor is an annual species of fern in the genus *Aleuritopteris*. It is an invasive plant having terrestrial or lithophytes habitat. *Aleuritopteris bicolor* mostly grow in cool shady places. After studying the available literature about *Aleuritopteris bicolor* revealing its phytochemical and ethnobotanical uses, the experimental methodology that has been adopted for the present study includes successive diffusion extraction using different solvents in increasing order of polarity, concentration of the extracts followed by their antimicrobial screening and the determination of the MIC value of the active extract against various pathogenic microbe. The findings of the present study were described under the following heads.

3.1. Appearance and yield of crude extracts

50 gm of the powdered plant material was subjected to successive solvent extraction. The extract was concentrated on distillation assembly and it was finally reduced to dryness to get dry extract. The appearance of the extracts after successive diffusion extraction varied in colour from light green to dark green, dark yellow and reddish brown depending upon the solvent which were used. Yield of crude extract ranges from 0.8% in ethyl acetate to 4.8% in chloroform solvent (table 3.1).

Table.no.3.1. Yield and Appearance of crude extracts from different solvent

S.No	Solvent used	Quantity of plant material (gm)	Appearance	Weight of Extract (gm)	Percentage Yield
1	Petroleum ether	50 gm	Dark Green	0.7	1.4
2	Benzene	50 gm	Light Green	0.6	1.2
3	Chloroform	50 gm	Blackish Green	2.4	4.8
4	Ethyl acetate	50 gm	Dark yellow	0.4	0.8
5	Acetone	50 gm	Light Green	0.5	1.0
6	Ethanol	50 gm	Reddish Brown	1.13	1.54
7	Aqueous	50 gm	Light brown	2.1	4.2

3.2. Result of preliminary antibacterial test

For thousands of years, nature has been a source of medicinal agent form where an impressive number of modern drugs have been isolated based on their use in traditional medicine. Historically, most of the medicinal preparations were derived from plants, the medicinal value of which lies in some chemical substances that produce a definite physiological action on the human body. The Pteridophytes possess an important role in folklore medicine although neglected in modern days. These plants have been successfully used in different systems of medicines like Ayurvedic, Unani, Homeopathic and other systems of medicines. All the seven extract i.e. petroleum ether, benzene, chloroform, ethyl acetate, acetone, ethanol, and aqueous of extract *Aleuritopteris bicolor* were subjected for their preliminary antibacterial screening at 1000mg/ml concentration against four gram positive and one gram negative bacterial strains. Different extract of *Aleuritopteris bicolor* showed antibacterial activity against all the studied bacterial strains that are pathogenic to human being causing several diseases. Among the studied assayed extract, ethyl acetate exhibited the maximum activity against studied gram negative and gram positive bacteria followed by acetone. In contrast to this benzene, chloroform, alcohol, petroleum ether, and aqueous extract showed negligible activity against all studied bacterial strains. Whereas the

studies conducted by Samir Kumar Pal (2013) showed similar type of antimicrobial activity of four selective ferns *Cyclosorus interruptus*, *Gleichenia microphylla*, *Microsorium pteropus*, *Athyrium filix-femina* against both Gram positive and Gram negative bacteria. The antimicrobial potential of some ferns has also been studied by Kumar and Kushik (1999), Parihar *et al.* (2010). The result of antibacterial activity of rhizome and frond extracts of three selective ferns *Cyclosorus interruptus*, *Gleichenia microphylla*, *Microsorium pteropus* show good antibacterial activity. *In vitro* antimicrobial activity of important ferns was performed by Parihar *et al.* (2010) against both Gram positive and Gram negative bacteria which showed inhibitory effect against the bacterial strains and some of the extracts were more competent than the selected antibiotic. Above studies indicates that the antibacterial substances present in rhizome and frond are in good amount. The epidermal glands present on the rhizome and on fronds (Manikam, 1984) contain substances like phenolic compounds, glycosides, flavonoids and alkaloids (Alcaraz *et al.*,2000; Cushnie and Lamb,2005; Yusuf, 1994). These substances are largely responsible for the antimicrobial activity and are being soluble in organic solvents easily extracted in methanol, ethanol and acetone but less soluble in water (Adedapo *et al.*,2009; Banerjee and Sen,1980).

Table.3.2. Zone of inhibition (mm) against the bacterial strain

S. No.	Bacterial strains	Zone of inhibition in mm						
		I ₁	I ₂	I ₃	I ₄	I ₅	I ₆	I ₇
Strain-1	<i>Streptobacilli</i> (Sw-5)	8	8	8	11	11	11	-
Strain-2	<i>Lactobacilli</i> (LB)	7	7	-	21	11	11	-
Strain-3	<i>Streptobacilli</i> (2A)	7	9	10	13	13	9	5
Strain-4	<i>Staphylococci</i> (2VJ)	7	10	9	20	12	12	-
Strain-5	<i>Streptobacilli</i> (SB)	8	7	7	15	-	-	-

Note: I₁-Petroleum ether, I₂- Benzene, I₃- Chloroform, I₄-Ethyl acetate, I₅-Acetone, I₆- Ethyl Alcohol, I₇-Distilled water

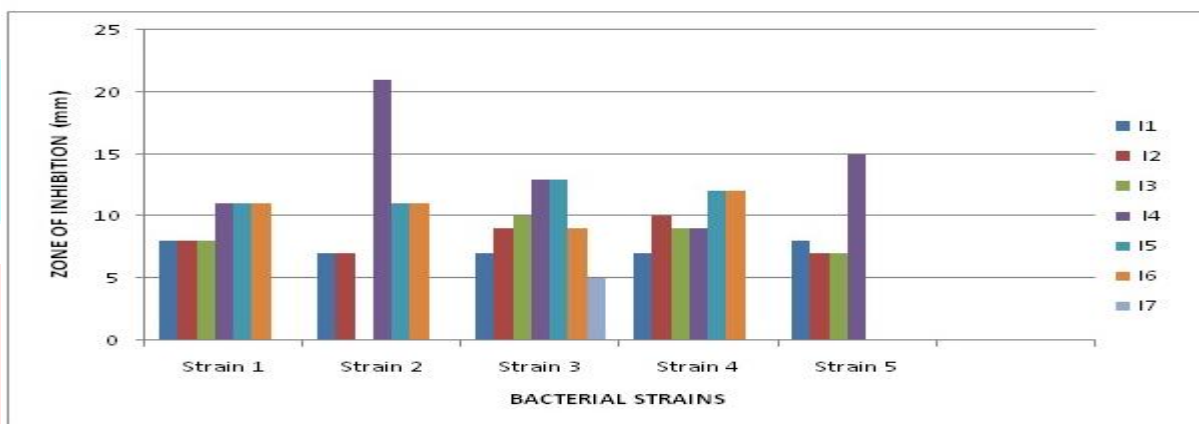


Figure 3.1. Graph of preliminary antibacterial assay showing Zone of Inhibition (mm)

3.3. Results of Minimum Inhibitory Concentration (MIC) analysis for ethyl acetate:

Minimum Inhibitory Concentration is important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A lower MIC is an indication of a better antimicrobial agent (Andrews, 2001; Turnidge *et al.*, 2003). MIC analysis was performed by serial dilution of the concentrated ethyl acetate extract in pure DMSO to achieve a decreasing concentration range of 500mg/ml to 31.25mg/ml. On performing MIC for the ethyl acetate extract of *Aleuritopteris bicolor*, the results revealed that all bacterial strains were sensitive against the 125 mg/ml concentration of the extract, there by exhibiting 125mg/ml as their MIC value. No zone of inhibition was obtained around the disc impregnated with 62.5 mg/ml and 31.25 mg/ml concentration interpreting that all bacterial strains could resist this concentration of the extract. Similar type of studies conducted on performing MIC for the acetonic extract of *Adiantum incisum* Forssk revealed that all selected bacterial strains i.e. *Staphylococcus aureus* MTCC-737, *E. coli* ATCC-433, *Pseudomonas aerogenosa* ATCC- 424, and *Listeria monocytogens* ATCC-657 were sensitive against the 125mg/ml concentration of the extract, thereby exhibiting 125mg/ml as their MIC value whereas MIC analysis for the ethanolic extract showed that *Staphylococcus aureus* MTCC-737, *E. coli* ATCC-433, *Pseudomonas aerogenosa* ATCC-424, *Bacillus pumilis* MTCC-1607, *Bacillus cereus* ATCC-11778, and *Streptoococcus mutans* ATCC-890 were sensitive against the 125 mg/ml concentration of the extract, thereby exhibiting 125mg/ml as their MIC value. These studies revealed that acetone and ethanol extract of *Aleuritopteris bicolor* possess good antibacterial activity against bacterial strains that cause various human infections like urinary tract infection, meningitis, food poisoning, fever, diarrhoea etc. (Singh *et al.* 2013). One ready to use antibiotic impregnated disc i.e. Amikacin positive control in order to check the sensitivity of the bacterial cultures. All of them showed clear zones of inhibition around the disc interpreting their high sensitivity towards antibiotics. In contrast to this, DMSO (99% pure) was used as a negative control.

Table.no.3.3. Minimum inhibitory concentration (MIC) of ethyl acetate extract

S. No.	Bacterial strains	Zone of inhibition in mm						Positive control	Negative control
		1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	Amikacin (30mcg)	DMSO
Strain-1	Streptobacilli sps (Sw-5)	11	8	8	7.5	7.5	-	29	-
Strain-2	Lactobacilli (LB)	21	21	11	-	-	-	30	-
Strain-3	Bacilli (2A)	13	11	9	9	8	-	26	-
Strain-4	Staphyloocci (2VJ)	20	11	7	6	-	-	15	-
Strain-5	Streptobacilli (SB)	15	14	13	10	8	-	25	-

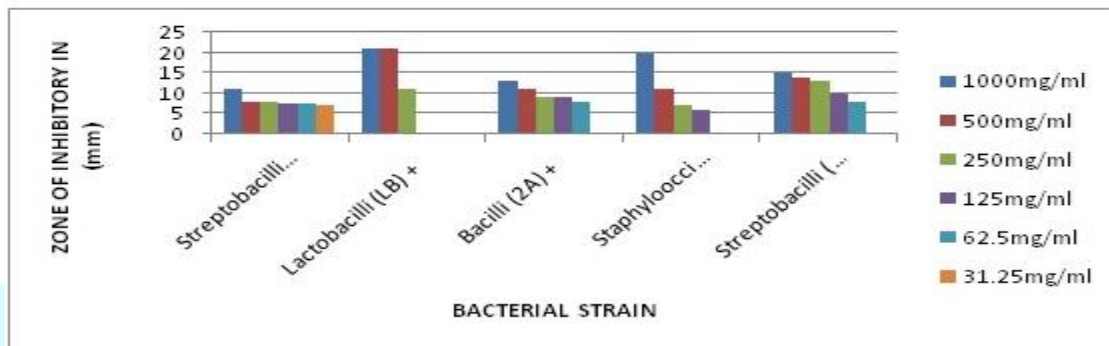


Figure no.3.2. Graph of Minimum Inhibitory Concentration(MIC) of Ethyl Acetate extract

IV. CONCLUSION

Out of the seven extracts of *Aleuritopteris bicolor* assayed the ethyl acetate extract was most active against all the studied gram positive and gram negative bacteria. Therefore, minimum inhibitory concentration (MIC) of the extract was determined against the selected bacteria showing zones of inhibition ≥ 10 mm. The MIC for different strains ranges between 125mg/ml to 62.50mg/ml. So, it can be concluded that ethyl acetate extract of *Aleuritopteris bicolor* possess good antibacterial activity against bacterial strains that cause infection. *Aleuritopteris bicolor* can be recommended in future for various biological activities such as antibacterial, antioxidant, anti-inflammatory.

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