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A RESEARCH ARTICLE ON ANTIBACTERIAL ACTIVITY OF EXTRACT OF *SELLAGINELLA BRYOPTERIS* L.

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ABSTRACT
The present study was performed to see the effect of extract of *Selaginella bryopteris* on Gram positive and gram negative bacteria by the agar well diffusion method. The aqueous and hydro alcoholic extracts of *Selaginella bryopteris* in varying amount i.e. 2,4,8,16,32,and 64 microgram were tested separately along with controls the results indicated that alcoholic extract is active against both Gram positive and Gram negative bacteria but more active against Gram negative bacteria at high concentration. The consequences of zone of inhibition of the alcoholic and hydro alcoholic extract in comparison with standard antibacterial drug (cefixime) was showed in result section. The present research work is significant on the aspect of antibacterial property estimation in an important medicinal pteridophyte species, so far least studied, It is traditionally used for curing wounds and irregular menstruation, uterine disorders and other internal injuries. It contains a variety of secondary metabolites such as alkaloids etc. due to which it can act as antioxidants, anti-inflammatory, anti-cancer, anti-allergic, antibacterial, antiviral etc.

KEYWORDS: *Selaginella bryopteris*, antibacterial, Zone of inhibition.

INTRODUCTION
Selaginella bryopteris (L.) usually known as "Sanjeevani', which translates as "One that infuses life" derives from the medicinal properties of the herb, It is a lithophyte with remarkable resurrection capabilities and lots of medicinal properties. *Selaginella* species have attracted attention of researchers all around the world due to the presence of high value bioactive chemicals such as flavonoid, biflavonoids, tannin, saponin, steroid and many other medicinally important chemical constituents. It is also used as a strength tonic to improve fitness and to extend lifespan by the local tribal communities of India. The herbs are extensively exploited and sold throughout the country in different markets. It is observed that this herb can with stand years of drought very effectively, and still there is no adverse effect in its vigor. The cause for this special power lies in its drought resistant gene. The detached fronds of Selaginella bryopteris (L.) have been said to have unique ability to survive desiccation similar to that of whole plant. The fronds curl, become dry and virtually dead. The herb forms a lush green velvety carpet like landscape during the raining season and in summer it undergoes extreme desiccation.

MATERIALS
1. Test Strains - Microorganisms Used
Standard cultures of:

Gram negative bacteria :	*Escherichia coli, Pseudomonas aeruginosa*
Gram positive bacteria :	*Staphylococcus epidermidis, Staphylococcus aureus*

The strains were obtained from **Microbiology Department, M. M. College of Medical Sciences and Research, M. M. University, Mullana, Ambala**. The microorganisms, which were maintained by sub-culturing, were used at regular intervals in nutrient agar medium.

2. Nutrient Broth
3. Alcoholic(SBA) and Hydro alcoholic(SBHA) extract of ***Selaginella bryopteris* L. *(Sanjeevani)***
4. Petri plates, glass syringes, cork borers, inoculation loop, cotton.
5. Standard Drug - Cefixime

METHODS

Method followed: cup-plate method.

AGAR DIFFUSION METHOD

This method gives the extent of growth of the microorganism, inoculated into a solid nutrient agar bed by the antimicrobial substance. The test substance is kept in a cup made of agar bed, diffuses into it and inhibits the growth of microorganism. The diameter of the zone of inhibition was measured in comparison with suitable drug substance, is considered as potency of that substance. The diameter of zone of inhibition is directly proportional to the concentration of the drug substances added into the cup, thickness of the agar bed, diffusion coefficient of the antimicrobial substance into the agar cup, sensitivity of the microorganism to the test substance and the temperature. The appropriate media is sterilized and cooled to 42 ℃, Incubated with the test organism, mixed uniformly and poured into petri plates and cooled. Bores are made into it; specified test solution is added and left at room temperature for 30 minutes. Incubate at 37 °C for 24 hours. The zone of inhibition is measured in mm.

WORKING PROCEDURE

Stock solution of the synthesized compounds and standard drug used were prepared in dimethyl formamide (DMF) taken in the concentration of 2000 µg/ml.

ANTIBACTERIAL ACTIVITY

PREPARATION OF INOCULUM

The suspensions of all the microorganisms were prepared in nutrient broth

Composition of Nutrient Broth

S. No.	Ingredients	Weight (g)
1	Beef extract	10.00
2	Peptone	10.00
3	Sodium chloride	5.00
4	Distilled water H = 7.4±0.2	1000.00 ml

- Above mentioned quantities of different ingredients were weighed and dissolved in appropriate amount of water.
- The mixture was dissolved with the aid of stirring and heating.
- pH of the solution was adjusted with 5 M sodium hydroxide.
- The mixture was boiled for 10 minutes to obtain clear solution and filtered if necessary.
- Sterilized by autoclave using 15 lb pressure at 115°C for 30 minutes.
- Sterilized nutrient broth was cooled and inoculated with microorganism under aseptic conditions.
- It was allowed to incubate overnight at 37 °C before use as 24 hours old culture was used.

PREPARATION OF ASSAY MEDIA

Composition of Nutrient Agar media [Table no.2.4].

S. No.	Ingredients	Weight (g)
1	Beef extract	10.00
2	Peptone	10.00
3	Sodium chloride	5.00
4	Agar	1-2%
5	Distilled water pH = 7.4±0.2	1000.00 ml

The above-mentioned quantities of different ingredients were accurately weighed and dissolved in appropriate amount of distilled water. Media so prepared was sterilized by autoclave using 15 lb pressure at 115 °C for 30 minutes.

In-vitro antibacterial activity: The anti-bacterial property of SBA and SBHA was determined via cup-plate (345-349). 30 µl of inoculum was inoculated in 150 ml of sterilized nutrient medium and poured in sterilized petri-plates. Sterile cork bore of 9 mm diameter was used for boring well, 50 µl of solution of concentration 2-64 µg/ml was loaded and plates were incubated at 37^0 C for 24 hours. The zone of inhibition and MIC was determined using antibiotic zone reader (Hi Antibiotic zone scale). MIC is defined as the lowest concentration of the compound that inhibited the growth of bacteria. DMSO (Dimethyl sulphooxide) was selected as a control and cefixime was used as reference compound. The results are presented in triplicates.

RESULT

Antibacterial activity of Aqueous Extract of *Selaginella bryopteris* L. Baker.

COMPOUND	Conc.in µg	Minimum inhibitory concentration (MIC) in µg/ml and Zone of Inhibition (mm)			
		EC	***PA***	***SE***	***SA***
Aqueous Extract of *Selaginella bryopteris* L. Baker (SANJEEVANI)	2	20	12.5	34	18
	4	34	25	100	18
	8	34	20	100	20
	16	84	6.5	200	30
	32	184	25	100	30
	64	200	25	50	20
Control	-	-	-	-	-
Cefixime	2	50	400	400	50

Where, *EC–Escherichia coli, PA – Pseudomonas aeruginosa*
SE–Staphylococcus epidermidis, SA – Staphylococcus aureus

Antibacterial activity of Hydro-Alcoholic Extract of *Selaginella bryopteris* L.

COMPOUND	Conc.in µg	Minimum inhibitory concentration (MIC) in µg/ml and Zone of Inhibition (mm)			
		EC	***PA***	***SE***	***SA***
Hydro-Alcoholic Extract of *Selaginella bryopteris L.* Baker *(SANJEEVANI)*	2	84	6.5	50	30
	4	84	12.5	50	34
	8	125	20	30	34
	16	168	25	200	34
	32	150	50	200	34
	64	180	25	84	34
Control	-	-	-	-	-
Cefixime	2	50	400	400	50

DISCUSSION

Antimicrobial activity was studied by Agar well diffusion method against two gram −ve & two gram +ve Bacteria. The observations of the MIC study has been showed in result section. From the data it is evident that the alcoholic extract is active against both Gram positive and Gram negative bacteria but more active against Gram negative at high concentration. The consequences of zone of inhibition of the alcoholic and hydro alcoholic extract in comparison with standard antibacterial drug (cefixime) were showed in result section. The result showed that both extract of *Selaginella bryopteris* displayed concentration dependent antibacterial activity. It indicates that *Selaginella bryopteris* shows antibacterial activity towards all four investigated phytopathogenic bacteria. But the maximum antibacterial activity was found towards, E. coli while it was less active against *Pseudomonas aeruginosa*.

CONCLUSSION

The result concluded that both extract of *Selaginella bryopteris* displayed concentration dependent antimicrobial activities.*Selaginella bryopteris* shows antibacterial activity towards all four investigated phytopathogenic bacteria. The maximum antibacterial activity was found towards E**.** *coli* while it was less active against *Pseudomonas aeruginosa.* The scholar also believes that more research must be carried out on ethno medicinal plants to enhance the area of herbal therapeutics. This is the preliminary study, further investigation on active principle identification related to antibacterial effects against this species needed.

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