Research Article

Evaluation of antimicrobial activity of *Selaginella bryopteris*

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Abstract

Objective: The goal of this study was to determine the preliminary antibacterial activity of Methanolic extract of *Selaginella bryopteris*, belonging to family selaginellaceae. **Materials and methods:** The antibacterial activity of the methanolic extract was done on some standard and wild pathogenic bacterial strains such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi*. The testing was done by the agar cup plate method. Zone of inhibition of extract (50, 100 and 150 mg/ml) was compared with standard Amoxicillin (0.5 and 1 mg/ml) prepared in DMSO. **Results and conclusion**: The extract shows prospective antibacterial properties comparable with that of standard amoxicillin against the organisms tested. The methanolic extract of *Selaginella bryopteris* displayed a concentration related antibacterial activity. The results show that the inhibition of the bacterial growth was more pronounced on *Escherichia coli* as compared to the other tested organisms.

***Keywords*:** *Selaginella bryopteris*, Antibacterial, E. coli, Zone of inhibition, aqueous infusion, aqueous decoction

Introduction

An antimicrobial is a substance that kills or inhibits the growth of microorganisms (Indian pharmacopoeia, 2007), like as bacteria, fungi, or protozoan and antimicrobial drugs either kill microbes or prevent the growth of microbes. Antibiotics are usually used to treat bacterial infections. The toxicity to humans and other animals from antibiotics is generally considered to below e.g. usage of antibiotic agents in viral respiratory tract infections (Evans et al., 2007; Kokate et al., 2007). The spread of multi-drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages, spices have evoked interest as sources of natural products for their potential uses as alternative remedies to heal many infectious diseases. The goal of this study was to determine the preliminary antibacterial activity of Methanolic extract of *Selaginella bryopteris*, belonging to family selaginellaceae In most Indian literature the Indian endemic resurrection plant, *Selaginella bryopteris* (L.) Bark, locally known as "Sanjeevani', along with Cressa cretica and a few more angiosperms are credited with the same meaning (Ganeshaiah et al., 2009). *Selaginella bryopteris* is a pteridophytic plant belongs to the family selaginellaceae and it is known for its remarkable resurrection capabilities. In Sanskrit it is known as sanjeevani booti. *Selaginella bryopteris* is a lithophytic which grows on the hills of tropical areas, particularly the arawali mountain terrain from east to west in India and the plants grow luxuriantly during rains exhibiting a lush green velvety landscap (Kholia et al., 2009). During summer the plants undergo extreme desiccation; fronds curl and become dry virtually dead. In this condition they look like closed fist hence often known in unani as punjemeriam or hathazori. The dry plants when left in water unfold their fronds, turn green and come back to active life. So far there has been no scientific report in literature about the antidepressant activity of *Selaginella bryopteris*, therefore the present study has been undertaken to investigate the effect of antibacterial effect on urinary tract infection (Kaur et al., 1994).

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Materials and methods

Plant collection and authentication of *Selaginella bryopteris* were collected during November from Botanical garden of

Guru Ramdas Khalsa Institute of Science and Technology Pharmacy Jabalpur mp and were identified by their Head of Pharmacognosy department. A voucher specimen has been deposited in the department of Pharmacognosy, Guru Ramdas Khalsa Institute of Science and Technology Pharmacy, Jabalpur, M.P.

Plant material extraction

The whole plants were dried well in sunlight and reduced to a coarse powder. Then the powder was subjected to soxhlet extraction with methanol for 72-80 hours at a temperature of 50-60ºC. The extract was concentrated and the solvent was completely removed by rotator evaporators. They were freeze dried and stored in the vacuum desiccators until further use (Charde et al., 2012; Bajaj et al., 2012).

Preliminary phytochemical screening

Preliminary phytochemical screening test performed. Following phyto-constituents are present like phytosterol, polyphenol, saponins, flavonoids and carbohydrates (Dev et al., 2015; Shukla et al., 2014).

Microorganisms

Standard cultures of following microorganisms were obtained from Peoples University Bhopal M.P. The microorganisms were identified by staining techniques. The organisms were maintained by sub culturing at regular intervals in nutrient agar medium. Gram +ve bacteria: *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis* Gram -ve bacteria: *Escherichia coli, Salmonella typhi*.

Preparation of inoculums

The suspension of all organisms were ready by inoculating one colony of the strain in 20 ml of nutrient broth in conical flask and incubated at 37°C for 24 hours to activate the strain. The suspension is attuned such that it contained approximately 1 x 106 cells/ml. It was obtained by calculating the cells by Neubers chamber. Nutrient agar (HiMedia) was arranged for the study.

Culture medium

The medium was prepared by dissolving 12 gm of nutrient broth in 1000ml of distilled water pH should maintained between (7.3 ± 0.2), and subjecting it to sterilization in an autoclave at 120-211°C for 15 min. Antimicrobial Agent, the reference standard amoxicillin was taken.

Determination of Minimum Inhibitory Concentration

The molten nutrient agar media was arranged and distributed in McCartney bottles, 20 ml each, prior to sterilization. A measured amount of the methanol extract of *Selaginella bryopteris* was added to each bottle in such a manner that the final concentration per ml of the agar medium was 0 (control), 5, 15, 25, 50 and 100 mg. the final mixture was poured individually into 100 mm sterile petri-plates properly. For uniform diffusion of the drug all over the medium, the nutrient agar plates contain different concentrations of the drug were chilled overnight at 4˚ C and then dried for 24h at 37˚ C before inoculation. One loopful (loop diameter – 2mm) of an overnight grown bacteriological culture of the test organism at concentration ~ 106 colony form units (cfu/ml) was positioned in all the petriplates marked by checkerboard technique. The spot inoculated plates were incubated at 37˚ C for 24h and then investigate for any growth of microorganisms. The minimum concentration of extract which avoid bacterial growth was taken as MIC (Table 1). The antibacterial growth was observed by formation of bacterial colony or turbidity around the inoculum's spot (Pandey et al., 2016; Gupta et al., 2015).

Table 1. Determination of MIC of methanolic fruit extract of *Selaginella bryopteris* against different bacteria

Name of bacteria in mg/ml	Growth in nutrient agar containing different concentration of extract					
	0	5	15	25	50	100
S. aureus	0	+	+	-	-	-
S. epidermidis	+	+	+	-	-	-
B. subtilis	+	+	+	+	-	-
B. cereus	+	+	+	+	-	-
E. coli	+	+	+	+	-	-
S. typhi	+	+	-	-	-	-
S. aureus	+	+	+	-	-	-

0' – Control (without extract); '+' – Growth; '-' – No growth

Determination of Zone of Inhibition by cup plate method

The antibacterial activity of methanolic of *Selaginella bryopteris* extract was performed using Agar cup-plate method. 20ml of sterile nutrient agar medium was poured into sterile petri-dishes and allowed to solidify. The petri dishes were incubated at 37°C for 24 hours to check for sterility. The medium was seeded with the organisms by pour plate method using sterile top agar (4 ml) contained 1 ml culture. Bores were made on the medium using sterile borer. Dried methanolic extract of *Selaginella bryopteris* was dissolved in Dimethyl sulfoxide (DMSO) to obtained different concentration (50, 100 and 150 mg/ml) and sterilized by filtration through a Whatman filter paper no. 1, and 0.1 ml of the different concentrations of extract were added to the respective bores. 0.1ml of Amoxicillin at a concentration of (0.5 mg/ml, 1mg/ml) was taken as standard reference drug. The plates were incubated overnight at 37oC with suitable positive and negative controls. The petri-dishes were kept in refrigerator at 4oC for ½ hour for diffusion. After diffusion the petri-dishes

were incubated at 37°C for 24 hours and zone of inhibition were observed and calculated. Dimethyl sulfoxide was used as the control (Tiwari et al., 2016).

Table 2. Antibacterial activity of Amoxicillin and fruits methanolic extract of *Selaginella bryopteris*

Microorganisms	Zone of Inhibition in mm				
	Extract in mg/ml			Amoxicillin in mg/ml	
	50	100	150	0.5	1
S. aureus	12±0.31	16±0.49	18±.032	20±0.24	26±0.99
S. epidermidis	14±0.51	15±0.81	16 ±0.98	22±0.45	24±0.71
B. subtilis	8±0.12	10±0.62	11±0.76	13±0.56	15±0.64
B. cereus	6±0.46	7±0.016	8±0.43	12±0.71	15±0.33
E. coli	20±0.13	21±0.023	23±0.31	21±0.44	25±0.14
S. typhi	14±0.67	17±0.017	21±0.13	19±0.59	23±0.86

Results and discussion

The observations of the MIC study has been showed in table 1 and it was found that the minimum inhibitory concentration for methanolic plant extract of *Selaginella bryopteris* against *E. coli* is 15 mg/ml, where as for *Salmonella typhi, Staphylococcus aureus* and *Staphylococcus epidermidis* it was 25 mg/ml and for, Bacillus cereus and *Bacillus subtilis* were repressed at 50 mg/ml. From the data it is evident that the methanolic extracts is active against both Gram positive and bacteria but more active against Gram negative at low concentration. The consequences of zone of inhibition of the methanolic fruit extract and comparison with standard antibiotic amoxicillin were showed in table 2. The result showed that the methanolic fruit extract of *Selaginella bryopteris* displayed concentration dependent antibacterial activities. It indicates that *Selaginella bryopteris* shows antibacterial activity towards all six investigated phytopathogenic bacteria. The maximum antibacterial activity was found towards *E. coli*, while it was less active against *S. aureus*. The compounds responsible for this antimicrobial property were not investigated. However preliminary phytochemical analysis of the methanolic extract exposed the presence of phytosterol, polyphenol, saponins, flavonoids and carbohydrates. The antimicrobial potency of the plant may be attributed to the single or combined effect of the above mentioned chemical groups. The methanolic fruit extract of *Selaginella bryopteris* had impressive antibacterial and could lead to the discovery of new antibiotics. This becomes more significant as the current antibiotics in use are fast loosing effectiveness due to emergence of resistant microorganisms. The isolation of components of fruits of *Selaginella bryopteris* methanol extract is in progress as very potent antimicrobial agents.

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