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Original Research Article

Antibacterial Activity of *Selaginella bryopteris* Fronds Extract on Bacteria Isolated from Mastititic Milk

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Keywords

Selaginella bryopteris, Antibacterial, Zone of inhibition

A B S T R A C T

The present study was performed to see the effect of extract of fronds of *Selaginella bryopteris* on *Staphylococcus aureus* and *Bacillus* sp. isolated from mastitis affected cow's milk by the agar well diffusion method. The methanolic extracts of *S. bryopteris* in varying amount i.e. 30, 40, 50 and 100 mg were tested separately along with controls. Results indicated that 50 mg methanolic extracts showed the maximum inhibitory effects against *S. aureus* (7.33±1.03 mm) and *Bacillus* sp. (5±0.89 mm). The present endeavor is significant on the aspect of antimicrobial properties estimation in an important medicinal pteridophyte, so far least studied, *S. bryopteris*.

Introduction

Selaginella species have attracted attention of researchers worldwide due to the presence of high value bioactive molecules such as flavonoid, biflavonoids, tannin, saponin, triterpene, steroid and many other secondary metabolites (Swamy *et al.*, 2006; Setyawan, 2011; Weng and Noel, 2013). The pharmacological properties of biflavonoids were well reported that includes antimicrobial, antiviral, anticancer, anti-inflammatory activities (Ma *et al.*, 2001; Tang *et al.*, 2003; Sah *et al.*, 2005). *S. bryopteris* is one of the Sanjeevani-like plants enriched with flavonoids and biflavonoids found mainly in hilly terrain of Indian states like Bihar, Jharkhand and Uttar Pradesh and southern India states, also reported in Indian folklore as herbal drug (Sah *et al.*, 2005; Swamy *et al.*, 2006; Sah, 2008; Ganeshaiah *et al.*, 2009). Natural habitat is the only source of plant material for use as local herbal drug as well as research purposes that led to its over-exploitation. These valuable plant needs attention for its conservation in its niche. 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. Earlier, *S. bryopteris* was investigated for its antibacterial effect on urinary tract infection (Kaur *et al.,* 1994)

Mastitis is a major disease of cattles causing huge economic loss to dairy industry globally (Nielsen *et al.,* 2010). These include losses in milk production, shortening of productive life, losses in milk quality, medicine costs, costs of veterinary care (Hogeveen *et al.,* 2011). The most frequent agents of infection in dairy cattle are *Staphylococcus aureus, Escherichia coli, Streptococcus uberis* and *Streptococcus dysgalactiae* (Schukken and Kremer, 2001). With the increasing use of antibiotics there is development of resistance to one or more antibiotics in mastitis causing bacterial strains which necessitates the search of new antimicrobial agents to combat this problem. Now-days there is surge in bioprospection activities on natural resources including plants for antimicrobial agents to deal with the issue of antibiotic resistance. Since, *S.bryopteris* has been reported to have antibacterial activity so in the present study, the effect of extract of fronds of this plant on *S. aureus* and *Bacillus* sp. isolated from mastitis affected cow's milk was determined by the agar well diffusion method (Magaldi *et al.,* 2004; Valgas *et al.,* 2007). The present endeavor is significant on the aspect of antimicrobial properties estimation in an important medicinal pteridophyte, so far least studied, *S. bryopteris*.

Materials and Methods

Plant Material

Plants of *S. bryopteris* were collected from its niche, Girihinda Hills in Sheikhpura district of Bihar) in the month of August. The collected plants were maintained in plastic pots containing soil and compost mixture (1:1) at Bihar Agricultural College, Sabour.

Sample Preparation

The fronds (2.5 g) ground with pestle and mortar in 100 ml of methanol and sonicated at 33 KHz for 30 min using Ultra-sound Sonicator (Qsonica, USA) and filtered by filter paper (Whatman No. 1). The equal volume (250 µL) of each samples containing 30, 40, 50 and 100 mg of *S. bryopteris* methanolic extracts used in the present study. Equivalent volume of methanol was also used as control to see the inhibitory effect due to methanol, if any. As a standard antibiotic, 100 µL (40 mg/mL) gentamicin was used as positive control.

Isolation and Identification of bacteria

A loopful of Mastitis affected milk sample was cultured on Nutrient agar media at 37 °C for 24 hours. Bacteria were identified based on morphological, gram staining, catalase and coagulase test followed by 16S ribosomal RNA sequencing. The 16S rDNA sequence was used to carry out BLAST alignment search tool of NCBI Genbank database. Based on maximum identity score first fifteen sequences were selected and aligned using multiple alignment software program ClustalW. Distance matrix was generated using RDP database and the Phylogenetic tree was constructed using MEGA5. Based on nucleotide homology were identified and submitted to NCBI GenBank database [*S. aureus* strain S22, accession no. KY432815; *Bacillus* sp. strain S41, accession no. KY435720)].

Agar well diffusion method

Agar well diffusion method, one of the widely used methods to evaluate the antimicrobial activity of plants or microbial

extracts was followed (Magaldi *et al.*, 2004; Valgas *et al.*, 2007). In brief, the Nutrient agar plate surface was inoculated by spreading 100 µL of the overnight grown inoculum of *S. aureus* strain S22 and *Bacillus* sp. strain S41 separately over the entire agar surface in Petridish. Then, a hole with a diameter of 4-5 mm was punched aseptically with a tip, and a volume (250 µL) of the methanolic extract solution at desired concentration was introduced into the well. Then, agar plates were incubated at 37 °C for 24 hours to activate the strain. The observations were recorded on minimum inhibitory concentration (MIC) and zone of Inhibition (in mm) after 24 and 48 h.

Results and Discussion

The discovery of new antibiotics is a very important in the light of concern related multidrug-resistance in bacteria. Bioactive compounds derived from prokaryotes, fungus, plants and other sources are one of the major sources of prospective drug-like novel molecules. There are several reports in recent past aimed at the investigation of plant and microbial extracts, essential oils, pure secondary metabolites and new synthesized molecules as potential antimicrobial agents (review by Balouiri *et al.*, 2016).

In the present study, the methanolic extracts in varying concentration (30, 40, 50 and 100 mg) were tested along with gentamicin (40 mg) and methanolic control. The observations of the study found that the minimum inhibitory concentration (MIC) for methanolic plant extract of *S. bryopteris* against *S. aureus* and *Bacillus* sp. were repressed at 30 mg (Table 1). We also found that methanolic extract (50 mg) showed the maximum inhibitory effects as evident by the zone of inhibition against *S. aureus* (7.33±1.03 mm) and *Bacillus sp.* (5±0.89 mm) after 24 h of incubation (Table 1 and 2). This inhibition zone decreased upon incubation for an extended period of 48 h [*S. aureus* (6.67±0.52 mm) and *Bacillus* sp. (4.67±0.52 mm)] (Table 3 and 4). Though, the maximum antibacterial activity was found towards, *S. aureus* than the *Bacillus* sp. Earlier, it was reported that Kembu-kembu and *Lantana camara* (both from Verbenaceae family) had similar effect to inhibit the growth of *Bacillus* sp., *E. coli*, and *S. aureus* (Rajakaruna *et al.*, 2002; Pasqua *et al.*, 2005).

Earlier, we performed phytochemical analysis of the methanolic extract which revealed that the frond of *S. bryopteris* is enriched with phenolics, flavonoids and anti-oxidants compounds. The antimicrobial potency of the plant may be attributed to the single or combined effect of the above mentioned chemical groups.

Therefore, it appears that *S. bryopteris* is a prospective medicinal plant in terms of antimicrobial effect against *S. aureus* and *Bacillus* sp., and could be the source of effective antibacterial compound.

Table.1 Zone of inhibition (mm) of methanolic extract of *S. bryopteris* against *S. aureus* and *Bacillus* sp. after 24 h of incubation

Name of bacteria	Methanolic extract of *S. bryopteris* (mg)			
	30	40	50	100
S. aureus	4.33±1.03	4.33±1.37	7.33±1.03	5.33±0.52
B. subtilis	2.67±0.52	3.67±0.52	5±0.89	4.33±0.52

Data represent mean ± SD

Table.2 Comparison of zone of inhibition (mm) in response to gentamicin and methanolic extract of *S. bryopteris* (50 mg) after 24 h of incubation

Name of bacteria	Gentamicin (40 mg/mL) (Control 1)	Equivalent concentration of methanol (Control 2)	Methanolic extract of *S. bryopteris* (50 mg)
S. aureus	11±0.89	4.33±0.52	7.33±1.03
B. subtilis	10.33±0.52	0	5±0.89

Data represent mean ± SD

Table.3 Zone of inhibition (mm) of methanolic extract of *S. bryopteris* against *S. aureus* and *Bacillus* sp. after 48 h of incubation

Name of bacteria	Methanolic extract of *S. bryopteris* (mg)			
	30	40	50	100
S. aureus	4.33±1.03	4.33±1.37	6.67±0.52	5.33±0.52
B. subtilis	2.67±0.52	3.67±0.52	4.67±0.52	4.33±0.52

Data represent mean ± SD

Table.4 Comparison of zone of inhibition (mm) in response to gentamicin and methanolic extract of *S. bryopteris* after 48 h of incubation

Name of bacteria	Gentamicin (40 mg/mL) (Control 1)	Equivalent concentration of methanol (Control 2)	Methanolic extract of *S. bryopteris* (50 mg)
S. aureus	11±0.89	4.33±0.52	6.67±0.52
B. subtilis	10.33±0.52	0	4.67±0.52

Data represent mean ± SD

In conclusion, *Selaginella* methanolic extracts (50 mg) showed promising antibacterial activity against *S. aureus and Bacillus* sp. Though, the maximum antibacterial activity was found towards, *S. aureus* than the *Bacillus* sp. This is the preliminary study, further investigation on active principle identification related to antibacterial effects against these species needed.

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