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PHYTO-CHEMICAL EXTRACTION AND ANTI-MICROBIAL ACTIVITY OF SELAGINELLA BRYOPTERIS

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ABSTRACT

The present study was performed to determine the preliminary anti-microbial activity of *Selaginella Bryopteris* belonging to family *Selaginellaceae*. The antibacterial activity of the methanolic extract was done some standard bacterial strains such as *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. The testing was done by the agar cup plate method. Zone of Inhibition of extract was compared with standard Gentamicin. Results indicate that 50 mg/ml methanolic extracts showed the maximum inhibitory effects against *E. coli* (7mm).

KEYWORDS: *Selaginella Bryopteris*, Antibacterial, *E. coli*, Zone of inhibition.

INTRODUCTION

The *Selaginella bryopteris* also known as Devanagari or Sanjeevani. It has medicinal use and it depends on decaying plants and rain water for its nutrients. The grow on rocks. Sanjeevani refers to "One that Infuses Life".[1] The nanoparticle of *Selaginella bryopteris* shows targeted delivery.[2] The nanotechnology platform could serve as customizable targeted drug delivery.[3] Nanotechnology involves a convergence of multiple areas of science and molecular biology.[4]

Selaginella species have attracted attention of researchers worldwide due to the presence of high molecules such as flavonoid, bioflavonoids, tannin, saponin, triterpene, steroid and many other secondary metabolites.[5,6,7] The pharmacological properties of bioflavonoids were well reported that includes antimicrobial, antiviral, anticancer, anti-inflammatory activities.[8,9,10] *S. bryopteris* is one of the Sanjeevani-like plants enriched with flavonoids and bioflavonoids 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.[11,12]

The name given to its because of its medicinal uses. This plant is used to treat several health problems like Heart stroke, Jaundice, Dysuria, Irregular Menstruation etc. Although it is used from ancient times these are not validated scientifically. Even now these are used by tribes in India.[1]

It is considered to be the divine plant in India as it is used to cure Lakshmana in the war of Ramayana. Various studies conducted on the plant by many researchers and found that is has high resistance towards drought conditions. The agricultural scientists started studying this character and are trying to transfer this gene to the agricultural plants to get good yield. It has a series of eleven bioflavonoids containing amentoflavone and hinokiflavone.[1]

An antimicrobial is a substance that kills or inhibits the growth of microorganisms[13], 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 infection.[14,15]

MATERIALS AND METHODS

Plant material was collected from the hilly areas.

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.[16]

MICRO ORGANISMS

The test organisms *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli* were cultured onto nutrient agar in order to determine their viability. The identity of each test organism was confirmed using standard cultural, morphological and biochemical techniques. Stock cultures were maintained as Glycerol stocks at 4ºC checking their viability time to time.

SAMPLE PREPARATION

The fronds (1.5g) ground with pestle and morter in 100ml of methanol and sonicated at 33KHz for 30 min using Ultra-sound Sonicator (Qsonica, USA) and filtered by filter paper

(Whatman No. 1). The equal volume (250µL) of sample containing 50 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.

CULTURE MEDIUM

The medium was prepared by dissolving 28.0 gm of nutrient agar in 1000ml of distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121ºC) for 15 minutes. Cool to 45-50ºC. The sterilized agar was then transferred into the petri-dishes and was allowed to solidify. Thereafter, the procedure was executed in laminar air flow to ensure proper aseptic conditions. Antimicrobial Agent, the reference standard Gentamicin was taken.

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.[17] In brief, the Nutrient agar plate surface was inoculated by spreading 100 µL of the overnight grown inoculum of S. aureus strain, Bacillus sp. Strain and E. coli Strain. Separately over the entire agar surface in Petri dish. 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 37ºC for 24 hours to activated the strain. The observations were recorded on Minimum Inhibitory Concentration (MIC) and Zone Of Inhibition (in mm) after 24 h.

RESULTS AND DISCUSSION

The antimicrobial activity of the plant extracts was examined Gram positive and Gram negative bacteria by measuring zone of inhibition.

The antimicrobial activity was performed by Agar disc diffusion method at concentration level of 50mg/ml. Gentamicin (antibacterial) as the standard drug at a concentration of 40mg/ml. LB Agar was used as the culture media for antibacterial activity. The results of the antimicrobial activity are shown in figures and tables.

Table: Comparison of zone of inhibition (mm) in response to Gentamicin and Methanolic extracts of S. bryopteris (50mg) after 24h of incubation.

Sr. No.	Name of bacteria	Gentamicin (40mg/ml)	Methanolic Extract of *S.B* (50mg/ml)
1	*S. aureus*	12mm	5mm
2	*E. coli*	13mm	7mm
3	*B. subtilis*	12mm	4.5mm

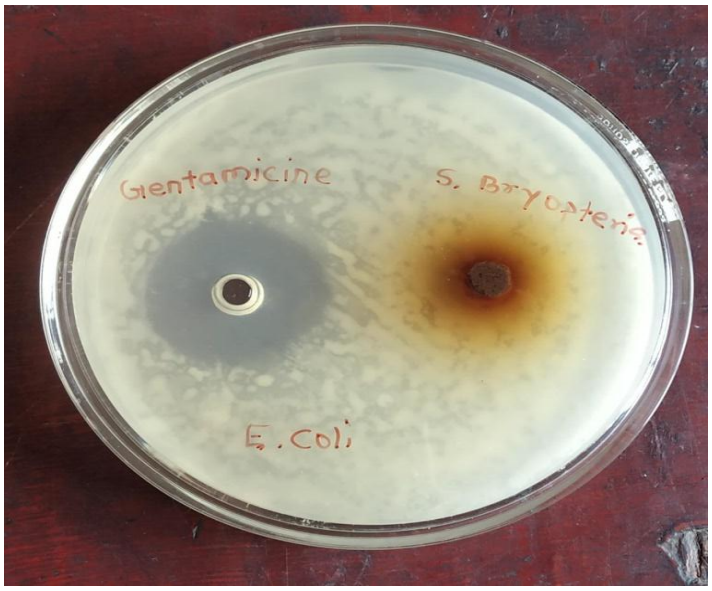

Fig. Zone of Inhibition of *S. bryopteris* VS Gentamicin *(E. coli)*.

The *Selaginella bryopteris* plant extract showed high activity against *E.coli* compared to *S. aureus and B. subtilis.* The zone of inhibition is calculated in mm.

The compounds responsible for this antimicrobial property were not investigated. However preliminary phytochemical analysis of 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.

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