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Phytochemical Extraction and Activity against *Salmonella Typhi* of *Selaginella Bryopteris*

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ABSTRACT

Selaginella Bryopteris is widely known medicinal plant in India. It has many medicinal use such as anti-bacterial, anti-fungal and anti-cancer activity. The present study is performed to determine the activity of *Selaginella Bryopteris* belongs to family *Selaginellaceae* against *Salmonella Typhi* (Non-pathogenic strain). The activity is done by using methanolic extract of *Selaginella Bryopteris* against standard strain, *Salmonella Typhi* (Non-pathogenic strain). The testing is done by agar cup plate method. The zone of inhibition of methanolic extract of *Selaginella Bryopteris* is compared with methanol blank as a standard. The 50mg/ml methanolic extract of *Selaginella Bryopteris* shows maximum inhibition (7mm) whereas the blank methanol does not show any kind of inhibition.

Keywords: *Selaginella Bryopteris*, *Salmonella Typhi*, Zone of Inhibition

INTRODUCTION

India is major bio-diversity country, which is rich in medicinal plant resource as well as great traditional knowledge about such medicinal plant. *Selaginella Bryopteris* is widely known Indian medicinal plant. It is also called Sanjeevani as well as Devanagari. It is considered to be the Holy plant in India as it is used to cure Lakshmana (Younger brother of Lord Ram) in the war of Ramayana^{1,2}. More than 80% of the world's population natural medicines and depends on medicinal plants for health care. About 90% of herbal raw drugs used in the manufacture of Ayurveda, Siddha, Unani, and Homeopathy systems of medicine is largely from the wild out of which 70% collection involves destructive harvesting³. Many biflavonoids, lignans, alkaloids and anthraquinones have been identified from genus *Selaginella*. However, few terpenoids were also reported⁴. Extracts of *S. bryopteris* have antioxidant, anticarcinogenic, antiplasmodial, and leishmanicidal activity. Six compounds were isolated from the acetone extract of *S. bryopteris*. These were identified as β -sitosterol, heveaflavone, (+)-syringaresinol, amentoflavone, vanillic acid, and β -sitosterol β -D-glucoside^{5,6}. Species of *Selaginella* have a fairly large spectrum of activity related to medication spanning cancer, cardiovascular

diseases, diabetes, gastritis, hepatitis, skin diseases and urinary tract infections⁷⁻⁹. Recent pharmacological studies proved that the ethanolic extract of *S. Doederleinii* could induce human nasopharyngeal carcinoma cell (CNE) apoptosis and because of abundant biflavonoids, the ethyl acetate extract had more powerful anti-cancer activities than the ethanolic extract for *S. doederleinii*^{10,11}. *Selaginella Bryopteris* is enriched with flavonoids and bioflavonoids found mainly in hilly area of Indian states like Bihar, Jharkhand and Uttar Pradesh and Southern India states, also reported in Indian folklore as herbal drug¹²⁻¹⁴.

MATERIAL AND METHOD

The Plant material is collected from Dongargaon which is located at rural village area 20km away from Nagpur city.

Plant Material Extraction:

The plant was dried at room temperature and it was reduced to a coarse powder by using grinder. Then the coarsed powder was subjected to soxhlet extraction with methanol till 30 cycles completed. The extract is removed from apparatus and it was concentrated by using distillation process and

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dried at room temperature in desiccator, stored in refrigerator for further use¹⁵.



Figure 1: Methanolic extraction of *Selaginella Bryopteris*.

Phyto-chemical Test:

Phytochemical test of extract shows the presence of saponins, phytosterol, polyphenol, carbohydrate and flavonoids.

Micro-organism:

Standard culture of *Salmonella Typhi* (Non-pathogenic strain) were obtain from Rajiv Gandhi Institute of Biotechnology, Nagpur, Maharashtra. The *Salmonella Typhi* is identified by Gram staining technique. The micro-organism was maintained by using sub-culturing at different regular time interval in nutrient agar media.

Sample Preparation:

The dried extract grounded with pestle and mortar and transfer in 50ml volumetric flask, then methanol was added to the mark and sonicated at 33KHz for 30 min using Ultra-sound Sonicator (Qsonica, USA), then it was filter through Whatman filter paper. The equivalent volume (250µl) of sample containing 50mg of *S. Bryopteris* methanolic extract were incorporated in present study. Equivalent volume of methanol was also used as control to identify the inhibitory effect due to methanol.

Culture Medium:

The agar medium was prepared by dissolving 28.0g of nutrient agar in 1000ml of distilled water. Heat is applied to boil to dissolve the agar completely into distilled water. Then media is Sterilize by autoclaving at 15 lb pressure (121°C)

for 15 minutes. Cool to 40-45°C. The sterilized agar was then transferred into the petri-dishes and was allowed to solidify. After that the procedure was completed in laminar air flow to ensure proper aseptic conditions¹⁶.

Agar well Diffusion Method:

The Agar well diffusion method is one of the widely used methods to evaluate the antimicrobial activity of plants or microbial extracts. Nutrient agar plate surface was inoculated by spreading 250µl of the overnight grown inoculum of *Salmonella Typhi* Non-pathogenic strain. Then, a hole is made with a diameter of 4-5 mm was punched aseptically with a tip, and a volume (250µl) of the methanolic extract solution was added in well. Then media is 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 24h is recorded^{17,18}.

RESULT AND DISCUSSION

The anti-microbial activity against *S.Typhi* of plant *Selaginella Bryopteris* extract was performed by measuring zone of inhibition.

The activity was performed by using agar disc diffusion method at level of concentration 50mg/ml. Blank methanol is taken as control against methanolic extract of *Selaginella Bryopteris*. Nutrient agar (Himedia) is used as a culture media for anti-microbial activity. The result of experiment is shown in table and figure.

Table 1: Comparison of zone of inhibition (mm) in response to blank methanol as control and Methanolic extracts of *Selaginella bryopteris* (50mg) after 24h of incubation.

Sr. No.	Name of Micro-organism	Methanol (Control) (Zone of Inhibition in mm)	Methanolic extract of S.B. 50mg/ml (Zone of Inhibition in mm)
1	Salmonella Typhi	Not detected	6
	(Non-pathogenic)	Not detected	6
		Not detected	7

The *Selaginella bryopteris* plant extract showed high activity against *S.Typhi*. The zone of inhibition is calculated in mm.

The compounds which is responsible for this anti *Salmonella Typhi* were not investigated. However preliminary phytochemical analysis of methanolic extract proves the presence of phytosterol, polyphenol, saponins, flavonoids and carbohydrates. The potency of the plant extract may

be attributed to the single or combined effect of the above-mentioned chemical groups.

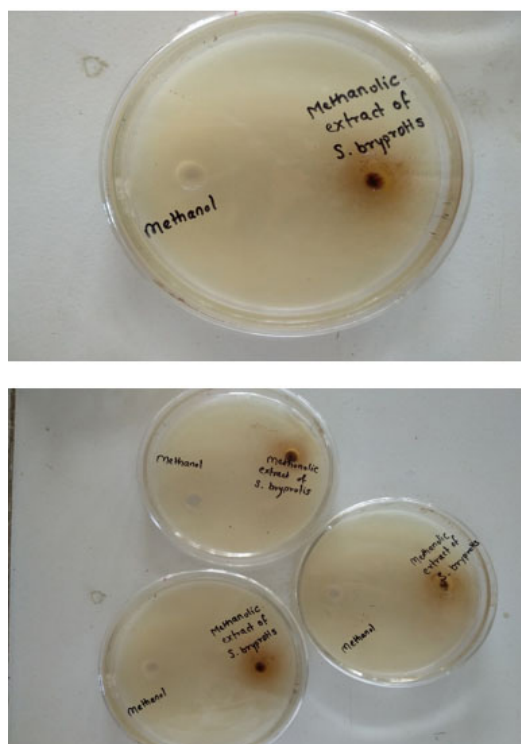


Figure 2: Zone of Inhibition of *S. bryopteris* VS methanol against *Salmonella Typhi*.

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