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## Unveiling the Therapeutic Potential of *Selaginella bryopteris*: Pharmacognostic and Formulation Approaches

Ruchi Singh<sup>1</sup>, Vikas Chandra Sharma<sup>2\*</sup>

<sup>1</sup>Faculty of Pharmacy, Bhagwant University, Sikar Road, Ajmer 305004, Rajasthan, India

<sup>2</sup>DDM College of Pharmacy, V. P. O. Gondpur Banehra (Upper), Tehsil-Ghanari, Dist- Una, Himanchal Pradesh, Pin Code 177213, India

**\*Corresponding author:** Dr. Vikas Chandra Sharma

Director cum Principal, DDM College of Pharmacy, V. P. O. Gondpur Banehra (Upper), Tehsil-Ghanari, Dist- Una, Himanchal Pradesh, Pin Code 177213, India

E-mail ID: [vikas.a.sharma08@gmail.com](mailto:vikas.a.sharma08@gmail.com)

Phone No. +91-7987716003, +91-9302040444

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### ABSTRACT

*Selaginella bryopteris*, commonly known as 'Sanjeevani,' is a medicinal plant highly esteemed in conventional treatment because of its robust therapeutic potential. This study delves into the pharmacognostic, physicochemical, pharmacological, and formulation aspects of *S. bryopteris*, aiming to establish a comprehensive scientific basis for its medicinal use. Pharmacognostic evaluation involved macroscopic and microscopic analyses to characterize the plant's morphological and anatomical features, providing crucial data for correct identification and quality control. Physicochemical studies, including determination of extractive values, moisture content, ash values, etc. were conducted to assess the purity and standardize the plant material. An analysis of the plant's phytochemical composition uncovered bioactive components that might be responsible for its many pharmacological effects, including tannins, saponins, alkaloids, flavonoids, and others. Pharmacological investigations were carried out to explore the antimicrobial properties of *S. bryopteris*, demonstrating significant activity that supports its traditional use in treating oxidative stress-related disorders, infections, and inflammation. Moreover, a novel formulation of *S. bryopteris* extract was developed, focusing on enhancing its bioavailability and therapeutic efficacy. The formulation was subjected to various physicochemical evaluations, including stability studies, to ensure its suitability for therapeutic application. This holistic approach not only reaffirms the traditional uses of *S. bryopteris* but also opens new avenues for its application in modern medicine. The research highlights the significance of combining traditional wisdom with evidence-based practices, providing a solid foundation for the future development of *S. bryopteris*-based therapeutics. The findings hold promise for further research and development of pharmaceutical formulations that harness the full potential of this remarkable plant.

**KEYWORDS:** *Selaginella bryopteris*, Pharmacognostic, Pharmacological, Formulation, Physicochemical, Characterization

## INTRODUCTION

*Selaginella bryopteris*, commonly known as "Sanjeevani," holds a prominent place in traditional medicine due to its reputed medicinal properties and resilience in extreme environmental conditions. This ancient pteridophyte, belonging to the family Selaginellaceae, has been revered in Ayurvedic and folk medicine for its purported ability to "bring life" to those on the brink of death. The unique ability of *S. bryopteris* to survive prolonged periods of desiccation and then revive upon rehydration has captivated scientists, leading to its classification as a "resurrection plant." The plant's resilience is not merely a botanical curiosity but is thought to be indicative of its potential therapeutic applications, particularly in stress-related disorders. Pharmacognostically, secondary metabolite richness makes *S. bryopteris* an intriguing research topic, including flavonoids, terpenoids, and phenolic compounds. These bioactive compounds are believed to contribute to the plant's medicinal properties, such as anti-inflammatory, adaptogenic effects, antioxidant, etc. The exploration of the pharmacognostic characteristics, including macroscopic and microscopic features, provides foundational knowledge necessary for the authentication and herbal quality control used in herbal formulations.

Physicochemical analysis is crucial in understanding the plant's chemical profile and establishing standards for its use in various formulations. When assessing the raw material's quality and purity, it is crucial to consider parameters like moisture content, extractive values, and ash levels. These parameters, along with chromatographic and spectroscopic analyses, help in the identification and quantification of key phytoconstituents, ensuring consistency in herbal preparations. The pharmacological potential of *S. bryopteris* is backed by a number of *in vitro* studies and *in vivo* studies, which have demonstrated its efficacy in managing oxidative stress, enhancing cognitive function, and providing neuroprotection. The plant's adaptogenic properties are particularly noteworthy, as they align with its traditional use in enhancing vitality and endurance. Modern research has begun to unravel the molecular mechanisms underlying these effects, providing a scientific basis for its traditional applications. From a formulation perspective, the incorporation of *S. bryopteris* into modern dosage forms presents both opportunities and challenges. The development of novel formulations, such as nanoparticles, capsules, and topical gels, aims to enhance the bioavailability and therapeutic efficacy of the plant's bioactive compounds. However, ensuring the stability and consistency of these formulations remains a critical concern. The integration of advanced delivery systems, such as solid lipid nanoparticles (SLNs) and phytosomes, could potentially overcome these challenges, leading to more effective therapeutic outcomes.

The study of *S. bryopteris* from pharmacognostic, physicochemical, pharmacological, and formulation perspectives offers a comprehensive understanding of its potential as a therapeutic agent. This manuscript aims to provide an in-depth analysis of each of these aspects, contributing to the scientific validation of *S. bryopteris* as a valuable resource in the development

of herbal medicines. Innovative uses of this historical plant in contemporary health care might be possible via the fusion of traditional knowledge with current research in science.

## **MATERIALS AND METHODS**

### **Instrumentation:**

Shimadzu® UV-Vis Spectrophotometer (UV-1800, Japan) and Shimadzu® Electronic Balance (AUW220D, Japan) were employed. The Transonic Digital S (Sonicator), USA, was used for the sonication process. The recordings were made using Scope Image 9.0 software and the microscopy was carried out using a trinocular microscope CosLab®HL-24(B).

### **Chemicals:**

A local vendor in Lucknow procured all the chemicals, consumables, and reagents for the assessment from Sigma-Aldrich (India) and HiMedia (Germany). The experiment used double distilled water equipment from Borosil® in India.

### **Collection of plant material:**

*S. bryopteris* leaves were collected from a plant at Shakti College of Pharmacy's medicinal plant garden in Balrampur city in the Indian state of Uttar Pradesh. A botanist from Department of Botany, Lucknow University in Bilaspur, Uttar Pradesh certified the plant.

### **Preparation of extract:**

We took the leaves from the tree, let them dry in the shade for a while, and then ground them up to the right consistency. Using 50 mL DW, 50 mL EA, 50 mL MET, and 50 mL PE, separately, the content (100 g, divided into many fewer portions) was heated to a temperature of 65-75°C and exposed to 32 cycles of continuous hot Soxhlet extraction. A rotating vacuum evaporator was used to extract the solvent while maintaining controlled temperature and lowered pressure. The results showed a hydroalcoholic thorn extract yield of 11.8% w/w for *S. bryopteris*<sup>6</sup>.

### **Pharmacognostic evaluations:**

We looked at the organoleptic, physicochemical, histological, and phytochemical properties of *S. bryopteris* leaves powder. Considerable attention was paid to the organoleptic aspects, including form, size, texture, colour, and fracture. The physicochemical variables, including water soluble ash, alcohol soluble extractive value, total ash content, acid insoluble ash, were investigated according to the methods given in the Indian Pharmacopoeia (2020). The loss on drying (LOD) at 105±1°C was also measured. Since too much water in plant materials encourages bacterial development, mould presence, and degradation via hydrolytic activity, the LOD determination is very important. Chalk powder, earthy silica minerals, lime, and other earthy stuff may be identified by looking at the total ash value. Earthy materials with a high concentration of calcium oxalate crystals in their cells could have been identified using acid-insoluble ash, while water-

exhausted materials might have been identified using water-soluble ash. When the drug's extractive values are soluble in alcohol, it indicates that there are adulterants, production errors, or low quality ingredients. Following the procedures specified in the USP Pharmacopoeia (2020), the powder's densities (bulk and tapped) were determined. A trinocular microscope was used to conduct thorough histological identifications on the transverse slice (TS) at a resolution of 30x. Sulfuric acid and phloroglucinol were used to stain the portion. We used a trinocular microscope with a 10x magnification to conduct powder microscopy after suitably staining the sample. Features that were crucial were identified and appropriately written down<sup>7</sup>.

### **Phytochemical evaluation:**

Alkaloids, Sugars, glycosides, proteins, tannins, steroids, flavonoids, terpenes, etc. were all identified by phytochemical screening of the extract, using the specified standard test protocols<sup>8</sup>.

### ***In vitro* antimicrobial activity:**

Researchers tested extract's antimicrobial properties *in vitro* against a number of harmful bacterial species, including *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Escherichia coli*. Similarly, extract was tested for its antifungal efficacy *in vitro* against *Aspergillus niger* and *Candida albicans*, two types of fungus strains. MIC values of several compounds were compared with two reference drugs, ciprofloxacin (anti-bacterial) and fluconazole (anti-fungal)<sup>9</sup>.

### **Antibacterial activity:**

We found out how effective the extract was against bacteria *in vitro* by using the disc diffusion method and Muller Hinton Agar medium. The organisms were cultivated in nutrient broth and incubated for 24 hours at  $37\pm 1^\circ\text{C}$  before being disseminated onto Muller Hinton agar plates in a longitudinal flow cabinet. After diluting the extract in dimethylsulfoxide (DMSO), it was completely saturated on Whatman filter paper No. 1 sterile discs (6 mm diameter). The discs were then placed in the incubator on top of the pre-made bacterial plates. The extract's inhibitory zone width was measured in millimeters after it was incubated for 24 hrs at  $37\pm 1^\circ\text{C}$ . A disc coated with dimethyl sulfoxide (DMSO) served as the negative control, while the activity was compared to that of the standard antibiotic ciprofloxacin. The experiments were repeated three times<sup>10</sup>.

### **Antifungal activity:**

To test the extract's antifungal activity *in vitro*, we performed the disc diffusion method under normal conditions using Potato dextrose agar medium. Agar plates were inoculated with a standardized solution of the microorganisms under study, and then sterile discs of Whatman filter paper No. 1 (6 mm diameter) were placed on top. Discs with specific amounts of the antifungal drug fluconazole (50  $\mu\text{g/mL}$ ) and extract (100  $\mu\text{g/mL}$ ) were embedded therein. Over the course of 72 hrs, the plates were kept at a temperature of  $28\pm 2^\circ\text{C}$  to determine their antifungal activity. As a control, we used a paper disc that had been soaked with dimethyl sulfoxide (DMSO)<sup>11</sup>.

**MIC Determination:**

To determine the extract's MIC, the agar streak dilution method was used. Before the evaluation, a DMSO extract stock solution was made. Then, the components to be tested were combined with a precise amount of sterile molten Muller Hinton agar. A particular quantity of medium containing the extract was used to fill a Petri dish to a depth of 3-4 mm and then let it firm. Following amplification of the microbial suspension to  $10^5$  CFU/mL, it was introduced to test plates that already contained extract in DMSO. Subsequently, the plates were placed in an incubator set at  $37\pm 1^\circ\text{C}$ . Upon completion of the incubation period, the MIC values were determined. Prior to using the average to arrive at the final result, each measurement was double-checked. One 100 mL volume of DMSO served as the negative control, while one 100  $\mu\text{g/mL}$  volume of the common antibiotic ciprofloxacin served as the positive control. At what concentration of the test sample did the test plate not show any signs of microbial or fungal growth<sup>12</sup>.

**Formulation development:**

The gel was made with the following ingredients: *S. bryopteris* extract, triethanolamine, ethanol, distilled water, and carbopol 940 (CP). The *S. bryopteris* extracts (aqueous, methanol, ethyl acetate, and petroleum ether) was mixed with hydroalcoholic content. Then, CP having fixed hydroalcoholic content, was added drop wise into the *S. bryopteris* extract mixture<sup>13</sup>. In order to get the desired gel consistency, the mixture was constantly churned. Addition of DMSO occurred immediately upon gel formation. Lastly, the gel was allowed to set (**Table 1**).

**Table 1.** Formulation Chart.

INGREDIENTS	F1	F2	F3	F4
<i>S. bryopteris</i> aqueous extract (g)	1	-	-	-
<i>S. bryopteris</i> ethyl acetate extract (g)	-	-	1	-
<i>S. bryopteris</i> methanol extract (g)	-	1	-	-
<i>S. bryopteris</i> petroleum ether extract (g)	-	-	-	1
Carbapol 940 (g)	1	1	1	1
Triethanolamine (mL)	1.8	1.8	1.8	1.8
Ethanol (mL)	18.7	18.7	18.7	18.7
DMSO (mL)	3	3	3	3
Distilled Water (mL)	76.5	76.5	76.5	76.5

**Evaluation parameters:**

The formulations were comprehensively evaluated for washability, physical evaluation, spreadability, skin irritation test, viscosity, pH, swelling index, accelerated stability studies, and extrudability, as per standard protocols<sup>14</sup>.

**Extrudability:**

To test the formulation's extrudability, 100 g of gels were initially placed into collapsible aluminium tubes with caps and sealed manually. The tubes, each holding a unique recipe, were securely clamped between two slides. Then, after 10 minutes, the extruded ribbon length was measured following the placement of a 500 g weight on top of the slides and, lastly, the removal of the cap.

**pH:**

A digital pH metre that had been calibrated was used to measure the dermal gel's pH. To get a consistent measurement, the glass electrode was submerged in a mixture of 1 g of the formulation and 25 mL of pure water. We took three separate pH readings for each formulation and averaged them together.

**Physical appearance:**

The developed herbal gel was examined visually for its transparency, colour, and overall look. By feeling the mixture between the fingers and looking for lumps, roughness, homogeneity, and smoothness, we were able to measure the gel's smoothness.

**Skin irritation test:**

A semi-occlusive bandage was used to cover the normally hairless skin for one hour after applying 0.5 g of the prepared gel over a 6 cm<sup>2</sup> region. When the placement time was over, the bandage was peeled off, the gel had been scraped away, and the area was looked for blisters or any other comparable symptoms. The duration of the test was seven days. Grades were used to express the outcomes.

**Spreadability:**

In order to determine the herbal dermal gel's spreadability, the slip-drag theory was used. The process included placing 2 g of the mixture onto a prepared ground slide, followed by a comparable glide slide with a hook attached to it. By pressing a big item against the slides, which released the trapped air, a uniform layer was created between them. What remained after scraping off the excess gel was the perimeter. Next, the top slide was fine-tuned such that it dragged with an intensity of 50 g. To determine how long it took for the upper slide to move 6 cm, the following formula was utilized:

$$S = M \times L / T$$

**Swelling index:**

Quickly after preparation, 5 mL of emulsion was added to plastic pots to assess the creaming index. After 4 hours, the amount of the cream that had developed was measured in order to estimate the creaming %. By dissolving 2 grammes of the dermal herbal gel in 10 millilitres of distilled water, the swelling index of the finished product was ascertained. After one hour, the formula that had inflated was transferred from the beaker to a petridish. After reweighing the contents, through applying the subsequent equation, we managed to ascertain the swelling index:

$$Si = Wt - Wo / Wo$$

**Viscosity:**

The viscosity of the formulation was measured using the Digital Brookfield Viscometer with spindle no. 6 set at 10 rpm and a temperature of  $25 \pm 1^\circ\text{C}$ . Before taking the measurements, the gel was allowed to settle for at least 30 minutes in a wide-mouthed container that was filled to the brim with enough to submerge the spindle.

**Washability:**

After applying the gel to the skin, we personally observed its impact and evaluated how easy it was to wash off with distilled water to determine the formulations' washability.

**Accelerated stability studies:**

The optimized recipe was placed in a controlled environment ( $40^\circ\text{C} \pm 2^\circ\text{C}$ ;  $75\% \pm 5\%$  relative humidity) for duration of 90 days. A PVC container was used to store the gel formulation that had been made, and it was covered with black foil. The crucial parameters that were previously discussed were reevaluated.

**Statistical analysis:**

We performed all of our experiments three times. The resultant results were presented as the average plus or minus the standard deviation (SD). When comparing the control and experimental groups for pharmacological activity, the t-test was used.

**RESULTS:****Physicochemical evaluations:**

The leaves were found to be conical in shape, with a rough texture and a variety of colors from light ash to grey-brown. Their sizes varied between 18 and 26 mm. The physicochemical examinations have shown that it is devoid of spoilage, browning, and mould development—all of which are mostly caused by water—and that it has a low water content. With a loss of just 0.43%, the product met the standards set by the pharmacopoeia. There was 6.56% water solubility, 1.98% acid insoluble, and 15.13 weight percent total ash. All of the contaminants tested were found to be within the acceptable limits set by the pharmacopoeia. According to

**Table 2**, the powder had an alcohol soluble extractive value of 9.82, a high % compressibility index of 42.85. This suggests that the bulk and tapped densities were 0.156 (g/cm<sup>3</sup>) and 0.273 (g/cm<sup>3</sup>), respectively.

**Table 2.** Physicochemical evaluations.

<b>PARAMETERS</b>	<b>DESCRIPTION</b>
<b>% compressibility index</b>	47.23
<b>Acid insoluble ash (% w/w)</b>	2.55
<b>Alcohol soluble extractive value</b>	8.02
<b>Bulk density (g/cm<sup>3</sup>)</b>	0.336
<b>Color</b>	Yellow-brown
<b>Loss on drying (%)</b>	0.31
<b>Shape</b>	Irregular
<b>Size</b>	22 – 26 mm
<b>Tapped density (g/cm<sup>3</sup>)</b>	0.294
<b>Texture</b>	Rough
<b>Total ash content (% w/w)</b>	14.49
<b>Water soluble ash (% w/w)</b>	7.11

### Phytochemical analysis:

Diterpenes, carbohydrates, alkaloids, phenol, glycosides, sterols, tannins, flavonoids, and triterpenes were identified by phytochemical screening of the extract (**Table 3**).

**Table 3.** Phytochemical analysis.

<b>Chemical constituent</b>	<b>Test performed</b>	<b>Observations</b>	<b>Inference</b>
Alkaloid	Hager's test	Yellow precipitate	<b>Alkaloid present</b>
Carbohydrate	Fehling's test	No Red precipitate	Carbohydrate absent
Diterpene	Copper acetate test	Eemerald green color observed	<b>Diterpene present</b>
Flavonoid	Shinoda's test	Pinkish-red color	<b>Flavonoid present</b>
Glycoside	Borntrager's test	No Faint pink color observed	Anthraquinone glycoside absent
Glycoside	Legal's test	No red color observed	Cardiac glycoside absent
Phenol	FeCl <sub>3</sub> test	Bluish-black color observed	<b>Phenol present</b>
Protein	Xanthoprotic test	No yellow color observed	<b>Protein absent</b>
Saponin	Froth formation test	Frothing for 5 min	<b>Saponin present</b>
Sterol	Libermann-Burchard's test	Brown-ring formation	<b>Sterol present</b>



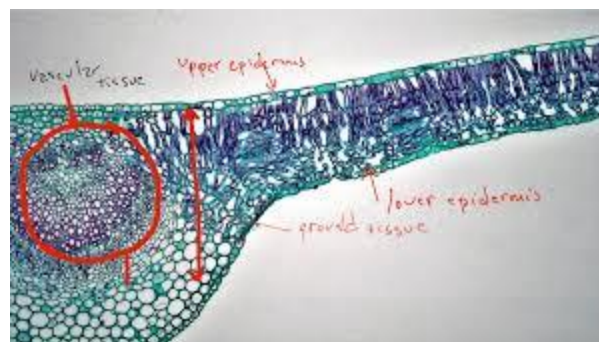
Tannin	Gelatin test	Green color appeared	<b>Tannin present</b>
Triterpene	Salkowski's test	Yellow color observed	<b>Triterpene present</b>

### Pharmacognostic study:

The pharmacognostic study of *S. bryopteris* reveals a complex and intriguing structural composition that contributes to its therapeutic potential. A cross-sectional examination of the leaves highlights various anatomical features, including the presence of starch grains, stone cells, and parenchymatous cells. Notably, the leaves exhibit prominent polygonal stone cells, which are sclerenchymatous in nature and provide mechanical strength to the plant structure. These cells are characterized by their rigid, thick-walled appearance, often isodiametric or polyhedral, indicating significant lignification that enhances their durability. In the epidermal layer, cork cells were observed to be suberin-impregnated, exhibiting a brick or polygonal arrangement. This adaptation is essential for protecting the plant against desiccation and environmental stress. The phloem was predominantly composed of parenchymatous cells, indicating a supportive role in nutrient transport and storage. Thick-walled parenchymatous cells were also prevalent in the xylem, contributing to the structural integrity and functionality of the vascular system.

Microscopic examination of the powdered plant material revealed several noteworthy features. The presence of heterogeneous rays, which varied from uni- to multi-seriate (1–11 seriate), consisted of procumbent cells and sheath cells that play a vital role in the plant's overall architecture. Starch granules of various sizes were abundant, indicating the plant's capability for energy storage. Additionally, fibers were observed, often interspersed with parenchyma strands, forming either aggregated or narrow banded structures. This arrangement highlights the plant's mechanical strength and potential resilience.

The study also identified a high concentration of tannins, which contribute to the characteristic brownish-reddish hue observed in the plant material. The tannins are known for their astringent properties. Furthermore, the fibers were arranged alternately with significant axial parenchyma, much of which was apotracheal, indicating a complex organization that is not readily visible to the naked eye. Overall, the pharmacognostic study of *S. bryopteris* underscores its rich anatomical and phytochemical profile, supporting its traditional uses in medicine and its potential as a source of bioactive compounds. These findings emphasize the need for comprehensive investigations into its pharmacological properties and mechanisms of action (**Figure 1**).



**Figure 1.** Powder Microscopy of *Selaginella bryopteris*.

### Biological activities:

#### Antimicrobial activity of extract:

Extract has modest antibacterial efficacy against *C. albicans*, *B. subtilis*, *E. coli*, *K. pneumonia*, and *A. niger*. *S. bryopteris* showed the most potent antibacterial activity against *B. subtilis*, with ZOI of 16.6 mm and a minimum inhibitory concentration (MIC) of 12.5 µg/mL. According to **Table 4**, it was lower than conventional ciprofloxacin, which had a MIC value of 6.25 µg/mL and a diameter of 26.6 mm. The anti-microbial effect of *S. bryopteris* against *K. pneumonia* was modest at 50 µg/mL, with a ZOI of 11.3 mm. The extract also showed some efficacy against *E. coli*, but not much, with a ZOI of 13.6 mm and a minimum inhibitory concentration (MIC) of 12.5 µg/mL. Results from tests for anti-fungal characteristics indicated that thorn extract significantly inhibited the development of *C. albicans* and *A. niger*. ZOI for *C. albicans* was 18.3 mm at a MIC of 25 µg/mL, while for *A. niger*, it was 17.3 mm at a MIC of 12.5 µg/mL. The extract showed more action against *A. niger* than *C. albicans*, however it is still relatively inactive when compared to fluconazole, which had a 31.6 mm zone of inhibition.

**Table 4.** Antimicrobial activity of *S. bryopteris* extract.

	<i>Candida albicans</i>	<i>Bacillus subtilis</i>	<i>Klebsiella pneumonia</i>	<i>Aspergillus niger</i>	<i>Escherichia coli</i>
<i>Selaginella bryopteris</i>	25.4 ± 0.88 (12.5)	19.3 ± (12.5)	22.4 ± (25)	19.6 ± (12.5)	21.9 ± 1.31*** (50)
<b>Ciprofloxacin<sup>#</sup></b>	-	26.6 ± 1.15*** (6.25)	27.3 ± 0.57*** (6.25)	-	29.6 ± 0.57*** (6.25)
<b>Fluconazole<sup>\$</sup></b>	32.3 ±	-	-	31.6 ±	-

1.15\*\*\*

0.66\*\*\*

(6.25)

(6.25)

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## Characterization of herbal gel formulation

### Extrudability

An impressive extrudability spanning from ++ to +++ was shown by the prepared herbal gels. Extrudability, which allows for simple extrusion from the collapsible tube, falls in tandem with formulation viscosity.

### pH

Herbal gel formulations (F1–F4) have pH values between 6.1 and 6.6, which is quite near to the skin's natural pH range (**Table 4**).

### Physical appearance

The colour, look, and transparency of the gel compositions were assessed visually. They varied in being semi-solid, greasy, a creamy white to colored, and somewhat transparent, regardless of the formula. Massage the ointment across your fingers to feel its consistency, softness, clumpiness, and greaseiness. The developed herbal gel formulations (F1-F4), although somewhat greasy, did not contain any particles, clumps, or aggregations.

### Skin Irritancy

Following a week of using herbal gel formulations (F1-F4), there were no noticeable rashes, swelling, redness, or inflammation detected in the skin irritation test.

### Spreadability

The spreadability of the ointment formulation was found to be rather limited, ranging from 13.76 to 17.81 g.cm/sec, for the herbal gel formulations (F1–F4). An analysis of the relationship between the two variables showed that the spreadability decreases dramatically when the formulation viscosity increases.

### Swelling index

A swelling index ranging from 106% to 115% was noted. Because the gel formulation is matrix-based, the swelling index indicated that the therapeutic material may be released in a controlled manner.

### Viscosity

An important component that affects the spreadability, extrudability, and pourability of pharmaceuticals is their viscosity. Formulations F1–F4 of herbal gels have viscosities between

49,500 and 55,000 cps. Research on rheology has shown that formulation viscosity drops as torque rises because shear stress grows so dramatically.

### Washability Test

All the herbal gel ointment formulations that were produced have an excellent washability feature. In terms of washability, formulation F1 was superior than formulation F3, which had the worst results.

### Accelerated Stability Testing

After short AST, the optimized formulation (F2) showed no significant difference in viscosity, pH, swelling index, spreadability, physical appearance, extrudability, etc. Significant changes of 0.3 units in pH, 1000 cps in viscosity, and 1.14 g.cm/sec in spreadability have been noted, having said that, the investigation did not reveal any alterations to the transparency, smoothness, or physical appearance (**Table 5**). The formulation was stable during the three months, and it will likely stay that way for much longer in tropical and subtropical climates.

**Table 5.** Characterization of developed herbal gel formulations.

Characteristics	F1	F2	F3	F4
Extrudability	++++	+++	++++	++++
pH	6.6	6.9	6.3	6.8
Skin Irritancy	NIL	NIL	NIL	NIL
Spreadability (g.cm/sec)	14.87	18.92	16.78	17.57
Swelling index (%)	113	119	114	121
Viscosity (cps)	56900	51700	53400	52800
Washability	Good	OK	Low	OK

### Antimicrobial study of formulations

**Table 6** displays the findings of the antibacterial screening conducted on various herbal extracts, gel formulations, conventional drugs, and commercially available herbal formulations. The antibacterial activity of the *S. bryopteris* methanol and ethyl acetate extracts was moderate, with an average MIC value against *E. coli* and *B. subtilis*, in contrast to the poor activity of the watery extract. The petroleum ether extract of *S. bryopteris*, on the other hand, showed the strongest antibacterial action, however it was not as strong as the gold standard antibiotic Clindamycin. Formulations (F1–F4) showed fewer efficacies than the commercially available herbal medicine, although they showed results similar to the extracts.

**Table 6.** Anti-microbial activity of herbal extract, gel formulations, standard drug, and marketed herbal formulation.

Components	<i>E. coli</i>	<i>B. subtilis</i>
<i>S. bryopteris</i> aqueous extract	13.6 ± 1.17*** (12.5)	11.7 ± 1.99*** (12.5)

<i>S. bryopteris</i> methanol extract	16.9 ± 1.43*** (12.5)	13.5 ± 1.63*** (12.5)
<i>S. bryopteris</i> ethyl acetate extract	14.2 ± 1.91*** (12.5)	15.8 ± 1.81*** (12.5)
<i>S. bryopteris</i> petroleum ether extract	19.3 ± 1.33*** (12.5)	17.6 ± 1.47*** (12.5)
Clindamycin #	29.9 ± 1.57 (6.25)	28.1 ± 1.15 (6.25)
F1	22.4 ± 1.37*** (25)	20.2 ± 1.47*** (25)
F2	24.1 ± 1.72*** (25)	23.6 ± 1.69*** (25)
F3	20.7 ± 1.44*** (25)	21.9 ± 1.31*** (25)
F4	23.9 ± 1.73*** (25)	22.3 ± 1.52*** (25)
Marketed herbal formulation	24.8 ± 0.96 (12.5)	25.4 ± 0.88 (12.5)

All results indicate mean ± SEM of n = 3; \*\*\*p<0.001. The test chemicals' zones of inhibition against microorganisms are measured in millimetres. The MIC is signified by the values included in the brackets. A reference for antimicrobial activity

## DISCUSSION

*S. bryopteris* has been extensively studied from several angles, including pharmacognostic, physicochemical, pharmacological, and formulation. This plant has a reputation for its traditional medicinal usage and remarkable resilience to adverse settings. The research provides support for the traditional uses of *S. bryopteris* and also indicates novel ways it may be used in contemporary therapeutic settings by combining these several fields of study. For accurate plant material identification and quality control, pharmacognostic studies of *S. bryopteris* have proved vital. In light of the increasing demand for herbal goods throughout the world, it is crucial to conduct thorough analyses of both macroscopic and microscopic traits, such as the shape of the leaves and the characteristics of the spores, to guarantee the plant's authenticity and prevent adulteration. To back this up, the physicochemical study determined important baseline characteristics including moisture content, ash levels, and extractive values—all of which are critical for keeping goods derived from *S. bryopteris* pure, high-quality, and stable. This plant has a long history of usage for improving health, and modern methods have verified the presence of important bioactive components including flavonoids, terpenoids, and phenolics that are thought to be responsible for these benefits.

In particular, *S. bryopteris* has been shown to have antioxidant, anti-inflammatory, and adaptogenic actions, according to pharmacological investigations. This research lends credence to the plant's long-held reputation for stress relief and general well-being, and it is in line with conventional wisdom. Given the involvement of oxidative stress in the onset of several chronic illnesses, the antioxidant activity of *S. bryopteris* is particularly remarkable. The plant's antioxidant and anti-oxidant properties make it a promising candidate for the treatment of neurological illnesses, cardiovascular problems, and age-related ailments. Its anti-inflammatory characteristics further highlight its utility in treating inflammatory disorders, which are often associated with oxidative stress. This study's adaptogenic results show that *S. bryopteris*

modulates the body's stress response by regulating stress hormones and increasing cellular defense systems, which might significantly improve endurance and reduce tiredness.

The study's formulation component has focused on creating new dosage forms, such as topical gels and NDDS, to increase the bioavailability and delivery of chemicals obtained from *S. bryopteris*. These innovations resolve typical problems with herbal remedies, including the inability of active ingredients to dissolve, remain stable, and be bioavailable. The therapeutic effects of *S. bryopteris* are maintained during storage and usage, as proven by stability tests, since these formulations retain their efficacy throughout time. This study's results provide credence to *S. bryopteris*'s historical applications while also paving the way for its potential use in contemporary medicine. To fully grasp *S. bryopteris*'s therapeutic potential, it is necessary to combine pharmacognostic, physicochemical, pharmacological, and formulation investigations. To confirm these results in human patients and investigate the molecular processes behind the reported pharmacological effects, clinical trials should be the focus of future study. For *S. bryopteris* to be widely used in contemporary healthcare, it is vital to establish standardized formulations and dosage forms. The natural products used to prevent and cure a variety of disorders, especially those associated with inflammation and oxidative stress, might benefit from *S. bryopteris*'s promising pharmacological profile. The future of herbal therapy is bright if this old plant is further studied within the context of contemporary science.

## CONCLUSION

The extensive study of *S. bryopteris* from several angles, including pharmacognostic, physicochemical, pharmacological, and formulation considerations, highlights the plant's great medicinal potential and its importance in modern medicine. In order to accurately identify and manage the quality of herbal materials, pharmacognostic analysis has given critical insights into the microscopic and morphological characteristics of the plant. The authenticity of *S. bryopteris* in herbal formulations may be assured thanks to this core knowledge, which reduces the hazards associated with adulteration. Important factors that are vital in determining the purity, quality, and stability of the plant material have been disclosed by physicochemical assessments, such as ash values, moisture content, and extractive values. The existence of bioactive substances such as phenolics, flavonoids, and terpenoids—responsible for the plant's medicinal effects—has been further established by the identification and quantification of important phytoconstituents, made possible by modern chromatographic and spectroscopic methods. *S. bryopteris* has shown a wide range of pharmacological actions, including antioxidant, anti-inflammatory, and adaptogenic mechanisms. These results are in line with the plant's long-established reputation for boosting energy, alleviating stress, and promoting mental clarity. As its molecular processes are better understood, the traditional uses of this compound may be supported by science, and its promise for treating illnesses associated with oxidative stress, protecting neurons, and improving general health can be realized. From a formulation standpoint, the work has shown that *S. bryopteris* may be made much more bioavailable and effective in therapy by creating new dosage forms such as topical gels and nanoparticles. Solid lipid nanoparticles (SLNs) and phytosomes are two

examples of modern delivery technologies that overcome stability and consistency issues to efficiently transport bioactive chemicals from plants to their intended locations. Finally, by integrating its extensive historical use with contemporary scientific evidence, *S. bryopteris* becomes an attractive prospect for the creation of herbal remedies. Our knowledge of *S. bryopteris* is enhanced and new opportunities for its novel use in modern healthcare are created via the combination of pharmacognostic, physicochemical, pharmacological, and formulation investigations. *S. bryopteris* has the potential to become a highly sought-after therapeutic agent on a worldwide scale if future studies concentrate on clinical trials and the creation of standardized formulations. The remarkable integration of ancient wisdom with cutting-edge scientific understanding showcases the power of medicinal plants to revolutionize healthcare and enhance human well-being.

### CONFLICT OF INTEREST

No conflict of interest is declared.

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Not required

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