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Phytopharmacological and toxicological evaluation of Selaginells bryopteris (Sanjeevani) for its anti-ulcer potential

Nishant Tyagi || Arvind kumar
||Sorav Ghosh
S.D college of pharmacy & vocational studies Muzaffarnagar 251001

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I. INTRODUCTION

Selaginellaceae Wilk family is a distinct plant family which includes the genus 'Selaginella'. This genus is found all around the world and is comprised of approximately 700 to 750 plant species. It has wide geographical distribution in tropical regions of America, African continent, Europe, Japan, New Guinea, Australian and Kamchatka peninsula in Russia. It has most abundant presence in Amazon basi with 31 species. Plants belonging to family Sellaginellaceae occur mostly as terrestrial and perennial herbs measuring lesser than 2 cm height. Its roots are characterized by dichotomous branching while its stem is either erect or is seen as a creeper. Its leaves measure roughly 0.5 to 1.0 cm length, have spiral arrangement and have quite often fourth rank on secondary as well as ulitimate branching system. However, variations in height of the plant may be seen with smaller species having 3 cm long stems while few species have lengths measuring between 50 cm to 1 m. [1, 2, 3] It is a dessication tolerant plant that has the capacity for undergoing regeneration characterized by rolling of leaves after coming in contact with humidity and undergoing revival. [4, 5] Selaginella plant is also known as "Spike moss" and is the last surviving species belonging to the fern family, Sellaginellaceae. Tribal populations belonging to Songhati situated in India have used it in paste form via oral application for treatment of beri-beri, diarrhea, dysentery and as a rejuvenating drink. In Madhya Pradesh, India, the Goud tribal people use it as a tonic for strength. In Chattisgarh, India, females in Bastar used the dried herb for curing irregularities of menstruation, leucorrhea and to reduce the pain of labour. [6, 7]

Traditionally, this plant has been used for treating variety of disorders, for example, cardiovascular diseases, diabetes, hepatitis, gastritis, diseases of skin, cancers and infections of urinary tract. [9, 10, 11] Selaginella is known for its hepato-protective nature due to wide variety of bioactive constituents. For example, deluaflavone, sumaflavone, kayaflavone, robustaflavone, podocarpusflavone A, isocryptomerin etc. [7, 8]

Few Selaginella species demonstrate anti-nociceptive, anti-inflammatory, anti-mutagenic, anti-spasmodic, cytotoxic, immunostimulatory and RNA reverse transcriptase inhibition.[12, 13, 14, 15]

Plants belonging to genus Selaginella are rich in steroids, alkaloids, secolignans, caffeoyl, biflavanoids, phenylpropanones, lignans and alkaloidal glycosidic compounds. [16, 17] Ethnomedicinal properties of Selaginella bryopteris can be utilized in treating a variety of conditions such as- heat stroke, burning sensation while micturition, gonorrhea, menstrual problem, uterine diseases, minimizing labour pain, jaundice, solar damage to skin, diseases of stomach, growth-related problems, anti-parasitic, anti-hemorrhoidal agent etc.[16, 17, 18, 19, 20, 21]

Ulcers are characterized by loss of tissue superficially from skin or mucosal surface. They can be of various types depending upon their site of occurrence as- oral ulcers, peptic or genital ulcers. Peptic ulcers are caused by erosion of gastric or duodenal lining. Gastric ulcers are found in stomach lining and are characterized by the presenceof pain. These are commonly found in older gaed individuals. The process of consuming food may result in increase in pain rather than relief. Other associated symptoms of gastric ulcers include- nausea, vomiting and loss of weight. Subjects with ulcers of gastric mucosa may have normal or reduced or even absence of stomach acid. [22]

Aphthous ulcers are the commonest of oral lesions with peak of incidence occurring between 35 to 44 years of age. The terminology "aphthous has derivation from Greek "aphtha" meaning ulcer. These are associated with pain initially. It gets covered by grayish-yellow pseudomemebrane and is surrounded by an erythematous halo. Most common site of occurrence is non-keratinized mucosa. For example, tongue, buccal and labial mucosa. Its etiology is unknown and is based upon its clinical presentation, they are of three types- major, minor and herpetic variety. The most common type is the minor recurrent aphthous ulcers which have a diameter of less than 1 cm. it undergoes healing within 10 to 14 days. This type of aphthous ulcer has prevalence of 70%. The major form of aphthous ulcer has a prevalence between 7 to 20% and it measures greater than 1 cm while the herpetic form of aphthous ulcer is characterized by presence of numerous small-

sized lesions. Its etiology is multi-factorial and include- psychological factors, immunological factors, trauma, family history, malnutrition, blood diseases and certain drugs such as- chemotherapeutic agents and barbiturates. [23, 24]

Thus, based upon various existing literature, this study was planned as an experimental in vitro analysis to analyze the biological active compounds in Selaginella bryopteris and evaluate it s anti-ulcer potential by assessing its anti-oxidative capacity.

II. REVIEW OF LITERATURE

Selaginella bryopteris (also known as 'Sanjeevani') is a pteridophyte demonstrating unique properties of resurrection. It has unique ability to survive even without water where it turns brown while curling up and regains its form soon after contacting water. In India, it is found in Himalayas over mounts KAilash and Rishabh; Dronagiri Hills, Garhwal, Kumaon, Arawali Mountain, Uttar Pradesh, in Satpura hill range in Mandla, Betul, Jabalpur and Hoshangabad. [25, 26, 27]

Classification of Selaginella species

Kingdom:	Plantae
Division:	Lycopodiophyta
Class:	Selaginellopsida
Order:	Selaginellales
Family:	Selaginellaceae
Genus:	*Selaginella*
Common name:	Spike moss

Phytochemistry of Selaginella:
There is very limited literature available on pharmacological activity of various constituents of Sellaginella.

Biological activities of various metabolites of Selaginella:
a) Chromones: Chromone derivatives of this plant- Uncinoside A and B have reported anti-viral activity against respiratory syncytial virus, parainfluenza virus. [28]
b) Lignin derivatives: Cytotoxic effect against murine-derived carcinoma cells has been demonstrated by only Selaginella doederleinii species. No other species of this plant has exhibited anti-carcinogenic properties. [29]
c) Flavanoid derivatives: These compounds have shown various activities such as- anti-bacterial, anti-malarial, anti-viral and anti-oxidant properties. [30, 31, 32, 33, 34]
However, further research is required for exploring all biological constituents of Selaginella and their activities.

Chemical constitution and its relation to properties of resurrection:

This plant has acquired various adaptive features such as- an increase in sugar-based contents like- galactinol, sucrose as well as raffinose which prevent the plant tissues from effects of dehydration and resultant osmotic changes. Also, there are numerous varieties of sugars such as well as sugar alcohol that can protect these plants from harmful effects of cellular dehydration. Also, this herb has demonstrated variations in its bioflavanoid constitution for instance, ametoflavone and hinokiflavones. [34] Additionally, various steroids have also, been isolated from Selaginella species. A steroid is a biologically-active compound containing four-ringed structure steroidal compounds likewise- 22-dehydrocampecterol, 24α-methyl-cholest-5-en-3β-ol, 24β-methyl-cholest-5-en-3β-ol, 24α-ethyl-cholesta-5,22-dien-3β-ol, β-setosterol etc. [36, 37]

Property of tolerating water stress:

Selaginella species can survive severe conditions of drought which is attributed to disaccharide molecule, trehalose and other metabolic products like- betaine (an osmoprotectant), flavanoids and aromatic amino acids. The rehydration process of this plant is due to rematabolization of nitrogen by glutathione metabolism. [38] Selaginaelll bryopteris can overcome drought stress by mechanical, destabilizing and oxidative stress by morphological changes such as curling of leaf, anti-oxidative activity by enzymes such as- superoxide dismutase and proline accumulation. [39] The mechanism of folding of leaf can limit damage due to photo-oxidation as a result of exposure to light by reducing area of transportation and hence, is an important adaptive feature of survival of dehydration. [40]

Healing of wound is a physiological process which restores damaged tissues of the body. Contraction of wound starts in the fibroblastic phase, followed by which it enters into phase of proliferation which is characterized by inflammation, angiogenetic pathway, degradation of collagen, formation of granulation tissue and process of epithelialization. [40, 41, 42, 43, 44, 45]

Bothe edema as well as inflammation are important cardinal signs of inflammation and are important parameters for evaluating anti-inflammatory activity of an agent. Lipid peroxidation is free-radical mediated

procedure and is also, a marker of early as ell as irreversible damage to tissies. Lipid peroxidation causes destruction of biological cell membranes that result in alterations in fluidity as well as permeability. [43] Nitric oxide is synthesized in sites of inflammation by inducible nitric oxide synthase or iNOS. High nitric oxide levels are observed in pathologies of inflammatory origin, circulatory shock as well as carcinogenesis. [44]

Paswan et al (2019) reported 10% greater recovery and healing their in vivo study on wound healing following administration of ethanolic extract of S. bryopteris. Clinical healing was confirmed by use of histopathology examination with dermis exhibiting proliferation of blood capillaries and replacement of skeletal muscle by cellular elements and collagen fiber bundles. [41]

Paswan et al (2017) in their experimental analysis reported no mortality arising from loral administration of Selaginella bryopteris in dosage of 250 to 2000 mg/kg body weight. Topical treatment with non-polar methanol extract (10 mg/20µl) was found to significantky reduce erythema (2.4±0.5); edema (30.4 ± 1) and lipid peroxidase level (32.3 ± 3.2). Similar reductions were noted in nitric oxide, TNF-α, IL-1β and IL-6 levels (8.07 ± 0.55, 69.6 ± 15.5, 7.7 ± 4.8 and 82.6 ± 5.9, respectively). [45]

Singh et al (2017) studied the efficacy of aquous extract of S. bryopteris by causing decrease in apoptosis, production of reactive oxygen species and heat shock protein expression in cryopreserved mesenchymal stem cell culture.[47]

Agrahari et al (2013) in their phytochemical analysis of S. bryopteris reported maximal inhibitory action against release of nitric oxide. [46]

de Seha et al (2012) in their preliminary analysis indicated that ethanolic extract of S. convolute is an effective analgesic agent. Its activity is mediated by inhibitory activity of various peripheral as well as central inhibitory mechanisms. [48]

The aqueous extract prepared from S. bryopteris demonstrates activities that enhance growth and also, provide protective activity against cytotoxic death. [44] Sah et al (2005) in their experimental study using 10% aqueous-based extract of Selaginella bryopteris demonstrated that pre-treating mammalian cells with this extract for a duration of one hour showed protective response against oxidative stress. Thus, there was suppression of thermally induced inhibition of cellular growth. It is particularly useful against heat shock proteins.

Table 1: Table illustrating physical and chemical constituents of aqueous extract of S. bryopteris

Properties	
I. Physical parameters:	
a. Color:	Pale yellow
b. Odor:	Aromatic
c. pH:	5.5 to 5.6
d. Specific gravity:	1.01
II. Chemical constitution:	
Chemical present:	
a. Protein:	19.75 mg
b. Hexoses:	61.85 mg

III. AIM AND OBJECTIVES

The aim of present study was phyto-pharmacological and Toxicological Evaluation of *Selaginella bryopteris* (Sanjeevani) for it's anti-ulcer potential.

Objectives included-

a) Collection and authentication of plant material collected from its geographical source.

b) Pharmacognostical studies to derive its extractive value.

c) Extraction and phytochemical screening of plant material with different solvents

d) Screening of plant for evaluation of its anti-ulcer activity by evaluating its anti-oxidant potential.

IV. MATERIALS AND METHODS

This was an experimental study which involved collection of plant material, authentication, preparation of various extracts, identification of phenolic and flavanoid compounds and assessment of its anti-oxidative potential which is a direct indicator of its anti-ulcer properties.

Following are the armamentarium used in performing the study:

I) Solvents used for extraction:

1) Petroleum ether
2) Carbon tetra-chloride
3) Butanol
4) Ethanol

5) Diethyl ether
6) Aqueous

II) Apparatus used:
1) Soxhlet apparatus
2) Rotary vaccum evaporator

(III) Chemical reagents used:
1) Zinc dust
2) 10% sodium hydroxide
3) Magnesium turnings
4) Concentrated sulphuric acid
5) 10% tannic acid
6) Gelatin
7) Ferric chloride
8) Concentrated hydrochloric acid
9) Mayer's reagent
10) Wegner's reagent
11) Acetic anhydride

(IV) Armamentarium used for quantitative analysis of flavanoids and phenolic compounds present in extracts:
a) **Phenolic derivatives:** Gallic acid (5mg/50 ml); 20% sodium carbonate and 50% Folin-Ciocalteau reagent
b) **Flavanoid derivatives:** 10% aluminium nitrate, 1M potassium acetae and 80% ethanol.

(V) Armamentarium used for HPTLC:
a) **Solvents:** methanol, ethyl acetate, water, silica gel, ready made TLC plates, 1% ethanolic aluminium chloride, iodine chamber, microscopic slides.

b) **Instruments used:** RP-HPLC and UV illumination spectrophotometer.

For determining antioxidant activity:
a) DPPH radical scavenging activity-
1) 1, 1-Diphenyl-2-picrylhydrazyl (DPPH)
2) Ethanol

b) Cartenoid/Linoleic acid assay
1) Carotene
2) Linoleic acid
3) Agar

Extraction

Air-dried leaves of the herb in powdered form in weight of 50g were taken and then, sequential extraction was performed using six solvents- petroleum ether, carbon tetrachloride, di-ethyl ether, butanol, ethanol and water using a Soxhlet apparatus. All the obtained extracts were concentrated by using a rotary vaccum dryer.

Phytochemical tests
I. Flavonoids:
a) Shinoda test: Magnesium turnings were added to the extract followed by drop-wise addition of concentrated hydrochloric acid (HCl). Appearance of scarlet or crimson-red color after few minutes was indicative of flavanoids

b) Alkaline reagent test:
Few drops of 10% sodium hydroxide (NaOH) solution was added drop by drop. There was development of yellow color which turned colorless on addition of few drops of diluted hydrochloric acid (HCl).

c) Zinc Hydrochloride test:

Zinc dust was added to the plant extract followed by drop-wise addition of concentrated hydrochloric acid. Red color will appear after some time (minutes).

Total flavanoid content estimation:

Various concentrations of rutin (ranging between 20 up to 100 µg/ml) were prepared in methanol. Test solution of 100µg/ml concentration was also prepared. An aliquot measuring 0.5ml of diluted sample was then mixed with 2 ml distilled water followed by 0.15 ml of 5% $NaNO_2$ solution. After 6 minutes, 0.15 ml of 10% Aluminium chloride solution was added and was allowed to stand for total of 5 minutes. Followed by this, 2 ml of 4% sodium hydroxide (NaOH) solution was added to this mixture. The final volume was then adjusted upto 5 ml using distilled water and was then, allowed to stand for 15 minutes. Absorbance of solutions were determined at 510 nm against water which was used as blank. Total flavonoid content was then calculated using the Standard regression curve of Rutin.

II. Alkaloids

10 mg of solvent-free extract was mixed with few millilitres of diluted hydrochloric acid and was then, filtered. Obtained filtrate was then tested using different alkaloidal reagents.

a) Mayer's test:

2 drops of Mayer's reagent was added by sides of test-tube to a small quantity of filtrate. Appearance of whitish or cream-colored precipitate confirmed a "positive" result.

b) Wagner's test:

Two drops of Wagner's reagent was added by sides of test-tube to small quantity of filtrate obtained. A positive test reaction was confirmed by appearance of a reddish-brown precipitate.

c) Tannic acid test:

2 to 3 drops of 10% Tannic acid was added to few ml of filtrate. A buff colored precipitate was confirmatory of a 'positive' test..

III. Tests for Steroids or Terpenoids:

a) Libermann-Burchard's test

10 mg of extract was dissolved in 2 milliliters of acetic anhydride. Then, one to two drops of concentrated sulfuric acid was slowly added along sides of the test-tubes. If the solution turns into red color which changes to blue color and finally, green confirms test as 'positive'.

b) Salkowski Reaction:

0.5 ml extract was dissolved in chloroform, then, drop-wise addition of concentrated sulphuric acid was added by side of test-tube. Appearance of red color in upper layer confirmed a 'positive' result.

IV. Tests for Phenolic compounds or Tannins:

a) Ferric chloride test:

10 mg of extract was dissolved in 1 millilites of distilled water to which few drops of neutral 5 % ferric chloride solution was added. Appearance of dark-green or bluish color is confirmatory of phenolic compounds.

b) Gelatin test:

10 mg of extract was dissolved in 1 millilitres of distilled water. To which, 2 ml of 1% gelatin solution which contained 10% sodium chloride was added. Presence of white precipitate was demonstrative of phenolic compounds.

c) Lead acetate test

The extract (10 mg) is dissolved in 1 ml of distilled water and 3ml of 10% lead acetate solution is added. A white precipitate indicates the presence of phenolic compounds.

Total phenolic content estimation:

Total amount of phenolic content within plant extracts was determined by using the 'Folin Ciocalteu reagent'. Various extract concentrations (from 20 to 100µg/ml) of gallic acid were prepared in methanol. 100 µg/ml concentration of plant extract was similarly prepared in methanol. 0.5ml of each sample was then mixed with 2 ml of 10 times diluted Folin Ciocalteu reagent and 4 ml of 7.5 % sodium carbonate. The test tubes were covered using parafilm . They were incubated at room temperature for a duration of 30 minutes with intermittent

shaking. Absorbance values were observed at 765 nm by using methanol as blank. Total phenolic content was calculated by standard regression curve of Gallic acid. Results were expressed as gallic acid equivalent (mg/g).

Thin Layer Chromatography (TLC) analysis:
Silica gel mixture (which was prepared by mixing 25 grams of silica in 50 ml of water) was poured onto microscope slides while forming a thin layer. These slides were then allowed to dry at room temperature for one day. Activation of slides was done at 120 degree Centigrade for a duration of 30 minutes.

Then the TLC chamber was saturated with mobile phase of Ethyl acetate prepared in a solution comprising of Methanol and Water in ratio of 10:1.65:1.35 for approximate period of 30 minutes before placement of TLC plate within the TLC chamber.

4 µl of extract sample were loaded at 1.5 cm distance base. Few minutes of air drying was allowed in between subsequent applications. Following this, TLC plate is then placed within the saturated chamber and was then, allowed to run. After apprximately 10 minutes, Thin Layer Chromatography plate was removed and then, air-dried.

For facilitating visualization, TLC plate was kept within Iodine chamber for a duration of 5 minutes. Brown colored spots appeared indicative of positive test results.

High Pressure Thin Layer Chromatography (HPTLC) analysis:
1 ml each of extract samples were prepared by dilution with Ethanol and Butanol, followed by centrifugation till 5 ml of solution (test so;ution) was obtained.
2 µl of these test solutions were then loaded on 5cm x 10cm Silica gel TLC
plate which was of 0.2 mm thickness).
The loaded plate was then kept in a TLC twin trough developing chamber along with
Ethyl acetate-Methanol-Water solution (10:1.65:1.35) for a total duration of 20 minutes. After this, TLC plate was eluted with mobile phase for up to 80 mm as a solvent.
Followed by this, plate was then dried using warm air for evaporating solvents from surface of the plate. The plate was then kept in Photo-documentation chamber and
images were captured under White and UV light at wavelengths of 254nm and 366nm.
The TLC plate was then sprayed with 1% ethanolic Aluminium chloride reagent and was than dried at a temperature of 120 degree Centigrade in a Hot-air oven for up to five minutes. Then, the plate was photographed at a Ultra-Violet light at a wavelength of 366 nm for Flavonoid detection.

Role in 1-Diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging:
The effectiveness of herbal extracts on DPPH radicals was done as follows-
19 mg of DPPH was dissolved in 500 ml of ethanol. 3.0 ml of the prepared solution was then added to test sample in different concentrations varying between 10µg/ml to 500µg/ml prepared from 1mg/ml of stock solution.
Total volume was prepared for up to 500 µl for various prepared concentrations. The reaction mixtures were then shaken to mix properly and were then incubated for up to 30 minutes at room temperature.
Absorbance value of resultant solution was analyzed at 517 nm while comparing against blank.
Inhibitory percentage of DPPH was calculated as follows-
Scavenging activity (%) = Absorbance (control) –Absorbance (sample) x 100
IC50 value (measured in µg/ml) was the concentration at which the scavenging activity was 50%.

V. RESULTS AND OBSERVATIONS

Table 2: Table showing the percentage yield of each of the extracts

Plant name	Percentage yield (in %)		
	Petroleum ether	Methanol	Ethyl acetate
Selaginella bryopteris	1.56	2.89	2.34

Graph 1: Graph showing percentage yield in each of the plant extract

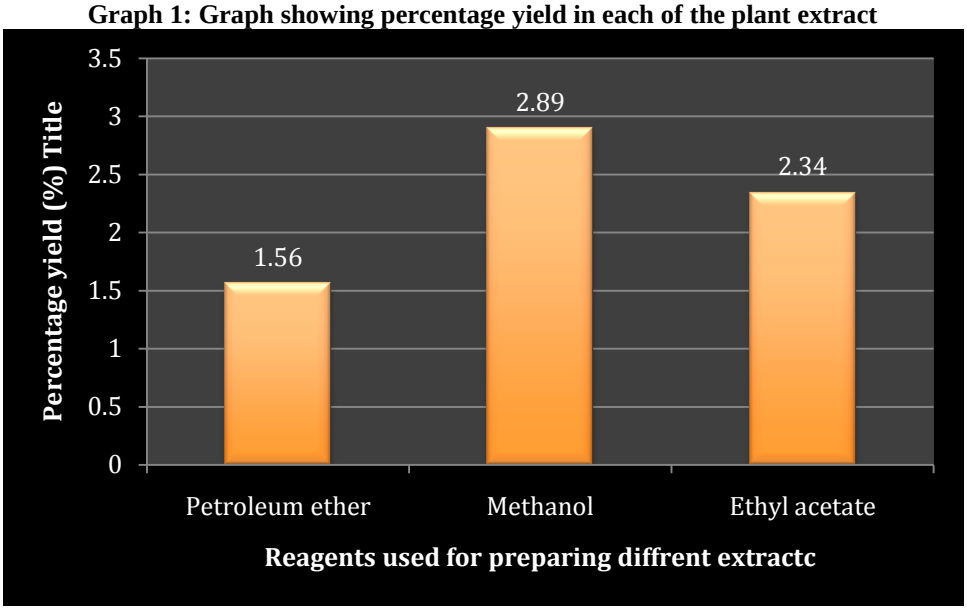


Table 3: Table illustrating total phenolic and flavonoid contents within extracts

Test	Methanolic extracts
Total phenolic content	186.33 ± 0.516mg/gm equivalent to Gallic acid
Total flavanoid content	89.59 ± 2.547mg/gm equivalent to Rutin

Graph 2: Graph showing total phenolic and flavonoid constituents within extracts

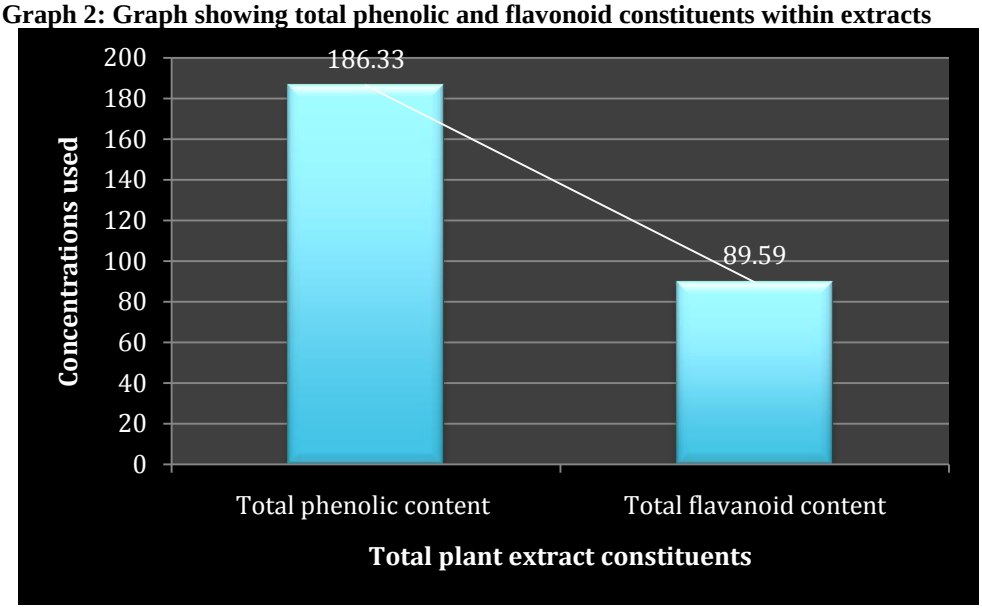


Table 4: Table showing quantitative estimation of phenols in various extracts

Different extracts	Concentration (µg/ml)
Petroleum ether	45
Methanol	39.32
Ethyl acetate	13.21

Graph 3: Graph illustrating quantitative estimation of phenols in various extracts

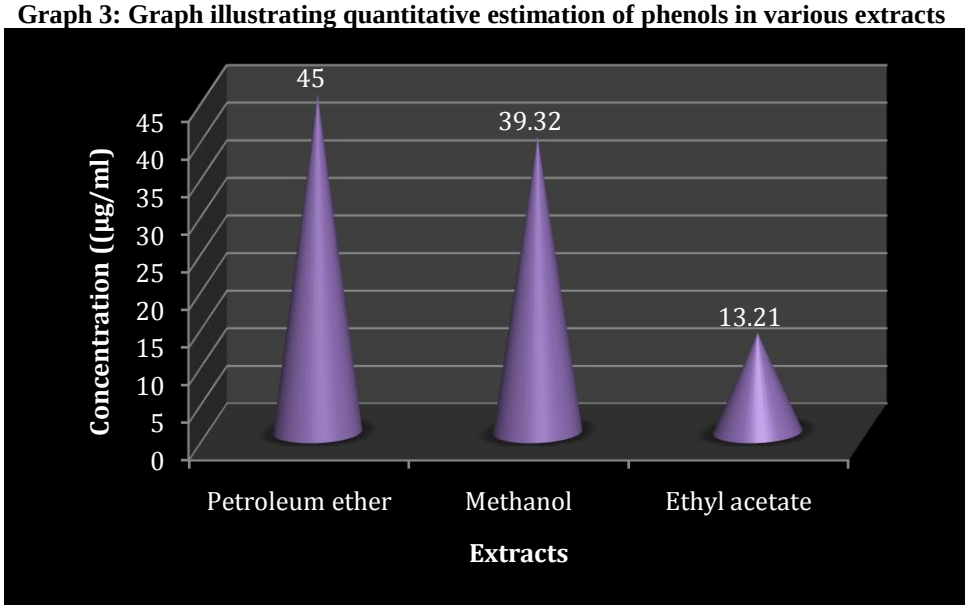


Table 5: Table showing quantitative estimation of flavanoids in various extracts

Different extracts	Concentration (µg/ml)
Petroleum ether	0.16
Methanol	0.45
Ethyl acetate	5.89

Graph 4: Graph demonstrating concentrations of flavanoids in different extracts

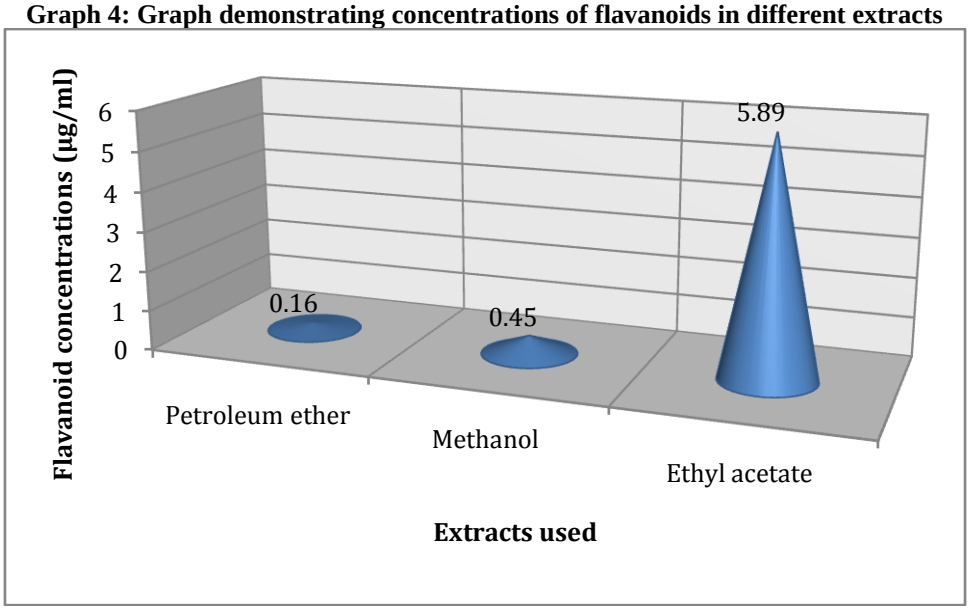
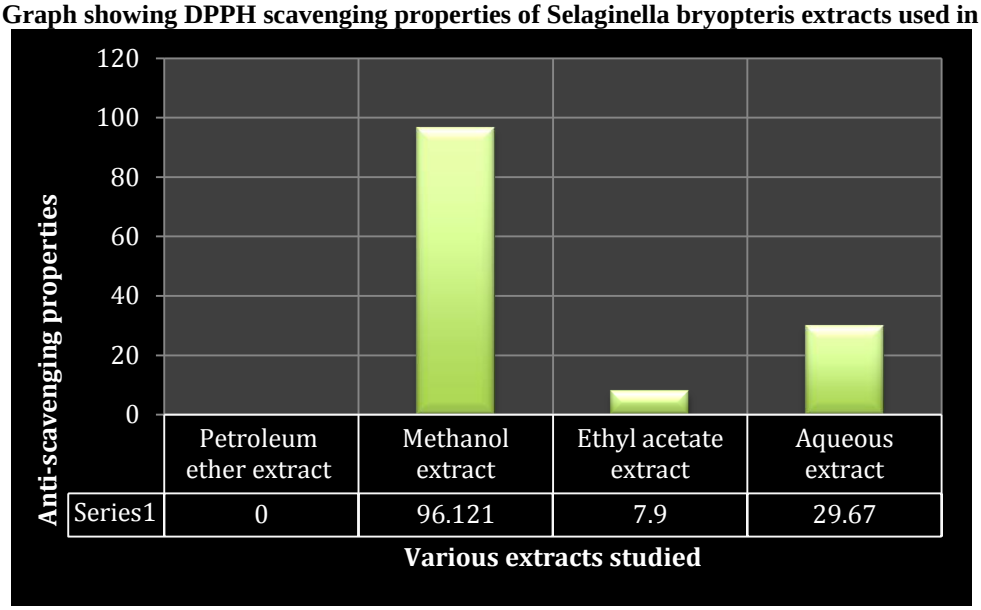


Table 6: Table showing the DPPH- Free radical scavenging properties of various *Selaginella bryopteris* extracts

Extracts prepared	IC50(µg/ml)
Petroleum ether extract	Nil
Methanol extract	96.121 ± 0.022
Ethyl acetate extract	7.9 ± 0.010
Aqueous extract	29.67 ± 0.063

IC50 (µg/ml): It is the amount of extract which is required for 50% reduction of DPPH after a duration of 30 minutes.

Graph 5: Graph showing DPPH scavenging properties of Selaginella bryopteris extracts used in the study



VI. DISCUSSION

Pteridophytes are important part of the diverse flora of found in the Indian subcontinent. Based upon diversity in species these are only next to Angiosperms. Greater than 1200 species of ferns have been reported from Indian soil. The medicinal importance of these Pteridophytes have been known to mankind for over 2,000 years. While comparing these with Angiosperms, they have very limited usage in modern medicine. Hence, the research work on anti-microbial and medicinal activities of pteridophyte plant group is still largely unexplored.

Selaginella species are primitive, seed-less and vascular plant forms with approximately 700 species showing a wide variety of features whuch enables them to be widely distributed predominantly in warmer as well as moist climatic zones. Most of the plants belonging to species of *Selaginella* have been known as 'resurrection plants' as they have the property of curling up in form of a brown ball-shaped structure during dry climate whereas they uncurl turning green in presence of humidity or moisture. They are hetero-sporous meaning that they can produce different spore types- a) micro-spores (male) and b) mega-spores (female).

The genus *Selaginella* is the most neglected group among Pteridophytes though it has several medicinal uses. Several *Selaginella* species are used in traditional medicine in various regions of the world to treat multiple diseases such as cancer, cardiovascular problems, diabetes, hepatitis, skin diseases and urinary tract infections. From over 60 species of *Selaginella* found in India, few species have been used medicinally while four *Selaginella* species, i.e., *S.tamariscina*, *S.chrysocaulos*, *S.rupestris* and *S.bryopteris* have been phytochemically analyzed.

Thus, this study focused upon exploring the biological constituents of Selaginella bryopteris, mainly the phenols and flavanoids as an anti-ulcer agent by means of laboratory analysis.

Crude extracts of plant products obtained after each successive soxhlation extraction procedure were concentrated using a water bath by evaporating the solvents completely for obtaining actual yield following extraction by using petroleum ether, ethyl acetate and methanol. Total yield percentage were found to be- 1.56 %, 2.89% and 2.34% in petroleum ether, methanol and ethyl acetate extracts, respectively (table 1 and graph 1).
The petroleum ether, ethyl acetate and methanol extract of *S. bryopteris* was subjected for screening of their phytochemical constituents. Quantitative phytochemical assay was performed by calculating total phenolic content (TPC) and total flavonoid content (TFC). The TPC was calculated with respect to gallic acid as standard and TFC was calculated with respect to rutin tas standard. The TPC and TFC in methanolic extract were found to be 186.33±0.516 mg/gm and 89.59 ± 2.547 mg/gm, respectively (table 2 and graph 2).

Quantitative estimation of phenolic constituents in various extracts:
The Ethyl acetate extract demonstrated highest concentration of phenols (45 µg/ml) when compared to methanolic extract (39.32 µg/ml) and Petroleum Ether extract (13.21 µg/ml) (table 3 and graph 3).

Quantitative estimation of flavonoid content:

The Ethyl acetate fraction showed highest flavanoid concentration (5.89 µg/ml) when compared with methanol. (0.45 µg/ml) and petroleum ether extracts (0.16 µg/ml). Petroleum ether showed minimal concentration of flavanoid content (table 4 and graph 4).

In present study, flavonoids showed significant positive correlation with the antioxidant activity of the *S. bryopteris* extracts. It was observed that total phenols and flavonoids were present in good amounts in the leaves of *Selaginella bryopteris*. Also, significant correlation was observed between total phenolic content and antioxidant activity of all the extracts. For this purpose, cartenoid-Linoleic acid assay was performed as a preliminary step for detection of anti-oxidant property of *S. bryopteris* extracts.

In present investigation, lower IC50 value (7.9 g/ml) in Ethyl acetate extract was observed suggesting that leaves of this plant may be used as a natural antioxidant in ulcer cases (table 5 and graph 5).

Carotene-Linoleic acid assay:

All extracts of *S. bryopteris* showed significant anti-radical scavenging properties. An antioxidant activity of mean zone of 14 mm of color retention was seen when compared to positive control- 'ascorbic acid' with a retention zone of 18 mm. This anti-oxidant activity of *S. bryopteris* extracts is due to flavonoids and other phenolic compounds present in it.

Thus, present study found significant phytomedicinal values in extract of Selaginella bryopteris as a potential agent for treating ulcers afflicting humans.

VII. CONCLUSION

Phytochemical compounds are biologically active chemical components that are found to occur naturally in various plants. They have been regarded as "secondary form of metabolites" as the plants synthesizing them have little or no requirement of them.

These agents have formed basis for traditional or old medicine and have also been used sometimes, in modern medicine. Thus, assessing any particular phytochemical by isolating, purification as well as characterization is very important as they are used as pharmaceutical product. Also, determining the presence of useful bio-active compounds from any plant is mainlyly dependent upon a particular type of solvent used within the extraction procedure . Thus, in this study various solvents- petroleum ether, methanol and ethyl acetate were used for preparing various extracts and their phenolic as well as flavanoid contents as well as presence was determined. The anti-oxidant property was evaluated by DPHH anti-scavenging method and it was concluded that the ethyl acetate extract od S. bryopteris possess most medicinal anti-ulcerative potential.

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