

Study of Phytochemical Activity and Pharmacological Action of Selaginella Bryopteris for Management of Oxidative Stress

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Abstract: This investigation seeks to identify the numerous phytoconstituents contained in dried plant material. Methanol, ethanol, and distilled water were used to extract the roots and stems for this investigation. Solvents that are water, if you will. Alkaloids, phenols, saponins, glycosides, steroids, flavonoids, amino acids, terpenoids, and carbohydrates were all identified using group-specific phytochemical screening methods. In many parts of the globe, people turn to the reviving and healing abilities of the mystical plant Selaginella bryopteris. Traditional medicine has long relied on S. bryopteris-based remedies for a broad variety of conditions, including spermatorrhea, colitis, epilepsy, leucorrhoea, urinary tract infections, fever, venereal disorders, constipation, beri-beri, and cancer. Numerous in vivo and in vitro models and clinical investigations have been used to examine the medicinal and pharmacological properties of S. bryopteris in recent years. Selaginella bryopteris has been the subject of extensive biochemical and pharmacological research, lending scientific credibility to many of its traditional uses. Antibacterial, growth-promoting, anti-protozoal, relief from heat stroke and the burning sensation during urination, prevention of stress-induced cell death, enhancement of memory, alleviation of stomach-aches, anti-hyperglycemic, and antioxidant properties are just some of the many biological activities it is involved in. Due to its immense pharmacological and therapeutic potential, S. bryopteris is without a doubt one of the most important plants.

Key Words: Selaginella bryopteris, Methanol, Alkaloids, phenols, saponins, glycosides, steroids, flavonoids, amino acids, terpenoids, and carbohydrates.

I. INTRODUCTION

Selaginella bryopteris is a pteridophytic plant which is known for its remarkable resurrection capabilities. The dry plants have been used as a working remedy for several human health complications since centuries in India, particularly in the tribal areas. Briefly, the medicinal uses of S. bryopteris include:

- (i) Relief from hot wave and burning sensation during urination;
- (ii) Restoration to normalcy of menstrual irregularities and given externally to pregnant women for an easy delivery; and
- (iii) For curing jaundice.

Selaginella is a lithophytic xerophyte that grows on the hills of tropical areas, particularly the

Arawali mountain terrains from east to west in India. The plants grow luxuriantly during rains exhibiting a lush green velvety landscape. During summer the plants undergo extreme desiccation. The fronds curl, become dry and virtually dead. In this condition, they look like a closed fist hence often known in Unani as 'punjemariam' or 'hathazori'. The dry plants when left in water unfold their fronds, turn green and come back to active life. Several species of Selaginella have been used as potential herbal medicines.

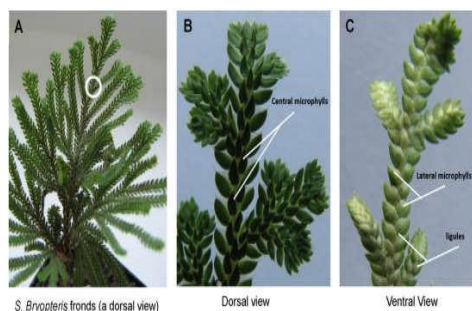


Figure 1: Fronds of *S. bryopteris* growing in a pot (A). Close dorsal view of the frond showing the alternate arrangement of the microphylls on the axis (B). Ventral view of the frond showing the lateral microphylls and the transparent ligule at its base (C).

The genus *Selaginella* is a prominent member of the lycophytes and is one of the oldest genera of vascular plants and includes some 750 species occurring mainly in tropical zones. Phylogenetic reconstructions indicate that the lycophytes are the sister group to the ferns and the seed plants. *Selaginella* species have small leaves or microphylls with a single unbranched vascular bundle and show considerable variation in the numbers, pattern and distribution of their stomata. Stomata first appeared in terrestrial plants about 400 million years ago as a possible adaptation to new and variable environments. The stomata play a central role in the pathways for both the loss of water from plants and the exchange of CO₂.

II. Medicinal Use of *Selaginella Bryopteris*

The traditional utilization of *Selaginella* based on field studies and literature review are presented respectively. It appears that the traditional use *Selaginella* in Java and other islands in Nusantara is still relatively rare, compared to the number of species that grow in this region. At least the local name given shows the little popularity of this plant in the community, this is certainly due to least utilization of the plants. The dominance of Javanese herbal medicine systems (jamu) in traditional medicine in Indonesia and Malaysia, which is generally made from raw materials of about 30 species of cultivated plants, especially rhizomes and spices seem to have put aside the potential use of *Selaginella*, whose availability in nature is affected by seasons. From the field studies, it is known that the *Selaginella* is useful to

treat wounds, menstrual disorders and for treatments before, during, and after giving birth, and to improve fitness and endurance of the body (tonic).

III. Mythology behind *Selaginella Bryopteris*

The popular name Sanjeevani which translates as "One that infuses life" derives from the medicinal properties of the plant. In Hindu mythology, Sanjeevani is a magical herb that has the power to cure any malady. It was believed that medicines prepared from this herb could revive a dead person. This herb is mentioned in the Ramayana when, Ravana (King of demons) hurls a powerful weapon at Lakshmana (brother of Lord Rama) who had killed his beloved son Indrajit. Lakshmana was badly wounded and was nearly killed by Ravana. When Lakshmana fell unconscious, near death, hit by an arrow from Ravana, Hanuman (beloved disciple of Lord Rama) approached the Lankan Royal Physician Sushena for advice. Sushena asked Hanuman to rush to Dronagiri Hills and fetch four plants: Mruthasanjeevani (restorer of life), Vishalyakarani (remover of arrows), Sandhanakarani (restorer of the skin), and Savarnyakarani (restorer of skin color).

IV. Chemical Composition of *Selaginella Bryopteris*

The plants/parts washed with distilled water were dried at 40°C for 72 hours subsequently the dried sample were powdered and stored separately in polyethylene bags for further study. Phytochemical screening of alkaloids, steroids, phenol, saponin and tannins was done following the method of Sinha (1980) and Mahadevan (1982).

Alkaloid: 2gm powdered sample was extracted & filtered. The filtrate was made by acidic by 1% HCl subsequently, the acidic extract was made alkaline with 28% ammonium hydroxide and was extracted with equal volume of Chloroform soluble fraction was tested with Dragendorff's, Mayer's & Wagner's reagent. Any turbidity or precipitation showed the presence of alkaloids.

Steroid: 1gm powdered sample was homogenized with 15 ml petroleum ether and filtered. The ether was evaporated and residue was added with acetic anhydride and few drops of concentrated sulphuric

acid. The pink and blue colour indicates the presence of steroids.

Phenol: Small amount of powdered sample homogenized with 80% ethanol was centrifuged. About 5ml of supernatant was treated with freshly prepared mixture of equal ferric chloride (0.3% FeCl₃ in NH₄Cl and potassium ferricyanide (0.3%). The bluish green or pink colour conferred the presence of phenol.

Tannin: The ethanolic extract of sample was evaporated upto dryness. The residue was dissolved in distilled water and added 5%head acetate. Milky turbidity or white precipitation showed the presence of tannin.

Saponin: The ethanolic extract in ethanol was evaporated then dissolved in water and shake vigorously. A honey comb froth persisting for half an hour indicated the presence of saponin. Confirmatory test was done by crushing about 2 gm powder in Chloroform and adding few drops of conc H₂SO₄ sulphuric acid to the filtrate. Subsequently 1ml of acetic anhydride was added to 1ml of iced filtered. The presence of blue or bluish green or reddish brown colour accompanied with the formation of pink ring confirmed the presence of Saponin.

Results of preliminary phytochemical testing have been presented in the Table 2.1. The result shows that concentration of alkaloid, steroid, phenol, tannin and saponin varied but these active ingredients were also detected in Selaginella plants. However the concentration of alkaloid, steroid and phenol were noted higher in comparison to tannin. But saponin was detected in trace amount. Similar works regarding the phytochemical screening of chemicals substances was done by several earlier workers like Kapoor et.al (1975), Kumari(1997), Prasad et.al(1995). Singh et. al(2014) also studied the phytochemical screening of medicinal plant and found similar results. Recently Kashyap et.al (2020) worked on phytochemical estimation of katarni rice particularly grown in Bhagalpur and Banka district of Bihar and also support the present findings.

Some noteworthy investigator including Ali et.al.(2018), Rimal et.al.(2012), Kibe et al.(2017) and Emmanuel et.al.(2017) have also studied on phytochemical and observed similar results. The

chemical screening of medicinal plants two give basic ideas of what type of chemical constituents present in it and further possible scope in research work.

Table 1: Chemical Screening of Selaginella bryopteris plants.

Chemical Constituents	Amount/Concentration
Alkaloid	+++ (High)
Steroid	+++ (High)
Phenol	+++ (High)
Tannin	++ (Moderate)
Saponin	+ (Trace)

V. Material and Methods

A. Phytochemical Studies

The fresh plant Selaginella bryopteris Wild was collected from international van mela organized by laghu vanopaj sangh in the month of November-December 2018. The plant material was washed thoroughly with running tap water and then leaves were separated and cut into small pieces. The leaves were shade dried at room temperature and dried leaves were crushed and powdered by mortar-pestle. And the powdered material was collected in air-tight plastic jars.

The dried powder of plant leaves of Selaginella bryopteris was weighed accurately. And weighed powdered material was successively extracted with Petroleum ether, Ethyl acetate and Methanol at a temperature range of 60-80 °C by Soxhlet apparatus. 500 gm powdered material macerated with petroleum ether (900 ml) for 48 hrs. And then the defatted powder material was extracted with ethyl acetate and finally methanol solvent by Soxhlet apparatus at low pressure and 40°C temperature.

The leaf powder of Selaginella bryopteris was analyzed to report the physico – chemical characteristics such as pH, moisture content, percentage of total ash content and acid soluble ash by following standard protocols.

Determination of Total Ash Value

Ash values are helpful to determine the quality as well as purity of a Selaginella bryopteris, especially when the plant is present in powdered form. The object of ashing powder is to remove the traces of organic matter which may be interferes in an

analytical determination. On incineration, the crude plant normally produce ash which is usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude plant reveals the care taken during its preparation. A higher limit of acid-insoluble ash is incorporated especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. Some researchers suggested mixing of acids like sulphuric acid with the powdered crude plant before ashing and making the ash sulphated which is normally less fusible than ordinary ash.

Chemicals: Dilute hydrochloric acid

Apparatus: Silica crucible, Dessicator, Ash less filter paper

Sample: leaf powder of *Selaginella bryopteris*

Boil the total ash obtained as the above procedure for 5 minutes and mix 25 ml of dilute hydrochloric acid. Filter and collect the insoluble matter on an ashless filter paper, after that wash the filter paper with hot water, ignite in tared crucible, cool and keep in desiccator. Weigh the obtained residue and calculate acid-insoluble ash of the crude plant (leaf powder of *Selaginella bryopteris*) with reference to the air-dried plant. The unpeeled variety of *Selaginella bryopteris* root must contain not more than 10% of total ash and 2.5% of acid-insoluble ash.

% of Total ash value = $(\text{Weight of total ash} / \text{wt. of total crude material}) \times 100$

Acid-Insoluble Ash

The acid insoluble ash is a measure of the sandy matter and plant body parts including calyx, leaves, etc., which contain higher content of non-combustible acid insoluble matter.

Acid insoluble ash is determined by dissolving ash in dilute hydrochloric acid (10% m/m), the liquid filtered through an ashless filter paper and thoroughly washed with hot water. The filter paper is then ignited in the original dish, cooled and weighed.

Apparatus

Flat-Bottom Dish - of stainless steel, porcelain, silica or platinum.

Muffle Furnace - maintained at $550 \pm 10^\circ\text{C}$.

Desiccator

Reagent

Dilute Hydrochloric Acid (5N)

To the ash contained in the dish, add 25 ml of dilute hydrochloric acid, cover with a watch-glass and heat on a water-bath for 10 minutes. Allow to cool and filter the contents of the dish through a Whatman filter paper No. 42 or its equivalent. Wash the filter paper with water until the washings are free from the acid and return them to the dish. Keep it in an oven maintained at $100 \pm 2^\circ\text{C}$ for about 3 hours. Ignite in a muffle furnace at $550 \pm 10^\circ\text{C}$ for one hour. Cool the dish in a desiccator and weigh. Heat the dish again at $550 \pm 10^\circ\text{C}$ for 30 minutes, cool in a desiccator and weigh. Repeat this process of heating for 30 minutes, cooling and weighing until the difference between two successive weighing is less than 1 mg. Record the lowest weight.

% of Acid insoluble ash = $(\text{Weight of acid insoluble ash} / \text{wt. of crude drug taken}) \times 100$

Phytochemistry of plants defining the chemical profiles of medicinal herbs and an understanding of analytical tests for identification of the herbs and for the qualitative analysis of any known active ingredients. The leaf extracts of the *Selaginella bryopteris* were subjected to phytochemical analysis using standard experimental procedures. Phytochemical investigation were carried out on the ethyl acetate and methanol extracts to identify the bioactive phyto-constituents utilizing standard methods of analysis. The methods used for detection of various phytochemicals were followed by qualitative chemical test to give knowledge regarding the nature of constituents present in crude extracts. Phytochemical analysis of leaves extracts of *Selaginella bryopteris*.

Test for Steroids

Leibermann's Reaction: 2 ml of acetic anhydride

was added to 0.5g of the plant extract and 2 ml of sulphuric acid. The colour change from violet to blue green of the sample indicates the presence of steroids and sterols.

Fest for Carbohydrates

Fehling's test: 1 ml of plant extract, 1 ml of Fehling's solution A and 1 ml of Fehling's solution B were added in a test tube and heated in the water bath for 10 minutes. Formation of yellow or red precipitate indicates the presence of carbohydrates.

Molish test: 2 ml of extract was treated with 2 drops of alcoholic α -naphthol solution in a test tube and then 1ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicated the presence of carbohydrates.

Benedict's test: Equal volumes of Benedict's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5-10 minutes. Solution appears green, yellow or red color in the solution which indicated the presence of reducing sugar.

Test for Tannins

Ferric chloride test: Few drops of 0.1% ferric chloride solution were added with the extract. Formation of blackish blue colour indicated the presence of tannins.

Gelatin test: To the extract 1% solution of gelatin containing 10% sodium chloride added. Formation of white precipitate indicates the presence of tannins.

Test for flavonoids

Shinoda test: To the extract 5ml of 95% ethanol and few drops of conc. HCl and 0.5g of magnesium turnings were added. Appearance of pink coloration or magenta-red colour indicated the presence of flavonoids.

Sodium Hydroxide test (NaOH): The increasing amount of sodium hydroxide added in the extract. Appeared yellow coloration and decolorized after addition of acid showed the presence of flavonoids.

Lead Acetate test: Lead acetate solution added in the small quantity of the extract. Formation of yellow precipitate may indicate the presence of flavonoids.

Alkaline reagent test: The extract was treated with

few drops of sodium hydroxide in a test tube. Formation of intense yellow colour, which becomes colourless on addition of few drops of dilute acid, indicates the presence of flavonoids.

Test for Glycosides

Keller-killiani test: 1-2ml of test solution, 3ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube and carefully 0.5ml of concentrated sulphuric acid added by the side of the test tube. Reddish brown color appeared at the junction of two liquid layers and upper layer turned bluish green indicating the presence of glycosides

Legal's test: 1ml of test solution was dissolved in pyridine. 1ml of sodium nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red color indicates the presence of glycosides.

Test for Alkaloids

To the extract dilute hydrochloric acid was added, shake it well and filtered. With the filtrate following tests were performed.

Dragendorff's test: To 2-3 ml of the filtrate few drops of Dragendorff's reagent was added in a test tube. Formation of red precipitate indicates the presence of alkaloids.

Mayer's test: To 2-3ml of filtrate, few drops of Mayer's reagent was added along sides of tube. Formation of white or creamy precipitate indicates the presence of alkaloids.

Hager's test: To 1-2 ml of filtrate, few drops of Hager's reagent was added in a test tube. Formation of yellow color precipitate indicates the presence of alkaloids.

Wagner's test: To 1-2 ml of filtrate, few drops of Wagner's reagent was added in a test tube. Formation of reddish-brown precipitate indicates the presence of alkaloids

Test for Fats and oils

To 5 drops of the sample was added 1 ml of 1% copper sulphate solution and a few drops of 10% sodium hydroxide. The formation of a clear blue solution confirmed the test.

Test for Triterpenoids & Steroids

Salkowski test: 2 ml of the plant extract was mixed with 2 ml of chloroform and conc. H₂SO₄, to form a layer. Appearance of reddish-brown coloration of the inter face was formed, to show the presence of terpenoids.

Liebermann-Burchard's test: The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two layers, if upper layer turned green, indicate the presence of steroids and formation of deep red colour indicates the presence of triterpenoids.

Test for Saponins

Foam test: The extract was diluted with distilled water and shaken in test tube for about 15 minutes, foam produced persists for 10 minutes. It indicates the presence of saponins.

Test for Phenolic compounds

Ferric chloride test: 2ml extract was dissolved in distilled water and 2ml of 5% ferric chloride solution was added. Formation of blue, green or violet colour the indicates presence of phenolic compounds.

Dilute iodine solution test: To 2-3ml of the extract, few drops of dilute iodine solution were added. Formation of transient red colour indicates the presence of phenolic compounds.

B. Pharmacological Studies

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.

Preparation of herbal extracts

The above said herbs were selected and procured from the specific place. They were washed with water and then powdered. The powder was taken

and extraction was carried out in large scale capacity reactor using 75% methanol and concentrated. The concentrated extract was spray dried and the dried powder was taken to check the antioxidant activity.

DPPH radical scavenging assay

DPPH radical scavenging activity was done using the reported method; the reaction mixture containing 1 mL of DPPH solution (0.1 mmol /L, in 95% ethanol v/v) with different concentrations of the extract was shaken and incubated for 20 min at room temperature and the absorbance was read at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and calculated using the following equation:

$$\text{Effect of scavenging (\%)} = [1 - A_{\text{sample (517nm)}} / A_{\text{control (517nm)}}] \times 100$$

Superoxide radical scavenging assay

The superoxide radical scavenging activity of the extracts was measured according to the literature method. The reaction mixture containing PMS (0.1 mmol/L), NADH (1 mmol/L), NBT (1 mmol/L) in phosphate buffer (0.1 mol/L, pH 7.4) with different concentrations of the extract was incubated at room temperature for 5 min and the color was read at 560 nm against a blank. The scavenging effect was calculated using the following equation:

$$\text{Effect of scavenging (\%)} = [1 - A_{\text{sample (560nm)}} / A_{\text{control (560nm)}}] \times 100$$

Inhibition of microsomal lipid peroxidation

Liver excised from adult male Wister rats, was homogenized (20 g /100 mL tris buffer) in 0.02 mol/L, tris buffer (pH 7.4). Microsomes were isolated by the calcium aggregation method. 100 µL of liver microsomal suspension (0.5 mg protein) was incubated with 1 mmol /L each of FeSO₄ and ascorbic acid with or without extract in a total volume of 1 mL in 0.1 mol/L phosphate buffer (pH 7.4). After incubation at 37 °C for 60 min, TBA (0.67g/100 mL water) was added to the reaction mixture and boiled for 15 min. The TBA reactive substances (TBARS) was calculated from the

absorbance at 535 nm where BHA was used as the positive control.

Measurement of reducing power

The extracts were taken in different concentrations in phosphate buffer (0.2 mol /L, pH 6.6) and incubated with potassium ferricyanide (1 g /100 mL water) at 50 °C for 20 min. the reaction was terminated by adding TCA solution (10 g /100 mL water), centrifuged at 3000 rpm for 10 min and the supernatant was mixed with ferric chloride (0.1 g/100 mL water), the absorbance measured at 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power.

VI. Results and Discussion

Isolation, purification and identification of pure compound AR – I from leaves extracts of *Selaginella bryopteris*. Ethyl acetate and methanolic leaves extracts of *Selaginella bryopteris* submitted for isolation of pure compound by column chromatography and purification by thin layer chromatography and structure elucidation by spectral analysis UV, IR, NMR and Mass spectroscopy.

Ethyl acetate leaves extract of *Selaginella bryopteris* was isolated by glass column chromatography, used silica gel (230 – 400 mesh size, Merck) with ethyl acetate: Methanol (80:20) solvent system ratio as mobile phase and compound AR – I was collected from mixed column of AE₃₂ – AE₄₁ and crystallized with methanol.

Column fractions were further purified by thin layer chromatography and pure compound was collected from silica gel 60 F₂₅₄ TLC plates with solvent system of toluene: acetic acid (60: 20) by volume. Three spots were observed in thin layer chromatography. The bands of developed plates were showed in UV – Inspection cabinet (Labcare) at 254 nm and 365nm were depicted and R_f value of the pure compound was 0.48 and yield 23mg.

Table 2: Showing IR Spectrum of compound AR – I form leaves extract of *Selaginella bryopteris*

S.No.	Wave	Functional groups
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	numbers (cm ⁻¹)	
1.	3454	-O – H (alcohol)
2.	1636	-C = C (aromatic)
3.	1402	-CH ₂ deformation vibrations
4.	677	Out of plane bending may be of O – H

The ¹HNMR spectra of compound AR – I measured in CDCl₃ using Bruker 400 MHz ¹HNMR spectrometer and TMS used as an internal standard. Chemical shifts were given in δ values and expressed in parts per million. The ¹HNMR spectra showed peaks were (δ ppm) δ 1.3 CH₂ at sugars, δ 3.5 O – H protons substituted on benzene, δ 4.0 O – H protons of sugar, δ 4.9 O – H proton of sugars and δ 6.9 aromatic protons.

Table 3: Showing ¹HNMR Spectra of compound AR – I

S.No.	Peak (δppm)	Interpretation
1.	1.3	- CH ₂ of sugars
2.	3.5	- O – H protons substituted on benzene
3.	4.0	- O – H protons of sugars
4.	4.9	- O – H protons of sugars
5.	6.9	Aromatic protons

Elucidated structure of compound AR – I

Name of compound: Quercetin – 3 – glucuronide

Molecular formula: C₂₁H₁₈O₁₃

Molecular weight: 478.36

Melting point : 193 – 195°C

The structure of compound AR – I was elucidated by employing spectroscopic analysis and drawn by ACD/ Chems sketch software version 12.0 trademark of Advanced Chemistry Development.

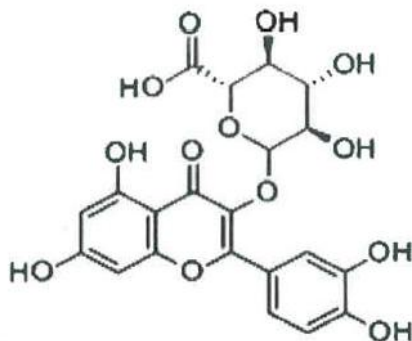


Figure 2: Structure of Quercetin – 3 – glucuronide

Physical Characterization of isolated compound AR – I

Colour	:	Yellow
State	:	Powder
Solubility	:	Ethyl acetate, Methanol and Water
Melting point	:	193 – 195°C
Rf Values	:	0.48

The results of physical, chemical and spectral analysis proved the compound AR – I was 6 – {[2 – (3, 4 - dihydroxyphenyl) – 5, 7 – dihydroxy – 4 – oxo – 4H – Chromen – 3 – yl]oxy} – 3, 4, trihydroxyonane – 2 – carboxylic acid or Quercetin – 3 – glucuronide present in leaves of *Selaginella bryopteris*.

Isolation, purification and identification of pure compound AR – II from leaves extract of *Selaginella bryopteris*.

Methanol extract of *Selaginella bryopteris* leaves was separated by column chromatography purified with thin layer chromatography and structure elucidated by spectral analysis (UV, IR, NMR and Mass spectroscopy).

Column Chromatography of Compound AR – II

Compound AR – II isolated by column chromatography used silica gel as stationary phase and mobile phase composed of CHCl_3 – MeOH with increasing polarity of solvents. Silica gel (230 – 400 mesh size, Merck) was successfully used for the separation of steroidal saponins.

Thin layer chromatography of compound AR – II

To determine the purity and relative to front (Rf) of isolated compound by thin layer chromatography was carried out on silica gel 60 F₂₅₄ plates with

CHCl_3 : MeOH: H_2O (5:3:2) solvent system as mobile phase. TLC of compound AR – II give only one spot. Developed plate visualized in UV and vanillin – sulphuric acid reagent. The Rf value of compound AR-II was 0.35 and the colour of spot was brown. Spot visualized in UV light and blue – violet colour in developing reagents.

UV – Visible Spectra of Compound AR – II

The UV spectra were recorded with a systronics PC based double beam Spectrophotometer 2202. UV – visible spectra showed in 200 – 900nm wavelength. The carbonyl group in steroidal saponins absorbs UV light and ethylene double bond appears at 195 – 198nm. Due to lack of strong chromosphere in some Saponins they do not absorb UV light. The maximum absorption of compound AR-II was 425m, and spectra.

VII. Conclusions

The present study was intended to investigate the phytochemical constituents present in the *Selaginella bryopteris*. To know the chemical constituents of *Selaginella bryopteris*, the histochemical, preliminary phytochemical analysis, fluorescence analysis, spectroscopic and chromatographic analysis such as UV-Vis, FTIR, TLC, HPLC, HPTLC and GC-MS were carried out. In addition, to know the biological potentials of *Selaginella bryopteris*, antioxidant, cytotoxicity using brine shrimp lethality, anticancer using MCF cell line culture, larvicidal, antidiabetic and hepatoprotective activities were also carried out. The histochemical analysis results revealed the variations in the occurrence of various metabolites viz., phenolics, tannins, cutin and suberin and lignin with varied quantities in different tissues of the studied *Selaginella bryopteris* stem. High concentration of lignin was observed in epidermis, hypodermis and xylem of the studied *Selaginella bryopteris*. High concentration of phenolic was occurred in ground tissue and xylem of the studied *Selaginella bryopteris*.

High concentration of tannin presence was observed in the epidermis of studied *Selaginella bryopteris*. The results of histochemical analysis clearly confirmed the occurrence and distribution

of tannin, lignin and phenolic compounds in the studied *Selaginella bryopteris*. Qualitative phytochemical screening of studied *Selaginella* species showed the occurrence of various secondary metabolites viz., steroids, alkaloids, phenolics, flavonoids, saponins, tannin, cardiac glycosides, aminoacids and anthraquinone in the studied three *Selaginella* species. Among the four extracts of *Selaginella bryopteris*, the ethanolic extract of *Selaginella bryopteris* showed the presence of more frequency (88%) of metabolites followed by chloroform extracts (55%) and acetone extract (44%). The least percentage of metabolites (22%) was observed in petroleum ether extracts of *Selaginella bryopteris*.

Among the tested extracts of *Selaginella bryopteris*, the ethanolic and chloroform extracts of *Selaginella bryopteris* showed highest percentage of metabolites (55%) existence. Subsequently, acetone extract showed the occurrence of metabolites with 44% and lowest percentage (11%) of metabolites presence was reported in the petroleum ether extracts of *Selaginella bryopteris*. Among the four screened extracts of *Selaginella bryopteris*, maximum frequencies (60%) of metabolites were observed in the ethanolic extracts of *Selaginella bryopteris*. Next to that the chloroform extracts of *Selaginella bryopteris* showed 55% percentage of metabolites occurrence, followed by acetone extracts (44%). The petroleum ether extracts of *Selaginella bryopteris* illustrated only eleven percentage of metabolites presence.

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