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StudyofPhytochemicalActivityandPharmacological Action ofSelaginella Bryopteris forManagementofOxidative Stress

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Abstract:*Thisinvestigationseekstoidentifythenumerousphytoconstituentscontainedindriedplantmaterial.Methanol, ethanol, and distilled water were used to extract the roots and stems for this investigation. Solvents that arewater,ifyouwill.Alkaloids,phenols,saponins,glycosides,steroids,flavonoids,aminoacids,terpenoids,andcarbohydrates 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 haslongreliedonS.bryopteris-basedremediesforabroadvarietyofconditions,includingspermatorrhea,colitis,epilepsy,leucorrhoea, urinary tract infections, fever, venereal disorders, constipation, beri-beri, and cancer. Numerous in vivoandinvitromodelsandclinicalinvestigations havebeenusedtoexaminethemedicinalandpharmacologicalproperties of S. bryopteris in recent years. Selaginella bryopteris has been the subject of extensive biochemical andpharmacologicalresearch,lendingscientificcredibilitytomanyofitstraditionaluses.Antibacterial,growth-promoting, anti-protozoal, relief from heat stroke and the burning sensation during urination, prevention of stress-inducedcelldeath,enhancementofmemory,alleviationofstomach-aches,anti-hyperglycemic,andantioxidantproperties are just some of the many biological activities it is involved in. Due to its immense pharmacological andtherapeuticpotential,S.bryopterisiswithoutadoubtoneofthemost importantplants.*

Key Words: *Selaginella bryopteris, Methanol, Alkaloids, phenols, saponins, glycosides, steroids, flavonoids, aminoacids, terpenoids,andcarbohydrates.*

I. INTRODUCTION

Selaginellabryopterisisapteridophyticplantwhichisknownforitsremarkableresurrectioncapabilities. The dry plants have been used as aworkingremedyforseveralhumanhealthcomplications since centuries in India, particularlyin the tribal areas. Briefly, the medicinal uses of S.bryopterisinclude:

(i) Relieffromhotwaveandburningsensationduringurination;
(ii) Restorationtonormalcyofmenstrualirregularitiesandgivenexternallytopregnantwomen foran easydelivery; and
(iii) Forcuringjaundice.

Selaginellaisalithophyticxerophytethatgrowsonthehillsoftropicalareas,particularlythe Arawali mountain terrains from east to west inIndia. The plants grow luxuriantly during rainsexhibiting a lush green velvety landscape. Duringsummer the plants undergo extreme desiccation.The fronds curl, become dry and virtually dead.Inthiscondition,theylooklikeaclosedfisthence often known in Unani as ‘punjemariam’ or‘hathazori’.The dry plantswhen left inwaterunfold their fronds, turn green and come back toactive life. Several species of Selaginella havebeen used aspotentialherbalmedicines.

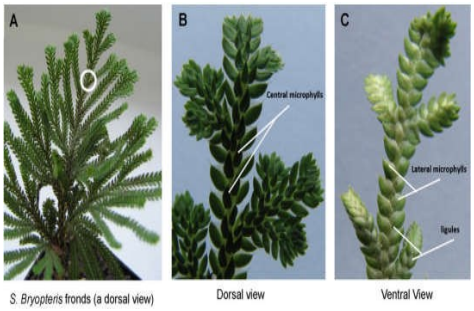

Figure 1: Fronds of S. bryopteris growing in apot (A). Close dorsal view of the frond showingthe alternate arrangement of the microphylls onthe axis (B). Ventral view of the frond showingthe lateral microphylls and the transparent liguleatitsbase(C).

The genus Selaginella is a prominent member ofthe lycophytes and is one of the oldest genera ofvascularplantsandincludessome750speciesoccurri ngmainlyintropicalzones.Phylogeneticre-constructions indicate that the lycophytes are thesistergrouptothefernsandtheseedplants.Selaginel laspecieshavesmallleavesormicrophyllswithasingle unbranchedvascularbundleandshowconsiderablevar iationinthenumbers, pattern and distribution of their stomata.Stomatafirst appearedinterrestrial plants about400 million years ago as a possible adaptation tonew and variable environments. The stomata play acentral role in the pathways for both the loss ofwaterfromplantsandtheexchangeofCO2.

II. MedicinalUseofSelaginellaBryopteris

The traditional utilization of Selaginella based onfieldstudiesandliteraturereviewarepresentedrespe ctively.ItappearsthatthetraditionaluseSelaginellainJ avaandotherislandsinNusantaraisstillrelativelyrare,c omparedtothenumberofspecies that grow in this region. At least the localname given shows the little popularity of this plantinthecommunity,thisiscertainlyduetoleastutiliz ationoftheplants.ThedominanceofJavaneseherbalm edicinesystems(jamu)intraditionalmedicineinIndon esiaandMalaysia,whichisgenerallymadefromrawma terialsofabout30speciesofcultivatedplants,especiall yrhizomes and spices seem to have put aside thepotential use of Selaginella, whose availability innatureisaffectedbyseasons.Fromthe fieldstudies,itisknownthattheSelaginellaisusefulto treatwounds,menstrualdisordersandfortreatments before, during, and after giving birth,and to improve fitness and endurance of the body(tonic).

III. MythologybehindSelaginellaBryopteris

The popular name Sanjeevani which translates as"One that infuses life" derives from the medicinalpropertiesoftheplant.InHindumythology,S anjeevani is a magical herb that has the power tocure any malady. It was believed that medicinesprepared from this herb could revive a dead person.ThisherbismentionedintheRamayanawhen,R avana (King of demons) hurls a powerful weaponatLakshmana(brotherofLordRama)whohad killedhisbelovedsonIndrajit.Lakshmanawasbadly wounded and was nearly killed by Ravana.When Lakshmana fell unconscious, near death, hitbyanarrowfromRavana,Hanuman(beloveddisciplеofLordRama)approachedtheLankanRoyalPhysicia nSushenaforadvice.SushenaaskedHanuman to rush to Dronagiri Hills and fetch fourplants:Mruthasanjeevani(restoreroflife),Vishal yakarani(removerofarrows),Sandhanakarani(restore roftheskin),andSavarnyakarani(restorerofskincolor) .

IV. Chemical Composition of SelaginellaBryopteris

The plants/parts washed with distilled water weredried at 40*C for 72 hours subsequently the driedsamplewerepowderedandstoredseparatelyinpo lyethylene bags for further study. Phytochemicalscreeningofalkaloids,steroids,phenol , saponinandtanninswasdonefollowingthemethodofSi nha (1980)and Mahadevan(1982).

Alkaloid: 2gm powdered sample was extracted &filtered. The filterate was made by acidic by 1%HClsubsequently,theacidicextractwasmadealkal ine with 28% ammonium hydroxide and wasextracted with equal volume of Chloroform solublefraction was tested with Dragendorff's, Mayer's &Wagnar'sreagent.Anyturbidityorprecipitationsho wed thepresenceof alkaloids.

Steroid: 1gm powdered sample was homogenizedwith 15 ml petroleum ether and filtered. The etherwas evaporated and residue was added with aceticanhydrideandfewdropsofconcentratedsulphuri

acid.Thepinkandbluecolourindicatesthepresenceofsteroids.

Phenol:Smallamountofpowderedsamplehomogenized with 80% ethanol was centrifuged.About 5ml of supernatant was treated with freshlypreparedmixtureofequalferricchloride(0.3% FeCl3inNH4Clandpotassiumferricyanide(0.3%). The bluish green or pink colour conferredthepresence of phenol.

Tannin:Theethanolicextractofsamplewasevaporated uptodryness.Theresiduewasdissolvedin distilled water and added 5%head acetate. Milkyturbidityorwhiteprecipitationshowedthepresenceoftannin.

Saponin:Theethanolicextractinethanolwasevaporatedthendissolvedinwaterandshakevigorously. A honey comb froth persisting for halfanhourindicatedthepresenceofsaponin.Confirmatorytestwasdonebycrushingabout2gmpowderinChloroformand addingfewdropsofconcH2SO4sulphuricacidtothefiltrate.Subsequently 1ml of acetic anhydride was added to1ml of iced filtered. The presence of blue or bluishgreen or reddish brown colour accompanied withthe formation of pink ring confirmed the presenceofSaponin.

Results of preliminary phytochemical testing havebeen presented in the Table 2.1. The result showsthatconcentrationofalkaloid,steroid,phenol,tanninandsaponinvariedbuttheseactiveingredients were also detected in Selaginella plants.However the concentration of alkaloid, steroid andphenol were noted higher in comparison to tannin.But saponin was detected in trace amount. Similarworksregardingthephytochemicalscreeningofchemicals substances was done by several earlierworkerslikekapooret.al(1975),Kumari(1997), Prasad et.al(1995). Singh et. al(2014) also studiedthe phytochemical screening of medicinal plant andfoundsimilarresults.RecentlyKashyapet.al(2020)workedonphyotochemicalestimationofkatarni rice particularly grown in Bhagalpur andBanka district of Bihar and also support the presentfindings. SomenoteworthyinvestigatorincludingAliet.al.(2018), Rimal et.al.(2012), Kibe et al.(2017)and Emmanueal et.al.(2017) have also studied onphytochemicalandobservedsimilarresults.The chemical screening of medicinal plants two givebasic ideas of what type of chemical constituentspresent in it and further possible scope in researchwork.

Table1:ChemicalScreeningofSelaginellabryopterisplants.

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

V. MaterialandMethods

A. PhytochemicalStudies

ThefreshplantSelaginellabryopterisWildwascollected from international van mela organized bylaghu vanopaj sangh in the month of November-December2018.Theplantmaterialwas washedthoroughly with running tap water and then leaveswereseparatedandcutintosmallpieces. Theleaves were shade dried at room temperature anddriedleaveswerecrushedandpowderedbymortar-pestle. And the powdered material was collected inair-tightplasticjars.

Thedriedpowderofplantleaves ofSelaginellabryopteris was weighed accurately. And weighedpowdered material was successively extracted withPetroleum ether, Ethyl acetate and Methanol at atemperaturerangeof60-80^{o}CbySoxhletapparatus.500gmpowderedmaterial maceratedwith petroleum ether (900 ml) for 48 hrs. And thenthedefattedpowder materialwas extractedwithethylacetateandfinallymethanolsolventbySoxhletapparatusatlowpressureand40^{o}Ctemperature.

TheleafpowderofSelaginellabryopteriswasanalyzed toreportthephysico–chemicalcharacteristicssuchaspH,moisturecontent,percentage of total ash content and acid soluble ashbyfollowingstandardprotocols.

DeterminationofTotalAshValue

Ash values are helpful to determine the quality aswell as purity of a Selaginella bryopteris, especiallywhen the plant is present in powdered form. Theobject of ashing powder is to remove the traces oforganicmatterwhichmaybeinterferesinan

analytical determination. On incineration, the crudeplantnormallyproduceashwhichisusuallyconsistingofcarbonates,phosphatesandsilicatesofsodium, potassium,calciumand magnesium.The total ash of a crude plant reveals the care takenduringitspreparation.Ahigherlimitofacid-insolubleashisincorporatedespeciallyincaseswhere silica may be present or when the calciumoxalatecontentofthedrugisveryhigh.Someresearcherssuggestedmixingofacidslikesulphuricacid withthepowderedcrude plantbefore ashing and making the ash sulphated whichisnormallylessfusible thanordinaryash.

Chemicals:Dilutehydrochloricacid

Apparatus:Silicacrucible,Dessicator,Ashlessfilterpaper

Sample:leafpowderofSelaginellabryopteris

Boil the total ash obtained as the above procedurefor 5 minutes and mix 25 ml of dilute hydrochloricacid. Filter and collect the insoluble matter on anashless filter paper, after that wash the filter paperwith hot water, ignite in tared crucible, cool andkeep in desiccator. Weigh the obtained residue andcalculate acid-insoluble ash of the crude plant (leafpowder of Selaginella bryopteris) with reference totheair-driedplant.TheunpeeledvarietyofSelaginella bryopteris root must contain not morethan 10% of total ash and 2.5% of acid-insolubleash.

% of Total ash value = (Weight of total ash / wt. oftotalcrude material)x100

Acid-InsolubleAsh

The acid insoluble ash is a measure of the sandymatter and plant body parts including calyx, leaves,etc.,whichcontainhighercontentofnon-combustibleacid insolublematter.

Acid insoluble ash is determined by dissolving ashin dilute hydrochloric acid (10% m/m), the liquidfilteredthroughanashlessfilterpaperandthoroughly washed with hot water. The filter paperisthenignitedintheoriginaldish,cooledandweighed.

Apparatus

Flat-BottomDish-ofstainlesssteel,porcelain,silica orplatinum.

MuffeFurnace-

maintainedat550±10°C.Desiccator

Reagent

DiluteHydrochloricAcid(5N)

Totheash contained inthe dish, add25 ml ofdilute hydrochloric acid, cover with a watch-glassand heat on a water-bath for 10 minutes. Allow tocool and filter the contents of the dish through aWhatmanfilterpaperNo.42oritsequivalent.Wash the filter paper with water until the washingsare free from the acid and return them to the dish.Keep it in an oven maintained at 100 ± 2°C forabout 3 hours. Ignite in a muffle furnace at 550 ±10°Cforonehour.Coolthedishinadesiccatorand weigh. Heat the dish again at 550±10°C for 30minutes,coolinadesiccatorandweigh.Repeatthisprocessofheatingfor30minutes,coolingandweighinguntilthedifferencebetweentwosuccessive weighing is less than 1 mg. Record thelowestweight.

%ofAcidinsolubleash=(Weightof acidinsolubleash/wt.ofcrudedrug taken)x100

Phytochemistryofplantsdefiningthechemicalprofiles of medicinal herbs and an understanding ofanalytical tests for identification of the herbs andforthe qualitativeanalysisofanyknownactiveingredients.TheleafextractsoftheSelaginellabryopterisweresubjectedtophytochemicalanalysisusingstandardexperimentalprocedures.Phytochemicalinvestigationwerecarriedout onthe ethyl acetate and methanol extracts to identifythe bioactive phyto-constituents utilizing standardmethodsofanalysis.Themethodsusedfordetection of various phytochemicals were followedbyqualitativechemicaltesttogiveknowledge regardingthenatureofconstituentspresentincrudeextracts. Phytochemical analysis of leaves extractsofSelaginellabryopteris.

TestforSteroids

Leibermann'sReaction:2mlofaceticanhydride

was added to 0.5g of the plant extract and 2 ml ofsulphuric acid. The colour change from violet toblue green of the sample indicates the presence ofsteroidsandsterols.

FestforCarbohydrates

Fehling'stest:1miofplantextract,1mlofFehling'ssolutionAand1mlofFehling'ssolutionBwereaddedinatesttubeandheatedinthewaterbathfor10minutes.Formationofyelloworredprecipitate indicates the presence of carbohydrates.Molishtest:2mlofextractwastreatedwith 2dropsofalcoholica-naphtholsolutioninatesttubeandthen1mlofconcentratedsulphuricacidwasaddedcarefullyalongthesidesofthetesttube.Formationofvioletringatthejunctionindicatedthepresence ofcarbohydrates.
Benedict'stest:EqualvolumesofBenedict'sreagent 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 redcolorinthesolutionwhichindicatedthepresenceofreducingsugar.

TestforTannins

Ferricchloridetest:Fewdropsof0.1%ferricchloridesolutionwereaddedwiththeextract.Formationofblackishbluecolourindicatedthepresenceoftannins.
Gelatin test: To the extract 1% solution of gelatincontaining 10% sodium chloride added. FormationofwhiteprecipitateindicatesthepresenceoftannIns.

Testforflavonoids

Shinoda test: To theextract Smt of 95% ethanolandfewdropsofcone. HCI and0.5gofmagnesiumturningswereadded.Appearanceofpinkcolorationormagenta-redcolourindicatedthepresenceofflavonoids.
SodiumHydroxidetest(NaOH):Theincreasingamount of sodium hydroxide added in the extract.Appeared yellow coloration and decolorized afteraddition of acid showed the presence of flavonoids.Lead Acetate test: Lead acetate solution added inthesmallquantityoftheextract.Formationofyellowprecipitatemayindicatethepresenceofflavonoids.
Alkalinereagenttest:Theextractwastreatedwith fewdropsofsodiumhydroxideinatesttube.Formation ofintenseyellowcolour,whichbecomescolourless onadditionoffewdrops ofdiluteacid,indicatesthepresence offlavonoids.

TestforGlycosides

Keller-killianitest:1-2mloftestsolution,3mlofglacialaceticacidand1dropof5%ferricchloridewereaddedinatesttubeandcarefully 0.5mlofconcentratedsulphuricacidaddedbythesideofthetesttube.Reddishbrowncolorappearedatthejunction oftwoliquid layersandupperlayerturnedbluish green indicating the presence of glycosidesLegal'stest:1mloftestsolutionwasdissolvedinpyridine.Imlofsodiumnitroprusidesolutionwasaddedandmadealkalineusing10%sodiumhydroxidesolution.Formationofpinktobloodredcolorindicatesthepresenceofglycosides.

TestforAlkaloids

To the extract dilute hydrochloric acid was added,shakeitwellandfiltered.Withthefiltratefollowing testswere performed.

Dragendroff's test: To2-3 ml of the filtrate fewdrops of Dragendroff's reagent was added in a testtube.Formationofredprecipitateindicatesthepresenceofalkaloids.
Mayer'stest:To2-3mloffiltrate,fewdropsofMayer'sreagentwasaddedalongsidesoftube.Formation of white or creamy precipitate indicatesthepresence ofalkaloids.
Hager's test: To 1-2 ml of filtrate, few drops ofHager's reagent was added in a test tube. Formationof yellow color precipitate indicates the presence ofalkaloids.
Wagner's test: To 1-2 ml of filtrate, few drops ofWagner'sreagentwasaddedinatesttube.Formationofreddish-brownprecipitateindicatesthepresence ofalkaloids

TestforFatsandoils

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 bluesolution confirmedthetest.

TestforTriterpenoids&Steroids

Salkowski test: 2 ml of the plant extract was mixedwith2mlofchloroformandconc.H_2SO4,toform a layer. Appearance of reddish-brown coloration ofthe inter face was formed, to show the presence ofterpenoids.

Libermann-Burchard's test: The extract was treatedwithchloroform.Tothissolutionfewdropsofac etic anhydride were added, boiled andcooled.Concentrated sulphuric acid was added through thesides of the test tube. Formation of brown ring atthe junction of two layers, if upper layer turnedgreen,indicatethepresenceofsteroidsandforma tion of deep red colour indicates the presenceoftriterpenoids.

TestforSaponins

Foam test: The extract was diluted with distilledwater and shaken in test tube for about 15 minutes,foam produced persists for 10 minutes. It indicatesthepresence of saponins.

TestforPhenoliccompounds

Ferric chloride test: 2ml extract was dissolved indistilledwaterand2mlof5%ferricchloridesolution was added. Formation of blue, green orvioletcolourtheindicatespresenceofphenoliccomp ounds.

Dilute iodine solution test: To 2-3ml of the extract,fewdropsofdiluteiodinesolutionwereadded.F ormationoftransientredcolourindicatesthepresenceo fphenoliccompounds.

B. PharmacologicalStudies

The fronds (2.5 g) ground with pestle and mortar in100 ml of methanol and sonicated at 33 KHz for 30min using Ultra-sound Sonicator (Qsonica, USA)and filtered by filter paper (Whatman No. 1). Theequal volume (250 µL) of each samples containing30, 40, 50 and 100 mg of S. bryopteris methanolicextractsusedinthepresentstudy.Equivale ntvolume of methanol was also used as control to seethe inhibitory effect due to methanol, if any. As astandard antibiotic, 100 µL (40 mg/mL) gentamicinwasusedaspositivecontrol.

Preparationofherbalextracts

The above said herbs were selected and procuredfrom the specific place. They were washed withwaterandthenpowdered.Thepowderwastaken andextractionwascarriedoutinlargescalecapacityrea ctorusing75%methanolandconcentrated. The concentrated extract was spraydried and the dried powder was taken to check theantioxidantactivity.

DPPHradicalscavengingassay

DPPH radical scavenging activity was done usingthereportedmethod;thereactionmixturecontain ing1mLofDPPHsolution(0.1mmol/L,in95%ethanol v/v)withdifferentconcentrationsofthe extract was shaken and incubated for 20 min atroom temperature and the absorbance was read at517 nm against a blank. The radical scavengingactivitywasmeasuredasadecreaseintheab sorbanceofDPPHandcalculatedusingthefollowing equation:

Effect of scavenging (%) = $[1\text{-}A_{sample\ (517nm)} /A_{control(517nm)}]\times100$

Superoxideradicalscavengingassay

The superoxide radical scavenging activity of theextracts was measured according to the literaturemethod. The reaction mixture containing PMS (0.1mmol/L), NADH (1 mmol/L), NBT (1 mmol/L) inphosphate buffer (0.1 mol/L, pH 7.4) with differentconcentrations of the extract was incubated at roomtemperature for 5 min and the color was read at 560nmagainstablank.Thescavengingeffectwascalcu latedusingthefollowingequation:

Effectofscavenging(%)=$[1\text{-}A_{sample(560nm)}/A_{control(560nm)}]\times100$

Inhibitionofmicrosomallipidperoxidation

LiverexcisedfromadultmaleWisterrats,washomogen ized (20 g /100 mL tris buffer) in 0.02mol/L,trisbuffer(pH7.4).Microsomeswereisolat edby thecalciumaggregation method. 100µLofliver microsomalsuspension(0.5mgprotein)was incubated with 1 mmol /L each of FeSO4 andascorbicacidwithorwithoutextractinatotalvolum e of 1 mL in 0.1 mol/L phosphate buffer (pH7.4). After incubation at 37 ^oC for 60 min, TBA(0.67g/100 mL water) was added to the reactionmixture and boiled for 15 min. The TBA reactivesubstances(TBARS)wascalculatedfromthe

absorbanceat535nm9whereBHAwasusedasthepositivecontrol.

Measurementofreducingpower

The extracts were taken in different concentrationsinphosphatebuffer(0.2mol/L,pH6.6)andincubatedwithpotassiumferricyanide(1g /100mL water) at 50 ^{o}C for 20 min. the reaction wasterminatedbyaddingTCAsolution(10g/100mL water), centrifuged at 3000 rpm for 10 min and thesupernatantwasmixedwithferricchloride(0.1g/100 mL water), the absorbance measured at 700nm.Theincreasedabsorbanceofthereactionmixturereindicated increasedreducingpower.

VI. ResultsandDiscussion

Isolation,purificationandidentificationofpurecompoundAR–IfromleavesextractsofSelaginellabryopteris.Ethylacetateandmethanolic leaves extracts of Selaginellabryopterissubmittedforisolationofpurecompoundby columnchromatography and purification by thinlayer chromatography and structure elucidation byspectralanalysisUV,IR,NMRandMassspectroscopy.

EthylacetateleavesextractofSelaginellabryopteriswasisolatedbyglasscolumnchromatography, used silica gel (230 – 400 meshsize, Merck) with ethyl acetate: Methanol (80:20)solventsystemratioasmobilephaseandcompoundAR–IwascollectedfrommixedcolumnofAE_{32}–AE_{41}andcrystallizedwithmethanol.

Columnfractionswerefurtherpurifiedby thinlayerchromatographyandpurecompoundwascollected from silica gel 60 F_{254}TLC plates withsolvent system of toluene: acetic acid (60: 20) byvolume. Three spots were observed in thin layerchromatography.Thebandsofdevelopedplateswere showed in UV – Inspection cabinet (Labcare)at 254 nm and 365nm were depicted and Rf valueofthepurecompoundwas0.48 andyield23mg.

Table 2: Showing IR Spectrum of compound AR – IformleavesextractofSelaginellabryopteris

S.No.	Wave numbers (cm^{-1})	Functionalgroups
1.	3454	-O–H(alcohol)
2.	1636	-C=C(aromatic)
3.	1402	-CH_2 deformation vibrations
4.	677	Outofplanebending

The ^{1}HNMRspectra of compoundAR – I measuredin$CDCl_3$usingBruker400MHz^{1}HNMR spectrometer and TMS used as an internalstandard. Chemical shifts were given in δvaluesand expressed in parts per million. The ^{1}HNMRspectra showed peaks were (δ ppm) δ 1.3 CH_2atsugars, δ 3.5 O – H protons substituted on benzene,δ4.0O–Hprotonsofsugar,δ4.9 O–Hprotonof sugarsandδ6.9aromatic protons.

Table 3: Showing ^{1}HNMR Spectra of compoundAR– I

S.No.	Peak (δppm)	Interpretation
1.	1.3	-CH_2ofsugars
2.	3.5	- O – H protons substitutedonbenzene
3.	4.0	-O–Hprotonsof sugars
4.	4.9	-O–Hprotonsof sugars
5.	6.9	Aromaticprotons

ElucidatedstructureofcompoundAR–I
Nameofcompound:Quercetin–3–glucuronideMolecularformula: $C_{21}H_{18}O_{13}$
Molecular weight: 478.36
Melting point : 193–195^{0}C
The structure of compound AR – I was elucidatedby employing spectroscopic analysis and drawn byACD/Chemsketchsoftwareversion12.0trademarkofAdvanced ChemistryDevelopment.

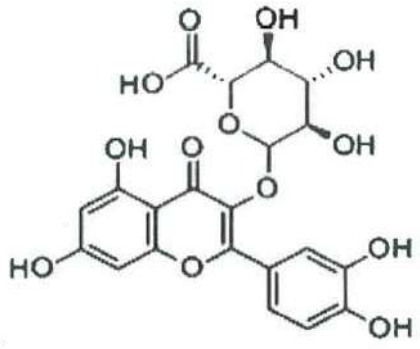

Figure2:StructureofQuercetin–3–glucuronide

PhysicalCharacterizationofisolatedcompoundAR– I

Colour	:	Yellow
State	:	Powder
Solubility	:	Ethyl acetate,Methanol andWater
Meltingpoint	:	193–195^{0}C
RfValues	:	0.48

Theresultsofphysical,chemicalandspectralanalysisprovedthecompoundAR–Iwas6–{[2–(3, 4 - dihydroxyphenyl) – 5, 7 – dihydroxy – 4 –oxo– 4H–Chromen–3–yl]oxy}–3,4,trihydroxyonane–2–carboxylicacidorQuercetin
– 3 – glucuronide present in leaves of Selaginellabryopteris.
Isolation,purificationandidentificationofpurecompoundAR–IIfromleavesextractofSelaginella bryopteris.

Methanol extract of Selaginella bryopteris leaveswas separated by column chromatography purifiedwiththinlayerchromatographyandstructureelucidated by spectral analysis (UV, IR, NMR andMassspectroscopy).

ColumnChromatographyofCompoundAR–II

CompoundAR–IIisolatedbycolumnchromatography used silica gel as stationary phaseandmobile phase composed of $CHCl_3$–MeOHwithincreasingpolarityofsolvents.Silicagel(230
– 400 mesh size, Merck) was successfully used forthe separationofsteroidalsaponins.

ThinlayerchromatographyofcompoundAR–II

To determine the purity and relative to front (Rf) ofisolated compound by thin layer chromatographywascarriedoutonsilicagel60F_{254}plateswith $CHCl_3$:MeOH:H_2O(5:3:2)solventsystemasmobile phase. TLC of compound AR – II give onlyone spot. Developed plate visualized in UV andvanillin – sulphuric acid reagent. The Rf value ofcompound AR-II was 0.35 and the colour of spotwas brown. Spot visualized in UV light and blue –violet colourindevelopingreagents.

UV–VisibleSpectraofCompoundAR–II

The UV spectra were recorded with a systronics PCbased double beam Spectrophotometer 2202. UV –visible spectra showed in 200 – 900nm wavelength.The carbonyl group in steroidal saponins
absorbsUVlightandethylenedoublebondappearsat195
– 198nm. Due to lack of strong chromosphere insome Sapogenins they do not absorb UV light. ThemaximumabsorptionofcompoundAR-IIwas425m,andspectra.

VII. Conclusions

The present study was intended to investigate thephytochemicalconstituentspresentintheSelaginellabryopteris.Toknowthechemicalconstituentsof Selaginellabryopteris,thehistochemical,preliminaryphytochemicalanalysis,fluorescenceanalysis,spectroscopicandchromatographic analysis such as UV-Vis, FTIR,TLC, HPLC, HPTLC and GC-MS were carriedout. In addition, to know the biological potentialsofSelaginellabryopteris,antioxidant,cytotoxicityusingbrineshrimplethality,anticancerusingMCFcelllineculture,larvicidal,antidiabetic and hepatoprotective activities werealsocarriedout.Thehistochemicalanalysisresults revealed the variations in the occurrenceofvariousmetabolitesviz.,phenolics,tannins,cutinandsuberinandligninwithvariedquantitiesindifferenttissuesofthestudiedSelaginella bryopteris stem. High concentrationof lignin was observed in epidermis, hypodermisand xylem of the studied Selaginella bryopteris.High concentration of phenolic was occurred ingroundtissueandxylemofthestudiedSelaginella bryopteris.

Highconcentrationoftanninpresencewasobserved in the epidermis of studied Selaginellabryopteris. The results of histochemical analysisclearlyconfirmedtheoccurrenceanddistribution

of tannin, lignin and phenolic compounds in thestudiedSelaginellabryopteris.Qualitativephyto chemical screening of studied Selaginellaspeciesshowedtheoccurrenceofvarious secondarymetabolitesviz.,steroids,alkaloids,phen olics, flavonoids, saponins, tannin, cardiacglycosides, aminoacids and anthraquinone in thestudied three Selaginella species. Among the fourextracts of Selaginella bryopteris, the ethanolicextractofSelaginellabryopterisshowedth epresence of more frequency (88%) of metabolitesfollowedbychloroformextracts(55%)a ndacetone extract (44%). The least percentage ofmetabolites(22%)wasobservedinpetroleumethe rextractsofSelaginellabryopteris.

AmongthetestedextractsofSelaginellabryopteris, the ethanolic and chloroform extractsofSelaginellabryopterisshowedhighestper centageofmetabolites(55%)existence.Subsequentl y,acetoneextractshowedtheoccurrence of metabolites with 44% and lowestpercentage(11%)ofmetabolitespresencewa sreportedinthepetroleumetherextractsofSelaginell a bryopteris. Among the four screenedextractsofSelaginellabryopteris,maximu mfrequencies (60%) of metabolites were observedin the ethanolic extracts of Selaginella bryopteris.Nexttothatthechloroformextractsof Selaginella bryopteris showed 55% percentage ofmetabolitesoccurrence,followedbyacetoneextra cts (44%). The petroleum ether extracts ofSelaginellabryopterisillustratedonlyelevenperce ntageofmetabolitespresence.

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