WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 9, 1370-1378. Research Article ISSN 2277– 7105

PRELIMINARY PHYTOCHEMICAL SCREENING OF DESICCATED FRONDS OF *SELAGINELLA BRYOPTERIS* (L) BAKER (PITTAKALU)

[1]P. Rupa* and [1]N.Lakshmi Bhavani

Plant Tissue Culture and Plant Molecular Genetics Lab
Department of Botany, University College of Science, Saifabad, Osmania University,
Hyderabad 500004, India.

Article Received on
15 September 2014,

Revised on 09 Oct 2014,
Accepted on 02 Nov 2014

***Correspondence for Author**
P. Rupa
Department of Botany, University College of Science, Saifabad, Osmania University, Hyderabad500004, India.

ABSTRACT

The present work has been carried on the fern *Selaginella bryopteris* (L) Baker which belongs to family Selaginellaceae. It shows high desiccation tolerance, during summer. The fronds are folded inside and form as fist, the desiccated plant rehydrates back with water availability.The present study aims to screen the presence of the various types of phytoconstituents in desiccated plant. For this study both root and stem were extracted using methanol, ethanol and distilled water as solvents. Specific phyto chemical screening tests for each group were adopted to find various phytoconstituents like alkaloids, phenols, saponins, glycosides, steroids, flavonoids, aminoacids, terpenoids, and carbohydrates. The present study revealed the presence of alkaloids, amino acids, tannins, glycosides, steroids, flavonoids and carbohydrates in both root and stem extracts. But phenols, anthraquinones and saponins are absent in both root and in stem extraction. Terpenoids are present only instemextraction (not screened in aquacious extracts). Phytochemicals are separated effectively in methanol solvent followed by ethanol and distilled water.

KEYWORDS: PhytoConstituents, *Selaginellabryopteris*, Root and Stem.

INTRODUCTION

Selaginella bryopteris is a Pteridophyte belonging to the family Selaginellaceae. It prefers to grow in rocky, shady habitats especially in dry hills. It is also distributed in arid and semi arid zones of southern India, Odessa and Occasionally found throughout the Telangana and Rayalaseema region of Andhra Pradesh. The plant grows laxatively during rainy season and

form as green pads on the surface of the rocks. The plant has adopted drought resistance. During summer the plant show high desiccation, the fronds are folded in side and looks as fist or folded legs of birds hence called as "Pittakala mokka" or "Pitta kalu".In the presence of moisture it rehydrates back. From ancient period several Pteridophytes are being used in Ayurveda and Unani medicine in India and Western Asia. The Pteridophytes are found to have ethno medicinal importance and are used by the local and tribal people. The Pteridophytes show various economic values towards food and fodder indicators, bio fertilizers, insect repellents, medicine and folk medicines (Ghosh et al., 2004).

Sellaginella Spp are traditionally used to cure several diseases like wounds, fever, cancer, pneumonia, tonsils, kidney stones, headache, hepatitis, fever, paralysis skin diseases, bone fractures, jaundice, toothache, blood coagulation, diarrhea, gastric ulcers, asthma, backache, blood purification, fatigue, and to neutralize the poison of snake and scorpion bites (Dixit and Bhatt 1974, Bouquet 1992 and Lee et al.1996, Batugal et al 2005, Setyawan and Darusman 2008 and Ahmad dwi Setyawan 2011). Recent researches proved that *Selaginella* spp have secondary metabolites like bioflavonoid (Silva etal., 1995 and Lee etal.,1996), tannins (Chickmavathi etal.,2008), glycosides (Reena Antony et al., 2011& Jithender Malviya etal.,2012)) alkaloids (Zheng et al.,2011) and Selaginellin (Zhang etal, 2007 : Cheng et al., 2008).

*S. bryopteris(L.)*Bark has been used in Indian herbal medicine from olden days. Extraction obtained from the stem of *S.bryopteris (L.)*Bak showed the antibacterial activity against *Niesseria gonorrhea* and the paste of leaves is used in Spermatorrhea, leucorrhoea, for stomachache and urinary tract inflammation in children (Jitender Malviya etal., 2012). Ethanolic extract of *S.bryopteris (L)* Bak can cure stomachache (Pandey etal.,1993). Water extract of *S.bryopteris (L.)*Bak reduces cell death caused by UV irradiation (Sah etal. 2005). The extract of *S.bryopteris (L.)* Bak increased cell growth and protected against dead cells induced by oxidative stress (Sah etal., 2005). *S.bryopteris (L.)*Bak is treated as anti-inflammatory and cures venal diseases (Agarwal and Singh 1999). Amentoflavone and hinkoflavne from *S.bryopteris (L.)*Bak have anti protozoan activity against *Plasmodium falciparum, Leismania donovani and Trypanosoma* spp (Kunert etal., 2008).

MATERIALS AND METHODS

Collection of the Plant Material

The desiccated plant material was collected in the month of December, 2013 from hill and rocky region located at 1.5 K.M far from Ekambary temple which is famous ancient Lord Siva Temple in Bhasheerabad mandal, Rangareddy District, Telangana, India. The GPS (Global Position System) of the location is Latitude 17^0 8'29-31" N,Longitude $77^0$27'10-22"E, Accuracy 15m -21m,Altitude- 417m and Alt-Accuracy-18m.

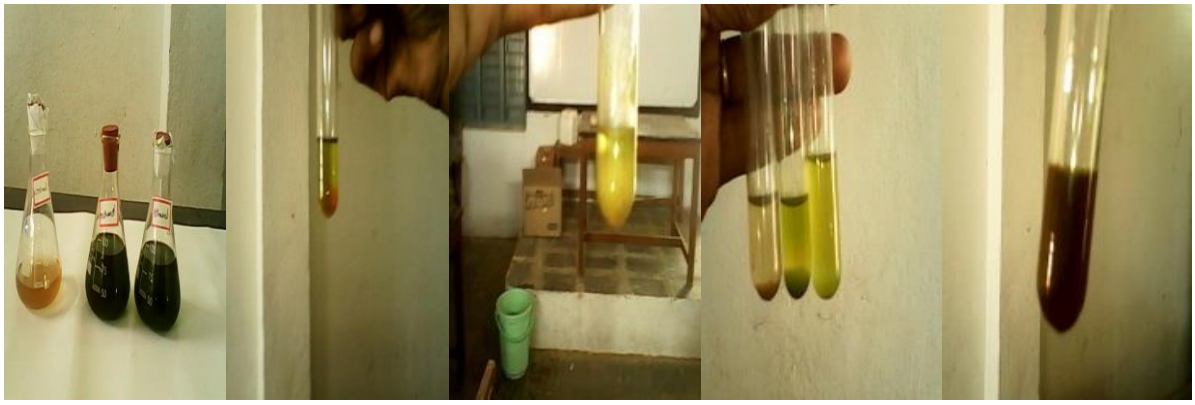
Identification of the Plant and Its Authentification

Identification and authentification of the Plant has made by **Dr. VastsavayaS. Raju**, Retired Professor, Former Head and Chairperson, BOS, Plant Systematics Laboratory, Department of Botany, Kakatiya University, Warangal, Telangana, India and He has identified the plant as ***Selaginellabryopteris* (L.) Baker** (Selaginellaceae). It is locally known as '***Pitta kalu***'.

Plant Extraction

Collected plant materials were separated as roots and stems, washed thoroughly in the tap water till the soil was clear and then allowed to dry in open air.After 3 days,dried materials were made as fine powder by mechanical grinding. About 10 gm of powder of stem was mixed separately with 100ml of methanol, ethanol and distilled water and subjected to 72 hours of continuous stirring under room temperature with the help of rotatormagnetic stirrer. After three days the plant extracts were filtered with No.42 what man filter paper twice or thrice. The filtrate was collected into air tight, sterilized and labeled bottles and preserved in 4^0C of temperature for future usage.The same procedure is followed for root extraction also.

(a) **(b)** **(c)** **(d)**



(e) (f) (g) (h) (i)

a&c: *Selaginellabryopteris* plant, b: Desiccated fronds of *Selaginellabryopteris*,d:Root &Stem fine powder,e: Extraction of *Selaginellabryopteris* and f-i:Test results.

Phyto Chemical Screening Tests

Followingphyto chemical tests were adopted from S.P.Dhanbal2003, JaliwalaT. Aetal, 2011 Jyothi Chaithanyaet al., 2013 and Dutta Jaya shree, 2013.

Detection of Alkaloids (Mayer's Reagent Test)

5 ml of 2N HCL was added to 5 ml of plant extract, boiled, then filtered; then 2 drops of Mayer's reagent is added to filtrate. Appearance of a cream colour precipitation indicates the presence of alkaloids.

Detection of Aminoacids (Ninhydrin Reagent Test)

About 2 drops of Ninhydrin solution was added to 2 ml of plant extract. Appearance of purple colour indicates the presence of amino acids.

Detection of Anthraquinones (Nitricacid Test)

5ml of plant extract was added with 2ml of nitric acid. Appearance of Pale Brown precipitation indicates presence of anthraquinones.

Detection of Carbohydrates (Fehling Test)

1ml of plant extract was added to 5 to 10 drops of Fehling's solution. The mixer was subjected to boiling for15minutes. Appearance of brick red Precipitation indicates the presence of carbohydrates.

Detection of Cardiac Glycosides (Keller –Killani Test)

0.5 ml of plant extract was added with few drops of glacial acetic acid (boil and cool), then

Few drops of $Fecl_3$ solution is added. Transfer the content slowly to the test tube containing 2 ml of Conc. H_2SO_4 from the walls of the test tube.Appearance of reddish brown ring at the junction of two solvents indicates the presence of Cardiac glycosides**.**

Detection of Flavonoids (Alkaline Reagent Test)

2ml of plant extract was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Detection of Phenols (Ferric Chloride Test)

2 ml of 5% ferric chloride solution was added to 2ml of plant extract. Appearance of bluish green colour solution indicates the presence of phenols.

Detection of Saponins (Foam or Forth Test)

A small quantity of plant extract was diluted with 20 ml of distilled water. The suspension was shaken vigorously in a graduated test tube for 15 minutes. A two centimeters layer of foam or forth which is stable for 10 minutes indicates the presence of saponins.

Detection of Steroids (Salkowski Test)

1ml of plant extract was added to 1ml of concentrated H_2SO_4. Appearance of wine red color indicates presence of steroids.

Detection of Tannins (Lead Acetate Test)

Tannins were tested by adding a few drops of 1%lead acetate to 5 ml of plant extract. Appearance of yellow precipitation indicates the presence of tannins.

Detection of Terpenoids (Acetic Anhydride Test)

1ml of the plant extract was dissolved in 1ml of chloroform, and then 1ml of acetic anhydride was added following the addition of 2ml of concentrated sulphuric acid, formation of reddish color indicates the presence of terpenoids.

RESULTS

Table-1 indicatesthe presence and absence of various phyto constituents in the stem extraction of *Selginellabryopteris* which revealed presence of alkaloids, amino acids, carbohydrates, cardiac glycosides, flavonoids, steroids, tannins and terpenoids (not found in distilled water extract) whereas anthroquinones, amino acids, phenols and saponins were

absent Table -2 denotes the results of various tests of phyto chemical analysis in the root extract of *Selaginellabryopteris*. This reveals that terpenoids, saponins, phenols and anthraquinones were absent, where as alkaloids, amino acids, flavonoids, carbohydrates, steroids, cardiac glycosides, tannins were present.

Table 1. Phyto constituents of desiccated stem extract of *Selaginella bryopteris*(L) Baker.

Sc.No.	Name of the Phytochemical	ethanol	Ethanol	Distilled water
1	Alkaloids	+++	++	+
2.	Amino acids	+++	++	+
3.	Anthraquinones	_	_	_
4.	Carbohydrates	++	+++	+
5.	Cardiac glycosides	+++	+++	+
6.	Flavonoids	+++	++	+++
7.	Phenols	_	_	_
8.	Saponins	_	_	_
9.	Steroids	++	+++	+
10.	Tannins	+++	++	+
11.	Terpenoids	+++	++	_

(+++) = Strongly present (++) = Moderately present

(+) = Poorly present (-) = Absent

Table2: Phyto constituents of desiccated root extract of *Selaginellabryopteris* (L) Baker.

S. No.	Name of the Phytochemical	Methanol	Ethanol	Distilled water
1	Alkaloids	+++	++	+
2.	Amino acids	+++	++	+
3.	Anthraquinones	_	_	_
4.	Carbohydrates	++	+++	+
5.	Cardiac glycosides	++	+++	+
6.	Flavonoids	+++	++	+
7.	Phenols	_	_	_
8.	Saponins	_	_	_
9.	Steroids	++	+++	+
10.	Tannins	+	++	_
11.	Terpenoids	_	_	_

(+++)= Strongly present (++) = Moderately present

(+)= Poorly present (-) = Absent

DISCUSSION

Selaginella is considered as the first vascular plant on the earth which existed before 300 million years. Many Spp of *Selaginella like S.ornata, S.plana, S.opaca, S.remotifolia, S.avistata,S. involensS. convoluta, S.moellendorffi, S.rupestris, S.lepidophylla, S.unicata, S.apoda* and *S. intermedia* have shown positive results towards various phyto chemical

screening like alkaloids, flavonoids, tannins, saponins and steroids, but hydro quinines were shown negative results (Tatik Chikmawati et al., 2012). Presence of bioflavonoids in *Selaginella* respond effectively to environmental conditions such as defense against pests, diseases, herbivores and competitions (AhamadDwiSetyawan 2011). *Selaginella* also contain valuable disaccharide, namely trehaloses (White and Tower 1967) which protect the plant from desiccation and allows survival in severe environmental stress (AhamadDwiSetyawan2011) This research paper revealed the presence of various phyto chemicals of *Selaginellabryopteris* (L) Baker**.** *Sellaginella* Spp are medicinally potential due to presence of various secondary metabolites which act as anti-bacterial, antioxidant, anti-inflammatory, anti-cancer, anti-allergic, antimicrobial, anti-fungal and anti-viral also (Reena Antony et al., 2011). Even though *Sellaginella* have potential benefits of the medicine, but the utilization of plants are very limited, so require exploring to further researches towards human beneficiary.

ACKNOWLEDGEMENT

Authors are very much thankful to the eminent taxonomist Dr.VastsavayaS.Raju, Retired Professor, Former Head and Chairperson, BOS, Plant Systematics Laboratory, Department of Botany, Kakatiya University, Warangal, Telangana, India for the identification of the plant.

REFERENCES

1. (a) Ghosh, S. R., Ghosh, B., Biswas, A. and Ghosh, R. K: *The Pteridophytes flora of Eastern India.* Flora of India. BSI Ser. 2004; 4(1): 1-591.
2. (b) Bouquet A cave A 1992. Paris R: *Plants medicinal DU Congo-Brazzaville (III).*Planets medicinalesetphytotherapie. Tome, 1971; 5(2): 154-158.
3. (c) Batugal, LA, da Silva LH, Fett-NetoAG, deAzevedoJr WF, de MoreiraIS, Palma MS, CalixoJB, Astolfi-FilhoS, dosSantosRR, soares MB, Santos D: *The use of biodiversity as source of new chemical entities against defined molecular targets for treatment of malaria, tuberculosis and T-cells mediate- A Review*: MemInstOswaldo Cruz, 2005; 100: 475-506.
4. Agarwal SS, Singh VK, *Immunomodulators: a Review of studies on Indian Medicinal Plants and Synthetic Peptides:* Part1.Medicinal Plants. Pinsa, 1999; 65(3&4):179-204.
5. AhamadDwiSetyawan Review: *Natural products from Genus Selaginella (Selaginellaceae):* BIOSCIENCE, March 2011; 3(1): 44-58.

6. AsimMandal&Amal Kumar Mondal; *Qualitative Analysis of free amino acids of some Pteridophytes with special reference to their Ethno medicinal uses in West Bengal, India;* international journal of science and nature; I.J.S.N, 2012; 3(4): 819-823. ISSN 2229 – 6441.
7. Cheng XL, MCa SC, Yu JD, Yang SY, Xiao XY, Hu JH, Lu Y, Shaw PC, But PPH, Lin RC. *SelaginellinA and B, two novel natural pigments isolated from Selaginellatamariscina*: Chem Pharm Bull, 2008; 56(7): 982-984.
8. Chikmawati T, Setyawan AD, Miftahudin. 2008. *Phytochemical constituent of plant extract of Selaginella in Java.* 8th Seminary and Congress of Indonesian Plant Taxonomy Association ("PTTI"), Cibinong Science Center, Bogor-Indonesia, October 2008; 21-23.
9. Dixit RD, BhattGK. *Ferns: A much neglected group of natural plants II*. J.Res Indian Med, 1974; 9; 59-68.
10. Dutta Jaya Sree: *PhytoChemical Analysis and TLC Fingerprinting of MethanolicExtracts of Three Medicinal Plants*; International Research Journal of Pharmacy, 2013; ISSN 2230-8407.
11. JaliwalaY.A, Panda P.K, Patro V.J, Chourasia Neha, Bhatt Neeraj kumar, Amitpandit, Mohanty P.K.(2011) *Pharmacognostic and preliminary phytochemical screniing of Ficusarnottianamiq*; Journal of current pharmaceutical Research, 2011; 6(1):21-27.
12. JitenderMalviya, Vaibhavi Joshi, Kiransingh: *Antimicrobial activity of Some –medicinl Plants used by Baiga Tribes from Amarkantak, India*: Advances in Life Science and Technology: ISSN 2224-7181(Paper) ISSN 2225-062X (online), 2014; 12: 128-129.
13. Lee HS, Oh WK, Kim BY, Ahn SC, Kang DO, Shin DI, Kim J, Mheen TI, Ahn JS. *Inhibition of phospholipase Cγ1 activity by amentoflavone isolated from Selaginella-tamariscina.* Planta Med. 1996; 62(4): 293-296.
14. P. Jyothichaithanya, R. Chandrashekar and N. Lakshmi Bhavani, AdPlant Sci; 2013; 24(I): 651-656, ISSN 0970-3586.
15. Rao, K.T., Reddy, K.N., Pattanaik.C. And Reddy,C.S. *Ethno medicinal importance of Pteridophytes used by Chenchus of Nallamalais, Andhra Pradesh, India.* 2006.
16. Reena Antony and Rini Thomas; *A mini review on medicinal properties of the resurrecting plantSelaginellabryopteris (Sanjeevani*); International Journal of Pharmacy and Life sciences, 2011; 0976-7126.
17. R. Chandrashekar, Angajala Kishore Kumar, Y.RamaReddy,P.Jyothichaithanya, N.Lakshmi Bhavaniand JalapathiPochampally; *Isolation of Gossypol and Analysis of*

Phytochemicals in Seed Exrtacts of Btand Non –BtVarities of Cotton: Journal of Pharmacognosy and Phytochemistry, 2013; 2(1): ISSN2278-4136.

18. Sah NK, Singh SNP, Sahdev S, Banerji S, Jha V, Khan Z, Hasnain SE. *Indian herb sanjeevani (Selaginellabryopteris) can promote growth and protect against heat shock and apoptotic activities of ultraviolet and oxidative stress*. J Biosci, 2005; 30(4): 499-505.
19. Setyawan AD, Darusman LK. Review: *Bioflavonoid Compounds of SelaginellaPal.Beauv and its benefit*: Biodiversitas, 2008; 9(1):64-81.
20. Silva GL, Chai H, Gupta MP, Farnsworth NR, Cordell GA, Pezzuto8 JM, Beecher CW, Kinghorn AD. *Cytotoxic biflavonoids from Selaginellawilldenowii:* Phytochem, 1995; 40(1): 129-13.
21. S.P. Dhanbal; *Evaluation of Therapeutic Activity and Development of Quality control profile for some antidiabetic herbal Drugs.*(Thesis), 2003,
22. Tatik Chikmawati, Ahmad Dwisetyawan and Miftahudin; *PhytoChemical Composition of SelaginellaSpp from Java Island Indonesia;* Makara Journal of Science, 2012; 16/2(2012)129-133.
23. White E, Towers GHN: *ComparativeBio Chemistry of Lycopods:* Phytochem, 1967; 6(5): 663-667.
24. Zhang LP, LiangYM, Wei XC, Cheng DL.2007. *A new unusual natural pigment from Selaginellasinensis and its noticeable physicochemical properties*. J.Org Chem, 72: 3921-3924.
25. Zhang Xian Chun; *Studies on the Chinese Species of Selaginella (I) Selaginella subgenus TetragonostaclysJermy*; ActaPhytoxonomicaSinica (Golden Jubilee issue), 2001; 39(4): 345~355.
26. Zheng XK, Zhang L, Wang WW, Wu YY, Zhang QB, Feng WS: *Anti-diabetic activity and potential mechanism of total flavonoids of Selaginellatamariscina (Beauv.) Spring in rats induced by high fat diet and low dose STZ. J Ethnopharmacol*, 2011; 137:662-668.