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PHARMACOGNOSTICAL STUDIES ON SELAGINELLA SP. AND EVALUATION OF ITS ANTIMICROBIAL PROPERTIES

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ABSTRACT

Selaginella, is one of the species of spike mosses (Selaginellaceae) which is touted to be a prospective natural product. It is full of various medicinal properties and also known as "Sanjeevani". This study is undertaken with a view to partially purify and characterize the antimicrobial compound from the species which in turn may prove useful for medicinal purposes.Because of the active components present in the species, Selaginella has been used in the treatment for urinary and kidney infections, chronic gastritis, and gastric carcinoma.Present study shows that physiochemical and preliminary phytochemical investigation is essential for the acceptability of herbal drugs and it provides valuable information for further investigations.

Keywords: Selaginella, Medicinal, Anti-Microbial, Phytochemical

I. INTRODUCTION

Selaginella, also known as spike moss, is the only surviving genus within the Selaginellaceae family. Within the spike mosses, the single genus Selaginella contains about 700 species that now exploit a diverse array of arctic, temperate, tropical and semi- arid habitats. It is a cosmopolitan genus which can grow in the most extreme habitat such as in cold tundra and alpine (S. selaginoides, S. rupestris) or in drought dessert (S.lepidophylla, S. sartorii); however most of them grows in tropical rain forest. Selaginella is the largest genus of heterospore fern, the sporangium is modified from reproductive leaves on tip of branch that forms loose, free and open groups called strobili. It has dichotomous branch and minute scale-like leaves that are generally in two different sizes, where median is smaller than lateral ones. Its roots are borne on wiry rhizopores which arise from forks in stems. Stems have air spaces around the protostele. The plant is heterosporous where microspore is much smaller than megaspore and usually has different color. Vascular bundle is simple, surrounded by a layer of phloem. The stellar arrangement is prostelic and xylem is exarch surrounded by phloem. Nusantara or Malesia (Malay Archipelago) is one of the biodiversity hotspot of Selaginella. S. lepidophylla often mentioned as, rose of Jericho is one of only a few species of spike mosses that has evolved desiccation tolerance as under stressful conditions the plant starts accumulating trehalose and sugars which acts as a substitute of water and maintains the native conformation of the phospholipid bi-layer and proteins, protecting them from degradation. Further the cell wall gets folded and becomes highly convoluted preventing the tearing of the plasmalemma. Accumulation of anthocyanin's and carotenoids occurs which protects the chlorophyll from excessive radiation. Members of the Selaginellales are well known for their use in traditional folk medicine. S. lepidophylla, also known as

'Sanjeevani' has been used as a diuretic, and as a treatment for urinary and kidney infections. Selaginella is touted to be a prospective natural resource and possess extraordinary potential for the pharmaceuticals.

II. MATERIALS AND METHODS

2.1Collection, Authentication and Preparation of Plant Material

The leaves of the plant were washed, dried and powdered by mechanical process. It was then stored in a suitable place.

2.1.1 Extraction

The dried powder plant material was extracted with petroleum ether, ethyl acetate, dichloromethane and methanol, by successive reflux and distillation method in increasing order of their polarity. The powdered drug was refluxed with each solvent for about 10 hours at a temperature according to the solvent followed by filtration using Whatmann filter paper and the filtrate so obtained was evaporated in a distillation unit for about 2 hours. The extracts were then stored in freezer.

2.2 Pharmacognostical Studies

a. Organoleptic Study: The powdered drug was subjected to the organoleptic evaluation such as colour and odour.

b. Fluorescence Analysis of Drug Powder: In this study, the fluorescence characteristics of powdered plant were studied under UV light. Many drugs fluorescence when their cut surface or the powder is exposed to ultraviolet radiation. This may prove an effective method to identify drug. For a few drugs UV light will provide information which cannot be obtained by other means such as macroscopy, microscopy as well as chemical tests.Here 0.5 gm of the powder drug was taken in a clean and dried test tube. To each tube 5 mL of different organic solvents like chloroform,picric acid,hydrochloric acid,nitric acid,1N NaOH,Methanol were added separately. Then,all the test tubes were shaken and they were allowed to stand for about 20-25 minutes.The solutions obtained were observed under visible and UV light for their characteristic colour reaction and were compared with a standard colour chart.

2.3 Preliminary Phytochemical Screening

Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, proteins, saponins and glycosides were carried out on powder drug and extracts using standard procedure.

2.4 Optical Density Method

This method was employed to check the antimicrobial properties of extracts derived from the plant in different solvents, namely ethyl acetate, dichloromethane and methanol. It involves the calculation of minimum inhibitory concentration using optical density measurement. For this, microbial stock was prepared for the following bacteria: Bacillus acidophilus, Bacillus Licheniformis, Bacillus methylotropus and Pseudomonas fluorescence, for which 25 µL of live microbe was added to 9 mL nutrient broth medium (autoclaved) and labelled working stock.

A set of solution was prepared for each extract and microbe. Each set comprised of a blank nutrient broth medium,which was used as a control, a solution containing medium and 15 µL of microbe, a solution containing

medium, microbe and 50 µL of solvent of extract and finally a solution containing medium, microbe and 50 µL extract. The test tubes were incubated and shaken thoroughly for 24 h at 34°C after which the optical density was measured using a digital colorimeter at 580 nm and percentage bacterial growth inhibition was calculated as formula given below.

$$\text{Percentage growth inhibition} = \frac{\text{OD of Control} - \text{OD of Test}}{\text{OD of Control}} \times 100$$

III. RESULTS AND DISCUSSION

3.1 Organoleptic Study

Observations were recorded regarding consistency and color of the extracts that were obtained through extraction as compiled under Table 1.It was observed that the extract of Ethyl Acetate had Jelly like consistency with a greenish black color while DCM's Extract was dark green in color with greasy consistency followed by Methanol's extract which was black in color.

Table 1. Color and Consistency of Extracts

Extract	Consistency	Colour
Ethyl Acetate	Jelly -like	Greenish Black
DCM	Greasy	Dark Green
Methanol	Purely liquid	Blackish

3.2 Fluorescent Study

Fluorescent study of the powder drug using different chemical reagents showed different coloration under visible light and UV light as shown under Table 2.Among various solvents tested HCl, HNO3, and Petroleum ether showed almost negligible fluorescence whereas picric acid showed characteristic coloration.Some of the substances may be often converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent, hence we can often assess qualitatively some crude drugs using fluorescence as it's the most important parameter of pharmacognostical evaluation.

Table 2.Fluorescence Analysis of Powder Drug

Reagents	**Fluorescence observed**
Powder as such	Dark Green
Powder + 1N NaOH in Methanol	Light Green
Powder + 1N NaOH in water	Yellowish Green
Powder + 50% HCl	Almost colourless
Powder + 50% H_2SO_4	Dark Green
Powder + 50% HNO_3	Almost colourless
Powder + Pet. Ether	Colourless
Powder +$CHCl_3$	Pale Green
Powder + Picric Acid	Fluorescent Green
Powder + 5% $FeCl_3$ Solution	Pale Green
Powder + CH_3OH	Light Green
Powder +(HNO_3 + NH_3)	Light Green

3.3 Phytochemical Screening

The results of the Phytochemical screening of powder drug for the three solvents viz. Ethyl acetate,DCM,Methanol extracts are given below in Table 3.The phytochemical analysis revealed the presence of phytosterols, glycosides, saponin,proteins and amino acids,fixed oil,Mucilage,Phenolic compounds,Carbohydrates,Alkaloids and flavonoids in the extracts.

3.4 Optical Density Method

Optical density readings of Bacillus Licheniformis were recorded along with percentage inhibition as shown in Fig.1 and Fig.2. It was observed that the extract with DCM as the solvent showed maximum inhibition as compared to other solvents used.

Table 3. Phytochemical Analysis

Compounds	Percentage (%)
Amino Acids	19
Carbohydrates	16
Lipids	13
Peptides	4
Nucleotides	5
Cofactors, Prosthetic groups, Electron Carriers	6
Unnamed compounds	34
Secondary Metabolism	3

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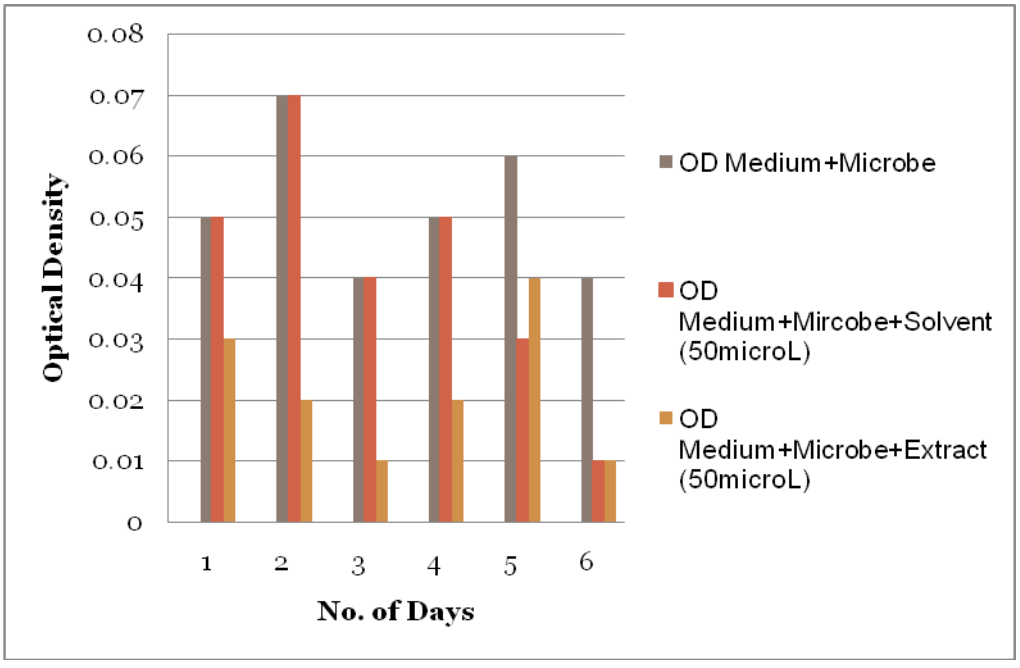

Fig.1 Optical Density Readings of Bacillus Licheniformis

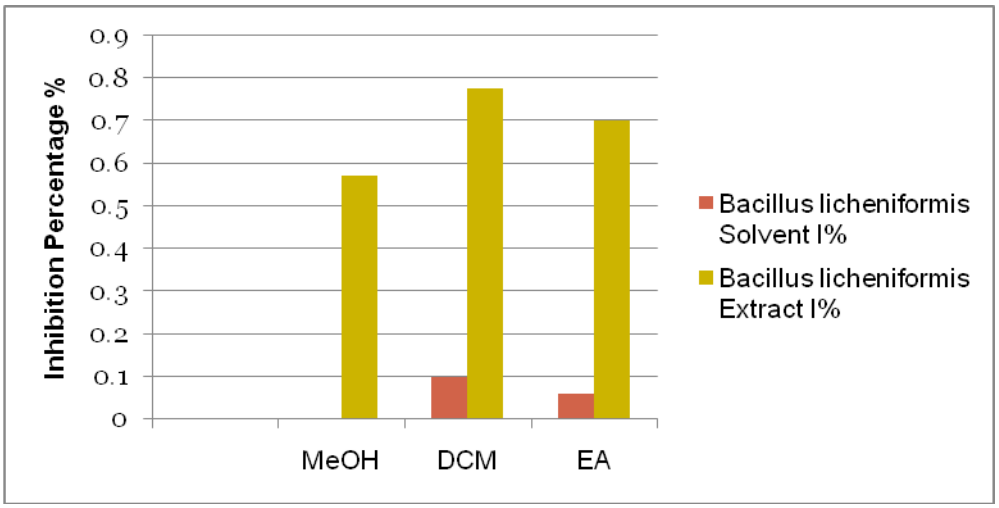

Fig.2 Percentage% Inhibition on Bacillus Licheniformis

IV. CONCLUSION

The pharmacognostical studies help in the correct identification of the crude drugs which is an essential prerequisite. In the current investigation, it can be inferred that extractive values of ethyl acetate solvent,DCM solvent and water as compared to methanol extract are better. This implies that Ethyl acetate,DCM and water are more effective solvents for extraction. Present study concludes that physiochemical and preliminary phytochemical investigation may be used for quality control, identification and to differentiate from other closely related species. Since scientific validation is essential for the acceptability of herbal drugs and it provides valuable information for further investigations.

As all the extracts show positive results for the test of flavonoids, and it has been already established that Selaginalla species have flavones and biflavonoids, it surely can be tested for the pharmacological properties including antimicrobial,anticancer,antiviral,anti inflammatory and anti-fibrillogenesis activities. Also the positive result for carbohydrate test indicates that disaccharides like trehalose, sucrose, glucose might be present in this species which are known to be responsible for resurrection properties. Thus compounds from Selaginella can act both as a potential antimicrobial drug as well as a source of natural compounds.

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REFERNCES

[1]. A.Yobi, B.W. Wone, W. Xu, D.C. Alexander, L. Guo, J.A. Ryals, M.J. Oliver, J.C and Cushman, Metabolomic Profiling in Selaginella lepidophylla at Various Hydration States Provides New In-sights into the Mechanistic Basis of Desiccation Tolerance, Molecular Plant, 6(2), 2013, 369-385.

[2]. K. Jeet, R. Thakur, A. K. Sharma and A. Shukla,Pharmacognostic and phytochemical investigation on whole aerial part of argyreia nervosa, International Journal of Biological & Pharmaceutical Research, 3(5), 2012, 713-717.

[3]. J.K Weng and J.P Noel, Chemodiversity in Selaginella: A reference system for parallel and convergent metabolic evolution in terrestrial plants,Frontiers in Plant Science. Review, 4(19), 2013, 1-11,

[4]. A.D Dikshit, D.K Pandey, and S. Nath, Antifungal activity of some bryophytes against human pathogens, Journal of Indian Botanical Society, 61, 1982, 447-448.

[5]. J.P. Frahm, Recent development of Commercial products from Bryophytes, The Bryologist, 107(3), 2004, 277-283.

[6]. P Kumar and B. L. Chaudhary, Antibacterial Activity of Moss Entodon Myurus (Hook) Hamp. Against Some Pathogenic Bacteria, The Bioscan, 5(4), 2010, 605-608.

[7]. B. L. Chaudhary and P. Kumar,Antibacterial activity and preliminary phytochemical screening of epiphytic moss stereophyllum ligulatum jaeg. , International Journal of Pharma and Bio Sciences, 2(4), 2011, 674-681.

[8]. R. Antony and R. Thomas , A mini review on medicinal properties of the resurrecting plant Selaginella bryopteris (Sanjeevani) , International journal of pharmacy & life sciences, 2(7), 2011, 933-939.

[9]. A. J. De Britto, D. H. S. Gracelin, P. Benjamin and J. R. Kumar, Phytochemical studies on five medicinal ferns collected from Southern Western Ghats, Tamilnadu, Asian Pacific Journal of Tropical Biomedicine. 2012, S536-S538.

[10]. T. Chikmawati, A. D. Setyawan and Miftahudin, Phytochemical composition of selaginella spp. From Java Island Indonesia, MakaraJournal of Science, 16/2, 2012, 129-133.