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Preliminary phytochemical and antimicrobial studies on a spike-moss *Selaginella inaequalifolia* (hook. & grev.) Spring

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ABSTRACT

Objective: To screen the anti-cancer spike-mosses for the presence of various bioactivities and to identify the important bioactive chemicals present in *Selaginella inaequalifolia (S. inaequalifolia)* (Hook. & Grev.) Spring. **Methods:** Preliminary phytochemical screening was done by following the method of Brindha *et al*. Antimicrobial study was carried out by disc diffusion method. **Results:** Results of preliminary phytochemical screening on five different extracts (petroleum ether, benzene, chloroform, ethanol and distilled water) of the spike-moss *S. inaequalifolia* show the presence steroids, triterpenes, phenolic group, tannin, sugars and catechin. Alkaloids, amino acids, anthraquinone and reducing sugar did not show any positive result. Among the five different extracts, ethanol and chloroform extracts show the presence of maximum number (4 each) of compounds. The results on antimicrobial studies show that all the three microbes [*Staphylococcus aureus (S. aureus)*, *Escherichia coli (E. coli)* and *Candida albicans (C. albicans)*] tested are resistant to the ethanol extract and susceptible to petroleum ether extract. The petroleum ether extract shows maximum inhibition with 45 mm of inhibition zone in *C. albicans*. The inhibition zone in *S. aureus* and *E. coli* are 26 mm and 22 mm respectively. **Conclusions:** The present study shows *S. inaequalifolia* having potent antibacterial and anticandidal activities.

1. Introduction

India is a mega-biodiversity country, which is not only rich in medicinal plant resource, but also rich in traditional knowledge about such medicinal plants. Sah[1] has explained the role and use of medicinal pteridophyte, particularly the fern ally *Selaginella* even in the famous ancient literature 'Ramayana'. The Sanjeevani booti is actually a heterosporous Indian Himalayan pteridophyte, which in botanical language known as *Selaginella bryopteris (S. bryopteris)*. There are several studies to prove the presence of various bioactivities and bioactive compounds in various spike-mosses and all such studies are based on the species from other countries. Indian species of *Selaginella* have not yet been subjected to such phytochemical screening to understand the medicinal values. It is evident from the fact that in 'Ayurvedic Pharmacopoeia of India' (Part I, Vol 3), out of hundred drugs, only one is of the fern (*Adiantum lunulatum* Burm.). In the data-base on Indian medicinal plants prepared by FRLHT, Bangalore, six species of *Selaginella* [*Selaginella involvens (S. involvens), Selaginella krausiana (S. krausiana), Selaginella repanda(S. repanda), Selaginella rupestris (S. rupestris) and Selaginella wildenowii (S. wildenowii)*] have been mentioned, but for none of the species, experimental data is available. There are about 700 species of *Selaginella* throughout the world[2]. Fifty nine species are present in India[3] and twelve species are present in south India[4]. Even with the presence of such a large number of anti-cancer spike-mosses, there is no detail study on phytochemistry or pharmacology on Indian spike-mosses. Preliminary studies on the immunomodulatory and antioxidant properties of *Selaginella* species have been done by Gayathri *et al*[5]. Recently, Duraiswamy *et al*[6] have studied the antimicrobial effect of *Selaginella inaequalifolia (S. inaequalifolia)* against poultry pathogens. So, in the very competitive world, it is an urgent need to screen the anti-cancer spike-mosses

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from India, for the presence of various bioactivities and to identify the important bioactive chemicals present in them. Thus the present study has been aimed to make preliminary phytochemical and antimicrobial studies on a rare species of *S. inaequalifolia* (Hook. & Grev.) Spring, which is confined to India and Burma[3,4,7]. Within India it is present only in Assam, Tamil Nadu and Kerala.

2. Materials and methods

Material was collected from Upper Kothayar, Kanniyakumar District, Tamil Nadu, India. The fresh materials were shade dried. Different extracts prepared from powdered materials were used for phytochemical and antimicrobial studies. Preliminary phytochemical screening was done by following the method of Brindha *et al*[8]. Antimicrobial study was carried out by disc diffusion method[9].

3. Results

3.1. Phytochemical screening

By preliminary phytochemical screening of eleven different chemical compounds (steroids, triterpenoids, alkaloids, phenolic groups, saponins, tannin, anthraquinone, sugars, catechin, amino acids and reducing sugars) were tested in five different extracts. Thus out of (5×11=55) tests for the presence or absence of the above compounds, only 15 gave positive results and the remaining 40 gave negative results. The 15 positive results show the presence of steroids, triterpenes, phenolic group, tannin, sugars and catechin (Table 1). Alkaloids, amino acids, anthraquinone and reducing sugar did not show any positive result for their presence in any of the five extracts tested.

Sugar shows the maximum presence in four different extracts followed by steroids and tannins 3 different extracts each. Among the five different extracts, ethanol and chloroform extract show the presence of maximum number (4 each) of compounds. Steroids, triterpenoids and sugars are present commonly in both the extracts, while phenolic group is present in ethanol extract and absent in chloroform extract. Reverse trend is seen with tannin which is absent in ethanol extract and present in chloroform extract. Saponin and catechin are present only in water extract in which no other compound has been detected.

3.2. Antimicrobial activity

In order to test the antibacterial anticandidal effects of chemicals present in *S. inaequalifolia* (Hook. & Grev.) Spring, two bacteria, namely *Staphylococcus aureus (S. aureus)* (gram positive) and *Escherichia coli* (*E. coli)* (gram negative) and one species of *Candida* [*Candida albicans* (*C. albicans*)] were tested. Based on the preliminary phytochemical screening, two extracts, one with minimum number of compounds (petroleum ether–2) and another one with maximum number of compounds (Ethanol–4) were used for antibacterial and anticandidal tests. The sterile discs immersed in 10% extract (10 g powder in 100 mL solvent) were placed in inoculated petri plates. The results as diameter of the inhibition zone have been given in the Table 2. The results show that all the three microbes tested are resistant to the control and ethanol, while all the three microbes are susceptible to petroleum ether extract with the inhibition zone between 33–45 mm. The petroleum ether extract shows maximum inhibition with 45 mm of inhibition zone in *C. albicans*. The inhibition zone in *S. aureus* and *E. coli* are 26 mm and 22 mm respectively.

Table 1

Results of preliminary phytochemical screening.

Name of the extract	Steroids	Ttriterpene	Alkaloids	Phonolic group	Saponin	Tannin	Anthraquinone	Sugars	Catechin	Amino acids	Reducing sugar
Petroleum ether	–	–	–	–	–	+	–	+	–	–	–
Benzene	+	–	–	–	–	+	–	+	–	–	–
Chloroform	+	+	–	–	–	+	–	+	–	–	–
Ethanol	+	+	–	+	–	–	–	+	–	–	–
Distilled water	–	–	–	–	+	–	–	–	+	–	–
Number of extracts with the chemical compound	3	2	–	1	1	3	–	4	1	–	–

No.: Number of extracts with the chemical compound.

Table 2

Inhibition zone as obtained from different extracts tested against different organisms.

S. No.	Name of the extract tested	Inhibition zone (mm) in different microbes tested		
		S. aureus	*E. coli*	*C. albicans*
1	Control	0	0	0
2	Ethanol extract	0	0	0
3	Petroleum ether extract	26	22	45

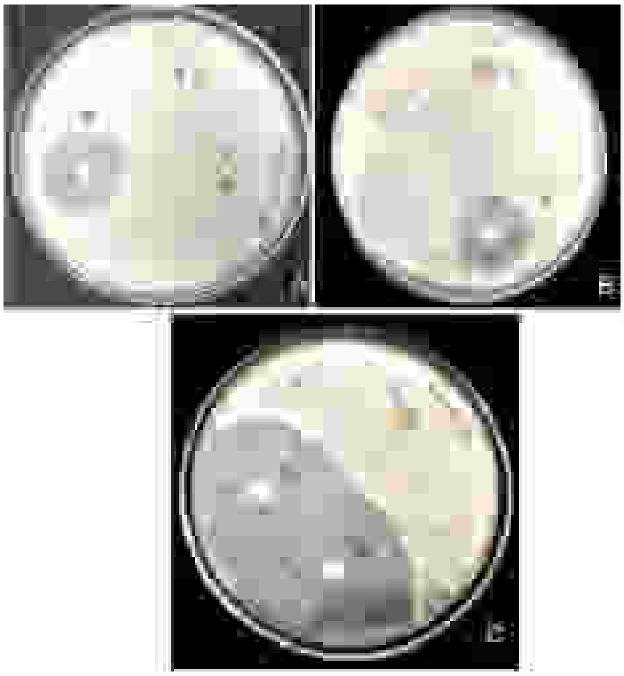

Figure 1. Antibacterial activity with *S. aureus* (A), *E. coli* (B), and *C. albicans*(C). 1-Control, 2-Ethanol extract, 3-Petroleum ether extract

4. Discussion

The preliminary phytochemical screening clearly tallies with the colour of the extracts which are all predominantly light or dark green or yellowish-green. These green colours are mainly due to the presence of different kinds of chlorophyll pigments (Chlorophyll a & b) along with other common pigments like carotenoids. But the ethanol extract and chloroform extract are little different in colour *i.e.* the ethanol extract is reddish-green under ordinary light and brownish green in UV light and the chloroform extract is orange-green under ordinary light. In the meantime it has to be noted that several isolated and purified secondary metabolites are yellow crystals, for example, the chromone glycoside (Unicoside A) from *Selaginella uncinata*[10] is yellow powder and so the presence of yellow colour of the extract of aerial parts of the plants is not strictly the indication for presence of different pigments and it may also be due to the presence of yellow coloured secondary metabolites. Thus in the present study the yellowish-green extract of petroleum ether shows the presence of more number of spots (one brown spot and two yellow spots) in thin layer chromatography. As far as *Selaginella* species is concerned, the presence of sugars in all the extracts needs a special mention. Sugars not only play an important role in providing energy (starch) and in building the plant body (cellulose), but also they play a number of ecological roles, particularly in plant-animal interactions[11]. Some kinds of sugars like trehalose play an important role in desiccation tolerance of several species of plants including *Selaginella*[12]. To evaluate the nature of desiccation tolerance in the resurrection plant *Selaginella tamariscina (S. tamariscina)*, Liu *et al*[13] have compared the composition of soluble sugars and saturation ratios of phospholipids (PLs) between hydrated and desiccated tissues of *S. tamariscina*. The results revealed that trehalose (at >130 mg/g DW) was the major soluble sugar, and low saturated fatty acid content in PLs (0.31) was maintained in both hydrated and desiccated tissues. The role of sugar-alcohols in osmotic-stress adaptation has been explained by Shen *et al*[14]. Although *S. inaequalifolia* (Hook. & Grev.) Spring is not a desiccation tolerant species, the presence or absence of the sugar trehalose needs further confirmation.

From the preliminary phytochemical screening of five different extracts from whole plants of *S. inaequalifolia* (Hook. & Grev.) Spring, it is concluded that the secondary metabolites like steroids, tannin and triterpenoids are moderately present, while phenolic groups, catechin and saponin are rarely present. As far as triterpenoids (6-polymers of isoprene or 3-polymers of monoterpenes), which have been detected in ethanol and chloroform extracts, are concerned, they are commonly present in polypodiaceous ferns and are of rare occurrence in other groups of pteridophytes. Imperato[15] in his review on 'Recent progress in phytochemistry of pteridophyta' enumerated about fifty different triterpenoids from 12 genera of ferns. Presence of triterpenoids has also been reported in Blechnum[16]. Recently, Paulraj[17] reported the presence of triterpenoids in the epidermal glands of six thelypteroid ferns from South India. Triterpenoids are said to be absent in South India fern genera like Pteris, Acrostichum[18], Histiopteris, Microlepia, Hypolepis, Pteridium and Cyathea[19,20]. Steroids and saponins are also the sub-groups of triterpenoids. Thus, the present reports for the presence of triterpenoids in the fern ally *S.inaequalifolia* (Hook. & Grev.) Spring is important. Tannins, which have been identified in chloroform, benzene and petroleum ether extracts of *S. inaequalifolia* (Hook. & Grev.) Spring, are glycosides containing polyhydroxyphenols or their derivatives. Chemically, they are colourless, non-crystalline compounds that form colloidal solutions in water. The anhydrous derivatives of tannins, the phlobapenes, are yellow, red or brown amorphous substances that are readily seen in sections of plant materials, as granular masses or variously sized bodies. Tannins also form water soluble co-polymers with proteins and due to this property are capable of transforming raw animal skins into leather. In plant cells, however, tannins are independent of proteins. The exact role of tannins is not clear. Since tannin-rich plant materials are of astringent taste, they may serve as barriers to herbivory.

In order to separate the compounds present in different extracts, thin layer chromatography was performed by running in different solvent-systems. Under ordinary light only one spot was detected from each extract, but with different colours. Thus the spot is yellow in colour in ethanol and benzene extract while in chloroform and petroleum ether extracts the colour of the spot is green and brown respectively. The number and colour of the spots developed from ethanol and benzene extracts in iodine vapour are similar as observed under ordinary light. While in chloroform and petroleum ether extracts, apart from the spots observed in ordinary light, two more yellow spots were observed in both the cases in iodine vapour.

It is to be noted that the extracts selected for the antimicrobial tests were based on the number of compounds *ie.* one extract with minimum number of compounds (Petroleum ether-2) and another one with maximum number of compounds (Ethanol-4) with the expectation that the extract with more number of compounds may have high degree of antimicrobial activity. But the obtained results are opposite to the expected results with nil activity in ethanol extract and maximum activity in petroleum ether extract. The possible reason for this result has to be analyzed critically. One possible reason may be analysed based on type of compounds present, instead of number of compounds present in the extracts based on the preliminary phytochemical screening. In both ethanol and petroleum ether extracts sugars are commonly present and primary metabolite may not be consider as antimicrobial agent. Other compounds present are: tannin in petroleum ether; steroids, triterpenoids and phenolic groups in ethanol extract. Since ethanolic extract did not show antimicrobial effect, it may be suggested that either the different types of compounds under these three categories may not have antimicrobial activity or such compounds may be present in the extract below the minimum concentration. The only secondary metabolite present in petroleum ether extract is tannin which may have antimicrobial effect with the presence of enough concentration. Since tannins are astringent it is considered as anti-herbivore chemical agent. It may also give protection for the plants against microbial pathogens. Several species of *Selaginella* have been proved to have high degree of antimicrobial activity, particularly anticandidal activity, due to the presence of different kinds of flavonoids.

Whether the active compound is tannin or any other compound, it is not a surprise result for the presence of high degree of antimicrobial activity in the presently studied species of *Selaginella*. There are several reports to show *Selaginella* species for having potent antimicrobial chemicals. Isocryptomerin, a biflavonoid, isolated from *S. tamariscina* has novel antibacterial, antifungal and synergistic properties[21,22]. Antifungal activities of isocryptomerin might be due to its membrane-disruption mechanism(s)[21]. Isocryptomerin shows potent antibacterial activity against gram-positive and gram-negative bacterial strains including clinical isolates of antibiotic-resistant species such as methicillin-resistant *S.aureus* (MRSA)[22]. Amentoflavone, which is present in several species of *Selaginella*, has also been proved as potent anticandidal agent with significant physiological changes inducing S-phase arrest in intracellular environment. Therefore, amentoflavone may be applied to a lead compound for the development of therapeutic agents, which can treat candidiasis resulted from *Candida* infections[23].

Thus the present study, along with previous studies, show that various species of *Selaginella*, including the presently studied species (*S. inaequalifolia*) are having potent antibacterial and anticandidal chemicals. The particular active compounds, whether biflavonoids or other, has yet to be identified in *S. inaequalifolia*.

Conflict of interest statement

We declare that we have no conflict of interest.

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