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Pharmacognostical studies on an endemic Spike-Moss *Selaginella tenera* (Hook. & Grev.) Spring from the Western Ghats, South India

S. Suganya, V. Irudayaraj and M. Johnson

Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India

ABSTRACT

Pharmacognostical studies on an endemic spike moss Selaginella tenera (Hook. & Grev.) Spring have been carried out on the materials collected from Tirunelveli hills, Western Ghats, South India. Results of detailed morpho-anatomical and physico-chemical studies have been presented. Morphologically this species is distinct in having erect habit, heterophyllous leaves both on vegetative and reproductive parts. The vascular bundles in stem, rhizophore and roots are protostele. The preliminary phytochemical screening shows the presence of phenolic group, steroids, triterpenoids, tanninsa and sugars. All the morpho-anatomical and physico-chemical characters this endemic species will be useful to distinguish the species from other spike mosses from Western Ghats.

Keywords: *Selaginella tenera;* Pharmacognosy; Phytochmeistry, Fern- allies.

INTRODUCTION

Plants have been a source of medicine and a major resource for health care since ancient times, with some traditional herbal medicines having been in use for more than 2,000 years. Next to flowering plants the dominant vegetation on the earth is formed by the non-flowering vascular plants called Pteridophytes. There are about 1200 species of Pteridophytes in India. Like flowering plants, pteridophytes are also used by tribals in India and other countries. Considerable number of pteridophytes is used in Chinese medicines. The Chinese herb *Selaginella doederleinii (Shi Shang Bai)* is used as an anticancer drug [1, 2] (The medicinal use of *Selaginella* in India goes back to the period of 'Ramayana'). Sah [3] has explained the role and

use of medicinal pteridophyte, particularly the fern ally *Selaginella* in the famous ancient literature '*Ramayana'*. In this world famous epic of Hindi poet *Tulsidas*, there is a description of a wonder herb called as *Sanjeevani booti* (Life giving herb), which according to the popular belief had given life to the dying Laxmana, the younger brother of Lord Shree Rama. It is believed that medicines prepared from this herb can even revive a dead person also. The *Sanjeevani booti* is actually a heterosporous Indian Himalayan Pteridophyte, which in botanical language known as *Selaginella bryopteris* **[**3].

According to the principles of traditional Chinese medicine, *Selaginella* has sweet, spicy, bitter and cold properties, and is associated with the Liver, Lung and Stomach meridians. Its main functions are to clear heat, reduce toxicity, and drain damp heat. Among the conditions *Selaginella* is used for are coughs, sore throats, and jaundice. *Selaginella* is also used to treat cancer of the liver and cirrhosis of the liver. More recent research has shown that *Selaginella* may be effective against both acute and chronic hepatitis. *Selaginella* may also be used externally to help stop bleeding and promote wound healing. The typical dose of *Selaginella* is between 15 and 30 grams. Larger doses (up to 60 grams per day) are considered if *Selaginella* is being used to treat cirrhosis or other liver disorders. *Selaginella* is available in a variety of forms. Whole, dried *Selaginella* may be purchased at some Asian markets and herbal shops. *Selaginell radiata* (Aubl.) Spring is sold in the market of Suriname [4]. Prepared *Selaginella* can be found in pill, powder and tablet forms. *Selaginella* should be taken with caution by patients diagnosed with cold deficiency. Taking large amounts of *Selaginella* may result in loss of appetite and abdominal discomfort. There are no known drug interactions associated with *Selaginella* [5]. *Selaginella doederleinii*, a popular anticancer herb, may contain an as yet unidentified substance that contributes to reversible bone marrow suppression [6]. These results suggest that *Selaginella tamariscina* could be a candidate chemopreventive agent against gastric cancer [7]. Recently antibacterial efficacy of *Selaginella inaequalifolia* and *S. involvens* against poultry pathogens and human pathogens have been studied by Duraiswamy *et al* [8] and Irudayaraj *et al.* [9].

Today biopiracy is an international problem in which the most victimized countries are those with rich bioresources and traditional knowledge. Adverse impact through bio-piracy has been believed to be a major threat to our country. So at this competitive stage, it is an urgent requirement to analyse and document our knowledge about our indigenous bioresources, particularly the medicinally important rare and endemic plants. Most of the information available on the medicinally important species of *Selaginella* is from other countries and there is very little information about species of *Selaginella* from India. In '*Ayurvedic Pharmacopoeia of India'* (Part I, Vol 3), out of hundred drugs, only one is of the fern (*Adiantum lunulatum* Burm.). In contrast, several value added natural medicines are introduced in the market from the developed countries. As mentioned above, numerous bioactive compounds, particularly biflavonoids with various bioactivities, particularly cytotoxic activity, have been reported in various species of *Selaginella* from other countries. Recently Janaki [10] has studied the pharmacognosy of *Selaginella inaequalifolia* (Hook. & Grev.) Spring. Without making pharmacological and pharmacognostical studies, it is not possible to know the medicinal values of Indian spike-mosses.

Selaginella consists of about 700 species. Alston [11] enumerated 58 species of *Selaginella* from India and Sri Lanka. Of these 45 species were reported from India. Eighteen species have been

reported from South India [12, 13]. Taxonomically it is a very difficult group of plants even for Pteridologists. If it is so with the whole plant itself, it will be very difficult to distinguish the substitute species of *Selaginella* in different forms of medicines such as powder, paste, decoction etc. So it is necessary to use the pharmacognostical standards like organoleptic and physic-chemical characters to distinguish the crude drugs of *Selaginella* species. In the present study, in order to document the pharmacognostical standards, the endemic species of *Selaginella tenera* (Hook. & Grev.) Spring from the Western Ghats, South India has been selected to make pharmacognostical analysis.

EXPERIMENTAL SECTION

Materials and Methods

Materials for the present study were collected from Kakachi (1100m) on Upper Kothayar on Tirunelveli Hills, where it grows commonly along shaded road-sides. Identification of the specimens was done based on "*Pteridophyte Flora of the Western Ghats, South India*" [13]. For anatomical studies fresh specimens were used. Entire plants were air dried and powdered and the powdered materials were used for further phytochemical and pharmacognsotical studies [14].

RESULTS AND DISCUSSION

Morpho-anatomical studies

Terrestrial-small herb of about 30 cm height growing along shaded roadsides at about 1100m near Kakachi – Tirunelveli Hills. The species grow as solitary plants without forming colonies. Stem erect or suberect, about 25 cm height, profusely branched, 2mm thick, pink in colour; ultimate branch may or may not bear cone (Plates I & III). Rhizophores borne on the basal one – fourth to one-third part of the main stem as stilt roots; basal most part of the rhizophore bears thin, branched, wiry roots; free rhizophores hanging from the stem without touching the soil usually do no bear roots. Roots about 1 mm thick, light pink in colour. Leaves dimorphic throughout, lateral leaves oblong-lanceolate, oblique with a single midvein; apex obtuse or sub-acute, margin entire throughout. Median leaves broadly ovate with aristate apex; margin entire except at the base which is denticulate. Cones borne at the tip of ultimate branches, about 1.5 to 2.0 cm, flattened; sporophylls dimorphic, lateral and median sporophylls more or less similar with lateral and median vegetative leaves in morphology; both the sporophylls bear globose or ovate sporangia. Microspores about 35 micrometer in diameter, megaspores about 55 micrometer in diameter, exine of the microspores prominently tubercled (Plate IV). Erect, pink colour stem with anisophyllous leaves both on vegetative parts and cones are the identifying characters for the species *S. tenera.*

The anatomy of stem of *Selaginella tenera* is simple with the single layered epidermis followed by few layered sclerenchymatous hypodermis. The remaining portion is covered by parenchymatous ground tissue. Single protestele is present with the central xylem surrounded by phloem. Uniseriate trabeculae are present around the vascular tissue. The anatomy of rhizophores and roots are similar to stem except for the presence of trabeculae in the vascular bundle. Thus there is an epidermis followed by sclerenchymatous hypodermis and parenchymatous ground tissue. Vascular bundle is of protostelic type. Usually the rhizophores are with two groups of xylem surrounded by phloem in contrast to single group of xylem in roots (Plate III). The

epidermis of the main stem is made up of long, narrow, compactly arranged cells without any stomata and epidermal appendages. But there is difference in size of the cells and in cell inclusions (Plate II).

The lower epidermis of the lateral vegetative leaf show morphologically different kinds of cells along the midvein and on other region. Thus the cells along the midvein are elongated and they are intermingled with stomata. The lower surface of the leaf other than the midvein is filled with somewhat rectangular cells without any stomata. Epidermal cells in all the area are with wavy undulated margin (Plate VIII & XVI).

Fluorescence analysis

The colour of the extracts from organic and inorganic solvents was observed both under ordinary light and UV light. There is little difference between different extracts and between the light sources. In various organic solvents the extracts are generally light green or pale green. The colour of chloroform and ethanol extracts of *S. tenera* under ordinary light is fluorescent green and dark green respectively. The colour of the extracts in inorganic solvents is usually light green or pale green, rarely light brown, grass green or dark green (Table – 1).

Table - 1: Results of Fluorescence analysis

Solvents	Colour under Ordinary light	Colour under U.V light
Water and Organic solvents		
Distilled Water	Light Green	Light Green
Benzene	Light Green	Light Green
Chloroform	Fluorescent Green	Light Green
Ethanol	Dark Green	Light Green
Petroleum ether	Light green	Light Green
Acetone	Pale Green	Dark Green
Inorganic solvents		
1N NaOH	Green	Dark Green
50% H_2SO_4	Dark Green	Dark Green
50% HNO_3	Light Brown	Light Green
1N HCl	Pale Green	Pale Green

Physical constants

Physical constants like moisture content-loss of weight, extractive values, ash values for the species have been given in table 2.

Table - 2: Results of Physical constants

S. No.	Characters	Amount (%)
1	Loss of weight	66.9
2	Total ash	30.5
3	Acid insoluble ash	0.95
4	Water soluble ash	0.13
5	Sulphated ash	2.3
6	Extractive values: Alcohol	7.5
	Water	5.8

Phytochemical screening

The results of preliminary phytochemical screening have been given in table – 3. Five different extracts were screened for the presence or absence of twelve different organic chemical compounds. Alkaloids, saponins, catechins, flavones- group, amino acids and reducing sugar did not show any positive result in all the five extracts. None of the chemicals screened is present in all the extracts. Steroids, phenolic group, tannin, sugar showed positive result for their presence in one or more extracts. Triterpenoids gave positive result only in petroleum ether extract. The benzene, chloroform and ethanol extracts of the species show the presence of sugar. Steroids, triterpenes, phenolic group, tannin, sugars, saponin and catechin have been reported from the spike-moss *Selaginella inaequalifolia* (Hook. & Grev.) Spring [10]. 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.

Table – 3: Results of preliminary phytochemical screening on *Selaginella tenera*

Name of the extract	Steroids	Ttriterpene	Alkaloids	Phonolic group	Saponin	Tannin	Anthraquinone	Flavone group	Sugars	Catechin	Amino Acids	Reducing sugar	Number of compounds present in each extract
Petroleum ether	_	+	_	_	_	+	_	_	_	_	_	_	2
Benzene	_	_	_	_	_	+	_	_	+	_	_	_	2
Chloroform	+	_	_	+	_	+	_	_	+	_	_	_	4
Ethanol	+	_	_	+	_	_	_	_	+	_	_	_	3
Distilled Water	_	_	_	_	_	+	_	_	_	_	_	_	1
Number of extracts with the chemical compounds	2	1	_	2	_	4	_	_	3	_	_	_	

(+) - sign indicates the presence of particular compound; (-) - sign Indicates the absence of particular compound

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 report for the presence of triterpenoids in the fern ally *Selaginella tenera* (Hook. & Grev.) Spring, is important.

Tannins, which have been identified in both the species of the present study, 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. Screening for the presence or absence of inorganic chemicals like chlorine, sulphur, phosphorous, iron and calcium shows the negative result for phosphorous and calcium and positive result for chlorine and iron in *Selaginella tenera* (Table - 4).

Table - 4: Results of mineral analysis

S. No	Minerals	Result
1	Chlorine	+
2	Sulphur	_
3	Phosphorous	_
4	Iron	+
5	Calcium	_

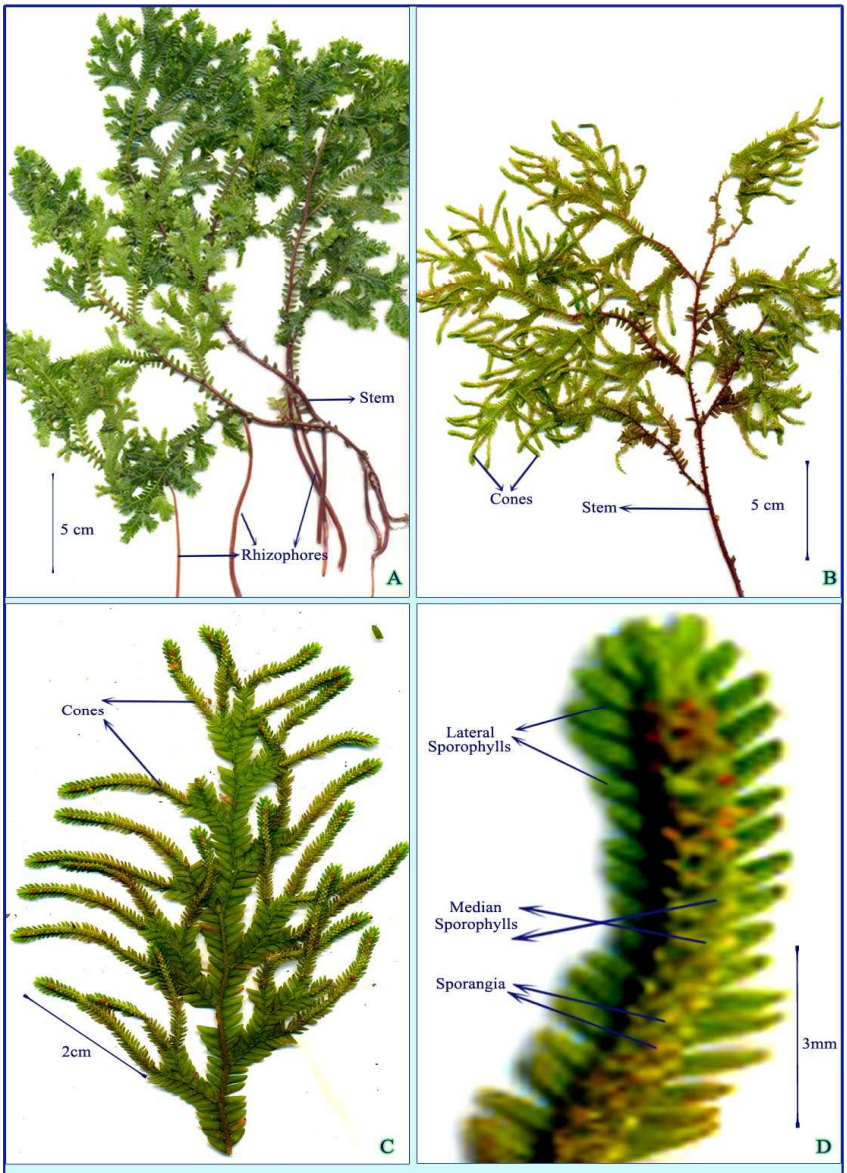
Results of quantitative estimation of sugar, protein and total phenol on the two species have been given in table - 5. In *S. tenera* 44mg/g sugar is present. Protein is present 4.8mg/g is present in *S. tenera*. Phenol is 3.6mg/g in *S. tenera*. It is to be noted that the Trehalose sugar accumulate in high concentration as osmoprotectant during drought in several plants including the drought tolerant *Selaginella* species [21].

Table - 5: Results of quantitative analysis of some organic compounds

S. No	Organic compounds	Result (mg /g)
1	Sugar	44.0
2	Protein	04.8
3	Phenol	03.6

Morpho-anatomical and physico-chemical studies have been carried out on the anticancer spike-moss *Selaginella tenera* which is endemic to South India. The macromorphological, micromorphological and anatomical characters of the species are distinct. How far the morphological characters of the *Selaginella* species are useful in pharmacognosy is questionable, because of the difficulties to differentiate various species of *Selaginella* even by the Pteridologists who use mainly the morphology of vegetative and reproductive parts. Thus morphological characters in spike-mosses are very limited use in pharmacognosy and they can be used only to differentiate different groups like, homeophyllous and heterophyllous spike-mosses. Several species are with heterophyllous leaves on vegetative part and homeophyllous species (eg. *Selaginella wightii*) are very rare. The same is true in the sporophylls on the spike also. Thus species with homeophyllous sporophylls are very common when compared to species with heterophyllous sporophylls. The endemic species *S. tenera* is with heterophyllous sporophylls. Anatomically also majority of the species are similar in having protostele in stem, rhizophore and root. Anyhow, the species of *Selaginella* can be distinguished easily from other plants in having the trabeculae around the vascular tissues in stem. This is the main reason and an urgent need for developing all the pharmacognostical standards for all the anticancer spike-mosses to identify the crude drugs by using mainly chemical or molecular markers other than morphological characters. So it is necessary to go for advanced phytochemical studies like HPLC or advanced molecular studies like protein profiling, isozyme studies or DNA analysis to know the molecular markers, if any, for the species of *Selaginella*.

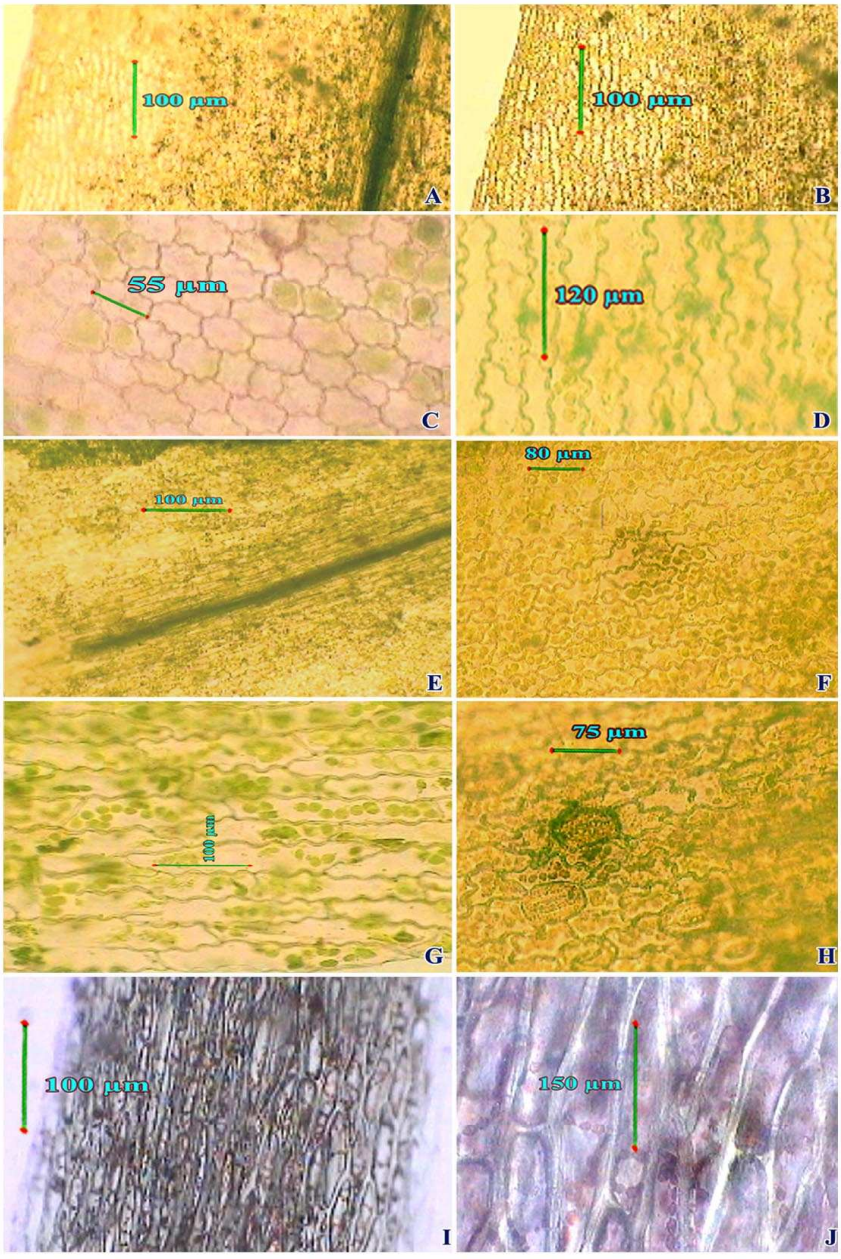
PLATE I



Figs. A - D: *Selaginella tenera* - General Morphology.

Fig. A - Habit, Fig. B. Mature plant with numerous mature cones.
Fig. C. Portion of a branch bearing many dorsiventral cones.
Fig. D. Portion of a cone enlarged showing the presence of dimorphic sporophylls.

PLATE II



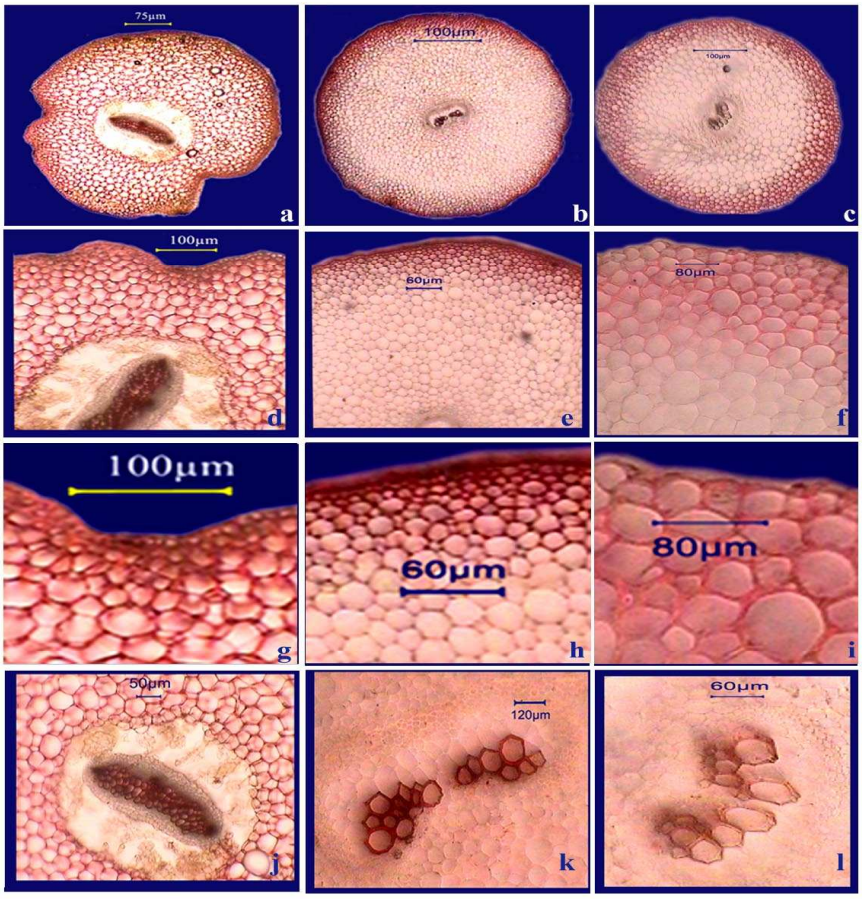
Figs. A - J: *Selaginella tenera* - Epidermal features.

Figs. A - D: Upper epidermis of lateral leaf of main branch
(C-Epidermal cells towards mid vein, D- Epidermal cells towards margin)

Figs. E - H: Lower epidermis of lateral leaf of main branch
(E - Portion of lower surface of the leaf, F-G - Lower epidermal cells towards margin of leaf, H - Stomata on the lower surface of the mid vein)

Figs. I - J: Epidermal cells of main stem at different magnification.

PLATE III



Figs. a -l: Anatomy of stem, rhizophore and root of *Selaginella tenera*

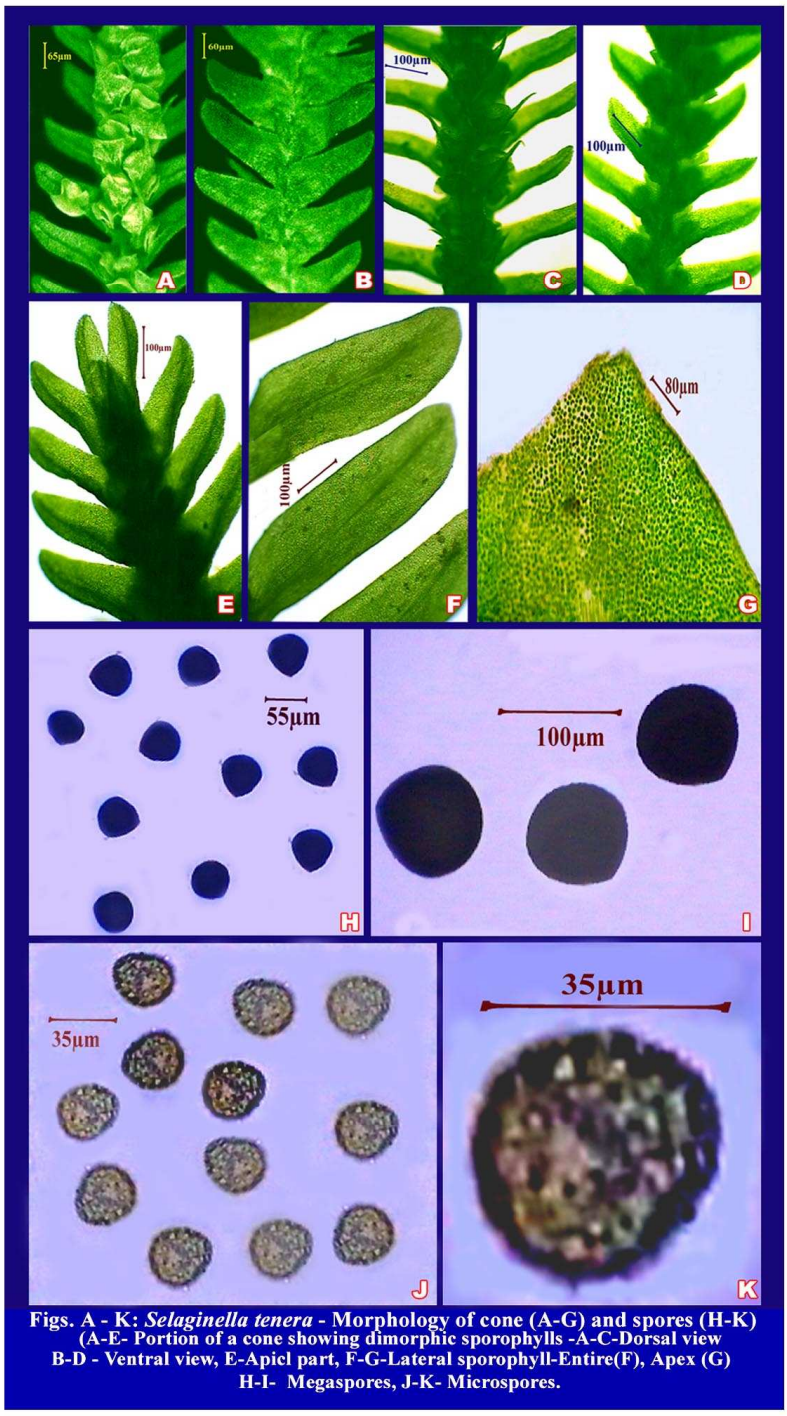
Figs. a, d, g, j - Stem; Figs. b, e, h, k - Rhizophore, Figs. c, f, i, l - Root

Figs. a, b, c - Entire view, Figs. d, e, f - Portion enlarged

Figs. g, h, i - Portion enlarged showing epidermis and ground tissue

Figs. j, k, l - Vascular bundle enlarged.

PLATE IV



Figs. A - K: *Selaginella tenera* - Morphology of cone (A-G) and spores (H-K)
(A-E- Portion of a cone showing dimorphic sporophylls -A-C-Dorsal view
B-D - Ventral view, E-Apicl part, F-G-Lateral sporophyll-Entire(F), Apex (G)
H-I- Megaspores, J-K- Microspores.

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