RESEARCH ARTICLE

Antioxidant, antimicrobial and phytochemical analysis of four species of *Selaginella* P. Beauv

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Abstract

Selaginella is a member of the family Selaginellaceae under the class Lycopodiopsida, which includes spike mosses and smaller clubmosses. They are said to be one of the oldest terrestrial plants with medicinal benefits. Four *Selaginella* species were selected for the present study. *Selaginella plana*, *S. radicata*, *S. wallichii* and *S. willdenowii* were collected from several districts of Kerala and subjected to antioxidant, antibacterial, antifungal analysis and phytochemical screening. Antioxidant analysis was done with DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Antifungal analysis against *Candida albicans*, *Aspergillus niger* and *Penicillium chrysogenum* and antibacterial analysis using two-gram negative bacteria, *E. coli* and *Vibrio cholerae* and two-gram positive bacteria, *Staphylococcus aureus* and *Streptococcus pyogenes* was done using agar well diffusion method. The phytochemical screening process comprises both qualitative and quantitative analysis with various solvents. Qualitative analysis of 10 selected phytochemicals was done using standard methods. The quantitative analysis includes estimation of alkaloids, flavonoids, phenols, glycoside and tannins. The aqueous extract of *S. radicata* shows the highest percentage (85.21 ± 0.02) of anti-oxidant activity. Chloroform extract of *S. plana* shows higher antibacterial activity against *Streptococcus pyogenes* with an inhibition zone of 10 ± 0.01mm. Likewise aqueous extract of *S.willdenowii* shows antifungal activity against *P.chrysogenum* with an inhibition zone of 13 ± 0.01mm. Phytocompounds such as steroids and tannins are present moderately, while terpenoids, quinones, fatty acids, and saponins are rarely present in all solvents. From the findings, it is revealed that *Selaginella* species have moderate antioxidant and antibacterial activity because of the presence of phytochemicals like flavonoids, alkaloids, phenols, tannins, etc.

Highlights

- The present study summarizes the bioactivities of the phytochemicals in selected species of Selaginella.
- This study provides quantification of phytocompounds, phenol, flavonoid, alkaloid, tannins and glycoside.
- The importance of the study lies in the pharmacological roles of the phytocompounds present in this plant and it can be used as a potent natural drug against various ailments.
- This study revealed the antioxidant and antibacterial activity of selected Selaginella species.

Keywords: *Selaginella* sp., antioxidant, antibacterial, antifungal, phytochemical screening

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Introduction

Pteridophytes have been used traditionally to combat many diseases, like cancer, bacterial infections, inflammation, etc. Some of the pteridophytes have been reported to have insecticidal and other pharmacological properties. Many studies reported that they have potential antibacterial and antioxidant properties, even against other drug-resistant pathogens (Baskaran *et al*., 2018). *Selaginella* species are the core essence of biodiversity assessment and play a major role in all disciplines of biological research (Ferroni *et al.,* 2021). Taxonomically, it is considered an ancient plant because it is thought to have originated roughly 383 million years ago (Khalid *et al*., 2018; Gupta *et al*., 2013). *Selaginella* P. Beauv. is the largest and only extant genus in Selaginellaceae family. It has around 800 species located world wide (POWO, 2023), with the largest diversity observed in tropical regions. In India, more than 65 species have been reported so far (E-flora India, 2023) of which 30 are reported from Kerala (E-flora Kerala, 2023). Some species from the *Selaginella* genus are utilized in secret formulations of folk medicines (Paswan *et al*., 2017). Further, *Selaginella* species are extensively exploited in many countries to treat a variety of ailments (Bautista *et al*., 2018). *Selaginella* is an important and unexplored genus with regards to its micromorphological structures, molecular systematics, and pharmacological properties.

In the present study, four species of *Selaginella*, namely *S.willdenowii* (Desv. ex Poir.) Baker, *S. wallichii* (Hook. & Grev.) Spring, *S. radicata* (Hook. & Grev.) Spring, and *S. plana* (Desv. ex Poir.) Hieron were collected for their phytochemical analysis, antioxidant and antimicrobial activity.

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Materials And Methods

Plant Collection

Fresh materials of *S.delicatula* were collected from Ponmudi hills (Thiruvananthapuram), *S. radicata* from Marayoor (Idukki), *S. plana and S. willdenowii* from TBGRI, Palode (Thiruvananthapuram). The specimens collected were identified with the help of authentic literature (Dixit 1992). Voucher specimens were deposited at the herbarium of Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Thiruvananthapuram (TBGT).

Plant Extract Preparation

The fresh plant specimen (about 100 gram) were rinsed briefly under running tap water for a few minutes, cut and dried in shade on blotting paper for two weeks. Then the dried materials were powdered and kept for future studies. One hundred grams of powdered samples were mixed with 500 mL of solvent and subjected to cold maceration for 72 hours. The extracts were filtered using Whatman filter paper and concentrated by drying. Sequential extraction was performed using four solvents (ethanol, water, petroleum ether and chloroform) of increasing polarity. The yield percentage of the organic solvents was determined. The percentage of yield was calculated using the following formula:

$$\% \text{ Yield} = W1/W2 \times 100$$

W1: weight of extract after the removal of solvent; W2: weight of powdered sample used for extraction

Antioxidant Analysis

The antioxidant activities of the extracts of *Selaginella* were evaluated on the basis of hydrogen donors and radical scavenging capabilities with 2, 2-diphenylpicrylhydrazyl (DPPH), a stable free radical (Benzie & Strain, 1999). Various aliquots of petroleum ether, chloroform, aqueous, and ethanolic extracts (50, 100, 200, 400, 800, and 1000 µg) were mixed with 5 ml of a 0.1 mM ethanolic DPPH solution, kept to rest for 20 minutes by 27°C at 517nm. Standard was ascorbic acid, while the negative control was a DPPH solution with no extract. Radial scavenging activity was measured with the below formula:

$$\%\, of inhibition = \frac{ControlAbsorbance - SampleAbsorbance}{ControlAbsorbance} \times 100$$

Antibacterial Assay

The antibacterial activity in test samples were determined using agar - well diffusion method (Magaldi *et al*., 2004; Valgas *et al*., 2007). Mueller-Hinton agar (15-20 millilitres) was spread on glass petri dishes of similar size and left to solidify. A standardized inoculum of organisms, such as *Escherichia coli* MTCC 443, *Vibrio cholerae* MTCC 3906, *Staphylococcus aureus* MTCC 87, and *Streptococcus pyogenes* MTCC 442, was consistently put over the surface of the plates with a clean cotton swab. Each plate has six 5mm-diameter wells (20 mm away) pierced in an aseptic manner using a clean cork borer. The sample to be tested (50µL) was added to the sample wells from the 10mg/mL stock solution. Gentamycin was used as the positive control (40 µL from 4mg/ml stock) and the solvent used for sample dilution was the negative control. Plates were set aside for 24 hours at 36 ± 1°C, aerobic conditions. After incubation, the inhibition zone was calculated in millimetres.

Antifungal Assay

The antifungal activity in test samples were determined using agar - well diffusion method (Magaldi *et al*., 2004; Valgas *et al*., 2007). *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 872, and *Penicillium chrysogenum* MTCC 5108 are the fungal strains used. Mueller-Hinton agar and Potato Dextrose Agar MH096 Himedia were placed in a 1:1 ratio onto the petri dishes of similar size and left to harden. Standardized inoculum of the test organism was consistently put over the surface of the plates with a clean cotton swab. Each plate has four 5mm-diameter wells (20 mm away) pierced in an aseptic manner using a clean cork borer. To test, add 50 µL of the 10 mg/mL to the sample wells. Clotrimazole was used as the positive control (40µl from 300 mcg/ml stock) and the solvent used for sample dilution as the negative control. Plates were set aside for 24 hours at 27°C ± 1°C, aerobic conditions. After incubation, the inhibition zone was calculated in millimetres.

Phytochemical Analysis

The presence or absence of phenols (Lead acetate Test), flavonoids (Ferric chloride Test), alkaloids (Wagner's Reagent), steroid (Liebermann-Burchard Test), terpenoid (Liebermann-Burchard Test), saponins (Foam Test), glycosides (Kellar-Killani Test), tannins (Ferric chloride Test), fattyacids (Filter paper test) and quinones (HCl method) were examined qualitatively using a conventional approach (Sofowora, 1993; Harborne, 1973). The quantity of alkaloids, flavonoids, phenols, glycosides and tannins were quantified using conventional procedures (Madhu *et al*., 2016).

Results

Yield Percentage

The water extract produces highest percentage yield in all species, with *S. willdenowii* achieving the highest percentage yield (Table 1).

Antioxidant Analysis

The antioxidant properties of four *Selaginella* species were studied with DPPH, and the extracts demonstrated a linear rise in scavenging activity with concentration. All the four species have the highest antioxidant activity in aqueous extract 52.45 ± 0.02 to 85.21 ± 0.02. (Table 2, demonstrated in Fig. 1).

Table 1: Percentage yield of *Selaginella* sp. using different solvents

Species	% yield			
	Petroleum ether	*Chloroform*	*Ethanol*	H_2O
S. plana	0.87	2.5	2.9	6.82
S. radicata	0.27	2.0	5.0	5.1
S. wallichii	0.33	5.07	4.27	5.60
S. willdenowii	0.93	1.86	3.8	11.06

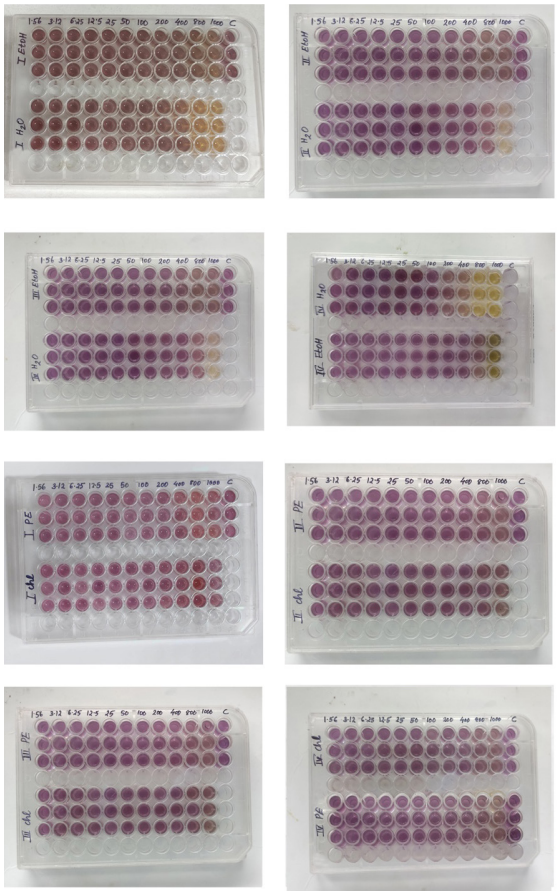

Fig. 1: Antioxidant assay of different extract of *S. willdenowii, S. plana, S. wallichii and S. radicata*

Antibacterial Activity of *Selaginella* species

The antibacterial activity of *Selaginella* species against potential bacterial pathogens was analysed and recorded (Table 3, demonstrated in Figs. 2 and 3). Chloroform extract of *S. plana* has high antibacterial activity (10 ± 0.01) against *Streptococcus pyogenes* MTCC 442, while the ethanol extract of *S.radicata* has high antibacterial activity (8 ± 0.01) against *E.coli* MTCC 443 and *Vibrio cholerae* MTCC87. The ethanol extract of *S.wallichii* also has high antibacterial activity (8 ± 0.01mm) against *E.coli* MTCC 443 .

Antifungal Activity

S. plana the aqueous extracts had the highest antifungal activity against *Aspergillus niger* MTCC872 (8 ± 0.01mm), *S. wallichii* petroleum ether extract had the highest antifungal activity against *Penicillium chrysogenum* MTCC 5108 (9 ± 0.01mm), and *S. willdenowii* aqueous extracts had highest antifungal activity against *Penicillium chrysogenum* MTCC 5108 (13 ± 0.01mm). (Table 4, demonstrated in Figs. 4 and 5)

Phytochemical Analysis

Alkaloids were detected in the ethanol extracts of all species. The presence of saponins and flavonoids, in all extracts except petroleum ether. (Table 5).

The ethanolic *S. willdenowii* extract had a maximum concentration of phenol (85.89 ± 0.02mg/g), the chloroform extract of *S. radicata* had the highest concentration of flavonoid

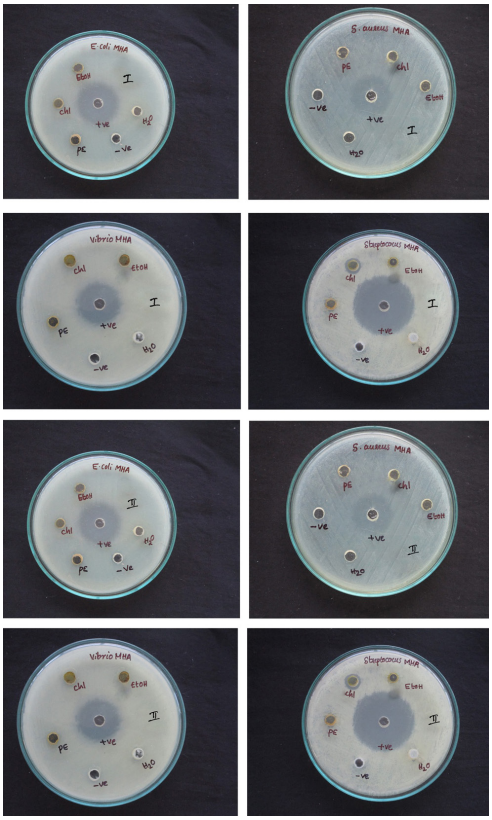

Fig. 2: Antibacterial activity of different extract of *S. willdenowii* and *S. plana* using different bacterial strains

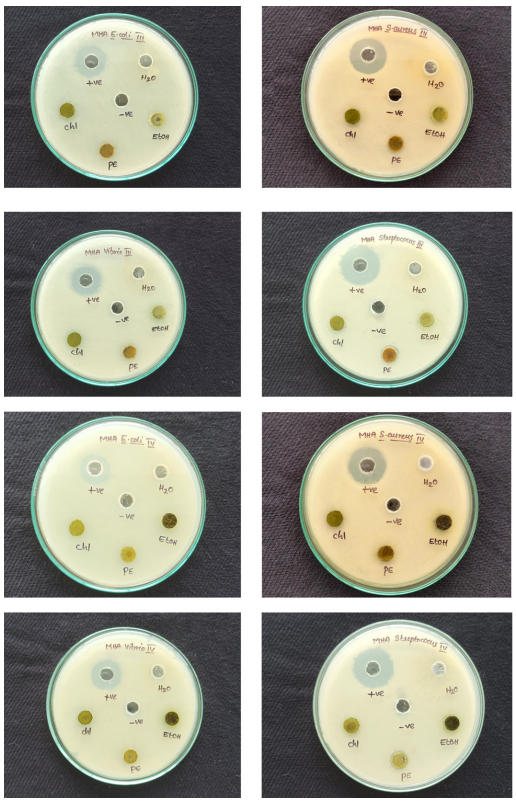

Fig. 3: Antibacterial activity of different extract of *S. willdenowii* and *S. radicata* using different bacterial strains

Table 2: Antioxidant assay of selected *Selaginella* species

Selaginella sp	*Concentration (µg)*	*Antioxidant activity of extract (%)*			
		Water	*Ethanol*	*Petroleum ether*	*Chloroform*
S. plana	50	31.07 ± 0.01	24.19 ± 0.01	19.62 ± 0.01	22.24 ± 0.02
	100	36.54 ± 0.01	30.20 ± 0.02	24.96 ± 0.01	25.76 ± 0.01
	200	40.57 ± 0.01	33.54 ± 0.01	28.88 ± 0.01	31.08 ± 0.01
	400	45.27 ± 0.02	37.47 ± 0.01	34.60 ± 0.02	36.17 ± 0.01
	800	50.92 ± 0.02	45.60 ± 0.02	39.63 ± 0.02	40.30 ± 0.01
	1000	52.45 ± 0.02	50.81 ± 0.01	42.38 ± 0.01	44.46 ± 0.01
IC_{50} µg/ml		220.00	187.456	210.74	215.20
S. radicata	50	38.51 ± 0.01	34.13 ± 0.01	27.24 ± 0.01	28.80 ± 0.01
	100	43.89 ± 0.02	38.60 ± 0.01	32.74 ± 0.02	32.91 ± 0.01
	200	55.78 ± 0.01	48.22 ± 0.01	36.45 ± 0.02	36.15 ± 0.02
	400	68.72 ± 0.01	57.62 ± 0.01	39.21 ± 0.01	41.89 ± 0.01
	800	81.14 ± 0.01	61.81 ± 0.02	42.89 ± 0.01	48.01 ± 0.01
	1000	85.21 ± 0.02	68.82 ± 0.02	48.72 ± 0.01	56.24 ± 0.02
IC_{50} µg/ml		95.134	134.98	242.98	181.871
*S. wallichi*i	50	32.07 ± 0.01	28.19 ± 0.02	22.62 ± 0.01	25.24 ± 0.02
	100	38.04 ± 0.02	33.00 ± 0.01	26.96 ± 0.01	27.76 ± 0.01
	200	45.57 ± 0.02	35.54 ± 0.01	31.88 ± 0.01	31.08 ± 0.01
	400	48.17 ± 0.02	37.47 ± 0.01	34.60 ± 0.02	34.17 ± 0.01
	800	54.12 ± 0.02	45.60 ± 0.02	39.63 ± 0.01	40.30 ± 0.02
	1000	57.90 ± 0.02	50.81 ± 0.01	42.38 ± 0.01	44.46 ± 0.01
IC_{50} µg/ml		189.013	223.709	246.096	250.67
S. willdenowii	50	35.51 ± 0.02	30.13 ± 0.01	25.24 ± 0.01	24.80 ± 0.01
	100	39.68 ± 0.02	34.60 ± 0.01	27.74 ± 0.01	29.91 ± 0.02
	200	47.76 ± 0.01	36.22 ± 0.01	32.45 ± 0.01	34.15 ± 0.01
	400	51.72 ± 0.01	38.62 ± 0.02	36.21 ± 0.02	36.89 ± 0.01
	800	58.24 ± 0.01	47.61 ± 0.02	40.89 ± 0.01	42.01 ± 0.01
	1000	65.12 ± 0.02	52.42 ± 0.02	43.51 ± 0.02	44.33 ± 0.02
IC_{50} µg/ml		163.649	223.295	254.673	250.504

Values are the average of triplicates (Mean ± SD)

(197.56 ± 0.01mg/g), the ethanolic extract of *S. radicata* has the highest concentration of alkaloids (189.18 ± 0.02mg/g), ethanolic *S. willdenowii* extract had a maximum concentration of tannin (79.66 ± 0.01mg/g (and the petroleum ether *S .radicata* extract had the of glycoside (35.66 ± 0.01mg/g). (Table 6).

Discussion

Antioxidant Activity

The antioxidant properties of the plant extract are mainly due to the redox characteristics of hydroxyl groups, which enable them to function as reducing agents. When antioxidants interact with DPPH, they reduce the free radical, neutralizing it. The degree of discoloration represents the antioxidant capacity of the plant extract (Gayathri *et al*., 2005) The antioxidant characteristics of different *Selaginella* species were studied using DPPH, and the results showed modest scavenging activities. The results suggested that antioxidant properties of extracts increased linearly with concentrations. *S. radicata* had the maximum antioxidant activity (85.21 ± 0.02) in the aqueous extract.

Antibacterial Activity

There were several reports that *Selaginella s*pecies contain powerful antimicrobial compounds (Lee *et al*., 2009; Juneyoung *et al*., 2009). The inhibition zone is shown in both the chloroform

Table 3: Antibacterial activity of *Selaginella* species against selected microorganism

Bacterial pathogens	*Solvents used*	*Zone of Inhibition (mm diameter) of Selaginella species*				
		S. plana	*S. radicata*	*S. wallichii*	*S. willdenowii*	*Standard (Gentamycin)*
E. coli MTCC 443	Water	7.5 ± 0.01	-	-	-	18 ± 0.01
	Ethanol	-	8 ± 0.01	8 ± 0.01	-	18 ± 0.01
	Petroleum ether	-	-	-	-	18 ± 0.01
	Chloroform	T	-	T	-	18 ± 0.01
Vibriocholerae MTCC 3906	Water	-	-	-	-	18 ± 0.01
	Ethanol	8 ± 0.01	8 ± 0.01	-	-	18 ± 0.01
	Petroleum ether	-	-	-	-	18 ± 0.01
	Chloroform	T	-	T	-	18 ± 0.01
Staphylococcus aureus MTCC 87	Water	-	-	-	-	22 ± 0.01
	Ethanol	8 ± 0.01	7.5 ± 0.01	T	T	22 ± 0.01
	Petroleum ether	-	-	-	-	22 ± 0.01
	Chloroform	T	T	-	-	22 ± 0.01
Streptococcus pyogenes MTCC 442	Water	-	-	-	-	24 ± 0.01
	Ethanol	9 ± 0.01	-	-	-	24 ± 0.01
	Petroleum ether	-	-	-	-	24 ± 0.01
	Chloroform	10 ± 0.01	T	T	T	24 ± 0.01

Values are the average of triplicates (Mean ± SD), Trace ≤7mm

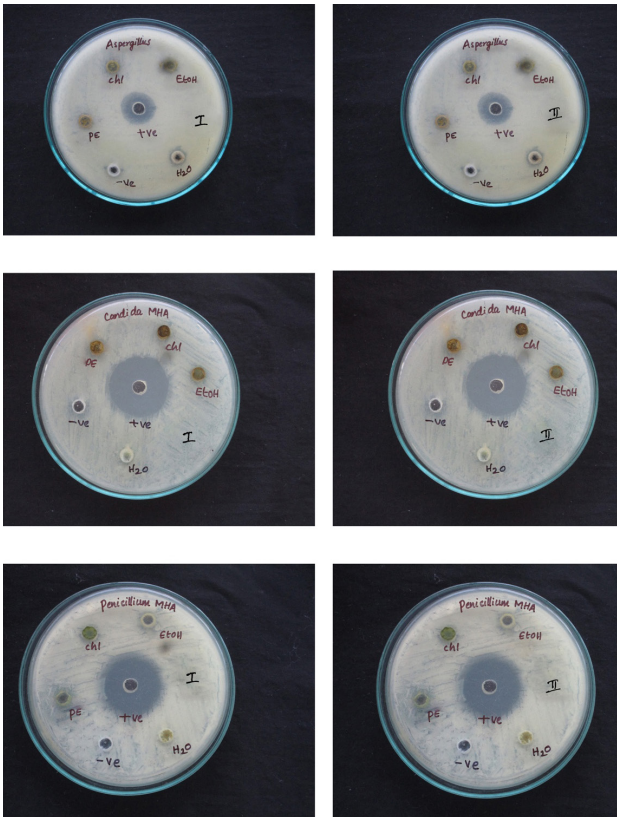

Fig. 4: Antibacterial activity of different extract of *S. willdenowii* and *S. plana* using different bacterial strains

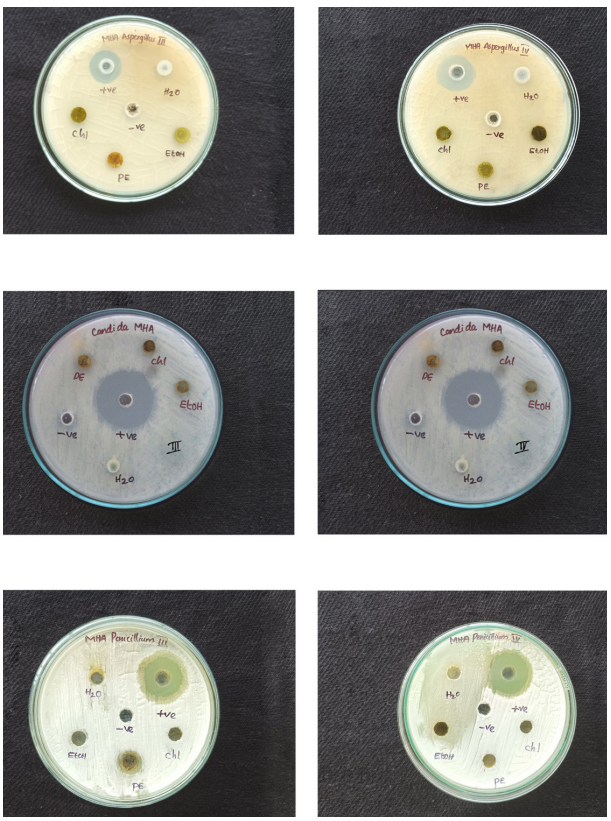

Fig. 5: Antifungal activity of different extract of *S. wallichii* and *S. radicata* using different fungal strains

Table 4: Antifungal activity of *Selaginella* sp.

Fungal pathogens	Solvents used	Zone of inhibition (mm diameter) of *Selaginella* species				
		S. plana	*S. radicata*	*S. wallichii*	*S. willdenowii*	*Standard Clotrimazole*
Candida albicans MTCC 227	Water	-	-	-	-	20 ± 0.01
	Ethanol	T	T	T	T	20 ± 0.01
	Petroleum ether	-	-	-	-	20 ± 0.01
	Chloroform	T	T	T	-	20 ± 0.01
Aspergillus niger MTCC 872	Water	8 ± 0.01	-	-	9 ± 0.02	18 ± 0.01
	Ethanol	T	T	T	8 ± 0.02	18 ± 0.01
	Petroleum ether	-	-	-	-	18 ± 0.01
	Chloroform	T	T	-	-	18 ± 0.01
Penicillium chrysogenum MTCC 5108	Water	-	-	-	13 ± 0.01	25 ± 0.01
	Ethanol	T	T	T	11 ± 0.01	25 ± 0.01
	Petroleum ether	-	-	9 ± 0.01	-	25 ± 0.01
	Chloroform	T	-	T	T	25 ± 0.01

Values are the average of triplicates (Mean ± SD), Trace ≤7mm

Table 5: Qualitative analysis of various phytochemical constituents in *Selaginella* species

Extract/Phytochemical		*Phenol*	*Tannin*	*Flavonoid*	*Saponin*	*Terpenoid*	*Alkaloid*	*Glycoside*	*Steroid*	*Quinone*	*Fatty acids*
Petroleum ether	*S.willdenowii*	++	++	++	+	+	+++	+++	+	+	+
	S. plana	++	++	++	+	+	+++	++	-	-	-
	S. wallichii	++	++	++	+	+	+++	++	+	+	+
	S.radicata	++	++	++	+	-	++	++	-	+	-
Chloroform	*S.willdenowii*	+	+	+++	-	-	++	++	+	-	+
	S. plana	+	++	+++	+	-	+++	++	-	-	-
	S. wallichii	++	++	++	+	+	+++	++	+	+	+
	S.radicata	+	+	++	-	+	+	+	+	-	+
Ethanol	*S.willdenowii*	++	++	+++	+	+	+	+	+	+	+
	S. plana	++	+	+++	+	-	+++	++	+	+	+
	S. wallichii	++	++	++	+	-	+++	++	+	+	-
	S. radicata	++	++	+	+	+	+	+	+	+	+
Water	*S.willdenowii*	++	++	+	+	+	++	+	+	+	+
	S. plana	++	++	++	+	+	++	+	+	+	+
	S. wallichii	++	++	++	+	+	+++	++	+	-	+
	S.radicata	+++	++	++	+	+	+	+	+	-	+

+++ strong positive, ++ positive, + trace, - negative

and ethanol extracts of *S. plana*. The presence of flavonoids may be the reason for antibacterial activity in the respective species.

Antifungal Activity

S.willdenowii shows an appreciable inhibition zone against *Aspergillus niger* and *Penicillium chrysogenum* in both aqueous and ethanol extracts. Both *S. plana* and *S. wallichii* also have an inhibition zone against *Aspergillus niger* and *Penicillium chrysogenum*, respectively. Presence of phenol may be the reason for the potential antifungal activity of *S. willdenowii*. A study conducted by Lee *et al*., (2009) suggests that a novel compound, isocryptomerin, from *S.tamariscina* shows energy-independent antifungal activity against *Candida albicans*. But in the present study, there was no activity seen in any of the solvents against *C. albicans*.

Table 6: Quantitative analysis of various phytochemical constituents in *Selaginella* species

Solvents	Sample	Phytocompounds				
		Phenol (mg/g)	*Flavonoid (mg/g)*	*Alkaloids(mg/g)*	*Tannin (mg/g)*	*Glycoside (mg/g)*
Petroleum ether	*S. plana*	22.5 ± 0.01	15.78 ± 0.01	29.91 ± 0.01	11.45 ± 0.01	35.58 ± 0.01
	S.radicata	32.5 ± 0.01	18.23 ± 0.01	48.27 ± 0.01	15.76 ± 0.01	35.66 ± 0.01
	S. wallichii	32.5 ± 0.01	45 ± 0.01	167.36 ± 0.02	27.36 ± 0.02	26.42 ± 0.01
	S.willdenowii	25.17 ± 0.02	19.23 ± 0.02	28.00 ± 0.01	12.35 ± 0.01	36.17 ± 0.02
Chloroform	*S. plana*	9.42 ± 0.01	175.51 ± 0.01	39.36 ± 0.02	9.12 ± 0.01	19.53 ± 0.01
	S.radicata	13.99 ± 0.01	197.56 ± 0.01	138.27 ± 0.01	18.73 ± 0.01	26.25 ± 0.01
	S. wallichii	32.09 ± 0.01	38.33 ± 0.01	173.73 ± 0.01	21.82 ± 0.01	9.36 ± 0.01
	S.willdenowii	10.63 ± 0.01	48.85 ± 0.02	12.82 ± 0.01	15.99 ± 0.01	11.95 ± 0.01
Ethanol	*S. plana*	43.30 ± 0.01	127.05 ± 0.01	21.91 ± 0.01	63.63 ± 0.01	24.99 ± 0.01
	S.radicata	32.61 ± 0.01	97.05 ± 0.01	189.18 ± 0.02	51.77 ± 0.01	15.50 ± 0.01
	S. wallichii	22.5 ± 0.01	30 ± 0.01	63.73 ± 0.01	12.01 ± 0.01	15.75 ± 0.01
	S.willdenowii	85.89 ± 0.02	71.41 ± 0.01	52.82 ± 0.02	79.66 ± 0.01	29.45 ± 0.01
Water	*S. plana*	25.28 ± 0.01	13.54 ± 0.02	44.64 ± 0.01	42.06 ± 0.01	7.26 ± 0.01
	S.radicata	34.25 ± 0.01	15.28 ± 0.01	66.45 ± 0.02	42.41 ± 0.01	7.93 ± 0.01
	S. wallichii	12.5 ± 0.01	10 ± 0.01	137.36 ± 0.01	19.51 ± 0.01	15.66 ± 0.01
	S.willdenowii	104.59 ± 0.01	64.23 ± 0.01	10.09 ± 0.01	73.29 ± 0.02	16.08 ± 0.01

Values are the average of triplicates (Mean ± SD)

Phytochemical Analysis

Based on the preliminary phytochemical analysis of four different extracts from selected species of *Selaginella*, secondary metabolites such as steroids and tannins are found in moderate amounts, while terpenoids, steroids, quinones, fatty acids, and saponins are rarely present. In *S. willdenowii*, phenols, tannins, and glycosides are moderately present, whereas flavonoids and alkaloids are highly abundant in all solvents. In *S. plana* and *S. wallichii*, phenols, tannins, flavonoids, and glycosides are present in moderate quantities, with alkaloids showing a high presence in all solvents. In *S. radicata*, phenols, tannins, flavonoids, alkaloids, and glycosides are moderately represented across all solvents.

In a study conducted by Irudayaraj et al., (2010), it was concluded that secondary metabolites such as steroids and tannins are somewhat prevalent, while phenolic compounds and saponins are rarely detected in all solvents of *S. inaequalifolia*. The alkaloid content was higher in the ethanolic extract of *S. radicata* and lower in the water extract of *S. willdenowii*. The chloroform extract of *S. radicata* exhibited a greater concentration of flavonoids compared to the water extract of *S. wallichii*. Higher concentrations of phenolic compounds and tannins were observed in *S. willdenowii*, with lower amounts found in *S. plana*. Glycosides were more abundant in the petroleum ether extract of *S. willdenowii* and less so in the aqueous extracts of *S. plana* and *S. radicata*.

Conclusion

The study shows that the antioxidant and antimicrobial properties of Selaginella species vary, suggesting that comprehensive scientific validation of their active components is necessary for medical use. The medicinal properties of these plants are linked to secondary metabolites such as tannins, phenols, flavonoids, alkaloids, and saponins. These phytochemicals are responsible for the therapeutic effects observed in the species examined. Although the plants demonstrate moderate activity, this is likely due to the presence of these compounds. Further research is essential to pinpoint the specific active ingredients responsible for their bioactivity.

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Authors Contribution

The first author conceived and designed the study, conducted the analysis, interpreted the data, and drafted the manuscript. The second author reviewed, provided critical revisions, and approved the final version for publication.

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