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Qualitative phytochemical analysis and antimycobacterial activity of *Selaginella involvens* against *Mycobacterium smegmatis*

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Abstract
Selaginella Involvens from *Selaginella*ceae family is one of the two qualified species listed in Chinese Pharmacopoeia that has long been used as a traditional Chinese medicine for promoting blood circulation. Numerous bioactive compounds including alkaloids, flavonoids, lignans, and *Selaginella* have been identified in this medicinal plant with broad biological activities like antivirus, antifungal, antibacterial, cytotoxic, and anti-inflammatory properties. *Selaginella* represent a type of characteristic constituent of *Selaginella*. So far, over one hundred *Selaginella* with different polyphenolic skeletons have been reported from this genus. From these some of *Selaginella* displays high anticancer activity. The study was conducted to detect possible phytoconstituents present in *Selaginella involvens* (*Selaginella*ceae) and investigate its antimycobacterial activity. The anti-mycobacterial activity of the extract was done using standard bacterial strain of *Mycobacterium smegmatis*. The testing was done by the agar well diffusion method. The crude extract was tested against isolated bacteria at concentrations of 4 and 8 mg. The gentamycin (80mcg) was taken as positive control and Dimethyl sulfoxide as negative control. Zone of Inhibition of extract was compared with standard drug Gentamycin. Results indicate that 8 mg of ethyl acetate extract showed the maximum inhibitory effects against *Mycobacterium smegmatis* (11 mm).

Keywords: *Selaginella* involvens Spring, Phytochemicals, Antimycobacterial, *Mycobacterium smegmatis*, agar well diffusion, Zone of inhibition

Introduction
The drug-resistance phenomenon of *Mycobacterium tuberculosis* is a major obstruction of allopathy treatment. An adverse side effect of allopathic treatment is that it causes serious health complications. The search for suitable alternatives of conventional regimens is needed, i.e., by considering medicinal plant secondary metabolites to explore anti-TB drugs, targeting the action site of *M. tuberculosis* [2].

In 1884, Sigmund isolated a novel species of the mycobacterium family which was named *Mycobacterium smegmatis* (*M.sm*) by Lehmann and Neumann in 1889. *M.sm* is a saprophytic, biofilm-forming bacterium that is non-infectious in mammals. *M.sm* is commonly found in water, soil and plants, endemic to sixteen states, Australia, Russia, Canada, and Switzerland [1]. Its doubling time (3h) is faster than other *Mycobacterium* species, and it grows in non-pigmented, velvety, yellow-colored colonies. It may survive through short anaerobic periods during certain stages of its disease course. There are several similarities between *M.sm* and other harmful infectious mycobacterial species, such as *Mycobacterium tuberculosis* (*M. tb*). The most apparent similarity between the two species is a common mycothiol biosynthesis pathway for the production of basic thiol, which is required for intra-bacterial homeostasis. The sharing of a key metabolic pathway, even this simple example, alludes to the commonalities between *M. tb* and *M.sm* at multiple functional levels [1, 3].

The pathogenicity and slow growth of around 3 to 4 weeks to form colonies – make *M. tuberculosis* extremely difficult to work with in the laboratory. The seminal breakthrough toward developing genetic approaches to study pathogenic mycobacteria was the isolation of a transformable derivative of *Mycobacterium smegmatis*. *M. smegmatis* is a nonpathogenic and fast-growing species (colonies in 3 days) and was historically used as a mycobacteriophage host. The development made *M. smegmatis* as a model for studying properties of all mycobacteria, including pathogens, such as *M. tuberculosis* and the nontuberculous mycobacteria (NTM) pathogens [3, 4].

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Medicinal plants are gifts of nature to cure limitless number of diseases among human beings. *Selaginella involvens* (SI) from Sellaginellaceae family is a well-known traditional medicine for the treatment of metabolic disorders, several inflammatory diseases, and various cancers in many Asian countries. It *is* used in folk medicine to treat the side effects of mental instability, tumor prevention and healing, renal function enhancement, stones, asthma, bronchial disease, and radiation therapy, also used to treat bleeding and maintain hemostasis. It is primarily found in tropical and subtropical areas. These species have been reported for numerous constituents like alkaloids, flavonoids, lignans, *Selaginella*, phenolics, and terpenoids [5-7].

Materials and Methods

1. Plant collection and Authentication

The whole plant of *Selaginella involvens* Spring (*Selaginella*ceae) were collected from Rayirath Garden, Pattikkad, Thrissur district, Kerala, India. The collected materials were taxonomically identified by Dr.P.S. UDAYAN, Assistant professor Department of Botany and Research centre, Sreekrishna College, Guruvayur, Kerala. The voucher specimen of *Selaginella involvens* (Sw.) spring (84) was submitted in the Herbarium.

2. Preparation of plant extract

The shade dried coarsely powdered whole plant of *Selaginella involvens* Spring (250 g) was extracted with four different solvents like petroleum ether, acetone, ethyl acetate, ethanol by using Soxhlet apparatus at 50-55 °C until complete exhaustion of drug. The extract was filtered through Whatman filter paper No.1. and concentrated in vaccum rotary evaporator to get the powdered form. The percentage yield was calculated [8, 9].

3. Qualitative Phytochemical Analysis

Phytochemical investigation was done for alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides, protein and carbohydrates by routine chemical tests [10, 11].

4. Antimycobacterial study

The inoculums were procured from The Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh. The test organism *Mycobacterium smegmatis*, (MTCC NO: 6) was cultured on to nutrient agar media in order to determine their viability.

5. Culture medium

Muller Hinton Agar medium (HIMEDIA- M173) was used for determination of susceptibility of microorganisms to antimicrobial agents. Suspended 38 grams in 1000 ml distilled water. Heat until it boils to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure, (121 °C) for 15 minutes. Cool to 45-50 °C. Mix well and pour into sterile Petri plates [12].

6. AGAR well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of the test sample against microorganisms. The determination of antibacterial activity of phytochemical studied by using Muller Hinton Agar medium (HIMEDIA- M173) [9].

The sterilized 15- 20 ml of Mueller-Hinton agar was poured on glass petri plate and allowed to solidify. After the solidification, the wells (4 wells/ plate) were made with a sterile cork borer of diameter 8 mm (20 mm apart from one another) were punched aseptically in each plate. The standardized inoculum of the test organism such as *Mycobacterium smegmatis* was uniformly spread on the surface of these solidified media using sterile cotton swab. The test volumes (4 mg & 8 mg) of the sample at desired concentrations were added to the first 2 wells, one well with 80 mcg of Gentamycin as positive control and other one with DMSO as negative control. Then, the agar plates were incubated in incubator under 37 °C for 24 hr. After incubation, clear zone was observed. Inhibition of the bacterial growth was measured in mm [13, 14, 15].

7. Results and Discussion

7.1 Percentage yield of *Selaginella involvens* extracts after solvent extraction

Sl. No	Extracts	Colour	Percentage yield (%w/v)
1.	Petroleum ether extract	Dark red	10.92
2.	Acetone extract	Dark brown	12.00
3.	Ethyl acetate extract	Dark brown	19.60
4.	Ethanolic extract	Dark green	16.06

Out of these 4 extracts the ethyl acetate extract shows maximum percentage yield. So, the Ethyl acetate extract was selected for further investigations.

7.1 Qualitative phytochemical analysis

The phytochemicals present in various extracts are given below:

	Petroleum ether	Acetone	Ethyl acetate	Ethanol
Alkaloids	+	+	+	+
Saponins	-	+	+	-
Anthraquinone Glycosides	-	-	+	-
Cardiac glycosides	-	-	-	-
Phenolic compounds	+	+	+	+
Flavonoids	-	+	+	+
Carbohydrates	+	+	+	+
Proteins and amino acids	+	-	+	-
Triterpenoids and steroids	-	-	+	-
Gums and mucilages	-	+	-	+
Resins	-	-	-	-

The above results shows that the ethyl acetate extract contains most of the important phytochemicals

7.2 Anti-mycobacterial activity

The antimycobacterial activity of the ethyl acetate extract of the plant was examined by measuring zone of inhibition. The antimycobacterial activity was performed at 4mg and 8mcg concentrations by Agar well diffusion method. Gentamycin 80 mcg was used as standard. The results of the antimycobacterial activity are shown below.

Sample extract	Organism	Zone of inhibition (mm)			
		Standard Gentamycin (80 mcg)	Negative control	Test 1 (T1) (4 mg)	Test 2 (T2) (8 mg)
Ethyl acetate	*Mycobacterium smegmatis*	14	-	4	11

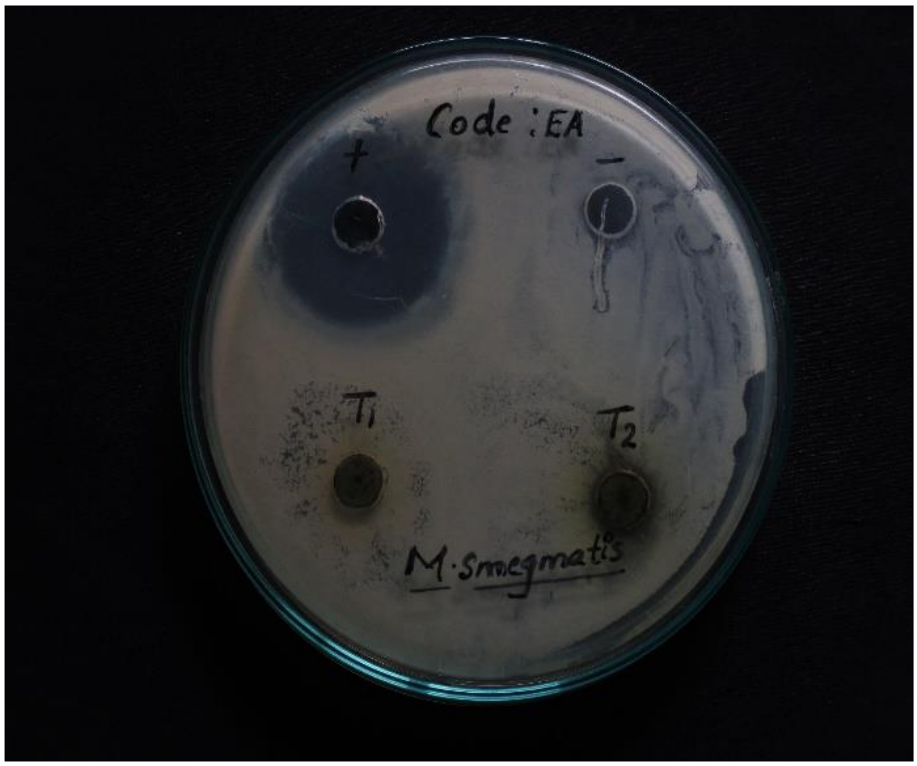

Fig 1: Zone of inhibition of *Selaginella involvens* Spring against Gentamycin

The *Selaginella involvens* plant extract exhibited maximum activity at 8mg concentration against mycobacterium. The zone of inhibition was calculated in mm. However preliminary phytochemical analysis of ethyl acetate extract was exposed the presence of terpenoids, phytosterol, polyphenol, saponins, flavonoids, carbohydrates and tannins. The antimicrobial potency of the plant may be attributed to the single or combined effect of the above-mentioned chemical groups.

8. Conclusion

Tuberculosis is one of the diseases with high prevalence rate in India. Current therapeutic approaches for TB have various side effects so it augments the opportunity to phytochemical study. Medicinal plants are rich in bioactive constituents with incredible pharmacological activities. The tremendous activities of these phytochemicals make the plant attractive for TB treatment. Plants represent new sources of anti-TB agents but only few plant species have been thoroughly investigated for their anti-TB potential and thus there is a renewed opportunity in utilizing phytomedical research for TB drug discovery from alternative sources.

Overall, the antimycobacterial activity of the *Selaginella involvens* extract against *Mycobacterium smegmatis* supports its efficacy in the treatment mycobacterial infection. This is the first research work on the anti-*mycobacterial* activity of *Selaginella involvens* extract in India. This particular study has a wide scope in future for the development of herbal drugs with less or no side effects.

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Conflict of interest

No conflict of interest.

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