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Phytochemical Screening and Microbicidal Activity of *Siegesbeckia orientalis* Against Human Bacterial Pathogens

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Abstract

Background: *Sigesbeckia orientalis,* commonly known as the "Chinese herb" has been utilized in traditional medicine for its various therapeutic properties. This plant is part of the Asteraceae family and is known for its anti-inflammatory, analgesic, and antimicrobial effects. The increasing prevalence of antibiotic-resistant pathogens has prompted researchers to explore alternative sources of antimicrobial agents, particularly from natural products.**Aim:** This study investigated the ability of *Sigesbeckia orientalis* to inhibit common human pathogens, including *Proteus vulgaris, Staphylococcus aureus, Klebsiella* sp., *Escherichia coli, Pseudomonas aeruginosa, Enterobacter aerogenes, Trichophyton rubrum,* and *Epidermophyton floccosum*. **Method:** Fifty grams of crushed material were treated with 300 ml each of chloroform, hexane, methanol, and ethanol for 24 hours at 23±2°C. The extracts were filtered through Whatman filter paper into a clean beaker. A second extraction was performed with the same solvents for another 24 hours, followed by filtration. The combined extracts were evaporated using a rotary flash evaporator and stored in sealed containers at 4°C until further use. **Results:** The ethanol extract showed significant antibacterial activity, particularly against *Pseudomonas aeruginosa* (15 mm) and *Staphylococcus aureus* (13 mm). In contrast, the chloroform extract was less effective, with minimal inhibition against several bacteria. Minimum inhibitory concentrations (MICs) were 0.75 mg/ml for S. aureus and 1.0 mg/ml for *P. aeruginosa*, while other bacterial isolates exceeded 2.0 mg/ml. MIC values for *Trichophyton rubrum* and *Epidermophyton floccosum* from the ethanol extract were higher than the standard antifungal agent, ketoconazole. **Conclusion:** These findings support the antimicrobial potential of phytoconstituents in traditional medicinal plants for developing new treatments for infections.

Major Findings: The antimicrobial activity observed in plant extracts may be attributed to the presence of one or more phytoconstituent active compounds, which could be functioning synergistically. According to the current study's findings, *S. orientalis* leaf preparations in ethanol contain a variety of pharmacologically potent substances.

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Keywords: Antimicrobial Activity, Human Bacterial Pathogens, Minimum Inhibitory Concentration, Phytochemical Constituents, *Siegesbeckia orientalis*

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1. Introduction

Sigesbeckia orientalis is an annual erect crop that ranges in height from 0.4 to 1.5 m. It typically has multiple branches and hollow, reddish-coloured stems. Leaflets can range in size from 2–17 cm and have a broadly lance-shaped shape, 1–7 cm wide, with a petiole up to 2 cm long, underside has small, yellow glands. This plant is showing anti-inflammatory, analgesic, anti-allergic, immunosuppressive, anti-hyperuricemia, neuroprotective, anti-Proliferative, cervical cancer, antifeedant, insecticidal, antimicrobial effect[1], and xanthine oxidase inhibitory activities. Indigenous doctors assert that their treatments are less expensive and more efficient than contemporary treatments. Folk medicine is used by low-income individuals in developing nations to treat common infections, including farmers, residents of remote small villages, and members of indigenous groups[2]. Alkaloids, Flavonoids, tannins, and phenolic chemicals are the most significant of these bioactive substances found in plants[3].

Drug susceptibility has emerged as a major danger to worldwide wellness, which impacts all significant pathogenic microbes and medications against bacteria[4]. It causes many different infectious diseases and is challenging to cure. In order to defend themselves from antimicrobial agents, bacterial and fungal pathogens have developed a variety of defence mechanisms. As a result, resistance to both old and novel medications is increasing. Due to the increased effectiveness of new herbal medicines and growing worries about the negative impacts of current medical therapy, there has been an increase in recent years in scientific research in medicinal plants. Therefore, it is necessary to search for novel biochemical forms lead substances in the plant world. This specific herb exhibits antimicrobial action against molds, gram +ve and -ve bacteria, and both[3-5].

2. Materials and Methods

2.1 Plant Collection

Leaves of *S. orientalis* were collected from the Araku hills in Visakhapatnam, Andhra Pradesh, and identified through their morphological characteristics. After washing with distilled water and air drying in the shade, the leaves were ground to a powder (100-120 grit) using an apex mill and dried in a hot air oven at 40°C for 48 hours before extraction.

2.2 Chemicals

The solvents used in this study, including ethanol, methanol, chloroform, and hexane, were of Analytical Reagent (AR) quality and sourced from Merck (Mumbai). Nutrient Medias were obtained from Himedia Laboratories (Mumbai). Additionally, all the chemicals were procured from SD Fine Chemicals (Mumbai).

2.3 Microbes Examined (6B + 2F)

The species utilized in the experiments included clinical isolates such as *Escherichia coli, Proteus vulgaris, Klebsiella* sp., *Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes, Trichophyton rubrum,* and *Epidermophyton floccosum*, which were obtained from IMTECH, Chandigarh. Both bacterial and fungal colonies were maintained on Sabouraud Dextrose Agar (SDA) and nutrient agar media, respectively.

2.4 Preparation of Aqueous Extract

In a blender, 50 gm of pulverized *S. oreintalis* leaves were macerated for 10 minutes with 100 millilitres of sterile distilled water. The squash was spun through a centrifuge at 4000 rpm for 30 minutes after passing through two layers of cotton fabric. After being thermally disinfected at 120°C for 30 minutes, the liquid was filtered using Whatman No. 1 filter paper. The extracted materials were stored in an aseptic manner at 4°C until they were used in brown vials[6].

2.5 Preparation of Solvent Extract

The extraction process was conducted following the method described by Okigbo *et al*. Initially, 50 grams of the crushed material were treated with 300 ml of chloroform, hexane, methanol, and ethanol, respectively, and allowed to process for 24 hours at a temperature of 23±2°C. The resulting concentrate was then filtered through Whatman No. 1 filter paper into a clean conical beaker. A second extraction was carried out using the same amount of solvent for an additional 24 hours at the same temperature, followed by filtration. The extracts were combined and placed in the sample holder of a rotary flash evaporator (Buchi Rotavapor R-124, Switzerland) to evaporate the solvents. The dehydrated extracts were subsequently stored in sealed containers at 4°C until needed for further use[7].

2.6 Antibacterial Screening

Antibacterial activity was evaluated using the disc diffusion method. Bacterial cultures were adjusted to 1 x 10^6 CFU/ml and spread on nutrient agar plates. After 15 minutes of drying, sterile 5 mm discs were placed on the agar and filled with 10 µl (1 mg/ml) of plant extracts. A negative control used 10 µl of solvent, while a positive control used a 10 µg streptomycin disc. Plates were incubated at 37°C for 18–24 hours and analysed for zones of inhibition[8], as shown in Figure 1.

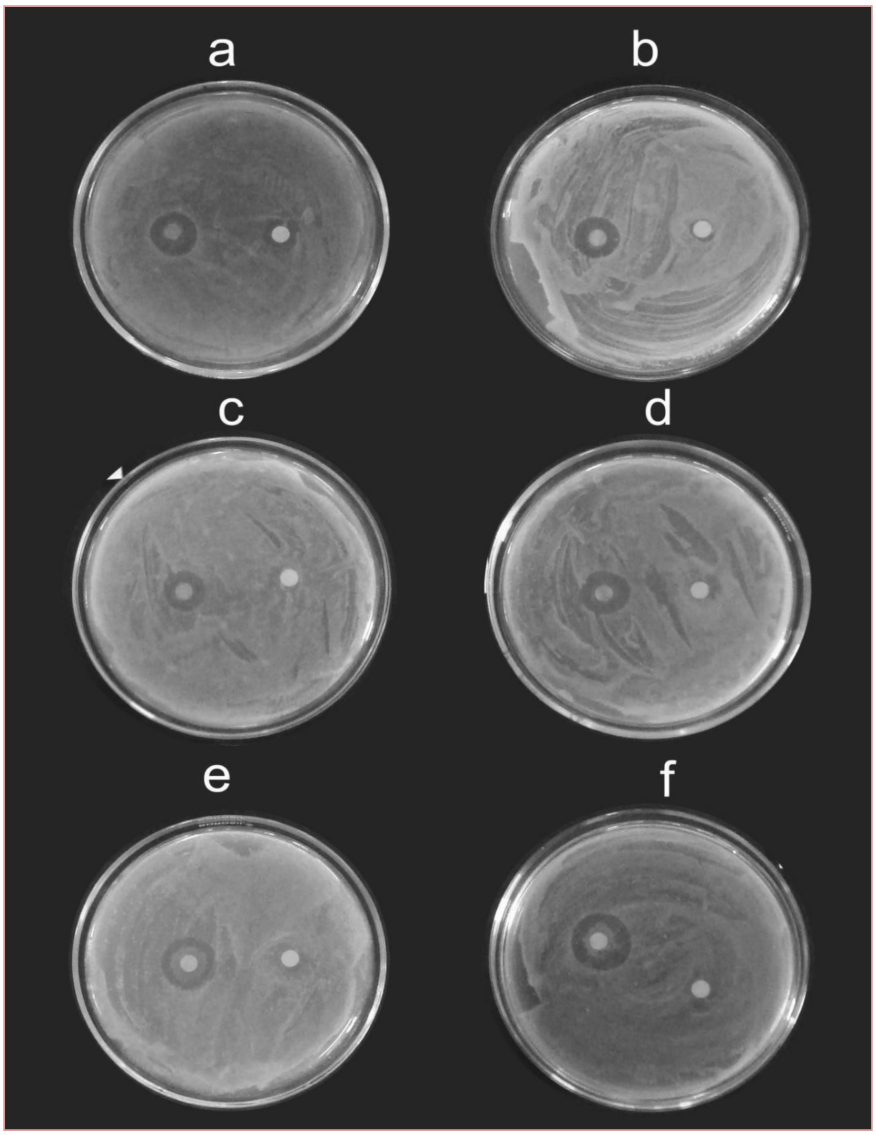


Figure 1. *Seizesbeckia oreintalis* leaf extract in ethanol has antibacterial action against the test bacteria (a) *Escherichia coli* (b) *Enterobacter aerogenes* (c) *Klebsiella* sp. (d) *Proteus vulgaris* (e) *Staphylococcus aureus* and (f) *Pseudomonas aeruginosa.*

2.7 Determination of Minimum Inhibitory Concentration

The Minimal Inhibitory Concentration (MIC) was determined using the broth dilution method. Experimental microbes were cultivated to a concentration of 1 x 10^6 CFU/ml in nutrient broth. A 0.5 ml extract (0.25-2 mg/ml) was combined with 4 ml of broth and 0.5 ml of bacterial suspension. Control tubes contained 4.5 ml of broth and 0.5 ml of culture, while uninoculated vessels had 5 ml of broth. After incubating for 18 hours at 37 °C, absorbance at 600 nm was measured to assess bacterial growth inhibition. The MIC was defined as the lowest concentration preventing bacterial development[9]. The percentage of growth inhibition was calculated using the appropriate equation.

$$Percent\ \ growth\ inhibition = [(Acontrol - Atest\ /\ Acontrol] \times 100$$

2.8 Antifungal Activity Assay

The mycelial dry weight method, following Rasooli and Abyaneh's technique, assessed the fungicidal activity of ethanol extracts at 1 to 10 mg/ml. After one week on SDA medium, 0.85% saline was applied to skin fungi. Conidia were counted with a haemocytometer and diluted to a concentration of 1x10^6 spores/ml in Sabouraud dextrose broth. An anti-dermatophytic test was conducted by adding 20µl of mold solution and extract concentrations to sterile Sabouraud broth in screw-capped containers, which were incubated at 30°C for a week. Apparent mycelial growth was monitored, and mycelia were filtered through Whatman No. 1 paper, dried at 60°C to a constant weight. Mycelial dry weights of the samples were compared to determine fungal growth inhibition, with ketoconazole used as a control[10]. The percent growth inhibition was calculated using a specific formula.

$$Percent\ \ growth\ inhibition = [(Acontrol - Atest\ /\ Acontrol] \times 100$$

2.9 Phytoconstituents Analysis

In order to determine the presence of phytoconstituents the ethanolic leaf extracts of *S. orientalis* were subjected to a qualitative phytonutrient study[11-14].

2.10 Statistical Analysis

The experimental results are represented by the mean and standard deviation of three independent measurements. To assess the significance of differences among group means, One Way Analysis of Variance followed by Tukey's test was employed. Pvalue of 0.05 or less was considered statistically significant[15,16]. The statistical analysis was conducted using Graph Pad statistical software.

3. Results

3.1 Phytochemical Screening

The ethanol extract, which demonstrated significant antibacterial and antifungal properties, was utilized for the screening of phytochemical compounds. The findings from the phytochemical analysis of *S. orientalis* leaf ethanol extracts are presented in Table 1.

3.2 Antibacterial Screening of Solvent Extracts

Different solvent extracts of *S. orientalis* leaf's antibacterial action against bacterial infections revealed varying degrees of inhibition. The findings were shown in Table 2 and Figure 2.

3.3 Determination of MIC

Using the broth dilution method, the MIC values for *S. oreintalis* ethanol leaf extract were calculated (Table 3). The values ranged from 0.25 to 2.0 mg/ml.

3.4 Antifungal Activity Assay

The ethanol extracts of *S. orientalis* leaves were evaluated for their Minimum Inhibitory Concentration (MIC) values against two dermatophytes, *T. rubrum*

Table 1. Chemical constituents of *S. orientalis* leaves concentrate in ethanol

Phytonutrients	Ethanol leaf extracts of *S. orientalis*
Phenols	+
Flavonoids	+
Tannins	+
Terpenoids	+
Alkaloids	+
Polysterols	-
Cardiac glycosides	+
Saponins	+
Carbohydrates	+
Protiens	+

Table 2. Zone of inhibitory activity of various *S. orientalis* leaf extracts against the test microorganisms

Solvent extract	*E.coli*	*E.aerogenes*	*Klebsiella* sp.	*P.vulgaris*	*S.aureus*	*P.aeruginosa*
Aqueous	2.0 ± 2.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.0	3.0 ± 1.73	4.0 ± 1.72
Ethanol	11.0 ± 1.0	10.0 ± 1.73	8.0 ± 3.0	9.0 ± 1.70	13.0 ± 2.64	15.0 ± 1.74
Methanol	8.0 ± 2.64	6.0 ± 1.73	5.0 ± 1.0	7.0 ± 2.0	9.0 ± 1.0	10.5 ± 1.80
Hexane	3.0 ± 1.0	1.0 ± 1.0	0.0 ± 0.0	2.0 ± 1.0	4.0 ± 1.73	4.5 ± 1.32
Chloroform	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 0.26	3.0 ± 1.0
Streptomycin (10µg/disc)	15.0 ± 2.0	14.0 ± 1.73	11.0 ± 1.72	12.0 ± 1.73	11.0 ± 2.0	14.0 ± 1.70

Extract used at a quantity of 1 mg/ml. Results suggest the average of three separate trials. As shown, average values with various superscripts are considerably different from one another by Turkey's HSD ($P \leq 0.05$)

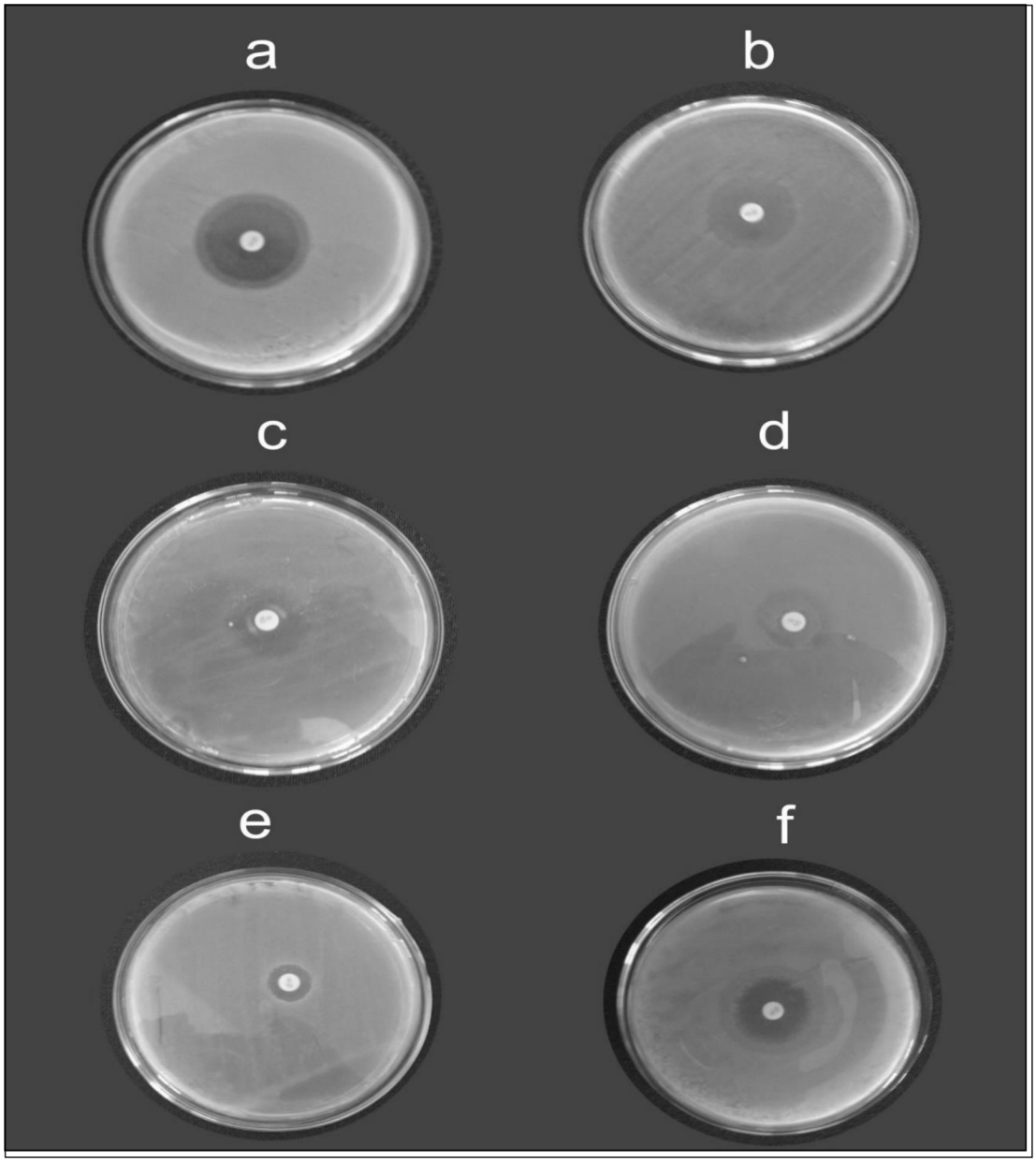


Figure 2. Streptomycin, a common antibiotic, had antibacterial action (10 g/disc) against the test bacteria **(a).** *Escherichia coli,* **(b).** *Enterobacter aerogenes,* **(c)**. *Klebsiella* sp. **(d).** *Proteus vulgaris,* **(e).** *Staphylococcus aureus* and **(f).** *Pseudomonas aeruginosa*.

Table 3. The minimum inhibitory concentration of *S. oreintalis* leaf ethanol extract

Bacteria	Control	Concentration (mg/ml)				
		0.25	0.5	0.75	1	2
E. coli	0.27±0.05	0.27±0.01	0.17±0.02	0.14±0.03	0.13±0.00	0.11±0.00
E. aerogenes	0.30± 0.02	0.29± 0.01	0.24± 0.01	0.19± 0.02	0.15± 0.01	0.14± 0.01
Klebsiella sp.	0.25± 0.00	0.24± 0.01	0.20± 0.02	0.20± 0.02	0.18± 0.02	0.16± 0.01
P. vulgaris	0.25± 0.01	0.24± 0.00	0.20± 0.00	0.19± 0.01	0.16± 0.00	0.15± 0.01
S. aureus	0.22± 0.04	0.21± 0.01	0.15± 0.00	0.09± 0.02	0.03± 0.01	0.02± 0.00
P. aeruginosa	0.14± 0.03	0.13± 0.02	0.06± 0.01	0.02± 0.01	0.02± 0.00	0.01± 0.01

The numbers represent absorbance at 600 wavelengths. Values represent the average of three separate trials. Turkey's HSD ($P \leq 0.05$) shows that mean values with various superscripts are considerably different from one another.

Table 4. Minimum inhibitory concentration of *S. oreintalis* leaf ethanol extracts against dermatophytes using mycelial dry weight method

	T. rubrum		*E. floccosum*	
	MIC (mg/ ml)	% Growth inhibition	MIC (mg/ ml)	% Growth inhibition
Ketaconazole	0.4 ± 0.01	90.3	0.25 ± 0.02	93
S. oreintalis	> 10 ± 0.06	70.9	1.75 ± 0.05	74.6

and *E. floccosum*, using the mycelial dry weight method. Table 4 presents the MIC values and the percentage of growth inhibition of *S. orientalis* ethanol leaf extracts in comparison to the antifungal agent Ketoconazole. Among the two pathogens, *E. floccosum* exhibited greater susceptibility to the ethanol extract of *S. orientalis*, with an MIC value of 1.75 mg/ml, resulting in a 74.6% reduction in growth. In contrast, the MIC for *S. orientalis* extract against *T. rubrum* was found to be higher than the recommended concentration of 10 mg/ml. Overall, the MIC values obtained for *T. rubrum* and *E. floccosum* using the ethanol extract of *S. orientalis* surpassed those of the standard antifungal medication Ketoconazole.

4. Discussion

Recent studies have revealed that leaf extracts of *S. orientalis,* when prepared with ethanol, contain a diverse array of pharmacologically active compounds. Phytochemical analysis of these ethanol extracts has identified the presence of phenols, flavonoids, saponins, tannins, terpenoids, cardiac glycosides, and phytosterols, all of which exhibit various biological activities. These active constituents may function independently or synergistically, contributing to the antimicrobial properties of the plant extracts, including both antibacterial and antifungal effects. Phenolic compounds represent one of the largest and most prevalent classes of plant metabolites. The antibacterial properties of polyphenols have been highlighted in research by Baravalia *et al*[17]. In addition to their antibacterial effects, polyphenols and flavonoids are known to have a range of biological impacts. Furthermore, Fatope[18] emphasized the significance of polyphenols as important fungitoxic phytochemicals.

As shown in Table 2, the alcoholic extracts demonstrated the most pronounced antibacterial activity, with the largest zones of inhibition observed against *P. aeruginosa* (15.0 mm) and *S. aureus* (10.0 mm). Moreover, these extracts exhibited a broad spectrum of activity, affecting the majority of the bacterial strains tested. In contrast, the chloroform extract displayed the weakest inhibitory effect, with no activity against *E. aerogenes*, *P. vulgaris*, or *Klebsiella* sp., and only minimal activity against *E. coli* (1.0 mm), *S. aureus* (2.2 mm), and *P. aeruginosa* (3.0 mm). The minimum inhibitory concentrations (MICs) for *S. aureus* and *P. aeruginosa* were determined to be 0.75 mg/ml and 1.0 mg/ml, respectively. Spectrophotometric analysis at 600 nm revealed that the alcoholic extracts inhibited the growth of *P. aeruginosa* and *S. aureus* by 83.3% and 84.8%, respectively, as compared to the control. For the remaining bacterial strains, the MIC values were found

to be greater than 2.0 mg/ml. The condition known as dermatomycoses is brought on by a class of fungus called dermatophytes. One of the most common mycoses is tinea, which affects keratinized skin tissue and is most common in immunosuppressed patients but also affects patients from all patient groups[19]. Ringworm is one of the most prevalent dermatomycoses, and it is brought on by Trichophyton, Epidermophyton, and *Microsporum* species, according to clinical studies done in India. Because of the humidity and temperature patterns that predominate in these regions, the disease is more common there and, despite the availability of a number of antimycotic medications, presents a therapeutic challenge. Most of these medications are effective against various fungal infections. In addition, these medications have been discovered to have a variety of side effects[20]. Recent research highlights the potential of higher plant-derived products as effective chemotherapeutic agents with significant fungicidal activity. Various studies have explored the antifungal properties of specific plant extracts, emphasizing their role in developing alternative treatments for fungal infections[21,22].

5. Conclusion

It is widely recognized that the capacity of plants to produce various chemically complex compounds via secondary metabolism correlates with their potential antimicrobial properties. In this study, antimicrobial activity observed in plant extracts may be attributed to the presence of one or more of the phytoconstituent active compounds, which could be functioning synergistically. According to the current study's findings, *S. orientalis* leaf preparations in ethanol contain a variety of pharmacologically potent substances. The pharmacological activities of extracts, which can contain the complete range of plant chemicals, are typically highlighted.

6. Acknowledgement

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Author Contribution

SPM and RS carried out the experiments and were involved in data analysis and drafting of the manuscript. SKY and SS contributed to the critical revision of the manuscript. ASD and SAG conceived and designed the study and performed data analysis. BB, MGP, and VS were responsible for drafting the manuscript. All authors have read and agreed to the published version of the manuscript.