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Research Paper

A Comparative Study on *Euphorbia Milii* and *Alstonia Scholaris* Latex for The Management of Onychomycosis

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ABSTRACT

The aim of the study was to determine the antifungal activity of latex obtained from the *A. scholaris* and *E. milii*. A comparative study was conducted to assess the antifungal efficacy of *A. scholaris* and *E. milii* Latex against Candida albicans. Zone of inhibition of plant extracts with respect to five concentrations viz. 0.06µg/ml, 0.12µg/ml, 0.25 µg/ml,0.5 µg/ml, and 1µg/ml have been done. Prior determining the antifungal activity phytochemical screening of plant latex are done**.** Phytochemical screening of plant extracts showed the presence of alkaloids, saponins, and carbohydrates. *A. scholaris* and *E. milii* exhibit promising potential as alternative treatment for onychomycosis. *E. milii* shows its antifungal activity at concentrations of 0.06µg/ml, 0.12µg/ml, 0.25µg/ml, 0.5µg/ml, and 1 µg/ml with the zone of inhibition of 11mm, 12mm, 15mm, and 13mm respectively and no zone of inhibition was obtained at 0.25µg/ml. *A. scholaris* shows its antifungal activity at concentrations of 0.06µg/ml, 0.12µg/ml, 0.25µg/ml, 0.5µg/ml, and 1µg/ml with a zone of inhibition of 25mm, 16mm, and 15mm respectively, while no zone of inhibition was obtained at 0.12µg/ml and 1µg/ml. The result demonstrates the antifungal potential of selected plant latexes from *E. milii* and *A. scholaris*. The *A. scholaris* was found to be more effective for its antifungal activity than *E. milii*.

INTRODUCTION

India is a vast country with more than a billion people and spread over an area of 3.3 thousand million sq. kilometers. Located in the tropics, receiving a heavy annual monsoon makes climatic conditions favorable for fungi to grow in the major part of the country. [1] Fungal infections, otherwise known as mycosis, are diseases caused by fungus. Fungal infections are most common on skin or nails. Onychomycosis is a nail infection by fungus, causing discoloration and thickening of the affected nail plate. The major causative agents of onychomycosis are dermatophytes. However, new research has revealed that non-dermatophyte

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molds are also a causative agent and are more prevalent, especially in warmer climates. Microscopy and fungal culture are the earlier diagnosing methods, and histology and PCR are done for more accurate diagnosis. Generally, involvement of the nail plate by Candida albicans is rare. Around 80%-90% of all nail infections are caused by dermatophytes, and studies in the UK found that Candida species (11%-15%) are also isolated from toenails. [2] The disease is reported to have an overall prevalence of 2% to 13%, but the prevalence is much higher in certain populations, such as older people and those with immunosuppressive conditions. [3] Around 0.5% of people under 18 were affected by this fungal nail infection. [3] Oral antifungals, topicals, and devices are the various treatment options. Oral antifungals such as terbinafine, itraconazole, and fluconazole have higher cure rates and shorter treatment periods than topical agents but have adverse side effects and drug interaction. New antifungals such as fosravuconazole are being evaluated. Topical treatments have serious side effects, but lower cure rates and much longer treatment regimens. New topical formulations are being evaluated in such a way as faster-acting alternatives to the currently available topical treatments. Laser devices are used to improve the cosmetic appearance of the nail, but their effectiveness for onychomycosis has yet to be sufficiently proven. Factors such as the growth of toenails, nail keratin thickness preventing the penetration of topicals and systemic drugs, and survival of fungi in the surrounding environment such as footwear for longer periods resulting in limited efficacy of current treatment. [4] Certain of these factors make the patient ineligible for oral antifungals and also the side effects associated with oral antifungals result in patient non-adherence and failure of the treatment.

In the 21st century, herbal medicine has been considered a promising future medicine for the management of health care. Overall, nowadays, the demand for plant-based medicines, health products, food supplements, and cosmetics has increased in both developing and developed countries, because natural products are nontoxic, have fewer side effects, and are easily available at affordable prices. [5] Phytochemical constituents present in medicinal plants are essential for the development of new drugs. For centuries, people have turned to natural remedies to cure self-limiting diseases such as colds, allergies, toothaches, etc. However the use of herbal medicines has been declined in the mid-20th century because they were not economically profitable as the newer synthetic drugs. [6] Later on, the toxic effects of conventional medicine resulted in the promotion of “natural health”, where there occur a shift in the universal trend from synthetic to herbal medicines. Undoubtedly the demand for plant-derived products has increased worldwide. Many species have been used in traditional and complementary medicine for the treatment of various microbial diseases such as fungus-causing onychomycosis. The utilization of *Euphorbia milii* and *Alstonia scholaris* has shown numerous traditional evidence. One of the largest and oldest plant families in the world, comprising approximately 300 genera and 8000 species is *Euphorbiacea*. *Euphorbia milii* is frequently used in folk medicine to treat warts, cancer, hepatitis, and trichiasis. [7] In the world around 60 species of *Alstonia* are present and in India around 6 species are found, where some of the species have medicinal importance. [8] It belongs to the Apocynaceae family. In AYUSH and non-codified drug system, the various parts of *Alstonia scholaris* are used for the treatment of malaria, jaundice, gastrointestinal troubles, cancer, etc. [8]

MATERIALS AND METHODS

Procurement of the Plant material

A. scholaris were collected from the nearby temple Sree Mariyamman Kovil, and *E. milii were* collected from Wayanad planters and prepared the herbarium. The plants were authenticated by a Botanist. Follicles of *A. scholaris* were collected and the follicles were cut with sharp blades and compressed to collect milky white latex in a container. Latex is stored at 0-4^{0}C in the refrigerator until used. Latex from the *E. milii* has collected aseptically. After a longitudinal cut in the plant stem, the latex was collected into glass tubes that were sealed, wrapped in aluminium foil, and evaporated to dryness. Dried samples of latex were stored in tightly closed glass vials that were kept protected from light in the refrigerator (4–6°C) until further use. Collected latex of *E. milii* and *A. scholaris* diluted with DMSO with concentrations 0.06, 0.12, 0.25, 0.50& 1 mg/L for antifungal activity.

Phytochemical screening

Different chemical tests such as Wagner's test, Molisch's test, ferric chloride test, frothing test and Ninhydrin test were conducted to detect the presence of Alkaloids, carbohydrates, phenolic compounds, saponins, and amino acids in *A. scholaris and E. milli*.

Antifungal study: Agar well diffusion method

For the culture of C. albicans, Potato Dextrose Agar (PDA) media was used. 20 ml of sterile culture media were poured into Petri plates. Then, 1 ml of inoculum suspension of C. albicans was spread over the medium. A well of 6 mm was made using a sterile cork borer. Then, 100 microlitres of extract were added. The Petri plates were incubated for 48 hours at 37°C. The zone of inhibition of each Petri plate was measured. The standard used in this study was Amphotericin B (0.25 mg/L as MIC). The Activity Index (AI) and Percentage Activity (PA) were determined by using the following Equations,

Activity Index= Zone of inhibition of test / Zone of inhibition of control

Percentage Activity= Activity Index 100

RESULT

Alstonia scholaris and *Euphorbia milii* were collected and authenticated. Latex from both plants were collected stored. Dilutions were made with concentrations of 0.06, 0.12, 0.25, 0.50 & 1 mg/L for antifungal activity.

QUALITATIVE PHYTOCHEMICAL SCREENING

Qualitative chemical tests were carried out in both the latex obtained from *Alstonia scholaris* and *Euphorbia milii.* The result of the chemical test for latex obtained was tabulated in the following table (Table:1).

SN	TESTS	RESULT	
		A. scholaris	***E.milii***
1	Test for alkaloids	+++	+++
2	Test for carbohydrates	+++	+++
3	Test for phenolic compound	++	+
4	Test for saponins	++	++
5	Test for amino acids	-	-

Table 1: Qualitative chemical test for *Alstonia scholaris* and *Euphorbia milii*

The latex obtained from *Alstonia scholaris* and *Euphorbia milii* were subjected to preliminary phytochemical analysis, revealing the presence of various chemical constituents such as alkaloids, phenolic compounds, carbohydrates, saponins, etc. The phytochemical screening confirms the presence of anti-fungicidal components, with alkaloids potentially contributing to antimicrobial activity.

ANTIFUNGAL ACTIVITY

Anti-fungal activity of latex obtained from *Alstonia scholaris* and *Euphorbia milii* has been carried out and tabulated in table: 2. Zone of inhibitions of latex of Alstonia scholaris of three concentrations such as 0.06 µg/ml, 0.25 µg/ml, and 0.5 µg/ml has done and the diameters observed were 25mm, 16mm, 15mm respectively. Zone of inhibitions of latex obtained from *Euphorbia milii* of four concentrations such as 0.06 µg/ml, 0.12 µg/ml, 0.5 µg/ml, and 1 µg/ml has been done and the diameters observed were 11mm, 12mm, 15mm, and 13mm respectively (Figure: 1). The standard drug Amphotericin B was taken in 50 mg/ml concentration and diameter was found to be 20mm. The zone of inhibition of *Alstonia scholaris* and *Euphorbia milii* were compared to that of standard drug Amphotericin B and were found to be comparatively less.

Table 2: Anti-fungal activity of *Alstonia scholaris* and *Euphorbia milii* latex

DILUTION (mg/L)	*A. scholaris*			*E. milii*		
	ZOI (mm)	Activity Index	Percentage Activity	ZOI (mm)	Activity Index	Percentage Activity
0.06	25	1.25	125	11	0.55	55
0.12	0	0	0	12	0.6	60
0.25	16	0.8	80	0	0	0
0.5	15	0.75	75	15	0.75	75
1	0	0	0	13	0.65	65
Undiluted latex	0	0	0	0	0	0
Standard	20	1	100	20	1	100

Figure 1: Zone of inhibition of C. albicans (A)different concentrations of *Alstonia scholaris* and *Euphorbia milii*, (B) by combination of latex of *Alstonia scholaris* and *Euphorbia milii*, (C) std. solution of Amphotericin B

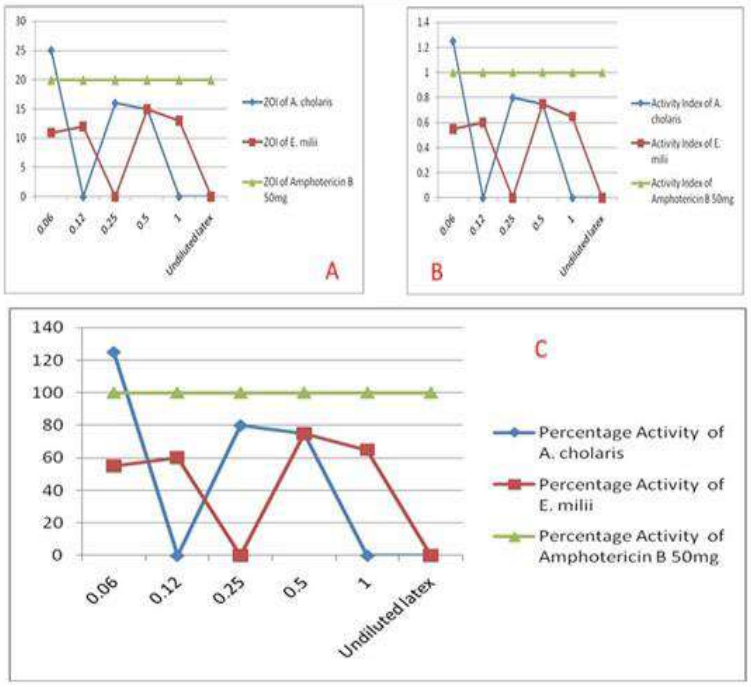

Figure 2: (A) ZOI of *Alstonia scholaris* and *Euphorbia milii* latex, **(B)** Activity Index of *Alstonia scholaris* and *Euphorbia milii* latex, **(C)** Percentage Activity of *Alstonia scholaris* and *Euphorbia milii* latex

COMPARATIVE ANALYSIS of *Alstonia scholaris* and *Euphorbia milii*

Zone of inhibitions of the latex of *Alstonia scholaris* of two concentrations 0.06 µg/ml and 0.5 µg/ml and the diameters observed were 25mm and 15mm respectively (Table: 3). Zone of inhibitions of latex obtained from *Euphorbia milii* of two concentrations like 0.06 µg/ml and 0.5 µg/ml has been done and the diameters observed were 11mm and 15mm respectively. The standard drug Amphotericin B was taken in 50 mg/ml concentration and the diameter was found to be 20mm. The zone of inhibition of *Alstonia scholaris* and *Euphorbia milii* were compared to that of the Amphotericin B and were found to be comparatively less (Figure: 3).

Table 3: Comparative analysis of Alstonia scholaris and Euphorbia milii

DILUTION (mg/L)	*A. scholaris*			*E. milii*		
	ZOI (mm)	Activity Index	Percentage Activity %	ZOI (mm)	Activity Index	Percentage Activity %
0.06	25	1.25	125	11	0.55	55
0.5	15	0.75	75	15	0.75	75
Combination of both latex	0	0	0	0	0	0
Standard	20	1	100	20	1	100

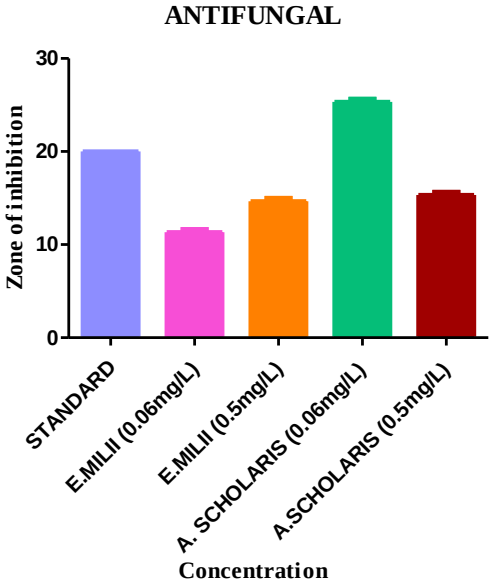

Figure 3: Comparative analysis of*Alstonia scholaris* and *Euphorbia milii*

DISCUSSION

Candida albicans, a prevalent opportunistic fungal pathogen, plays a crucial role in causing various infections, particularly in immunocompromised individuals. Its ability to switch between different morphological forms and its arsenal of virulence factors make it a formidable adversary in the clinical setting. Moreover, the rise in antifungal resistance poses a serious challenge in the management of these infections. Therefore, understanding the biology and pathogenicity of Candida albicans, along with the mechanisms of action and limitations of current antifungal therapies, is essential to develop effective

strategies to combat this medically significant fungal pathogen. Amphotericin B is effective against the majority of the Candida species, including *Candida albicans*, which was chosen as the control in the study and showed a MIC value of 0.25 mg/L. This study was aimed at determining the antifungal activity of the latex obtained from *Euphorbia milii* and *Alstonia scholaris* against Candida albicans by the agar well diffusion method. Different phytochemicals with biological activity can be found in a variety of herbs and herbal extracts, offering therapeutic value. The latex obtained from *Alstonia scholaris* and *Euphorbia milii* was subjected to preliminary phytochemical analysis, revealing the presence of chemical constituents such as alkaloids, phenolic compounds, carbohydrates, saponins, etc. This was in accordance with Deepak Ganjewala [9] and Amal Z Hassan, [10] were they also reported the same phytochemical profile for the both plants.

The agar well diffusion assay is a standard method widely used for the rapid screening of natural products for antimicrobial activity. Plant latexes were screened using this very convenient assay method. The results indicate that caution is needed, since the latexes may have different diffusion rates on the agar plate, and this may contribute to variations in the size of the inhibitory zones, leading to erroneous conclusions regarding their antifungal activity. *Euphorbia milii* shows its antifungal activity at concentrations of 0.06 mg/L, 0.12 mg/L, 0.25, 0.5mg/L, and 1mg/L with zone of inhibition of 11mm, 12mm 15mm, and 13mm respectively, while *Alstonia scholaris* shows its antifungal activity at concentrations of 0.06 mg/L, 0.12 mg/L, 0.25 mg/L, 0.5 mg/L, and 1 mg/L with a zone of inhibition of 25mm, 16mm, and 15mm respectively. Fungal activity is considered a desirable quality for antifungal agents since it can eliminate the fungus from tissues. The phytochemical screening confirms the presence of phenolic compounds which exhibit anti-fungal activity. [11] In the comparative study of both plants, it was found that *Euphorbia milii* shows its antifungal activity at a concentration of 0.5 µg/ml with a zone of inhibition of 15mm, while *Alstonia scholaris* shows its antifungal activity at a concentration of 0.06 µg/ml with a zone of inhibition of 25mm. The *Alstonia scholaris* was found to be more effective for its antifungal activity than *Euphorbia milii*. Amphotericin B and Ampicillin are known to be very effective against human pathogenic fungi and bacteria and despite their severe side effects, may require prolonged use. It is encouraging to note that both the latex obtained from *Euphorbia milii* and *Alstonia scholaris* in the study were fungicidal at low concentrations. Until now not much information was available about the mode of action of natural products that inhibit Candida growth, hence further studies focused on the method of action could be beneficial to understand the mechanism of both drugs on antifungal activity.

CONCLUSION

In conclusion, the comparative study of latexes obtained from *Euphorbia milii* and *Alstonia scholaris* for the management of onychomycosis has provided valuable insights into the potential of these natural sources as alternative treatments for fungal nail infections. Through the investigation, it was found that both *Euphorbia milii* and *Alstonia scholaris* possess antifungal properties, which can be attributed to the presence of bioactive compounds within their latex. These compounds have demonstrated inhibitory effects on the growth of fungi responsible for onychomycosis, making them potential candidates for the development of antifungal medications. However, further research is necessary to determine the specific mechanisms of action and efficacy of these natural latex extracts against onychomycosis. Additionally, studies on the safety, dosage, and potential side effects of these treatments should be conducted to ensure their

suitability for clinical applications. Nonetheless, this comparative study serves as a foundation for future investigations and highlights the importance of exploring natural alternatives for the management of onychomycosis. The findings contribute to the growing body of knowledge on natural products as potential sources of novel antifungal agents, offering potential benefits for patients suffering from fungal nail infections.

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