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# Phytochemical Analysis and Antifungal Activity of Some Medicinal Oil Plants Against Human pathogens Causing skin Infections

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**Abstract :** Phytochemical and antifungal activity of hexane seed extracts of *Argemone mexicana, Derris indica* and *Santalum album* against superficial fungal pathogens was investigated. Phytochemical analysis of all seeds extracts was determined by adapting standard methods. Antifungal activity was evaluated by inhibition of spore germination and growth kinetics assay by using standard procedure. Phytochemical screening revealed the presence of steroids, glycosides, flavonoids, alkaloids, and saponins in all the extracts. Exposure of fungal spores to 156**,** 312, 625, 1250, 2500 and 5000µg/ml concentration of the oil for a period of 30–180 minutes showed varying degree of inhibition of spore germination. Among the oil tested, *A. mexicana* seed oil showed 60-80% inhibition of spore germination on 180 min of exposure. *A. mexicana* seed oil strongly inhibits the members of the *T. rubrum* and *C. albicans* than tested with *D.indica* and *S. album* seed oil on spore germination and time dependent growth kinetics inhibition of tested microbes. *A. mexicana* seed oil may use as antifungal drug against human pathogens which cause certain superficial fungal infections of the skin.
**Keywords:** Medicinal oil plants, phytochemical analysis, antifungal activity, seed extracts.

## Introduction

Since time immemorial, natural products have been a major part of phytomedicines contain active components, have various medicinal properties that forms main source of new pharmaceuticals and healthcare products[1]. Natural products derived from any part of the plant such as bark, leaves, flowers, roots, fruits, seeds, etc, have importance as they provide amazing source of new drugs and new chemical entities for drug development[2-4]. Plants contain a large amount of bioactive compounds and even combinations of plants become more complexity which gives one of the most important challenges. Natural antimicrobial compounds derived from secondary metabolite play an important role which serve as defence mechanisms against many microorganisms[5-8].

In recent years, people of all ages are affected by fungal infections range from superficial to deeply invasive or dispersed have dramatically increased in immunocompromised patients particularly involving the skin and mucosal surfaces, are most common in tribal population due to lack of sanitation, potable water and awareness of hygienic food habits [9,10]. As the population of immunocompromised patients continues to increase, infectious diseases have become a serious problem not only in developing countries, but also in the United States[11,12]. An important group of these skin pathogens are the fungi, among which dermatophytes and *Candida*species are prominent [13-15]. Dermatophytes have the capacity to invade keratinized tissues, such as hair,

skin and nails to produce dermatophytosis 3, 4. Dermatophytes causes various types of diseases such as tinea corporis, tinea pedis, capitis, barbae, cruris, manum and onychomycosis. Nowadays, treatments for these infections are still restricted to a few antifungal drugs. However, these drugs have limited in use due to having high toxicity and the emergence of drug resistance in their antifungal activities [16-19].

Plant products traditionally being used in ethnomedicine as effective antifungal agents, are considered to be a part of the preformed defense system of higher plants and therefore, expected to deliver active antimicrobial compounds against infectious diseases[20,21]. In these perspective, there is an increasing demand for novel and effective antifungal agents, justifying searching for new drugs. Therefore *in vitro* study is examined to check the efficacy of selected medicinal oils against *T. rubrum* and *C.albicans.*

## Materials and Methods

### Collection of plant material

Seeds of *Argemone mexicana, Derris indica* and *Santalum album* were collected from plants growing around Gulbarga University, Kalaburagi, Karnataka, India and initially rinsed with distilled water to remove soil and other contaminants and dried on paper towels in the laboratory at $37^0$C.

### Preparation of plant extracts

The dried seeds were finely ground to semi-powdered state by using a blender. About 250g powdered seeds were extracted successively withhexane in Soxhlet extractor for 48h. The fractions obtained were combined into calibrated flasks, evaporated to dryness and weighted in order to determine the extraction's efficiency. The oils were stored in a sealed glass vial (bijoux bottle) in the refrigerator at $4^0$C until they were used. Further, all the extracted oils were screened for phytochemical and antifungal activity.

### Test organisms

Microbial strains used in the present study were *Trichophyton rubrum* and *Candida albicans. T. rubrum* obtained from Mahdevappa Rampure medical college, Kalaburagi, Karnataka, and *C. albicans* MTCC 3017 from Institute of Microbial Technology, Chandigarh, India. The microbial cultures were maintained in SBA (Sabourauds agar, Difco) and YPDA (Yeast peptone dextrose agar) plates at $4^0$C.

### Preparation of the spore suspension and test sample

The *T. rubrum* were grown on Sabourauds agar (SBA) and *C. albicans* on Yeast peptone dextrose agar (YPDA) plates in dark at 28 ± 2 $^0$C for 7–9 days, after which time spores were harvested from sporulating colonies and suspended in sterile distilled water. The concentrations of spores in suspension were determined using a hematocytometer and adjusted to $1.0 \times 10^8$ spores/ml.

The test solutions were prepared, hexane seed oil extracts were dissolved in 5% dichloromethane, respectively. To prepare the stock solutions with their respective known weights, which were further diluted to prepare test samples, where the final concentration of the solvent was 0.5% (v/v).

### Phytochemical analysis

All the extracts were subjected to phyochemical analysis by dissolving them in respective solvents. The extracts were screened for the presence of Phenol, flavonoid, alkaloid, steroid, glycoside, saponin, lignin, tannin and diterpenoid by adapting standard methods [22-25].

### Spore germination assay

Spore germination assay of the extract were determined by using the standard procedure[26]. Test samples of oil (5µl) were dissolved in 5% dichloromethane to obtain 156, 312, 625, 1250, 2500 and 5000µg/ml concentrations of the oil. The samples were inoculated with spore suspension of each fungal pathogen containing $1.0 \times 10^8$ spores/ml. From this, aliquots of 10 µl spore suspension from each were placed on separate glass slides in triplicate. Slides containing the spores were incubated in a moisture chamber at $30^0$C for 24 h.

Each slide was then fixed in lacto phenol-cotton blue and observed under the microscope (40X) for recording of spore germination. The spores generated germ tubes were enumerated and percentage of spore germination was calculated by using the formula [27].

The control (0.5%) dichloromethane was tested separately for spore germination of fungi.

$$\% \text{ of growth inhibition} = \frac{(C-T)}{C} \times 100$$

Where,
C = growth in the control
T = growth in the treatment

***In vitro* growth kinetics assay**

*Argemone mexicana* seed oil which appeared to be effective in spore germination assay was chosen for kinetic study and evaluation of antifungal activity. 10 µl spore suspension ($1.0 \times 10^8$ spores/ml) of fungal pathogen was inoculated to 156, 312, 625, 1250, 2500 and 5000µg/ml concentrations of oil in a test tube and a homogenous suspension was made by inverting the test tubes 3–4 times. After the specific intervals, i.e., 30, 60, 90, 120, 150 and 180 min, the reaction mixture was filtered through Whatman No. 1 filter paper and the retained spores were washed two or three times with sterile distilled water. The filter was then removed and spores were washed off into 10 ml of sterile distilled water. From this 10 µl of spore suspension was taken onto the glass slide and incubated at $30^0$C for 24 h. The spores generated germ tubes were enumerated and percentage of spore germination was calculated. Control sets were prepared in 0.5% dichloromethane with sterile distilled water. All experiments were conducted in triplicate.

## Results and Discussion

There is evidence that natural products such as essential oils and extracts of medicinal plants are considered as non-phytotoxic compounds and responsible to have less deleterious side effects than related synthetic drugs used throughout the world effective for treatment of many skin and other diseases [28-30]. Therefore, natural oil products obtained from medicinal plants can be used as a natural therapy to inhibit infectious pathogens which are responsible for causing superficial fungal infections in human beings. Also, the resurgence of interest in treating skin diseases and increasing demand for effective, safe, natural products, that quantitative data on plant oils and extracts are required.

In recent years, several researchers have reported that the alkaloids, phenols, triterpenoids, glycosides and tannins, have high potential that could be developed as antimicrobial compounds against pathogenic microorganisms[31,32]. Various secondary metabolites have been reported from medicinal oil yielding plants. In the present investigation, phytochemical analysis of *S. album*, *D. indica* and *A. mexicana* hexane seeds extract was carried out to determine the presence of various secondary metabolites responsible for antimicrobial activity. Phytochemical screening revealed the presence of steroids, glycosides, flavonoids, alkaloids, and saponins in all the extracts. *S. album* and *A. mexicana* extract, revealed positive result to tannins where as *D. indica* extract revealed negative result to tannins and phenols test (Table 1). Similar results were found with chloroform extract by Suryakant *et al*. [33]. Santosh *et al*. detected the presence of steroids and tannins in *A. mexicana* hexane and water extracts [34]. The present result supports to the above researchers findings. All the seeds extract did not respond positive to phenols and lignin may be because of their occurrence in trace amounts or may be even absent in these seeds (Table 1).Presence of phenols and absence of saponins and lignins found by Prasad *et al*. in *S. album* leaf extract[35]. Thus the phytochemical tests are helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compounds.

**Table 1: Phytochemical analysis of various compounds present in *Argemone mexicana, Santalum album* and*Derris indica* seed extracts.**

| Components | Name of the test | *A. mexicana* | *D. indica* | *S. album* |
|---|---|---|---|---|
| Phenolics | Ellagic acid test | - | - | - |
| | Phenol test | - | - | - |
| Steroids | Salkowski test | + | + | + |
| | Liebermann-Burchard's test | + | + | + |
| Flavonoids | Flavonoid test | + | + | + |
| | Shinoda test | + | + | + |
| | Ferric chloride test | + | + | + |
| | Lead acetate test | + | + | + |
| | Pew's test | + | + | + |
| | NaoH test | + | + | + |
| Alkaloids | Mayer's test | + | + | + |
| | Wagner's test | + | + | + |
| | Dragendorff's test | + | + | + |
| Glycosides | Killer-Killani test | + | + | + |
| | Conc. $H_2SO_4$ test | + | + | + |
| | Molisch's test | + | + | + |
| Saponins | Foam test | + | + | + |
| Lignins | Labat test | - | - | - |
| | Lignin test | - | - | - |
| Tannins | Gelatin test | + | - | + |
| | Ferric chloride test | + | - | + |

+, positive; -, negative.

Throughout the world, essential oils and extracts have long been known and used for treatment of many conditions, including skin conditions. There is evidence on natural products such as essential oil and extracts might be inclined to have less lethal side effects than synthetic drugs[30]. In recent years, development of safer antifungal agents such as plant-based essential oils and extracts, interests have been generated to control severe fungal diseases[29,36]. Also, developing interest in effective, safe, natural products and quantitative data on plant oils and extracts requisite of human infectious fungal pathogens. Previously, our research group has documented the antimicrobial activity of various essential oils against human pathogens [37].

The results obtained from inhibition of spore germination, *A. mexicana, D. indica* and *S. album* seed oilsignificantly inhibits the fungal spore germination at the varied concentrations. Oil exhibited a potent inhibitory effect on the spore germination of *T. rubrum* and *C. albicans* at concentrations ranging from 156-5000µg/ml (Fig 1 and Fig 2). Dichloromethane (0.5%, v/v) as a control did not inhibit the spore germination.In the current study, the oil of *A. mexicana* showed potential antifungal effect against *C. albicans*. This research work also describes the complex effect of seed oil on fungal spore germination and exhibited a wide range of antifungal activity. It appeared that exposure time of the oil had a little effect on the fungicidal activity at lower concentration but as the concentration increases, the fungicidal action was very rapid. During the inhibition of spore germination study, at 5000µg/ml concentration of *S. album* and *D. indica* seed oil, showed 40-50% inhibition of spore germination whereas *A. mexicana* seed oil showed 50-60% inhibition against *T. rubrum*. With the same concentration of *S. album* and *D. indica* seed oil, showed 50-60% inhibition of spore germination whereas *A. mexicana* seed oil showed 70-80% inhibition against *C. albicans*.These activities could be attributed to the presence of phytoconstituents present in the oil. In this study, it has become clear that *A. mexicana* seed oil have great potential to strongly inhibit the members of the *T. rubrum* and *C. albicans* compare with *D.indica* and *S. album* seed oil, which cause certain superficial fungal infections of the skin.

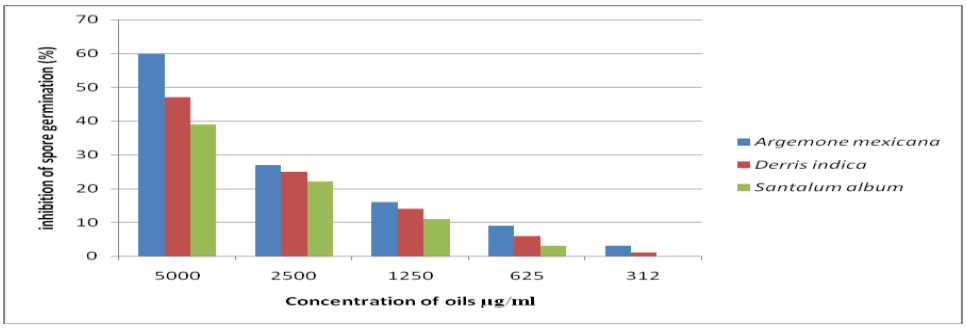


**Fig. 1: Effects of different concentration (µg/ml) of the selected oils on inhibition of spore germination against *Trichophyton rubrum.***

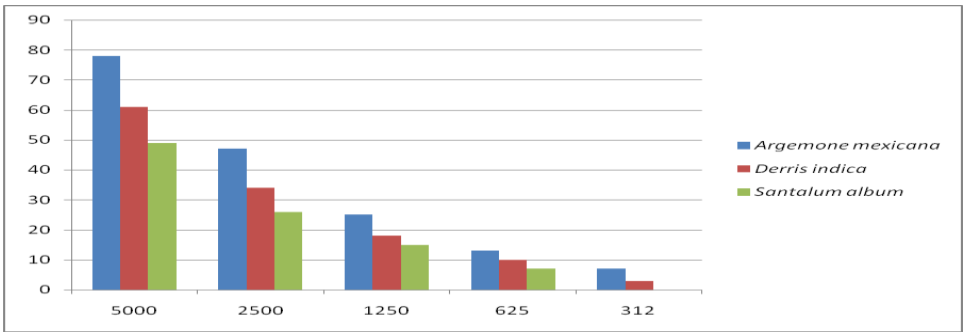


**Fig. 2: Effects of different concentration (µg/ml) of the selected oils on inhibition of spore germination against *Candida albicans.***

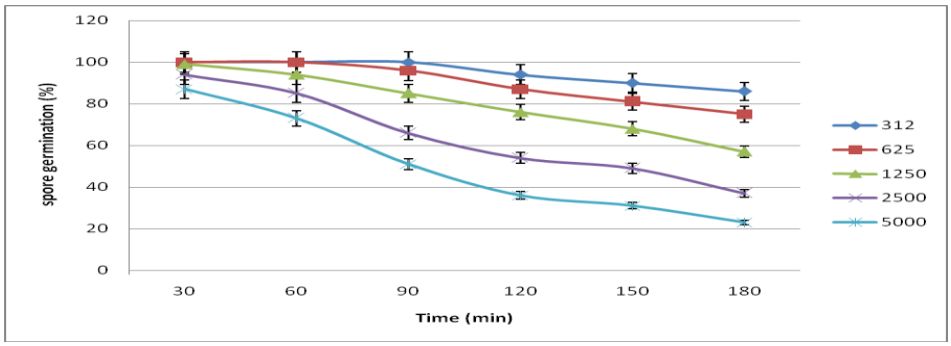


**Fig. 3: Kinetics of inhibition of *C.albicans* spores by the seed oil of *Argemone mexicana.***

For growth kinetic assay *A. mexicana* seed oil which appeared to be more effective in inhibition of fungal spore germination assay, was chosen for kinetic study and evaluation of antifungal activity against *C. albicans* (Fig 3). Exposure of spores to 156**,** 312, 625, 1250, 2500 and 5000µg/ml concentration of the oil for a period of 30–180 min showed varying degree of inhibition of spore germination. An increase in fungicidal activity was observed with increase in exposure time. The seed oil at 156**,** 312, 625, 1250µg/ml concentrations showed least antifungal activity but not rapid killing and about 20–30% inhibition was observed at exposure time of 120 and 150 min. However, there was a marked increase in the killing rate at 2500and 5000µg/ml after 90 min of exposure time and 60-80% inhibition of spore germination was observed on 180 min of exposure. This antifungal effect might be exerted due to presence of bioactive compounds in hexane extract of *A. mexicana* as evident by the finding of other researcher's isolated alkaloids, such as dehydrocorydalmine and oxyberberine from *A. mexicana*, found to inhibit spore germination [38,39]. A similar study was also carried out by the same group with a mixture of quaternary alkaloids and some phenolic acids[40]. This observation suggest that using seed oil at higher concentration have been found to be promising fungi toxic against human pathogens such as *T. rubrum* and *C. albicans* responsible forcausing superficial skin infection.

## Conclusion

In view of above results it might be concluded that, in future as the fast and reliable alternatives, *A. mexicana* seed oil may potentially contribute in the field of medical mycology as the supplement to control skin infectious fungal pathogens. As this oil found to be effective against *C. albicans* compare to *T. rubrum* and to know the phytochemicals responsible for the antifungal activity was further subjected for isolation of active pure compounds and *in vivo* studies was further conducted with the isolated compounds so as to confirm the present *in vitro* findings as the diameter of the zone of inhibition is not only affected by sensitivity of the microorganisms alone but also the concentration of the extract is very important.

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