Broad spectrum antymycotic drug for the treatment of ringworm infection in human beings

Infection caused by dermatophytic fungi in man and animals is common throughout the world. Dermatophytoses pose a serious concern to the sociologically backward and economically poor population of India. Fungi cause both superficial and internal mycoses. The mycoses, though normally not lethal, are unpleasant and difficult to cure, and cause considerable economic loss. Majority of superficial infections are caused by a closely related group of keratinophilic fungi called dermatophytes, which cause ringworm infection or Tinea infection. Clinical surveys carried out in India have shown ringworm as one of the most common dermatomycoses caused by the species Epidermophyton, Microsporum and Trichophyton. In dermatophytic infection of the skin, the fungus remains confined to the stratum corneum, while pathogenic changes are produced in the deeper layer of the epidermis and dermis, according to medical observation. These fungi produced a ring-shaped lesion of infected skin. The disease begins as a small, round, swollen area of inflammation and due to the different rates of spread, initial small lesions become oval or oblong in shape. The disease is predominant in tropical and subtropical countries due to their prevailing moisture and temperature regimes, and poses a therapeutic problem despite several antymycotic drugs available in the market. But due to various side effects, long duration of treatment and high cost of the drugs, treatments have not been successful in some cases.

In recent years, there has been a gradual revival of interest in the use of medicinal plants in developed as well as in developing countries, because herbal medicines have been reported to be safe and without any adverse side effect. Thus, a search for new drugs with better and cheaper substitutes from plant resources are a natural choice. Recently, some products of higher plant origin have been shown to be an effective source of chemotherapeutic agents, without undesirable side effects and with strong fungicidal activity. These findings promoted us to explore other plant products which could be exploited as effective antymycotics.

We report here the detailed antifungal study of the essential oil obtained from the seeds of Trachyspermum ammi, in vitro as well as in clinical trials against ringworm infection in human beings.

For in vitro experiments, we extracted the essential oil from the seeds of T. ammi by hydrodistillation using Clevenger’s apparatus. These oils were evaluated for their antifungal properties against the four selected test pathogens, namely Trichophyton rubrum, T. simii, Chrysosporium indicum and C. tropicalis.

T. rubrum and T. simii were isolated from infected skin scrapings of Tinea patients at the S.M.S. Hospital, Jaipur, while C. tropicalis and C. indicum were isolated from soil samples through To. Ka. Va. hair-baiting technique.

The filter paper disc method of Gould and Bowie was used for screening the essential oils against test fungi. Standard size Whatman No. 1 filter paper discs, 6.0 mm in diameter, sterilized by dry heat at 140°C in an oven for one hour were used to determine antifungal activity. SDA medium for agar disc diffusion test was prepared. After sterilization, it was poured into sterilized petri plates and allowed to solidify. Then sterile suspension of each of the fungi was prepared from 8 to 10-day-old cultures separately. The suspension was vortexed and 0.1 aliquots were spread over the respective agar medium plates. Sterilized filter paper discs were soaked in neat, undiluted (100%) as well as in diluted oil (25, 50, 75% concentration) of T. ammi. Dilution has been done in acetone. An oil-saturated disc was placed on an agar plate containing fungal spore suspension. Similarly, solutions of standard antibiotics (Grisofulvin/Ketoconazole, 1000 µg/ml) for antifungal activity were prepared and impregnated in the filter-paper discs. These discs were then placed over the plates preceded with respective microorganisms. These plates were incubated at 37°C for 48–72 h in an incubator. Five replicates were kept in each case and the average values were determined and inhibition zones observed. The antifungal activity was determined by measuring the inhibition zones around the discs. The activity of oils was measured by the following formula:

\[
\text{Activity Index} = \frac{\text{Inhibition zone of sample}}{\text{Inhibition zone of standard}}
\]

For the in vitro studies, two concentrations (1 and 2%) of T. ammi oil (ajwain oil) were prepared in petroleum jelly and labelled as ointments A1 and A2.

In our previous work, a comparative efficacy of T. ammi with different antymycotic drugs like Grisofulvin, Itraconazole, Ketoconazole and Zacon was carried out and all concentrations of T. ammi oil were found to be more effective than standard drugs. A survey was also conducted for concentration of active ingredient of antifungal ointments and drugs. It was observed that usually all standard ointments and drugs possess minimum concentration (1%) of effective antifungal compound. Shahi et al. also used 1% concentration of Eucalyptus oil against dermatophytes. Hence, as a result of the above studies and also a suggestion from V. N. Saxena, S.M.S. Hospital, Jaipur, we used 1 and 2% concentrations of oil for preparing the ointments.

Before applying these ointments on Tinea patients, the patch test of Roxburgh and Borrie was followed to find out whether these ointments have any irritant activity or not. For this purpose, people of both the sexes and of different ages were selected randomly. A circular area of 6 cm² on the lower glabrous and upper hairy surface of palms, and behind the neck region of each individual was cleaned with distilled water and 70% ethyl alcohol, respectively. After drying the skin, these ointments were applied on each individual separately. The qualitative observation was recorded after 24 h.

For in vivo investigation, patients of both the sexes and of different age groups (infant to 50 year-old) were selected. A total of 30 patients were selected for the study to see the clinical response of ointments (A1 and A2) of T. ammi. These were applied topically on patients to control the fungal infection. Among these, 20 were treated with A1 and 10 with A2. Medication was applied twice a day for three weeks as advised by the skin specialist. Patients were not
allowed to take any other systemic or topical therapy during the course of the present study.

Patients of both the sexes were diagnosed for different clinical types like Tinea corporis, Tinea capitis, Tinea manum and Tinea barbae based on site and type of infection. The diagnosis was further confirmed by microscopic examination of scrapings (from infected area) treated with 10% potassium hydroxide (KOH). Only KOH-positive cases were enrolled for the study. Patients having onychomycosis were excluded from the study. Patients were examined just before the start of therapy and at the end of each week of the treatment, up to complete cure. During each visit of the patient, the same reference lesion was scraped for fungal culture to identify the organism and to demonstrate the presence of hyphae by microscopic examination of the scrapings which were covered with 10% KOH preparation. Signs and symptoms of inflammation such as erythema, scaling, itching, maceration, vesication and pustulation were recorded as mild, moderate, severe or absent.

Additional information of patients regarding their profession, living conditions, along with clinical diagnosis – site of infection, primary or secondary infection, sex, age were also recorded. Usually, patients with secondary infection were avoided for the study. Although the cutaneous fungal disease manifested itself in several body areas and all the affected areas were treated, only one was selected and designated as the reference lesion.

During each visit of the patient, overall clinical improvement was reported as none, partial, significant or completely clear, by comparing the present state of their infection with the state at the time of their initial visit. Adverse systemic or local reactions were noted (at each visit) and recorded as mild, moderate or severe.

Table 1 shows antidermatophytic activity of T. amni oil against test fungi. All the four concentrations of T. amni oil exhibited more potent antifungal effect against T. simii and C. tropicum. Griseofulvin (as standard) was not found effective against C. indicum, therefore Ketoconazole was taken as the standard antifungal drug. In the case of C. indicum, all the four concentrations showed significantly larger inhibition zones (22, 32, 37 and 45, respectively) than the standard (IZ = 17 mm). All the concentrations also exhibited excellent properties in case of T. rubrum and T. simii. Zone of inhibition produced by 25% T. amni oil was slightly lesser (32 mm) than standard Griseofulvin in case of C. tropicum, but concentration above 25% showed excellent inhibition zone (Figure 1).

Table 2 shows effect of the ointments A1 and A2 on Tinea patients. Both ointments showed absence of any adverse effect or any irritation during the complete treatment period. Data incorporated in Table 2 show that all the 30 patients of Tinea infection recovered completely with the timely application of these ointments. Both ointments possess excellent and broad antifungal properties (Figure 2).

A complete treatment period of Tinea corporis differs (4-6 week) with different allopatic drugs. Out of 12 patients treated with ointment A1, two showed complete relief within one week, seven were completely cured during the second week and the remaining three showed complete cure during the third week of treatment.

Ointment A2 was found to be much better than A1. It was applied on six patients, among which two were completely cured at the end of the first week, three after the second week and one after the third week of treatment.

In the case of Tinea capitis a total of six patients were treated with ointments A1 and A2 separately. Among the four patients treated with A1, two showed complete improvement after the second week of treatment and another was cured completely after the third week of treatment. A2 showed excellent performance within the second week.

A complete treatment period of Tinea manum was 6-8 weeks. Out of six patients, four were treated with ointment A1. They showed complete cure during their third visit. Two patients treated with ointment A2 obtained complete relief after the second week of treatment.

Ointment A2 with 2% concentration cured the infection much faster than ointment A1 without any adverse side effect. Both ointments, when tested for irritation activity on experimental animals, did not show any irritation effect. The same result was also observed during the patch test method on human beings.

Hence the oil of T. ammi, owing to its strong antifungal activity inhibiting heavy doses of inocula having fungicidal properties and with no irritation on the human skin can be used successfully in the form of a broad spectrum antymycotic drug for the control of superficial fungal infection in human beings. It has been found to be a better and cheaper substitute for curing the disease without any adverse side effect. It is easy to apply and responds much faster than synthetic allopatic preparations.

Table 1. Comparison of efficacy of Trachyspermum ammi oil with commercial antifungal drug

<table>
<thead>
<tr>
<th>Concentration of oil (%)</th>
<th>Test fungus</th>
<th>IZ</th>
<th>AI</th>
<th>IZ</th>
<th>AI</th>
<th>IZ</th>
<th>AI</th>
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<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
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<td></td>
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<td>25</td>
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<td>1.294</td>
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<td>50</td>
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<td>75</td>
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<td>100</td>
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</table>

IZ, Inhibition zone, including 6 mm diameter of filter paper disc; AI, Activity Index; Inhibition zones of standard Griseofulvin against T. rubrum = 28 mm, T. simii = 24 mm, C. tropicum = 35 mm; Inhibition zones of Ketoconazole against C. indicum = 17 mm.
Figure 1. Comparative efficacy of *Trachyspermum ammi* oil with standard allopathic drug. *a*, Inhibition zone against *Chrysosporium indicum* at (A) 100, (B) 75, (C) 50 and (D) 25% concentration of oil. *b*, Inhibition zone against *Trichophyton rubrum* at (A) 100, (B) 75, (C) 50 and (D) 25% concentration of oil. *c*, Inhibition zone against *T. simii* at (A) 100, (B) 75, (C) 50 and (D) 25% concentration of oil. *d*, Inhibition zone against *C. tricicum* at (A) 100, (B) 75, (C) 50 and (D) 25% concentration of oil. *e–h*, Inhibition zone of standard against *e*, *C. indicum* (f) *T. simii* (g) *T. rubrum* and (h) *C. tricicum*.
Figure 2. Treatment of different clinical types with ointments A1 and A2. 

Table 2. Response of ointment A1 and A2 on Tinea infection.

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Fungal species isolated</th>
<th>No. of patients</th>
<th>No. improvement</th>
<th>Partial</th>
<th>Significant</th>
<th>Completely cured</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Ist Week</td>
<td>Ilnd Week</td>
<td>IIIrd Week</td>
<td>Ist Week</td>
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<tr>
<td>Ointment A1</td>
<td></td>
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<tr>
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<td>2</td>
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<td>2</td>
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<tr>
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<td>3</td>
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<tr>
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<tr>
<td></td>
<td>T. mentagrophytes</td>
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<td>T. rubrum</td>
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<tr>
<td>Tinea manu</td>
<td>T. rubrum</td>
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Isolation and identification of active ingredient of T. ammi oil which is responsible for antidermatophytic activity is in progress.


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Identification of Phytophthora species affecting plantation crops by RFLP of PCR-amplified internal transcribed spacer regions of ribosomal RNA

Areacanut, coconut, cocoa, rubber, black pepper, betel vine and vanilla are the most important plantation crops that have been seriously affected by Phytophthora species. Destruction of black pepper by P. capsici; heavy loss of cocoa due to black pod and bud rot, and immature nut fall of coconut caused by P. palmivora; fruit rot of areacanut and cardamom, and abnormal leaf fall in rubber due to P. meadii are some of the destructive diseases of plantation crops caused by Phytophthora. Some species cause the same symptoms on a particular host (e.g. P. palmivora, P. megakarya, P. capsici and P. citrophthora cause black pod on cocoa), while others have a wide host range (e.g. P. meadii infects rubber, cocoa, areacanut and cardamom). As the plantation crops are grown together under high-density, multi-species cropping systems or in the vicinity of each other, rapid identification of Phytophthora species is of utmost importance. P. areacae (P. palmivora), P. capsici, P. meadii and P. nicotianae are the major species that affect the plantation crops industry. These species were mainly identified based on morphological features. It is well recognized that identification of species is a highly specialized, time-consuming and difficult task. The limited number and plasticity of morpho-