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Utilization of Essential Oil as a Bio-Chemotherapeutant Against Tinea capitis

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ABSTRACT

Tinea capitis is a disease caused by superficial fungal infection commonly involving the scalp, eyebrows and eyelashes. It is also called ringworm of scalp and usually caused by members of genera *Microsporum* and *Trichophyton*. These fungal genera are controlled by utilizing essential oils as bio-chemotherapeutant. During antifungal screening of the essential oils of some angiospermic plants, oil of *Artemisia maritima* exhibited the strongest activity, completely inhibiting the mycelia growth of common scalp infecting fungi (Tinea capitis) *viz. Microsporum canis*, *Trichophyton rubrum and T. mentagrophytes* at 0.5µl/ml, 0.3µl/ml and 0.4µl/ml respectively. The essential oil was found to be fungicidal at 0.7µl/ml, 0.4µl/ml and 0.5µl/ml concentrations. The fungicidal activity of the oil was found to be thermo stable up to 70^{0}C, with no decrease in activity up to 24 months of storage. The oil also showed a broad fungi toxic spectrum, inhibiting the mycelia growth of other dermatophytic fungi. *viz. Aspergillus flavus, A. fumigataus, A. ustus, Candida albicans, Epidermatophyton floccosum, Microsporum gypseum, M. canis, M. nanum, Rhizopus nigricans, Trichophyton tonsurans and T. violaceum*. Moreover, it did not exhibit any adverse effect on mammalian skin up to 5% concentration. As such, the oil has a potential use as an effective herbal chemotherapeutic after undergoing successful clinical trials.

Key words *Antidermatophytic; Artemisia maritima; essential oil; Tinea capitis, Microsporum, Trichophyton*

Fungal infection of scalp caused by dermatophytes is known as Tinea capitis. It is also called ringworm of scalp. The disease is considered to be form superficial mycosis or dermatophytosis. Tinea captis is caused by fungi of species of genera *Microsporum* and *Trichophyton*.(Rippon 1985)[1] Causative agents of tinea captis include keratinophilic fungi termed dermatophytes. These molds are usually present in nonliving cornified layers of skin and some times are capable of invading the outermost layer of skin, stratum corneum, or other keratinized skin derived from epidermis, such as hair and nails. From the site of inoculation, the fungal hyphe grow centrifugally in the stratum corneum. The fungus continues downward growth in to the hair, invading keratin as it formed. It is the most common pediatric dermatophyte infection worldwide[2]. The fungi grow well in warm, moist areas. The tinea captis infection is more likely if minor scalp injuries, do not bathe or wash hair and wet skin for a long time such as from sweating. It most often affects children and goes away at puberty. However it can occur at any age. Ringworm may involve part or the entire scalp. The affected areas are bald with small black dots due to hair that has broken off, round scaly areas of skin that are red or swollen(inflamed), pus-filled scores called kerions and may be very itchy[3]. Both the skin surface and hairs are involved .Infection of hairs may be described as ectothrix (sheath of arthroconidia formed on the outside of hair shaft) or endothrix (arthroconidia formed within the hair shaft). Mostly synthetic antifungal are used to treat these infections. The leading antifungal oral medication for ringworm is griseofulvin and terbinafine hydrochloride. These are largely non-renewable, non-biodegradable and residual toxicity. Both have common side effects, including diarrhea, fatigue, headache, itching and upset stomach. The medicated shampoo contains the active antifungal ingredient ketoconazole or selenium sulfide. Medicated shampoo helps prevent the fungus from spreading, but it does not fungus. Thus, in a meaningful search for new treatments with better and cheaper substitutes, plant resources are the natural choice. Naturally occurring fungi toxicants described to date are mostly biodegradable[4]and are devoid of side effects compared with commercially available antifungal. Recently, essential oils of higher plant origin have been shown to be an effective source of chemotherapeutic agents without undesirable side effects and with strong fungicidal activity.[5-9] The findings prompted us to explore other plant products (essential oils), which could be utilize as effective fungi toxicants. We here report on the result of our investigation of the essential oil of *Artemisia maritima* L. *a* member of the family Compositae (Asteraceae) as an effective antifungal against the *Microsporum* and *Trichophyton* causing Tinea captis.

MATERIALS AND METHODS

Plant samples were collected during the month of September from the Kullu Valley of Himanchal Pradesh, India. The essential oil was extracted from the aerial parts of *Artemisia maritima* by hydro distillation using a Clevenger apparatus.[10]A clear light-yellow-colored oily layer was obtained on the top of the aqueous distillate, which was separated from the latter and dried over anhydrous sodium sulphate.

In *in vitro* studies, the minimum inhibitory concentrations (MICs) of the oil against test pathogens

Table 1. Physicochemical properties of the oil of *Artemisia maritima*

Properties studied	Observations
Plant height (cm)	Up to 1.0 m
Oil yield (%)	0.60
Specific gravity at 29.5^{0}C	0.8835 – 0.9457
Refractive index at 25^{0}C	1.4799 – 1.4925
Optical rotation	+4^{0}25' - 16^{0}75'
Acid value	7.535 – 27.07
Ester value	71.0 – 175.0
Carbonyl value	75.0
Solubility	Acetone
Cineole (%)	14.0 – 22.0

were determined following the poisoned food technique[11] with slight modification.[8] The requisite quantity of the oil samples were mixed in acetone (2% of the required quantity of the medium) and then added in pre-sterilized sabouraud dextrose agar (SDA) medium, pH 5.6. In control sets, sterilized water (in place of the oil) and acetone were used in the medium in appropriate amounts. Mycelial discs of 5mm diameter, cut out from the periphery of 7 day old cultures, were aseptically inoculated upside-down on the agar surface of the medium. Inoculated Petri plates were incubated at 27±1^{0}C and the observations were recorded on the seventh day. Percentage of mycelial growth inhibition (MGI) was calculated according to the formula:

$$MGI = (dc - dt) \times 100/dc$$

Where dc = fungal colony diameter in control sets. dt = fungal colony diameter in treatment sets.

The minimum fungi static /fungicidal concentrations of the oil at minimum inhibitory concentrations (MICs) were ascertained by the method of Garber and Houston.[12] This was done by reinoculated the inhibited fungal discs at MICs on SDA medium. Observations were recorded after 7 days of incubation. Fungal growth on the seventh day indicated a fungi static nature, while the absence of fungal growth denoted fungicidal action of the oil. The effect of inoculums density (increased progressively up to 30 discs in multiples of five, each of 5 mm diameter) of the test pathogens on MICs of the oil was determined following the procedure outlined by Dikshit and Dixit.[13] The effect of physical factors viz. temperature and expiry of toxicity during storage of the oil, was evaluated according to Shahi *et al*.[8] Five lots of oil were kept in small vials, each containing 5 ml oil; these were exposed to different temperatures (30, 50 and 70^{0}C) in an incubator for 1 h. Antifungal activity was then tested at MICs by the poisoned food technique.[11] Expiry of toxicity of the oil was determined by storing the oil at room temperature and testing antifungal activity at MICs at regular intervals of 60 days up to 24 months, following the poisoned food technique.[11]

The minimum killing time (MKT) of the oil was determined by the mycelial disc killing technique (MDKT) of Shahi *etal*.[8] Two treatment sets were maintained, one with pure oil (PO) and the other with the minimum fungicidal concentrations (MCCs) of the oil. The treatment set using MCCs of the oil was prepared by mixing the required quantity of the oil samples in acetone (5% of the total quantity of the treatment solution) and then adding this to the appropriate quantity of distilled water. Simultaneously, controls were maintained using sterilized water (in place of the oil) and acetone, adding into the distilled water in appropriate quantities.

Mycelial discs of 5 mm diameter, cut out from the periphery of 7 day-old cultures of the test pathogens, were aseptically placed in the culture tubes of different treatment and control sets. These mycelial discs were taken out of

Table 2. Mycelial growth inhibition from the oil of against test pathogens.

Concentration (µl/ml)	Mycelial growth inhibition (MGI) (%)		
	Microsporum canis	*Trichophyton rubrum*	*Trichophyton mentagrophytes*
1.0	100^c	100^c	100^c
0.9	100^c	100^c	100^c
0.8	100^c	100^c	100^c
0.7	100^c	100^c	100^c
0.6	100^s	100^c	100^c
0.5	80	100^c	100^c
0.4	55	100^c	100^s
0.3	28	100^s	62.2
0.2	30	87.7	46.6
0.1	-	65.7	16.6

^{c} Fungicidal, ^{s} Static.

Table 3. Minimum killing time of the oil of *Artemisia maritima* against test pathogens:

Minimum killing time(MKT)	Mycelial growth inhibition (MGI) (%)					
	Microsporum canis		*Trichophyton rubrum*		*T. mentagrophytes*	
	PO	*MCCs*	*PO*	*MCCs*	*PO*	*MCCs*
120m	100	100	100	100	100	100
80m	100	100	100	100	100	100
70m	100	80	100	100	100	100
60m	100	76	100	100	100	89
50m	100	56	100	97	100	78
40m	100	35	100	88	100	63
30m	100	-	100	78	100	50
60s	100	-	100	—	100	—
30s	100	-	100	—	100	—
20s	100	-	100	—	100	—
10s	100	-	100	—	100	—
5s	95	-	93	—	89	—
1s	87	-	58	—	52	—

PO (pure oil), MCCs (minimum fungicidal concentration)

the tubes at different time intervals and washed immediately in the washing solution (containing acetone: sterilized distilled water, ratio 1:2) to remove the treatment solution. These washed mycelial discs were aseptically transferred upside-down to the SAD medium (pH 5.6) in the Petri plates. The same procedure was followed with the control sets. The inoculated Petri plates were incubated at 27±1^{0}C and the observations recorded as an average value of five replicates on the seventh day. The percentage of fungal growth inhibition (FGI) was calculated by the formula of Shahi *et al*.[8]All the experiments were repeated twice, each containing five replicates, and the data presented here are based on their mean values.

To determined the maximum tolerable concentrations (MTCs) and long-term toxicity for irritant activity, if any, of the oil by their topical application on human skin and nails, we followed the patch test method as described by Shahi *et al*.[8]

People of both sexes aged 10-30 years were selected randomly and a group of 30 individuals of each sex was constituted. Circular areas of 5 cm^2 on upper hairy and lower glabrous surface of the palms, nail and 3 cm^2 of neck region of each individual were first washed with distilled water followed by 70% ethyl alcohol and then allowed to dry for 5 min. Five drops of the graded concentrations of testing solution were applied to each individual separately for 3 weeks. The volunteers were not allowed to wash the applied area. Qualitative observations were recorded afterwards at intervals of 24 h up to 3 weeks.

RESULTS AND DISCUSSION

The essential oil was extracted from the aerial parts of *Artemisia maritima* by hydro distillation using Clevenger apparatus.[10]A clear light-yellow-colored oil on hydro distillation, yielded 0.6 % essential oil. The physicochemical properties of the oil are shown in Table 1. The MIC of the oil of *Artemisia maritima* as a fungicide was found to be 0.7µl/ml, 0.4µl/ml, 0.5 µl/ml against *Microsporum canis*, *Trichophyton rubrum*, *Trichophyton mentagrophytes,* respectively (Table 2). The oil inhibited heavy doses of inocula which exhibited 100% mycelial growth at their respective fungicidal concentrations. The activity of the oil did not expire even up to 24 months storage and persisted up to 70^{0}C. The pure oil (100%) killed the fungi in just 10 seconds (s) while at its minimum fungicidal concentration it required 80, 60, and 70 minutes (m) against *Microsporum canis*, *Trichophyton rubrum* and *Trichophyton mentagrophytes,* respectively (Table 3). The oil also exhibited a broad range of antifungal activity, inhibiting some other fungi, e.g. *Aspergillus flavus, A. fumigates, A.niger, Candida albicans*, *Malassezia, Epidermophyton floccosum, M. gypseum, M. nanum, T. violaceum* in range of 0.6- 1.2 µl/ml concentration. When tested for irritant activity and long-term toxicity on human skin and nails, the oil did not show any irritation or adverse effect at 5% concentration up to 3 weeks.

The essential oil of *Artemisia maritima* exhibiting strong toxicity against the test fungi causing Tinea capitis, it is superficial fungal infection commonly involving the scalp, eyebrows and eyelashes. It is also called ringworm of scalp. The oil appears to possess wide range of antifungal activity. The findings suggest that they may be used for the cure of Tinea capitis diseases of animals and human beings after suitable clinical trials.

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