Research Paper

Chemical Composition, Toxicity and Antidermatophytic Activity of Essential Oil of *Trachyspermum ammi*

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Present study was designed to explore the essential oil of *Trachyspermum ammi* and its fractions against fungi causing dermatophytoses in humans along with toxicological evaluation on mice. The chemical composition of *T. ammi* essential oil analysed by gas chromatography and gas chromatography-mass spectrometry presented 20 compounds. Thymol was found to be the major compound (58.88 %) followed by p-cymene (24.02 %), γ-terpinene (13.77 %) and β-pinene (1.90 %). Antidermatophytic activity was determined by disc diffusion method and minimum inhibitory concentration. Maximum zone of inhibition was observed against *Chrysosporium tropicum* (63.83±0.166 mm) followed by *Trichophyton simii* (57±0.288 mm), *Trichophyton rubrum* (51.33±0.333 mm) and *Chrysosporium indicum* (45±0.577 mm). Minimum inhibitory concentration of *T. ammi* oil ranged from 0.025 to 0.5 µl/ml against test fungi. *T. ammi* oil was further subjected for fraction separation through Buchii's glass oven equipment. Five fractions were separated at different temperature conditions labelled as TA^{I}-TA^{V}. Maximum effects were seen in case of TA^{IV} and TA^{V} fractions. Excellent results of TA^{V} was observed against *Microsporum gypseum* (0.015±0.002 µl/ml) followed by *Microsporum canis* (0.017±0.002 µl/ml), *Trichophyton rubrum* (0.02±0.000 µl/ml) and *Candida albicans* (0.05±0.003 µl/ml). No work on *T. ammi* essential oil fractions, their antidermatophytic activity and toxicological behaviour on Swiss albino mice were reported till date. Acute dermal irritation assay was applied for toxicological studies on albino mice. Low concentration up to 3 % did not show any irritation on mice skin. At 5 % concentration 3 mice showed mild erythema, while on 7 % concentration all five mice exhibited well defined erythema. Present study concluded that essential oil of *T. ammi* and its fractions have strong antidermatophytic properties with no side effect at low concentrations and thus could produce alternative therapeutics to current antibiotics.

Key words: Dermatophytes, MIC, mice, toxicology, essential oil, *Trachyspermum ammi*

Trachyspermum ammi L belonging to family Apiaceae is an important commercial ingredient for the food and flavouring industry. It is a known as a popular aromatic herb and spice that grows in Egypt, Persia, Bangladesh, Afghanistan, Ethiopia and India[1]. Now a days, the *Ajwain* fruit oil is chiefly employed for flavouring sausages, meat, canned goods, perfumes, mouth preparation and liquors. The *T. ammi* fruits have many folk medicinal activities like cytotoxicity[2], antimicrobial[3-5], antiinflammatory, antioxident[6], antipyretic, analgesia, diuretic, antitermitic[7], antiviral[8], anticipative, anticandidial activity[9], anthelmintic[10] and carminative effects. Its effectiveness against kidney stones was studied by Ahsan *et al*.[11].

Essential oil of aromatic plants has been recognized for many years as a major source of pharmaceutical agents and food additives. In recent years there has been a gradual revival of interest in the use of medicinal products such as essential oil and other botanical products in response to the ever increasing incidence of adverse side effects associated with conventional drugs, high cost medicines, long duration of treatment time and emergence of resistance to antifungal drugs especially in case of dermatophytoses. Dermatophytoses are superficial infections of keratinized tissue caused by organism of three genera of fungi known as

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dermatophyton[12]. The dermatophytes represent more than 40 closely related species classified in three genera: *Microsporum*, *Trichophyton* and *Epidermophyton*. The skin infection due to dermatophytes has become a significant health problem affecting children, adolescent and adults. Recently, there has been an increase in the incidence of fungal infections in developing countries. This may be owing to frequent usage of antibiotics, environmental condition, immunosuppressive drugs and various other conditions like organ transplantation, lymphomas, leukaemia and human immunodeficiency virus[13]. Fungal infections in human are frequently observed during those seasons of the year when the environmental temperature and relative humidity are pretty high[14]. With its temperature exceeding even 46°, Jaipur has a dry climate in summer. Its humidity level during monsoon is also very high. These climatic conditions are amenable to the incidence of the fungi and consequently the disease[15]. Present work deals with the chemical analysis of *T. ammi* essential oil, separation of essential oil fractions, screening of antidermatophytic activity and toxicological behaviour on Swiss albino mice.

MATERIAL AND METHODS

Oil extraction method:

Fruits of *T. ammi* were brought from an authorized Ayurvedic store of medicinal plants at Jaipur in India. For extraction of essential oil, fruits were macerated with a small quantity of distilled water and the thus produced slurry was distilled with the hydro-distillation unit (Clevenger's apparatus) for 7-8 h. Essential oil collected in tubes was dried with anhydrous sodium sulphate. The moisture free oil was then stored in amber colored bottles and kept in a refrigerator. Essential oil of *T. ammi* was further subjected in the glass bulbs of Buchii's glass oven equipment for the separation of its ingredients at different temperature intervals. First fraction was separated at 160°. Remaining ones were separated after 20-25° temperature interval to the preceding fraction.

Microorganism for *in vitro* studies:

Antifungal properties of *T. ammi* were investigated against most commonly reported and isolated dermatophytes, keratinophilic fungi and *Candida albicans* in Jaipur area. *Trichophyton rubrum*, *T. verrucosum*, *Microsporum gypseum* and *C. albicans* were isolated from infected skin scrapings of tinea patients from the SMS Hospital, Jaipur during survey of dermatophytoses while *M. fulvum*, *M. canis*, *Fusarium verticilloides*, *Chrysosporium tropicum* and *C. indicum* were isolated from soil samples through To-Ka-Va hair baiting technique[16]. All these fungi were identified through standard protocols. These fungi were maintained on Sabouraud's dextrose agar medium.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis:

Quantitative analysis of the essential oil was carried out using a Shimadzu GC-2010. Nitrogen was used as carrier gas at 10 psi inlet pressure with FID and Omega SPTm column (30.0 m×0.25 mm ID, film thickness 0.25 μm). Injector and detector temperatures were 270° and 280°, respectively. Column temperature programmed from 80° (2 min hold), 80° to 180° at 4°/min and 180° to 230° at 6°/min with-hold time of 6 and 19 min, respectively. The flow rate of carrier gas was 1.21 ml/min and split ratio was 1:80. The data were processed on GC solutions software for oil composition.

GC-MS data was also obtained on a Shimadzu GCMS-QP-2010 plus system using same column. Helium was used as carrier gas. Injector, mass detector, ion source temperatures, column temperature and other condition were same as given in GC. EI source and mass range were 70 eV and 40-850 AMU, respectively. Compounds were identified by using Willey, NIST and Perfumery libraries. Compounds were identified by using Willey, NIST and Perfumery libraries. The compound identification was finally confirmed by comparison of their relative retention indices with literature values.

Screening of oil:

The filter paper disc method[17] was employed for screening the essential oils against dermatophytes. Standard size Whatman No. 1 filter paper discs of 6.0 mm in diameter, sterilized by dry heat at 140° in an oven for 1 h were used to determine antifungal activity. Twenty millilitres of sterilized Sabouraud's dextrose agar medium was taken in each autoclaved Petri-dish and allowed to solidify. Fungal spore suspension was prepared in sterilized distilled water by transferring a loopful of 15 d old culture. The 1 ml of spore suspension of approximately 0.5 to 5×10^4 colony-forming unit (CFU)/ml was 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 and 75 % concentrations). Dilution was done

in acetone. An oil saturated disc was placed on an agar plate containing fungal spore suspension. Griseofulvin, ketoconazole and itraconazole were used as standard drugs. These plates were incubated at 37° for 72 h. Five replicates were kept in each case and the average values of each one was determined and inhibition zones were observed. The antifungal activity was determined by measuring the inhibition zone around the disc. The activity of oil was measured using the following Eqn., activity index= inhibition zone of the sample/inhibition zone of the standard.

Semisolid agar antifungal susceptibility method:

Semisolid agar antifungal susceptibility testing method of Provine and Hadley[18] was carried out in brain heart infusion agar (BHIA, HiMedia) for the determination of minimum inhibitory concentration (MIC). BHIA was prepared according to manufacturer's instruction. Sterile swab dipped into sterile Tween-80 was used to pick the pure colony of yeast. This was then suspended in 3-4 ml of sterile normal saline and vortexed. The turbidity of the homogenous suspension was adjusted to ~0.5 McFarland standard. Similarly inoculum was prepared for filamentous fungi (3-7 d old slant at 37° on potato dextrose agar).

The semisolid agar tubes containing known concentrations of test oil as well as oil-free control, prepared in triplet, were inoculated with one loopful (HiMedia Flexiloop 4) of 0.5 McFarland adjusted culture by inserting the loop deep within the semisolid agar. The tubes were incubated at 37° for 48 h for *C. albicans* and 72 h for dermatophytes. For present investigation, 0.025 to 5 µl/ml concentrations of pure oil and its fractions were tested.

End point determination was carried out according to the Clinical and Laboratory Standards Institute (NCCLS/CLSI) guidelines, M27-A and M38-A. The growth was compared to that of oil-free control and scored by visual inspection as follows: +4 growth same as control; +3 slight decrease in growth; +2 significant reduction in growth (reduction 80% in yeast and 50 % in filamentous); +1 slight growth or few visible hyphal fragments; 0: no growth.

Toxicological studies of essential oil:

The acute dermal irritation assay of Draize[19] was applied for toxicological studies of the essential oil. All this work was done in accordance with the animal ethical guidelines. The permission for which was obtained from the institutional animal ethics committee (1678/GO/9/12/CPCSEA). A group of 20-25 Swiss albino mice was screened for this study. All the mice selected for the study were good in health. The mice exhibiting sniffles, hair loss, loose stools or apparent loss were rejected and replaced. Only the mice considered suitable for the use were selected for the study. Prior to treatment initiation, all mice were weighted. Their weight ranged from 20-25 g. Within 24 h before the test, the fur was removed from the dorsal area of the trunk of each mice, caring to avoid abrading of the skin. Observation was recorded one hour after the application of oil, at 24, 48 and on 72 h after application. The scoring system examined the skin for the presence of erythema and edema. The former was graded as 0 for no erythema, with erythema scores of 1 for very slight, 2 for well defined, 3 for moderate to severe and 4 for severe to eschar formation. Edema was scored in a similar manner with 0 indicating none, 1- very slight, 2- slight, 3- moderate and 4- severe. A score for each animal was determine using the immediate, 24, 48 and 72 h observations for calculations and dividing by four. The primary irritation index (PII) is the sum of the scores for all of the animal scores that is divided by number of observations primary dermal irritation index (PDII): 0.0- non irritation, >0.0-0.5- negligible irritant, >0.5-2.0- mild irritant, >2.0-5.0- moderate irritant, >5.0-8.0- severe irritant.

Statistical analysis:

Statistical analysis was carried by one way ANOVA following multiple comparison tests Tukey's method. Data is expressed as mean and SEM.

RESULTS AND DISCUSSION

In present investigation, GC and GC-MS analysis of *T. ammi* essential oil showed presence of 20 compounds for 100 % of total oil as listed in their elution (fig. 1). Thymol was found as a major compound (58.88 %) followed by p-cymene (24.02 %), γ-terpinene (13.77 %), β-pinene (1.90 %), α-thujene (0.31 %) and myrcene (0.35 %). Other compounds include α-pinene (0.16 %), α-terpinene (0.17 %), terpinen-4-ol (0.08 %) and α-terpineol (0.08 %; Table 1). Percent occurrence of eugenol was found 0.01.

The data of disc diffusion technique incorporated in Table 2 shows that in the case of *T. rubrum,* the maximum inhibition zone (51.33±0.333 mm) was found when undiluted oil was used. Other concentration like 75, 50 and 25 % oils also exhibited

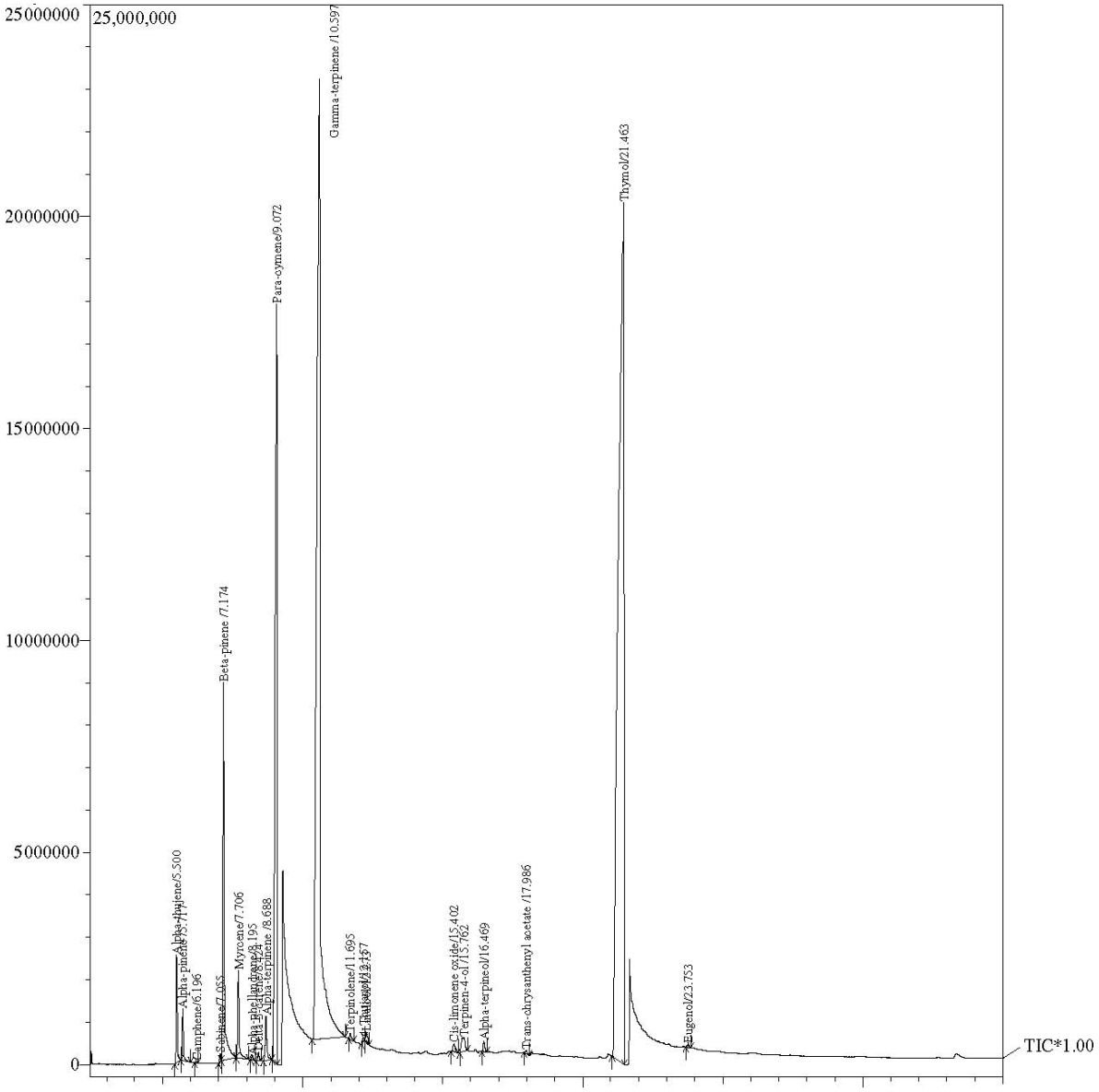

Fig. 1: GC-MS analysis of *T. ammi* essential oil
Thymol was found as a major compound (58.88 %) followed by p-cymene (24.02 %), γ-terpinene (13.77 %), β-pinene (1.90 %), α-thujene (0.31 %) and myrcene (0.35 %)

more promising activity (inhibition zone=47±0.577, 39.33±0.333 and 29.66±0.666 mm, respectively) than that of griseofulvin (inhibition zone=28±0.577 mm). In the case of *T. simii,* all the four concentration of oil showed prominent antifungal activity as compared to griseofulvin (24±0.577 mm). The maximum inhibition zone 57±0.288 mm was formed when undiluted oil was used. *Chrysosoprium indicum* was found to be most susceptible fungus to *T. ammi* oil than standard ketoconazole. The maximum inhibition zone 45±0.577 mm and activity index (2.64±0.033) were observed when undiluted *T. ammi* was used. Twenty five percent oil too showed immense inhibition action (inhibition zone=22±1.154 mm; AI=1.29±0.068) as compared to ketoconazole (IZ=17±0.577 mm).

Undiluted *T. ammi* exhibited broad spectrum activity against selected dermatophytes and other related keratinophilic fungi (Table 3). MIC was determined by modified micro broth dilution method. Its maximum activity was noticed against *T. verrucosum* and *T. rubrum* (MIC 0.025±0.000 µl/ml) followed *C. albicans* (0.20±0.000 µl/ml), *F. verticilloides* (0.233±0.033 µl/ml), *M. fulvum* (0.266±0.033 µl/ml) and *M. gypseum* (0.5±0.000 µl/ml).

Five different fraction of *T. ammi* were separated and labeled as TA, TA^{II}, TA^{III}, TA^{IV} and TA^{V} (Table 4). These fractions were separated on the basis of boiling point of compounds. Among all these fractions, TA^{V} was found to be an excellent antidermatophytic fraction. Its maximum activity was found against

M. gypseum (0.015±0.002 µl/ml) and *M. canis* (0.017± 0.002 µl/ml) followed by *T. rubrum* (0.02±0.000 µl/ml) and *C. albicans* (0.05±0.003 µl/ml). TA^{IV} fraction also showed marvellous activity against all the test fungi. MIC of this fraction was found to be 0.133± 0.033 µl/ml against *M. canis,* 0.026±0.002 µl/ml against *M. gypseum*, 0.050±0.000 µl/ml against *T. rubrum* and 0.053±0.003 µl/ml against *C. albicans*.

TABLE 1: CHEMICAL COMPOSITION OF *T. AMMI* ESSENTIAL OIL

Peak#	R. Time	RI	Area	Area %	Name
1	7.243	925	428373	0.3091	α-Thujene
2	7.494	932	231036	0.1667	α-Pinene
3	8.034	946	7823	0.0056	Camphene
4	8.961	971	24359	0.0176	Sabinene
5	9.121	975	2641134	1.9056	ß-Pinene
6	9.670	991	498781	0.3599	Myrcene
7	10.267	1004	17221	0.0124	α-Phellandrene
8	10.475	1010	49177	0.0355	Δ-3-Carene
9	10.769	1016	248690	0.1794	α-Terpinene
10	11.362	1025	33296066	24.0228	p-Cymene
11	12.830	1061	19093349	13.7757	γ-Terpinene
12	13.954	1087	34845	0.0251	Terpinolene
13	14.427	1098	55083	0.0397	4-Thujanol
14	14.537	1101	71163	0.0513	Linalool
15	17.598	1170	17976	0.0130	Cis-limonene oxide
16	18.390	1178	123717	0.0893	Terpinen-4-ol
17	19.508	1194	119369	0.0861	α-Terpineol
18	20.634	1228	18885	0.0136	Trans-chrysanthenyl acetate
19	24.402	1306	81612379	58.8826	Thymol
20	25.893	1360	12423	0.0090	Eugenol
			138601849	100.00	

MIC of TA^{III} fraction was found to be 0.050± 0.000 µl/ml against *T. rubrum*, 0.053±0.003 µl/ml against *M. gypseum,* 0.133±0.033 µl/ml against *M. canis* and 0.233±0.033 µl/ml against *C. albicans*. MIC of TA^{II} fraction was found to be 0.1±0.033 µg/ml against *M. gypseum*, 0.233±0.033 µg/ml against *T. rubrum*, 0.333±0.033 µl/ml against *M. canis* and 0.533±0.033 µl/ml against *C. albicans*. TA^{I} fraction showed slightly less activity as compared to other *T. ammi* fractions.

Acute dermal irritation assay, the toxicological studies involved five concentration of oil on albino mice. Each concentration was applied on five individual healthy mice. Low concentration up to 3 % did not show any irritation on mice skin. At 5 % concentration 3 mice showed mild erythema, which disappeared on 72 h observation whereas on 7 % concentration, all five mice exhibited well defined erythema up to 72 h of the observation (Table 5). Therefore 7 % oil was placed in grade III category of allergenicity rating (Table 6).

T. ammi is an important medicine plant, which possesses both medicinal and therapeutic properties. *T. ammi* oil was analyzed using GC and GC-MS and the components thereof were identified on the basis of their RI values as well as by comparison of their mass spectra with those reported in literature. The essential oil is composed of fatty acid as well as many polar and nonpolar compounds. Such a high antimicrobial activity of essential oil may be due to presence of high concentration of polar compounds[20]. Chemical analysis of *T. ammi* oil showed thymol as a major component. Similar results were observed by various researchers[21-23]. Antimicrobial activities are mostly

TABLE 2: COMPARISON OF EFFICACY OF *T. AMMI* OIL WITH COMMERCIAL ANTIFUNGAL DRUGS

Concentrations of oil	Test of fungi													
	Trichophyton rubrum				*Trichophyton simii*				*Chrysosporium indicum*		*Chrysosporium tropicum*			
	IZ	AI			IZ	AI			IZ	AI	IZ	AI		
		TC/G	TC/I	TC/K		TC/G	TC/I	TC/K		TC/K		TC/G	TC/I	TC/K
25 %	29.66 ±0.666	1.05 ±0.023	1.41 ±0.032	0.58 ±0.012	32 ±0.557	1.33 ±0.024	1.6 ±0.028	0.86 ±0.015	22 ±1.154	1.29 ±0.068	32 ±0.577	0.91 ±0.016	1.88 ±0.034	0.82 ±0.015
50 %	39.33 ±0.333	1.40 ±0.011	1.88 ±0.010	0.77 ±0.004	37 ±1.154	1.54 ±0.048	1.85 ±0.057	0.99 ±0.031	32 ±0.866	1.88 ±0.050	50.83 ±0.166	1.45 ±0.004	2.99 ±0.019	1.30 ±0.004
75 %	47 ±0.577	1.67 ±0.020	2.23 ±0.027	0.92 ±0.011	45 ±1.154	1.87 ±0.048	2.25 ±0.014	1.21 ±0.031	38 ±0.577	2.23 ±0.034	55.33 ±0.333	1.58 ±0.009	3.25 ±0.010	1.41 ±0.008
100 %	51.33 ±0.333	1.83 ±0.012	2.45 ±0.010	1.01 ±0.004	57 ±0.288	2.37 ±0.011	2.85 ±0.014	1.54 ±0.007	45 ±0.577	2.64 ±0.033	63.83 ±0.166	1.82 ±0.005	3.74 ±0.01	1.63 ± 0.004

IZ=Inhibition zone including 6 mm diameter of filter paper disc; AI=activity index; TC=test compound, inhibition zones of standard griseofulvin (G) against *T. rubrum*=28±0.577 mm; *T. simii*=24±0.577 mm; *C. tropicum*=35±1.154 mm. Inhibition zones of standard itraconazole (I) against *T. rubrum*=21±0.577 mm; *T. simii*=20±0.577 mm; *C. tropicum*=17±0.577 mm. Inhibition zones of standard ketoconazole (K) against *T. rubrum*=51±0.577 mm; *T. simii*=37±1.154 mm; *C. tropicum*=39±1.154 mm; *C. indicum*=17±0.577

attributable to the presence of phenolic compounds such as thymol and to hydrocarbons like g-terpinene and p-cymene[24]. p-Cymene (24.02 %) was found to be the second most common and abundant component of essential oil followed by γ-terpinene (13.77 %). According to Delgado[25], cymene does not have antimicrobial activity, but it increases the activity of phenolic compounds including thymol by swelling of the cytoplasmic membrane. Sharifzadeh *et al.*[26] also reported thymol as a major component of *T. ammi* essential oil. Similar reports were also observed by Moazeni *et al.*[22], Thangam and Dhananjayan[21]. Sharma and Swati[27] also studied the GC-GC/MS analysis of *T. ammi* oil and found thymol as a major component.

The antidermatophytic activity of *T. ammi* essential oil was evaluated through the measurement of zone of inhibition, activity index and MIC. The correlation between two different methods examined was to be the larger inhibition zone correlated with lower MIC value. In present investigation, all the four concentration exhibited excellent antidermatophytic activities as compared to standard allopathic drugs itraconazole, ketoconazole and griseofulvin. MIC ranged from 0.025 to 0.5 µl/ml against selected fungi. Fraction separation of essential oil on the basis of boiling temperature of compounds through the glass oven equipment is a new field of work. No work has been reported on screening of essential oil fractions against any bacteria and fungi. The oil fractions obtained from the glass oven

TABLE 3: MIC OF ESSENTIAL OILS AGAINST SELECTED FUNGI

Fungi	*T. ammi* MIC (µl/ml)
Candida albicans	0.20±0.000
Microsporum gypseum	0.5±0.000
Microsporum canis	0.05±0.000
Trichophyton rubrum	0.025±0.000
Trichophyton verrucosum	0.025±0.000
Microsporum fulvum	0.266±0.033
Fusarium verticilloides	0.233±0.033

TABLE 4: MIC OF *T. AMMI* FRACTIONS AGAINST DERMATOPHYTES

Fraction/fungi	TA^{I}	TA^{II}	TA^{III}	TA^{IV}	TA^{V}
Candida albicans	0.433±0.033	0.533±0.033	0.233±0.033	0.053±0.003	0.05±0.003
Microsporum gypseum	0.233±0.033	0.1±0.033	0.053±0.003	0.026±0.002	0.015±0.002
Microsporum canis	0.333±0.033	0.333±0.033	0.133±0.033	0.133±0.033	0.017±0.002
Trichophyton rubrum	0.233±0.033	0.233±0.033	0.050±0.000	0.050±0.000	0.020±0.000

TABLE 5: THE ACUTE DERMAL IRRITATION ASSAY ON ALBINO MICE

No. of mice	1 h		24 h		48 h		72 h	
	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
3 % *T. ammi* oil								
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
5 % *T. ammi* oil								
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	1	0	1	0	0	0	0	0
4	1	0	2	0	1	0	0	0
5	1	0	2	0	1	0	0	0
7 % *T. ammi* oil								
1	1	0	2	0	2	0	1	0
2	1	0	2	0	2	0	1	0
3	2	0	2	0	2	0	1	0
4	2	0	2	0	2	0	1	0
5	2	0	2	0	2	0	1	0

Erythema was scored as follows; no erythema=0, very slight erythema (barely perceptible)=1, well defined erythema=2, moderate to severe erythema=3, severe erythema (beet redness) to slight eschar formation (injuries in depth)=4. Edema formation was scored as follows no edema=0, very slight edema=1, sight edema (edges of area well-defined by definite raising)=2, moderate edema (raised approximately 1 mm)=3, severe edema (raised more than 1 mm and extending beyond area of exposure)=4

TABLE 6: ALLERGENICITY RATING (ERYTHEMA) WITH *T. AMMI* OIL

Concentration	Grade	Classification
1 %	I	Non-irritant
2 %	I	Non-irritant
3 %	I	Non-irritant
5 %	II	Negligible irritation
7 %	III	Mild irritation

equipment were further subjected for MIC also showed excellent antidermatophytic properties. Five different fractions of *T. ammi* were separated and labelled as TA^{I}-TA^{V}. Among all these fractions, TA^{V} showed more prominent fungicidal activities. TA^{V} was separated at 240° temperature. This fraction contained high concentration of thymol with other trace compounds. Boling point of thymol is 232.9°. Presence of thymol enhanced the antimicrobial activity of this fraction augmented manifold. Previous studies showed that thymol has a high microbicidal and antiaflatoxigenic effects due to the presence of phenolic -OH group[28]. TA^{I} fraction was separated at 160° contains less effective components like α pinene (boiling point 155) and β pinene (boiling point 155-156°). Therefore TA^{I} was found to be less effective as compared to any other fractions.

No work on acute dermal irritation of *T. ammi* has been reported till date. During present investigation, five concentrations of *T. ammi* oil were applied superficially on the skin of Swiss albino mice. Up to 3 % concentration of oil no adverse side effect was observed. Slightly inflammation and redness were observed at 5 % concentration, which disappeared at later hours. PDII score of 5 % oil was found to be 0.5 while in presence of 7 % oil 1.65 PDII score was recorded. Allergenicity rating classified 1-3 % oil as non-irritating category, 5 % as negligible irritation and 7 % as mild irritation category with grade III. This study unfolds that low concentration of oil had no toxic effects. No work on *T. ammi* dermal toxicity on mice and other animals has been reported till date. Craig *et al.*[29] studied the western juniper oil and Port Orford cedar oil for possible dermal toxic effects on mice and rabbits. They used 0.5 % and 5 % concentration on shaved skin. Acute dermal irritation study using rabbits had a PII of 3.3 with 100 % Port Orford cedar oil extract. This was reduced to a PII of 0.625 when diluted 1:1 with olive oil. Undiluted western juniper oil extract had a PII score of 2.7 while a 5.0 % solution had a PII score of 0.3, a 0.5 % solution of western juniper oil was a non-irritant. Abdel *et al.*[30] studied the acute toxicity and irritancy of the essential oil of the leave of *Vitex simplicifolia* Oliv. and results thereof suggested that leaf essential oil of *V. simplicifolia* possessed slightly toxic and slightly irritancy activity. Very low MIC ranges of pure oil and its fractions and toxicity testing results reveal that up to 3 % *T. ammi* essential oil is safe for the preparation of ointment for the superficial treatment of fungal infection.

In summary, this study showed that low concentrations of oil exhibited no toxic side effects. Further, *T. ammi* oil did not elicit any hypersensitivity reactions nor would an acute skin irritation occur at the low concentration to which animals bedded on these materials be exposed. *T. ammi* oil exhibited excellent antidermatophytic activity as compared to standard allopathic drugs. Therefore, oil can be used for the treatment of tinea or ring worm infections as an alternate therapy.

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Conflict of interest:

No conflict of interest.

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