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Chemical Characterization and Antidermatophytic Activity of *Thuja orientalis* Collected from Jaipur District, Rajasthan

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Abstract

Thuja orientalis commonly known as white cedar was collected from Rajasthan university campus, Jaipur District, Rajasthan, India. GC/GC-MS analysis of *T. orientalis* essential oil showed the presence of 38 compound for 100% of total oil. Delta-3-carene (30.37%) was the main component of thuja oil followed by alpha -pinene (13.11%), limonene (10.07%), alpha- terpinene (8.44%), alpha -terpinyl acetate (2.96%), trans-caryophyllene (4.48%), alpha -humulene (3.98%), cedrol (5.65%), myrecene(5.88%), beta pinene (2.67%), sabinene(1.39%), germacrene D (1. 08%) and bornyl acetate (1.00%). In present investigation antidermatophytic activity of *T. orientalis* essential oil was also screened against selected dermatophytes and *candida albicans* through disc diffusion technique and minimum inhibitory concentration evaluating method. Maximum zone of inhibition 20± 0.29mm was reported against *Trichophyton rubrum* MTCC 296 (AI=0.67) followed by 14±1.00 mm against *Trichophyton tonsurans* MTCC 8475 (AI=0.7). MIC was ranging from 0.7 μl/ml to>2.5 μl/ml. *Candida albicans* (MTCC 3018) was found to be most resistant strain. *Thuja* essential oil exhibited excellent antifungal activity against all pathogen except *C. albicans.* These results suggest the potential therapeutically effect of *T. orientalis* essential oil against dermatophytic fungi.

Key words: *T. orientalis*, essential oil, dermatophytes, Delta-3-carene,MIC

Introduction:

Thuja orientalis L. (syn. *Biota orientalis Endl*) commonly known as Morpankhi belonging to the Family Cupressaceae is an ever green monoecious shrubs used in various forms of homeopathy and traditional medicines in various methods. It is cultivated as an ornamental tree in cool and moist places for its extremely beautiful dense foliage and bush like habit of growth. The shoot are flat, leaves are scale like. The phytoconstituents of *T. orientalis* such as flavonoids and terpenoids showed the biological activities (1). Biological activities of *T. orientalis* such as antimicrobial(2), antioxident(3), antibacterial (4), anti-inflammatory (5), insecticidal (6), nematicidal (7), anticancer (8) are well studied. Hair growth promoting activity of hot water extract of *Thuja* was studied by Zhang et al (9). Essential oil derived from the *Thuja* have some toxic properties. Ingestion of *Thuja* leave oil even can cause death (10).

Dermatophytoses possess a serious concern to sociologically backward and economically poor population of India. The dermatophytes represent more than 40 closely related species classified in three genera: *Microsporum*, *Trichophyton* and *Epidermophyton*. High humidity and high temperature condition of Jaipur city specially in summer, favour the incidence of fungi and consequently the diseases (11-13). Skin infection due to dermatophytes has become a significant health problem affecting children, adolescent and adults(14). This may be

the result of frequent usage of antibiotics, environmental condition, immuno-suppressive drugs and various conditions, like organ transplantation, lymphomas, leukemia and human immunodeficiency virus (15).

The present investigation deal with the screening of *T. orientalis* leaves essential oil and their antidermatophytic activity against some selected dermatophytes and *Candida albicans*.

Material and Methods

Collection of plant and extraction of oil: *T. orientalis* leaves were collected from Rajasthan University campus. Identification was done by Botanist Prof. S. Misra, Herbarium (Voucher number RUBL 21183), Department of Botany ,University of Rajasthan, Jaipur, India. Leaves were shade dried and cut into small pieces. The semi-crushed leaves were hydrodistilled in a Clevenger's apparatus for 7-8 hours. Essential oil collected in tubes were dried with anhydrous sodium sulphate. Moisture free oil was than stored in amber coloured bottles and kept in the refrigerator.

Gas chromatography : The quantitative analysis of the essential oils were 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 x 0.25 mm ID, film thickness 0.25 μm). Injector and detector temperatures were 270°C and 280°C respectively. Column temperature programmed from 80°C (2 mins hold), 80°C to 180°C at 4°C/min and 180°C to 230°C at 6°C/min with hold time of 6 min 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 Analyses : GC-MS data was obtained on a Shimadzu GCMS-QP-2010 plus system using Omega SPTm column (30.0 m x 0.25 mm ID. film thickness 0.25 um). Helium was used as carrier gas. Injector, Mass detector and Ion source temperatures were 270°C, 280°C and 250°C respectively. Column temperature programmed from 80°C (2 mins hold), 80°C to 180°C at 4°C/min and 180°C to 230°C at 6°C/min with hold time of 6 min and 19 min. respectively. The flow rate of carrier gas was 1.21 ml/min and split ratio was 1:80. EI source and mass range were 70 eV and 40-850 amu respectively. Compounds were identified by using Willey, NIST and Perfumery libraries.

Fungal culture: For antidermatophytic studies five fungi and one yeast procured from the Imtech Chandigarh are *Trichophyton rubrum* (MTCC 296), *T. mentagrophytes* (MTCC 7687), *T. tonsurans* (MTCC8475), *Microsporum canis*(MTCC2820), *Candida albicans* (MTCC3018) and *Microsporum fulvum*(MTCC2837). These selected fungi are maintained on Sabouraud's dextrose agar media and Potato dextrose agar media.

Screening of oil of their antifungal activity:

Disc diffusion method: The filter paper disc method Wannisorn *et al.*(16)was used for evaluation of antifungal activity of essential oil. 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. 20 ml sterilized Sabouraud's dextrose agar medium was taken in each autoclaved petridish and allowed to solidify. Fungal spore suspension was prepared in sterilized distilled water by transferring a loopful of 15 day-old culture. 1 ml of spore suspension of approximately 0.5 to 5×10^4 (cfu/ml) was spread over the respective agar medium plates. Sterilized filter paper were soaked in neat undiluted oil. An oil saturated disc was placed on an agar plate containing fungal spore suspension. These plates were incubated at 37ºc for 72 hours. Five replicates were kept in each case and the average values were determined and inhibition zone were observed. Ketoconazole was used as a standard drug. The antifungal activity was determined by measuring the inhibition zone around the disc. The activity of oil was measured by the following formula.

$$\text{Activity Index} = \frac{\text{Inhibition Zone (IZ) of the sample}}{\text{Inhibition Zone (IZ) of the Standard}}$$

Semisolid agar antifungal susceptibility method : Minimum inhibitory concentration was determined by Semisolid agar antifungal susceptibility testing method(17). For this experiment Brain Heart Infusion Agar (Hi-media) was used. BHIA was prepared according to manufacturer's instruction.

Inoculum preparation: Sterile swab dipped into sterile tween 80 was used to pick the pure colony of test organism. 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.

Inoculation of drug containing tubes : The semisolid agar tubes containing known concentrations of test oils as well as oil-free controls, prepared in triplet, were inoculated with one loopful (HimediaFlexi loop 4) of 0.5 McFarland adjusted culture by inserting the loop deep within the semisolid agar. A loopful of the inoculum suspension was streaked onto Sabouraud dextrose agar to check for purity and viability. The tubes were incubated at 37°C for 72 hours

End point determination: End point determination was carried out according to the NCCLS/CLSI guidelines, M27-A and M38-A. 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.

Statistical analysis: Each parameter was tested in triplicate. Conventional statistical methods were used to calculated means and standard deviations. Statistical analysis (T-test) was applied to the data to determine differences ($p < 0.05$).

Results and Discussion

GC/ GC-MS analysis of *T. orientalis* essential oil showed the presence of 38 compounds for 100% of total oil which listed in their elution(Fig.1,2). Delta-3-carene (30.37%) was the main component of *Thuja* oil followed by alpha -pinene (13.11%), limonene (10.07%), alpha-terpinene (8.44%), alpha -terpinyl acetate (2.96%), trans- caryophyllene (4.48%), alpha -humulene (3.98%), cedrol(5.65%), germacrene D (1. 08%) and bornyl acetate (1.00%). Other compounds are alpha thujene (0.75%), alpha-phellandrene (0.59%), alpha -terpine (0.29%), gamma- terpinen (0.55%), terpinen-4-ol (0.76%), alpha -terpineolt (0.33%), ocimenyl acetate (0.61%), beta- elemene (0.20%), beta- cedrene (0.53%), alpha-selinene (0.19%), gamma -muurolene(0.27%), delta-cadinene (0.71%), elemol (0.28%), caryophyllene oxide (0.65%), viridiflorol (0.52%), humulene epoxide II (0.49%), tau- muurolor (0.24%) and alpha-cadinol (0.41%).Similar result was obtained by Nickavar et al.(18). They studied the fruit and leaf essential oils of *T. orientalis*in Iran and found alpha pinene (21.9%)as major constituent of leaf oil followed by alpha cedrol (20.3%), delta 3 carene (10.5%) and limonene(7.2%). Guleria *et al.*(19) studied the essential oil of *T. orientalis* collected from western Himalayan region. Leaf oil contained alpha-pinene (29.2%), delta-3-carene (20.1%), alpha-cedrol (9.8%), caryophyllene (7.5%), alpha-humulene (5.6%), limonene (5.4%), alpha-terpinolene (3.8%) and alpha-terpinyl acetate (3.5%) as major constituents.

Tsiri *et al.*(20) investigated the chemical composition of the essential oils of four varieties of *Thuja*species like *T. occidentalis 'globosa'*, *T. occidentalis 'aurea'*, *T. plicata*and *T. plicata 'gracialis* cultivated in Poland. The main constituents in all samples were the monoterpene ketones *á*- and *â*-thujone, fenchone and sabinene, as well as the diterpenesbeyerene and rimuene. They also checked the antimicrobial activity of these oil against selected bacteria and *Candida* sp. Jirovetz *et al.* (21) studied the chemical composition and antimicrobial activity of *Salva* sp. and *Thuja* sp. essential oils.

Antidermatophytic activity of *Thuja* essential oil was studied by disc diffusion method and minimum inhibitory concentration determination. Maximum Zone of inhibition was found to be 20±0.288mm against *T. rubrum* MTCC 296 (AI=0.67), but maximum activity index 0.73 was

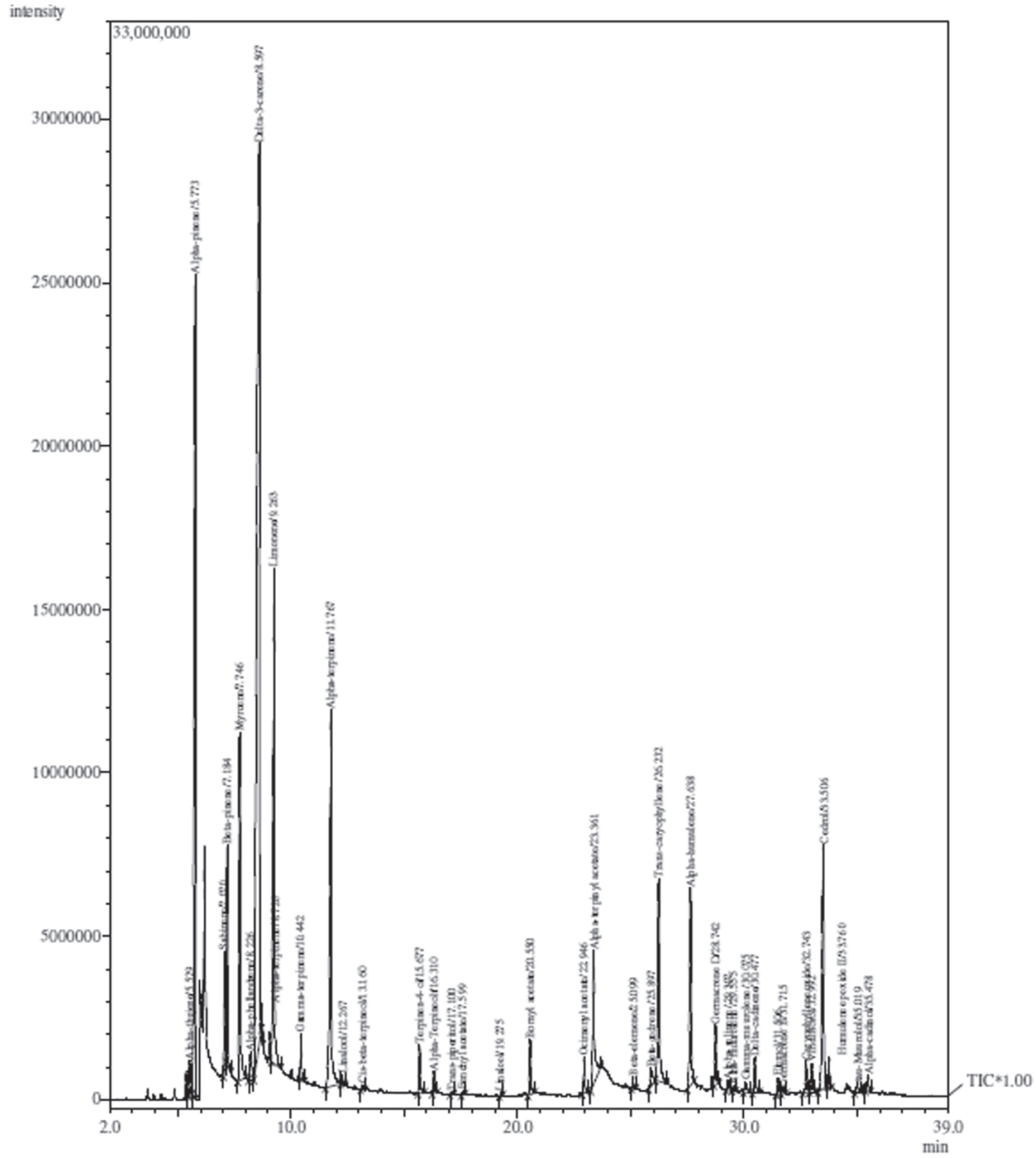

Fig. 1. GCMS analysis of *Thuja orientalis*

Fig. 2. Chemical compositions of *Thuja orientalis*

Peak#	R.Time	Area	Area%	RI	Name
1	5.529	5654678	0.75	926	Alpha-thujene
2	5.773	99279048	13.11	933	Alpha-pinene
3	7.070	10532112	1.39	972	Sabinene
4	7.184	20212491	2.67	975	Beta-pinene
5	7.746	44539565	5.88	992	Myrcene
6	8.226	4463801	0.59	1005	Alpha-phellandrene
7	8.597	229961085	30.37	1014	Delta-3-carene
8	8.726	2169635	0.29	1017	Alpha-terpinene
9	9.263	76250386	10.07	1030	Limonene
10	10.442	4178735	0.55	1058	Gamma-terpinene
11	11.767	63941559	8.44	1089	Alpha-terpinene
12	12.267	983255	0.13	1101	Linalool
13	13.160	563719	0.07	1121	Cis-beta-terpineol
14	15.677	5791080	0.76	1176	Terpinen-4-ol
15	16.310	2495738	0.33	1191	Alpha-Terpineolt
16	17.100	275300	0.04	1208	Trans-piperitol
17	17.599	405468	0.05	1219	Fenchyl acetate
18	19.275	235879	0.03	1257	Linalool
19	20.550	7550788	1.00	1285	Bornyl acetate
20	22.946	4611929	0.61	1341	Ocimenyl acetate
21	23.361	22448055	2.96	1350	Alpha-terpinyl acetate
22	25.099	1494033	0.20	1391	Beta-elemene
23	25.897	3976515	0.53	1410	Beta-cedrene
24	26.232	33937017	4.48	1418	Trans-caryophyllene
25	27.638	30168221	3.98	1453	Alpha-humulene
26	28.742	8204553	1.08	1480	Germacrene D
27	29.307	1473653	0.19	1493	Alpha-selinene
28	29.555	550832	0.07	1500	Alha-muurolene
29	30.075	2081752	0.27	1507	Gamma-muurolene
30	30.477	5339706	0.71	1512	Delta-cadinene
31	31.506	2139791	0.28	1526	Elemol
32	31.715	974739	0.13	1528	Germacrene B
33	32.743	4906070	0.65	1542	Caryophyllene oxide
34	32.992	3943967	0.52	1545	Viridiflorol
35	33.506	42806479	5.65	1552	Cedrol
36	33.760	3698369	0.49	1555	Humulene epoxide II
37	35.019	1838680	0.24	1572	tau-Muurolol
38	35.478	3138623	0.41	1578	Alpha-cadinol
		757217306	100.00		

observed against *Microsporum canis* MTCC 2820(IZ=10.3±0.58mm) followed by AI=0.7against *T. tonsurans* MTCC 8475(IZ=14± 1.00 mm) (Table1). Zone of inhibition 10±0.00 mm was observed against *M. fulvum* MTCC 2837 (AI=0.34), 9±0.58mm against *T. mentagrophytes* MTCC 7687(AI=0.36) and 6±0.00 mm against *Candida albicans* MTCC 301(AI=0.21).*Candida albicans* was found to be most resistant strain from *T. orientalis* essential oil.MIC was determined by semi solid agar antifungal susceptibility testing method with visual end point determination according to NCCLS recommendations. Maximum effect was seen against *T. mentagrophytes* MTCC 7687(0.7 µl/ml) followed by *M. canis* MTCC 2820(0.9 µl/ml),*T. rubrum* MTCC 296 (0.9 µl/ml), *M. fulvum* MTCC 2837(1.1 µl/ml) and *Candida albicans* MTCC 3018(>2.5 µl/ml).(Table. 2)

Jain and Garg (2)studied antimicrobial activity of *T. orientalis* essential oil against six bacteria and five plant pathogenic fungi through disc diffusion technique. The oil was found moderately activity against all six bacteria. Maximum 21mm inhibition zone was observed against *Curvularia lunata.* Most of work has been done on antibacterial activity and on plant pathogenic fungi of *Thuja* plant extracts(3-4,22-25). There are no studies on antidermatophytic properties of *T. orientalis* so far and the present study is the new report.

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Table 1: Antifungal activity of *T. orientalis* essential oil and compared with standard drug

Fungi	*C. lemon* IZ	Ketoconazole IZ	AI
*Candida albicans*MTCC 3018	6±0.00	28±0.58	0.21
*Microsporum canis*MTCC 2820	10.3±0.58	14±0.00	0.73
*Microsporum fulvum*MTCC 2837	10±0.00	29.1±1.15	0.34
*Trichophyton rubrum*MTCC 296	20±0.29	30±0.58	0.67
*Trichophyton mentagrophytes*MTCC 7687	9±0.58	25±0.00	0.36
*Trichophyton tonsurans*MTCC 8475	14±1.00	20±0.58	0.70

IZ = Inhibition zone including 6 mm diameter of filter paper disc;
AI=Activity Index

Table 2 : MIC (µl/ml) of essential oils against selected dermatophytes

Test Organisms	MIC
1. *Candida albicans* MTCC 3018	>2.5
2. *Microsporum fulvum*MTCC 2837	1.1
3. *Microsporum canis*MTCC 2820	0.9
4. *Trichophyton rubrum*MTCC 296	0.9
5.*Trichophyton mentagrophytes*MTCC 7687	0.7

Conflict of Interest: No conflict of interest.

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