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ESSENTIAL OIL CONSTITUENTS AND ANTIMICROBIAL POTENCY OF *THUJA ORIENTALIS* GROWN IN RAJASTHAN

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ABSTRACT

The chemical composition of essential oils obtained from *Thuja orientalis* L. (Cupressaceae) grown in Rajasthan was determined. Their essential oil was determined by hydro-distillation, analysed by GC/MS and GC-FID. The analyses of plant resulted in the identification of thirty two compounds, representing 88.5 % of the total oil. Out of 32 components, the major component was α-thujone (52.43 %) and other dominant components analyzed were β-Thujone (4.89 %), Camphor (4.36 %), Sabinene (4.21 %), Fenchone (3.57 %). Oil of T. orentalis exhibited the antibacterial (MIC values 1.32–1.83 mg/mL) as well as antifungal (MIC 1.86–2.87 mg/mL) activity. In all of the selected pathogens S. aureus bacterial strains (MIC 1.32 mg/mL) and A. alternate fungal strain (MIC 1.86 mg/mL) was found to be most sensitive to T. Orentalis essential oil. Isolated α- and β-Thujone from the plant also show high antibacterial (MIC values 0.064–0.091 mg/mL) as well as antifungal activity (MIC values 0.45–0.86 mg/mL).

Keywords: *Thuja orientalis, essential oil, α- β thujone, Antimicrobial activity.*

INTRODUCTION

Recently, multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Service, 1995) making it a global growing-problem. Isolation of microbial agents less susceptible to regular antibiotics and recovery of increasing resistant isolates during antibacterial therapy is rising throughout the world which highlights the needs of new principles. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of actions (Barbour et al, 2004; Hamil et al, 2003).

Thuja orientalis L. (Cupressaceae) is an evergreen species widely cultivated as a common ornamental plant (Assadi, 1998). It is widely distributed around China, Iran, Japan, America, Nagaland, Korea and India (Bucur, 1995, Asili et al., 2007; Jaiswal et al., 2011and Bansal et al., 2011).

T. orientalis plant is commonly used in herbals (Duke and Ayensu, 1985) as a haemostatic, expectorant, and cough remedy (Kuo and Chen., 1990). The stem of this plant is used in the treatment of coughs, colds, dysentery, rheumatism and parasitic skin-diseases (Nahed *et al.*, 2010). The root bark is used for treatment of burns and scalds, and seeds are used internally in the treatment of palpitations, insomnia, nervous disorder and constipation in the elderly (Duke and Ayensu, 1985). Dried leaves of *P. orientalis* have been used as a haemostatic, expectorant and hypo tensor in Korean folk medicine (Koo *et al.*, 2002). Fresh leaves of that plant are used as an anti inflammatory drug (Pantong *et al.*, 1986). A yellow dye is obtained from the young branches (Grieve, 1984). Seeds are used for bronchitis, insomnia and as antitussive (Nishiama *et al.*, 1995). *Thuja* is also occasionally used for treating diseases of skin, blood, gastrointestinal tract, kidney, brain, warty excrescences, spongy tumors (Biswas *et al.*, 2011). It is used internally in the treatment of coughs, hemorrhages, excessive menstruation, bronchitis, asthma, skin infections, mumps, bacterial dysentery, arthritic pains and premature blandness (Bown, D. 1995 and Greenberg *et al*., 1978). The chemical constituents of *P. orientalis* such as terpenoids and flavonoids showed the pharmacological or biological activities (Hassanzadeh *et al.*, 2001). Many chemical components had been isolated by the different parts of *P. orientalis* like sesquiterpenoids and diterpenoids from the heartwood (Erdtman *et al.*,1956 ; Dev *et al.*,1964; Tomita *et al.*, 1968 and Tomita *et al.*, 1969), Flavonoids from leaves, mono and sesquiterpenoids in essential oils of different parts of the plant (Pelter *et al.*, 1970 and Yan-hua *et al.*,2006), four bisnor and trinorlabdan type diterpenoids from seeds (Inoue *et al.*, 1985), two monolognol derivatives from pollens (Ohmoto *et al.*,1988), some labdane and isopimarane diterpenoids from pericarpes and leaves (Kuo and Chen, 1990; Koo *et al*., 2002). The most prominent constituents of the oil are thujone, isothujone, fenchone and carnphor (Asili *et al.*, 2007). Thujone was a weak inhibitor of acyl-CoA: lysophosphatidylcholine acyl-transferase activity in mouse brain synaptosomes compared to psychoactive cannabinoids (Greenberg *et al.*, 1978). The essential oil which obtained by leaves is toxic. α-thujone is useful as an insecticide and an antihelminthic agent for the treatment of parasitic worms. However, α -thujone is a toxic substance that disrupts neurological signals in the brain. Ingestion of the essential oils of *Thuja* leaves can cause death (Hold *et al.*, 2000).

The chemical constituents of *T. orientalis* oil has not been much studied except for those of Chen *et al.* and Li *et al.* where they have reported α-pinene and α-cedrol as the major constituents of *T. orientalis*, respectively (Chen *et al.*, 1984; Li and Liu, 1997).

The aim of this study was to identify and evaluation of antimicrobial activity of essential oil constituents of the plant *T. orientalis* grown in Rajasthan.

METHODOLOGY

Plant material: Plant *T. orientalis* was collected from the University botanical garden and shade dried. Specimen (No.) of the plant was deposited at the herbarium of Department of Botany, University of Rajasthan, Jaipur.

ISOLATION OF THE ESSENTIAL OIL

Shade dried fresh plant parts of *T. orientalis* were subjected to hydro distillation. The distillate was then extracted by using petroleum ether as solvent. The resulting extract was dried over anhydrous sodium sulfate. Petroleum ether was removed carefully under vacuum and a pale yellow color essential oil was obtained. Further the essential oil was used for antimicrobial activity.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS

The extract and the standard samples were analyzed by GC-MS of Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with using Agilent 7890A/5975C GC HP-5. Capillary column (phenyl methyl siloxane, 25 m×0.25 mm i.d) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was 100 °C (3 min) to 280 °C at 1 to 40 °C/min;

detector temperature, 250 to 280°C; carrier gas, He (0.9 mL/min). Retention indices were determined by using retention times of samples that were injected under the same chromatographic conditions. The components of the standard and plant samples were identified by comparison of their mass spectra and retention time with those given in literature and by comparison with the mass spectra of the Wiley library or with the published mass spectra.

ISOLATION OF TERPENOIDS ANTIMICROBIAL ACTIVITY ASSAYS

Microorganisms: All the test organisms, bacterial isolates of *Escheriachia coli*, *Bacillus subtilius*, *Staphylococcus aureus* and *S. epidermidis* and fungal isolates viz *Candida spp.*, *Aspergillus niger, Penicillium spp.* and *Alternaria alternate* were obtained from Division of Biosciences, Seminal Applied Sciences Pvt Ltd, Jaipur, Rajasthan. Antimicrobial activity of the essential oils and the isolated compounds was determined using the agar dilution technique (Janssen et al., 1987). Standard antibiotics Streptomycin and Ketokenozol were used in order to control the tested bacteria and fungi. For each strain, the growth conditions and the sterility of the medium were checked and the plates were incubated at 37 °C and the MICs were determined as the lowest concentrations preventing visible growth.

STATISTICAL ANALYSIS

Results of the analyses were compared by one way analysis of variance (ANOVA). The significance between pairs of variable means were analysed using least significant difference (LSD) test at 5 % level of significance (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The hydrodistillation of the shade dry parts of the plant *T. orientalis* gave a pale yellow oil with 0.89% yield. The essential oils components of the studied plant with their percentages and their retention indices are listed in Table 1, while the antibacterial and antifungal activities of the essential oils and their components are given in Table 2.

As a result of GC analyses, 32 components were identified representing 88.4% of the total oil. The plant *T. orientalis* was found to have α-thujone (52.43 %), β-Thujone (4.89 %), Camphor (4.36 %), Sabinene (4.21 %), Fenchone (3.57 %) as major constituents and followed by 27 other essential oil constituents (i.e. 19 %). (Table 1)

Antimicrobial activity of *T. Orentalis* essential oil and isolated compounds shown inhibitory activity against selected bacterial and fungal strains and comparable to control (streptomycin sulphate and ketokenozol). (Table 2)

In the screening of antimicrobial activity, the oil of *T. orentalis* exhibited the antibacterial (MIC values 1.32–1.83 mg/mL) as well as antifungal (MIC 1.86–2.87 mg/mL) activity. In all of the selected bacterial strains *S. aureus* (MIC 1.32 mg/mL) was found to be most sensitive to *T. Orentalis* essential oil and followed by *B. subtilius* (MIC 1.36 mg/mL), *S. epidermidis* (MIC 1.44 mg/mL) and *E. coli* (MIC 1.83 mg/mL). Fungi *A. alternate* (MIC 1.86 mg/mL) was most sensitive among all fungal strains and followed by *A. niger* (MIC 2.13 mg/mL), *Penicillium spp.* (MIC 2.45 mg/mL) and *Candida spp.* (MIC 2.87 mg/mL).

The antimicrobial activity of isolated α- and β-Thujone from the plant also high antibacterial (MIC values 0.064–0.091 mg/mL) as well as antifungal activity (MIC values 0.45–0.86 mg/mL).

S. aureus (MIC 0.064 mg/mL) was found to have minimum MIC value and most sensitive to the compounds and followed by *S. epidermidis* (MIC 0.074 mg/mL), *E. coli* (MIC 0.082 mg/mL) and *B. subtilius* (MIC 0.091 mg/mL). Fungi *Candida spp.* (MIC 0.45 mg/mL) was most sensitive and followed by *Penicillium spp.* (MIC 0.76 mg/mL), *A. alternate* (MIC 0.78 mg/mL) and *A. niger* (MIC 0.86 mg/mL).

The most prominent constituents of the oil are thujone, isothujone, fenchone and carnphor (Asili *et al.,* 2007). High antibacterial and antifungal activity is due to high content of α- and β-thujone, which also exhibited strong microbial activities against the selected pathogens and are well known as the main active compounds in many essential oils from the plant having similar antimicrobial activity (Baser et al, 2002; Sivropoulou et al., 1997).

The present study reveals that the plant *Thuja occidentalis* is found to have strong antibacterial and antifungal activity. The chemosystematic value of the total ketone content, especially of thujone isomers, is confirmed from this study. Pathogens are economically damaging human and animal health, agriculture and food. Thus, an approach towards the discovery of lead compounds has been made using an essential oil from *T. orientalis*. Sensitivity of pathogens towards active constituents of *T. orientalis* has indicated the need for the application of natural biocides in the field and during post-harvest storage. Present findings could support the potential use of plant essential oils, which could be of economical benefit.

Table 1. Components (%) of the volatiles of *Thuja Orentalis* essential oils

Peak no.	Compounds a	*T. Orentalis*	K.I.
1	*α*-Thujene	0.27	928
2	*α*-Pinene	1.42	932
3	Camphene	1.29	951
4	Sabinene	4.21	969
5	Myrcene	0.58	986
6	*α*-Terpinene	0.58	1002
7	*p*-Cymene	0.15	1019
8	Limonene	2.14	1022
9	γ-Terpinene	0.94	1055
10	Fenchone	3.57	1081
11	*α*-Thujone (*cis*)	52.43	1098
12	*β*-Thujone (*trans*)	4.89	1110
13	Camphor	4.36	1139
14	Borneol	0.28	1158
15	Terpinen-4-ol	2.81	1169
16	*meta*-Methylacetophenone	0.11	1178
17	*α*-Terpineol	0.54	1185
18	*endo*-Fenchyl acetate	0.66	1219
19	Couminalaldehyde	0.06	1236
20	Carvone	0.24	1237
21	Piperitone	0.13	1252
22	Cyclofenchone	0.38	1266
23	(-)-Bornyl acetate	0.32	1290
24	Carvacrol	0.24	1297
25	Geranyl acetate	0.31	1383
26	*trans*-Cinnamyl acetate	0.12	1391

27	δ-Cadinene	0.1	1525
28	(-)-Caryophyllene oxide	0.68	1576
29	Rimuene	2.35	1893
30	Beyerene	0.7	1929
31	(+)-Beyerene-19- ol	0.74	2220
32	trans-Totarol	0.89	2313
	Total (%)	**88.49**	

Kovats Indices (KI)

Table 2. Antimicrobial activities (MIC mg/mL) of *T. Orentalis* essential oils and their main components

Antimicrobial activity of	***Escherichia coli***	***Bacillus subtilius***	***Staphylococcus aureus***	***S. epidermidis***	***Candida spp.***	***Aspergillus niger***	***Penicillium spp.***	***Alternaria alternata***
T. Orentalis Essencial oil	1.83	1.36	1.32	1.44	2.87	2.13	2.45	1.86
α- and β-Thujone	0.082	0.091	0.064	0.074	0.45	0.86	0.76	0.78
Streptomycin	$3.5 * 10^{-3}$	$3 * 10^{-3}$	$4 * 10^{-3}$	$4.2 * 10^{-3}$	-	-	-	-
Ketokenozol	-	-	-	-	$1.2 * 10^{-3}$	$0.5 * 10^{-3}$	$1.4 * 10^{-3}$	$0.8 * 10^{-3}$

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