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Research Article

Chemical composition, Antioxidant and Antibacterial activity of thuja orientalis essential oil

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ABSTRACT

Essential oils derived from many aromatic plants are well known to possess cytotoxic, antioxidant, antifungal, insecticidal and antimicrobial activities. *Thuja orientalis* (family: Cupressaceae) is widely cultivated as a common ornamental plant. It possesses anti-plasmodial, antioxidant and elastase inhibitory activities. Chemical composition and pharmacological potential of hydro distillate from *Thuja orientalis* are reported in this study. Fresh fruits were subjected to conventional hydrodistillation. Antioxidant activity was assessed as free radical scavenging capacity (RSC) towards 2, 2-diphenyl-1-picrylhydrazil (DPPH) radicals and antibacterial activity was evaluated against six test bacteria by agar well diffusion method. Qualitative and Quantitative analysis of *Thuja orientalis* hydrodistillate by gas chromatography coupled with mass spectrometry revealed the presence of nineteen constituents, representing 94.6% of the total oil. The major constituents of oil were alpha-pinene (83%), sabinene (2.6%), delta-3-carene (2.5%). The oil showed appreciable antibacterial effect against all Gram-positive and Gram negative bacteria tested with MIC values between 12.8-25.6 mg/ml. Therefore this oil could be used in the formulation of antimicrobial and antioxidant agents.

Keywords: Thuja orientalis, essential oil, antimicrobial, antioxidant, chemical composition.

INTRODUCTION

Thuja orientalis (family: Cupressaceae) is an evergreen species, which grows naturally in china, korea, japan and iran. Also this species is widely cultivated as a common ornamental plant [1]. Essential oils derived from many aromatic plants are well known to possess cytotoxic, antioxidant [2], antifungal, insecticidal [3] and antimicrobial activities [4].

Previous phytochemical investigations of this plant resulted in the isolation of many chemical constituents such as flavoniods [5] [6], terpenes [7],[9] and phenolics [10]. Its crude extracts have exhibited anti-plasmodial activity [11]. Several biflavonoids and flavonoid glycosides have been isolated from the fruits of *Thuja orientalis*. Also their estimation of antioxidant and elastase inhibitory activities is reported [12]. Antifungal potential of *Thuja orientalis* is reported [13]. Phytochemical studies of its essential oil showed the nineteen and twenty-eight constituents from the fruit and leaf oil respectively. Alpha-pinene (52.4%), delta-3-carene (14.2%), alpha-cedrol(6.5%) and beta-phellandrene were major components in fruit oil, while alpha-pinene (21.9%), alpha-cedrol (20.3%), delta-3-carene(10.5%) and limonene(7.2%) were the main constituents in the fruit oil [14]. Antifungal activity of leaf essential oil isolated from *Thuja orientalis* against Alternaria alterata is known [15]. Therefore the aim of this study was phytochemical analysis, antioxidant activity and antimicrobial activity of this essential oil against different microorganisms.

MATERIALS AND METHODS

Plant material: The *Thuja orientalis* plant material was collected from University of Kashmir, Srinagar. The plant was properly identified and the voucher specimen of *Thuja orientalis* bearing specimen no.1910 was deposited at herbarium in Centre of plant Taxonomy, University of Kashmir, Srinagar, J & K, India.

Essential oil isolation: The essential oil of the fresh fruits of *Thuja orientalis* was obtained by hydrodistillation using a clevenger-type apparatus for three hours. The oil sample was dried over

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anhydrous sodium sulphate and kept in glass vials at – 4°c prior to analysis.

Chemical composition:

GC–MS analysis: GC–MS analysis was carried on a Varian Gas Chromatograph series 3800 fitted with a VF-5 ms fused silica capillary column (60 m × 0.25 mm, film thickness 0.25 µm) coupled with a 4000 series mass detector under the following conditions: injection volume 0.5 µl with split ratio 1:60, helium as carrier gas at 1.0 ml/min constant flow mode, injector temperature 230 ^{0}C, oven temperature was programmed from 60 to 280 ^{0}C at 3 ^{0}C/min. Mass spectra: electron impact (EI+) mode, 70 eV and ion source temperature 250 ^{0}C. Mass spectra were recorded over 50–500 a.m.u range.

Antimicrobial assay

Microbial strains and culture media: The antibacterial activity of the essential oil of *Thuja orientalis* were tested against a panel of six bacterial strains obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The bacterial strains used were Bacillus subtilis (MTCC-441), P. aeruginosa (MTCC-1688), S. aureus (MTCC 96), K. pneumonia (MTCC-19), E. coli (MTCC-443) and P. vulgaris (MTCC-1771). Bacterial strains were grown on nutrient agar plates at 37 ^{0}C and maintained on nutrient agar slants. Cell suspension of microorganisms in 0.9% NaCl was adjusted at 0.5 McFarland to obtain approximately 10^6 cfu/ml.
Antimicrobial activity and determination of minimum inhibitory concentration (MIC): The antibacterial susceptibility tests were carried out using the agar well diffusion assay/microdilution assay with some modification. First Muller Hinton medium was prepared and 0.5% of tween-20 was dissolved per 100 ml of agar medium in order to facilitate proper diffusion of agar in the oil. 20 ml aliquot was transferred in to each boiling tube. After this sterilization of boiling tubes was carried out in autoclave. Temperature of tubes was regulated upto 38^{0}C and oil samples were added in the concentration range of 0.2-25.6 mg/ml.

The contents of the tube were transferred into plates which were kept under laminar flow and allowed to dry for 30 minutes. Finally bacteria were inoculated from fresh cultures into the broth and its turbidity was adjusted in the range of .08-0.13 at 625nm. Later 3µl of inoculums of each bacteria was added into the plates. Streptomycin sulphate (1000mg/l) was used as positive control for bacteria. The MIC of oil was determined by the microdilution method, recommended by the National Committee for Clinical Laboratory Standards (NCCLS) as described previously [16] The oil was dissolved in dimethyl sulphoxide and added to the medium, and then diluted in order to obtain concentrations in the range of 0.25– 25.6 mg/ml. Inoculum suspension with a final concentration of 10^6 cfu/ml was added to plate. The MIC was defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate any visible growth after incubation at 37 ^{0}C for 24 h.

Antioxidant activity

DPPH free radical-scavenging activity: DPPH free radical scavenging activity was evaluated by measuring the scavenging activity of the essential oil on stable 2,2-diphenyl- 1-picryl hydrazyl radical. A 0.5 mM solution of DPPH in methanol was prepared and a stock solution of oil sample (1 mg/ml) in methanol was prepared. Various concentrations (20–100 µg/ml) were added to 1ml (0.5 mM DPPH) and final volume was made to 3 ml with methanol. The mixture was shaken thoroughly and kept standing at room temperature for 10 min. Then, the absorbance of the mixture was measured at 517 nm on a spectrophotometer. A decrease in the absorbance indicates an increase in DPPH-radical scavenging activity.

The percentage inhibition was calculated by the following equation:
DPPH radical scavenging= [(Ac- As)/Ac)×100]
Where, Ac is the absorbance of the control and As is the absorbance of the sample.
L-ascorbic acid served as positive control.

RESULTS AND DISCUSSIONS

The chemical composition of the essential oil isolated from the fruits of *thuja orientalis* analysed by gas chromatography-mass spectrometry are presented in the **table 1**, given below. The analysis revealed the presence of nineteen constituents representing 96.4% of the total oil. The order of elution of various constituents from RTx-5 cpolumn are shown in **figure1**. The major constituents of the oil were alpha-pinene (83%), sabinene (2.6%), delta-3-carene (2.5%). The percentage yield of oil was found to be .13 % (v/w), as per their fresh weight. The components of oil were classified into monoterpenes, accounting 94.7% and sesquiterpene hydrocarbons accounting 0.8%. Monoterpenes are represented by oxygenated monoterpenes 0.4% while as oxygenated sesquiterpenes are weakly represented by 0.5%.

The in vitro antimicrobial activity of essential oil was qualitatively and quantitatively assessed by the presence or absence of inhibition zones, zone diameters and minimum inhibitory concentration (MIC) values. Results from antimicrobial activity

by agar well diffusion method are presented in **table 2**. The essential oil of *Thuja orientalis* showed significant antibacterial effect against the entire test microorganisms used for screening. This oil was mainly effective against P. vulgaris and E.coli with highest inhibition zones of 24 and 22 mm respectively. Streptomycin sulphate was used as a positive control which showed inhibition zones between 20 -30 mm against different microorganisms tested. Therefore the antibacterial activity of *Thuja orientalis* essential oil seems closer to reference antibiotic. The MIC value of all tested bacteria was found between 12.8-25.6 mg/ml. As can be clearly seen from the photographs **(Figure 2)** that growth of all the six bacteria was observed on the plates between the concentration range of 0.4-12.6 mg/ml. While as no growth of bacteria was observed on the plate at a concentration of 25.6 mg/ml of oil. The previous reports on antimicrobial activity of Thuja orientalis essential oil against different microorganisms support our result [17]. The antimicrobial activity of this oil against K. pneumonaie, P. Aeroginosa and P. Vulgaris is reported first time.

DPPH(1,1-diphenyl-2-picryl hydrazyl free radical) free radical scavenging capacity of the essential oil was measured by DPPH assay under in-vitro conditions. The ability of the examined extract to act as donor for hydrogen atoms in the transformation of DPPH radical into its reduced form DPPH2 was investigated. The examined oil was able to reduce the stable purple coloured DPPH radical into yellow coloured DPPH2. The aforementioned oil showed promising radical scavenging activity at concentration of 100µl/ml. The results are plotted in the form of graph **(Figure.3).** This plant oil exhibited prominent DPPH free radical scavenging activity of 49.8% in comparison to ascorbic acid and α-tocopherol standard which showed the activity of 67.95 and 71.2%, respectively. The DPPH radical scavenging assay is commonly employed in evaluating the ability of antioxidants to scavenge free radicals. This method has been used extensively to predict the antioxidant activity because of the relatively short time required for analysis. The change in absorbance at 517 nm is used as a measure of the scavenging effect of a particular sample for DPPH radicals. The more rapidly the absorbance decreases, the more potent the antioxidant activity of the sample in terms of its hydrogen atom-donating capacity. It should be noted that our study showed major constituent α-Pinene 83.0% instead of 52% reported in literature. Antioxidant potential is also first time reports from the essential oil of *Thuja Orientalis.*

Table1: Chemical composition of the essential oil of *Thuja Orientalis*

S. No	Compound	% Peak Area	Methods of identification
1	**α-Pinene**	**83.0**	**MS, RI, Std**
2	Camphene	0.5	MS, RI
3	**β-Phellandrene**	**1.0**	MS, RI
4	**Tricyclene**	**2.2**	MS, RI, Std
5	Sabinene	2.6	MsS, RI
6	α-Phellandrene	0.2	MS, RI
7	**δ-3-Carene**	**2.5**	MS, RI, Std
8	**α-Terpinene**	**Tr**	MS, RI
9	P-Cymene	Tr	MS, RI
10	Limonene	1.1	MS, RI
11	1,8-cineole	0.2	MS, RI
12	γ-Terpinene	0.1	MS, RI
13	α-Terpinolene	1.5	MS, RI
14	4-Terpineol	0.2	MS, RI
15	β-Fenchol	Tr	MS, RI
16	β-Caryophyllene	0.3	MS, RI
17	α-Caryophyllene	0.4	MS, RI
18	Germacrene D	0.1	MS, RI
19	Cedrol	0.5	
Total (%)		**96.4**	
Monoterpene hydrocarbons		**94.7**	
Oxygenated monoterpenes		**0.4**	
Sesquiterpene hydrocarbons		**0.8**	
Oxygenated sesquiterpenes		**0.5**	

Table 2: In Vitro Anti Microbial Activity of *Thuja Orientalis* Essential Oil and reference antibiotic determined with Agar well Diffusion Method

S.No.	Test bacteria	Zones of inhibition (in mm)	Zone of inhibition of antibiotic (in mm)	MIC (in mg/ml)
Gram-Positive Bacteria				
2	*B.subtilis MTCC 441*	15	30	12.8-25.6
3	*K.pneumoniae MTCC 19*	16	17	12.8-25.6
Gram-Negative Bacteria				
4	*E.coli MTCC 443*	22	20	12.8-25.6
5	*P.aeroginosa MTCC 1688*	19	30	12.8-25.6
6	*P.vulgaris MTCC 426*	24	20	12.8-25.6

FIG: 1 Order of elution of various constituents of analysed essential oil

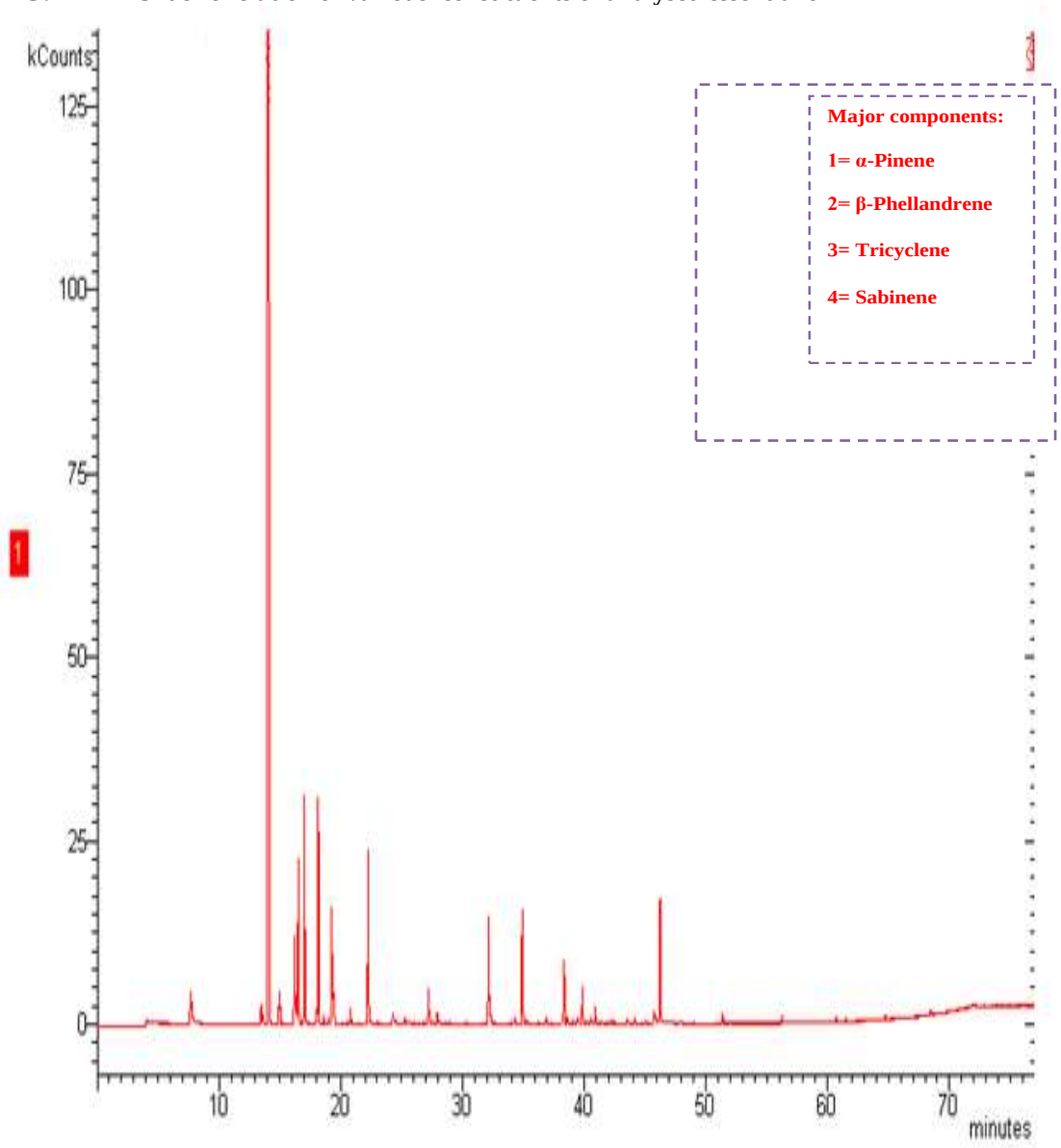


Fig 2 Plates shows antimicrobial activity of Essential oil.

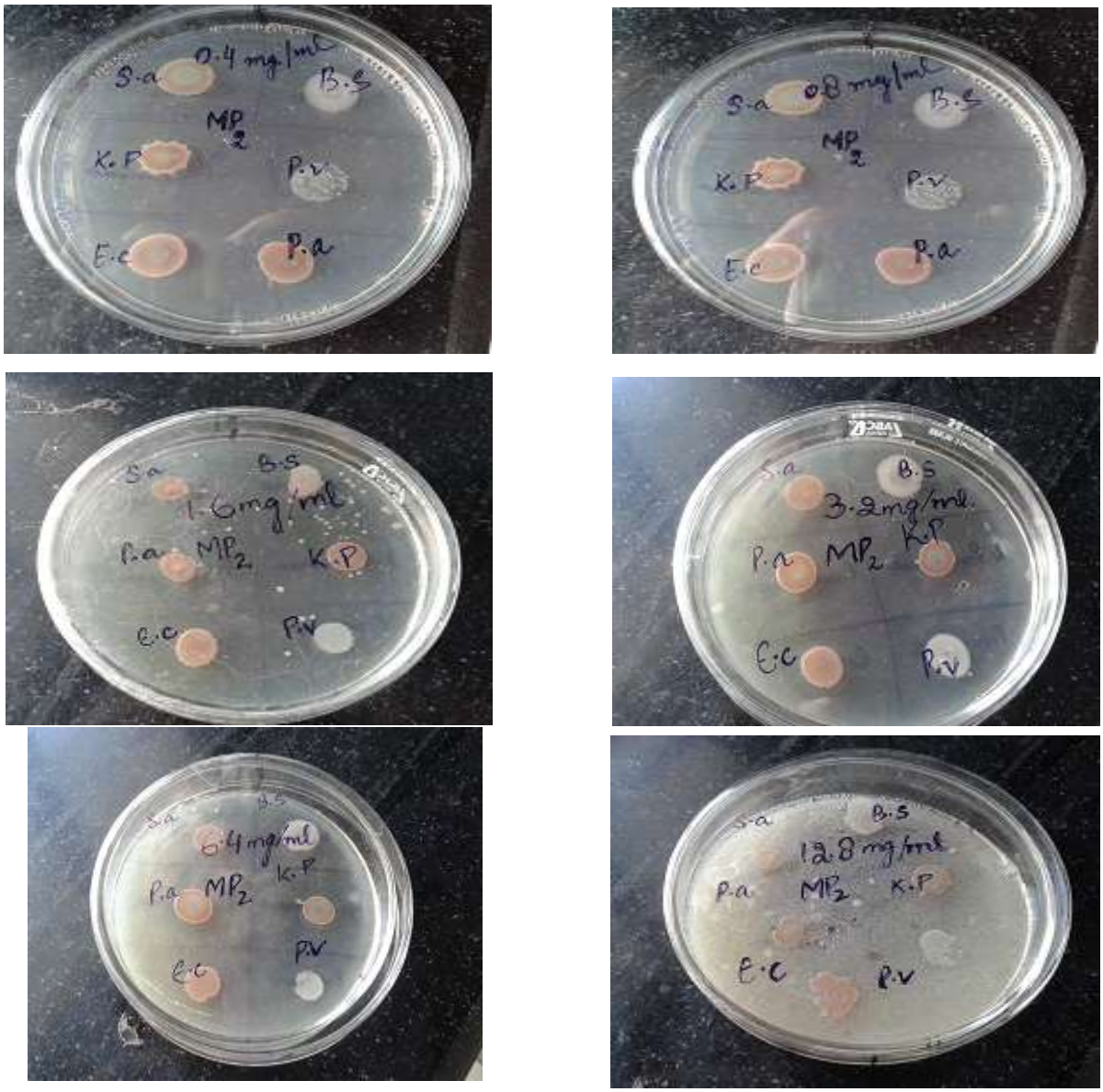
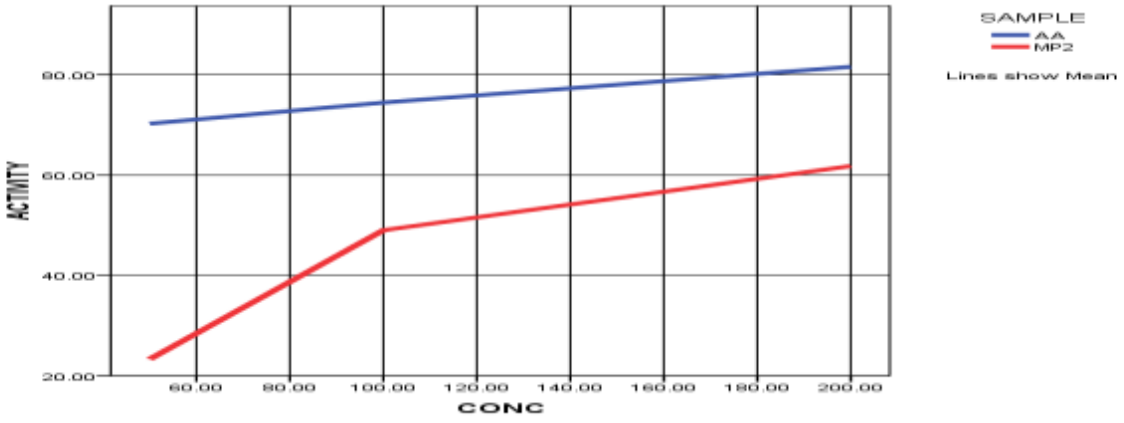


Fig 3 Antioxident potential of essential oil from *Thuja Orientalis*



MP2; Essential oil, AA; Ascorbic acid, conc; concentration (µg/ml).

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