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Research Article

Antibacterial and Phytochemical Investigation of *Thuja orientalis* (L.) Leaves Essential Oil from Syria

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ABSTRACT

Thuja Orientalis (L) (*Platycladus orientalis*) belongs to the family of *Cupressaceae*. This species is an important herb in oriental world medicine as used in treatment of so many diseases. In this work the essential oil content variation and antimicrobial activity have been determined. Fresh leaves of *Th. Orientalis*, grown in the south Syria were subjected to hydro distillation using a Clevenger-type apparatus and the chemical composition of the essential oil have been studied using gas chromatography-mass spectrometry (GC-MS). Mono and sesquiterpenoids in essential oil were reported as chemical constituents of this plant. Twenty-four components (97.59%) were identified. The major components were α-pinene (22.25%), 3-carene (20.65%), cedrol (18.71%), β –Caryophyllene (6.13%), α-Humulene (5.68%), Terpinolene (4.53%), and Limonene (3.35%). The present study led to investigate the antimicrobial activity of the essential oil against some pathogenic microorganisms. The essential oil showed appreciable antibacterial effect against some Gram-positive less than Gram negative bacteria. Minimum inhibitory concentration (MIC) revealed the lowest activity against *Streptococcus pyogens* and *Vibrioparahaemplyticus* (1.6 mg/mL) while the highest activity was against *Bacillus subtilis and Staphylococcus aureus* (0.4 mg/mL). The MBC activity was from (1.6 mg/mL) to (3.2 mg/mL).

Keywords: *Platycladus orientalis, Thuja*, Essential oil, GCMS, Antibacterial.

INTRODUCTION

Medical plants are an important source of natural compounds with biological properties, including antimicrobial effects, Due to the occurrence of resistance to antimicrobials and the incidence of infectious diseases, there is a need to search for new sources of antimicrobial compounds that may inhibit microorganisms by different mechanisms than those in current use[1,2]. Aromatic plants are considered of great interest for their flavors and for their medicinal properties, along with human consumption, and ornamental uses; thus, they are especially suitable for multifunctional sustainable crop models[3]. For centuries, aromatic herbs and spices have been added to different foods to improve the flavor and organoleptic properties. The use of aromatic plants in phytotherapy is mostly related to different activities of their essential oils, such as antimicrobial, spasmolytic, carminative, hepato protective, antiviral, anticarcinogenic activities[4,5], etc. Furthermore, many studies are interested antioxidant activities of aromatic plants and their essential oils[6]. Antioxidant activities are also confirmed for most of the phenolic compounds present in different herbs[6,7]. *Th. Orientalis* (L.) is being used as herbal medicine from ancient times and categorized as one of the fundamental herbs in traditional medicine where it is mainly used in treating conditions such as antitussive, expectorant, anti-inflammatory, antibacterial, antifungal, antioxidant activity, and used in homeopathy both internally and externally[8-10]. *Thuja* is a distinct genus of coniferous tree in the *cypress* family *Cupressaceae*, containing only one species, *Th. Orientalis*, also known as *Oriental arborvitae*. The plant is indigenous of Korea, Japan, north of China and Iran, commonly known as arborvitae or tree of life. It is an evergreen, monoecious trees or shrubs growing to 10-30 m tall. The leaves are arranged in flattened fan shaped growing with re sine glands[11]. It introduced in Syria from Northeast Asia. Also, this species is widely cultivated as a common ornamental plant in Syria and other countries. In this work, the composition of the essential oil isolated by hydro distillation from the fresh leaves of *Th. Orientalis*, grown in the south Syria is reported along with the antibacterial activity of its bioactive compounds against of some human bacteria.

MATERIALS AND METHODS

Aerial parts of *Th. Orientalis* used in this study were collected early in the morning in June 2015 from plants growing in the Garden of faculty of science in Damascus University, and authenticated by the taxonomist of Department of Botany. The dirt was removed with tap water. The aerial parts were cut to smaller pieces by scissors to increase the efficiency of the extraction and thereby increasing the yield to be obtained.

Essential oil extraction

The fresh leaves (200 g) were subjected to hydro distillation for sex hours using a Clevenger-type apparatus, ac

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Table 1: Quantities (area %) of components of essential oil fresh leaves *Th. Orintalis*

S. NO.	RI	R. I[A]	Compounds	Area (%)
			Monoterpene Hydrocarbons	56.28
1	935	932	α -Pinene	22.25
2	949	946	Camphene	1.36
3	971	969	Sabinene	0.55
4	976	974	β -Pinene	0.90
5	993	988	β -Myrcene	2.18
6	1014	1008	3-Carene	20.65
7	1022	1020	P-Cymene	0.51
8	1026	1024	Limonene	3.35
9	1090	1086	Terpinolene	4.53
			Oxygenated Monoterpenes	4.14
10	1175	1174	Terpinen-4-ol	0.61
11	1283	1284	Bornyl acetate	0.78
12	1347	1346	α-Terpinyl acetate	2.75
			Sesquiterpene hydrocarbons	16.09
13	1388	1389	β -Elemen	0.39
14	1411	1410	α-Cedrene	0.82
15	1418	1417	β -Caryophyllene	6.13
16	1454	1455	α-Humulene	5.68
17	1476	1478	γ-Muurolene	0.20
18	1479	1484	Germacrene D	1.62
19	1511	1513	γ -Cadinene	0.41
20	1521	1522	δ -Cadinene	0.84
			Oxygenated Sesquiterpenes	21.08
21	1576	1577	Spathulenol	1.11
22	1581	1582	Caryophyllene oxide	0.60
23	1602	1600	Cedrol	18.71
24	1653	1652	α-Cadinol	0.66
		Total		97.59

RI: retention index (Kovalts) relative to n-alkanes (C8–C20) on a non-polar DB-5 column.
RI[A]: retention index in the literature on a non-polar DB-5 column (Adams, 2007).

cording to the literature[12]. The sample was added to distilled deionizer water (1L) in a 2 L around bottomed flask and heated to boiling, after which the essential oil was evaporated together with water vapor and finally collected in a condenser. The resulting product was then dried over anhydrous Na_2SO_4. Pale yellow colored essential oil was obtained in the yield of (0.27% v/w). The oil was then kept in a sealed dark glass vial at 4°C until required.

Qualitative and quantitative analysis

GC/MS analyses

Qualitative analysis was performed using an Agilent 6890 N gas chromatograph (GC) equipped with Agilent 5973 mass selective detector (MSD), Agilent Auto sampler 7683 and Agilent DB-5MS capillary column (30 m, 0.25 i.d., 0.25 µm film thickness) (Agilent Technologies, Santa Clara, CA, USA). The MS detector was operated in electron impact (EI) mode at 70 eV with interface temperature of 280°C; the scan range was 50–550 amu. The injection port temperature was set at 250°C. GC was performed in split less mode; carrier gas was helium at a constant flow rate of 1 mL/min. The column temperature was programmed as follows: an initial temperature of 60°C increased to 280°C at rate of 3°C/min. The injection volume was 1.0 µL.

Identification of Components

Relative percentage amounts were calculated from peaks total area using apparatus software. The identification of individual compounds was based on comparison of their mass spectra with those obtained from the NIST/NBS, Wiley Libraries spectra, stored in the GC-MS database. Further confirmation was done from Retention Index data generated from a series of alkane's retention indices (relatives to C8 - C20 on the DB-5MS column), and (Adams 2007).

Antibacterial Assay

Bacterial cultures collection

The organisms used in this study were obtained from the Medical Microbiology and Parasitology Department, Faculty of Medicine, Damascus University. The cultures include; four gram-positive bacteria (*Bacellus subitus, Staphylococcus aureus, Streptococcus pyogens, Micrococus luteus*) and four gram-negative bacteria (*Salmonella typhimurium, Klebsellia pneumonia, Proteus vulgaris, Vibrio parahaemplyticus*). The bacteria cultures were maintained on nutrient agar slant at 37°C for 24 h. The organisms were preserved at 4°C. The essential oil was tested for antibacterial activity by the agar disc diffusion method[13]. The microorganisms were grown overnight at 37°C in 20 mL of Mueller-Hinton broth. The cultures were adjusted with sterile saline solution to obtain turbidity comparable to that of McFarland no. 5 standard (1.0×10^8) CFU/mL. Petri dishes containing sterilized Mueller-Hinton agar were inoculated with these microbial suspensions. Sterile Whatman No.1 (6 mm) discs papers were loaded with 20 µL of the essential oil and placed on the inoculated plates and, after staying at 4°C for 2 h, the plates were incubated at 37°C for 24 h. Antimicrobial activities studied with four concentrations 25%, 50%, 75% and100% of the essential oil respectively. After 24 h incubation at 37°C, the inhibition zone diameters were measured, and expressed by mm. Negative control was prepared using DMSO solvent, and amoxicillin as positive control. Each assay in this experiment was replicated three times.

Minimum inhibitory concentration and minimum bactericidal concentration

The MIC of essential oil was tested by the method[14,15]. The essential oil was incorporated into nutrient broth medium in a tube and adjusted to a final concentration in the range of 0.2 to 6.2 mg/mL. 20µL of standardized suspension of each test organism (10^8 CFU/mL) was transferred to the tubes, and incubated at 37°C for 24 h. The MIC was defined as the lowest concentration of the essential oil at which the bacteria does not show visible growth. The microorganism growth was indicated by turbidity. To determine MBC, broth was taken from the MIC, where the organisms quantitatively indicate the minimum concentration when no viable organism appears in the culture and inoculated in Mueller Hinton agar for 24 h at 37°C. The MBC is defined as the lowest concentration of the essential

Table 2: Antibacterial activity of the essential oils (diameter of Zone of inhibition in mm) of Th. Orintalis fresh leaves

Test Organisms	Different concentrations (percentages) of essential oil				amoxicillin
gram-positive bacteria					
Bacillus subtilis	10.9 ± 0.16	15.12 ± 0.13	30.14 ± 0.14	39.4± 0.11	40 ± 0.11
Micrococus luteus	NA	NA	NA	NA	17 ± 0.21
Streptococcus pyogens	15.4 ± 0.12	18.9 ± 0.16	22.9 ± 0.18	33.9 ± 0.11	NA
Staphylococcus aureus	22.21 ±0.15	25.11 ± 0.11	35.34 ± 1.12	40.12 ± 1.14	45± 0.13
gram-negative bacteria					
Klebsiella pneumoniae	NA	NA	NA	NA	NA
Vibrio parahaemplyticus	23.17 ± 0.14	30.14± 0.17	30.14± 0.17	42.04 ± 0.18	30± 0.32
Proteus vulgaris	NA	NA	NA	NA	45± 0.12
Salmonella Typhimurium	NA	NA	NA	NA	16± 0.31

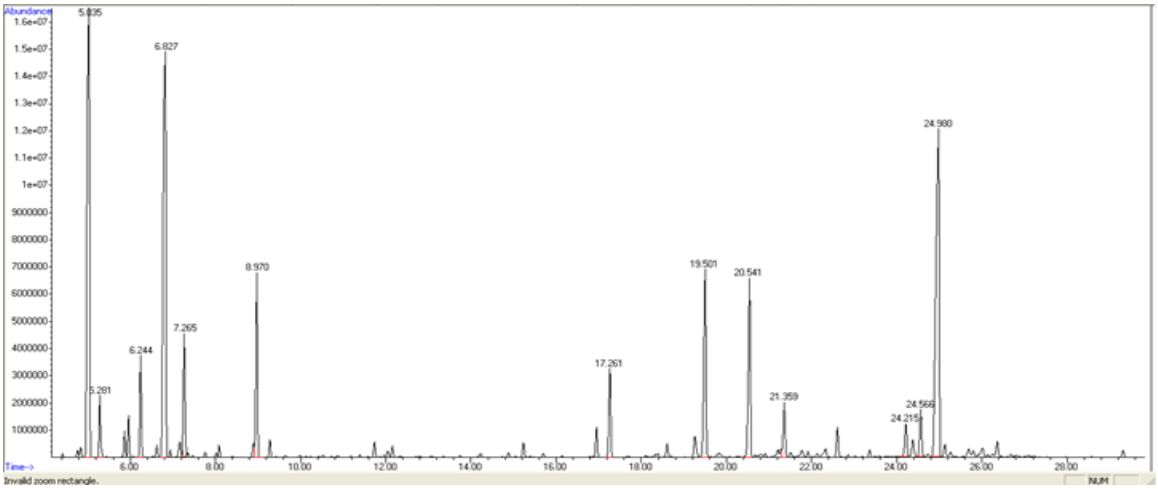

Figure 1: GC/MS Chromatogram of essential oil Th. Orientalis leaves

Table 3: (MIC) and (MBC) of the Th. Orientalis essential oil (Values in mg/mL).

Microorganism	MIC	MBC
gram-positive bacteria		
Bacillus subtilis	0.4	3.2
Micrococus luteus	NA	NA
Streptococcus pyogens	1.6	1.6
Staphylococcus aureus	0.4	1.6
gram-negative bacteria		
Klebsiella pneumoniae	NA	NA
Vibrio parahaemplyticus	1.6	3.2
Proteus vulgaris	NA	NA
Salmonella Typhimurium	NA	NA

oil at which inoculated bacteria was totally killed. DMSO solution served negative control.

Statistical Analysis

All determinations in this article were carried out in triplicates and SPSS Statistics 19.0 Software was used to evaluate one-way analysis of variance (ANOVA) at $p \leq 0.05$. Canonical Discriminate Analysis was also used to establish differences between samples, and to evaluate the importance of different variables on discrimination.

RESULTS AND DISCUSSIONS

The hydro distillation of the Fresh Leaves *Th. Orientalis* gave a yellow essential oil color. The percentage yield and specific gravity was 0.27% (v/w) and 0.93 respectively. The essential oil was soluble in ethanol, methanol and tween 80 solution. In this essential oil, 24 components were identified, this amount to 97.59% of the total essential oil composition and represent four different groups of hydrocarbon namely; monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and Oxygenated Sesquiterpenes. The monoterpene hydrocarbons constituted the most dominant chemical group (56.28%) and among which α–Pinene (22.25%) is most predominant followed by 3-Carene (20.65), Terpinolene (4.53), Limonene (3.53), β-myrcene (2.18), Camphene (1.36). The oxygenated monoterpenes (4.14%) is α-Terpinyl acetate (2.75). The sesquiterpenes hydrocarbons (16.09%) include β –Caryophyllene (6.13), α-humulene (5.68), Germacrene D (1.62). Cedrol (18.71) and Spathulenol (1.11) were the major components Oxygenated Sesquiterpenes and account for 21.08% of the essential oil composition. (Table 1) shows the constituents identified percentage composition and (fig 1) shows the GC/MS Chromatogram. The composition of the essential oil showed some similarities with the previous studies, but with differences in their percentage depending distinctly on the region in which they are grown. An earlier report from China[16] reported that the yield of leaf oil of *Th. orientalis* from China was 0.3% and had 38 constituents. The major components were α-pinene (4.3%), Δ-carene (6.1%) and cedrol (22.3%). In Vietnam[17] Monoterpenes (55.1%) and sesquiterpenes

(39.8%) are classes of compounds identified in the 69 constituents of the oil of *Th. Orientalis*. The main compounds were α-pinene (34.1%) and α-cedrol (16.5%), β-caryophyllene (5.4%), β-selinene (5.2%), limonene (4.9%) and α-terpinene (3.7%). In Nigeria[18] The main constituents of the leaf essential oil are α-pinene (15.92%), α-caryophyllene (10.42%), Trans-β- ocimene (8.71%), limonene (8.25%) and patchoulane (7.46%). Other compounds found are Fenchene (4.90%), Aromandendrene (3.73%), Cedrol (2.64%), Germacrene D (2.43%) and β-myrcene (2.28%). In India in the North-Western Himalaya[19] Twenty-two compounds representing 94.0% of the total essential oil was identified. The leaf essential oil contained α-pinene (29.2%), δ -3-carene (20.1%), α-cedrol (9.8%), caryophyllene (7.5%), α-humulene (5.6%), limonene (5.4%), α-terpinolene (3.8%) and α-terpinyl acetate (3.5%) as major constituents. In Iran[20] S. Hashemi and S. Safavi, reported the main components of leaves oils were α-pinene (35.2%), α-cedrol (14.6%) and δ -3-carene (6.3%), Limonene (6.1%), β-Caryophyllene (5.8%). In the another study in Iran[21] B. Nickavar and his group reported the leaf essential oil contained α-pinene (21.9%), α-cedrol (20.3%), δ -3-carene (10.5%) and limonen (7.2%) as the main components. In Pakistan[22] the major components were α -pinene (40.6 %), β -caryophyllene (6.8%), cedrol (10.7 %), alloaromadendrene (7.8 %), and β -myrcene (3.7 %) and R-+-limonene (3.2 %). Lastly In Egypt[23] the main components were α-pinene (21.83%), β- pinene (6.71%), β-caryophyllene (12.07), α-cedrol (6.86%), β- selinene (6.15%), Limonine (5.49%). In comparison with published data, the essential oils of *Th. Orientalis* could be classified as follows: group characterized by a predominant α-pinene, δ -3-carene and cedrol [this study], group dominated by cedrol, δ -3-carene and β-caryophyllene, group with contents of cedrol, β-caryophyllene and α-caryophyllene, and group whith α-pinene, δ -3- carene and sabinene. The in vitro antimicrobial activity of *Th. Orientalis* essential oil against the microorganisms and its potentials activity were qualitatively and quantitatively assessed by microdilution methodology. The inhibition zones and disc diameters of the essential oil against the tested microorganisms are shown in (Table 2). The results which were obtained from this method for the essential oil revealed that the volatile oil have the highest activity against the *Vibrio parahaemplyticus* (42.04 ± 0.18) (gram negative), and *Staphylococcus aureus* (40.12 ± 1.14), *Bacillus subtilis* (39.4± 0.11) *and Streptococcus pyogens* (33.9 ± 0.11) (gram positive). while it showed no activity against the *Klebsiella pneumonia*, *Proteus vulgaris* and *Salmonella Typhimurium* (gram negative) and *Micrococus luteus* (gram positive). The results of the MIC and MBC tested are shown in (Table 3) which is indicated that the E. oil has varying degrees of growth inhibition against the bacterial strains (i.e. MIC values varies between: 0.4 - 1.6 mg/mL and MBC values varies between: 1.6 - 3.2 mg/mL. The Gram-positive strains showed more susceptibility to the tested essential oil than the Gram-negative. On the other hand, no activity was registered against *Klebsiella pneumonia*, *Proteus vulgaris* and *Salmonella Typhimurium* (gram negative) a n d *Micrococus luteus* (gram positive). The previous reports on antimicrobial activity of *Th. Orientalis* essential oil against different microorganisms support our result[24,25].

CONCLUSION

The present study is demonstrated that the essential oil of *Th. Orientals* leaves has significant inhibitory effects against some gram-positive and gram-negative bacteria, which are associated with clinical diseases. The study therefore not only reveals the plant excellent natural antibacterial to be utilized nutritionally and pharmaceutically, but also provides good scientific justification for increased in traditional use of the plant. The percentage of α-pinene, 3-Carene and α-cedrol which exist inside the oil was calculated using GC-MS analysis. These percentage shows that the oil of *Th. Orientals* possesses a potential for use in the many medical applications. As result, *Th. Orientals* plant is known as (The tree of life), and more additional studies are recommended to explore deeply.

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