13- GC/MS ANALYSIS AND BIOCHEMICAL STUDIES OF THE ESSENTIAL OIL OF *THUJA ORIENTALIS* L. GROWING IN EGYPT.

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Abstract

The hydrodistilled essential oils of the fresh fruits and leaves of Thuja orientalis L. Syn. Biota orientalis (L.) Endl (Platycladus orientalis L.) Family: Cupressaceae were subjected to GC and GC/MS analysis. Twenty-four and twenty-one compounds had been identified in the essential oils of fruits and leaves respectively. The major components were identified as α – pinene (33.05%), α- phellandrene (10.39%), α–terpinene (7.73%) and camphene (5.47%) in fruit oil, while α - pinene (21.83%), benzyl benzoate (19.12%), caryophyllene (12.07%) and α - cedrol (6.86%) were detected in leaf oil. Both oils were tested for their diuretic and antioxidant activities.

INTRODUCTION

Thuja orientalis L. Family: Cupressaceae is indigenous to temperate regions of Asia and America and grows wildly in parts of western Himalayas [(1)]. It is highly aromatic and resinous shrub that widely cultivated in gardens located in temperate and semi-temperate areas [(2)].

Thuja orientalis L. was cultivated in Egypt as an ornamental plant. *Thuja* was an old remedy for delayed menstruation also it is a stimulant to smooth muscles such as those of uterus and bronchial passages so it is used for treatment of bronchitis [(3)] and also as cough suppressant in traditional Chinese medicine [(4)]. Externally it is used as a wash for infectious skin disease such as impetigo and scabies [(5)].Previous work on *Thuja orientalis* L. has focused on the terpenoid and flavonoid composition [(6-10)].

A number of investigators from different countries have examined the chemical composition of the essential oil of *Thuja orientalis* L. [(11-14)]. The present study was conducted to determine the volatile components of oils of both leaves and fruits of this species growing in Egypt by using modern chromatographic techniques and screening for their diuretic and antioxidant activities.

EXPERIMENTAL

I. Material:

a. Plant Material: Leaves and fruits of *Thuja orientalis* L. Family: Cupresseaceae were collected from the shrubs cultivated in Helwan University Gardens, Cairo, Egypt in April-September 2003.Identification of the plant was confirmed by Dr. Wafaa M. Amer, Ass. Prof. of Botany, Botany Dept., Faculty of Science, Cairo University, Giza, Egypt. Voucher specimens are kept in herbarium, Pharmacognosy Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt.

b. Material for pharmacological studies:

- Animals; Male Albino Sprague Dawely rats of weights ranging from 170-200 g from the animal house colony of the Nile Company for Pharmaceutical Industries, Cairo, Egypt. They were kept on standard laboratory diet having the following composition: 1% vitamin mixture, 4% mineral mixture, 10% corn oil, 20% sucrose, 10.5% casein pure (95%), 0.2% cellulose, 54.3% starch, and water, was provided *ad lib.*
- Moduretic tablets; El-Kahira Company for Pharmaceutical Industries, Cairo, Egypt. Each tablet contains combination of the two diuretic drugs amiloride hydrochloride 5 mg and hydrochlorothiazide 50 mg.
- Alloxan monohydrate ; Sigma company For Pharmaceuticals (USA).
- Standard glutathione (GSH) and 5,5-dithiobis-(2 nitrobenzoic acid); Sigma company For Pharmaceuticals (USA).
- Vitamin-E(dl-α-tocopheryl acetate); Pharco Pharmaceutical Company. It is available in the form of soft gelatinous capsules each contains 400 mg vitamin-E.

II. Methods:

a. Preparation of the oil: The oils were separately prepared by hydro-distillation of fresh leaves and fruits. In each case the oil was dried over anhydrous sodium sulfate and kept refrigerated until analysis. Percentage yield was determined according to Egyptian pharmacopoeia 1984 [(15)].

b. *GC/MS*: GC/MS analysis was performed with SHIMDZU GC/MS – QP5050 A system. Column DBI, 30m, 0.53mm ID, 1.5 µm film (J&W Scientific); Helium was used as carrier gas at flow rate of 2 ml/min.; injector temperature 250^0C, detector temperature 280^0C, oven temperature program; 40^0 (1 min), 150^0 (1 min) at 7.5^0C/min, 250^0C (2 min) at 1.2^0C, ionization mode EI, ion source 70 eV.

c. Identification of components; The constituents of the oils were identified by comparing their retention times, mass fragmentation patterns with those data of the available reference samples, GC/MS spectral database and with published data [16]. The percent composition of components in each oil was determined by computerized peak area measurements using internal normalization method.

d. Determination of LD_{50} of the oils; The LD_{50} of the oils were determined using Karber method [17]. This study is a trial to assess *in vivo* their acute toxicity and determination of the safe therapeutic doses of the oils administered orally by gavage in rats.

e. Testing for diuretic activity; four groups, six rats each, were used. The animals were housed in metabolic cages and fasted for 18 hours before the experiment. The first group was considered as normal control, the second group was orally administered Moduretic (4.5mg/kg. b. wt.) as a positive control (standard) and each of the remaining groups administered orally 500mg/kg.b.wt. of one of the essential oils of the fruits and leaves as emulsion using few drops of Tween-80. Immediately after treatment, each animal was housed in a metabolic box. 24 Hours latter, urine was collected, the volume of the urine was measured, sodium and potassium levels were estimated spectro-photometrically and compared with those of the control group[18]. Results were evaluated and analyzed using Students't' test according to Sendecor and Cocheran [19].

f. In vivo testing for antioxidant activity; The antioxidant effect of the essential oils of the fruits and leaves of *Thuja orientalis* L. (at dose level 500 mg/kg.b.wt. administered orally as emulsion using few drops of Tween-80) was done by determination of glutathione in blood of alloxan-diabetic rats using Bulter *et al.* method[20] and using vitamin E (35mg/kg.b.wt.) as a positive control.

RESULTS AND DISCUSSION

The fresh fruits and leaves of *Thuja orientalis* L. yielded 2.2% and 1.2% v/w essential oils, respectively. Qualitative and quantitative variations in the components of the two oils are complied in Table (I).

GC/MS analysis of the fruit oil of *Thuja orientalis* L revealed the presence of oxygen containing compounds 9.7% and hydrocarbons 88.21%. The hydrocarbons were determined as 75.37% monoterpenes and 12.82% sesquiterpenes. The major components were identified as α – pinene (33.05%),α-phellandrene (10.39%), α – terpinene (7.73%) and camphene (5.47%). In leaf oil the amount of oxygen containing compounds was 29.85% while, the hydrocarbons were represented by 44.74% monoterpenes and 24.35 sesquiterpenes. α - pinene (21.83%), benzyl benzoate (19.12%), caryophyllene (12.07%) and α - cedrol (6.86%) were found to be the major components of the leaf oil.

The LD_{50} of the fruit and leaf oils were 5 and 4.5 g/kg.b.wt. for rats respectively.

The essential oils of the fruits and leaves were tested for their diuretic activity Table (II) and Figures (I&II). The oils revealed a remarkable increase in urinary excretion as well as a significant rise in sodium excretion compared with control group without inducing hypokalemia at dose level (500mg/kg.b.wt.), so *Thuja* oils are considered as ideal diuretic which promotes sodium excretion and reduces the body water contents without causing sever hypokalemia, which plays an important role in neuromuscular activity [21].

The oils of both fruits and leaves were also tested for their antioxidant activity by measuring the blood glutathione levels in the blood of alloxan-diabetic rats after oral administration of 500 mg/kg.b.wt. of each oh the tested oils Table (III), Figure (III).

The reduced levels of glutathione in alloxan-diabetic rats were greatly restored by the tested essential oils. They showed a comparable activity to that of vitamin E so, they are both considered as antioxidants.

Table (I): Essential oil constituents of *Thuja orientalis* (*Platycladus orientalis*) fruits and leaves:

No.	Compound	RT	Base Peak	M^+	% Fruits	% Leaves
1	α- pinene	11.48	93	136	33.05	21.83
2	Sabinene	12.42	93	136	11.71	0.08
3	Camphene	12.51	93	136	5.47	-----
4	β- pinene	12.73	93	136	1.45	6.71
5	α-phellandrene	12.92	93	136	10.39	2.60
6	Δ^3- carene	13.08	93	136	-----	0.93
7	β- myrecene	13.24	93	136	2.17	-----
8	Limonine	13.49	93	136	-----	5.49
9	*Cis*- ocimene	14.10	93	136	2.20	-----
10	2- carene	14.31	93	136	0.20	-----
11	α- terpinene	14.70	93	136	7.73	-----
12	γ- terpinene	15.44	93	136	0.49	-----
13	Terpinolene	15.85	93	136	0.53	3.11
14	Myrtenol	16.31	79	152	-----	0.98
15	Linalyl propionate	16.50	59	136	0.25	3.11
16	4- terpineol	16.74	71	154	2.17	-----
17	*Trans*-2,4-Decadienol	16.96	79	----	0.94	0.21
18	α- terpineol	17.05	59	154	0.79	0.46
19	Bornyl acetate	19.32	95	196	3.71	1.05
20	α-terpenyl acetate	21.75	43	196	1.84	1.46
21	(-)-β- elemene	23.55	81	204	0.72	0.72
22	β-caryophyllene	25.72	41	204	7.31	12.07
23	Widdrene	26.19	119	204	0.68	3.75
24	*Cis*-β-farnsene	26.29	69	204	0.37	-----
25	β- selinene	27.25	93	204	2.86	6.15
26	Zingibrene	27.55	93	204	0.58	-----
27	Germacene-B	27.74	93	204	0.30	0.39
28	α-longipinene	28.10	119	204	----	1.46
29	Calarene	30.29	41	204	----	0.38
30	α-cedrol	35.82	95	222	----	6.86
31	Benzyl benzoate	45.36	105	212	----	19.12
	Identified compounds:				97.91	98.95
	Unidentified compounds:				2.09	1.05
	Total hydrocarbons:				88.21	69.09
	- Monoterpenes:				75.37	44.74
	- Sesquiterpenes:				12.82	24.35
	Total oxygenated compounds :				9.70	29.85

Table (II): Diuretic activity of the essential oils of *Thuja orientalis* L. (*Platycladus orientalis*) fruits and leaves at dose level 500mg/kg.b.wt.:

Sample	Urine volume	Na+ ppm	K+ ppm
Normal control	7.3±0.3	273.5±10.32	543.5±25.36
Standard	17.8±0.8**	550±16.3**	778.5±32.3**
Fruits oil	15.3±0.8**	377.6±16.2**	422.5±28.5
Leaves oil	14.2±0.9**	426.3±21.5**	468.3±30.8

Table (III): Antioxidant activity of the essential oils of *Thuja orientalis* L. (*Platycladus orientalis*) fruits and leaves at dose level 500 mg/kg.b.wt.:

Group	Blood glutathione (mg%)	% change from control
Control	37.4±1.8	------
Diabetic (non- treated)	27.3±0.9*	27
Vitamin-E	36.8±0.7*	1.6
Fruits oil	36.4±1.1	2.6
Leaves oil	36.2±1.3	3.2

Figure (I): Diuretic activity of the essential oils of *Thuja orientalis* L. (*Platycladus orientalis*) fruits and leaves:

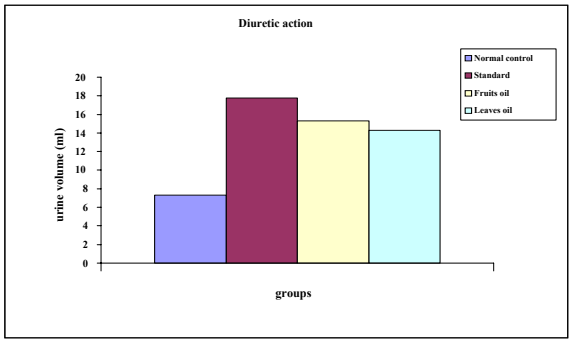


Figure (II): Sodium and Potassium levels in the urine of rats treated with of the essential oils of *Thuja orientalis* L. (*Platycladus orientalis*) fruits and leaves:

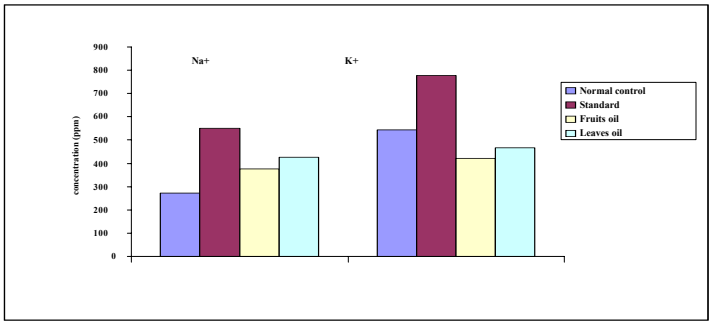
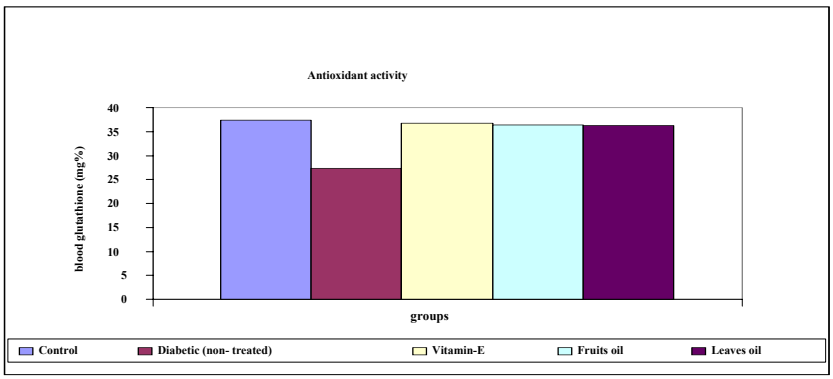


Figure (III): Effect of the essential oils of *Thuja orientalis* L. on the levels of blood glutathione in alloxan-diabetic rats at a dose level 500 mg/kg.b.wt.:



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تحليل الزيوت الطيارة لنبات ثوجيا أورينتالس الـ رع في مصر بواسطة كروماتوجرافيا الغاز ومطياف الكتلة ودراسة تأثيرها الحيوي

السادة الدكاترة

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*** قسم العقاقير و**قسم الكيمياء الحيوي –كلية الصيدلة (بنات)- جامعة الأزهر**

لقد تم في هذا البحث اجراء دراسة علي الزيوت الطيارة لكل من الثمار و الاوراق لنبات ثوجيا اورينتالس الـ رع في مصـر و ذلك لاهميته البيولوجية سعيا لمعرفة مكونات الزيوت كل علي حدة و معرفة المكونات المشتركة بينهم .

و قد تم تحضير الزيوت الطيارة للنبات محل الدراسة بطريقة التقطير المائي لكل من الثمار و الاوراق كل علي حدة بعد جمعهمـا مباشرة . و قد اسفرت عملية التقطير عن تقدير نسبة الزيوت الطيارة للثمار بنسبة ٢,٢% و للاوراق بنسبة ١,٢% .

و باستخدام كروماتوجرافيا الغاز المتصل بمطياف الكتلة تم التعرف علي ٢٤ مركبا في زيت الثمار تمثل ٩٧,٩% و ٢١ مركبـا في زيت الاوراق وهي تمثل ٩٨,٩٥% من الزيت و قد وجد ان المركبات ذات النسبة الاعلي في الثمار هي الفابينين ٣٣,٠٥% و الفـا فلاندرين ١٠,٣٩% و الفاتيربينين٧,٧٣% اما في الاوراق فقد وجد ان المركبات ذات النسبة السائدة هـي الفـابينين ٢١,٨٣% و بـ يل بـ وات ١٩,١٢% و كاريوفيللين ١٢,٠٧% .

و قد ثبت ان للزيوت الطيارة المحضرة من الاوراق و الثمار للنبات تاثيرا مدرا للبول دون حدوث انخفاض في نسبة البوتاسـيوم و اثبتت الدراسة ايضا ان لها تاثيرا مضادا للاكسدة.