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Comparative Study of Antioxidant and Anticancer Activity of *Thuja orientalis* Growing in Egypt and Saudi Arabia

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Authors' contributions

This work was carried out in collaboration between all authors. Author ERE designed the study and performed the collection of plant sample and extraction of essential oil. Author HA performed the GC-mass analysis. Author AERMD performed antioxidant activity. Author ERE managed the analyses of the study results, the literature searches, wrote the first draft of the manuscript, corrected the manuscript and approved the final submission. All authors read and approved the final manuscript.

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ABSTRACT

Cancer is a life threatening diseases, caused by many factors including the oxidative stress. Some medicinal plants are rich with volatile oil represent an important source of antioxidant and anticancer drugs. This study explored the compositions of the essential oils of the flowering aerial parts of *Thuja orientalis* (cupressaceae) and evaluated antioxidant and cytotoxic activity against different tumors cell lines. The compositions of the essential oils obtained by hydro-distillation of

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the flowering aerial parts was determined by GC-MS analysis, the antioxidant activities of essential were determined by DPPH radical scavenging method and, In-vtro cytotoxic activities were evaluated against different tumors cell lines, MCF7 (breast carcinoma cell line), PC3 (human prostate carcinoma), HCT116 (human colon carcinoma), A549 (lung carcinoma cell line), and Hep-G2 (liver carcinoma cell line), by the MTT method. The results showed that essential analyzed sample, very rich in Phellandrene, Terpenyl acetat, and β -Caryophyllene with high amounts. The results showed that essential oil extracted from Saudi plant exhibited higher antioxidant activity. Saudi oil extract also possess highest cytotoxic activities against MCF7, followed by, PC3 and, Hep-G2 while the least activity was recorded against lung carcinoma cell line. The highest antioxidant and cytotoxic activity of *Thuja* plant growing in Saudi Arabia were correlated with its high content of some compounds which are rich in Saudi plant and absence in Egyptian plant. These findings revealed that plant in two regions could be considered as a great potential source for natural health products, where Saudi plant more adapted to drastic condition by accumulation of higher amount of essential oil.

Keywords: Thuja orientalis; antioxidant; anticancer; essential oil.

1. INTRODUCTION

Thuja oriental belonging to family Cupressaceae, are an evergreen tree growing to 15 m. The seeds ripen from September to October. The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by wind. It can grow in semi-shade (light woodland) or no shade. This plant is commonly used in Chinese herbalist, where it is considered to be one of the 50 fundamental herbs [1]. The leaves are antibacterial, antipyretic, antitussive, astringent, diuretic, refrigerant and stomachic [2].

Platycladus oriental (Thuja orientals) is a medicinal plant whose fresh leaves have been used as an anti-inflammatory [3], while the dried leaves have been used to treat cough [4], and has also been used in Chinese medicine for the treatment of gout, rheumatism, diarrhea, and chronic tracheitis [5]. Ethanolic extract of Thuja occidentalis was used as homeopathic mother tincture to treat various ailments, particularly tumors, and also used in various other systems of traditional medicine. Anti-proliferative and apoptosis-inducing properties of the thujone-rich fraction separated from ethanol extract, have been evaluated for their possible anti-cancer potentials in the malignant melanoma cell line A375. by S-diphenyltetrazolium bromide assay method [6]. Many plants emit a considerable amount of organic material, mostly the carriers of the plant's odor, called essential oils. Additionally, large amounts of these essential oils are deposited within the plant itself. It has been deduced that these essential oils do not provide an energy source for the plant. We can assume this, because they remain in the leaves in plants that lose their leaves. Starch, or carbohydrate stores, are moved into the stem before the leaves drop. Therefore, what is the function of the essential oil? One idea is that in some cases the oils produce a scent attractive to certain animals and insects, aiding in pollination. In other cases the scent is noxious, acting as a repellent; or irritating, also functioning as a repellent. Other ideas are that, the oils act as an energy reserve, act to seal wounds, or as a varnish to prevent evaporation of water [7].

Phytochemical investigation of the essential oil of *Thuja orientalis* resulted in the isolation and identification of three new sesquiterpenes, 3α-methoxy-4α-epoxythujopsane, Δ3,15-4β-epoxythujopsene, and Δ3,4-thujopsen-2,15-diol, together with eight known sesquiterpenoids. The structures of these new compounds were elucidated based on spectroscopic data analyses including extensive 2D‐NMR data and HR-ESIMS. The full assignments of ^{1}H and ^{13}C NMR chemical shifts for thujopsadiene were obtained by 2D‐NMR for the first time. All compounds showed antiproliferative activities against the SK‐OV‐3 and SK‐MEL‐2 cell lines with IC50 values of 5.85– 28.64 μM. [8].

The aim of this study was to investigate adaptability of the *Thuja orientalis* plant to different environmental condition. Plants have been collected from to different regions from Saudi Arabia and Egypt one of theme (Saudi Arabia) is the most drastic environmental condition than the other, in which high temperature and very arid condition. The goal

was to measure the quantitative and qualitative of essential oil, as adaptability indicator, and to proof the statement that the plants answer on environmental stress by production of essential oil**.** Also the study of anticancer and antioxidant activity.

2. MATERIALS AND METHODS

2.1 Plant Materials

The aerial parts of all the plants, *Thuja orientalis* were collected from two region North Region (Arar) Saudi Arabia and National Research Centre (Research and production Station Nubaria) during spring season, two sample from both region are collect approximately in the same age. The plant samples were identified in Taxonomy& Botany Department, Northern Border University. The plant materials were air-dried in the shade and ground to a fine powder in order to carry out phytochemical and biological investigations.

2.2 Extraction of Essential Oil

The air-dried fruit (250 g) of *Thuja orientalis* from two regions were cut into small pieces, volatile oils were isolated from fresh plant material by wet steam distillation for 4 h. The essential oils were separated from the aqueous layer by diethylether and were dried over. The oils, taken in 2 mL of capillary GC grade *n*-pentane and dried over anhydrous sodium sulphate, were subsequently analyzed by GC and GC-MS and stored at –20℃. The percent yield of oils was 2.6%. and 3.4% of Egypt oil extract and Saudi oil Extract respectively.

2.3 GC-MS Analysis of the Essential Oil

Hewlett Packard gas chromatograph model (5890) series II plus, equipped with a carbowax 20 M capillary column, flame ionization detector (FID), helium as carrier gas at a flow rate of 1 mL/min, initial column temperature 60℃ increasing to 200℃ at a rate of 3℃ /min and held at 200℃ for 40 min, injector and detector temperatures 200 and 250℃, respectively. Hewlett Packard mass spectrometry model 5970. Temperature ionization detector (TIC) was used, carbowax 20 M capillary column (50 mm x.32 mm i.d.), temperature increasing from 60 to 200 ℃ by 3℃ /min, and MS ionization voltage 70 eV. The identification of the components was based on comparison of their mass spectra with those of the Wiley and NBS Libraries and those described by Adams [9].

2.4 Antioxidant Activity Determination by DPPH and ABTS Antioxidant Assay

DPPH free radical scavenging activity was determined using the method (Nile SH et al), [10,11]. DPPH (100 µM) stock solution was prepared by dissolving in 96% ethanol. 1ml of DPPH solution was added to 1 mg/ mL of C1, C2 extracts separately with 3 ml of ethanol. Than the mixture was vigorously shaken and keep in the dark for 10 min at room temperature. The resulting solution was observed at 517 nm at 10 min. The results were expressed in µM trolox/100 g dry weight. For the ABTS free radical-scavenging activity, ABTS (7 µM) stock solution was prepared by dissolving in water. The ABTS stock solution reacts with 2.45 µM potassium persulfate and before use kept in the dark at room temperature for 12–16 h. ABTS solution diluted with redistilled water to an absorbance of (0.7 ±0.02) at 734 nm and equilibrated and the blank reading was taken (Ab). After addition of 3.0 mL of diluted ABTS solution (A734 nm = 0.7±0.02) to 30 µg/mL of C1, and C2 extracts separately, the absorbance reading was exactly 6 min after initial mixing (ABTS). The results were corrected for dilution and expressed in µM trolox per 100 g dry weight (dw). All determinations were performed in triplicate.

2.5 Cytotoxic Effect on Human Cell Line (HPG 2 – MCF7 – HCT116- PC3 - A549)

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [12].

Procedure: All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium for HePG2-MCF7 and HCT116 – DMEM for A549. The media are supplemented with 1% antibiotic-antimycotic mixture (10,000 U/ml Potassium Penicillin, 10,000 µg/ml Streptomycin Sulfate and 25 µg/ml Amphotericin B), 1% L-glutamine and 10% fetal bovine serum and kept at 37℃ under 5% CO_2.

Cells were batch cultured for 10 days, then seeded at concentration of $10x10^3$ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37℃ for 24 h under 5% CO2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100-50-25-12.5-6.25-3.125-0.78 and 1.56 ug/ml). After 48 h of incubation, medium was aspirated, 40 ul MTT salt (2.5 µg/ml) were added to each well and incubated for further four hours at 37℃ under 5% CO2. To stop the reaction and dissolving the formed crystals, 200 µL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37℃. A positive control which composed of 100µg/ml was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions [13,14].

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula:

((Reading of extract / Reading of negative control) -1) x 100

A probit analysis was carried for IC50 determination using SPSS 11 program.

3. RESULTS AND DISCUSSION

The present study introduce, anticancer and antioxidant of the volatile oil components of plant collected from two regions and study the comparative analysis between two oil extract qualitatively and quantitatively.

3.1 Chemical Composition

Identification of the components of the essential oils by GC-MS revealed the existence of twenty-eight components of oils (Table 1). In previous works, the characteristic compounds of *Thuja* oil were camphene, phellandrene, pinene and Sabinene [15-16,17]. Our results (Table 1) are in agreement with these previous work, it has been found that the main components of the essential oil of Egyptian plant were, α-Phellandrene, caryophllene and terpenyl acetat, where the chemical composition of the Saudi plant oil contain limonene, and α-phellandrene, the major components of the oil extracts in two region are β -Caryophyllene with high amounts (24% and 14 %) and α-Cedrol with moderate amounts (8.5%, 6.5%) of Egyptian and Saudi plant respectively.

3.2 Screening of Antioxidant Activity

Antioxidant of volatile oil was in vitro screening DPPH and ABTS antioxidant assay. DPPH free radical scavenging activity was determined using the method (Nile SH et al.). The results were corrected for dilution and expressed in µM trolox per 100 g dry weight (dw). The DPPH radical of C1 (Saudi plant oil) and C2 (Thuja Egypt plant oil) is shown in Table 3 and Fig. 2 both extract showed a stronger DPPH radical scavenging activity (283.52 and 188.99 respectively). Free radical scavenging ability by hydrogen donation is a known mechanism for ant oxidation. In another experiment the DPPH of the extract of Thuja twigs was found to be 73.35 ± 1.04% at 300 µg/ml [18]. In present work, the obtained DPPH radical scavenging activity of both extract indicated it could be a good candidate in the search of natural, effective substances with antioxidant activity. The higher antioxidant activity of plant growing in Saudi Arabia may be due to the present of some compounds which not found in Egyptian plant, Limonene and Bisabolol.

3.3 Cytotoxic Activities

Both oils extract of Egyptian plant and Saudi plant, possess good activities against all five different tumor cell lines tested. Cytotoxic Activities of oils are shown in Table 2, and Fig. 1, Saudi oil extract possess the highest activities against breast carcinoma cell line (**47.6** and 35.5 µg/ml, respectively), followed by human colon carcinoma cell line, and liver carcinoma cell line, while the least activity was recorded against lung carcinoma cell line (**11.2** µg/ml), for Saudi plant and human prostate carcinoma cell line (**8.4** µg/ml) for Egyptian plant. Fig. 1 showed that the activities of the oil against lung carcinoma, liver tumor and breast carcinoma cell lines.

Table 1. Quantities (%) of components of the volatiles of *Thuja orientalis* essential oils

No	RI	Components	Oil extract % (Saudi Arabia)	Oil extract % (Egypt)
1	928	α-Thujene	0.27	0.2
2	977	β-Pinene	0.9	0.1
3	936	α-Pinene	0.30	0.25
4	971	Sabinene	0.08	2.1
5	1005	α-Phellandrene	1.6	2.1
6	1032	Limonene	5.4	--
7	969	Beta.-Sesqui- Phellandrene	1.18	----
8	1697	z-alpha.-Farnesene	0.37	-
9	1570.0	caryophllene oxide	----	2.25
10	1503.0	Alpha-muurolene	0.11	0.15
11	1058	γ-terpinene	0.49	0.4
12	1063	Terpenyl acetat	1.8	2.1
13	1101	Linalool	0.05	0.07
14	1241	α-Terpineol	0.46	0.07
15	1285	Bornyl acetate	1.05	0.25
16	1390	β-Elemene	-----	0.7
17	1451.3	Patchulane	-----	0.09
18	1423	β –Caryophyllene	14	24
19	1469	alpha.-Amorphene	-----	0.11
20	1483	Germacrene -D	0.35	0.11
21	1578.9	Globulol	---	0.08
22	1597.1	α-Cedrol	6.8	8.07
23	1651	α-Cadinol	----	1.00
24	1439.0	Isoaromadendrene epoxide	----	1.00
25	1449	Z-.alpha.-Bisabolene epoxide	---	0.35
26	1463	Bisabolol	1.2	---
27	1568.	Isomenthol	--	0.28
28	1577.1	Velleridol	---	2.3

Saudi plant were showed higher activity than those of leaf oil Egyptian, except lung carcinoma. The high activities of these oils may be accounted for by the presence of high ratio of monoterpenes in their composition Limonene and Bisabolol [18-20], referred the activity of the essential oil of *Myrcianthes sp*. against liver tumor cell line to the presence of α-pinene, β-pinene and limonene. This may explain the higher activities of Saudi oils than Egypt leaf oil against lung carcinoma, liver tumor and breast carcinoma cell lines since the former contained higher percentages of total monoterpenes, α-pinene and β-pinene [21] attributed the activity of *Croton flavens* leaf oil against lung carcinoma cell line to its contents of elemene and α-humulene which are constituents of both tested oils. Elemene was found to inhibit proliferation, stimulate apoptosis and induce cell cycle arrest in malignant cell. Limonene and perilla alcohol have been shown to inhibit protein prenylation in cultured cells [22], both Limonene and perilla alcohol inhibited chemically induced carcinogenesis from chemical carcinogens [23-26]. Dubey and Batra [27,28] reported that the hepato-protective activities and antioxidant activity of *Thuja occidentalis,* anti-proliferative and apoptosis- inducing properties of thujone-rich fraction (TRF) separated from *Thuja occidentalis,* and this result come in agreement of our result where thujone is from a major component of oil.

Table 2. The cytotoxic activity of essential oil in both region

	Cytotoxicity % at 100 ppm				
Samples	**HePG 2**	**PC3**	**MCF-7**	**HCT116**	**A549**
C1	24.8%	10.4%	47.6%± 3.56	32 ± 3.41	11.2%
C2	21.3%	8.4%	35.4%± 0.56	29.5%	13.2 %

C1,(Saudi Arabia oil extract) and C2 (Egypt oil extract)

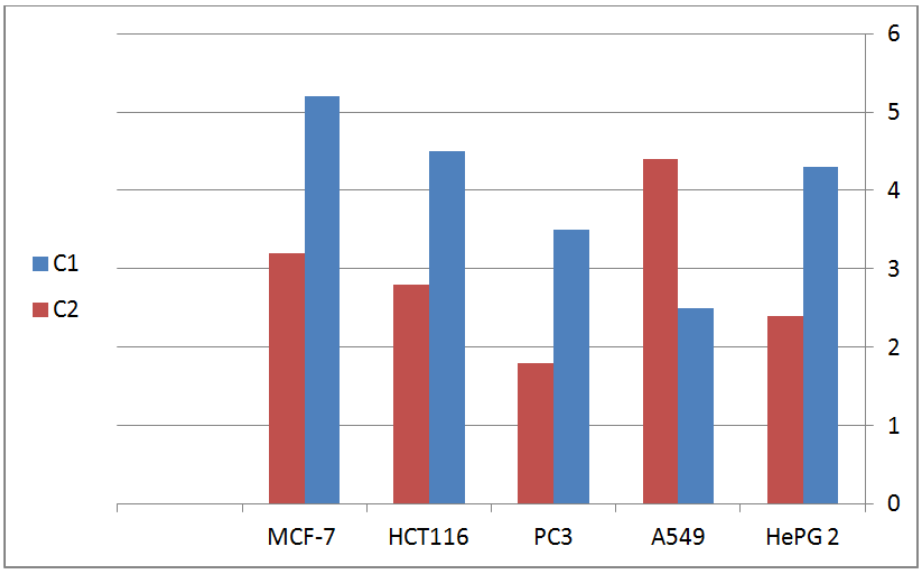

Fig. 1. The cytotoxic activity of essential oil in both region

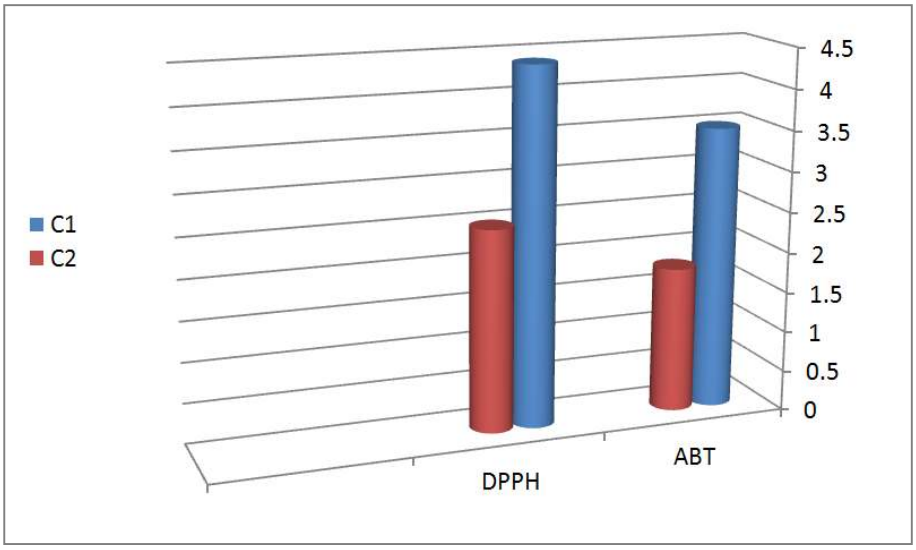

Fig. 2. In-vitro antioxidant activity of *Thuja orientalis* essential oils

Table 3. In-vitro antioxidant activity of *Thuja orientalis* essential oils

Concentration µg/ml	TEAC (µM trolox/100 g dw)	
	DPPH	ABTS
C1	283 ± 3.56	110 ± 3.41
C2	188 ± 0.56	75± 0.68

C1,(Saudi Arabia oil Extract) and C2 (Egypt oil Extract)

Environmental adaptability of the plants can be tested through from essential oil contents. Essential oil of Thuja plants are compounds with various therapeutically uses.

Production of essential oil is an indicator of plant adaptation on habitat conditions. It helps to easily submit plant environmental stress conditions, drought, intense radiation, high temperature, heavy metal contents [29]. Essential oils are not constant in the qualitative and quantitative terms. Quality of essential oil depends on the external environmental condition. Ecological aspects of the role of essential oils are reflected in the interaction of

plants with environmental factors. Natural selection favors survival of the population with the composition of essential oil has a higher adaptive value [30].

Thuja, is common widespread plant in Egypt and Saudi Arabia. However, it is principally used in tradition medicine as antihypertensive remedy. Also, it is used as an antispasmodic, so study the effect of environmental condition will affect the content of essential oil isolated, So we found some high variation qualitatively and quantitatively of volatile oil content, The total content of identified essential essential oil in Saudi plants was 34%, while Egypt plants was 26%. The main compound in plants was found in both localities, while some compounds as limonen and Bisabolol were found in Saudi plant, and absence in Egyptian plant, some compound present in high concentration in Egyptian plant, caryophllene this depended on environmental condition. Point out that the values obtained in this paper are compatible with other published reviews [15-17].

4. CONCLUSIONS

Thuja oriental is plants, are grow and survive in a different habitats and environment, two habitat Egypt and Saudi Arabia are very differs in environment, so plant can adapt themselves by accumulation of some metabolic products (volatile oil). And these accumulation of different compounds of essential oil reflect on the biological activity of the plant which also are different, where we found the Saudi plant possess the highest activities against breast carcinoma cell line, followed by human colon carcinoma, and liver carcinoma cell line, while the least activity was recorded against lung carcinoma cell line. Similar result for antioxidant activity, Saudi plant oil have higher activity, so the volatile oil of C1 has the better medicinal application prospect and deserves more attention in the pharmaceutical industry, this related to the different of chemical compositions of oil.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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