GRASAS Y ACEITES 66 (4)
October–December 2015, e110
ISSN-L: 0017-3495
http://grasasyaceites.revistas.csic.es

Fé de Erratas/Erratum

The manuscript:
Essential oils from Egyptian aromatic plants as antioxidant and novel anticancer agents in human cancer cell lines. *Grasas Aceites* **66** (2): e080. doi: http://dx.doi.org/10.3989/gya.0955142.
Its correct form is as follow:

Essential oils from Egyptian aromatic plants as antioxidant and novel anticancer agents in human cancer cell lines

M.M. Ramadan[a], M.M. Ali[b,✉], K.Z. Ghanem[c,d] and A.H. El-Ghorab[a,e]

[a]Chemistry of Flavour and Aroma Department National Research Centre, Dokki 12622, Giza, Egypt
[b]Biochemistry Department, National Research Centre, Dokki 12622, Giza, Egypt
[c]Food Science and Nutrition Department, National Research Centre, Dokki 12622, Giza, Egypt
[d]Clinical Nutrition Department, Faculty of Applied Medical Science, Jazan University, KSA
[e]Chemistry Department, Faculty of Science, Aljouf University, KSA

✉Corresponding author: mmali1999@yahoo.com

GRASAS Y ACEITES 66 (2)
April–June 2015, e080
ISSN-L: 0017-3495
doi: http://dx.doi.org/10.3989/gya.0955142

Essential oils from Egyptian aromatic plants as antioxidant and novel anticancer agents in human cancer cell lines

M.M. Ramadan[a], M.M. Ali[b,✉], K.Z. Ghanem[c,d] and A.H. El-Ghorabe[a,f]

[a]Chemistry of Flavour and Aroma Department National Research Centre, Dokki 12622, Giza, Egypt
[b]Biochemistry Department, National Research Centre, Dokki 12622, Giza, Egypt
[c]Food Science and Nutrition Department, National Research Centre, Dokki 12622, Giza, Egypt
[d]Clinical Nutrition Department, Faculty of Applied Medical Science, Jazan University, KSA
[f]Chemistry Department, Faculty of Science, Al-GohfUniversity, KSA

✉Corresponding author: mmali1999@yahoo.com

Submitted: 28 September 2014; Accepted: 26 January 2015

SUMMARY: Inhibitors of tumor growth using extracts from aromatic plants are rapidly emerging as important new drug candidates for cancer therapy. The cytotoxicity and *in vitro* anticancer evaluation of the essential oils from thyme, juniper and clove has been assessed against five different human cancer cell lines (liver HepG2, breast MCF-7, prostate PC3, colon HCT116 and lung A549). A GC/MS analysis revealed that α-pinene, thymol and eugenol are the major components of Egyptian juniper, thyme and clove oils with concentrations of 31.19%, 79.15% and 82.71%, respectively. Strong antioxidant profiles of all the oils are revealed *in vitro* by DPPH and *β*-carotene bleaching assays. The results showed that clove oil was similarly potent to the reference drug, doxorubicin in prostate, colon and lung cell lines. Thyme oil was more effective than the doxorubicin in breast and lung cell lines while juniper oil was more effective than the doxorubicin in all the tested cancer cell lines except prostate cancer. In conclusion, the essential oils from Egyptian aromatic plants can be used as good candidates for novel therapeutic strategies for cancer as they possess significant anticancer activity.

KEYWORDS: *Anticancer; Antioxidant; Clove Juniper; Thyme*

RESUMEN: ***Aceites esenciales de plantas aromáticas egipcias como novedosos agentes anticancerígenos y antioxidantes en líneas celulares de cáncer humano***. Los inhibidores de crecimiento de tumores usando extractos de plantas aromáticas están emergiendo con rapidez como nuevos e importantes medicamentos para el tratamiento del cáncer. La citotoxicidad y la acción anticancerígena *in vitro* de aceites esenciales de tomillo, enebro y clavo han sido evaluadas en cinco líneas celulares de cáncer humano (hígado HepG2, mama MCF-7, próstata PC3, colon HCT116 y pulmón A549). Los análisis de GC/MS mostraron que α-pineno, timol y eugenol son los principales componentes de los aceites egipcios de enebro, tomillo y clavo, con concentraciones de 31,19%, 79,15% y 82,71%, respectivamente. Se demuestra, mediante ensayos *in vitro* de blanqueo de DPPH y β-caroteno, el enérgico perfil antioxidante de todos los aceites. Los resultados mostraron que el aceite de clavo fue similar de potente al fármaco de referencia, doxorrubicina en las líneas celulares de próstata, colon y pulmón. El aceite de tomillo fue más efectivo que la doxorrubicina en las líneas celulares de mama y de pulmón, mientras que el aceite de enebro fue más eficaz que la doxorrubicina en todas las líneas celulares de cáncer ensayados, excepto en la de cáncer de próstata. En conclusión, los aceites esenciales de plantas aromáticas egipcias se pueden utilizar como buenos candidatos para nuevas estrategias terapéuticas para el cáncer al poseer una significativa actividad anticancerígena

PALABRAS CLAVE*: Anticancerígeno; Antioxidante; Clavo; Enebro; Tomillo*

Citation/Cómo citar este artículo: Ramadan MM, Ali MM, Ghanem KZ, El-Ghorabe AH. 2015. Essential oils from Egyptian aromatic plants as antioxidant and novel anticancer agents in human cancer cell lines. *Grasas Aceites* **66** (2): e080. doi: http://dx.doi.org/10.3989/gya.0955142.

1. INTRODUCTION

Throughout history, natural products have afforded a rich repository of remedies with diverse chemical structures and bioactivities against several heath disorders including cancer. The use of herbs as complementary and alternative medicine has increased dramatically in the last 20–25 years, herbs and spices have enjoyed a rich tradition of use for their flavor enhancement characteristics and for their medicinal properties. The search therefore continues to develop the drugs which selectively act on tumor cells without diverse side effects (Ali and Sohair, 2007). The screening of plant extracts/essential oils and natural products for anti-oxidative activity has revealed the potential of higher plants as a source of new antioxidant agents. The oil could be an excellent alternative to a number of synthetic antioxidants such as butylated hydroxyl toluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ), because there are concerns that these synthetic antioxidants may exhibit carcinogenic properties (Hamedo and Abdelmigid, 2009). Essential oil is a volatile, natural and complex compound present in a variety of aromatic plants and mostly extracted by steam or hydro-distillation from the plants (Jo *et al*., 2012). The rising prevalence of chronic diseases worldwide and the corresponding rise in health care costs is propelling interest among researchers and the public due to the multiple health benefits related to these food items, including a reduction in cancer risk and modifications in tumor behavior. A growing body of epidemiological and preclinical evidence points to culinary herbs and spices as minor dietary constituents with multiple anticancer characteristics (Paul *et al*., 2010). In the same direction and in a continuing effort to find more potent and selective anticancer compounds, we examined the effect of Egyptian thyme, juniper and clove essential oils for their anticancer activity against five different human cancer cell lines including liver HepG2, breast MCF-7, prostate PC3, colon HCT116 and lung A549. The most common cancers are lung, colon, liver and prostate, whereas breast cancer (23% of all new cancer cases) is the second leading cause of death in women worldwide and these days in Egypt as well. According to the Egyptian National Research Institute biostatistics (2011) liver, breast, colon, lung and prostate cancers are important public health problem, 37% of all new cancer cases in Egypt are breast cancer (Elattar, 2003). Chemotherapy for cancer, a devastating cancer with increasing worldwide incidence and mortality rates, is largely ineffective. The discovery and development of effective chemotherapeutics is urgently needed. In our effort to search for local herbal medicines with promising activity against cancer, the present work is the first step, and aims to use local aromatic plants for the discovery of natural, cheap and safe Egyptian drugs that will be followed by continuous steps to achieve this goal. In this study, the antioxidant activity as well as cytotoxicity and *in vitro* anticancer evaluation of the essential oils from thyme, juniper and clove have been assessed against five different human cancer cell lines (liver HepG2, breast MCF-7, prostate PC3, colon HCT116 and lung A549).

2. MATERIALS AND METHODS

2.1. Raw Materials and Chemicals

2.1.1. Plants

Egyptian dry thyme leaves (*Thymus vulgaris*), dry juniper fruits (*Juniperus communis*) and dry clove buds (*Syzygium aromaticum*) were obtained and identified from the department of medicinal and aromatic plants, ministry of agriculture, Egypt. The aromatic plants have been chosen because of their high content in essential oil, they are inexpensive and locally available.

2.1.2. Chemicals

Fetal bovine serum (FBS) and L-glutamine, were obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium was provided by Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and Sulfo-Rhodamine-B stain (SRB) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were obtained from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade and purchased from Sigma-Aldrich chemical Co. (St. Louis, MO, USA).

2.2. Isolation of Essential Oils

The essential oils (EOs) of thyme, juniper and clove were extracted according to the hydrodistillation method using Clevenger's apparatus for 3 hours (Lamaty *et al*., 1987). The yield of volatile oils was weighed and calculated in g·100 g^{-1} dry plant.

2.2.1. Identification of Essential Oils

2.2.1.1. Gas chromatography (GC) analysis About five µL of each pure volatile oil was used. A GC analysis was performed using a Hewlett-Packard model 5890 equipped with a flame ionization detector (FID). A fused silica capillary column DB-5 (60 m ×0.32 mm. id) was used. The oven temperature was maintained initially at 50 °C for 5 min., and then programmed from 50 to 250 °C at a rate of 4 °C·min^{-1}. Helium was used as the carrier gas, at flow rate of 1.1 mL·min^{-1}. The injector and detector

temperatures were 220 and 250 °C, respectively. The retention indices (Kovats index) of the separated volatile components were calculated using hydrocarbons (C7-C21, Sigma-Aldrich Co.) as references (Adams, 1995).

2.2.1.2. Gas chromatographic-mass spectrometric analysis (GC/MS) The analysis was carried out using a coupled gas chromatography Hewlett-Packard model (5890) / mass spectrometry Hewlett-Packard-MS (5970). The ionization voltage was 70 eV, mass range m/z 39-400 a.m.u. The isolated peaks were identified by matching with data from the library of mass spectra (National Institute of Standard and Technology, NIST) and compared with those of authentic compounds and published data. The quantitative determination was carried out based on peak area integration. Identification of the GC components also was confirmed with NIST mass spectra library data, as well as on comparison of their retention indices with those of authentic compounds (Adams, 1995).

2.3. Determination of Antioxidant Activity

2.3.1. β-carotene-linoleate scavenging assay

The antioxidant activities of thyme, juniper and clove volatile oils were evaluated using the β-carotene-linoleate scavenging assay (Gülçin *et al*., 2007). A volume of 0.1 mg β-carotene in 0.2 mL chloroform, 10 mg of linoleic acid and 100 mg of Tween-20 were mixed. The solvent was removed at 40 °C under vacuum and the resulting mixture was diluted with 10 mL of water and mixed well. 20 mL of oxygenated water were added to this mixture. Four milliliter aliquots were pipetted into different test tubes containing 200 µL of each volatile oil (20, 40, 60 and 80 $\mu g \cdot mL^{-1}$) and TBHQ (20, 40, 60 and 80 $\mu g \cdot mL^{-1}$) in ethanol. TBHQ, a standard synthetic antioxidant, was used for comparative purposes. A control containing 200 µL of ethanol and 4 mL of the above emulsion was prepared. The tubes were placed at 50 °C in a water bath and the absorbance at 470 nm was taken at time zero (t =0). The measurement of the absorbance continued to until the color of β-carotene disappeared in the control tubes (t =60 min) at an interval of 15 min. A mixture prepared as mentioned above without β-carotene served as a blank. All determinations were carried out in triplicate. The antioxidant activities (A.A.) of the essential oils were evaluated in terms of bleaching of β-carotene using the following formula:

$$\% \text{ of Inhibition} = [(A_B - A_A)/A_B] \times 100$$

Where: A_B: absorption of blank sample (t =0 min). $A_{A:}$ absorption of sample solution (t =60 min). The results were expressed in % basis in preventing the bleaching of β-carotene.

2.3.2. DPPH radical scavenging activity assay

Antioxidant activity was also determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay using a spectrophotometer at 517 nm (Gülçin, 2006). Each volatile oil of different concentrations (20, 40, 60 and 80 $\mu g \cdot mL^{-1}$) and TBHQ (20, 40, 60 and 80 $\mu g \cdot mL^{-1}$) was collected in different test tubes. Four milliliters of 0.1 mM methanolic DPPH were added to these tubes and they were shaken vigorously. The tubes were allowed to stand at room temperature for 30 min. The control was prepared without any extract and methanol. The changes in the absorbance of the prepared samples were measured at 517 nm (Gülçin, 2006). Radical scavenging activity was estimated as the inhibition percentage and was calculated using the following formula:

$$\% \text{ of Inhibition} = [(A_B - A_A)/A_B] \times 100$$

Where: A_B: absorption of blank (t =0 min). $A_{A:}$ absorption of sample solution (t =30 min).

2.4. Anticancer Activity

2.4.1. Cell lines and culturing

Anticancer activity screening for the thyme, juniper and clove volatile oils was carried out and 5 different human cancer cell lines including liver HepG2, breast MCF-7, prostate PC3, colon HCT116 and lung A549 were obtained from the American Type Culture Collection (Rockville, MD, USA). The tumor cells were kept in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 $U \cdot mL^{-1}$) and streptomycin (100 µg·mL) at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were grown at a concentration of 0.50×10^6 were grown in 5 mL of a complete culture medium.

2.4.2. In vitro antiproliferative assay

The antiproliferative activity was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the previously reported standard procedure (Skehan *et al*., 1990). Briefly, the cells were inoculated in a 96-well microliter plate (10^4 cells/ well) for 24 h before treatment with the tested volatile oils to allow the cells to attach to the wall of the plate. The thyme, juniper and clove volatile oils were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compounds under test and the reference drug, doxorubicin (0–100 $\mu g \cdot mL^{-1}$) were

added to the cells. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the volatile oils for 48 h at 37 °C and in an atmosphere of 5% CO_2. After 48 h, the cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and the attached stain was recovered with a Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between the surviving fraction and the concentration of volatile oils was plotted to get the survival curve for each cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table 3.

2.5. Statistical analysis

The results are reported as Mean ± Standard Error (S.E.) for the experiments carried out at least in triplicate. Statistical differences were analyzed by one way ANOVA test.

3. RESULTS AND DISCUSSION

3.1. Total yield and chemical composition of essential oils

The total yield of the essential oil from juniper fruits, thyme leaves and clove buds (relative to the amount of dried plant used) was 6.22±0.57, 4.87±0.33 and 9.32±0.61% (w/w) as shown in Figure 1. Our results reveal that the Egyptian aromatic plants under investigation are an excellent source of essential oils with relatively high yield compared with those cultivated in other countries. Lee and Shibamoto (2001) reported that the total yield of essential oil from American clove buds was 2.73%. The oil yield ranged from 0.70 to

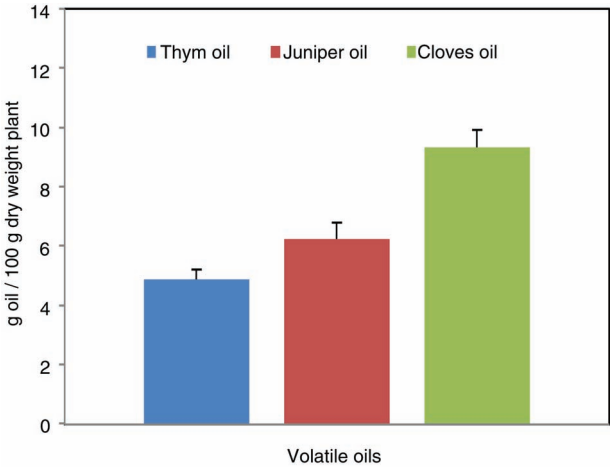

FIGURE 1. Total yield of essential oils (g oil·100 g^{-1} dry weight plant) from Egyptian thyme, juniper and clove.

2.1% of juniper cultivated in Estonia (Orav *et al.*, 2010) while the yield was 0.99% for Iranian thyme (Ghomi *et al.*, 2009). The results obtained from the GC and GC–MS chemical analysis of thyme, juniper and clove EOs are presented in Table 1 and Figure 2. In total, 39 compounds were identified. Regarding the groups of chemical constituents represented, the three essential oils mainly consisted of monoterpene hydrocarbons (M), light oxygenated compounds (LOC), heavy oxygenated compounds (HOC) and sesquiterpene hydrocarbons (S). As shown in Figure 1. Light oxygenated compounds (LOC) were the major portion of thyme and clove EO samples, while juniper oil was found to be rich in monoterpene (M) and sesquiterpene (S) compounds. HOC were the minor portion in all tested essential oils, and represent 2.84, 0.33 and 4.83% for juniper, thyme and clove respectively. These findings are in agreement with the results of Nikoli, *et al.* (2014), where LOC were the major volatile portions of thyme cultivated in Serbia. Pepeljnjak, *et al.* (2005) reported that the content in essential oil in juniper cultivated in Croatia ranges from 0.5 to 2.50% and its main compounds are terpene hydrocarbons such as α- and β-pinene, sabinene and thujone, *etc.* Essential oil also contains sesquiterpene hydrocarbons (caryophyllene, cadinene, elemene). The chemical composition of the investigated essential oils is shown in table 1;twenty-seven compounds were identified in juniper oil, which accounted for 90.84% of the total oil; the major constituent was α-pinene (31.19%). Thyme and clove oils showed twenty-two and seven compounds, respectively, which accounted for 98.68% and 97.93% of the total oil; the main constituent was thymol (79.15%) and eugenol (82.71%) for thyme and clove, respectively. Our results on the chemical profiling of three Egyptian EOs are in agreement with many studies (Orav *et al.*, 2010; Teixeira *et al.*, 2013; Nikoli *et al.*, 2014) which reported that a GC/MS analysis revealed α-pinene, thymol and eugenol as the major components of juniper, thyme and clove, respectively. Some other previous studies found similar chemical compositions for juniper, clove and thyme essential oils, but in different concentrations (Pepeljnjak *et al.*, 2005). β-caryophyllene was a common volatile compound present in three essential oils, which represent 9.90, 3.72 and 9.64% in juniper, thyme and clove respectively. Caryophyllenes, including β-caryophyllene, α-caryophyllene (α-humulene) and γ-caryophyllene (isocaryophyllene) are sesquiterpenes present in various essential oils. Natural bicyclic β-caryophyllene and *iso*-caryophyllene are *trans* and *cis* double isomers, respectively, while α-humulene is a ring-opened isomer.

In essential oils, β-caryophyllene is frequently found mixed with iso-caryophyllene and/or α-humulene. Essential oils are widely used in aromatherapy to

TABLE 1. Chemical composition of essential oils.

Peak No.	Constituents[a]	RI	Type of compound	Relative area %			Methods of identification[b]
				Clove	Thyme	Juniper	
1	α-Thujene	924	M	0.60	1.90	–	RI, MS
2	α-Pinene	930	M	31.19	1.20	–	RI, MS & St
3	Camphene	939	M	–	0.90	–	RI, MS & St
4	Sabinene	961	M	0.80	0.70	–	RI, MS & St
5	*β*-pinene	969	M	–	0.40	–	RI, MS & St
6	*β*-Myrcene	989	M	1.18	1.30	–	RI, MS
7	α-Phellandrene	1000	M	1.83	–	–	RI, MS
8	α-Terpinene	1010	M	0.48	0.80	–	RI, MS & St
9	*p*-Cymene	1016	M	–	10.33	–	RI, MS
10	Limonene	1019	M	–	0.60	–	RI, MS & St
11	1,8-Cineol	1024	LOC	–	1.20	–	RI, MS & St
12	*Cis*-Sabinen hydrate	1070	LOC	0.35	0.22	–	RI, MS
13	Linalool	1093	LOC	3.16	0.81	–	RI, MS & St
14	Trans-pinocarveol	1130	LOC	0.63	–	–	RI, MS
15	Camphor	1146	LOC	0.85	–	–	RI, MS & St
16	Borneol	1158	LOC	–	1.76	–	RI, MS & St
17	*p*-Mentha-1,5-dien-8-ol	1162	LOC	0.93	–	–	RI, MS
18	*α*-Terpineol	1206	LOC	2.85	2.15	–	RI, MS
19	Thymol	1292	LOC	–	59.15	–	RI, MS & St
20	Carvacrol	1299	LOC	–	7.56	–	RI, MS & St
21	α-terpinenyl acetate	1321	LOC	1.55	0.17	–	RI, MS
22	Neo-menthole	1339	LOC	0.33	–	–	RI, MS
23	Thymol acetate	1346	LOC	–	0.45	–	RI, MS
24	Eugenol	1355	LOC	–	–	82.71	RI, MS & St
25	Carvacrol acetate	1365	LOC	–	0.32	–	RI, MS
26	Linalyl acetate		LOC	2.79	–	–	RI, MS
27	α-Copaene	1369	S	0.32	–	–	RI, MS
28	Elemene	1391	S	1.26	–	0.14	RI, MS
29	*β*-Caryophyllene	1409	S	9.91	3.72	9.64	RI, MS
30	α-Humulene	1449	S	4.83	–	0.51	RI, MS
31	α-Muurolene	1477	S	2.53	–	–	RI, MS
32	Germacrene-D	1488	S	3.84	–	–	RI, MS
33	γ-Cubebene		S	8.85	–	–	RI, MS
34	γ-Cadinene	1500	S	1.39	2.31	0.09	RI, MS
35	δ-Cadinene	1515	S	5.58	–	–	RI, MS
36	Eugenol acetate	1528	HOC	–	–	4.70	RI, MS
37	Germacrene-B	1556	S	0.91	–	–	RI, MS
38	(-)-Caryophyllene oxide	1572	HOC	1.35	0.33	0.13	RI, MS
39	Torreyol	1646	HOC	0.58	–	–	RI, MS
Monoterpenes (M)				**36.08%**	**18.13%**	**0%**	
Light Oxygenated Compounds (LOC)				**13.44%**	**73.79%**	**82.71%**	
Heavy Oxygenated Compounds (HOC)				**2.84%**	**0.33%**	**4.83%**	
Sesquiterpenes (S)				**38.51%**	**6.03%**	**10.38%**	
Total Identified %				**90.84%**	**98.00%**	**97.93%**	

a: Compound listed in the order of elution from a DB_5 column;
RI: Retention indices relative to C_7–C_{20} *n*-alkanes on the DB-5MS column;
b: Identification based on retention index; MS, identification based on comparison of mass spectra;
- : Absent

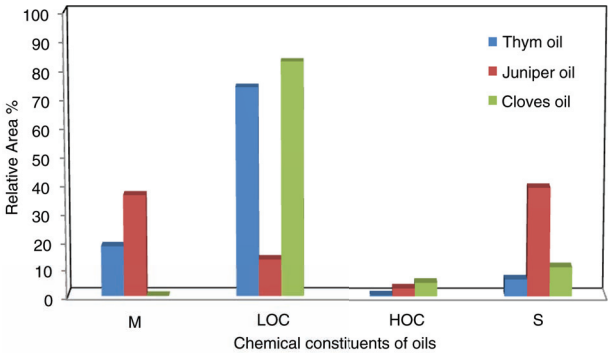

FIGURE 2. Relative area % of groups of chemical constituents of essential oils. The chemical constituents are monoterpene hydrocarbons (M), light oxygenated compounds (LOC), heavy oxygenated compounds (HOC) and sesquiterpene hydrocarbons (S).

alleviate symptoms of stress-induced anxiety; mild mood disorders and cancer pain. A number of active constituents are thought to be responsible for the medicinal action of essential oils. The essential oils of aromatic plants contain monoterpene and sesquiterpene hydrocarbons (such as limonene, γ-terpinene, α- and β-pinene, β-myrcene, sabinene…) and oxygenated derivatives (such as linalool, thymol, carvecrol, eugenol…) which play a very important role as antioxidants (Dugo *et al*., 2000).

3.2. Antioxidative activity

The essential oils were investigated for their radical-scavenging activity. Two different assays were conducted in order to evaluate their antioxidant properties; scavenging activity on DPPH radicals and inhibition of lipid peroxidation in a β-carotene–linoleate system. The results of the DPPH assay are shown in Table. 2. It is evident that all concentrations of the tested essential oils (20, 40, 60 and 80 μg·mL^{-1}) exhibited an excellent anti-oxidative potential compared with the standard antioxidant TBHQ, especially at their highest concentrations (80 μg·mL^{-1}), the thyme, juniper and clove oils exhibited inhibitions of 90.12, 85.63 and 93.24%, respectively, as shown in Figure 3. The excellent antioxidant activity is attributed to volatile constituents; these components could change free radicals such as DPPH$^{\cdot}$ to non-radical DPPH-H (Ramadan *et al*., 2013a). Numerous and diverse techniques are available to evaluate the antioxidant activities of specific compounds or complex mixtures such as EOs; however a single procedure cannot identify all the possible mechanisms characterizing an antioxidant. Therefore, Table 2 also shows the results of the β-carotene bleaching assay based on the loss in the yellow color of β-carotene due to its reaction with radicals produced during linoleic acid oxidation. The high inhibition effect of the oxidation of linoleic acid and the subsequent bleaching of β-carotene compared to TBHQ at the same concentration may be attributed to the presence of terpene hydrocarbons and oxygenated volatile compounds (Ramadan *et al*., 2013a). In both assays, the radical scavenging capacity of the tested EOs increased in a concentration dependent manner. The volatile extracts from some Egyptian spices possess excellent radical scavenging activity in DPPH and ABTS scavenging assays (Ramadan *et al*., 2013b; Ramadan *et al*., 2014). It is well known that oxygenated terpenes such as (thymol and eugenol) exhibited a higher antioxidant power in comparison to the other identified classes. Clove oil, rich with eugenol, was the superior oil as antioxidant in the DPPH as well as in the β-Carotene assay, when compared to the synthetic antioxidant TBHQ as shown in Table 2. The highest concentration (80 μg·mL^{-1}) of thyme, juniper and clove oils exhibited very good scavenging activity and a high level of inhibition of lipid peroxidation in a β-carotene–linoleate system compared with TBHQ. The % inhibition values were 84.61, 80.51 and 91.23%, respectively (Figure 3). The order of % inhibition of free radicals offered by the essential oils was clove > thyme > juniper. The strong antioxidant profiles of thyme, Juniper and clove oils have been proved in several studies. The results obtained from this study arein agreement with Lesjak, *et al*. (2011), who reported that juniper extract has shown a significant DPPH scavenger activity, which was still notably lower than the synthetic antioxidant

TABLE 2. Antioxidant activity (A.A.) of essential oils by two different methods.

	Inhibition % at different concentrations of essential oils							
	A.A. by DPPH free radical assay (μg·mL^{-1})				**A.A. by β- carotene/ linoleic acid assay (μg·mL^{-1})**			
	20	**40**	**60**	**80**	**20**	**40**	**60**	**80**
Thyme	46.77±2.50	68.88±2.10	81.32±3.10	90.12±2.70	40.93±2.20	67.34±1.90	75.98±2.70	84.61±2.70
Juniper	29.14±2.10	48.12±1.70	64.22±3.90	85.63±4.10	38.97±1.50	57.94±1.70	78.99±2.00	80.51±2.70
Clove	59.11±2.60	69.28±3.10	84.25±3.30	93.24±3.90	54.13±1.90	77.54±2.90	85.88±2.90	91.23±3.50
TBHQ	61.13±1.80	72.15±2.30	86.96±2.60	96.00±2.20	58.33±2.00	74.00±1.80	88.27±3.10	94.78±3.30

TBHQ: Tert –butyl hydroquinone, standard synthetic antioxidant.
Each value represents the mean ± S.E (Standard Error) of three repeated experiments.

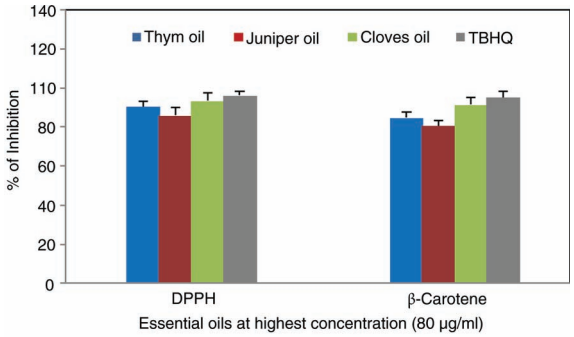

FIGURE 3. Antioxidant activity of essential oils at (80 µg·mL^{-1}) in DPPH and β-carotene bleaching assays.

TBHQ. Gülçin, *et al*. (2012) reported that the DPPH free radical scavenging activity of clove oil increased with an increasing concentration of clove oil. The antioxidant activities of the basic components of the essential oils of thyme (carvacrol and thymol) have been demonstrated (Jordan *et al*., 2013). The study of some antioxidants used in cancer treatment is a rapidly improving area. Antioxidants have been extensively studied for their ability to prevent or treat cancer in humans. Also, a regular intake of natural antioxidants is associated with reduced risks of cancer. The antioxidant activity exhibited by the tested EOs justifies traditional uses of Egyptian herbs. The observed antioxidant potential should be attributed to the phenolic oil constituents, while the oil chemoprotective efficacy against oxidative stress-mediated disorders is mainly due to its free radical scavenging properties. Recent studies have demonstrated that α-pinene (2,6,6-tri-methyl-bicyclo [3.1.1] hept-2-ene), the main component in Egyptian juniper, is present naturally in the essential oils of many aromatic plants and has antioxidant properties according to DPPH radical, hydroxyl radical, superoxide anion, malonaldehyde and β-carotene bleaching methods (Aydin *et al*., 2013). α-pinene was reported tohave a broad spectrum of biological activities, i.e. antioxidant and anticancer activities (Wang *et al*., 2012). Plant extracts, especially volatiles and phenolic extracts, as natural antioxidant, showed suppressing action against proliferation of human cancer cells. The degrees of anti-proliferation were time and dose-dependent.

3.3. *In vitro* antiproliferative and cytotoxic activity

The antiproliferative activities of the Egyptian thyme, juniper and clove essential oils were evaluated against 5 different human cancer cell lines including liver HepG2, breast MCF-7, prostate PC3, colon HCT116 and lung A549 using an SRB assay, in comparison with doxorubicin as the reference drug. The antiproliferative activities were expressed by median growth inhibitory concentration (IC_{50}) and provided in Table 3. From the results it is evident that although thyme oil displayed potent growth inhibitory activity against MCF-7, PC3, HCT116 and A549, it had no activity against the HepG2 cell line. The thyme oil exerted antiproliferative activity with IC_{50} values of 22.60±3.00, 23.00±4.20, 24.60±2.60 and 22.90±3.30 µg·mL^{-1} in MCF-7, PC3, HCT116 and A549 respectively. It is clear that, while thyme oil had an antiproliferative effect in PC3 and HCT116 cell lines closed to the doxorubicin, it was more effective than the doxorubicin in MCF-7 and A549 cell lines. For juniper oil, the results revealed that juniper oil shows potent growth inhibitory activity against HepG2, MCF-7 and A549 cell lines with no activity against PC3 and HCT116 cell lines. The IC_{50} was 18.00±2.40, 22.80±3.50 and 21.75±2.80 µg·mL^{-1} in HepG2, MCF-7 and A549 cell lines, respectively. It is clear that the antiproliferative effect of juniper oil was more potent than the doxorubicin in HepG2, MCF-7 and A549 cell lines. For clove oil the result revealed that while treatment with clove oil had no effect on HepG2 or MCF-7 cell lines, the IC_{50} was 24.00±3.90, 19.00±2.25 and 21.80±3.75 µg·mL^{-1} in PC3, HCT116 and A549 cell lines, respectively. It is clear that the antiproliferative effect of clove oil was similarly potent to the doxorubicin in PC3, HCT116 and A549 cell lines. The abundance of various components in each essential oil, comprising a complex mixture of mono and sesquiterpenes, accounts for the cytotoxic activity of each essential oil. The investigation of tumor growth inhibitors is a major obstacle in the medical field. For these reasons, the development of novel anticancer drugs

TABLE 3. Cytotoxicity of Egyptian thyme, juniper and cloves essential oils against different types of human malignant cell lines.

	IC_{50} (µg·mL^{-1})				
Compound	**HepG2**	**MCF-7**	**PC3**	**HCT116**	**A549**
Doxorubicin	20.10±2.00	24.00±2.50	18.00±2.00	19.25±2.00	25.50±2.70
Thyme oil	NA	22.60±3.00	23.00±4.20	24.60±2.66	22.90±3.30
Juniper oil	18.00±2.40	22.80±3.50	NA	NA	21.75±2.80
Cloves oil	NA	NA	24.00±3.90	19.00±2.25	21.80±3.75

Data are expressed as means ± S.E. of four separate experiments.
NA is no activity.

is still necessary and is in high demand. This work is an attempt to examine the essential oils for their cytotoxicity activity against five human cancer cell lines. The results revealed that the Egyptian essential oils under investigation possessed significant anticancer activity. The presence of many volatile compounds that possess antioxidant activity may be responsible for enhancing the antioxidant activity *in vitro* as well as protecting human organs through the scavenging free radicals (Abd-Algader *et al*., 2013). β-Caryophyllene, which is a common volatile component of all tested essential oils, has been reported to potentiate the anticancer activity of many tumor cell lines (Rosato *et al*., 2008). The anti-oxidative activity of natural compounds is frequently accompanied by cytoprotection. Nevertheless, comparative evaluation of the cytotoxicity and the anti-oxidative activity of the oil of cloves (*Syzygium aromaticum*) and its components (generally recognized as safe) showed that this type of oil and its major component eugenol were highly cytotoxic against human fibroblasts and endothelial cells even at low concentrations (Prashar *et al*., 2006). Our results showed that Egyptian clove oil, which contain 82.71% eugenol was similarly potent to the doxorubicin drug in PC3, HCT116 and A549 cell lines. The reason for the cytotoxicity of eugenol is probably the induction of apoptosis. It is interesting to examine the findings of Pisano *et al*. (2007), who demonstrated that dimeric forms (biphenyls) of eugenol elicited specific antiproliferative and pro-apoptotic activity on neuroectodermal tumor cells, possibly indicating their anticancer effect. Eugenol showed cytotoxic effects and acted as a genotoxicant in VH10 human fibroblasts and in Caco-2 human colonic cells, but not in HepG2 human hepatoma cells. Until now, various authors have reported on the antitumor activities of EOs and their constituents. Our results revealed that thyme oil had an anti-proliferative effect in PC3 and HCT116 cell lines closed to the doxorubicin; it was more effective than the doxorubicin in MCF-7 and A549 cell lines. According to AitM'barek *et al.* (2007), the thyme oil, which contains thymol as its major constituent, has an important *in vitro* cytotoxic activity against tumor cells. Our thyme EO inhibited the viability of several tumor cell lines. The activity of the oil is frequently attributed to the specific oil constituents, Tsukamoto *et al.* (1989) reported that thymol, which is the major constituent in our EO, might be involved in the stimulation of the active proliferation of pulp fibroblasts. Whether the thymol alone, or in combination with other oil constituents is responsible for the observed cytotoxicity against tumor cells still remains to be revealed, and presents an important limitation of the study. Strong antioxidant and antitumor activity supports the traditional use of thyme, which showed the strongest biological activity. In addition to their use in food and cosmetics, the thyme oil represents a great potential in anti-cancer treatments and certainly deserves further study (Nikoli *et al*., 2014). The antitumor activities of juniper oil and its constituents showed good and moderate levels of tumor inhibition. Our results showed that Egyptian juniper oil, which contains 26.19% α-pinene, was more potent than the doxorubicin drug in HepG2, MCF-7 and A549 cell lines. Wang *et al*. (2012) has mentioned that α-pinene demonstrated strong cytotoxicity towards human ovarian cancer cell lines (SK-OV-3 and HO-8910) and the human hepatocellular liver carcinoma cell line (Bel-7402). Also, Matsuo *et al.* (2011) revealed that α-pinene was able to induce apoptosis evidenced by early disruption of the mitochondrial potential and production of reactive oxygen species. The exact mechanisms of the cytotoxic action of α-pinene are not known, but oxidative stress is thought to be the main responsible mechanism in its cellular toxicity. In addition to oxidative stress, previous studies reported that different mechanisms have been linked to plant products' cytotoxicity, including: (i) proteasome inhibition; (ii) topoisomerase inhibition; (iii) inhibition of fatty acid synthesis; (iv) accumulation of p53; (v) induction of cell cycle arrest; (vi) inhibition of phosphatidyl-inositol 3-kinase; or (vii) enhanced expression of c-fos and c-myc (Brusselmans *et al*., 2005). Aydin *et al*. (2013) indicated that α-pinene is neither genotoxic nor mutagenic on healthy neurons or N2a NB cells and demonstrates that pure α-pinene possesses weak antioxidant and cytotoxic activity in cultured primary rat neurons. In addition, pure α-pinene has weak antioxidant properties and little anticancer potential on rat N2a NB cell line and suggests that α-pinene is of a limited therapeutic use as an anticancer agent. This may be revealed in the importance of synergism among all the components in essential oil. Our results in Table 1 showed that, beside the main volatile component of Egyptian juniper (α-pinene), there were other considerable amounts of volatile antioxidants such as, β-caryophyllene (9.91%), γ-Cubebene (8.85%), δ-Cadinene (5.58%), α-Humulene (4.83%), D-Germacrene (3.84%), Linalool (3.16%), α-Muurolene (2.53%), α-Terpineol (2.85%) as well as other minor constituents which together may have a synergistic effect. Thus, the oil is more effective than its pure, main component.

4. CONCLUSIONS

To summarize, we concluded that Egyptian thyme, juniper and clove essential oils are potential candidates for further development as an adjuvant in the modern chemotherapeutic treatment of different types of cancers.

REFERENCES

Abd-Algader NN, El-Kamali HH, Ramadan MM, Ghanem KZ, Farrag AH. 2013. *Xylopia aethiopica* volatile compounds protect against panadol-induced hepatic and renal toxicity in male rats. *World Appl. Sci. J*. **27**, 10–22.

Adams RP. 1995. Identification of essential oil components by gas chromatography/mass spectrometry; Allured Publishing: Carol Stream, IL, USA.

AitM'barek L, Ait Mouse H, Jaâfari A, Aboufatima R, Benharref A, Bénard J, El Abbadi N, Bensalah M, Gamouh A, Chait A, Dalal A. 2007. Cytotoxic effect of essential oil of thyme (*Thymus broussonettii*) on the IGR-OV1 tumor cells resistant to chemotherapy. *Braz. J. Med. Res*. **40**, 1537–1544. http://dx.doi.org/10.1590/S0100-879X2007001100014.

Ali MM, Sohair AH. 2007. Role of some newly synthesized tetrahydro-naphthalin-thiazol derivatives as anticancer complexes. *Int. J. Cancer Res*. **3**, 103–110. http://dx.doi.org/10.3923/ijcr.2007.103.110.

Aydin E, Türkez H, Geyikoğlu F. 2013. Antioxidative, anticancer and genotoxic properties of α-pinene on N2a neuroblastoma cells. *J. Biol*. **68**, 1004–1009.

Brusselmans K, Vrolix R, Verhoeven G, Swinnen J. 2005. Induction of cancer cell apoptosis by flavonoids is associated with their ability to inhibit fatty acid synthase activity. *J. Biol. Chem*. **280**, 5636–5645. http://dx.doi.org/10.1074/jbc.M408177200.

Chen D, Daniel KG, Chen MS, Kuhn DJ, Landis-Piwowar KR, Dou QP. 2005. Dietary flavonoids as proteasome inhibitors and apoptosis inducers in human leukemia cells. *Biochem. Pharmacol*. **69**, 1421–1432. http://dx.doi.org/10.1016/j.bcp.2005.02.022.

Dugo P, Mondello L, Dugo L, Stancanelli R, Dugo G. 2000. LC–MS for the identification of oxygen heterocyclic compounds in citrus essential oils. *J. Pharmaceut. Biomed. Anal*. **24**, 147–154. http://dx.doi.org/10.1016/S0731-7085(00)00400-3.

Elattar I. 2003. National Cancer Institute Egypt. Magnitude of liver cancer in Egypt.

Ghomi JS, Ebrahimabadi AH, Bidgoli ZD, Batooli H. 2009. GC/MS analysis and *in vitro* antioxidant activity of essential oil and methanol extracts of *Thymus caramanicus* Jalas and its main constituent carvacrol. *Food Chem*. **115**, 1524–1528. http://dx.doi.org/10.1016/j.foodchem.2009.01.051.

Gülçin I, Elmastas M, Aboul-Enein HY. 2007. Determination of antioxidant and radical scavenging activity of basil (*Ocimum basilicum*) assayed by different methodologies. *Phytother Res*. **21**, 354–361.

Gülçin I, Elmastas M, Aboul-Enein HY. 2012. Antioxidant activity of clove oil a powerful antioxidant source. *Arab J Chem*. **5**, 489–499. http://dx.doi.org/10.1016/j.arabjc.2010.09.016.

Gülçin I. 2006. Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). *Toxicol*. **217**, 213–220. http://dx.doi.org/10.1016/j.tox.2005.09.011.

Hamedo HA, Abdelmigid HM. 2009. Use of antimicrobial and genotoxicity potentiality for evaluation of essential oils as food preservatives. *Open Biotechnol. J*. **3**, 50–56. http://dx.doi.org/10.2174/1874070700903010050.

Jo JU, Park S, Parki Y, Chae YZ, Lee GH, Park GY, Jang BC. 2012. *Pinus densiflora* leaf essential oil induces apoptosis via ROS generation and activation of caspases in YD-8 human oral cancer cells. *Inter. J. Oncol*. **40**, 1238–1245.

Jordan MJ, Maria VL, Rota C, Loran S, Sotomayor JA. 2013. Effect of bioclimatic area on the essential oil composition and antibacterial activity of *Rosmarinus officinalis* L. *Food Control* **30**, 463–468. http://dx.doi.org/10.1016/j.foodcont.2012.07.029.

Lamaty G, Menut C, Bessiere JM, Zollo JM, Fekam PHA. 1987. Aromatic plants of tropical Central Africa: I. Volatile components of two annonaceae from Cameroon: *Xylopia aethiopica* (Dunal) A. Richard and *Monodora myristica* (Gaerth.) Dunal. *Flavour. Frag. J*. **2**, 91–94. http://dx.doi.org/10.1002/ffj.2730020302.

Lee KG, Shibamoto T. 2001. Antioxidant property of aroma extract isolated from clove buds *Syzygium aromaticum*. *Food Chem*. **74**, 443–448. http://dx.doi.org/10.1016/S0308-8146(01)00161-3.

Lesjak MM, Beara IN, Orcic DZ, Anackov GT, Balog KJ, Franciškovic MM. 2011. *Juniperus sibirica* Burgs dorf. as a novel source of antioxidant and anti-inflammatory agents. *Food Chem*. **124**, 850–856. http://dx.doi.org/10.1016/j.foodchem.2010.07.006.

Matsuo AL, Figueiredo CR, Arruda DC, Pereira FV, Scutti JAB, Massaoka MH, Travassos LR, Sartorelli P, Lago JHG. 2011. α-Pinene isolated from *Schinus terebinthifolius* Raddi (Anacardiaceae) induces apoptosis and confers antimetastatic protection in a melanoma model. *Biochem. Biophys. Res. Commun*. **411**, 449–454. http://dx.doi.org/10.1016/j.bbrc.2011.06.176.

Nikoli M, Glamo J, Isabel C, Ferreira FR, Calhelha RC, Fernandes Â, Markovi T, Markovi D, Giweli A, Sokovi M. 2014. Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. and Reut and *Thymus vulgaris* L. essential oils. *Ind. Crop Prod*. **52**, 183–190. http://dx.doi.org/10.1016/j.indcrop.2013.10.006.

Orav J, Koel M, Kailas T, Müürise M. 2010. Comparative analysis of the composition of essential oils and supercritical carbon dioxide extracts from the berries and needles of Estonian juniper (*Juniperus communis* L.). *Procedia Chem*. **2**, 161–167. http://dx.doi.org/10.1016/j.proche.2009.12.023.

Paul S, De Castro AJ, Lee HJ, Smolarek AK, So JY, Simi B, Wang CX, Zhou R, Rimando AM, Suh N. 2010. Dietary intake of pterostilbene, a constituent of blue berries, inhibits the β-catenin/p65 downstream signaling pathway and colon carcinogenesis in rats. *Carcinogenesis* **31**, 1272–1278. http://dx.doi.org/10.1093/carcin/bgq004.

Pepeljnjak S, Kosalec I, Kalodera Z, Blazevic N. 2005. Antimicrobial activity of juniper berry essential oil (*Juniperus communis* L., *Cupressaceae*). *ActaPharmaceut*. **55**, 417–422.

Pisano M, Pagnan G, Loi M, Mura ME, Tilocca MG, Palmieri G, Fabbri D, Dettori MA, Delogu G. 2007. Antiproliferative and pro-apoptotic activity of eugenol-related biphenyls on malignant melanoma cells. *Mol. Cancer* **8**, 234–242.

Prashar A, Locke IC, Evans CS. 2006. Cytotoxicity of clove (*Syzygium aromaticum*) oil and its major components to human skin cells. *Cell Proliferat*. **39**, 241–248. http://dx.doi.org/10.1111/j.1365-2184.2006.00384.x.

Ramadan MM, Abd-Algader NN, El-kamali HH, Ghanem KZ, Farrag AH. 2013a. Volatile compounds and antioxidant activity of the aromatic herb *Anethum graveolens*. *J. Arab Soc. Medical Res*. **8**, 79–88. http://dx.doi.org/10.4103/1687-4293.123791.

Ramadan MM, Abd Algader NN, El-kamali HH, Ghanem KZ, Farrag AH. 2013b. Chemo preventive effect of *Coriandrum sativum* fruits on hepatic toxicity in male rats. *World J. Med. Sci*. **8**, 322–333.

Ramadan MM, Yehia HA, Shaheen MS, Abed EL, Fattah MS. 2014. Aroma volatiles, antibacterial, antifungal and antioxidant properties of essential oils obtained from some spices widely consumed in Egypt. *American-Eurasian J. Agric. Environ. Sci*. **14**, 486–494.

Rosato A, Vitali C, Gallo D, Balenrano L, Mallamaci R. 2008. The inhibition of Candida albicans by selected essential oils and their synergism with amphotericin-B. *Phytomed*. **15**, 635–638. http://dx.doi.org/10.1016/j.phymed.2008.05.001.

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Nat. Cancer Instit*. **82**, 1107–1112. http://dx.doi.org/10.1093/jnci/82.13.1107.

Teixeira B, Marques A, Ramos C, Neng NR, Nogueir JMF, Saraiva JA, Nunes ML. 2013. Chemical composition and antibacterial and antioxidant properties of commercial essential oils. *Ind. Crop Prod*. **43**, 587–595. http://dx.doi.org/10.1016/j.indcrop.2012.07.069.

Tsukamoto Y, Fukutani S, Takeuchi S, Okamoto T, Mori M. 1989. Some phenolic compounds stimulate the proliferation of human pulpal fibroblasts. *Shika Kiso Igakkai Zasshi* **31**, 357–362. http://dx.doi.org/10.2330/joralbiosci1965.31.357.

Wang W, Li N, Luo M, Zu Y, Efferth T. 2012. Antibacterial activity and anticancer activity of *Rosmarinus officinalis* L. essential oil compared to that of its main components. *Molecules* **17**, 2704–2713. http://dx.doi.org/10.3390/molecules17032704.