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Research Article

Antioxidant and cytotoxic activities of *Artemisia monosperma* L. and *Tamarix aphylla* L. essential oils

Ramy M. ROMEILAH[1,3], Hossam S. EL-BELTAGI[2,3]*, Emad A. SHALABY[3], Kareem M. YOUNES[4,5], Hani EL MOLL[1], Saravanan RAJENDRASOZHAN[1], Heba I. MOHAMED[6]

[1]*Hail University, College of Science, Department of Chemistry, Hail, Saudi Arabia; r.romeilah@uoh.edu.sa; h.elmoll@uoh.edu.sa; s.rajendrasozhan@uoh.edu.sa*
[2]*King Faisal University, College of Agriculture and Food Sciences, Agricultural Biotechnology Department, P.O. Box 420, Al-Ahsa 31982, Saudi Arabia; helbeltagi@kfu.edu.sa (*corresponding author)*
[3]*Cairo University, Faculty of Agriculture, Department of Biochemistry, Giza, 12613, Egypt; dremad2009@yahoo.com*
[4]*Hail University, College of Pharmacy, Department of Pharmaceutical Chemistry, Hail, Saudi Arabia; k.younes@uoh.edu.sa*
[5]*Cairo University, Faculty of Pharmacy, Department of Analytical Chemistry, Cairo, Egypt*
[6]*Ain Shams University, Faculty of Education, Biological and Geological Sciences Department, Cairo, Egypt; hebaibrahim79@gmail.com*

Abstract

Essential (volatile) oil from leaves of *Artemisia monosperma L.* belonging to family Asteraceae, and aerial parts of *Tamarix aphylla* L. (Athel) belonging to family Tamaricaceae were collected from the desert of Ha'il region, northern region of Saudi Arabia, hydro distilled by Clevenger apparatus and analysed by means of GC-MS techniques. Antioxidant activities of essential oils of *A. monosperma* and *T. aphylla* compared with ascorbic acid and butylated hydroxytoluene (BHT) as reference antioxidant compound were determined by method of DPPH radical scavenging assay and ABTS assay. *In vitro* screening of potential cytotoxicity of essential oils was also evaluated against human promyelocytic leukaemia cell lines (HL60 and NB4). The GC/MS analysis of *A. monosperma* essential oil resulted in identification of 61 components predominated mainly by β-Pinene as principal component (29.87%) and *T. aphylla* resulted in identification of 37 components of essential oil predominated mainly by 6,10,14- trimethyl-2-pentadecanone (21.43%) as principal component. Antioxidant activity as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and 2,2 -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) increased with increasing essential oil concentrations of *A. monosperma* and *T. aphylla* (25, 50, 75, 100 and 200 µg mL^{-1}). The most pronounced increases detected in the high concentrations of the two essential oils. Biologically, essential oil extracts exhibited cytotoxicity effects in dose dependent manner against human promyelocytic leukaemia cell lines (HL60 and NB4). In conclusion, *A. monosperma* and *T. aphylla* essential oils could be valuable source for cytotoxic agents with high safety and selective cytotoxicity profiles.

Keywords: antioxidant activity; *Artemisia monosperma*; cytotoxic activity; essential oil; *Tamarix aphylla*

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Introduction

In the Ancient history there were used plants and other natural products for a range of purposes such as food, decorations, clothing, soaps, cosmetics, and medicinal uses (Pandey and Tripathi, 2011). Essential oil-bearing plants were observed to be useful in traditional medicine for treatment and/or prevention of various diseases (Mohamed *et al.*, 2018a, b; El-Beltagi *et al.*, 2018; 2019a, b). These plants recently produced a total yearly output of oils to above 45000 tones, produced by more than 100 precious essential oils used in market terms, as antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, cytotoxic, and many other effects (Sikka and Bartolome, 2018; El-Beltagi *et al.*, 2020a, b; Dawi *et al.*, 2021). Vast quantities of them are also used for the smell in perfumery field as well as in the pharmaceutical and food sector (Malik, 2018).

Furthermore, aromatherapy, a major complementary and alternative medicine industry is very commonly used in essential oils (CAM). It is used for a series of diseases for treatment, it is utilized in variety of activities, including inhalations, oral administrations, mouthwashes, and even trans-dermal processes (Bae *et al.*, 2018). However, essential oils contain various chemical classes such as terpenoids, alcohols, aldehydes, ketones, oxides, phenolic ethers, and hydrocarbons (Aly *et al.*, 2013; Dhif *et al.*, 2016; Hamed *et al.*, 2019).

Antioxidants are active ingredients capable of preventing or postponing process of oxidation and autoxidation by reducing generation of deleterious free radicals (Mohamed *et al.*, 2009; Abdel-Rahim and El-Beltagi, 2010; Abd El- Rahman *et al.*, 2012; El-Beltagi *et al.*, 2017; Abd El-Maksoud *et al.*, 2018). These radicals can cause severe organ damage to cells, tissues, and even entire organs in some circumstances, which can, as a result, cause lethal diseases (Shallan *et al.*, 2010a, b; Apostolova and Victor, 2015). Many phytochemicals and other microelements like ascorbic acid, tocopherol, carotenoids, flavonoids, anthocyanins, have antioxidant properties (Helmi and Mohamed, 2016; *Guo et al.*, 2017; Akladious and Mohamed, 2017). They based their mechanism of action, however, mostly on DNA oxidation inhibition, which prevents lipid oxidation, build up of atherosclerotic plaques, and low-density lipoprotein oxidation (LDL), preventing the risks of various types of cancer and different cardiovascular diseases (Reis *et al.*, 2016; Afify and El-Beltagi, 2011; Afify *et al.*, 2012).

Therapeutic, cosmetic, and aromatic properties of Asteraceae family are recognized. The key uses of this family are astringent, cholesteric, anti-haemorrhagic, antimicrobial, antioxidant, diuretics, analgesics and antispasmodic (Fabri *et al.*, 2011; Maham *et al.*, 2014). Asteraceae family is indented with essential oiled plants in plant kingdom, in which Artemisia genus takes its lead for bio-prospection. *A. monosperma* Del is a bushy, leafy tree with long small leaves containing scattered hair (Abad *et al.*, 2012). In Saudi Arabia, it is famous, where it goes under the local name of Aader or Selikah (Khan *et al.*, 2012). The flower spikes have tiny green bracts and round green bud-like flowers. It is a perennial fragrant plant that grows high in the Deserts of Arabia, up to 1 meter in height (Khan *et al.*, 2012). *A. monosperma* is also found in the desert of other Arabian countries such as Iraq, Kuwait, Egypt, and Saudi Arabia (Khan *et al.*, 2012). Most of the species have strong scents and bitter tastes, that impede the herbivory, because of presence of terpenoids and sesquiterpene lactones (Bora and Sharma, 2011). A test was carried out in laboratory in essential oils and chemical components of many different species of Artemisia to avoid oxidative harm by preventing or loosening free radicals or reactive species of oxygen. They have proven to be an alternative to synthetic antioxidants (Singh *et al.*, 2015).

Tamaricaceae is a large family comprising of several genera, many of whose are tiny shrubs and trees described as exotic, deciduous, or invasive. Previous studies have detected presence of different types of secondary metabolites in plant extract of several genera of *Tamaricaceae* like flavonoids (El Ansari *et al.*, 1976), phenolics (Souliman *et al.*, 1991), tannins (Orabi *et al.*, 2015), and alkaloids (Yusufoglu *et al.*, 2015). *Tamarix aphylla* is one of species that comes from this family that lives naturally in Asia, North Africa, south-eastern Europe and Saudi Arabia (Al Sobeai, 2018; Jasiem *et al.*, 2019). Several countries have used *T. aphylla* in traditional medicine. Its leaves were used for wounds and abscess healing, as astringent, and for rheumatism and joint pain (Marwat *et al.*, 2009; Mahfoudhi *et al.*, 2016). *T. aphylla* extract caused inhibition of insect growth because of presence of phenolic compounds like ellagic acid, tannin and may be used as an insecticidal activity (Klocke *et al.*, 1985). Aphyllin is also the isolated isoferulic acid derivative, which shows separate

operation for radical scavenging and increases viability of human keratinocytes (Nawwar *et al.*, 2009). In Saudi Arabia, alcohol extract of *T. aphylla* leaves have antioxidant, anti-inflammatory, and wound-healing functions because of the existence of active phytochemical compounds such as flavonoids or polyphenols (Yusufoglu and Alqasoumi, 2011).

Many studies have examined the potential roles of various *T. aphylla* extracts in prevention and/or treatment of many ailments (Mahfoudhi *et al.*, 2016; Qadir *et al.*, 2014) however, there has been very little research focusing on studying the volatile essential oil composition or assessing the antiproliferative effects against certain cancer cell lines. So that the present study aimed to essential oil hydro distilled from leaves of *A. monosperma*, and aerial parts of *T. aphylla* L. wild grown in desert of Ha'il region, northern region of Saudi Arabia was analysed by gas chromatography-mass spectrometry (GC-MS) and the antioxidant activity was measured. *In vitro* cytotoxic activities of aqueous extract against human promyelocytic leukaemia cell lines (HL60 and NB4) were also assessed.

Materials and Methods

Plant material

The leaves of *Artemisia monosperma* belonging to family Asteraceae, and aerial parts of *Tamarix aphylla L* (Athel) belonging to family Tamaricaceae were collected from desert of Ha'il region, northern region of Saudi Arabia. Dr. Mohamed Osama El-Segaee, Professor of Taxonomy, Faculty of Agriculture, Cairo University, kindly named plant samples.

Essential oil extraction

Five hundred grams of *Artemisia monosperma* dried leaves and dried aerial parts of *Tamarix aphylla* were hydro distilled in Clevenger type apparatus for 4h and following standard procedure described in literature (European Pharmacopoeia, 2005). The essential oils were dried over anhydrous sodium sulphate, stored in a dark glass bottle, and kept at 4 °C until analysis and amount of oil obtained from plant material was calculated as:

$$Oil\ (\%\ v/w) = \frac{Observed\ volume\ of\ oil\ (mL)}{Weight\ of\ sample\ (g)} x100$$

GC/MS analysis of essential oils

Essential oils were analysed by GC-MS according to Adams (1989). The GC/MS analysis was performed on rmoquest-Finnigan Trace GC-MS equipped with DB-5 (5% phenyl) methylpolysiloxane column (60 m \ 0.25 mm i.d., film thickness 0.25 μm). The injection temperature was 220 °C and oven temperature was raised from 40 °C (3 min hold) to 250 °C at a rate of 5 °C /min, then held at 250 °C for 2 min; transfer line temperature was 250 °C. One microliter of the sample was injected and helium was used at flow rate of 1.0 ml/min as carrier gas. Mass spectrometer with ionizing voltage of 70 eV was scanned over 40 to 500 m/z and identification was based on standard mass library used by National Institute of Standards and Technology (NIST Version 2.0) and Wiley libraries to detect the possibilities of essential oil components.

DPPH free radical scavenging assay

Radical scavenging activity of plant essential oils against stable DPPH radical was determined spectrophotometrically (Brand-Williams *et al.*, 1995). On UV/visible light spectrophotometer, colorimetric shifts (from deep-violet to light-yellow) were calculated at 517 nm when DPPH• was reduced. In terms of hydrogen donation or radical scavenging potential, antioxidant activity of essential oils was calculated using stable radical DPPH. Fifty microliters of different concentrations (25, 50, 75, 100 and 200 µg mL^{-1}) of essential

oils containing 5, 10, 20, 40 and 60 µg mL^{-1} of dimethyl sulphoxide (DMSO) as well as ascorbic acid and butylated hydroxytoluene (BHT) (as standard antioxidant compounds) were placed in suitable tubes and 5 mL of 0.004% methanolic solution of DPPH• was added to each tube to give final concentrations of 25, 50, 75, 100 and 200 µg mL^{-1}). Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined after 1 h for all samples. Methanol was used to zero the spectrophotometer. Absorbance of DPPH radical without antioxidant, *i.e.* the control, was measured. Care was taken to minimize DPPH radical stock solution's loss of free radical activity. Radical scavenging activity percentage inhibition was calculated in accordance with formula of Yen and Duh (1994):

$$\text{DPPH scavenging effect (\%)} = (A_{C(o)} - A_{A(t)}) / A_{C(o)}) \times 100$$

Where $A_{C(o)}$ is absorbance of control at t = 0 min and $A_{A(t)}$ is absorbance of antioxidant at t = 1 h.

Simple regression analysis was used to derive IC_{50} value (corresponding to concentration of 50% of inhibition).

ABTS radical scavenging activity

With some modifications, ABTS radical scavenging activity was determined according to technique described by Re *et al.* (1999). ABTS solution was prepared by dissolving ABTS (2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) in water to 7 mM concentration. ABTS radical cation ($ABTS^{\bullet+}$) was also developed by reacting with 2.45 mM potassium persulfate (final concentration) ABTS solution and allowing mixture to stand in dark for 12-16 h before use at room temperature. Then, absorbance of final ABTS radical solution was adjusted to 0.7 at 734 nm. The essential oils with concentrations of 25, 50, 75, 100, 200 µg mL^{-1} as well as ascorbic acid and butylated hydroxytoluene (BHT) standards with concentrations of 5, 10, 20, 40 and 60 µg mL^{-1} were combined with stabilized radical solution and incubated at 30°C. After 30 min, the absorbances were spectrophotometrically measured at 734 nm. Percentage inhibition of radical scavenging activity was calculated according to formula of Yen and Duh (1994):

$$\text{ABTS scavenging effect (\%)} = ((A_{C(o)} - A_{A(t)}) / A_{C(o)}) \times 100$$

Where $A_{C(o)}$ is absorbance of control at t = 0 min and $A_{A(t)}$ is absorbance of antioxidant at t = 1 h.

Simple regression analysis was used to derive the IC_{50} value (corresponding to concentration of 50% of inhibition).

Cytotoxic activity of essential oils

Human promyelocytic leukaemia cell lines (HL60 and NB4) obtained from American Type Culture Collection (ATCC). All these cells were maintained in RPMI-1640 supplemented with 10% FBS, 2 mmol/L L- glutamine, penicillin (100 U/ml), and streptomycin (100 mg mL^{-1}) in a humidified atmosphere of 5% CO_2 at 37 °C for 24 h after that the cell counts were determined. After this period the cell viability was evaluated using trypan blue technique. The viability percentage of cancer cells were measured by modified cytotoxic trypan blue-exclusion technique of Bennett *et al.* (1976). 2 x 105 cells/ml were seeded on 96-well plate prior to assay and then viability percentage of cancer cells was determined by treatment with different amounts of essential oils examined to give final concentrations of 25, 50, 75, 100, 200 µg ml^{-1}. The plate was incubated at 37 °C for 24h under 5% CO_2. Final volume in each experiment was made up to 100 µl with media containing 1% dimethyl sulphoxide (DMSO). Control cells were treated with equivalent amount of vehicle DMSO and then an equal volume of 0.4% trypan blue was added to each experiment and left to stand for 5 minutes at room temperature. 10 µl of stained cells were added in a hemocytometer slide and number of viable (unstained) and dead (stained) cells were counted. Each experiment was carried out in triplicate. Percentage of viable cells is [number of viable cells] divided by total number of [dead plus viable cells], multiplied by 100.

% viable cells = 100 x [live cells] / [dead + live cells]

The percentage of dead cells of each cell line were plotted against essential oil concentrations to obtain the "50% lethal concentration" IC_{50}, defined as essential oil concentration necessary to cause 50% death.

Statistical analysis

Statistical analyses were done using SPSS (version 25) program. Mean and standard error were descriptive measures of quantitative data using analysis of variance test (ANOVA) for independent samples. P-values <0.05 were considered significant.

Results and Discussion

Oil composition by GC/MS

The hydro distillation of *A. monosperma* leaves yield oil about 1.04% (v/w). The essential oils were analysed by GC/MS for determination of their components and results are given in Tables 1 as a relative peak area of each constituent. As shown in Table 1, GC-MS analysis of *A. monosperma* essential oil resulted in identification of 61 components predominated mainly by β-Pinene as principal component (29.87%), α-Terpinolene (8.56%), Bornyl acetate (7.63%), cis-β-Ocimene (7.11%) and Limonene (5.65%) as major ones. Oil composition of present study was similar to results of Khan *et al.* (2012) who found that β-pinene and α-terpinolene prevailed among components of Saudi leaf and stem essential oils. Oil composition determined in present study differed significantly from those reported earlier. Soliman (2007) found that propenoic acid, 3-phenylethyl ester, 2-pinene-4-one, *p*-cymene and geraniol as major constituents of *A. monosperma* oil. While Saleh (1985) observed that 3-methyl-3-phenyl-1,4-pentadiyne and capillen as major constituents of *A. monosperma* leaf oil. *Artemisia scoparia, Artemisia judaica* and *Artemisia sieberi* growing in northern region of Saudi Arabia were investigated regarding their volatile oil contents. Yield of oil ranged between 0.30 and 0.41%, (w/w). *A. monosperma* showed highest number of compounds with 30 components representing 93.78% of oil composition. Nevertheless, *A. judaica* showed the lowest number of compounds with just 16 components containing 87.47% of essential oil. *A. scoparia* and *A. sieberi* are both composed of 17 components, comprising 97.14 and 94.2% of total oil composition. *A. sieberi* and *A. judaica* were dominated by spathulenol (30.42 and 28.41%, respectively). For *A. monosperma*, butanoic acid (17.87%) was a major component (Guetat *et al.*, 2017).

The hydro distillation of *T. aphylla* aerial parts yield oil about 0.22% (v/w). The essential oils were analysed by GC/MS for determination of their components and results are given in Table 2 as a relative peak area of each constituent. As shown in Table 2, the GC-MS analysis of *T. aphylla* essential oil resulted in identification of 37 components predominated mainly by 6,10,14-trimethyl-2-pentadecanone as principal component (21.43%), 1,8-cineole (15.75%), ledol (8.24%), α-pinene (7.31%), and trans-pinocarveol (6.81%) as major ones. Similar findings were reported by Alhourani *et al.* (2018) who found that GC-MS analysis of *T. aphylla* EO revealed its richness in nonterpenoid nonaromatic hydrocarbon (52.39%), with predominance of 6,10,14-trimethyl-2-pentadecanone as principal component.

Different compositions of the essential oil from different *Tamarix* species were also reported in literature. As described by Orfali (2009), bicyclo[2.2.2]octan-2-one was found to be the major compound (46.09%) in *T. nilotica* of Saudi Arabia. Hexadecanoic acid methyl ester was reported as major principle in *T. chinensis* fruit (Mahemuti *et al.*, 2015). Hexadecanoic acid (in aerial parts and stems), 2,4-nonadienal (in flowers), and germacrene D (in leaves) were, however, reported as majors of *T. boveana* (Saïdana *et al.*, 2008). Like in *T. chinensis*, nonaromatic hydrocarbons approached abundant group of *T. aphylla* aerial sections, while fatty acids and fatty esters are majors in *T. boveana* leaves. On the other hand, among terpenes, oxygenated and hydrocarbon sesquiterpenes are prevalent in *T. aphylla* and *T. boveana* (Saïdana *et al.*, 2008) respectively.

Table 1. Chemical composition of *A. monosperma* leaves essential oils

No.	RT	RI	Compound name	Area sum %
1	4.55	921	α-Thujene	0.21
2	5.11	935	α-Pinene	3.98
3	5.34	944	Camphene	0.06
4	5.56	974	Sabinene	4.14
5	5.87	992	β-Pinene	29.87
6	5.98	997	β-Myrcene	0.32
7	6.07	1001	Mesitylene	0.07
8	6.19	1004	α-Phellandrene	0.11
9	6.57	1019	α-Terpinene	0.53
10	6.84	1023	p-Cymene	0.09
11	7.01	1029	Limonene	5.65
12	7.17	1032	γ-Terpinene	0.37
13	7.22	1045	trans-β-Ocimene	2.13
14	7.31	1049	cis-β-Ocimene	7.11
15	7.66	1052	α-Terpinolene	8.56
16	8.29	1079	cis-Sabinene hydrate	5.32
17	8.61	1110	Linalool	1.34
18	9.04	1123	p-Mentha-1,3,8-triene	0.04
19	10.21	1127	β-Thujone	0.08
20	10.96	1142	trans-p-Menth-2-en-1-ol	0.09
21	11.56	1155	Camphor	1.12
22	12.07	1186	Terpinen-4-ol	1.12
23	12.23	1192	p-Cymene-8-ol	1.61
24	12.32	1197	α-Terpineol	1.11
25	12.89	1225	Fenchyl acetate	0.06
26	13.02	1229	Carvotanacetone	0.12
27	13.57	1244	Piperitone	0.21
28	14.11	1278	Bornyl acetate	7.63
29	14.33	1295	Thymol	2.01
30	14.88	1307	Carvacrol	0.13
31	15.34	1335	Piperitenone	0.04
32	15.87	1349	α-Terpenyl acetate	0.09
33	15.96	1353	Citronellyl acetate	0.18
34	16.65	1390	β-Elemene	0.13
35	17.02	1409	Cinerone	0.08
36	17.55	1431	Davana ether	0.03
37	18.03	1456	β-Vinylnaphthalene	3.67
38	18.22	1464	α-Humulene	0.25
39	19.00	1485	Germacrene D	0.15
40	19.22	1496	α-Curcumene	0.08
41	19.75	1502	cis-Methyl isoeugenol	0.05
42	20.23	1509	Bicyclogermacrene	0.06
43	20.86	1512	α-Muurolene	0.16
44	21.26	1516	Citronellyliso-valerate	0.07
45	21.87	1519	Spathulenol	0.07
46	22.07	1521	Shyobunone	0.03
47	22.88	1529	γ-Cadinene	0.09
48	23.45	1532	δ-Cadinene	0.12
49	23.89	1534	α-Cadinene	0.04

No.	RT	RI	Compound name	Area sum %
50	24.01	1548	Nerolidol-epoxyacetate	0.23
51	24.73	1563	Longicamphenylone	2.08
52	25.11	1577	Spathulenol	0.91
53	25.43	1583	Neryl isovalerate	0.07
54	25.87	1594	Davanone	1.06
55	27.86	1643	α-Muurolol	0.06
56	28.96	1654	β-Eudesmol	0.12
57	29.07	1659	α-Eudesmol	0.42
58	29.76	1663	trans-Caryophyllene	1.03
59	30.23	1666	α-Cadinol	0.21
60	31.08	1689	Geranyl tiglate	0.23
61	31.97	1798	Farnesyl acetate	0.08
Total identified				97.08
% Essential oil yield (V/W)				1.04

RT=Retention time; RI= Retention index; Area sum %; Values were expressed as [area percentage]

Table 2. Chemical composition of *T. aphylla* aerial parts essential oils

No.	RT	RI	Compound name	% content
1	5.04	935	A-pinene	7.31
2	5.74	992	β-pinene	1.01
3	7.32	1005	1,8-cineole	15.75
4	7.99	1023	O-cymene	0.82
5	8.45	1032	γ-terpinene	0.57
6	8.94	1052	Terpinolene	1.51
7	9.87	1109	α-thujone	2.77
8	10.43	1136	Trans-pinocarveol	6.81
9	11.58	1198	Fenchyl alcohol	0.61
10	13.96	1215	β-cyclocitral	0.44
11	14.66	1223	Cis-carveol	1.29
12	16.98	1244	Carvone	0.61
13	18.05	1281	Ledene	0.22
14	19.65	1405	Dodecanal	0.64
15	21.76	1440	Neryl acetone	1.43
16	22.07	1446	Aromadendrene	0.55
17	24.35	1465	β-ionone	3.55
18	25.88	1507	Farenal	0.12
19	26.12	1509	Tridecanal	1.01
20	28.92	1529	Γ-cadinene	0.62
21	29.47	1561	Ledol	8.24
22	30.77	1604	Viridiflorol	4.61
23	32.65	1615	Tetradecanal	0.31
24	34.79	1663	Caryophyllene	1.77
25	36.35	1715	Farnesal	0.52
26	37.21	1766	Tetradecanoic acid	2.09
27	38.89	1811	Farnesoic acid	0.85
28	40.05	1847	6,10,14-trimethyl-2-pentadecanone	21.43
29	40.58	1883	Farnesyl acetone	2.03
30	42.06	1980	Hexadecanoic acid	4.52
31	43.98	2200	Docosan	2.76
32	44.08	2300	Tricosane	0.81

No.	RT	RI	Compound name	% content
33	44.65	2400	Tetracosane	0.09
34	45.76	2500	Pentacosane	0.11
35	45.97	2600	Hexacosane	0.05
36	46.32	2700	Heptacosane	0.16
37	46.87	2800	Octacosane	0.04
Total identified				98.03
% Essential oil yield				0.22

RT=Retention time; RI= Retention index; Area sum %; Values were expressed as [area percentage]

Antioxidant activity

Antioxidants are ingredients that protect living cells from the impairment created by unstable molecules called free radicals. Advantages of this antioxidant are necessary for conditions of free radicals, to function and to strengthen free radicals. Free radical damage can lead to the development of cancer (El-Beltagi *et al.*, 2018). A molecule of antioxidants may prevent the oxidation or retardation of other molecule production. Oxidation covers chemical reaction in which electrons are transferred from the one substance to another. Free radicals are created by oxidation reactions that cause chain reactions that damage cells. Antioxidants limit these chain actions by eliminating free radical interim and decreasing other oxidation reactions (El-Beltagi *et al.*, 2019a).The effect of antioxidants on DPPH radical scavenging and ABTS was thought to result from their hydrogen donating ability. In order to become stable diamagnetic molecule, DPPH is stable free radical and accepts an electron or hydrogen radical. In this study, the antioxidant activities of essential oils of *A. monosperma* and *T. aphylla* compared with ascorbic acid and butylated hydroxytoluene (BHT) as a reference antioxidant compound were determined by method of DPPH radical scavenging assay and ABTS assay and the results are summarized in Table 3.

The scavenging of DPPH radicals and ABTS increased with increasing essential oil concentrations (25, 50, 75, 100 and 200 µg mL^{-1}) (Table 3). The IC_{50} value of DPPH radicals and ABTS of *A. monosperma* essential oil was 74.12 and 64.31 µg mL^{-1} concentration respectively. IC_{50} values indicate the concentration of test sample required to inhibit 50% of the free radicals. The high concentration of *A. monosperma* essential oil (200 µg mL^{-1}) gave the highest % inhibition of DPPH and ABTS about (91.96% and 96.05% respectively).

In addition, scavenging of DPPH radicals and ABTS of *T. aphylla* essential oils were increased with increasing extract concentration from 25, 50, 75, 100 and 200 µg mL^{-1} (Table 3). The most pronounced increases in DPPH radicals and ABTS were detected in the high concentrations of oil (200 µg mL^{-1}) about 27.32% and 83.03% respectively. IC_{50} value of DPPH radicals and ABTS of *T. aphylla* essential oils was 134.9 and 109.23 µg mL^{-1}concentration respectively.

IC_{50} values indicated that antioxidant activity of *A. monosperma* essential oil was higher than *T. aphylla* essential oil. The high antioxidant activity of *A. monosperma* essential oil in this study may be due to presence of bornyl acetate (7.63%) and α-pinene (3.98%) which considered as good antioxidant. These results are in accordance with Horváthováa *et al.* (2009) who found that *A. monosperma* essential oil contain both borneol and bornyl acetate, which are considered as major contributors in antioxidant activity of essential oils. In addition, *A. monosperma* showed high ratios of α-pinene and terpinen-4-ol which are known to have noticeable antioxidant activities (Elansary *et al.*, 2012). In *T. aphylla, the* main identified volatile principle, 6,10,14-trimethyl-2-pentadecanone, is a nonaromatic oxygenated hydrocarbon (ketone) and has slightly fatty aroma with reported antimicrobial (Iyapparaj *et al.*, 2014) and antioxidant (Xu *et al.*, 2016) properties. Also, the radical scavenging effect of *T. aphylla* bark extract from Saudi Arabia, which had a greater quantity of total phenolic compounds and also determined to be stronger in DPPH and H_2O_2 assays (Suleiman, 2019).

Table 3. Antioxidant activities of *A. monosperma* and *T. aphylla* essential oils as well as ascorbic acid against DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ at different concentrations

Treatment	Concentration of essential oil (µg mL^{-1})	DPPH assay		ABTS assay	
		% Inhibition of DPPH$^{\bullet}$	IC_{50} (µg mL^{-1})	% Inhibition of ABTS$^{\bullet+}$	IC_{50} (µg mL^{-1})
A. monosperma	25	13.51^{ij} ± 3.16	74.12	19.17^{h} ± 1.21	64.31
	50	25.43^{gh} ± 2.29		39.21^{f} ± 3.42	
	75	50.24^{c} ± 2.03		66.54^{d} ± 1.92	
	100	62.92^{d} ± 2.39		82.36^{c} ± 2.26	
	200	91.96^{b} ± 2.23		96.05^{ab} ± 1.17	
T. aphylla	25	9.94^{j} ± 2.01	134.90	14.68^{h} ± 3.49	109.23
	50	20.06^{hi} ±1.31		27.18^{g} ± 3.31	
	75	30.17^{g} ± 1.94		39.87^{f} ± 0.58	
	100	38.29^{f} ± 2.44		48.48^{e} ± 0.44	
	200	72.32^{c} ± 2.82		83.03^{c} ± 1.39	
Ascorbic acid	5	13.50^{ij} ± 2.16	29.35	26.31^{g} ± 1.95	20.41
	10	27.66^{g} ± 1.83		39.36^{f} ± 2.02	
	20	38.89^{f} ± 1.53		49.39^{e} ± 0.90	
	40	61.04^{d} ± 3.53		91.88^{b} ± 0.90	
	60	92.96^{b} ± 2.03		100.00^{a} ± 0.00	
BHT	5	22.08^{gh} ± 1.81	18.12	36.34^{f} ± 0.64	9.93
	10	35.93^{f} ± 3.08		51.18^{e} ± 1.36	
	20	62.21^{d} ± 1.64		97.55^{a} ± 0.83	
	40	93.43^{ab} ± 1.67		100^{a} ± 0.00	
	60	100^{a} ± 0.00		100^{a} ± 0.00	
LSD 0.05		6.63		5.00	

- Each value represents the mean ± SE.
- Means in the same column followed by the same letter are not significantly different at ($P<0.05$).

Cytotoxic effect

The cytotoxic activity of *A. monosperma* and *T. aphylla* essential oils were tested *in vitro* against human promyelocytic leukaemia cell lines (HL60 and NB4) using viability test. Viability percentage of HL60 and NB4 cells after incubation with different concentrations of *A. monosperma* and *T. aphylla* essential oils (25, 50, 75, 100 and 200 µg mL^{-1}) were recorded in Table 4. Data showed that the incubation of cancer cells with different concentrations (25, 50, 75, 100 and 200µg mL^{-1}) for 24 h of *A. monosperma* and *T. aphylla* essential oils significantly reduced viability of those cells when compared to untreated cells (control). The dead cells (HL-60 and NB4) were significantly increased with increasing essential oils concentrations. The results showed that, anticancer effects of *A. monosperma* and *T. aphylla* essential oils on NB4 cells were more than anticancer effects on HL-60 cells.

On the other side, anticancer effects of *A. monosperma* essential oil on HL-60 and NB4 cells were more than anticancer effects of *T. aphylla* essential oil. The highest dead cells percentage of HL-60 was recorded *A. monosperma* and *T. aphylla* essential oils (92.76% and 56.08%) respectively, for concentration of 200 µg mL^{-1}. In the same trend, the highest dead cells percentage of NB4 was recorded by *A. monosperma* and *T. aphylla* essential oils (100% and 68.25%) respectively, for concentration of 200 µg mL^{-1}. *A. monosperma* essential oil showed potent cytotoxic effects with IC_{50} values of 93.39 µg mL^{-1} in HL-60 cell line and 55.73 µg mL^{-1} in NB4 cell line, whereas *T. aphylla* essential oil gave IC_{50} values of 164.62 µg mL^{-1} in HL-60 cell line and 84.83 µg mL^{-1} in NB4 cell line. IC_{50} values indicated that anticancer activity of *A. monosperma* essential oil was higher than *T. aphylla* essential oil against HL-60 and NB4 cell lines.

Essential oil from aerial section of *A. herba-alba* collected at floral stage had greater cytotoxic effect on HUVEC cells than herba-alba collected at seed stage, with cell viability of 20% at 80 µM. (Jaouadi *et al.,* 2014). *Artemisia absinthium* oil contains *trans*-caryophyllene, 3,6-dihydrochamazulene, and *cis*epoxyocimene as main compounds and showed cytotoxic activity against A549, HCT116, MCF7, H292, SK-MEL-5 and HS5 cells with the IC_{50} value of 51.1 ± 1.8 to 98.6 ± 5.2 µg/mL. Both of the obtained oils from aerial parts of *A. persica* and *A. turcomanica* contain β-thujone, 1,8- cineol, camphor, and filifolone as their main compounds, which

exhibited a significant cytotoxic effect on MCF-7 cells with IC_{50} value of 0.15 and 0.1 µg/mL, respectively (Nikbakht *et al.*, 2014). Essential oil of *A. indica* contains germacrene B, artemisia ketone, borneol, and *cis*-chrysanthenyl acetate as main constituents, which demonstrated cytotoxic activity with IC_{50} values of 10 to 19.5 µg/mL against THP-1, HEP-2, A-549 and Caco-2 cancer cell lines (Rashid *et al.*, 2013). The essential oil of *A. scoparia* contains iacetylenes 1-phenyl-2,4-pentadiyne, capillene, β-pinene, methyl eugenol as major components and was inactive against MCF-7 cells (Sharopov and Setzer, 2011). *Artemisia dubia* leaves oil contains chrysanthenone, coumarin, and camphor as major components at concentration of 100 µg/mL, which completely killed MCF- 7 cells (Satyal *et al.*, 2012).

Table 4. Cytotoxic effect of *A. monosperma* and *T. aphylla* essential oils on the viability of HL-60 and NB4 cells

Treatment	Concentration of essential oil (µg mL^{-1})	HL-60 cells			NB4 cells		
		% of dead cells	% of viable cells	IC_{50} (µg mL^{-1})	% of dead cells	% of viable cells	IC_{50} (µg.mL^{-1})
Control	0	0	100		0	100	
A. monosperma	25	$13.08^{f} \pm 2.55$	86.92	93.39	$29.04^{fg} \pm 2.16$	70.96	55.73
	50	$25.51^{e} \pm 1.98$	74.49		$37.90^{e} \pm 1.76$	62.10	
	75	$43.08^{d} \pm 3.02$	56.92		$66.12^{c} \pm 2.67$	33.88	
	100	$67.90^{b} \pm 2.95$	32.10		$87.11^{b} \pm 2.02$	12.89	
	200	$92.67^{a} \pm 0.85$	7.33		$100^{a} \pm 0.00$	0.00	
T. aphylla	25	$4.00g \pm 0.58$	96.00	164.62	$25.06^{g} \pm 2.16$	74.94	84.83
	50	$16.59^{f} \pm 2.42$	83.41		$35.50^{ef} \pm 2.59$	64.50	
	75	$27.49^{e} \pm 1.08$	72.51		$41.42^{e} \pm 4.35$	58.58	
	100	$38.65^{d} \pm 1.90$	61.35		$55.44^{d} \pm 4.46$	44.56	
	200	$56.08^{c} \pm 1.53$	43.92		$68.25^{c} \pm 1.09$	31.75	
$LSD_{0.05}$		6.07			7.82		

- Each value represents the mean ± SE.
- Means in the same column followed by the same letter are not significantly different at ($P<0.05$).

Similar results showed that aqueous and ethanolic extract of *T. aphylla* had potent cytotoxic activity against some cancer cell line specially MCF-7 cell line with IC_{50} values (Yusufoglu and Al-qasoumi, 2011). Also, essential oils were extracted from aerial parts of *Tamarix aphylla* L., a wild plant in Jordan. Aqueous (AE) and ethanolic (EE) extracts were prepared from *T. aphylla* and their cytotoxicity against breast adenocarcinoma (MCF-7), colorectal adenocarcinoma (Caco-2), and pancreatic carcinoma (Panc-1) cancer cell lines was evaluated. The lowest IC_{50} (2.17 ± 0.10 µg mL^{-1}) was recorded for the AE of *T.aphylla* against MCF-7, they found that *T.aphylla* has antitumor activity comparable with cisplatin however, more selective to cancer cells since its IC_{50} against fibroblast was 79.99 ± 4.90 µg mL^{-1} (Alhourani *et al.*, 2018). In addition, *T. aphylla* leaf extracts inhibited the proliferation of MCF-7 cells in a dose-dependent manner with significant cytotoxic effect at 24 h with a concentration of 50 mg mL^{-1}. MCF-7 cells in this study were exhibited anticancer activities in the concentration-dependent manner at 500 and 1000 µg mL^{-1} (Al Sobeai, 2018). Moreover, recent study on methanol extract of *T. aphylla* had investigated its potential cytotoxicity using brine shrimp method and revealed 70% mortality rate at concentration of 500 µg mL^{-1} (Muhammad *et al.*, 2017).

The essential oil and their constituents target multiple pathways in cancer cells was reported by Gautam *et al.* (2014) who demonstrated that, essential oils (EOs) are permeable and participate in different pathways involving cellular targets with respect to their cell membrane permeability. The EO's increase intracellular level, or those in reactive nitrogen cells the reactive (ROS/RNS) which generates apoptosis in cancer cells. Inhibition of protein Kinase B (Akt), mammalian target of rapamycin (Mtor) and Mitogen-Activated Protein Kinases (MAPK) pathways at different steps by EOs leads to corresponding up-/down regulation of various key biomolecules and corresponding genes. Alteration in expression of Nuclear factor kappa-light- chain-enhancer of activated B cells (NF-KB) by EOs and further binding of NF-KB to DNA result in apoptosis in cancer cells. Akt dephosphorylation results in overexpression of p21 through action of EOs, which either causes apoptosis by increasing amount of caspases or results in cell cycle arrest by binding to cyclines. In addition, mitochondrial

stress caused by EOs leads to activation of Bcl-2 gene and depolarisation of membrane, resulting in increased release of cytochrome-C to cytoplasm, causing apoptotic cell death in cancer cells. The EOs also modulate DNA repair mechanisms by acting as DNA polymerase inhibitors and lead to poly (ADP-Ribose) polymerase (PARP) cleavage which also results in apoptosis in cancer cells.

Conclusions

From the data presented in the current stud, can be concluded that the *A. monosperma* and *T. aphylla* that is widely dispersed in the deserts of Saudi Arabia has a different chemotype and can be used as a cheap source for the commercial isolation of *β*-pinene, *α*-terpinolene, 6,10,14-trimethyl-2-pentadecanone, 1,8-cineole and limonene. Moreover, the detailed chemical profiling of the volatile components of the leaf of *A. monosperma* and aerial parts of *T. aphylla* essential oils in the present study could be useful in the chemo-taxonomic classification of the *Artemisia* and *Tamarix* species that grow wild in the agro-climatic conditions of Saudi Arabia. These findings of antioxidant activities enhanced the capacity of *A. monosperma* and *T. aphylla* extracts in the reported traditional medicinal uses and suggest that these plants may be considered potential sources of new antioxidant drugs.

Authors' Contributions

Conceptualization: R.M.R., H.S.E. and E.A.S.; Data curation: K.M.Y., H.E. and S.R.; Formal analysis: R.M.R., K.M.Y., H.E. and S.R.; Funding acquisition: R.M.R. and E.A.S.; Investigation: R.M.R., H.S.E., E.A.S. and H.I. M.; Methodology: R.M.R., E.A.S. and H.S.E; Project administration: R.M.R., E.A.S. and H.S.E; Resources: R.M.R., K.M.Y., H.E. and S.R.; Software: R.M.R., and H.S.E; Supervision: R.M.R., H.S.E. and E.A.S.; Writing-original draft preparation: H.S.E. and H.I.M.; writing-review and editing, H.S.E. and H.I.M All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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