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@kfupm.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 predominant 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*
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 450000 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; Aify and El-Beltagi, 2011; Aify 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:

\[
\text{Oil (\% v/w)} = \frac{\text{Observed volume of oil (mL)}}{\text{Weight of sample (g)}} \times 100
\]

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):

\[
\text{DPPH scavenging effect (\%) = } \left( \frac{A_{C(o)} - A_{A(t)}}{A_{C(o)}} \right) \times 100
\]

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 (2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) in water to 7 mM concentration. ABTS radical cation (ABTS\(^{•+}\)) 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):

\[
\text{ABTS scavenging effect (\%) = } \left( \frac{A_{C(o)} - A_{A(t)}}{A_{C(o)}} \right) \times 100
\]

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 = \( \frac{100 \times [\text{live cells}]}{[\text{dead + live cells}]} \)

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* (Saidana *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* (Saidana *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-Myrccene             | 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-Terpineene           | 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-Terpinoles           | 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-Menthan-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| Piperitron             | 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-Vinylphenalene       | 3.67       |
| 38  | 18.22| 1464| α-Humulene            | 0.25       |
| 39  | 19.00| 1485| Germacrone 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-Murolene             | 0.16       |
| 44  | 21.26| 1516| Citronellyls-lavater   | 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  | α-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-cyclocitrinal           | 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-cadinen                 | 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 | Farnesol                  | 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 IC₅₀ value of DPPH radicals and ABTS of *A. monosperma* essential oil was 74.12 and 64.31 μg mL⁻¹ concentration respectively. IC₅₀ 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. IC₅₀ value of DPPH radicals and ABTS of *T. aphylla* essential oils was 134.9 and 109.23 μg mL⁻¹ concentration respectively.

IC₅₀ 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 Horváthová *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 H₂O₂ assays (Suleiman, 2019).
showed potent cytotoxic effects with IC\(_{50}\) values of 19.17 ± 1.21, 39.21 ± 3.42, and 66.54 ± 1.92 for HL-60, NB4, and 200 μg mL\(^{-1}\) in HL-60 cell line, whereas anticancer effects of A. monosperma and T. aphylla essential oils 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. (Jouadi et al., 2014). Artemisia absinthium oil contains trans-caryophyllene, 3,6-dihydrochamazulene, and cisepoxyocimene as main compounds and showed cytotoxic activity against A549, HCT116, MCF7, H292, SK-MEL-5 and H55 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

Table 3. Antioxidant activities of A. monosperma and T. aphylla essential oils as well as ascorbic acid against DPPH\(^{-}\) and ABTS\(^{-}\) at different concentrations

| Treatment         | Concentration of essential oil (μg mL\(^{-1}\)) | % Inhibition of DPPH\(^{-}\) | IC\(_{50}\) (μg mL\(^{-1}\)) | % Inhibition of ABTS\(^{-}\) | IC\(_{50}\) (μg mL\(^{-1}\)) |
|-------------------|-----------------------------------------------|-----------------------------|-----------------------------|----------------------------|-----------------------------|
| A. monosperma     | 25                                            | 13.51 ± 3.16                | 74.12                       | 19.17 ± 1.21               | 64.31                       |
|                   | 50                                            | 25.43 ± 2.29                | 39.21 ± 3.42                | 39.21 ± 3.42               | 66.54 ± 1.92                |
|                   | 75                                            | 50.24 ± 2.03                | 66.54 ± 1.92                | 39.21 ± 3.42               | 66.54 ± 1.92                |
|                   | 100                                           | 62.92 ± 2.39                | 82.36 ± 2.26                | 66.54 ± 1.92               | 66.54 ± 1.92                |
|                   | 200                                           | 91.36 ± 2.23                | 82.36 ± 2.26                | 96.05 ± 1.17               |                             |
| T. aphylla        | 25                                            | 9.94 ± 2.01                 | 14.68 ± 3.49                | 14.68 ± 3.49               | 109.23                      |
|                   | 50                                            | 20.06 ± 1.31                | 27.18 ± 3.31                | 27.18 ± 3.31               | 27.18 ± 3.31                |
|                   | 75                                            | 30.17 ± 1.94                | 39.87 ± 0.58                | 39.87 ± 0.58               | 39.87 ± 0.58                |
|                   | 100                                           | 38.29 ± 2.44                | 48.48 ± 0.44                | 48.48 ± 0.44               | 48.48 ± 0.44                |
|                   | 200                                           | 72.32 ± 2.82                | 83.03 ± 1.39                | 83.03 ± 1.39               | 83.03 ± 1.39                |
| Ascorbic acid     | 5                                             | 13.50 ± 2.16                | 26.34 ± 1.95                | 26.34 ± 1.95               | 26.34 ± 1.95                |
|                   | 10                                            | 27.66 ± 1.83                | 39.36 ± 2.02                | 39.36 ± 2.02               | 39.36 ± 2.02                |
|                   | 20                                            | 38.89 ± 1.53                | 49.39 ± 0.90                | 49.39 ± 0.90               | 49.39 ± 0.90                |
|                   | 40                                            | 61.04 ± 3.53                | 91.89 ± 0.90                | 91.89 ± 0.90               | 91.89 ± 0.90                |
|                   | 60                                            | 92.96 ± 2.03                | 100.00 ± 0.00               | 100.00 ± 0.00              | 100.00 ± 0.00               |
| BHT               | 5                                             | 22.08 ± 1.81                | 36.34 ± 0.64                | 36.34 ± 0.64               | 36.34 ± 0.64                |
|                   | 10                                            | 35.93 ± 3.08                | 51.18 ± 1.36                | 51.18 ± 1.36               | 51.18 ± 1.36                |
|                   | 20                                            | 62.21 ± 1.64                | 97.55 ± 0.83                | 97.55 ± 0.83               | 97.55 ± 0.83                |
|                   | 40                                            | 93.43 ± 1.67                | 100 ± 0.00                  | 100 ± 0.00                 | 100 ± 0.00                  |
|                   | 60                                            | 100 ± 0.00                  | 100 ± 0.00                  | 100 ± 0.00                 | 100 ± 0.00                  |

- 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.
exhibited a significant cytotoxic effect on MCF-7 cells with IC\textsubscript{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\textsubscript{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 icacetelynes 1-phenyl-2,4-pentadiyne, capillene, β-pinene, methyl eugenol as major components and was inactive against MCF-7 cells (Sharopov and Setter, 2011). *Artemisia dubia* leaves oil contains chrysanthene, 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\textsuperscript{-1}) | HL-60 cells | NB4 cells |
|----------------------|-------------------------------------------------------------|-------------|-----------|
|                      | % of dead cells | % of viable cells | IC\textsubscript{50} (µg mL\textsuperscript{-1}) | % of dead cells | % of viable cells | IC\textsubscript{50} (µg mL\textsuperscript{-1}) |
| Control              | 0 | 100 | - | 0 | 100 | - |
| *A. monosperma*      | 25 | 10.08 ± 2.55 | 88.92 | 29.04 ± 2.16 | 70.96 | 55.73 |
|                      | 50 | 25.51 ± 1.98 | 74.49 | 37.90 ± 1.76 | 62.10 | - |
|                      | 75 | 43.08 ± 3.02 | 56.92 | 66.12 ± 2.67 | 33.88 | - |
|                      | 100 | 67.90 ± 2.95 | 32.10 | 87.13 ± 2.02 | 12.89 | - |
|                      | 200 | 92.67 ± 0.85 | 7.33 | 100* ± 0.00 | 0.00 | - |
| *T. aphylla*         | 25 | 4.00 ± 0.58 | 96.00 | 25.06 ± 2.16 | 74.94 | 84.83 |
|                      | 50 | 16.59 ± 2.42 | 83.41 | 35.50 ± 2.59 | 64.50 | - |
|                      | 75 | 27.49 ± 1.08 | 72.51 | 41.42 ± 4.35 | 58.58 | - |
|                      | 100 | 38.65 ± 1.90 | 61.35 | 55.44 ± 4.46 | 46.59 | - |
|                      | 200 | 56.08 ± 1.53 | 43.92 | 68.25 ± 1.09 | 31.75 | - |
| LSD\textsubscript{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\textsubscript{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\textsubscript{50} (2.17 ± 0.10 µg mL\textsuperscript{-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\textsubscript{50} against fibroblast was 79.99 ± 4.90 µg mL\textsuperscript{-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\textsuperscript{-1}. MCF-7 cells in this study were exhibited anticancer activities in the concentration-dependent manner at 500 and 1000 µg mL\textsuperscript{-1} (Al Sobaei, 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\textsuperscript{-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 study, it 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 chemotaxonomic 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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