

Full Length Research Paper

Chemical composition, antimicrobial activity, antioxidant and total phenolic content within the leaves essential oil of *Artemisia absinthium* L. growing wild in Iran

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Water-distilled essential oil from the leaves of *Artemisia absinthium* L. collected from Ardabil, north-western Iran, was analyzed by Gas chromatography and mass spectrometry (GC and GC-MS). In the leaf oil of *A. absinthium*, 19 components, which represented 100% of the total composition were identified. 1,8-Cineole (36.46%), borneol (25.99%) and camphor (10.20%) were the major components in this oil. The leaves of *A. absinthium* was investigated to analyze their antimicrobial activity, antioxidant activity and total phenolic content. The present study revealed that the leaf essential oil of *A. absinthium* indicated significant activity against *Candida albicans*. Killing kinetics of various microorganisms treated with leaf oil of *A. absinthium* indicated that *C. albicans* is the most vulnerable. The total phenol contents of the leaf oil of *A. absinthium* was determined to be 168.67 ± 9.50 µg gallic acid equivalent/mg sample. Antioxidative properties of the leaves essential oil of *A. absinthium* was determined by 3 methods: The Ferric-reducing antioxidant power (FRAP), radical-scavenging capacity of the oil or bleaching of 2,20-diphenylpicrylhydrazyl (DPPH) and β-Carotene-linoleic acid assay. The ferric reducing power of the essential oils was determined to be 10.67 ± 0.45 gallic acid equivalent (mg/g). The leaf essential oil of *A. absinthium* reduced the concentration of DPPH free radical ($61.4 \pm 1.4\%$, 10 mg/ml of essential oil) with an efficacy lower than that of reference oil *Thymus x-porlock* (69.3% inhibition). IC₅₀ for DPPH radical-scavenging activity was 5.85 µg/ml. In β-carotene-linoleic acid test system, oxidation of linoleic acid was effectively inhibited by *A. absinthium* oil ($58.56 \pm 2.5\%$, amount of essential oil 0.625 mg/ml). The results suggest application of *A. absinthium* oil as a natural antioxidant agent.

Key words: *Artemisia absinthium*, essential oil, 1,8-cineole, antimicrobial activity, antioxidant, total phenolic content.

INTRODUCTION

Artemisia is a genus of small herbs or shrubs found in northern temperate regions. It belongs to the important family compositae (Asteraceae) (Rechinger, 1986).

Within this family, *Artemisia* is included into the tribe Anthemideae and comprises itself of over 500 species (Mozaffarian, 1996). The genus *Artemisia* has always been of great botanical and pharmaceutical interest and is useful in traditional medicines for the treatment of a variety of diseases and complaints (Rustaiyan and Masoudi., 2011; Firouzni et al., 2008). Some *Artemisia* species has been investigated chemically and the

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presence of monoterpenes, sesquiterpenes, specially sesquiterpene lactones and chemical composition of essential oil reported.

Among them, *Artemisia absinthium* (*absinthium*, absinthe wormwood, wormwood, common wormwood, green ginger or grand wormwood) is a species of wormwood native to temperate regions of Eurasia and northern Africa (Linnaeus, 1753; Wyk and Wink, 2004). It grows naturally in wide regions of Iran. *A. absinthium* is used medicinally as a tonic, stomachic, antiseptic, anti-spasmodic, carminative, cholagogue, febrifuge and anthelmintic. The components of leaves and flowering of *A. absinthium* include silica, two bitter substances (absinthin and anabsinthine), thujone, tannic and resinous substances, malic acid, and succinic acid. The bitter taste of wormwood is from sesquiterpene lactones (0.15 to 0.4%) (Anonymous, 2003); absinthin and artabsin being the main ones (Thomsen, 2005; Mills and Bone, 2005) and guainolides (Anonymous, 2003). Bitter tonic (Mills and Bone, 2005), aromatic bitter (Wichtl, 2004), anthelmintic (Mills and Bone, 2005), stomachic (Anonymous, 2003). Antiparasitic (Mills and Bone, 2005) antiseptic and choloretic (Thomsen, 2005) carminative, anti-inflammatory and mild antidepressant (Hoffmann, 2003; Mahmoudi et al., 2009).

The effectiveness of wormwood as an aromatic bitter and its antimicrobial properties come from the bitter compounds and its essential oil. Extracts of the plant have shown to exhibit strong antimicrobial activity, especially against Gram-positive pathogenic bacteria (Fiamegos et al., 2011). The oil of the plant can be used as a cardiac stimulant to improve blood circulation. Pure wormwood oil is very poisonous, but with proper dosage poses little or no danger (Lust, 1979). The oil is a potential source of novel agents for the treatment of *leishmaniasis* (Tariku et al., 2011). Although *A. absinthium* have been used as folk remedies to treat various ailments in medicine, as yet there has been little attempts made to study the antioxidant and antimicrobial potential of these plants against a wide range of microorganisms. As such, the aims of this study were to estimate the total phenolic content, antioxidant activity and antimicrobial activity of *A. absinthium*. The present study deals with the chemical composition, antibacterial, antioxidative and radical-scavenging properties of the essential oil of *A. absinthium* obtained by steam-distillation.

MATERIALS AND METHODS

General

The major equipment types used were a clewenger apparatus, GC (Schimadzu 15A), GC/MS (Hewlett-Packard 5973 with a HP-5MS column), microbial culture media (Merck), Shimadzu UV-2501PC spectrophotometer. Chemicals were of analytical grade.

Plant

The leaves of *Artemisia absinthium* L. were collected from Namin, Province of Ardabil, after Heyran ghaut, in north-western Iran in July 2011. Voucher specimens have been deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

Isolation of the essential oil

The leaves of *A. absinthium* were dried at room temperature for several days. Air-dried leaves of *A. absinthium* (110 g) were separately subjected to hydrodistillation using a clewenger-type apparatus for 3 h. After decanting and drying of the oil over anhydrous sodium sulfate, the oil was recovered. Results showed that essential oil yield was 1.05% (w/w).

Analysis of the essential oil

The composition of the essential oil obtained by hydrodistillation from the leaves of *A. absinthium* was analyzed by GC and GC/MS. Identification of the constituents of oil was achieved by comparison of their mass spectra and retention indices with those reported in the literature and those of authentic samples (Adams, 2001).

Gas chromatography

GC analysis was performed on a Schimadzu 15 A gas chromatography equipped with a split/splitless injector (250°C) and a flame ionization detector (250°C). Nitrogen was used as carrier gas (1 ml/min) and the capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The column temperature was kept at 60°C for 3 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min. Relative percentage amounts were calculated from peak area using a Schimadzu C-R4A chromatopac without the use of correction factors.

Gas chromatography-mass spectroscopy

GC-MS analysis was performed using a Hewlett-Packard 5973 with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). The column temperature was kept at 60°C for 3 min and programmed to 220°C at a rate of 5°C/min and kept constant at 220°C/min for 5 min. The flow rate of Helium as carrier gas was 1 ml/min. MS were taken at 70 eV. The retention indices for all the components were determined according to the Van Den Dool method, using n-alkanes as standards. The compounds were identified by (RRI, DB5) with those reported in the literature and by comparison of their mass spectra with the Wiley library or with the published mass spectra (Adams, 2001).

Oil dilution solvent

Bacterial strains were streaked on Mueller Hinton agar plates using sterile cotton swabs. Five microlitres of dimethylsulphoxide (DMSO), loaded on sterile blank disks, were placed on the agar plates and were incubated at 37°C for 24 h. There was no antibacterial activity on the plates and hence DMSO was selected as a safe diluting agent for the oil. Five microlitres from each oil dilution, followed by sterilization, using a 0.45 µm membrane filter,

were added to sterile blank discs. The solvent also served as control.

Microbial strain and growth media

Escherichia coli (ATCC25922), *Staphylococcus aureus* (ATCC25923), *Pseudomonas aeruginosa* (ATCC8830), *Candida albicans* (ATCC 5027) and *Acinetobacter baumannii* (ATCC 17978) were employed in the study. Nutrient agar was used. Bacterial suspensions were made in brain heart infusion (BHI) broth to a concentration of approximately 10^8 cfu/ml. Subsequent dilutions were made from the above suspension, which were then used in the tests.

Oil sterility test

In order to ensure sterility of the oils, geometric dilutions ranging from 0.036 to 72.0 mg/ml of the essential oil, were prepared in a 96-well microtitre plate, including one growth control (BHI + Tween 80) and one sterility control (BHI + Tween 80 + test oil). Plates were incubated under normal atmospheric conditions, at 37°C for 24 h. The contaminating bacterial growth, if at all, was indicated by the presence of a white "pellet" on the well bottom.

Disc diffusion method

The agar disc diffusion method was employed for the determination of antimicrobial activities of the essential oils in question. Briefly, 0.1 ml from 10^8 cfu/ml bacterial suspension was spread on the Mueller Hinton Agar (MHA) plates. Filter paper discs (6 mm in diameter) were impregnated with 5 µl of the undiluted oil and were placed on the inoculated plates. These plates, after remaining at 4°C for 2 h, were incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters. All tests were performed in triplicate.

Determination of minimum inhibitory (MIC) and bactericidal (MBC) concentrations

All tests were performed in brain heart infusion (BHI) broth supplemented with Tween 80 detergent (final concentration of 0.5% (v/v)). Test strains were suspended in BHI broth to give a final density of 10^7 cfu/ml and these were confirmed by viable counts. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were assessed according to our modified procedure (Rasooli and Mirmostafa, 2003). MIC was determined by a broth dilution method in test tubes as follows: 40 µl from each of various dilutions of the oils were added to 5 ml of brain heart infusion (BHI) both in tubes containing 10^7 cfu/ml of live bacterial cells. The tubes were then incubated on an incubator shaker to evenly disperse the oil throughout the broth in tubes. The highest dilution (lowest concentration), showing no visible growth, was regarded as the MIC. Cell suspensions (0.1 ml) from the tubes showing no growth were subcultured on BHI agar plates in triplicate to determine if the inhibition was reversible or permanent. MBC was determined as the highest dilution (lowest concentration) at which no growth occurred on the plates (Wayne, 2008; Akomo et al., 2009).

Bactericidal kinetics of the oil

Forty microlitres of each oil at the dilution determined by MBC was

added to each 5 ml of brain heart infusion (BHI) broth in tubes containing bacterial suspension of 10^7 cfu/ml and were then incubated at 37°C in an incubator shaker. Samples (0.1 ml) were taken after 5, 10, 15, 20, 25, 30, 45, 90, 120, 150, 180, 210 and 240 min. The samples were immediately washed with sterile phosphate buffer, pH 7.0, centrifuged at 10,000 rpm/1 min, resuspended in the buffer and were then spread-cultured on BHI agar for 24 h at 37°C. Phosphate buffer was used as diluent when needed. Bactericidal experiments were performed three times. Microbial colonies were counted from triplicates after the incubation period and the mean total number of viable cells per ml was calculated. The mean total number of viable bacteria from bactericidal kinetics experiments at each time interval was converted to \log_{10} viable cells using routine mathematical formulae. The trend of bacterial death was plotted graphically (Yadegarinia et al., 2006)

Ferric-reducing antioxidant power (FRAP) assay of the oil

The FRAP assay was carried out according to the procedure employed by (Lim et al., 2009). One millilitre of the extract dilution was added to 2.5 ml of 0.2 M potassium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated for 20 min at 50°C, after which 2.5 ml of 10% trichloroacetic acid was added. The mixture was then separated into aliquots of 2.5 ml and mixed with 2.5 ml of deionised water. Then, 0.5 ml of 0.1% (w/v) FeCl_3 were added to each tube and allowed to stand for 30 min. Absorbance for each tube was measured at 700 nm. The FRAP was expressed as gallic acid equivalents (GAE) in mg/g of samples used ($y = 16.66x + 0.003$; $r^2 = 0.999$).

Radical-scavenging capacity of the oil

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-coloured methanol solution of 2,2-diphenylpicrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent (Burits and Bucar, 2000; Cuendet et al., 1997). Fifty microlitres of 1:5 concentrations of the essential oils in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. Trolox (1 mM) (Sigma-Aldrich), a stable antioxidant, was used as a synthetic reference. The essential oil from *Thymus x-porlock* was used as a natural reference. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (%) was calculated in following way:

$$\% = (\text{A}_{\text{blank}} - \text{A}_{\text{sample}} / \text{A}_{\text{blank}}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Tests were carried out in triplicate.

β -carotene-linoleic acid assay

Antioxidant activity of essential oils was determined using the β -carotene bleaching test (Taga et al., 1984). Approximately 10 mg of β -carotene (type I synthetic, Sigma-Aldrich) was dissolved in 10 ml of chloroform. The carotene-chloroform solution, 0.2 ml, was pipetted into a boiling flask containing 20 mg linoleic acid (Sigma-Aldrich) and 200 mg Tween 40 (Sigma-Aldrich). Chloroform was removed using a rotary evaporator at 40°C for 5 min and, to the residue, 50 ml of distilled water were added slowly with vigorous

Table 1. Composition of the leaf oil of *A. absinthium*.

Compound	*RI	Percentage
1-Octen-3-ol	978	0.49
1,8-Cineole	1033	36.46
<i>p</i> -Menth-2-en-1-ol	1121	6.2
Chrysanthenone	1123	0.62
Terpine-1-ol	1134	4.42
<i>trans</i> -Pinocarveol	1139	1.59
Camphor	1143	10.2
<i>trans</i> -Verbenol	1144	0.87
Pinocarvone	1162	0.82
Borneol	1165	25.99
4-Terpineol	1177	2.72
α -Terpineol	1189	1.17
<i>cis</i> -Piperitol	1193	1.65
Myrtenal	1193	0.51
<i>trans</i> -Piperitol	1205	2.25
Carvone	1242	0.86
Piperitone	1252	2
Bornyl acetate	1285	0.67
Carvotanacetone-6-hydroxy	1304	0.52
Monoterpene hydrocarbons		-
Oxygenated monoterpenes		99.51
Sesquiterpene hydrocarbons		-
Oxygenated sesquiterpenes		-
Others		0.49
Total		100

*RI, Retention indices were as determined on a DB-5 column using the homologous series of n-alkanes.

agitation to form an emulsion. Five millilitres of the emulsion were added to a tube containing 0.2 ml of essential oil solution prepared (Choi et al., 2000) and the absorbance was immediately measured at 470 nm against a blank consisting of an emulsion without β -carotene. The tubes were placed in a water bath at 50°C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm over a 60 min period. Control samples contained 10 μ l of water instead of essential oils. Butylated hydroxy anisole (BHA; Sigma-Aldrich), a stable antioxidant, was used as a synthetic reference (Miraliakbari and Shahidi, 2008). The antioxidant activity was expressed as inhibition percentage with reference to the control after 60 min of incubation, using the following equation:

$$AA = 100(DR_C - DR_S) / DR_C$$

Where AA = antioxidant activity, DR_C = degradation rate of the control = $[\ln(a/b) / 60]$, DR_S = degradation rate in presence of the sample = $[\ln(a/b) / 60]$, a = absorbance at time 0, b = absorbance at 60 min.

Total phenolic content assay

Total phenol content was estimated as gallic acid equivalents

(GAE; mg gallic acid/g extract) as described earlier. In brief, a 100 μ l aliquot of dissolved extract was transferred to a volumetric flask containing 46 ml distilled H_2O , to which was subsequently added 1 ml Folin-Ciocalteu reagent. After 3 min, 3 ml of 2% Na_2CO_3 was added. After 2 h of incubation at 25°C, the absorbance was measured at 760 nm. Gallic acid (Sigma Co, 0.2 to 1 mg/ml gallic acid) was used as the standard for the calibration curve, and the total phenolic contents were expressed as mg gallic acid equivalents per gram of tested extracts ($Y = 0.001x + 0.0079$; $r^2 = 0.9967$) (Kahkonen et al., 1999)

RESULTS

Chemical composition of the essential oil

In the leaf oil of *A. absinthium* collected from Ardabil, north-western Iran, 19 components, which represented 100% of the total composition were identified. 1,8-Cineole (36.46%), borneol (25.99%) and camphor (10.20%) were the major components in this oil (Table 1).

Antibacterial Activity

As can be seen in Table 2, the essential oils were found to have good to moderate antimicrobial activities against all microorganisms tested. The leaf essential oil of *A. absinthium* indicated significant activity against *Candida albicans* and moderate inhibitory activity against *Staphylococcus aureus*. This oil has been reported to be weakly inhibitory against *E. coli*. Results from the disc diffusion method and determination of minimal inhibitory and bactericidal concentrations (MIC and MBC) indicate that *C. albicans* is the most sensitive microorganism, with the lowest MBC value (1 mg/ml). Other sensitive microorganism is *S. aureus*. Killing kinetics of various microorganisms treated with leaf oil of *A. absinthium* indicated that *C. albicans* is the most vulnerable while *E. coli* was found least vulnerable.

Total Phenolic and Content Antioxidant

The total phenol contents of the leaf oil of *A. absinthium* was determined to be 168.67 ± 9.50 μ g gallic acid equivalent/mg sample. Antioxidative properties of the leaves essential oil of *A. absinthium* was determined by 3 methods: The Ferric-reducing antioxidant power (FRAP), Radical-scavenging capacity of the oil or bleaching of 2,20-diphenylpicrylhydrazyl (DPPH) and β -carotene-linoleic acid assay. The ferric reducing power of the essential oils was determined 10.67 ± 0.45 gallic acid equivalent (mg/g). The leaf essential oil of *A. absinthium* reduced the concentration of DPPH free radical ($61.4 \pm 1.4\%$, 10 mg/ml of essential oil) with an efficacy lower than that of reference oil *T. x-parlock* (69.3% inhibition).

Table 2. Antimicrobial activities of the leaf oil of *A. absinthium*.

Microorganisms	<i>A. absinthium</i>			D value (min)
	IZ	MIC	MBC	
<i>Escherichia coli</i> ATCC 25922	15	5	10	18.24
<i>Staphylococcus aureus</i> ATCC 25923	32	1	2.5	8.21
<i>Pseudomonas aeruginosa</i> ATCC 8830	21	2.5	5	16.52
<i>Candida albicans</i> ATCC 5027	60	0.5	1	4.36
<i>Acinetobacter baumannii</i> ATCC 17978	18	2.5	5	17.14

IZ – inhibition zone (mm); MIC – minimum inhibitory concentration (mg/mL); MBC – minimum bactericidal concentration (mg/mL); D-value-decimal reduction time (minutes).

Table 3. Antioxidant activity and total phenolics of leaf essential oil of *A. absinthium*.

Parameters	Values
DPPH effect (%) (amount of essential oil)	61.4 ± 1.4 (10 mg/ml)
DPPH (IC ₅₀)	5.85 (µg/ml)
β-carotene-linoleic acid assay (amount of essential oil)	58.56 ± 2.5 (0.625 mg/ml)
Ferric-reducing antioxidant power (FRAP) (gallic acid equivalent (mg/g))	10.67 ± 0.45
Total phenolic content GAE (µg gallic acid/mg sample)	168.67±9.50

IC₅₀ for DPPH radical-scavenging activity was 5.85 µg/ml. In β-carotene-linoleic acid test system, oxidation of linoleic acid was effectively inhibited by *A. absinthium* oil (58.56 ± 2.5%, amount of essential oil 0.625 mg/ml). The results suggest application of *A. absinthium* oil as a natural antioxidant agent.

DISCUSSION

Chemical composition of the essential oil

The composition of the essential oil obtained by hydro-distillation from the leaves of *A. absinthium*, from Iran analyzed by GC and GC/MS, is listed in Table 1. The percentage and retention indices of components are given. As it is shown in Table 1, in *A. absinthium* oil, 19 components represented about 100% of the total oil, were identified. 1,8-Cineole (36.46%), borneol (25.99%), camphor (10.20%) were the major component in this oil. The other main components of leaf oil of *A. absinthium* were p-menth-2-en-1-ol (6.20%), terpine-1-ol (4.42%) and 4-terpineol (2.72%). As it is shown in Table 1, the largest part of the leaf oil of *A. absinthium* essential oil was formed by oxygenated monoterpenes (99.51%). The results proved that chemotype of the studied wormwood essential oil was specific and different from other wormwood essential oil chemotypes, which have been reported so far.

In another investigation on chemical composition of

essential oil from the leaves of *A. absinthium* collected from the region of Guigou and Errachidia, α-Thujone (39.69), sabinyl acetate (10.96) and β-thujone (7.25) were the major components in this oil (Derwich et al., 2009). On the other hand, the oils of *A. absinthium* of French origin contained (Z)-epoxyocimene and chrysanthenyl acetate as major components while the oils of croatian *A. absinthium* contained mainly (Z)-epoxyocimene and β-thujone (Juteau et al., 2003). Four chemotypes were found to be characteristic of *A. absinthium* growing in different geographical areas of Europe: sabinene and myrcene rich oil, α- and β-thujone rich oil, epoxyocimene rich oil, and (E)-sabinyl acetate rich oil. Some mixed chemotypes were also found (Orava et al., 2006).

The essential oil of *A. absinthium* from western Canada was characterized by high amounts of myrcene (10.8%), trans-thujone (10.1%) and trans-sabinyl acetate (26.4%) (Lopes-Lutz et al., 2008). Bornyl acetate (23.02%) was the major constituent in the essential oil of *A. absinthium* collected from Cuban origin (Pino, 1997). On the other hand, sabinene (17.56%) was the main constituent in essential oil from the aerial part of *A. absinthium* collected from Turkey (Erel et al., 2012).

As shown, the concentration of the active constituents is seasonally and geographically different and some genotypes are characterised by particularly high contents of active essential oil constituents. Oxygenated monoterpenes (99.51%), were the main components in the the leaves of *A. absinthium* collected from Iran.

Antibacterial activity

The antibacterial activities of *A. absinthium* oil was assayed against five bacteria and results presented in Table 2. The antibacterial activities of the essential oils were evaluated by disc diffusion method using Muller-Hinton Agar for bacteria with determination of inhibition zones (IZ), minimal inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and decimal reduction time (D-value). The present study revealed the leaf essential oil of *A. absinthium* collected from Iran, indicated significant activity against *C. albicans* and moderate inhibitory activity against *S. aureus*. On the other hand, antimicrobial screening was performed on samples of French origin and showed that *A. absinthium* oil inhibited the growth of both tested yeasts *C. albicans* and *Saccharomyces cerevisiae* var. *chevalieri* (Juteau et al., 2003), while the extract of *A. absinthium* collected from Eastern Anatolia region of Turkey, also showed antibacterial activity against all tested microorganisms (6 to 19 mm inhibition zone), apart from *Alcaligenes faecalis* and *Aspergillus niger* (Erel et al., 2012).

Bactericidal kinetics of the oils

Table 2 shows reduction times of *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans* and *A. baumannii*, respectively, after exposure to the MBC levels of the oils. It can be concluded that *C. albicans* is the most vulnerable to the oil under study. These values suggest the duration of time required for complete bactericidal effects of the oils.

Total phenol contents

As shown in Table 3, the total phenol contents (TPC) of the leaf essential oil of *A. absinthium* was determined to be 168.67 ± 9.50 μg gallic acid equivalent/mg sample (GAE/mg). Phytochemical investigation determined that extract of *A. absinthium* collected from Golestanak protege area central Elburz showed high phenolic and flavonoid contents (Mahmoudi et al., 2009). The high contents of total phenolic compounds (25.6 mg g^{-1}) and total flavonoids (13.06 mg g^{-1}) indicated that these compounds contribute to the antiradical and antioxidative activity (Canadanovic-Brunet et al., 2005).

Antioxidant activity

Antioxidative properties of the extract was determined by bleaching of β -carotene or 2,20-diphenylpicrylhydrazyl (DPPH). The Ferric-reducing antioxidant power (FRAP) was expressed as gallic acid equivalents or known Fe(II) concentration for *A. absinthium* essential oil. The DPPH

radical-scavenging activities of the leaf essential oil of *A. absinthium* are shown in Table 3. The leaf essential oil of *A. absinthium* notably reduced the concentration of DPPH free radical. IC₅₀ for DPPH radical-scavenging activity was 5.85 $\mu\text{g/ml}$. In phytochemical investigation, the antiradical activity of *A. absinthium*, collected from Serbia, was tested by measuring their ability to reactive hydroxyl radical during the Fenton reaction trapped by 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), using electron spin resonance (ESR) spectroscopy. Results demonstrated that the antiradical activity depend on the type and concentration of applied extracts and increased in the order ethyl acetate > methanol > n-butanol > chloroform > petroleum ether > remaining water extracts (Canadanovic-Brunet et al., 2005).

Ferric-reducing antioxidant power (FRAP) assay of the oil

The FRAP assay was expressed as gallic acid equivalents (GAE) in mg/g of samples used ($y = 16.66x + 0.003$; $r^2 = 0.999$). The ferric reducing power of the essential oils was determined 10.67 ± 0.45 gallic acid equivalent (mg/g).

Free radical-scavenging capacities of the oils

The DPPH radical-scavenging activities of the essential oil are shown in Table 3. The leaf essential oil of *A. absinthium* reduced the concentration of DPPH free radical ($61.4 \pm 1.4\%$, 10 mg/ml of essential oil) with an efficacy lower than that of reference oil *T. x-parlock* (69.3% inhibition). IC₅₀ for DPPH radical-scavenging activity was 5.85 $\mu\text{g/ml}$.

β -carotene-linoleic acid assay

The lipid peroxidation inhibitory activities of the essential oil was assessed by the β -carotene bleaching test. Results of the reference oil (*T. x-parlock*) were almost consistent with data obtained from the DPPH test. In β -carotene-linoleic acid test system, oxidation of linoleic acid was effectively inhibited by *A. absinthium* oil ($58.56 \pm 2.5\%$, 0.625 mg/ml of essential oil).

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REFERENCES

- Pino AJ (1997). Aristides Rosado & Victor Fuentes, Chemical Composition of the Essential Oil of *Artemisia absinthium* from Cuba. *J. Essent. Oil Res.* 9(1):87-89.
- Adams RP (2001). Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publ. Corp. Carol Stream, IL.
- Akomo EFO, Zongo C, Karou SD, Obase LC, Savadogo A, Atteke C, Traore AS (2009). *In vitro* antiplasmodial and antibacterial activities of *Canthium multiflorum schum* and *thonn* (Rubiaceae) extracts. *Pak. J. Biol. Sci.* 12: 919-923.
- Anonymous (2003). ESCOP Monographs: The Scientific Foundation for Herbal Medicinal Products. 2nd edition. Exeter, U.K, European Scientific Cooperative on Phytotherapy.
- Burits M, Bucar F (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytother. Res.* 14:323-328.
- Canadanovic-Brunet JM, Djilas MS, Cetkovic GS, Tumbas VT (2005). Free-radical scavenging activity of wormwood (*Artemisia absinthium*) extracts. *J. Sci. Food Agric.* 85:265-272.
- Choi HS, Song HS, Ukeda H, Sawamura M (2000). Radicals scavenging activities of citrus essential oils and their components: detection using 1,1-diphenyl-2-picrylhydrazyl. *J. Agric. Food Chem.* 48:4156-4161.
- Cuendet M, Hostettmann K, Potterat O (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helv Chim. Acta* 80:1144-1152.
- Derwich E, Benziane Z, Boukir A (2009). Chemical composition and insecticidal activity of the essential oils of three plants *Artemisia* sp: *Artemisia herba-alba*, *Artemisia absinthium* and *Artemisia pontica* (Morocco). *J. Environ. Agric. Food Chem.* 8(11):1202-1211.
- Erel SB, Reznicek G, Gokhan Şenol S (2012). Antimicrobial and antioxidant properties of *Artemisia* species from western Anatolia. *Turk. J. Biol.* 36:75-84.
- Fiamegos YC, Kastritis PL, Exarchou V, Han H, Bonvin AMJJ, Vervoort J, Lewis K, Hamblin MR, Tegos GP (2011). Antimicrobial and Efflux Pump Inhibitory Activity of Caffeoylquinic Acids from *Artemisia absinthium* against gram-positive pathogenic bacteria. 6(4):e18127. DOI:10.1371/journal.pone.0018127.
- Firouzni A, Vahedi H, Sabbaghi F, Bigdeli M (2008). Composition of the essential oil of *Artemisia ciniformis*, *A. kopetdaghensis*, and *A. khorasanica* in Iran. *Chem. Nat. Comp.* 44:804-806.
- Hoffmann D (2003). Medical Herbalism - The Science and Practice of Herbal Medicine. Rochester, Vermont: Healing Arts Press. F.
- Juteau F, Jerkovic I, Masotti V, Milos M, Mastelic J, Bessi ere JM, Viano J (2003). Composition and antimicrobial activity of the essential oil of *Artemisia absinthium* from Croatia and France. *Planta Med.* 69(2):158-161.
- Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* 47:3954-3962.
- Lim TY, Lim YY, Yule CM (2009). Evaluation of antioxidant, antibacterial and anti- tyrosinase activities of four Macaranga species. *Food Chem.* 114:594-599.
- Linnaeus C (1753). Species plantarum: exhibentes plantas rite cognitatas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas. 2. Holmiae (*Laurentii Salvii*). p.848. Archived from the original on 4 October 2008.
- Lopes-Lutz D, Alviano DS, Alviano CS, Kolodziejczyk PP (2008). Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils. *Phytochemistry* 69(8):1732-1738.
- Lust-John ND (1979). The Herb Book. Bantam Books. ISBN 978-0-553-26770-9.
- Mahmoudi M, Ebrahimzadeh, MA, Ansaroudi F, Nabavi SF, Nabavi SM (2009). Antidepressant and antioxidant activities of *Artemisia absinthium* L. at flowering stage. *Afr. J. Biotechnol.* 8 (24):7170-7175.
- Mills S, Bone K (2005). The Essential Guide To Herbal Safety. St Louis, Mo: Elsevier Churchill Livingstone.
- Miraliakbari H, Shahidi F (2008). Antioxidant activity of minor components of tree nut oils. *Food Chem.* 111:421-427.
- Mozaffarian VA (1996). Dictionary of Iranian Plant Names. Farhang Moaser, Tehran.
- Orava A, Raalb A, Arakb E, M uriseppa M, Kailasa T (2006). Composition of the essential oil of *Artemisia absinthium* L. of different geographical origin. *Proc. Estonian Acad. Sci. Chem.* 55(3):155-165.
- Rasooli I, Mirmostafa SA (2003). Bacterial susceptibility to and chemical composition of essential oils from *Thymus kotschyanus* and *Thymus persicus*. *J. Agric. Food Chem.* 51:2200-2205.
- Rechinger KH (1986). *Artemisia*. In: *Flora Iranica Compositae* No. 158, (Eds.), Rechinger KH and Hedge IC. (eds). Akademische Druck und Verlagsanstalt, Gras, Austria.
- Rustaiyan A, Masoudi S (2011). Chemical composition and biological activities of Iranian *Artemisia* species. *Phytochem. Lett.* 4:440-447.
- Taga MS, Miller EE, Pratt DE (1984). Chia seeds as a source of natural lipid antioxidant. *J. Am. Oil Chemists Soc.* 61:928-931.
- Tariku Y, Hymete A, Hailu A, Rohloff J (2011). *In vitro* Evaluation of Antileishmanial Activity and Toxicity of Essential Oils of *Artemisia absinthium* and *Echinops kebericho*. *Chem Biodivers.* 8(4): 614-623.
- Thomsen M (2005). Phytotherapy Desk Reference. 3rd edition. Michael Thomsen.
- Wichtl M (2004). Herbal Drugs and Phytopharmaceuticals: A Handbook for Practice on a Scientific Basis. 3rd edition. Stuttgart, Germany: CRC Press.
- Wyk B-E, Wink M (2004). Medicinal Plants of the World. Arcadia, South Africa: Briza Publications.
- Wayne PA (2008). NCCLS: Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard. 3rd Edn. National Committee for Clinical Laboratory Standards. ISBN: 1-56238-666-2.
- Yadegarinia D, Gachkar L, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I (2006). Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils. *Phytochemistry.* 67: 1249-1255.
- Zongo C, Akomo EFO, Savadogo A, Obase LC, Koudou J, Traore AS (2009). *In vitro* antibacterial properties of total alkaloids extract from *Mitragyna inermis* (Willd). Kuntze O, a West African traditional medicinal plant. *Asian J. Plant Sci.* 8:172-177.