

Asian Pacific Journal of Tropical Biomedicine



11

2020

Vol 10 No 11 Nov





Original Article

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.org



doi: 10.4103/2221-1691.294089

Impact Factor: 1.90

Phytochemical profile, antioxidant activity and wound healing properties of *Artemisia absinthium* essential oilA. Benkhaled¹, A. Boudjelal¹✉, E. Napoli², F. Baali³, G. Ruberto²✉¹Department of Microbiology and Biochemistry, Faculty of Sciences, University of M'sila, Algeria²Istituto del CNR di Chimica Biomolecolare, Catania, Italy³Department of Biological Sciences, Faculty of Nature and Life Sciences, University of BBA, Algeria

ABSTRACT

Objective: To evaluate chemical compositions, antioxidant and wound healing properties of Algerian *Artemisia absinthium* essential oil.

Methods: The chemical composition of the essential oil from *Artemisia absinthium* was analyzed by a combination of GC-FID and GC/MS. The antioxidant capacities including the total antioxidant capacity, DPPH[•] and ABTS^{•+} scavenging capacities were measured. The wound healing potential was assessed by the excision wound model of rats. The wounds were treated daily with an ointment prepared with two concentrations (5% and 10%) of *Artemisia absinthium* essential oil. The percentage of wound contraction was determined and wound healing was also evaluated by histological examination of the healed skin.

Results: The main component of *Artemisia absinthium* essential oil was camphor (48%) followed by chamazulene (10%) that was responsible for the dark blue color of the oil. *Artemisia absinthium* essential oil exhibited moderate antioxidant activity compared with BHT and Trolox. All preparations showed significant effects on wound contraction and the ointment prepared with 10% of essential oil was effective as the reference drug Cicatryl.

Conclusions: The essential oil of *Artemisia absinthium* shows moderate antioxidant activity. The 10% ointment enhances skin wound re-epithelialization and speeds up the healing process. The essential oil of *Artemisia absinthium* may be used as an alternative drug for wound healing.

KEYWORDS: *Artemisia absinthium*; Essential oil; Antioxidant; Wound healing

1. Introduction

Artemisia absinthium (*A. absinthium*) popularly known as common wormwood belongs to the family Asteraceae (Compositae). The genus *Artemisia* consists of over 500 different species and is distributed in almost all temperate zones. Many *Artemisia* species have been studied for their phytochemical profiles and pharmacological properties. *A. absinthium* has a long history as a medicinal plant[1,2] and is native to temperate regions of Eurasia and Northern Africa but is rather rare in Algeria[3]. *A. absinthium* contains an essential oil that is characterized by a particular color ranging from green to dark blue. One of the most peculiar features of this oil is the presence of large amounts of thujone, an oxygenated monoterpene, which can account for 40%-90% of the oil. This compound is normally present in two isomeric forms named α - and β -thujone and the latter is more abundant. Other important constituents of *A. absinthium* are the non-volatile sesquiterpene lactones, particularly absinthin, which are responsible for the bitter taste of its extracts.

✉To whom correspondence may be addressed. E-mail: amel.boudjelal@univ-msila.dz (Amel Boudjelal); giuseppe.ruberto@icb.cnr.it (Giuseppe Ruberto)

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2020 Asian Pacific Journal of Tropical Biomedicine Produced by Wolters Kluwer-Medknow. All rights reserved.

How to cite this article: Benkhaled A, Boudjelal A, Napoli E, Baali F, Ruberto G. Phytochemical profile, antioxidant activity and wound healing properties of *Artemisia absinthium* essential oil. Asian Pac J Trop Biomed 2020; 10(11): 496-504.

Article history: Received 2 January 2020; Revision 11 February 2020; Accepted 7 May 2020; Available online 9 September 2020

Besides the use as a medicinal plant, *A. absinthium* was used between the end of the 19th and the beginning of the 20th century for the production of the spirit absinthe, whose production and consumption grew very largely during the aforesaid periods[4–6]. However, this alcoholic beverage was successively banned since the presence of thujone was considered as the cause of the so-called ‘absinthism’ syndrome, including convulsions, hallucinations and mental disorders. However, no correlation was found between thujone and health. Then the limit of thujone content has been set as 35 mg/L in alcoholic beverages[2,7].

As a medicinal plant, *A. absinthium* has been used as an antipyretic, antispasmodic anti-inflammatory and memory-improving agent and was used to treat abscesses, wounds and other skin diseases[1,8]. In a recent study, the petroleum ether extract of *A. absinthium* collected in Turkey, which was particularly rich in volatile components, showed positive effects on *in vivo* induced pseudopregnancy[9].

A recent ethnopharmacological survey performed in Algeria reports that various phytotherapeutic preparations from *Artemisia* spp. (including *A. absinthium*) possess antidiabetic, antihypertensive, and analgesic properties[10]. Furthermore, *A. absinthium* essential oil also showed pesticide, insecticidal, and antimicrobial activities[8,11–13].

The main aim of the present study was to evaluate the antioxidant efficacy and the *in vivo* wound healing properties of *A. absinthium* essential oil and to provide a reference for sustainable exploitation of Algerian flora.

2. Materials and methods

2.1. Reagents and standards

All solvents used were high-purity American Chemical Society solvents from VWR (Milan, Italy); reagents and reference standards were purchased from Sigma-Aldrich Products (Merck KGaA, Darmstadt, Germany), Extrasynthese (Lyon, France) and Fluka (Milan, Italy).

2.2. Plant material

The flowering aerial parts of *A. absinthium* L. were collected in May 2018, in M’sila (situated in the central part of Algeria) at 35°12' 36.97" N latitude and 4°10' 46.08" E longitude. The plant was authenticated by Dr. Sarri DJ, Department SNV/ M’sila University, and a specimen (AB-13) was deposited at the herbarium of the Department.

2.3. Essential oil extraction

The essential oil was extracted by hydrodistillation using a Clevenger-type apparatus (Clevenger D04000201, Somiver, Algeria)

according to the European Pharmacopoeia[14]. The oil was dried with anhydrous sodium sulfate, weighed, and stored at 4°C until use.

2.4. Essential oil analysis

Gas chromatographic (GC) analyses were performed on a Shimadzu gas chromatograph, Model 17-A (Shimadzu, Japan) equipped with a flame ionization detector (FID) and with an operating software Class VP Chromatography Data System version 4.3 (Shimadzu). Analytical conditions: SPB-5 capillary column (15 m×0.10 mm×0.15 µm), helium as carrier gas (1 mL/min). Injection in split mode (1:200), injected volume 1 µL (4% essential oil/CH₂Cl₂ v/v), injector and detector temperature 250 and 280°C, respectively. Linear velocity in column 19 cm/s. The oven temperature was held at 60°C for 1 min, then, programmed as reported previously[15]. Percentages of compounds were determined from their peak areas in the GC-FID profiles.

Gas-chromatography-mass spectrometry (GC-MS) was carried out in the fast mode on a Shimadzu GC-MS mod. GCMS-QP5050A, with the same column and the same operative conditions used for analytical GC-FID and GCMS solution version 1.02 operating software (Shimadzu). Ionization voltage 70 eV, electron multiplier 900 V, ion source temperature 180°C. Mass spectra data were acquired in the scan mode in the *m/z* range 40–400. The same oil solutions (1 µL) were injected with the split mode (1:96).

2.5. Identification of components of essential oil

The identity of components was based on their GC retention index (relative to C₉–C₂₂ *n*-alkanes on the SPB-5 column), computer matching of spectral MS data with those from NIST MS libraries[16], the comparison of the fragmentation patterns with those reported in the literature[17] and, whenever possible, co-injections with authentic samples.

2.6. Total antioxidant capacity (TAC)

The TAC of *A. absinthium* essential oil was evaluated by the phosphomolybdenum method[18]. An aliquot of 0.3 mL of the essential oil was mixed with 3 mL of the reagent solution (0.6 mol/L of sulfuric acid, 28 mmol/L of sodium phosphate and 4 mmol/L of ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min and the absorbance measured at 695 nm. Butylated hydroxytoluene (BHT) was used as standard antioxidant. The antioxidant activity was expressed in µg equivalent of BHT per mg of essential oil (µg EBHT/mg essential oil).

2.7. DPPH radical scavenging activity

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging

capacity was measured according to Li *et al*[19]. A total of 1 mL of the freshly prepared 0.1 mM DPPH in methanol was added to the 3 mL of EO solution at different concentrations (0.05-1.5 mg/mL). The reaction mixture was left to stand at room temperature in the dark for 30 min and the absorbance was recorded at 517 nm. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_c - A_s) / A_c] \times 100$$

where A_c and A_s are the absorbances of the control and the sample, respectively. BHT was used as the reference compound. The assay was done in triplicate.

2.8. Scavenging activity of ABTS radical

The scavenging activity of ABTS radical (ABTS^+) has been evaluated following the method of Re *et al*[20]. An aliquot of 1 mL of essential oil was mixed with 2 mL of diluted ABTS^+ , after 30 min of incubation; the absorbance was measured at 734 nm. The percentage of ABTS^+ inhibition was calculated using the following formula:

$$\text{ABTS scavenging effect (\%)} = [(A_c - A_s) / A_c] \times 100$$

where A_c and A_s are the absorbances of the control and the sample, respectively. Trolox was used as the reference antioxidant. All measurements were performed in triplicate.

2.9. Wound healing activity

2.9.1. Preparation of the ointment

The *A. absinthium* essential oil (AEO) was mixed with petroleum jelly (PJ) (Unilever, France) in two concentrations of 5% and 10% to obtain the essential oil ointments OAEO 5% and 10%, respectively[21]. Cicatryl-Bio (CIC) (Pierre Fabre, France) was used as a reference drug.

2.9.2. Animals

Healthy adult male Wistar albino rats, weighing between 200-220 g, were obtained from Pasteur Institute of Algeria. Animals were fed with commercially pelleted rat diet and water *ad libitum*.

2.9.3. Experimental protocol

The rats were weighed, marked and divided into 5 groups of 5 rats each: Untreated group (UT group), Cicatryl-treated group (CIC group), ointment artemisia essential oil 5%-treated group (OAEO 5% group), ointment artemisia essential oil 10%-treated group (OAEO 10% group), and petroleum jelly-treated group (PJ group). An area of 250 mm² on the back of the rat was shaved. The animals were left in their cages 24 hours to verify the absence of irritation of the shaved zone[22].

2.9.4. Skin irritation test

The OAEO 5% and 10% were applied separately on the back of the rats. After 4 h, the skin of the animals was evaluated to detect the

presence of inflammation signs[23].

2.9.5. Healing activity

Animals were anaesthetized using intraperitoneal injection of ketamine (90 mg/kg)-xylazine (10 mg/kg)[24]. A circle approximately 2.5 cm in diameter was drawn on the skin of the lumbar region and was then excised. The animals were placed in individual cages with clean litters.

Preparations (CIC, OAEO 5%, OAEO 10%, and PJ) were applied locally at an amount of 0.5 g per rat of the different groups once per day until complete re-epithelialization[25]. The dimensions of excision wounds were measured every 3 days during the trial period by tracing the wounds on transparent paper and measuring through the graph paper. The percentage of the evolution of wound contraction was calculated using the following formula[26]:

$$\% \text{ Wound contraction} = [(\text{Initial wound size} - \text{Specific day wound size}) / \text{Initial wound size}] \times 100$$

2.9.6. Histological sections

At the end of the experimentation, the rats were sacrificed, the skin healed and 0.5 cm of healthy skin were removed for histological study[25]. The tissue slices were fixed in formalin (10%) for 72 h. These samples were dehydrated by passing it through three successive baths of ethanol. Then they were thinned in two baths of xylene and embedded in paraffin by two successive baths at 60°C each one. The obtained paraffin blocks were then cut by microtome, rehydrated, stained with haematoxylin-eosin[27] and analyzed by Optika B-500 microscope (Optika s.r.l., Italy) at $\times 10$ and $\times 40$ magnifications.

2.10. Statistical analysis

All statistical analyses were performed using GraphPad Prism 7.0. Data were expressed as mean \pm SD. Statistical significance between groups was analyzed by one-way ANOVA followed by Dunnett's test. Values for $P < 0.05$ were considered statistically significant.

2.11. Ethical statement

All experimental protocols were approved by the National Committee for Evaluation and Programming of University Research of Algerian Ministry of Higher Education and Scientific Research (Registration N : DO1N01UN280120150001), according to International Council for Laboratory Animal Science[28].

3. Results

3.1. Yield and chemical composition

The extraction yield of essential oil from the flowering aerial parts

of *A. absinthium* collected in Algeria was $(0.29 \pm 0.08)\%$ as v/w. The oil showed an intense dark blue color.

A total of 75 components were identified in the oil (Table 1). Oxygenated monoterpenes were quantitatively the main constituents accounting for 59.64% of the total oil and including 19 components. Camphor with ca. 47.59% was the main compound of this class and also of the total oil, followed by terpinen-4-ol (ca. 6.36%). While linalool and *cis*-sabinene hydrate also exceeded 1%, the content of all other components was below this value. The second class of components was represented by sesquiterpenes accounting for 16.67% of the oil. It included more classes with 24 components.

Chamazulene, which is responsible for the blue color of the oil reached a higher concentration (10.35%). Among the other components, only caryophyllene oxide and a dihydrochamazulene isomer slightly exceeded 1%. Monoterpene hydrocarbons accounted for 14.93% of the oil and included 13 components. The main compounds were γ -terpinene, camphene, *p*-cymene, α -pinene, and α -terpinene. The last group was represented by the so-called 'other compounds', with a total content of 3.73% and 18 components. The composition was rather variegated and the main component was geranyl *p*-cymene. A typical GC profile of *A. absinthium* essential oil with some of the main components is shown in Figure 1.

Table 1. Chemical composition of the essential oil of *Artemisia absinthium* from Algeria.

No ^a	RI ^b	Class/Compound	% ^c	No ^a	RI ^b	Class/Compound	% ^c
Monoterpene hydrocarbons (n=13)				53	1455	α -Humulene	0.089±0.005
2	927	Tricyclene	0.084±0.002	54	1474	7- <i>epi</i> -1,2-dehydro-Sesquicineole	0.042±0.001
3	930	α -Thujene	0.180±0.002	55	1478	γ -Gurjunene	0.040±0.001
4	938	α -Pinene	2.157±0.018	56	1480	γ -Murolene	0.042±0.003
5	954	Camphene	2.677±0.022	57	1494	Germacrene D	0.504±0.003
7	977	Sabinene	0.137±0.001	58	1525	Dihydrochamazulene 1 ^d	0.853±0.010
8	981	β -Pinene	0.137±0.001	59	1530	Dihydrochamazulene 2 ^c	0.141±0.002
10	990	Myrcene	0.844±0.005	60	1562	Elemol	0.434±0.003
11	1006	α -Phellandrene	0.202±0.001	61	1578	<i>E</i> -Nerolidol	0.024±0.003
12	1019	α -Terpinene	1.576±0.012	63	1595	Spathulenol	0.133±0.001
13	1028	<i>p</i> -Cymene	2.644±0.021	64	1601	Caryophyllene oxide	1.475±0.112
14	1032	Limonene	0.643±0.003	66	1631	Humulene epoxide II	0.113±0.004
17	1063	γ -Terpinene	2.878±0.016	67	1634	Dihydrochamazulene 3 ^f	1.014±0.036
20	1091	Terpinolene	0.768±0.003	68	1649	β -Eudesmol	0.099±0.004
Oxygenated monoterpenes (n=19)				69	1706	γ - <i>E</i> -Atlantone	0.033±0.002
15	1037	1,8-Cineole	0.236±0.002	70	1758	Chamazulene	10.346±0.152
18	1073	<i>cis</i> -Sabinene hydrate	1.311±0.010	71	1800	Dehydrochamazulene 4 ^g	0.228±0.005
19	1078	Camphenilone	0.018±0.002	73	1884	Hexahydrofarnesyl acetone	0.037±0.006
21	1100	Linalool	1.418±0.015	74	1911	<i>Z</i> -Nuciferol acetate	0.055±0.001
23	1110	<i>trans</i> -Sabinene hydrate	0.090±0.001	Diterpenes (n=1)			
24	1132	<i>cis-p</i> -Menth-2-en-1-ol	0.479±0.007	72	1841	Neophytadiene	0.049±0.002
25	1159	Camphor	47.592±0.208	Others (n=18)			
26	1161	Camphene hydrate	0.280±0.265	1	854	2- <i>E</i> -Hexenal	0.052±0.002
28	1175	Borneol	0.350±0.000	6	966	Benzaldehyde	0.031±0.001
29	1186	Terpinen-4-ol	6.359±0.042	9	987	6-methyl-5-Hepten-2-one	0.078±0.002
31	1197	α -Terpineol	0.414±0.003	16	1049	4,6-dimethyl-5-Hepten-2-one	0.125±0.001
34	1108	<i>trans</i> -Piperitol	0.067±0.002	22	1103	3-methyl-butyl 2-methyl Butanoate	1.080±0.045
35	1205	Verbenone	0.033±0.001	27	1162	2-ethyl Hexyl acetate	0.040±0.000
39	1248	Carvone	0.076±0.001	30	1191	<i>p</i> -methyl-Acetophenone	0.185±0.003
40	1273	Perillaldehyde	0.177±0.004	32	1200	Methyl salicylate	0.344±0.004
44	1305	Carvacrol	0.411±0.003	33	1202	Methyl chavicol	0.085±0.003
52	1459	Neryl acetone	0.022±0.002	36	1233	2-methyl-3- <i>Z</i> -Hexenyl butanoate	0.058±0.001
62	1584	Geranyl butanoate	0.090±0.001	37	1235	3-methyl-3- <i>Z</i> -Hexenyl butanoate	0.058±0.003
65	1623	Geranyl isovalerate	0.221±0.001	38	1237	Cumin aldehyde	0.061±0.001
Sesquiterpenes (n=24)				41	1290	<i>E</i> -Anethole	0.084±0.001
46	1386	α -Copaene	0.184±0.001	42	1293	2-Undecanone	0.967±0.009
47	1391	β - <i>E</i> -Damascenone	0.030±0.001	43	1295	Tridecane	0.180±0.010
48	1396	β -Bourbonene	0.194±0.002	45	1364	Eugenol	0.194±0.001
50	1434	β -Caryophyllene	0.622±0.014	49	1407	γ -4-dimethyl-Benzenebutanal	0.111±0.006
51	1442	β -Copaene	0.029±0.002	75	1946	Geranyl <i>p</i> -cymene	2.430±0.015

^aThe numbering refers to elution order; ^bRetention index (RI) relative to standard mixture of *n*-alkanes on SPB-5 column; ^cValues (relative peak area percent) represent averages of three determinations with \pm SD; ^dIsomer not determined, electron impact mass spectrometry (EIMS): 186 (86%), 171 (36%), 157 (100%), 142 (56%), 128 (28%), 115 (25%), 91 (7.5%); ^eIsomer not determined, EIMS: 186 (100%), 171 (75%), 157 (65%), 143 (65%), 129 (45%), 115 (28%), 91 (15%); ^fIsomer not determined, EIMS: 186 (100%), 171 (85%), 157 (55%), 142 (65%), 128 (45%), 115 (25%), 91 (20%); ^gIsomer not determined, EIMS: 182 (100%), 167 (66%), 152 (55%), 141 (10%), 128 (6%), 115 (15%), 91 (10%).

3.2. *In vitro* antioxidant activity

The antioxidant activity of *A. absinthium* essential oil was evaluated by total antioxidant capacity, and DPPH and ABTS radical scavenging assays, using BHT and Trolox as standards.

The results showed that the total antioxidant capacity of the essential oil was significantly ($P < 0.001$) lower than that of the reference compound BHT [(228.99±6.06) µg EBHT/mg and (417.99 ± 1.31) µg EBHT/mg, respectively].

A. absinthium essential oil was able to reduce the stable free radical DPPH with an IC_{50} of (139.93±7.81) µg/mL, but was significantly less active ($P < 0.001$) than the synthetic antioxidant BHT [IC_{50} (14.60±0.71) µg/mL].

In the ABTS assay, the anti-radical activity of the essential oil of *A. absinthium* showed higher IC_{50} in comparison with the IC_{50} of Trolox [(32.96±0.71) and (3.15±0.02) µg/mL, respectively] ($P < 0.001$).

3.3. Skin irritation test

No edema, erythema or signs of inflammation were observed after topical application of *A. absinthium* essential oil formulations on rats.

3.4. Evolution of healing process of wounds

During the healing period, and according to a specific interval of time of three days, the wounds were regularly measured. The

evolution of the surface of each wound excision was assessed and the results are represented in Figure 2, which showed that the OAEO 10% treatment had the best results.

All treated animals showed a significant reduction in wound area when compared to the untreated group ($P < 0.05$). There was no significant difference between groups treated with the *A. absinthium* essential oil ointment and the reference drug Cicatryl. An improved healing pattern with wound closure was observed in treated groups within 9-12 days (Table 2).

Daily visual observations indicated the presence of signs of inflammation around the wound in rats of different groups on the first day after the excision of the skin. These signs disappeared rapidly in the treated groups (CIC, OAEO 5% and 10%) and persisted for a few days for the rest of the groups (UT and PJ). There was a complete closure of wounds in the treated groups with OAEO and Cicatryl in comparison with the untreated group and the group treated with petroleum jelly.

3.5. Histological sections

Histology of the skin area of the excised wound on day 21 post wounding showed incomplete epithelization with more inflammatory cells, poorly formed granulation tissue and sparse distribution of collagen fibers for the UT and PJ groups. A wound gap was observed in the epidermis layers in the two groups. These observations explain the delay in the wound healing process (Figure 3A&B).

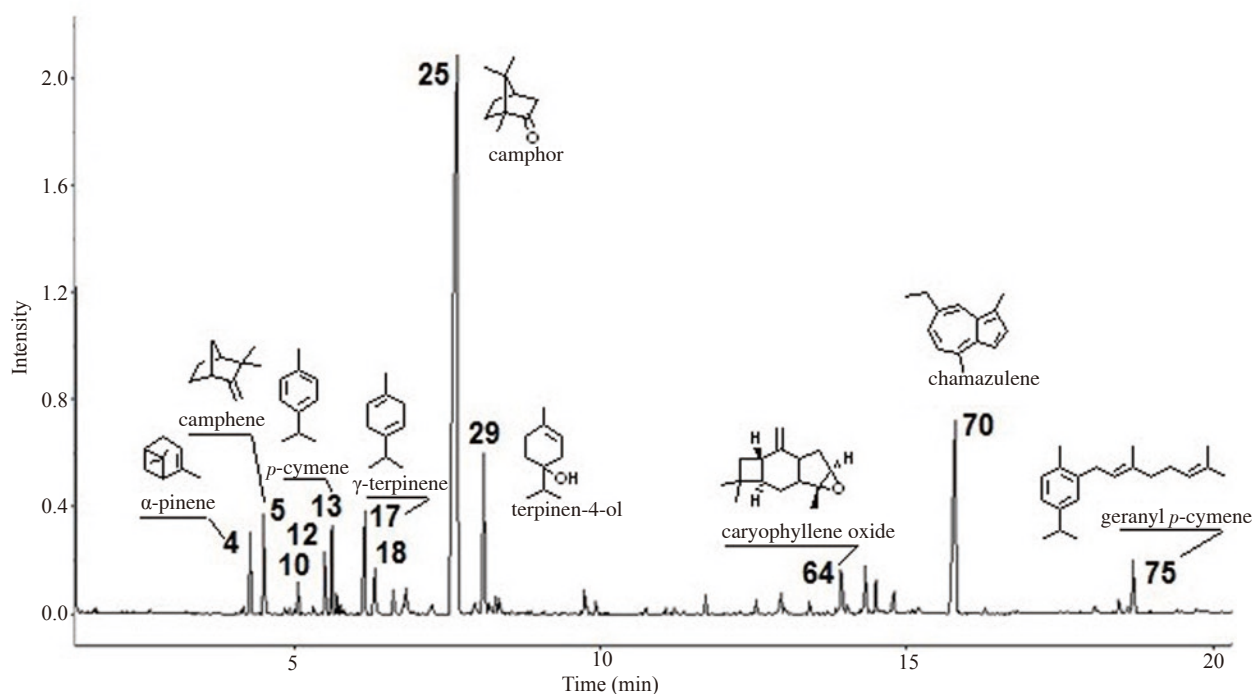


Figure 1. GC profile of the essential oil of *Artemisia absinthium* from Algeria and some of the main components (for numbering see Table 1).

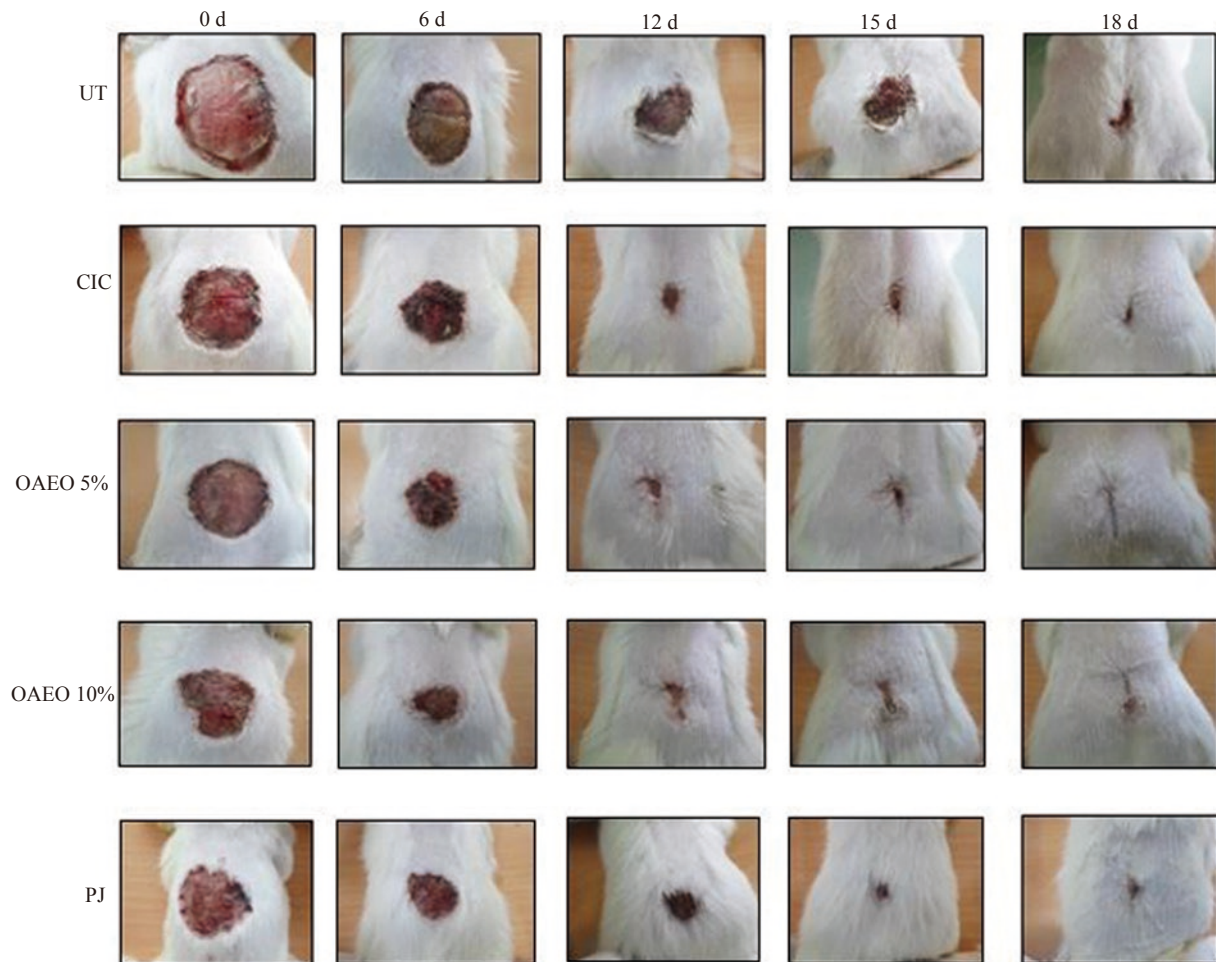


Figure 2. Chronology of excision wound healing in different groups. UT: Untreated group, CIC: Cicatryl-treated group, OAEO 5%: ointment artemisia essential oil 5%-treated group, OAEO 10%: ointment artemisia essential oil 10%-treated group, and PJ: petroleum jelly-treated group.

Table 2. Effect of different treatments on the evolution of the healing process of excision wounds in Wistar albino rats.

Groups	Days						
	3	6	9	12	15	18	21
UT	9.58±1.67	17.82±1.20	19.77±2.83	20.54±2.39	22.32±1.10	27.54±1.20	36.58±2.00
CIC	9.18±1.83	29.81±3.29*	49.91±1.55***	60.53±1.19***	75.87±1.10***	84.52±2.80***	96.00±1.36***
OAEO 5%	8.13±1.31	34.64±2.76***	52.77±2.32***	60.09±1.93***	68.75±1.34***	80.45±2.80***	91.81±2.01***
OAEO 10%	9.54±2.05	29.34±4.57*	44.74±3.57**	56.65±4.01***	72.00±4.64***	83.33±1.97***	97.23±3.25***
PJ	8.99±2.27	24.79±1.60	34.85±1.61**	39.51±1.37***	44.75±1.85***	50.56±1.52***	60.55±2.46***

Values are expressed as mean ± SD (n=5); *P<0.05, **P<0.01, and ***P<0.001 (when treated groups are compared to the UT group); UT: Untreated group, CIC: Cicatryl-treated group, OAEO 5%: ointment artemisia essential oil 5%-treated group, OAEO 10%: ointment artemisia essential oil 10%-treated group, and PJ: petroleum jelly-treated group.

The histological sections of all treated groups (CIC, OAEO 5% and OAEO 10%) showed better healing resulting in more regular cell layers and more epidermal ridges. There was a complete re-epithelialization with a visible thick neo-formative epidermis, abundant granulation tissue and higher collagen deposition. These histopathological observations provide additional evidence for the contraction value of wound areas in treated groups (Figure 3C, D, E).

4. Discussion

The essential oil yield in our study was slightly higher than literature data which report a yield range between 0.2% and 1.5%[4,29–31]. The chemical composition of *A. absinthium* oil can explain the biological activity of this oil and justify the use of this plant in folk medicine. *A. absinthium* from Algeria can be defined as a camphor chemotype, and in comparison with literature data, this sample appears partially similar to plants collected in Brazil and Tunisia, which show

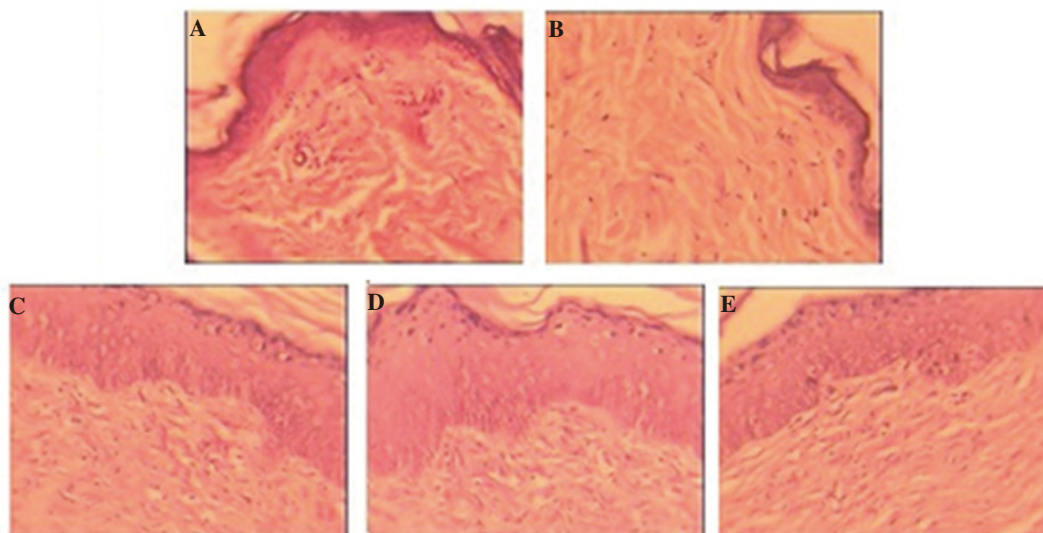


Figure 3. Histological evaluation of wound skin sections stained with hematoxylin and eosin (40× magnification). A and B: UT and PJ treatments, respectively, showing fewer collagen fibers and more inflammatory cells. C, D, and E: animals treated with OAE0 5%, OAE0 10%, and CIC drug reference, respectively, showing better healing and complete re-epithelialization.

the following main components: camphor>caryophyllene>1,8-cineole>germacrene-D> α -cadinol in the Brazilian sample, and chamazulene>camphor>bornyl acetate>chrysanthenyl acetate> γ -terpinene in the Tunisian sample[11,32].

Oxygenated monoterpenes such as camphor and terpinen-4-ol, which are representative components in the investigated oil, were reported to exhibit many biological activities, comprising anti-leishmania, insecticidal and repellency activity, as well as antimicrobial, anti-inflammatory and anti-respiratory tract infections[32–34]. Among the minor components, γ -terpinene, camphene, *p*-cymene, gernanyl *p*-cymene and α -pinene are of an amount over 2%.

On the basis of a literature survey, many other important compounds have also been identified in *A. absinthium* oil from different origins, including epoxyocimene, 1,8-cineole, myrcene, *trans*-sabinyl acetate, sabinene, viridiflorol, and thuja-2,4(10)-diene[2,30,32,35]. The predominance of one or two of the aforesaid components defines pure or mixed chemotypes[2,36].

The antioxidant/anti-radicals activity of *A. absinthium* essential oil was rather modest and probably ascribable to the presence of chamazulene.

Camphor is endowed with various pharmacological properties such as anti-bacterial, anti-fungal, and wound-healing activities[37]. A recent study has shown the effect of camphor on skin health by inducing fibroblast proliferation, maintaining or recovering collagen and elastin production in UV exposed skin, and preventing thickening of the epidermis and subcutaneous fat layer[38]. Camphor also showed a significant effect on epithelization and neovascularization on second-degree burn wounds in rats[39].

Therefore, the presence of camphor in *A. absinthium* essential oil may explain the wound healing activity of the OAE0 10% ointment group.

The wound healing property of *A. absinthium* essential oil was also remarkable in comparison with the standard drug Cicatryl. This suggests that *A. absinthium* essential oil could be developed as a potential effective wound healing ingredient for topical medicaments.

Conflict of interest statement

We declare that there is no conflict of interest.

Funding

This work was supported by Algerian Ministry of Higher Education and Scientific Research/ Algeria (CNEPRU: DO1N01UN280120150001) and Consiglio Nazionale delle Ricerche, Rome, Italy.

Acknowledgments

The authors are grateful to Mr. Antonio Greco (ICB-CNR, Catania) for his skilful technical support.

Authors' contributions

Amel B. and GR conceived and designed the experiments, supervising all steps of research; EN conducted the essential oil analyses, organized and wrote the relative experimental section; Amel B., AB and FB performed the essential oil extraction, the antioxidant activity evaluation, the *in vivo* experiments and wrote the pertinent portion; GR and Amel B. wrote the article with the contribution from all co-authors. All authors approved the final version of the manuscript.

References

- [1] Bora KS, Sharma A. The genus *Artemisia*: A comprehensive review. *Pharm Biol* 2017; **49**: 101-109.
- [2] Nguyen HT, Németh ZE. Sources of variability of wormwood (*Artemisia absinthium* L.) essential oil. *J Appl Res Med Arom Plants* 2016; **3**: 143-150.
- [3] Quezel P, Santa S. *Nouvelle flore d'Algérie et des régions désertiques méridionales*. Paris: Editions du Centre National de la Recherche Scientifique; 1963, p. 741-743.
- [4] Lechenmeier DW, Walch SG, Padosch SA, Kröner LU. Absinthe – A review. *Crit Rev Food Sci Nutr* 2006; **46**: 365-377.
- [5] Lechenmeier DW. Assessing the authenticity of absinthe using sensory evaluation and HPTLC analysis of bitter principle absinthin. *Food Res Int* 2007; **40**: 167-175.
- [6] Bach B, Cleroux M, Saillen M, Schönenberger P, Burgos S, Ducruet J, et al. A new chemical tool for absinthe producers, quantification of α - β -thujone and the bitter components in *Artemisia absinthium*. *Food Chem* 2016; **213**: 813-817.
- [7] Lechenmeier DW, Nathan-Maister D, Breaux T, Luarté J, Emmert J. Absinthe, absinthin and thujone – New insight into the spirit's impact on public health. *Open Add J* 2010; **3**: 32-38.
- [8] Goel R, Singh V, Kumari R, Kumari R, Srivastava S, Mallavarapu GR, et al. *Artemisia* (Asteraceae) essential oils: Compositional variation and mechanisms of its origin, biosynthesis of constituents, correspondence between biological activities and ethnomedicinal usage and repurposing prospects. *Proc Indian Natn Sci Acad* 2019; **4**: 723-790.
- [9] Demirel MA, Suntar I, Ceribaşı S, Zengin G, Ceribaşı AO. Evaluation of the therapeutic effects of *Artemisia absinthium* L. on pseudopregnancy model in rats. *Phytochem Rev* 2018; **17**: 937-946.
- [10] Hamza N, Berke B, Umar A, Cheze C, Gin H, Moore N. A review of Algerian medicinal plants used in the treatment of diabetes. *J Ethnopharm* 2019; **238**: 111841.
- [11] Viera TM, Dias HJ, Medeiros DCT, Grundman CO, Groppo M, Heleno VCG, et al. Chemical composition and antimicrobial activity of the essential oil of *Artemisia absinthium* Asteraceae leaves. *J Essent Oil Bear Plants* 2017; **20**: 123-131.
- [12] Rizvi SAH, Ling S, Tian F, Xie F, Zeng X. Toxicity and enzyme inhibition activities of the essential oil and dominant constituents derived from *Artemisia absinthium* L. against adult Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). *Ind Crops Prod* 2018; **121**: 468-475.
- [13] Naghizadeh S, Rafiee-Dasjerdi H, Naseri B, Golizadeh A, Esmailpou B. Insecticidal activity of essential oils from *Artemisia absinthium* L., *Artemisia dracunculus* L., and *Achillea millefolium* L. against *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae). *J Crop Prot* 2019; **8**: 479-489.
- [14] EDQM. Determination of essential oils in herbal drugs. In: *European pharmacopoeia 8.0*. Council of Europe, Strasbourg, France: EDQM; 2008, p. 251–252.
- [15] Saija A, Speciale A, Trombetta D, Leto C, Tuttolomondo T, La Bella S, et al. Phytochemical, ecological and antioxidant evaluation of wild Sicilian thyme: *Thymbra capitata* (L.) Cav. *Chem Biodiv* 2016; **13**: 1641-1655.
- [16] National Institute of Standard and Technology (NIST), U.S. Department of Commerce, Mass Spectral Library, 1998. [Online] Available from: <http://nist.gov/about-nist> [Accessed on April 2020].
- [17] Adams RP. *Identification of essential oil components by gas chromatography/quadrupole mass spectrometry*. 4th edition. Carol Stream, Illinois: Allured Publishing Co; 2007.
- [18] Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal Biochem* 1999; **269**: 337-341.
- [19] Li H, Hao Z, Wang X, Huang L, Li J. Antioxidant activities of extracts and fractions from *Lysimachia foenum-graecum* Hance. *Biores Techn* 2009; **100**: 970–974.
- [20] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad Biol Med* 1999; **26**: 1231-1237.
- [21] Megha J, Sharma V, Ganesh N. Antioxidant and wound healing potential of *Pistia stratiotes* L. *Asian Pac J Trop Dis* 2012; **579**: S584.
- [22] Hwisa NA, Katakam P, Chandu BR, Abadi EG, Shefha EM. Comparative *in vivo* evaluation of three types of honey on topical wound healing activity in rabbits. *J Appl Pharm Sci* 2013; **3**: 139-143.
- [23] Chowdhary N, Kaur M, Singh A, Kumar B. Wound healing activity of aqueous extracts of *Ficus religiosa* and *Ficus benghalensis* leaves in rats. *Ind J Res Pharm Biotech* 2014; **2**: 1071-1081.
- [24] Mashreghi M, Rezazade Bazaz N, Shahri M. Topical effects of frog, “*Rana ridibunda*” skin secretions on wound healing and reduction of wound microbial load. *J Ethnopharm* 2013; **145**: 793-797.
- [25] Pipelzadeh MH, Pipelzadeh MR, Husseinzadeh P. A study on the effects of modulation of intracellular calcium on excisional wound healing in rabbit. *Iranian Biom J* 2003; **7**: 161-166.
- [26] Tamri P, Hemmati A, Boroujerdnia MG. Wound healing properties of quince seed mucilage: *In vivo* evaluation in rabbit full-thickness wound

- model. *Int J Surg* 2014; **12**: 843-847.
- [27]Marque V. *Manuel de techniques d'anatomo-cytopathologique*. Elsevier-Masson, 2010;
- [28]International Council for Laboratory Animal Science (ICLAS). [Online] Available from: <http://iclas.org> [Accessed on April 2020].
- [29]Nguyen HT, Radácsi P, Gosztola B, Rajhárt P, Németh EZ. Accumulation and composition of essential oil due to plant development and organs in wormwood (*Artemisia absinthium* L.). *Ind Crops Prod* 2018; **123**: 232-237.
- [30]Janačković P, Rajčević N, Gravičević M, Novaković J, Giweli A, Stešević D, et al. Essential oil composition of *Artemisia* (Compositae) species in regards to chemophenetics. *Biochem System Ecol* 2019; **87**: 103960.
- [31]Msaada K, Salem N, Bachrouh O, Bousselmi S, Tammar S, Alfaify A, et al. Chemical composition and antioxidant and antimicrobial activities of wormwood (*Artemisia absinthium* L.) essential oils and phenolics. *J Chem* 2015; 804658.
- [32]Mathlouthi A, Belkessam M, Sdiri M, Diouani MF, Souli A, El-Bok S, et al. Chemical composition and anti-leishmania major activity of essential oils from *Artemisia* spp. grown in central Tunisia. *J Essent Oil Bear Plants* 2018; **21**(5): 1186-1198.
- [33]Cansian RL, Astolfi V, Cardoso RI, Paroul N, Roman SS, Mielniczki-Pereira AA, et al. Insecticidal and repellent activity of the essential oil of *Cinnamomum camphora* var. *linaloolifera* Y. Fujita (Ho-Sho) and *Cinnamomum camphora* (L.) J Presl. var. *hosyo* (Hon-Sho) on *Sitophilus zeamais* Mots. (Coleoptera, Curculionidae). *Rev Bras Pl Med Campinas* 2017; **17**(4) supl. I : 769-773.
- [34]Horváth G, Ács K. Essential oils in the treatment of respiratory tract diseases highlightong their role in bacterial infections and their anti-inflammatory action: A review. *Flav Fragrance J* 2015; **30**(5): 331-341.
- [35]Mohammadi A, Ahmadzadeh ST, Ameri AA, Imani I, Golmakani E, Kamali H. Seasonal variation in the chemical composition, antioxidant activity, and total phenolic content of *Artemisia absinthium* essential oils. *Pharmacog Res* 2015; **7**(4): 329-334.
- [36]Kordali S, Cakir A, Mavi A, Kilic H, Yildirim A. Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish *Artemisia* species. *J Agric Food Chem* 2005; **53**: 1408-1416.
- [37]Ao H, Wang J, Chen L, Li S, Dai C. Comparison of volatile oil between the fruits of *Amomum villosum* Lour. and *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen based on GC-MS and chemometric techniques. *Molecules* 2019; **24**: 1663.
- [38]Tran TA, Ho MT, Song YW, Cho M, Cho SK. Camphor induces proliferative and anti-senescence activities in human primary dermal fibroblasts and inhibits UV-induced wrinkle formation in mouse skin. *Phytother Res* 2015; **29**: 1917-1925.
- [39]Vaghardoost R, Gholam SRM, Tebyanian H, Babavalian H, Malaei L, Niazi M, et al. The healing effect of sesame oil, camphor and honey on second degree burn wounds in rat. *World J Plast Surg* 2018; **7**: 67-71.