

Phytochemical composition, antioxidant and wound healing activities of *Teucrium polium* subsp. *capitatum* (L.) Briq. essential oil

Sarra Chabane , Amel Boudjelal , Edoardo Napoli , Abderrahim Benkhaled & Giuseppe Ruberto

To cite this article: Sarra Chabane , Amel Boudjelal , Edoardo Napoli , Abderrahim Benkhaled & Giuseppe Ruberto (2020): Phytochemical composition, antioxidant and wound healing activities of *Teucrium polium* subsp. *capitatum* (L.) Briq. essential oil, Journal of Essential Oil Research, DOI: [10.1080/10412905.2020.1842260](https://doi.org/10.1080/10412905.2020.1842260)

To link to this article: <https://doi.org/10.1080/10412905.2020.1842260>



Published online: 23 Nov 2020.



Submit your article to this journal [↗](#)



Article views: 39



View related articles [↗](#)



View Crossmark data [↗](#)



Phytochemical composition, antioxidant and wound healing activities of *Teucrium polium* subsp. *capitatum* (L.) Briq. essential oil

Sarra Chabane^a, Amel Boudjelal^b, Edoardo Napoli^c, Abderrahim Benkhaled^b and Giuseppe Ruberto^c

^aDepartment of Life and Nature Science, Faculty of Science, Mohamed Boudiaf University, M'sila, Algérie; ^bDepartment of Microbiology and Biochemistry, Faculty of Sciences, Université Mohamed Boudiaf, M'sila, Algérie; ^cIstituto di Chimica Biomolecolare, CNR, Catania, Italy

ABSTRACT

Teucrium polium is widely used in Algerian folk medicine as to treat wounds. The aim of this study was to evaluate the chemical composition, antioxidant and wound healing properties of *Teucrium polium* essential oil. The composition was obtained by a combination of GC-FID and GC-MS analyses. The antioxidant activity was evaluated by *in vitro* assays (total antioxidant capacity, DPPH and bleaching of β -carotene). The *in vivo* wound healing potential of an ointment containing 10% of *T. polium* essential oil was investigated. The main components were in this order: β -pinene, germacrene, α -pinene, myrcene, limonene, bicyclogermacrene, *trans*- β -guaiene, spathulenol and β -bourbonene. *Teucrium polium* essential oil displayed a moderate antioxidant activity. The *in vivo* experiments showed that 10% OEO accelerated the wound healing process in comparison with controls. This study provides a scientific rationale for the use of *Teucrium polium* essential oil in the treatments of wounds.

ARTICLE HISTORY

Received 13 July 2020
Accepted 21 October 2020

KEYWORDS

Teucrium polium; essential oil; chemical composition; antioxidant; wound healing

Introduction

Lamiaceae is one of the largest families of flowering plants comprising about 250 genera and over 7,000 species. Most of the plants of this family are aromatic and therefore important source of essential oils (EOs); Lamiaceae are widely used as culinary herbs and reported as medicinal plants in several folk traditions (1). *Teucrium* genus comprising more than 300 species is the largest of the Lamiaceae family in the Mediterranean area (2,3).

Algeria is one of the major countries in Africa with a remarkable floristic richness related to its ecosystem and landscape diversity. The number of taxa of its flora is estimated at about 4000 including 300 endemic taxa of which approximately 90% are present in the north of the country (4). Unfortunately, notwithstanding this large patrimony of Algerian flora, until now, only a few species have been studied.

In Algeria, *Teucrium polium* is represented by 12 subspecies including the most common *T. polium* L. subsp. *polium* and *T. polium* L. subsp. *capitatum* (5,6). The latter is a perennial, pubescent, aromatic plant, 20–50 cm high, white or grey hairs on stems, with green-grayish leaves and white flowers. *T. polium* is widely used in Algerian folk medicine as antidiabetic, antihypertensive and to treat wounds (7). In addition, many biological activities have been ascribed to different extracts of this plant, such as

antioxidant, hepatoprotective, anti-cancer, antimicrobial, antinociceptive, and analgesic activities (3). According to a recent review (8) which analyzed about 270 papers dealing with the chemical composition and the antimicrobial activity of *T. polium* essential oil, α -pinene, β -pinene, spathulenol, verbenene, β -myrcene were individuated as the main components. These oils showed a mosquitocidal, repellent and insecticidal activities (9,10), and antimicrobial properties (11,12).

The aim of the present study has been to report the chemical composition of the essential oil *T. polium* subsp. *capitatum* collected in Algeria, and, for the first time to establish the antioxidant and wound healing properties by *in vitro* and *in vivo* studies, as well as the safety of its dermal traditional use.

Materials and methods

Plant material

The flowering aerial parts of *Teucrium polium* subsp. *capitatum* were collected in May 2018, from M'sila (Algeria). The plant was identified and authenticated taxonomically by Sarri D. (Department of Nature Sciences and Life, University of M'sila). A voucher specimen of the plant is deposited in the herbarium (AB-13, 2018) of the same Department.

Essential oil isolation

One-hundred grams of air-dried aerial parts of the plant were subjected to hydrodistillation using a Clevenger apparatus according to the current European Pharmacopoeia (13) until there was no significant increase in the volume of oil collected (3 h). The oil was dried over anhydrous sodium sulphate and stored under N₂ in a sealed vial until required.

Essential oil analysis

Gas chromatographic (GC) analyses were run on a Shimadzu gas chromatograph, Model 17-A 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 x 0.10 mm x 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 e 280°C, respectively. Linear velocity in column 19 cm/sec. The oven temperature was held at 60°C for 1 minute, then, programmed as reported previously (14). 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, operating software GCMS solution version 1.02 (Shimadzu). Ionization voltage 70 eV, electron multiplier 900 V, ion source temperature 180°C. Mass spectra data were acquired in the scan mode in *m/z* range 40–400. The same oil solutions (1 μL) were injected with the split mode (1:96).

Identification of components of essential oils

The identity of components was based on their GC retention index (relative to C₉-C₂₂ *n*-alkanes on the SPB-5column), computer matching of spectral MS data with those from NIST MS libraries (15), the comparison of the fragmentation patterns with those reported in the literature (16) and, whenever possible, co-injections with authentic samples.

Total antioxidant capacity (TAC) assay

The TAC of *T. polium* EO was evaluated by the phosphomolybdenum method (17). An aliquot of 0.3 mL of the EO was combined with 3 mL of the reagent solution (0.6 M of sulfuric acid, 28 mM of sodium phosphate and

4 mM of ammonium molybdate). The tubes were incubated in a water bath at 95 °C for 90 min. After the samples were cooled at room temperature and the absorbance was measured at 695 nm. The total antioxidant activity was calculated by the following equation:

$$TAC (\%) = \left(\frac{[A_{sample} - A_{control}]}{A_{blank}} \right) \times 100$$

where A_{sample} is the absorbance of the sample mixed with the reagent solution, A_{control} is the absorbance of deionized water mixed with the sample and A_{blank} is the absorbance of the reagent solution mixed with deionized water. The antioxidant activity was expressed in μg of Ascorbic Acid Equivalent per mg of EO (μg AAE/mg EO). All tests were carried out in triplicate.

DPPH scavenging assay

The free radical scavenging activity of samples was measured (18). Different concentrations of EO were mixed with the freshly prepared 0.1 mM DPPH in methanol. The 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:

$$DPPH \text{ scavenging capacity } (\%) = \left[\frac{(A_{blank} - A_{sample})}{A_{blank}} \right] \times 100$$

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. The sample concentration providing 50% inhibition (IC₅₀) was calculated by plotting inhibition percentages against concentrations of the sample. BHT was used as the reference compound. All tests were carried out in triplicate.

β-carotene/linoleic acid assay

The ability of the EO to inhibit the lipid peroxidation has been evaluated using the β-carotene/linoleic acid assay (19). The solution of β-carotene/linoleic acid mixture was prepared by dissolving 0.5 mg of β-carotene in 1 mL of chloroform with 25 μL of linoleic acid and 200 mg of tween 40. After complete evaporation of chloroform, 100 mL of distilled water saturated with oxygen (30 min) was added to the mixture under vigorous stirring. 2.5 mL of the emulsion was added to 350 μL of EO at different concentrations. BHT was used as positive control and the methanol and distilled water as negative control. The absorbance was measured at 490 nm after 24 hours of incubation at room

temperature in the dark. The antioxidant activity (AA%) was calculated, using the following equation:

$$AA\% = (AE / AE_{t_0}) \times 100$$

where AE: Absorbance in the presence of the EO after 24 h and AE_{t_0} : absorbance in the absence of the EO at 0 h. All measurements were performed in triplicate.

Animals

New Zealand albino rabbits weighing (1.9–2.1 kg) were purchased from Pasteur Institute of Algiers (Algeria), they were fed *ad libitum* with water and kibble diet. Animal studies have been authorized by the Institutional Ethics Committee (Registration N°: DO1N01UN280120150001) and all procedures were performed according to the International Council for Laboratory Animal Science (20).

Before the experimental procedure, an area on the back of the rabbits was shaved with an electric razor. The animals were left in their cages 24 hours to verify the absence of irritation of the shaved zone (21).

Preparation of the ointment

The essential oil of *T. polium* was incorporated in petroleum jelly (PJ) (Unilever, France) at a concentration of 10% to obtain the Ointment Essential Oil OEO 10%.

Namely, 10 g of essential oil was blended with 100 g of petroleum jelly previously melted in a water bath. The formulation was manually mixed to obtain a homogeneous mixture. This is a traditional preparation used by local herbalists to treat wounds (7).

Cicatryl-Bio (CIC), an allantoin-based pharmaceutical preparation (Pierre Fabre, Paris, France) was used as a reference drug.

Acute dermal irritation

The study was conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines 404 (22). The OEO 10% was applied topically on the back of the animals at an amount of 0.5 g per rabbit. The animals were observed for mortality and any toxic or deleterious effects with special attention given to the first 4 h and then once daily for a period of 14 days following the topical application. At the application sites, the skin was observed for signs of erythema, edema and local injury. The body weight and food intake were also recorded.

Evaluation of wound healing activity

The rabbits were randomly divided into four groups of four rabbits as follows: the first group was untreated (UT), the second group treated with the reference drug (CIC), the third group with OEO 10% and the fourth group with petroleum jelly (PJ).

Animals were anaesthetized using intraperitoneal injection of ketamine (90 mg/kg)-xylazine (10 mg/kg) (23). A circle of 2.5 cm in diameter was drawn on the skin of the lumbar region, which was then excised. Excisional wounds were immediately treated, and the animals were placed in individual cages with clean litters. Preparations (CIC, OEO 10% and PJ) were topically applied at an amount of 0.5 g per rabbit once per day for 16 days (24).

The dimensions of excision wounds were measured every 4 days during the trial period by tracing the wounds on a transparent paper and measuring through the graph paper. The percentage of the evolution of wound contraction was calculated using the following formula (25):

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

Histological section

At the end of the experimentation, the rabbits were sacrificed. The tissue slices were fixed in formalin (10%) for 72 h. The samples were dehydrated by passing them 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 paraffin blocks obtained were then cut with a microtome, rehydrated and stained with haematoxylin-eosin (26) and examined by Optika B-500 microscope.

Statistical analysis

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference (GRAPH PAD). The results are presented as means \pm SD. The inter-group significance was analyzed using the Tukey test and differences were considered significant at $p \leq 0.05$.

Results

Extraction yield and chemical composition of essential oils

The hydrodistillation of the aerial parts of *T. polium* subsp. *capitatum* gave an oil with a yield of 0.53% \pm 0.05% (v/w). The chemical composition was determined

by a combination of GC-FID and GC-MS analyses. Table 1 lists the 83 components identified in the oil, which have been subdivided into four classes: monoterpene hydrocarbons (MH), oxygenated monoterpenes (OM), sesquiterpenes (S) and others (O), being the last class representative of not terpenoid components.

T. polium subsp. *capitatum* EO was found to be rich in MH (ca. 60% and 11 compounds), S was the second class (ca. 30% of total and 27 compounds), a low content of OM (ca. 6% and 31 compounds), finally the O class with a total amount largely below 1% with 13 compounds.

The main components identified in the EO were in this order: β -pinene (ca. 33%), germacrene D (ca. 17%), β -pinene (ca. 10%), myrcene (ca. 8%), limonene (ca. 7%), bicyclogermacrene (ca. 3%), *trans*- β -guaiene (ca. 1.7%), spathunelol (ca. 1.6%) and β -bourbonene (ca. 1.3%). All other compounds comprising also all oxygenated monoterpenes and the other class were below 1%. Figure 1 shows the typical GC profile of this essential oil.

In vitro antioxidant activity

The *in vitro* antiradical activity of *T. polium* essential oil was evaluated by TAC, DPPH and bleaching test of β -carotene.

The experimental results obtained by the total antioxidant capacity test show clearly that the studied essential oil is significantly ($p \leq 0.001$) less powerful antioxidant than the reference standard ($508.91 \pm 7.56 \mu\text{g EAA/mg}$ and $417.98 \pm 1.85 \mu\text{g of EAA/mg}$, respectively).

In the DPPH-free radical method, the essential oil and the BHT depleted the initial DPPH concentration by 50% but at different concentrations. The IC_{50} of essential oil in compared with BHT was very significantly low ($p \leq 0.001$) ($5550.33 \pm 0.10 \mu\text{g/mL}$ and $14.6 \pm 0.71 \mu\text{g/mL}$, respectively).

In the case of inhibition of β -carotene bleaching assay, the antioxidant capacity is determined by inhibiting the formation of the conjugated diene hydroperoxides arising from linoleic acid oxidation. The essential oil was not able to effectively complete inhibit the linoleic acid oxidation, and only $53.52\% \pm 1.48$ inhibitions were achieved at 2 mg/mL concentrations, which were significantly ($p \leq 0.001$) far below the positive control BHT which showed a value of $89.10\% \pm 0.55$ at the concentration of 2 mg/mL.

Acute dermal irritation

The animals were divided into the following four groups: untreated group (UT); treated with Cicatryl-Bio group (CIC); 10% essential oil ointment group (OEO 10%);

petroleum jelly group (PJ). They were observed frequently during the 14 days following the topical application of 0.5 g of OEO 10%. No poisonous signs or mortality have been observed. The rabbits did not show any critical changes in behavior and breathing, any disability in feeding and water utilization, or postural irregularities and loss of hair. There were no irritation signs, no erythema, eschar, edema, or any other reactions on the skin of all animals after topical application.

Evolution of the healing process of wounds

During the healing period, and according to a specific interval of time of 4 days, the wounds were regularly measured and photographed. The assessment of the evolution of the surface of each wound excision was performed on the treated and untreated animals; the comparison between the different groups is indicated in Table 2 and Figure 2.

There was a progressive and time-dependent decrease of the wound surface area. All treated animals showed a significant reduction in wound area when compared to the untreated group ($p < 0.05$). A very high significant difference ($p < 0.001$) was observed between all treated groups and the untreated group at the end of the experimentation. The treated group with OEO 10% produced greater wound contraction compared with the other treated groups (CIC and PJ). There was no significant difference between the treated group with the OEO 10% and the group treated with the drug reference Cicatryl regarding the percentage of wound contraction during all the periods of healing.

Histological sections

The results of histopathological examination are showed in Figure 3, which allows comparing cicatricial zones of rabbits (treated or not treated) to a healthy zone on the same histological cut of the same sample.

The histological sections belonging to the untreated and PJ treated groups showed an inflamed dermis, infiltrant epidermal and incomplete epithelization with poorly formed granulation tissue and sparse distribution of collagen fibers and a plenty of inflammatory cells. These observations were in accordance with the wound healing process delay (Figure 3(a,b)). On the contrary, animals topically treated with CIC and OEO 10% showed a better re-epithelization resulting in more regular cell layers and more epidermal ridges with abundant granulation tissue formation and higher collagen content (Figure 3(c,d)). These histopathological observations provided additional evidences of the wound healing activity of OEO-based formulation.

Table 1. Chemical composition of *Teucrium polium* essential oil^a.

#	RI ^b	RI ^c	Class/Compounds	%	SD
Monoterpene Hydrocarbons (10) 59.7 0.003					
3	930	931	α -Thujene	0.1	0.000
4	939	941	α -Pinene	9.7	0.004
5	954	954	Camphene	0.3	0.000
6	960	959	Thuja-2.4 (9)-diene	0.1	0.000
7	979	987	β -Pinene	32.8	0.016
8	990	996	Myrcene	7.8	0.005
9	1017	1020	α -Terpinene	t	0.000
10	1026	1028	<i>o</i> -Cymene	0.1	0.000
11	1029	1036	Limonene	7.3	0.005
12	1037	1041	β -Z-Ocimene	0.2	0.000
14	1050	1052	β -E-Ocimene	1.1	0.001
18	1088	1091	Terpinolene	0.2	0.005
Oxygenated Monoterpenes 30 6.2 0.007					
16	1072	1078	<i>cis</i> -Linalool oxide	t	-
17	1082	1088	Camphenilone	t	-
19	1096	1101	Linalool	0.2	0.005
20	1105	1105	β -Fenchocamphorone	0.1	0.006
22	1116	1113	endo-Fenchol	0.1	0.001
23	1126	1127	β -Campholenal	t	-
24	1140	1142	Nopinone	0.3	0.001
25	1139	1145	<i>trans</i> -Pinocarveol	0.8	0.006
26	1142	1148	<i>trans</i> -Sabinol	0.1	0.006
27	1143	1151	<i>cis</i> -Sabinol	0.3	0.000
28	1158	1162	Nerol oxide	t	-
29	1164	1167	Pinocarvone	0.6	0.001
30	1169	1172	Borneol	0.2	0.000
31	1177	1182	Terpinen-4-ol	0.1	0.020
32	1188	1189	β -Terpineol	t	-
33	1195	1199	Myrtenal	0.8	0.013
34	1195	1201	Myrtenol	0.6	0.016
36	1205	1214	Verbenone	0.1	0.000
37	1216	1224	<i>trans</i> -Carveol	0.1	0.001
38	1229	1233	Nerol	0.2	0.000
39	1241	1245	Cumin aldehyde	0.1	0.001
40	1243	1248	Carvone	0.1	0.000
41	1267	1275	Geranial	t	0.001
42	1271	1279	Perilla aldehyde	0.1	0.001
43	1285	1289	Bornyl acetate	0.3	0.000
44	1290	1296	Thymol	0.4	0.068
45	1298	1303	<i>trans</i> -Pinocaryvl acetate	0.1	0.000
47	1326	1330	Myrtenyl acetate	t	-
51	1361	1368	Neryl acetate	0.1	0.000
52	1381	1383	Geranyl acetate	0.1	0.047
58	1436	1452	Neryl acetone	0.1	0.0
Sesquiterpenes (26) 30.0 0.170					
49	1338	1343	δ -Elemene	0.1	0.001
53	1388	1392	β -Bourbonene	1.3	0.004
54	1390	1397	β -Elemene	0.1	0.004
56	1420	1426	β -Ylangene	0.3	0.000
57	1430	1442	β -Copaene	t	-
59	1454	1457	α -Humulene	0.1	0.001
60	1466	1469	<i>allo</i> -Aromadendrane	0.5	0.000
61	1479	1475	γ -Muurolene	0.1	0.000
62	1481	1496	Germacrene D	16.6	0.016
63	1489	1498	β -Selinene	0.5	0.081
64	1500	1507	Bicyclogermacrene	3.2	0.205
65	1502	1513	<i>trans</i> - β -Guaiene	1.7	0.157
66	1502	1517	γ -Patchoulene	0.3	0.063
67	1513	1521	γ -Cadinene	0.3	0.014
68	1523	1528	δ -Cadinene	0.5	0.177
69	1538	1540	α -Cadinene	0.1	0.000
70	1561	1566	Germacrene B	0.2	0.008
71	1575	1568	Germacrene D-4-ol	0.2	0.005
72	1578	1588	Spathulenol	1.6	0.006
74	1592	1609	Viridiflorol	0.1	0.004
76	1640	1631	<i>epi</i> - α -Cadinol	0.1	0.005
77	1642	1651	<i>epi</i> - α -Muurolol	0.4	0.001
78	1654	1665	α -Cadinol	0.6	0.001
79	1676	1684	Cadalene	t	-
80	1688	1697	Eudesma-4 (14)7-dien-1 β -ol	0.2	0.001
81	1700	1704	Eudesm-7 (10)7-en-4-ol	0.6	0.001

(Continued)

Table 1. (Continued).

#	RI ^b	RI ^c	Class/Compounds	%	SD
82		1849	Perhydro farnesyl acetone ^d	0.4	0.001
Others (12) 0.5 0.001					
1	855	855	2 <i>E</i> -Hexenal	0.1	0.000
2	902	923	Heptanal	t	-
13	1042	1048	Benzene acetaldehyde	t	-
15	1055	1063	Pentyl iso butanoate	0.1	0.003
21	1112	1110	1-Octen-3-yl acetate	t	-
35	1201	1207	Decanal	t	-
46	1317	1319	3 <i>E</i> -Hexenyl tiglate	t	-
48	1332	1333	Hexyl tiglate	t	-
50	1359	1363	Eugenol	0.1	0.000
55	1400	1410	Tetradecane	t	-
73	1600	1604	Hexadecane	0.1	0.002
75	1612	1620	Tetradecanal	0.1	0.004
83	1900	1866	Nonadecane	t	0.000

^aThe numbering refers to elution order, and values (relative peak area % \pm SD) represent averages of 3 determinations (t = trace, < 0.05%); ^bLiterature Retention Index (RI); ^cRetention index (RI) relative to standard mixture of *n*-alkanes on SPB-5 column; ^dTentatively identified by MS data only.

Discussion

The yield (0.53% \pm 0.05, v/w) of *T. polium* belongs to the interval of values reported in the literature ranging between 0.14% and 0.6% (6). Chromatographic data showed that *T. polium* EO is mainly constituted by monoterpene hydrocarbons and characterized by β -pinene (33%) as the most leading component followed by sesquiterpenes with a high amount of germacrene D (17%) and a low content of oxygenated monoterpenes. This profile is more or less similar to those previously reported (5,6,27–29), but very different from other studies reporting the preponderance of sesquiterpenes in the chemical characterization of EOs derived from other ecotypes (Jordan, France, Algeria, Serbia, the Balkans, and Iran) (8). On the basis of a literature survey, many other compounds have also been identified in *T. polium* oil including δ -cadinene and α -cadinol (30), undecane, dodecane, tridecane, lycopersene (9), α -pinene, verbenol, α -terpineol (31), spathulenol and epizonaren (11), germacrene D, ocimene, β -pinene (12), limonene and camphor (32).

The difference in the quality or quantity of the composition of volatile oils may be due to genetic, differing chemotype, drying conditions, mode of distillation and/or extraction and geographic or climatic factors (28,31).

Results obtained in this study showed that *T. polium* EO have a moderate antioxidant activity against several charged radicals with the highest efficiency in hydrogen atoms transfer-based (HAT) and mixed-mode electron transfer (ET/HAT) assays and weak efficiency in inhibition of lipid peroxidation. These results were similar to those reported by Mahmoudi and Nosratpour (28) and Bendjabeur et al. (5).

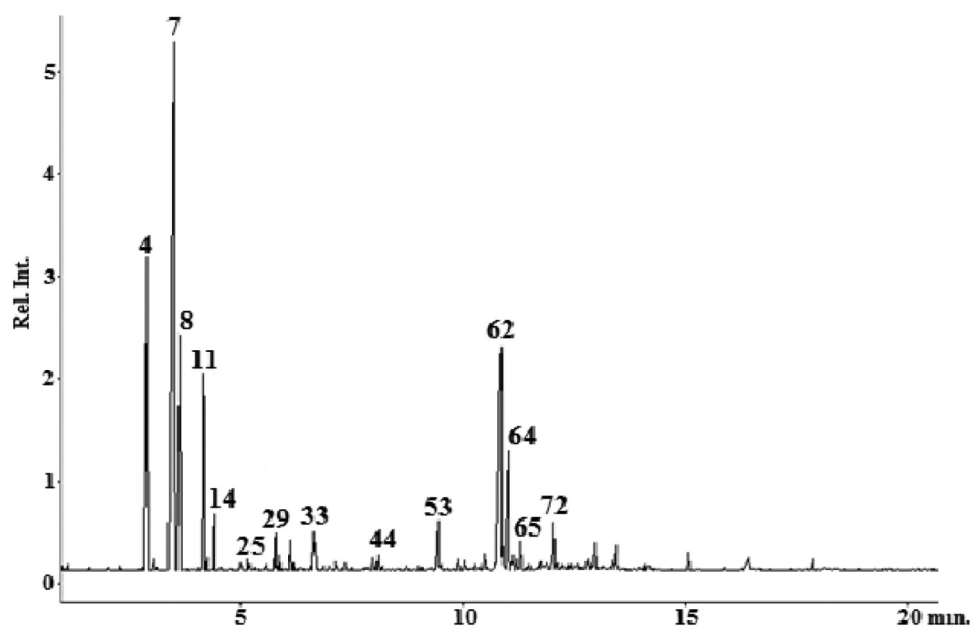


Figure 1. GC profile of *Teucrium polium* essential oil, for the peak numbering, see Table 1.

Table 2. Effect of different treatments on the evolution of the healing process of excision wounds.

Group	Wound contraction (%)			
	Number of days			
	4	8	12	16
UT	15.73 ± 0.07	23.93 ± 0.21	25.57 ± 0.50	42.62 ± 0.48
CIC	19.87 ± 0.23	29.48 ± 0.28	58.33 ± 0.24**	85.25 ± 0.02***
OEO 10%	5.63 ± 0.23	24.03 ± 0.12	62.90 ± 0.28***	89.61 ± 0.17***
PJ	17.91 ± 0.12	26.86 ± 0.21	32.83 ± 0.15*	67.16 ± 0.14**

Values are expressed as mean ±SD (n = 4), *p < 0.05, **p < 0.01, ***p < 0.001 when treated groups are compared to the UT group. UT: untreated group; CIC: group treated with Cicatryl-Bio; OEO 10%: group treated with essential oil ointment; PJ: group treated with petroleum jelly.

Several molecules, among those identified in the essential oil under examination, are endowed with various pharmacological properties such as antimicrobial, antioxidant, anti-inflammatory and analgesic effects (33–35).

Acute dermal toxicity corresponds to the adverse effects occurring within a short time of dermal application of a single dose of a test substance (36). In our study, no signs of dermal toxicity were observed after the application of the *T. polium* ointment. Based on our data, short-term treatment with EO-based formulation appears safe. The OEO 10% significantly improved the wound healing process after excision in albino rabbits. On histological examination, the treated groups (Cicatryl-Bio, OEO 10%) showed higher collagen deposition and complete re-epithelialization. The best results were obtained with OEO 10%. The treatment with the ointment had a strong impact on the granulation and epithelialization of wounds, accelerated tissue repair and reduced the duration of this process. This may be due to the combined effects of the bioactive constituents, mainly terpenes. The dermal absorption

of EO-based substances, as these terpenes increase the percutaneous absorption of drugs and other compounds due to their lipophilic characteristics. According to Cal and Sopala (37), the maximum concentration of terpenes in the stratum corneum and epidermis was obtained within 15 min of application. This bioavailability of the active molecules stimulates the inflammatory cell production (macrophage type-2) which is a key regulation step of the wound healing process. The anti-inflammatory effect is essential to shorten the healing period as well as to reduce pain and scarring (38).

Our data confirm results obtained in previous studies performed with extracts in other animal models (39), reported that the treatment with an extract from callus tissue derived from *T. polium* had a strong impact on the granulation and epithelialization of wounds, accelerated tissue repair, and reduced the duration of the wound healing process in rats. Ansari *et al.* (40) also demonstrated the effectiveness of a 2% *T. polium* extract with 91.5% of wound contraction against 75.3% for the reference

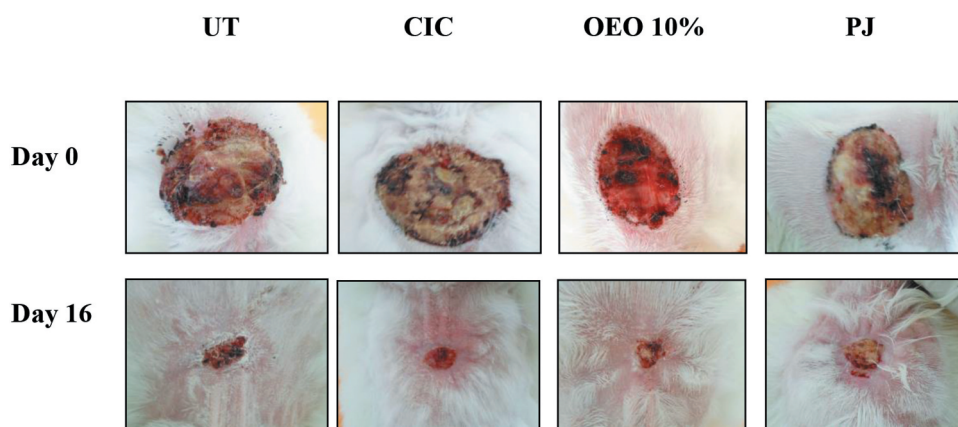


Figure 2. Chronology of excision wound healing in different groups. UT: Untreated group, CIC: Cicatryl-treated group, OEO 10%: ointment essential oil 10%-treated group and PJ: petroleum jelly-treated group.

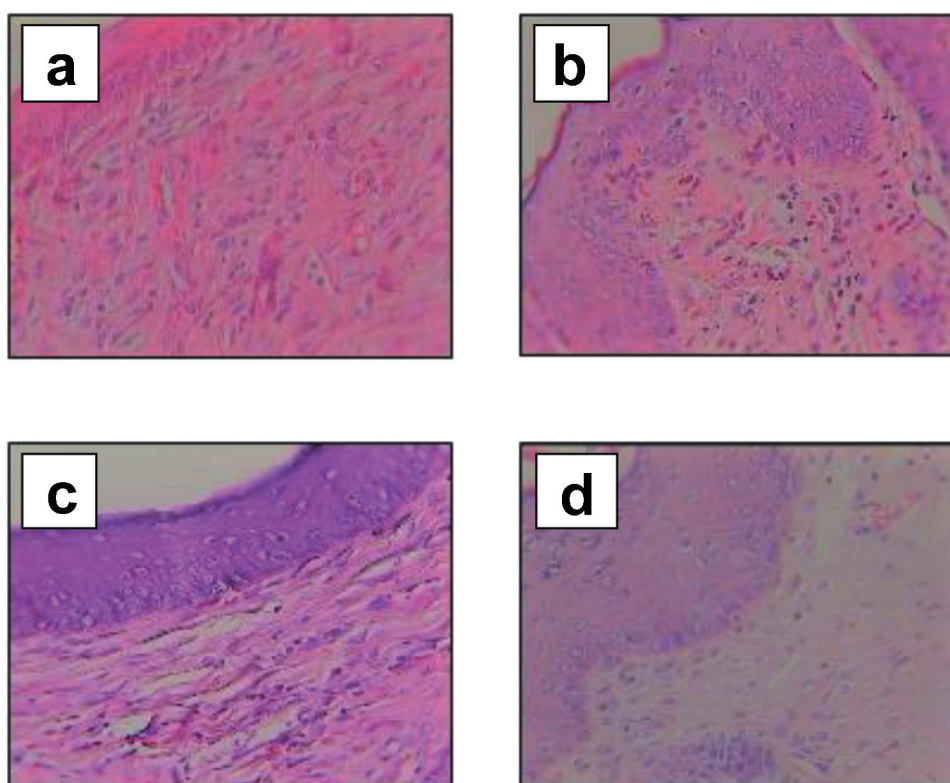


Figure 3. Histological evaluation of wound skin sections stained with hematoxylin and eosin (40 X magnification). **a** and **b**: UT and PJ-treatment, respectively, showing fewer collagen fibers and a plenty of inflammatory cells; **c** and **d**: animals treated with OEO 10% and CIC drug reference, respectively, showing better healing and complete re-epithelialization.

drug silver sulfadiazine cream on experimental second-degree burns in mice.

Conclusion

Results of this study showed that the 10% ointment obtained from the essential oil of *T. polium* is significantly effective in wound healing and could accelerate

the wound-healing process in the excision model on rabbits. Moreover, acute dermal toxicity assessment in albino rabbits indicated that the ointment essential oil of *T. polium* is potentially safe over a two-week treatment period corresponding to a typical application time in the therapy of wounds. The present study corroborates scientifically the traditional claims of *T. polium* in wound healing.

Acknowledgments

We wish to thank Mr. Antonio Greco and Ms. Tonia Strano (ICBCNR, Catania), for their skillful technical assistance and Dr. Sarri D. for the plant identification.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Algerian Ministry of Higher Education and Scientific Research (MESRS) [CNEPRU/DO1N01UN280120150001 Project].

References

1. E. Napoli, L. Siracusa and G. Ruberto, *New tricks for old guys: recent developments in the chemistry, biochemistry, applications and exploitation of selected species from the Lamiaceae family*. *Chemistry & Biodiversity*, **17**(3), e1900677 (2020).
2. T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentin and D.A. Webb, *Flora Europaea*. Cambridge University Press, Cambridge (1972).
3. I. Saleh, A. Abd-El Gawad, A.E.N. El Gendy, A. Abd, E. Aty, T. Mohamed, H. Kassem, F. Aldosri, A. Elshamy and M. E. Hegazy, *Phytotoxic and antimicrobial activities of Teucrium polium and Thymus decussatus essential oils extracted using hydrodistillation and microwave-assisted techniques*. *Plants*, **9** (716), 1–15 (2020). doi:10.3390/plants9060716
4. A. Sassoui, N. Hendel, D. Sarri, M. Maggi, F. Maggi, M. Bruno, D. Romano, A. Canale, R. Pavela and G. Benelli, *Essential oils from three Algerian medicinal plants (Artemisia campestris, Pulicaria arabica, and Saccocalyx satureioides) as new botanical insecticides?* *Environmental Science and Pollution Research*, **27**, 26594–26604 (2020). doi:10.1007/s11356-020-09064-w
5. S. Bendjabeur, O. Benchabane, C. Bensouici, M. Hazzit, A. Baaliouamer and A. Bitam, *Antioxidant and anticholinesterase activity of essential oils and ethanol extracts of Thymus algeriensis and Teucrium polium from Algeria*. *Journal of Food Measurement and Characterization*, **12** (4), 2278–2288 (2018). doi:10.1007/s11694-018-9845-x
6. H. Bendif, M. Lazali, N. Souilah, M.D. Miara, R. Kazernavičiūtė and R. Baranauskienė, *Supercritical CO₂ extracts and essential oils from Teucrium polium L. growing in Algeria: chemical composition and antioxidant activity*. *Journal Essential Oil Research*, **30**(6), 488–497 (2018).
7. A. Boudjelal, C. Henchiri, M. Sari, D. Sarri, N. Hendel, A. Benkhaled and G. Ruberto, *Herbalists and wild medicinal plants in M'Sila (North Algeria): an ethnopharmacology survey*. *Journal of Ethnopharmacology*, **148**, 395–402 (2013). doi:10.1016/j.jep.2013.03.082
8. R. Mahmoudi, M. Kazemini and A. Kaboudari, *Review on composition and antimicrobial effects of Teucrium (Teucrium polium L.) grown in Iran and a comparison with around the world*. *Journal of Babol University of Medical Sciences*, **19**, 54–64 (2017).
9. A. Ebadollahi and E. Taghinezhad, *Modeling and optimization of insecticidal effects of Teucrium polium L. essential oil against red four beetle (Tribolium castaneum Herbst) using response surface methodology*. *Information Processing in Agriculture*, **7**, 286–293 (2020). doi:10.1016/j.inpa.2019.08.004
10. M. Shahriari, N. Sahebzadeh and A. Zibae, *Effect of Teucrium polium L. (Lamiaceae) essential oil and α -pinene on detoxifying- and intermediary engaged enzymes of Ephestia kuehniella Zeller, 1879 (Lep.: pyralidae)*. *Acta Agriculturae Slovenica*, **113**, 251–261 (2019). doi:10.14720/aas.2019.113.2.6
11. N. Sadrizadeh, S. Khezri, P. Dehghan and R. Mahmoudi, *Antibacterial effect of Teucrium polium essential oil and Lactobacillus casei probiotic on Escherichia coli O157:H7 in Kishk*. *Applied Food Biotechnology*, **5**, 131–140 (2018).
12. N. Fertout-Mouri, A. Latreche, Z. Mehdadi, F. Toumi-Bénali and M.B. Khaled, *Composition chimique et activité antibactérienne de l'huile essentielle de Teucrium polium L. du mont de Tessala (Algérie occidentale)*. *Phytothérapie*, **15**, 346–353 (2017). doi:10.1007/s10298-016-1048-1
13. European Pharmacopoeia 8.0, *Determination of essential oils in herbal drugs*, 2.8. 12, 251–252 (2008).
14. F. Baali, S. Boumerfeg, E. Napoli, A. Boudjelal, R. Nadjat, A. Deghima, A. Baghiani and G. Ruberto, *Chemical composition and biological activities of essential oils from two wild medicinal Algerian plants: mentha pulegium L. and Lavandula stoechas L.* *Journal of Essential Oil Bearing Plants*, **22**, 821–837 (2019). doi:10.1080/0972060X.2019.1642800
15. NIST National Institute of Standard and Technology, *Mass Spectral Library* (1998).
16. R.P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectrometry*, 4th edn. Allured Publishing Co., Carol Stream, Illinois (2007).
17. P. Prieto, M. Pineda and M. Aguilar, *Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the determination of vitamin E*. *Analytical Biochemistry*, **269**, 337–341 (1999).
18. B. Tepe, D. Daferera, A. Sokmen, M. Sokmen and M. Polissiou, *Antimicrobial and antioxidant activities of essential oil and various extracts of Salvia tomentosa Miller (Lamiaceae)*. *Food Chemistry*, **90**(3), 333–340 (2005). doi:10.1016/j.foodchem.2003.09.013
19. H. Li, Z. Hao, X. Wang, L. Huang and J. Li, *Antioxidant activities of extracts and fractions from Lysimachia foenum-graecum Hance*. *Bioresources Technology*, **100**, 970–974 (2009). doi:10.1016/j.biortech.2008.07.021
20. International Council for Laboratory Animal Science (ICLAS). <http://iclas.org> (April 2020).
21. N.A. Hwisa, P. Katakam, B.R. Chandu, E.G. Abadi and E. M. Shefha, *Comparative in vivo evaluation of three types of honey on topical wound healing activity in rabbits*. *Journal of Applied Pharmaceutical Sciences*, **3**, 139–143 (2013).
22. The Organization of Economic Co-Operation and Development (OECD), *Test No. 404: Acute Dermal Irritation/Corrosion*. Guideline for testing of chemicals, Section 4, OECD Publishing, Paris (2015).

23. M. Mashreghi, M.R. Bazaz, N.M. Shahri, A. Asoodeh, M. Mashreghi, M.B. Rasouli and S. Golmoham madzadeh, *Topical effects of frog "Rana ridibunda" skin secretions on wound healing and reduction of wound microbial load*. Journal of Ethnopharmacology, **145**, 793–797 (2013). doi:10.1016/j.jep.2012.12.016
24. M.H. Pipelzadeh, M.R. Pipelzadeh and P. Husseinzadeh, *A study on the effects of modulation of intracellular calcium on excisional wound healing in rabbit*. Iranian Biomedical Journal, **7**, 161–166 (2003).
25. P. Tamri, A. Hemmati and M.G. Boroujerdnia, *Wound healing properties of quince seed mucilage: in vivo evaluation in rabbit full-thickness wound model*. International Journal of Surgery, **12**, 843–847 (2014). doi:10.1016/j.ijssu.2014.06.016
26. V. Marque, *Manuel de techniques d'anatomo-cytopathologique*. Paris: Elsevier Masson (2010).
27. L. Kerbouche, M. Hazzit, M.A. Ferhat, A. Baaliouamer and M.G. Miguel, *Biological activities of essential oils and ethanol extracts of Teucrium polium subsp. capitatum (L.) Briq. and Origanum floribundum Munby*. Journal of Essential Oil Bearing Plants, **18**(5), 1197–1208 (2015). doi:10.1080/0972060X.2014.935065
28. R. Mahmoudi and S. Nosratpour, *Teucrium polium L. essential oil: phytochemical component and antioxidant properties*. International Food Research Journal, **20**(4), 1697–1701 (2013).
29. A. Kabouche, Z. Kabouche, A. Ghannadi and S.E. Sajjadi, *Analysis of the essential oil of Teucrium polium ssp. aurasiacum from Algeria*. Journal of Essential Oil Research, **19** (1), 44–46 (2007). doi:10.1080/10412905.2007.9699227
30. H. Nikpour, M. Mousavi and H. Asadollahzadeh, *Qualitative and quantitative analysis of Teucrium polium essential oil components by GC-MS coupled with MCR and PARAFAC methods*. Phytochemical Analysis, **29**, 590–600 (2018). doi:10.1002/pca.2772
31. Y. Maizi, B. Meddah, A.T.T. Meddah and J.A. G. Hernandez, *Seasonal variation in essential oil content, chemical composition and antioxidant activity of Teucrium polium L. growing in Mascara (North West of Algeria)*. Journal of Applied Biotechnology Reports, **6**, 151–157 (2019). doi:10.29252/JABR.06.04.04
32. B. Salehi, S. Upadhyay, I.E. Orhan, A.K. Jugran, S.L. D. Jayaweera, D.A. Dias, F. Sharopov, Y. Taheri, N. Martins, N. Baghalpour, W.C. Cho and J. Sharifi-Rad, *Therapeutic potential of α - and β -pinene: a miracle gift of Nature*. Biomolecules, **9**(738), 1–35 (2019). doi:10.3390/biom9110738
33. R. Komakech, M.G. Matsabisa and Y. Kang, *The wound healing potential of Aspilia africana (Pers.) C. D. Adams (Asteraceae)*. Evidence-Based Complementary and Alternative Medicine, **1–12** (2019). doi:10.1155/2019/7957860
34. N. Primadina, A. Basori and D.S. Perdanakusuma, *Phytochemical screening and gas chromatography-mass spectrometry analysis of bioactive compounds present in karo traditional oil, an Indonesian traditional herbal medicine*. Asian Journal of Pharmaceutical and Clinical Research, **13**(2), 204–208 (2020). doi:10.22159/ajpcr.2020.v13i2.36736
35. The Organization of Economic Co-Operation and Development (OECD), *The OECD Guideline for Testing of Acute Dermal Toxicity 402*. OECD, Paris (2017).
36. K. Cal and M. Sopala, *Ex-vivo skin absorption of terpenes from Vicks VapoRub ointment*. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, **14**, 19–23 (2008).
37. I. Tümena, E.K. Akkolc, H. Taştand, I. Süntarc and M. Kurtcab, *Research on the antioxidant, wound healing, and anti-inflammatory activities and the phytochemical composition of maritime pine (Pinus pinaster Ait.)*. Journal of Ethnopharmacology, **211**, 235–246 (2018). doi:10.1016/j.jep.2017.09.009
38. H. Meguellati, S. Ouafi, S. Saad and N. Djemouai, *Evaluation of acute, subacute oral toxicity and wound healing activity of mother plant and callus of Teucrium polium L. subsp. Geyrii Maire from Algeria*. South African Journal of Botany, **127**, 25–34 (2019). doi:10.1016/j.sajb.2019.08.023
39. R. Ansari, N. Sahinfard, A. Namjou, M. Rafeian, H. Shirzad and M. Rafeian-Kopaei, *Ameliorative property of Teucrium polium on second degree burn*. Journal of Herbmed Pharmacology, **2**, 9–11 (2013).