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

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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.

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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.

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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 N_2 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 Date 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/CH_2Cl_2 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 C_9-C_{22} *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:

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

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:

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

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:

$$AA\% = (AE / AEt_0) \times 100$$

where AE: Absorbance in the presence of the EO after 24 h and AEt_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 allanthoin-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):

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

Histological section

At the end of the experimentation, the rabbits were sacrified. 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 examinated 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 ± 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% ± 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 ± 7.56 μg EAA/mg and 417.98 ± 1.85 μg 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 ± 0.10 μg/mL and 14.6 ± 0.71 μg/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% ± 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% ± 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	*o*-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	*cis*-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	*trans*-Pinocarveol	0.8	0.006
26	1142	1148	*trans*-Sabinol	0.1	0.006
27	1143	1151	*cis*-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	*trans*-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	*trans*-Pinocarvyl 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	*allo*-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	*trans*-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	*epi*-α-Cadinol	0.1	0.005
77	1642	1651	*epi*-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-1β-ol	0.2	0.001
81	1700	1704	Eudesm-7 (10)7-en-4-ol	0.6	0.001
82		1849	Perhydro farnesyl acetone[d]	0.4	0.001
			Others (12)	**0.5**	**0.001**
1	855	855	2*E*-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*E*-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

(*Continued*)

Table 1. (Continued).

[a]The numbering refers to elution order, and values (relative peak area % ± SD) represent averages of 3 determinations (t = trace, < 0.05%); [b]Literature Retention Index (RI); [c]Retention index (RI) relative to standard mixture of *n*-alkanes on SPB-5 column; [d]Tentatively identified by MS data only.

Discussion

The yield (0.53% ± 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).

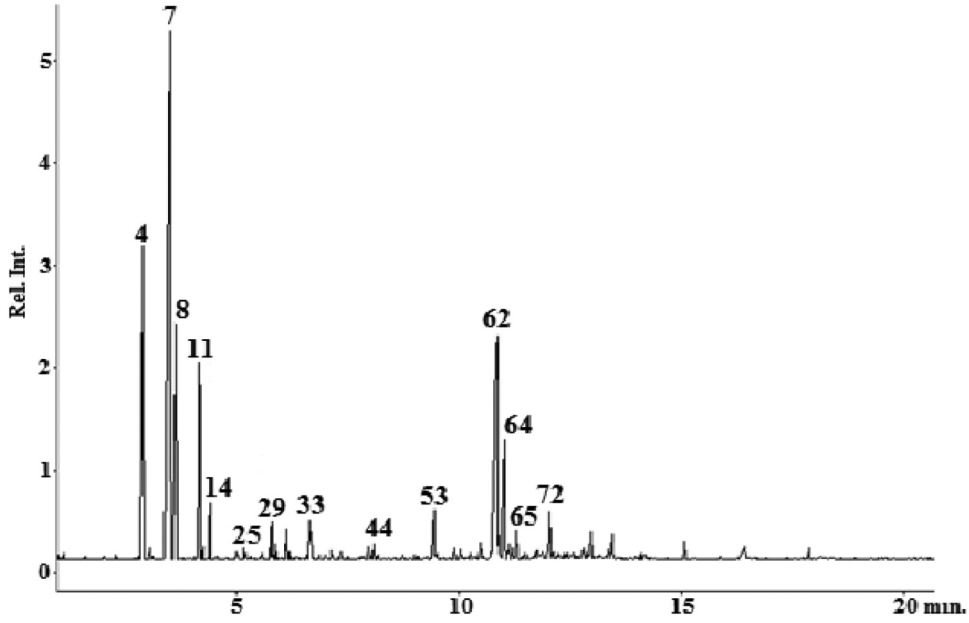

Figure 1. GC profile of *Teucrium polium* essential oil, for the peak numbering, see Table 1.

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

	Wound contraction (%)			
	Number of days			
Group	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

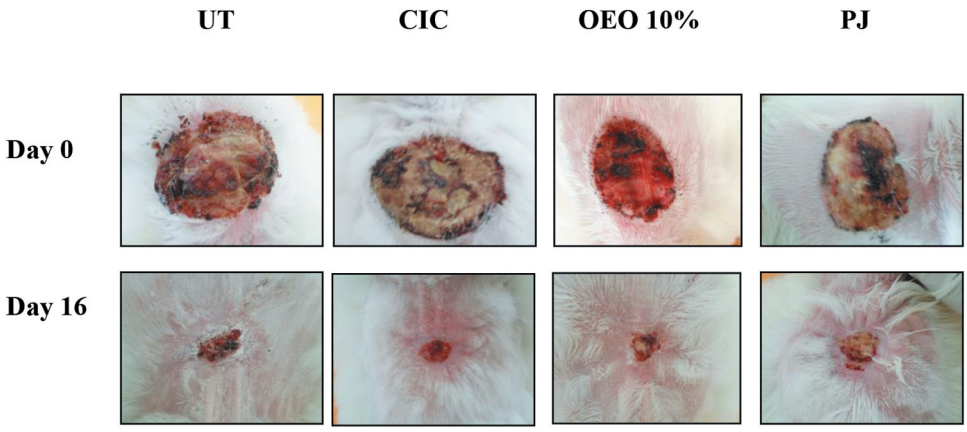

Figure 2. Chronology of excision wound healing in different groups. UT: Untreated group, CIC: Cicatryl-treated group, OEO 10%: ointment essential oil 10%-treated group and PJ: petroleum jelly-treated group.

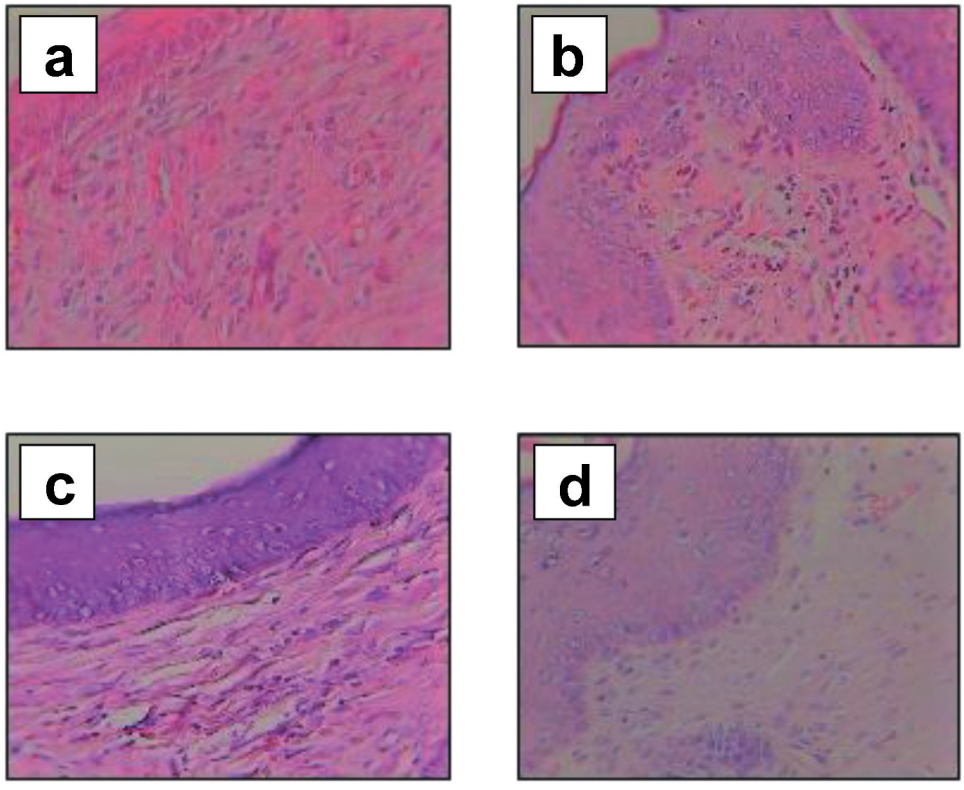

Figure 3. Histological evaluation of wound skin sections stained with hematoxylin and eosin (40 X magnification). **a** and **b**: UT and PJ-treatment, respectively, showing fewer collagen fibers and a plenty of inflammatory cells; **c** and **d**: animals treated with OEO 10% and CIC drug reference,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

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Disclosure statement

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