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Teucrium polium - wound healing potential, toxicity and polyphenolic profile

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ABSTRACT

The wound healing properties of *Teucrium polium* L., a plant used in the Algerian traditional medicine for the treatment of wounds, have been investigated using an excision wound model in rabbits. An ointment was prepared with two concentrations (5 and 10%) of a methanolic extract of the aerial parts. Both preparations showed significant effect on the wound contraction when compared to the control and the group treated with petroleum jelly. In addition, acute dermal and oral toxicity was assessed in animal models. The absence of signs of toxicity on the skin of rabbits indicated the safety of the ointment. After oral administration in mice at doses of 1000 and 2000 mg/kg b.wt, no signs of liver and kidney toxicity were detected by analysis of biochemical parameters and by histological examination. The composition of the methanolic extract was investigated by HPLC-PDA-MS analysis, and a comprehensive profile of phenolic compounds was established, with 14 flavonoids and phenylethanoid glycosides identified as the main polyphenolic constituents. Overall, the data support the use of *T. polium* as a wound healing agent in the Algerian traditional medicine.

1. Introduction

The genus *Teucrium* (family Lamiaceae) comprises approximately 300 species and is mainly distributed in Europe, North Africa and in the temperate parts of Asia (Bahramikia and Yazdanparast, 2012; Dehshiri and Azadbakht, 2012). Several species including *T. chamaedrys, T. montanum*, and *T. polium* are used in traditional medicinal systems (Stankovic et al., 2011). *T. polium* L., known popularly as felty germander (jâada or khayatit-lajrah in Arabic), is a deciduous shrub native to the western Mediterranean region abundantly growing in rocky places of the hills and deserts of Mediterranean countries up to South Western Asia (Bahramikia and Yazdanparast, 2012). The plant which includes many subspecies and varieties is very common in Algeria where it grows particularly in the Algerian and Oranian high plateaus and in the Oranian Saharian Atlas (Quézel and Santa, 1963). In Algerian traditional medicine, the aerial parts of the plant are used for the treatment of diabetes, hypertension and, in the form of a powder mixed with petroleum jelly or beeswax, as a wound healing agent (Boudjelal et al., 2013).

Extracts of *T. polium* have been shown to possess various biological activities including antioxidant, antibacterial, antiviral, antifungal, cytotoxic, antimutagenic, antiinflammatory, analgesic, antispasmodic, hypolipidemic, hypoglycemic, hepatoprotective, antiulcer, and anticonvulsant effects (Jaradat, 2015). Wound healing properties have been investigated in mice (Ansari et al., 2013) and rats (Meguellati et al., 2019; Huseini et al., 2020).

Flavonoids, phenylethanoid glycosides, and various terpenoids including neoclerodane diterpenes, sterols, and iridoids, have been isolated from the plant which also contains an essential oil rich in sesquiterpenes (Bahramikia and Yazdanparast, 2012; Basudan and Abu-Gabal, 2018; Elmasri et al., 2015).

The aim of the present study was to determine the polyphenolic composition of a methanolic extract of *T. polium* and to investigate its wound healing properties for the first time in rabbits. Furthermore, the acute cutaneous and oral toxicity were assessed. Wound excision was selected as a representative model to study the traditional use of the plant in the treatment of open wounds.

2. Materials and methods

2.1. Plant material and extraction

The flowering aerial parts of *Teucrium polium* (Subsp. *capitatum*) were collected in May 2018, in M'sila, Algeria, at 35° 12′ 36.97″ N

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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-92) was deposited at the herbarium of the Department. The plant material was rinsed and dried in the shade at room temperature and finely ground into a powder with a grinder (sieve pore size 200 μm).

The methanolic extract was obtained with a Soxhlet extractor. The vegetable powder (50 g) was extracted with 500 ml of methanol for 6 h. The extract was filtered and evaporated under reduced pressure to yield an oily residue (17.5%).

2.2. General experimental procedures

Medium pressure liquid chromatography (MPLC) was performed on a Premium Flash-Prep LC system PuriFlash® 4100 (Interchim, Montluçon, France). The sample was prepared as a dry load and adsorbed on 25 g silica gel 60. Preparative HPLC was carried out on a Preparative LC/MSD System (Agilent Technologies, Santa Clara, CA, USA) consisting of a quaternary pump (1200 Series, 1290 Infinity II 1260 Prep Bin Pump), a PDA detector (1100 Series), and a 6120 Quadrupole LC/MS. A SunFire Prep C18 OBD column (5 μm, 30 × 150 mm i.d., Waters, Milford, MA, USA), equipped with a C18 Prep Guard Cartridge (10 × 30 mm i.d.) was used. The flow rate was 20 ml/min. Data acquisition and processing was performed using ChemStation software (Agilent Technologies). For injection a 1290 Infinity II 1290 Valve Drive manual injection system (Agilent Technologies) was used. Semi-preparative HPLC was carried out on an Agilent 1100 Series instrument equipped with a PDA detector. Separations were carried out on a SunFire C18 column (5 μm, 150 × 10 mm i.d., Waters) equipped with a guard column (10 × 10 mm i.d.). A flow rate of 4 ml/min was applied. Data acquisition and processing was performed using ChemStation software. NMR spectra were recorded on a Bruker Avance III spectrometer (Rheinstetten, Germany) operating at 500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C. ^{1}H NMR and 2D NMR spectra were measured in DMSO-d6 or $CDCl_3$ (ARMAR Chemicals) with a 1 mm TXI probe at 23 °C. Data were analyzed using Topspin (Bruker) and Spectrus Processor (ACD/Lab, Toronto, Canada) softwares.

Silica gel 60 (0.040–0.063 mm) used for MPLC was from Merck KGaA (Darmstadt, Germany). TLC was performed on silica gel 60 F254 precoated plates (ALUGRAM Xtra Nano-SIL G, Macherey-Nagel, Düren, Germany); detection under UV 254 nm and after spraying with vanillin-sulfuric acid reagent followed by heating at 100 °C. Ultrapure water was obtained from a Milli-Q water purification system (Merck Millipore, Darmstadt, Germany). HPLC-grade acetonitrile was purchased from Avantor Performance Materials (Radnor Township, PA, USA). Methanol for extraction was from Honeywell (Offenbach, Germany). Solvents used for liquid/liquid partition and column chromatography were from Scharlau (Barcelona, Spain). Diosmin (Alexis Biochemicals, San Diego, CA, USA), luteolin (AdipoGen, San Diego, CA, USA), hyperoside (Carl Roth GmbH, Karlsruhe, Germany), isoquercitrin (Carl Roth), and luteolin 7-O-glucoside (Extrasynthèse, Gernay, France) were used as chromatographic reference substances.

2.3. Total phenolic and flavonoid contents

Total Phenolic Content (TPC) of the extract was determined using the Folin-Ciocalteu reagent according to the method of Singleton and Rossi (Singleton and Rossi, 1965), with gallic acid as a standard. 200 μl of each sample dissolved in methanol was added to 1 ml of Folin-Ciocalteu reagent (1:10 dilution in distilled water). The mixture was shaken and, after 4 min, 800 ml of Na_2CO_3 (75 mg/ml) solution were added. The mixture was kept for 2 h at room temperature and the absorbance measured at 760 nm. Total phenolic content was expressed in mg gallic acid equivalents per gram dry extract (mg GAE/g DE) using a calibration curve with gallic acid. All measurements were performed in triplicate.

Total Flavonoid Content (TFC) of the extract was measured according to the aluminum chloride colorimetric method based on the formation of a flavonoid-aluminum complex (Carocho et al., 2014) using a quercetin calibration curve: 1 ml of methanol extract was mixed with 1 ml of 2% $AlCl_3$ methanol solution. After 10 min, the absorbance was determined at 430 nm. The results were expressed as mg quercetin equivalent per gram of dry extract (mg QE/g DE). All measurements were performed in triplicate.

2.4. HPLC-PDA-MS analysis

HPLC-PDA-MS analysis was performed on a chromatographic system consisting of a degasser, quaternary pump (LC-20AD), a column oven (CTO-20AC), a PDA detector (SPD-M20A), and a triple quadrupole mass spectrometer (LCMS-8030) (Shimadzu, Kyoto, Japan). Separation was carried out on a SunFire C18 column (3.5 μm, 3.0 × 150 mm i.d., Waters) equipped with a guard column (3.0 × 10 mm). The mobile phase consisted of water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B). A gradient of 5–80% B in 30 min followed by 80–100% B in 5 min was applied. The flow rate was 0.4 ml/min. The extract was dissolved in DMSO at a concentration of 10 mg/ml and 10 μl were injected. The LabSolutions software (Shimadzu) was used for data acquisition and processing.

2.5. Compound isolation

The methanolic extract of *T. polium* (32 g) was suspended in 500 ml water and then successively partitioned with EtOAc (3 × 500 ml) and *n*-BuOH saturated with H_2O (4 × 500 ml) to provide a EtOAc-soluble fraction (8.2 g), a *n*-BuOH-soluble fraction (10.8 g), and the H_2O-soluble fraction (12.6 g). The EtOAc-soluble fraction was fractionated by MPLC on a silica column (47 × 5 cm, i.d.) with a *n*-hexane/EtOAc/MeOH gradient [*n*-hexane/EtOAc 98:2 (0–10 min), *n*-hexane/EtOAc 2–70% EtOAc (10–420 min), *n*-hexane/EtOAc 70–100% EtOAc (420–480 min), EtOAc/MeOH 0–20% MeOH (480–540 min), 100% MeOH (540–600 min)] at a flow rate of 20 ml/min. In total, 540 fractions were collected, which were combined based on TLC analysis into 22 fractions (Fr.1-Fr.22).

Fr. 11 (85 mg) afforded **14** (1.3 mg, t_R =29.3 min) after preparative HPLC with a gradient of 5–80% acetonitrile in water (both containing 0.1% formic acid) in 30 min. Compounds **10** (12.5 mg, t_R = 11.9 min), **11** (2.0 mg, t_R = 12.6 min), and **13** (16.9 mg, t_R = 19.3 min) were isolated from Fr. 14 (327 mg) by preparative HPLC with a gradient of 45–60% acetonitrile in water (both containing 0.1% formic acid) in 30 min. Separation of Fr. 21 (247 mg) by preparative HPLC with a gradient of 5–80% acetonitrile in water (both containing 0.1% formic acid) in 30 min afforded **9** (6.1 mg, t_R = 20.8 min) and **12** (3.0 mg, t_R = 23.9 min). Separation of Fr. 22 by preparative HPLC with a gradient of 15–50% acetonitrile in water (both containing 0.1% formic acid) in 30 min gave compound **2** (11.4 mg, t_R = 11.3 min) and a fraction (25.6 mg) which was further purified by semi-preparative HPLC with 16% aq. acetonitrile containing 0.1% formic acid to provide **3** (3.5 mg, t_R = 21.4 min), a mixture of **4** and **5** (4.8 mg, t_R = 23.4 min), and **6** (3.3 mg, t_R = 29.8 min). A portion (1.0 g) of the *n*-BuOH-soluble fraction was separated by preparative HPLC with a gradient of 5–80% acetonitrile in water (both containing 0.1% formic acid) in 30 min to give **1** (373 mg, t_R =11.6 min).

2.6. Animals

All animals (Swiss albino mice weighing 31–33 g and New Zealand albino rabbits weighing 1.9–2.1 kg) were obtained from Pasteur Institute of Algiers. They were fed *ad libitum* with water and kibble diet.

All experimental protocols were in accordance with the European Community Council Directive (86/609/EEC) and approved by the

National Committee for Evaluation and Programming of University Research of Algerian Ministry of Higher Education and Scientific Research (Registration N°: DO1N01UN280120150001).

2.7. Preparation of the ointment

The methanolic extract of *T. polium* was mixed with petroleum jelly (PJ) (Unilever, France) at a concentration of 5% and 10% to obtain the methanolic extract ointments OME 5% and OME 10%, respectively. Cicatryl-Bio (CIC) (Pierre Fabre, Paris, France) was used as reference drug.

2.8. Acute dermal irritation

The acute dermal irritation assay was carried out on New Zealand albino rabbits. The study was conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines 404 (OECD 2002a). OME 5% and 10% were 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 hrs 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.

2.9. Acute oral toxicity

The acute oral toxicity was assessed in healthy young adult female Swiss albino mice, nulliparous and non-pregnant. The study was conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines 423 (OECD 2002b).

Twelve Swiss albino mice were divided into four groups of three animals and treated orally with different doses of *T. polium* extract for 14 days: Group I, control, received distilled water; Group II received 1000 mg/kg b.wt; Group III received 2000 mg/kg b.wt and Group IV received 5000 mg/kg b.wt as suspension by gavage. The treated mice were observed individually. The main observations and evaluations were external physical aspects (appearance and hair loss), behavioral changes (posture, scraping, aggressiveness, sensitivity to noise and light, hypersalivation) and measurable clinical signs (changes in heart and respiratory rhythms, abdominal contraction, diarrhea) (Hussain Mir et al., 2013).

At the end of the experiment, mice were sacrificed. Blood samples were collected to explore biochemical parameters (transaminases, alkaline phosphatase, serum total protein, creatinine, urea and uric acid). The parameters were measured by enzymatic colorimetric methods using commercially available kits (Spinreact, Girona, Spain). The livers and kidneys were excised from all experimental mice for histopathological examinations (Gandhare et al., 2013).

2.10. Evaluation of wound healing activity

An area of 500 mm^2 on the back of the New Zealand albino rabbit was shaved with an electric razor. The animals were left in their cages 24 h to verify the absence of irritation of the shaved zone (Hwisa et al., 2013). The animals were randomly divided into 5 groups of 4 rabbits as follows: first group was untreated (UT), second group treated with the reference drug (CIC), third group with OME 5%, fourth group with OME 10%, and fifth group with petroleum jelly (PJ).

2.10.1. Wound healing assay

Animals were anaesthetized using intraperitoneal injection of ketamine (90 mg/kg)-xylazine (10 mg/kg) (Mashreghi et al., 2013). 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 after surgical operation and the animals placed in individual cages with clean litters. Preparations (CIC, OME 5%, OME 10% and PJ) were applied topically at an amount of 0.5 g per rabbit once per day till complete healing (Pipelzadeh et al., 2003).

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 (Tamri et al., 2014):

$$\%\text{wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

2.10.2. Histological sections

At the end of the experiment, the animals were sacrificed. The tissue slices (scarred skin and 0.5 cm of healthy skin) 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 (Marck, 2010).

2.10.3. Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) for determining the significant difference (GraphPad, version 7). The results are presented as means ± SEM. The inter group significance was analyzed using by Dunnett's or Tukey test whenever applicable and differences were considered significant at $p \leq 0.05$.

3. Results

3.1. Polyphenolic profile

The methanolic extract of *T. polium* aerial parts had a total phenolic content of 86.63 ± 0.03 mg GAE/g DE, and a total flavonoid content of 24.43 ± 0.01 mg QE/g DE.

The polyphenolic profile of the extract was investigated by HPLC-UV-MS (Fig. 1), and revealed the presence of several peaks corresponding to flavonoids and caffeic acid derivatives. Compounds **7** and **8** were identified from their UV and MS data, and by chromatographic comparison with commercial reference samples as diosmin (Al Bahtiti, 2012) and luteolin (D'Abrosca et al., 2013; Elmasri et al., 2015), respectively. Further compounds were identified by ^{1}H and 2D NMR analysis after isolation. They included poliumoside (**1**) (De Marino et al., 2012; Oganesyan et al., 1991), acteoside (**2**) (Elmasri et al., 2015), hyperoside (**3**) (Rudakova et al., 2014), isoquercitrin (**4**) (Shammas and Verykokidou-Vitsaropoulou, 1987), luteolin 7-O-β-D-glucopyranoside (**5**) (D'Abrosca et al., 2013; De Marino et al., 2012), luteolin 7-O-(5-O-syringoyl-β-D-apiofuranosyl)-(1→2)-β-D-glucopyranoside (**6**) (D'Abrosca et al., 2013), cirsiliol (**9**), cirsimaritine (**10**) (Elmasri et al., 2015; Stefkov et al., 2011; Verykokidou-Vitsaropoulou and Vajias, 1986), cirsilineol (**11**) (Stefkov et al., 2011), eupatorin (**12**) (Verykokidou-Vitsaropoulou and Vajias, 1986), 5-desmethylsinensetin (**13**) (Alwahsh et al., 2015; Harborne et al., 1986; Kisiel et al., 2001; Topcu et al., 1996), and salvigenin (**14**) (Elmasri et al., 2014) (Fig. 2). All compounds had been previously reported in *T. polium* except flavonoid **13**. The latter compound had been previously described in other species of the genus *Teucrium*. To our knowledge, this study represents the first report of a detailed polyphenolic profile of *T. polium*.

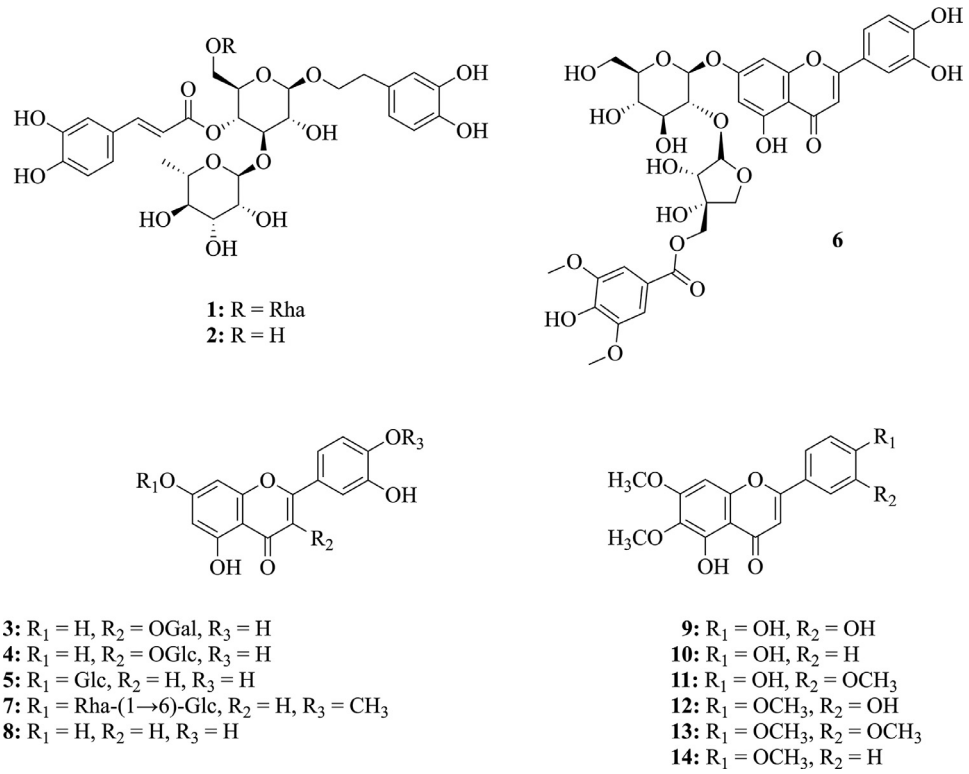

Fig. 1. Structures of the compounds identified in the methanolic extract of *T. polium*.

3.2. Acute oral toxicity

Acute oral toxicity was tested in mice at doses of 1000 to 5000 mg/kg b.wt. Even at the highest dose no mortality or specific signs of toxicity were observed after oral administration of the extract. In addition, there were no respiratory, nervous, cutaneous or gastrointestinal symptoms. According to the toxicity scale of Hodge and Sterner (1949) for mice and rats, the extract of *T. polium* can be classified as a "practically nontoxic" substance ($LD_{50} > 5000$ mg/kg) (Lu, 1992).

As to the biochemical parameters (Table 1), the mice treated with the three doses showed non-significant differences compared to the control group. The extract appeared to be well tolerated, as it did not affect liver parameters and had no effect on transaminase protein levels. The extract did not induce any significant changes in the renal parameters (urea, uric acid and creatinine concentrations) in the treated mice compared to the control group.

The different histological sections of liver and kidney from the treated mice were compared to the section of the normal control (Fig 3). The extract did not induce any changes, and the observations revealed a typical normal kidney and hepatic lobule for all treated mice. Slight dilation of the portal vein was observed with the doses of 2000 and 5000 mg/kg b.wt. While the reasons for this histological peculiarity remain unclear, this should not be considered as a sign of toxicity in the absence of any other abnormalities.

3.3. Acute dermal irritation

The animals were observed frequently during the 14 days following the topical application of 0.5 g of OME 5% or OME 10%. No signs of toxicity or mortality were seen. The rabbits were normal and did not show any critical changes in behavior and breathing, or any disability in feeding and water utilization, or postural irregularities and loss of hair. There were no signs of cutaneous irritation, no erythema, eschar, edema, or any other reactions on the skin of all animals after topical application.

3.4. Wound healing

3.4.1. Evolution of the wound healing process

During the healing period, the wounds were measured every 4 days. The evolution of the surface of each wound excision was assessed in the treated and untreated animals. The results are presented in Table 2. The photographic documentation of the wound healing process is included as Supplementary Material. All treated animals (CIC, OME 5% and OME 10%) showed very significant and significant reduction in wound area when compared to untreated and PJ groups ($p < 0.001$and $p < 0.01$, respectively). There was no significant difference between groups treated with the ointment prepared with the two concentrations (5 and 10%) of the extract and the reference drug Cicatryl-Bio. The OME 10% ointment proved its effectiveness in the healing process with 92.00 ± 0.14% of contraction of excision wounds in rabbits, which was better than 85.25 ± 0.18% obtained with the reference drug Cicatryl-Bio.

The daily visual observations indicated the presence of signs of inflammation (redness and fever) around the wound in rabbits of the different groups the first days after the excision of the skin. These signs disappeared quickly in the treated groups (CIC, OME 5% and OME 10%) and persisted in the rest of the groups for a few days (UT and PJ).

3.4.2. Histological sections

Histopathological examination was performed at the end of the experiment. Cicatricial zones of rabbits (treated or not treated) were compared to a healthy zone on the same histological cut of the same sample (Fig. 4).

The histological sections showed better healing and complete re-epithelialization in animals treated with the reference drug (Cicatryl-Bio), OME 5% and OME 10% compared to the untreated group and the group treated with petroleum jelly. The healed skin showed normal epithelialization, with thick mature epidermis and granulation tissue, and also higher collagen deposition in treated groups with CIC, OME 5%, and OME 10%.

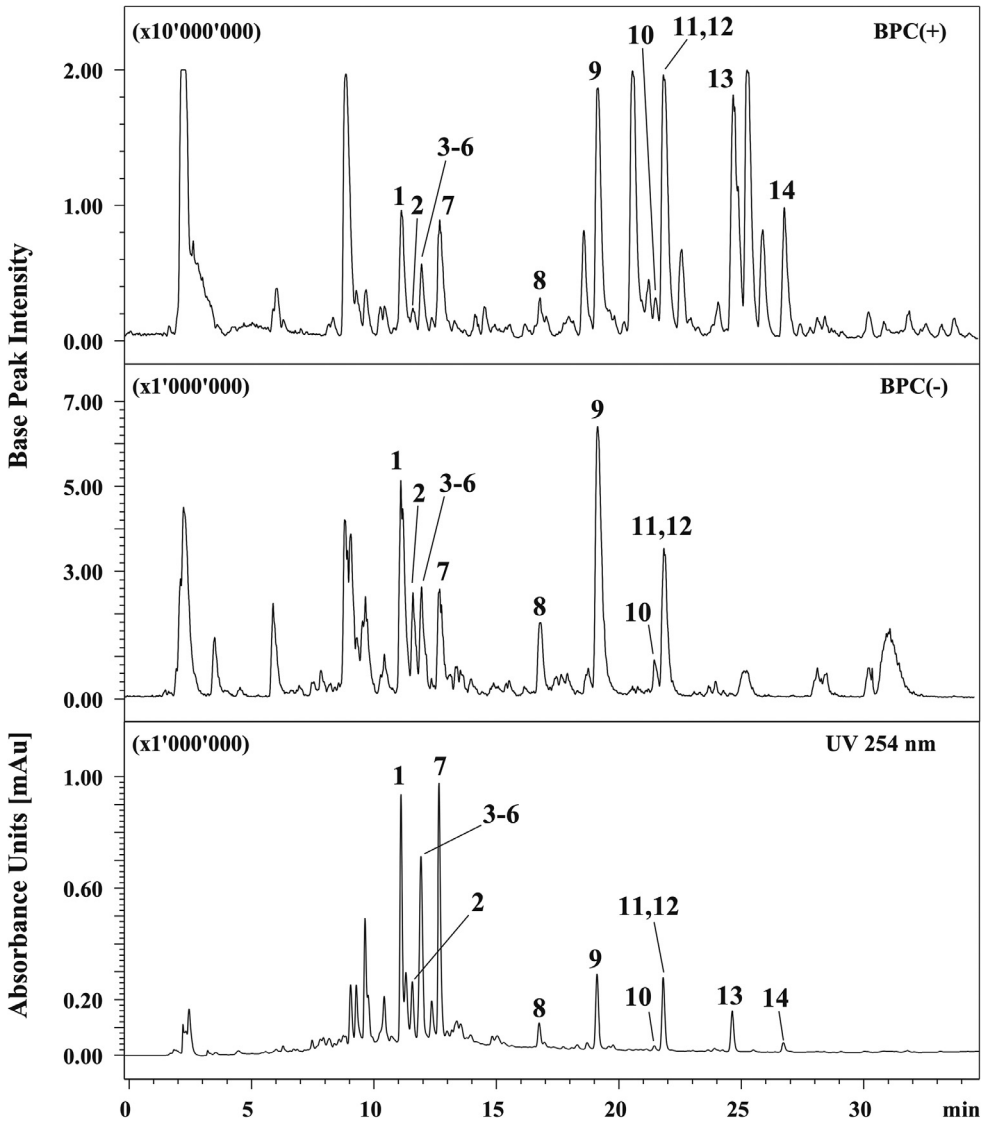

Fig. 2. HPLC-UV-MS analysis of the methanolic extract of *T. polium*. BPC: base peak chromatogram. Peak numbers refer to the identified compounds (Fig. 1).

Table 1
Biochemical parameters of the mice treated with the methanolic extract of *T. polium* at different doses.

Parameters	Treatment (mg/kg b.wt)			
	Control	1000	2000	5000
Urea (mg/l)	0.88 ± 0.02	0.91 ± 0.07	0.92 ± 0.03	0.93 ± 0.01
Creatinine (mg/l)	4.09 ± 0.15	5.43 ± 0.36	4.29 ± 0.47	4.05 ± 0.15
Uric Acid (mg/dl)	41.90 ± 4.49	42.32 ± 3.80	51.89 ± 7.63	25.98 ± 0.74
AST (IU/l)	40.66 ± 3.97	39.16 ± 3.83	43.16 ± 8.77	40.00 ± 2.31
ALT (IU/l)	11.00 ± 0.61	16.00 ± 1.00	15.66 ± 2.55	14.67 ± 1.02
ALP (IU/l)	54.67 ± 3.57	42.83 ± 3.51	53.20 ± 2.94	49.00 ± 1.29
Total Protein (g/dl)	60.00 ± 1.61	70.50 ± 3.43	72.00 ± 1.53	70.60 ± 2.50

Values are expressed as means ± SEM (n = 3).

The histological sections of the UT and PJ groups showed the presence of fleshy bud with more inflammatory cells, less collagen deposition and incomplete maturation of the dermis or epidermis. This indicates that, despite the contraction of the wound, petroleum jelly does not have therapeutic properties. Indeed, during the healing process, petroleum jelly is capable of inhibiting the evaporation of water from the wound. The formation of a wet physiological environment in the wound promotes skin repair and regeneration of damaged tissue. However, therapy with petroleum jelly can cause alteration and tissue maceration (Djerrou et al., 2011).

4. Discussion

Acute dermal toxicity corresponds to the adverse effects occurring within a short time of dermal application of a single dose of a test substance (OECD, 2017). Assessment of a single dermal dose toxicity

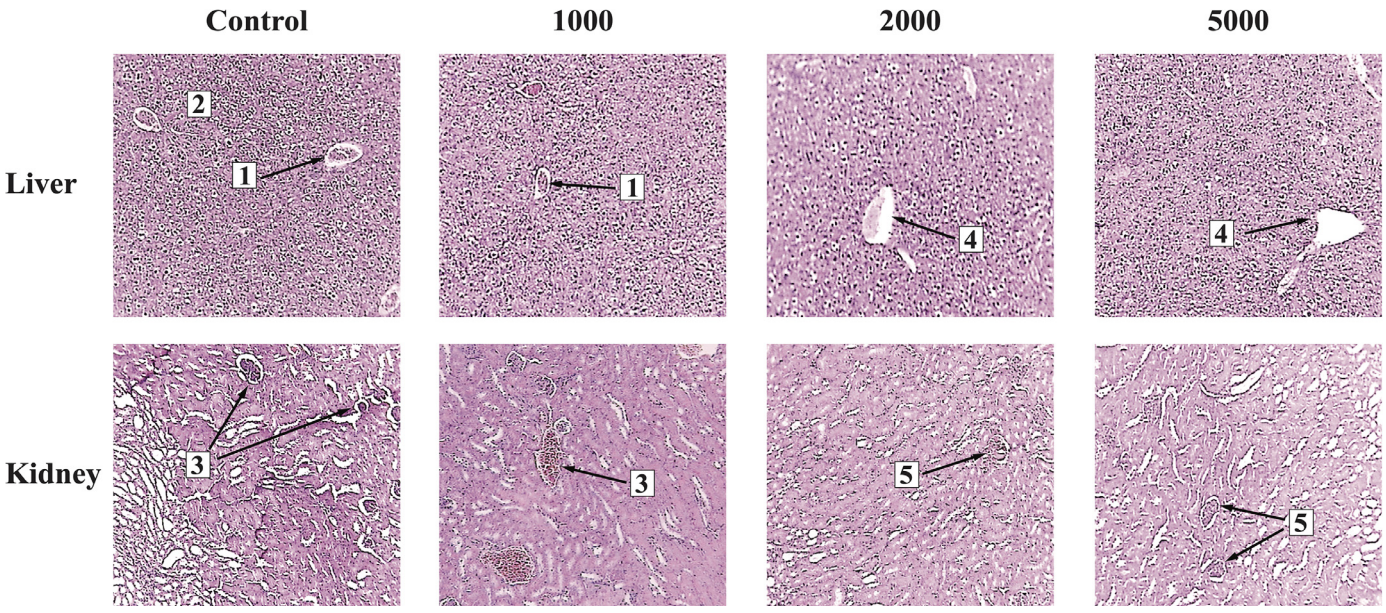

Fig. 3. Histopathological changes in liver and kidney of animals treated with *T. polium* methanolic extract at different doses (magnification x 10). 1: central vein; 2: surrounding hepatocytes; 3: glomerulus; 4: dilation of the portal vein; 5: tubular.

Table 2
Effect of different treatments on the evolution of the percentage of excision wound contraction in New Zealand albino rabbits.

Groups	Wound contraction (%)			
	Number of days			
	4	8	12	16
UT	15.73 ± 0.37	23.93 ± 0.24	25.57 ± 0.50	42.62 ± 0.38
CIC	19.87 ± 0.13	29.48 ± 0.28	58.33 ± 0.24**	85.25 ± 0.18***
OME 5%	11.33 ± 0.31	36.33 ± 0.64*	61.33 ± 0.70***	78.00 ± 0.95 ***
OME 10%	15.63 ± 0.85	38.18 ± 0.64*	81.81 ± 0.47***	92.00 ± 0.14***
PJ	17.91 ± 0.25	26.86 ± 0.17	32.83 ± 0.47*	65.16 ± 0.32**

Values are expressed as mean ± SEM, (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; OME 5% / 10%: groups treated with methanolic extract ointment; PJ: group treated with petroleum jelly.

is an important part of any toxicology program for new pharmaceutical or cosmetic products to be applied on the skin (Vinardell and Mitjans, 2008). In our study, no signs of dermal toxicity were observed after application of the *T. polium* ointment. As to the oral acute toxicity, the methanolic extract of *T. polium* did not produce any signs of toxicity in mice even at the highest dose (5000 mg/kg b.wt), and none of the animals died after 14 days of observation. Also, no significant changes were observed in biochemical parameters or histological sections of liver or kidney except a slight dilation of the portal vein. Similar observations have been reported in the study of Meguellati et al. (2019). At the same time, it should be mentioned that hepatotoxic and nephrotoxic effects of *T. polium* have been shown in many case reports and experimental studies where vacuolization, destruction and degeneration of liver and kidney were the most frequently described events (Aktürk Esen et al., 2019; Rafieian-Kopaei and Baradaran, 2013). The absence of toxicity observed in our study may be explained by the relatively short duration of administration (2 weeks). Indeed, the toxic effects of *T. polium* have been related to the dose and duration of use (Bachtarzi et al., 2016; Dağ et al., 2014). Based on our data, short-term treatment with a *T. polium* extract appears safe.

The 5% and 10% ointments (OME 5% and OME 10%) prepared from the methanolic extract of *T. polium* significantly improved the wound healing process after excision in albino rabbits. On histological examination, the treated groups (Cicatryl-Bio, OME 5%, and OME 10%) showed higher collagen deposition and complete re-epithelialization. The best results were obtained with OME 10%. Cicatryl-Bio contains allantoin as active ingredient. In an open wound model, allantoin was able to ameliorate and accelerate the repair of the skin. The wound healing effect of allantoin occurred via the regulation of inflammatory response and stimulation of fibroblastic proliferation and extracellular matrix synthesis (Araújo et al., 2010). The treatment with the ointment produced a similar effect that the control drug and 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 polyphenolic constituents, mainly flavonoids and caffeic acid derivatives. For example, the flavonoid fraction from *Ginkgo biloba* has been shown to enhance proliferation of normal human skin fibroblasts *in vitro* (Kim et al., 1997). Fibroblasts are responsible for the synthesis of collagen fibers and healing activity. The caffeic acid derivative acteoside (**2**) has been recently reported to increase the activation of promatrix metalloproteinase-2 and the expression of membrane type-1-matrix metalloproteinase, thereby possibly facilitating a remodeling of extracellular matrices (Si et al., 2018).

Our data confirm results obtained in previous studies performed in other animal models. Meguellati et al. (2019) reported that the treatment with an extract from callus tissue derived from *T. polium*

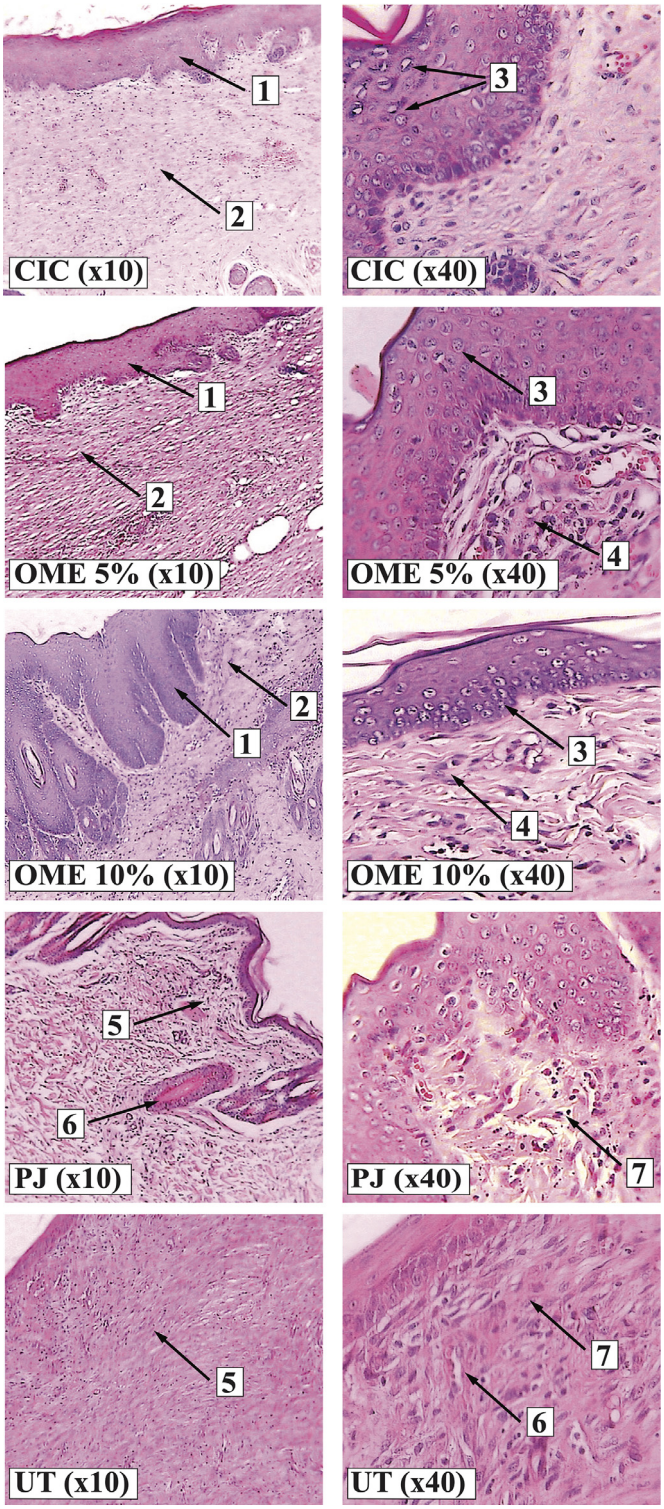

Fig. 4. Histological evaluation of wound skin sections stained with hematoxylin and eosin (10 and 40 x magnification) of various groups (CIC, OEM 5%, OEM 10%, PJ, and UT). UT: untreated group; CIC: group treated with Cicatryl-Bio; OME 5% / 10%: groups treated with methanolic extract ointment; PJ: group treated with petroleum jelly. 1: thick mature dermis; 2: collagen; 3: granulosa cells; 4: epidermis; 5: fleshy bud; 6: angiogenesis 7: incomplete epidermis.

had a strong impact on the granulation and epithelialization of wounds, accelerated tissue repair, and reduced the duration of wound healing process in an excision wound model in rats. Huseini et al., 2020 found that a 10% *T. polium* ointment accelerated the wound healing process in diabetic rats. Ansari et al. (2013) also demonstrated the effectiveness of a 2% *T. polium* extract with 91.5% of wound contraction against 75.3% for the reference drug silver sulfadiazine cream on experimental second degree burns in mice.

5. Conclusion

The 10% ointment obtained from the extract of *T. polium* showed significant wound healing properties in our wound excision model on rabbits, which were superior to those of the reference drug Cicatryl-Bio.

Moreover, acute oral toxicity studies in albino mice, and acute dermal toxicity assessment in albino rabbits indicated that the aerial

parts of *T. polium* are potentially safe over a two-week treatment period corresponding to a typical application time in the therapy of open wounds. Overall, our data confirm the potential of *T. polium* extract for the treatment of wounds and support the traditional use of this plant as a wound healing agent. Further investigations with *in vitro* models such as scratch assays are warranted to identify the active constituents and unravel the mechanism of action. In addition, the potential of *T. polium* for the treatment of other types of wounds such as closed wounds and diabetic ulcer wounds could also be explored.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Supplementary materials

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References

Aktürk Esen, S., Kahvecioğlu, S., Gül, C.B., Aktaş, N., Esen, İ., 2019. Toxic effects of herbal medicines: *Teucrium polium* and acute kidney injury. Eur. Research J. 5, 1028–1030 .

Al Bahtiti, N.H., 2012. "*Teucrium polium*" Extracts Jordanian Ja'adeh. Asian J. Agricult. Sci. 4, 379–382.

Alwahsh, M.A.A., Khairuddean, M., Chong, W.K., 2015. Chemical constituents and antioxidant activity of *Teucrium barbeyanum* Aschers. Records Natural Prod. 9, 159–163.

Ansari, R., Sahinfard, N., Namjou, A., Rafieian, M., Shirzad, H., Rafieian-kopaei, M., 2013. Ameliorative property of *Teucrium polium* on second degree burn. J. HerbMed Pharmacol. 2, 9–11.

Araújo, L.U., Grabe-Guimarães, A., Mosqueira, V.C.F., Carneiro, C.M., Silva-Barcellos, N.M., 2010. Profile of wound healing process induced by allantoin. Acta Cirurgica Brasileira 25 (5) doi.org/10.1590/S0102-86502010000500014.

Bachtarzi, K., Hilmi, S., Laouar, H., Belkheiri, A., Pacha, Y.H., 2016. The chronic toxic effect of *Teucrium polium* aqueous extract on some blood parameters in rat. Der Pharma Chemica 8, 384–387.

Bahramikia, S., Yazdanparast, R., 2012. Phytochemistry and medicinal properties of *Teucrium polium* L. (Lamiaceae). Phytother. Res. 26, 1581–1593.

Basudan, N., Abu-Gabal, N.S., 2018. Phytochemistry and biological properties investigation of *Teucrium polium* L. Int. J. Pharm. Biol. Sci. 8, 660–670.

Boudjelal, A., Henchiri, C., Sari, M., Sarri, D., Hendel, N., Benkhaled, A., Ruberto, G., 2013. Herbalists and wild medicinal plants in M'Sila (North Algeria): an ethnopharmacology survey. J. Ethnopharmacol. 148, 395–402.

Carocho, M., Barreiro, M.F., Morales, P., Ferreira, I.C.F.R., 2014. Adding molecules to food, pros and cons: a review on synthetic and natural food additives. Compr. Rev. Food Sci. Food Saf. 13, 377–399.

D'Abrosca, B., Pacifico, S., Scognamiglio, M., D'Angelo, G., Galasso, S., Monaco, P., Fiorentino, A., 2013. A new acylated flavone glycoside with antioxidant and radical scavenging activities from *Teucrium polium* leaves. Nat. Prod. Res. 27, 356–363.

Dağ, M., Özturk, Z., Aydinli, M., Koruk, I., Kadayifçi, A., 2014. Postpartum hepatotoxicity due to herbal medicine *Teucrium polium*. Ann. Saudi Med. 34, 541–543.

Dehshiri, M.M., Azadbakht, M., 2012. Anatomy of Iranian species *Teucrium polium* (Lamiaceae). J. Biol. Today's World 1, 48–52.

De Marino, S., Festa, C., Zollo, F., Incollingo, F., Raimo, G., Evangelista, G., Iorizzi, M., 2012. Antioxidant activity of phenolic and phenylethanoid glycosides from *Teucrium polium* L. Food Chem. 133, 21–28.

Djerrou, Z., Hamdi-Pacha, Y., Belkhiri, A.M., Djaalab, H., Riachi, F., Serakta, M., Boukeloua, A., Maameri, Z., 2011. Evaluation of *Pistacia lentiscus* fatty oil effects on glycemic index, liver functions and kidney functions of New Zealand rabbits. Afr. J. Tradit. Complement Altern. Med. 8, 214–219.

Elmasri, W.A., Hegazy, M.-E.F., Aziz, M., Koksal, E., Amor, W., Mechref, Y., Hamood, A.N., Cordes, D.B., Pare, P.W., 2014. Biofilm blocking sesquiterpenes from *Teucrium polium*. Phytochemistry 103, 107–113.

Elmasri, W.A., Yang, T., Tran, P., Hegazy, M.-E.F., Hamood, A.N., Mechref, Y., Pare, P.W., 2015. *Teucrium polium* phenylethanol and iridoid glycoside characterization and flavonoid inhibition of biofilm-forming *Staphylococcus aureus*. J. Nat. Prod. 78, 2–9.

Gandhare, B., Kavimani, S., Rajkapoor, B., 2013. Acute and subacute toxicity study of methanolic extract of *Ceiba pentandra* (Linn.) Gaertn. on rats. J. Sci. Res. 5, 315–324 .

Harborne, J.B., Tomas-Barberan, F.A., Williams, C.A., Gil, M.I., 1986. A chemotaxonomic study of flavonoids from European *Teucrium* species. Phytochemistry 25, 2811–2816.

Huseini, H.F., Abdolghaffari, A.H., Ahwazi, M., Jasemi, E., Yaghoobi, M., Ziaee, M., 2020. Topical application of *Teucrium polium* can improve wound healing in diabetic rats. Int. J. Low Extrem. Wounds 19, 132–138.

Hussain Mir, A., Sexena, M., Malla, M.Y., 2013. An acute oral toxicity study of methanolic extract from *Tridex procumbens* in Sprague Dawley's rats as per OECD guidelines 423. Asian J. Plant Sci. Res. 3, 16–20.

Hwisa, N.T., Katakam, P., Chandu, B.R., Abadi, E.G., Shefha, E.M., 2013. Comparative *in vivo* evaluation of three types of honey on topical wound healing activity in rabbits. J. Appl. Pharmaceut. Sci. 3, 139–143.

Jaradat, N.A., 2015. Review of the taxonomy, ethnobotany, phytochemistry, phytotherapy and phytotoxicity of germander plant (*Teucrium polium* L.). Asian J. Pharmaceut. Clin. Res. 8, 13–19.

Kim, S.J., Lim, M.H., Chun, I.K., Won, Y.H., 1997. Effects of flavonoids of *Ginkgo biloba* on proliferation of human skin fibroblast. Skin Pharmacol. Physiol. 10, 200–205.

Kisiel, W., Stojakowska, A., Piozzi, F., Rosselli, S., 2001. Flavonoids from *Teucrium fruticans* L. Acta Soc. Botanicorum Poloniae 70, 199–201.

Lu, F.C., 1992. Toxicologie : données Générales, Procédures d'Evaluation, Organes Cibles, Toxicologie : Données Générales, Procédures d'Evaluation, Organes Cibles, Evaluation du Risque. 1st ed. Elsevier Masson, Paris.

Marck, V., 2010. Manuel De Techniques D'anatomo-Cytopathologie, Manuel de Techniques d'Anatomo-Cytopathologie. 1st ed. Elsevier Masson, Paris.

Mashreghi, M., Rezazade Bazaz, M., Mahdavi Shahri, N., Asoodeh, A., Mashreghi, Mansour, Behnam Rassouli, M., Golmohammadzadeh, S., 2013. Topical effects of frog "Rana ridibunda" skin secretions on wound healing and reduction of wound microbial load. J. Ethnopharmacol. 145, 793–797.

Meguellati, H., Ouafi, S., Saad, S., Djemouai, N., 2019. Evaluation of acute, subacute oral toxicity and wound healing activity of mother plant and callus of *Teucrium polium* L. subsp. *geyrii* Maire from Algeria. S. Afr. J. Bot. 127, 25–34.

OECD (Organization of Economic Co-Operation and Development), 2002a. Test No. 404: Acute Dermal Irritation/Corrosion. OECD Publishing, Paris.

OECD (Organization of Economic Co-Operation and Development), 2002b. Test No. 423: Acute Oral Toxicity - Acute Toxic Class Method. OECD Guidelines for the Testing of Chemicals. OECD Publishing, Paris.

OECD (Organization of Economic Co-Operation and Development), 2017. Test No. 402: Acute Dermal Toxicity. OECD Publishing, Paris.

Oganesyan, G.B., Galstyan, A.M., Mnatsakanyan, V.A., Shashkov, A.S., Agababyan, R.V., 1991. Phenylpropanoid glycosides of *Teucrium polium*. Khimiya Prirodnykh Soedinenii 630.

Pipelzadeh, M.H., Pipelzadeh, M.R., Husseinzadeh, P., 2003. A study on the effects of modulation of intracellular calcium on excisional wound healing in rabbit. Iran. Biomed. J. 7, 161–166.

Quézel, P., Santa, S., 1963. Nouvelle Flore de l'Algérie et des Régions Désertiques Méridionales, 1st ed. Éditions CNRS, Paris, pp. 741–743.

Rafieian-Kopaei, M., Baradaran, A., 2013. *Teucrium polium* and kidney. J. Renal Inj. Prev. 2, 3–4.

Rudakova, Y.G., Senchenko, S.P., Popova, O.I., 2014. The study of phenolic compounds of herb *Teucrium polium* L. Vopr. Biol. Meditsinskoi i Farmatsevticheskoi Khimii 34–37.

Shammas, G., Verykokidou-Vitsaropoulou, E., 1987. Flavonoid heterosides of *Teucrium polium* L. Plantes Médicinales et Phytothérapie 21, 144–148.

Si, N., Kanazawa, H., Okuyama, K., Imada, K., Wang, H., Yang, J., Zhao, H., Bian, B., Ito, A., Sato, T., 2018. Involvement of catechols in acteoside in the activation of promatrix metalloproteinase-2 and membrane type-1-matrix metalloproteinase expression via a phosphatidylinositol-3-kinase pathway in human dermal fibroblasts. Biol. Pharm. Bull. 41, 1530–1536.

Singleton, V.L., Rossi, J.A.J., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.

Stefkov, G., Kulevanova, S., Miova, B., Dinevska-Kjovkarovska, S., Moolgaard, P., Jaeaeger, A.K., Josefsen, K., 2011. Effects of *Teucrium polium* spp. *capitatum* flavonoids on the lipid and carbohydrate metabolism in rats. Pharm. Biol. 49, 885–892.

Tamri, P., Hemmati, A., Boroujerdnia, M.G., 2014. Wound healing properties of quince seed mucilage: *in vivo* evaluation in rabbit full-thickness wound model. Int. J. Surgery 12, 843–847.

Topcu, G., Eris, C., Kurucu, S., Ulubelen, A., 1996. A new flavanone from *Teucrium alyssifolium*. Turk. J. Chem. 20, 265–267.

Verykokidou-Vitsaropoulou, E., Vajias, C., 1986. Methylated flavones from *Teucrium polium*. Planta Med. 52, 401–402.

Vinardell, M.P., Mitjans, M., 2008. Alternative methods for eye and skin irritation tests: an overview. J. Pharm. Sci. 97, 46–59.