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Comparative pharmacological and phytochemical investigation on the wound-healing effects of the frequently used essential oils

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Essential oils from several plants have been used for the first aid treatment of wounds, abscess, and burns. For this reason, we aimed to evaluate the wound-healing potential of some essential oils, obtained from *Pimpinella anisum* L., *Eugenia caryophyllus* (Spreng.) Bullock & S.G. Harrison, *Cuminum cyminum* L., *Foeniculum vulgare* Mill., *Laurus nobilis* L., *Lavandula angustifolia* Mill. ssp. *angustifolia*, and *Melissa officinalis* L., that have been frequently used in aromatherapy. In the present study, *in vivo* wound-healing activities of the selected essential oils were investigated by using linear incision and circular excision wound models. Moreover, the oils which are active in these test systems were analyzed by GC/MS for the identification of the major components. Essential oil obtained from *L. angustifolia* ssp. *angustifolia* and *L. nobilis* were found to be the most active among the tested samples. This research confirms the traditional usage of the active oils.

Keywords: essential oil; excision; GC; incision; tensiometer; wound healing

Introduction

The increase of the uses of complementary and alternative medicines for various diseases has led to the scientific evaluation of the plant extracts and other plant products believed to possess beneficial effects in dermatology, especially in the area of wound healing and burns (1–7). Essential oils are a group of natural products reported to be useful in a wide range of cosmetic and therapeutic conditions including digestive disorders, gynecological problems, dyspnea, spasmodic cough, and flatulent colic. Moreover, these secondary metabolites are used as antiseptic, anticonvulsant, antiasthmatic, expectorant, antispasmodic, antioxidant, and carminative and also reported to be useful for wound healing and reduction of wound infections (8–10). Volatile oils are a complex mixture of compounds that consist of monoterpenes, sesquiterpenes, and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols, and and oxides) (11). It is well known that geraniol, citral, nerol, menthol, thymol, carvacrol, and other terpenoids are responsible for the biological activities of the essential oils. Particularly, essential oils consisting of aromatic phenols, terpenic ketones, and alcohols display significant antibacterial, antifungal, and antioxidant effects. Antioxidant agents promote wound healing by preventing radical oxygen species and lipid peroxidation (12). The prevention of microbial contamination of the wound area also helps the healing process (13). Therefore, the present work was undertaken to assess unexplored wound-healing activity of essential oils obtained from various herbs from the local market.

Experimental

Plant material

The plants which are frequently used in aromatherapy were purchased from local markets in Turkey. The origins of the plants are from South of Turkey, except for the *Eugenia caryophyllus* (Spreng.) Bullock & S.G. Harrison, which is from India. All the samples were authenticated by Prof. H. Duman, who is the eminent scientist on Botany, from the Department of Biology, Faculty of Science, Gazi University, Ankara/Turkey. The plant parts and the yields of the oil which are obtained from each part of the samples are given in Table 1.

Isolation of the essential oil

The plant samples were subjected to hydro-distillation for 3 hours using a Clevenger-type apparatus. At the end of the distillation, oil for each sample was obtained, and then the water was removed carefully. The yields of the oils were given on dry weight basis (v/w) in Table 1.

Analysis of the essential oils

The volatile compounds of the active species (Figure 1) among the other tested species (Tables 2 and 3) were

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Table 1. The essential oils used in aromatherapy and their oil percentage.

Essential oils	Species	Plant parts	Essential oil yield %
Anisi aetheroleum	*Pimpinella anisum* L.	Fruit	1.2
Caryophilli aetheroleum	*Eugenia caryophyllus* (Spreng.) Bullock & S.G. Harrison	Flower	2.7
Carvi aetheroleum	*Cuminum cyminum* L.	Fruit	0.7
Foeniculi aetheroleum	*Foeniculum vulgare* Mill.	Fruit	2.9
Lauri aetheroleum	*Laurus nobilis* L.	Leaf	1.1
Lavandulae aetheroleum	*Lavandula angustifolia* Mill. ssp. *angustifolia*	Flower	6.0
Melissae aetheroleum	*Melissa officinalis* L.	Leaf	0.9

identified using a gas chromatograph (GC) coupled to a mass spectrometer (MS) and a Saturn 2000 MS Varian Chrompack with ZB-1 GC (Phenomenex) column (30 m × 0.25 µm film × 0.25 mm ID). The MS was equipped with an ion-trap analyzer set at 1508 for all analyses with an electron multiplier voltage of 1350 V. Scanning (1 scan s^{-1}) was performed in the range of 39–400 m/z using an electron impact ionization at 70 eV. The analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL min^{-1} in a split ratio of 1:20 and the following program: 60°C at the beginning and hold 3 min; 3°C/min–120°C; 15°C/min–300°C (Figure 1). The injector was held at 200°C. Most of the compounds were identified by using three-analytical methods: Retention indices (RI), GC/MS RI/authentic chemicals-standards (S), and mass spectra [authentic chemicals and NIST05 spectral library collection (MS)]. The retention index standards used in this study consisted of a mixture of aliphatic hydrocarbons ranging from C-5 through C-20 dissolved in methanol. The quantification of the volatile compounds was performed on a GC, Shimadzu 2010, with a flame ionization detector. The column and chromatographic conditions were those previously reported for the GC/MS analysis. The injector temperature was 200°C and hydrogen was used as carrier gas (1 mL min^{-1}).

Biological activity tests

Animals

Male, Sprague–Dawley rats (160–180 g), and Swiss albino mice (20–25 g) were purchased from the animal breeding laboratory of Saki Yenilli (Ankara, Turkey).

The animals were left for 3 days at room conditions and maintained on a standard pellet diet and water *ad libitum*. The animals were processed according to the suggested European ethical guidelines for the care of laboratory animals.

Preparation of test samples for bioassay

For the *in vivo* wound models, test samples were prepared in an ointment base (vehicle) consisting of glycol stearate, 1,2 propylene glycol, and liquid paraffin (3:6:1) in 1% concentration. Each test ointment (0.5 g) was applied topically on the wounded site. The animals of the vehicle group were treated with the ointment base only, whereas the animals of the reference drug group were treated with 0.5 g of Madecassol® (Bayer) which contains 1% extract of *Centella asiatica* (14).

Wound-healing activity

Linear incision wound model

Animals, seven rats in each group, were anaesthetized with 0.05 cc Xylazine (2% Alfazine®) and 0.15 cc Ketamine (10% Ketasol®). The hairs on the dorsal part of the rats were shaved and the skin was cleaned with 70% alcohol. Two 5 cm-length linear-paravertebral incisions were made with a sterile blade through the shaved skin at the distance of 1.5 cm from the dorsal midline on each side. Three surgical sutures were placed 1 cm apart from each other.

The ointments were topically applied on the wounds. On day 9, all the sutures were removed and on day 10, all the animals were killed. Tensile strength of skin samples was measured by using a tensiometer (Zwick/Roell Z0.5, Germany) (14–16).

Circular excision wound model

Each group of animals (seven animals in each) was anaesthetized with 0.02 cc Xylazine (2% Alfazine®) and 0.08 cc Ketamine (10% Ketasol®). The back hairs of the mice were shaved. The circular wound was created on the dorsal interscapular region of each animal with a 5 mm biopsy punch (Nopa instruments, Germany); wounds were left open (17). Test samples were applied topically once a day till the wounds completely healed. The progressive changes in wound area were monitored by a camera (Fuji, S20 Pro, Japan) every other day. Wound areas were evaluated by using AutoCAD program. Wound contraction was

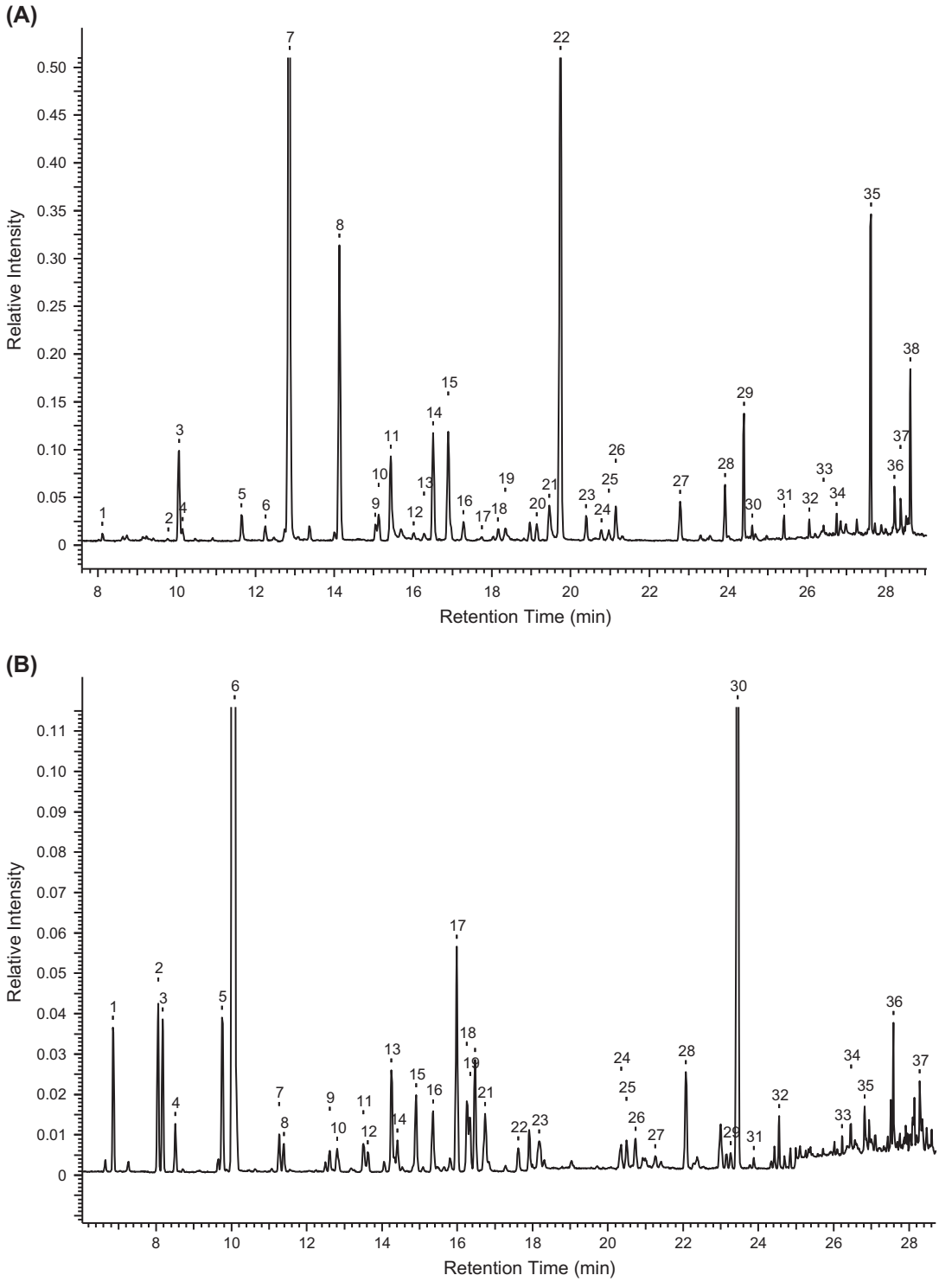

Figure 1. Gas chromatogram of the active essential oils. (A) *Lavandula angustifolia* Mill. ssp. *angustifolia* and (B) *L. nobilis* L.

calculated as percentage of the reduction in wounded area. A specimen sample of tissue was removed from the healed skin of each group of mice for the histopathological examination (14, 18).

Histopathology

The skin specimens from each group were collected at the end of the experiment. Samples were fixed in a 10% buffered formalin; processed and blocked with paraffin;

Table 2. Essential oil composition of *L. angustifolia* Mill. ssp. *angustifolia*.

No.	Peak name	*t*R (min)	RI exp.	RI lit.[a]	Identification	Area (%)
1	3-Octanone	8.12	969	969	MS, RI	0.1
2	p-Cymene	9.79	1011	1015	MS, RI, S	tr
3	Eucalyptol	10.06	1019	1024	MS, RI, S	3.1
4	Limonene	10.15	1021	1025	MS, RI, S	3.1
5	*trans*-Linalool oxide	11.64	1059	1058	MS, RI	0.8
6	*cis*-Linalool oxide	12.26	1073	1071	MS, RI	0.2
7	**Linalool**	12.87	1086	1086	MS, RI	**33.3**
8	**Camphor**	14.13	1114	1123	MS, RI, S	**9.8**
9	Isobutyric acid, hexyl ester	15.04	1136	1136[b]	MS, RI	0.5
10	*cis*-Thujol	15.13	1139	1145	MS, RI	0.8
11	Borneol	15.43	1145	1150	MS, RI, S	2.9
12	Terpinen-4-ol	16.01	1159	1164	MS, RI, S	0.1
13	Myrtenal	16.28	1164	1172	MS, RI, S	0.1
14	α-Terpineol	16.51	1169	1176	MS, RI, S	3.5
15	*n*-Hexyl butanoate	16.89	1177	1176[b]	MS, RI	3.9
16	γ-Terpineol	17.28	1185	1188	MS, RI, S	0.6
17	*trans*-Carveol	17.74	1195	1200	MS, RI	tr
18	Nerol	18.16	1204	1210	MS, RI, S	0.2
19	*n*-Hexyl *iso*-valerate	18.34	1208	1204[b]	MS, RI	0.2
20	*n*-Hexyl *n*-valerate	19.14	1228	1226[b]	MS, RI	0.3
21	Geraniol	19.46	1236	1235	MS, RI, S	1.4
22	**Linalyl acetate**	19.75	1243	1239	MS, RI, S	**19.7**
23	*trans*-Anethole	20.39	1257	1262	MS, RI, S	0.7
24	Isobornyl acetate	20.78	1266	1270	MS, RI	0.2
25	Thymol	20.97	1270	1272	MS, RI, S	0.1
26	Lavandulyl acetate	21.14	1274	1275	MS, RI	0.9
27	Hexyl tiglate	22.78	1314	1321[b]	MS, RI	1.3
28	Neryl acetate	23.92	1354	1342	MS, RI	1.4
29	Geranyl acetate	24.4	1370	1362	MS, RI	2.6
30	*n*-Hexyl hexanoate	24.61	1377	1378[b]	MS, RI	0.1
31	Caryophyllene	25.42	1408	1409	MS, RI, S	0.3
32	(E)-β-Farnesene	26.06	1451	1446	MS, RI	0.2
33	α-Amorphene	26.42	1475	1477	MS, RI	0.1
34	Valencene	26.74	1496	1494	MS, RI	0.3
35	**Caryophyllene oxide**	27.62	1575	1578	MS, RI	**6.2**
36	τ-Cadinol	28.22	1632	1633	MS, RI	0.6
37	Bisabolol oxide B	28.37	1647	1653	MS, RI	0.4
38	α-Bisabolole	28.63	1673	1673	MS, RI, S	2.9

Notes: Bold values indicate the main compounds in the oils.[a]Retention indices according to MassFinder (DB-1 column).[b]Retention indices according to Nist Webbook (http://webbook.nist.gov/chemistry/name-ser.html).tr: traces (less than 0.05).

and then sectioned into 5 μm sections and stained with hematoxylin & eosin (HE) and Van Gieson (VG) stains. Histopathologic examinations were performed by a pathologist which blinded to the groups. On histopathologic examination, numerical, volumetric, and colorimetric comparisons were used. The tissues were examined by a light microscope (Olympus CX41 attached Kameram® Digital Image Analyze System) and graded as mild (+), moderate (++), and severe (+++) for epidermal or dermal remodeling. Reepithelization or ulcus in epidermis; and fibroblast proliferation, mononuclear and/or polymorphonuclear cells, neovascularization, and collagen depositions in dermis were analyzed to score the epidermal or dermal remodeling. Van Gieson-stained sections were analyzed for collagen deposition. At the end of the examination, all the wound-healing processes were combined and staged for wound-healing phases as inflammation, proliferation, and remodeling in all groups (14).

Statistical analysis of the data

The data on percentage anti-inflammatory and wound healing were statistically analyzed using one-way analysis of variance. The values of $p \leqslant 0.05$ were considered statistically significant. Histopathologic data were considered to be nonparametric; therefore, no statistical tests were performed (14).

Results and discussion

It was reported that approximately 3000 essential oils are known, of which 300 are commercially important

Table 3. Essential oil composition of *L. nobilis* L.

No.	Peak name	tR (min)	RI exp	RI lit.[a]	Identification	Area (%)
1	*α*-Pinene	6.85	932	940	MS, RI, S	1.6
2	*β*-Pinene	8.05	967	981	MS, RI, S	2.0
3	*β*-Myrcene	8.17	970	987	MS, RI, S	1.8
4	2,3-Dehydro-1,8-cineole	8.51	979	991	MS, RI	0.3
5	*p*-Cymene	9.75	1010	1015	MS, RI, S	2.2
6	**Eucalyptol**	10.08	1019	1024	MS, RI, S	**61.6**
7	*γ*-Terpinene	11.27	1050	1051	MS, RI, S	0.3
8	*trans*-Sabinene hydrate	11.39	1053	1053	MS, RI	0.2
9	*cis*-Sabinene hydrate	12.61	1080	1082	MS, RI	0.1
10	Linalool	12.81	1084	1086	MS, RI, S	0.1
11	Campholenal	13.51	1098	1105	MS, RI	0.2
12	*cis-p*-Menth-2-en-1-ol	13.62	1101	1108	MS, RI	0.1
13	*trans*-Pinocarveol	14.25	1117	1123	MS, RI, S	1.5
14	Sabina ketone	14.41	1121	1132	MS, RI, S	0.4
15	*trans-β*-Terpineol	14.9	1133	1137	MS, RI	1.2
16	*cis-β*-Terpineol	15.36	1144	1141	MS, RI	0.9
17	**Terpinen-4-ol**	15.98	1158	1164	MS, RI, S	**3.1**
18	Myrtenal	16.25	1164	1172	MS, RI, S	1.0
19	Dihydrocarveol	16.34	1166	1175	MS, RI	0.8
20	*α*-Terpineol	16.47	1169	1176	MS, RI, S	1.5
21	Myrtenol	16.74	1174	1178	MS, RI, S	1.1
22	*trans*-Carveol	17.62	1192	1200	MS, RI	0.1
23	Carvone	18.18	1207	1214	MS, RI, S	0.2
24	*trans*-Anethol	20.35	1256	1262	MS, RI, S	0.1
25	Cuminic alcohol	20.49	1259	1260[b]	MS, RI	0.2
26	Bornyl acetate	20.74	1265	1270	MS, RI, S	0.2
27	Carvacrol	21.26	1276	1278	MS, RI, S	0.1
28	*cis*-Pinocarvyl acetate	22.07	1293	1300	MS, RI	1.3
29	Eugenol	23.27	1331	1331	MS, RI, S	0.1
30	**Terpinyl acetate**	23.46	1338	1335	MS, RI	**13.8**
31	Carvyl acetate	23.88	1350	1345	MS, RI	0.1
32	Methyleugenol	24.55	1375	1369	MS, RI	0.3
33	Isoeugenol methyl ether	26.22	1462	1468[b]	MS, RI	0.1
34	*γ*-Muurolene	26.46	1478	1475	MS, RI	0.1
35	*γ*-Cadinene	26.83	1503	1502	MS, RI	0.3
36	Caryophyllene oxide	27.59	1572	1578	MS, RI, S	0.9
37	*β*-Eudesmol	28.28	1638	1641	MS, RI	0.3

Notes: Bold values indicate the main compounds in the oils.[a]Retention indices according to MassFinder (DB-1 column).[b]Retention indices according to Nist Webbook (http://webbook.nist.gov/chemistry/name-ser.html).

especially for the pharmaceutical, agronomic, food, sanitary, cosmetic, and perfume industries. Essential oils or some of their components are used in perfumes and make-up products, in sanitary products, in dentistry, in agriculture, as food preservers and additives, and as natural remedies. Moreover, essential oils are used in massages as mixtures with vegetal oil or in baths but most frequently in aromatherapy as much as with medicinal properties. The use of essential oils in dermatology is also rapidly developing worldwide (10, 11). Because of the new attraction for natural products like essential oils, it is important to investigate for their potential on wound healing for new applications in human health.

Wound-healing activities of the essential oils obtained by hydro-distillation from *Pimpinella anisum*, *E. caryophyllus*, *Cuminum cyminum*, *Foeniculum*

Table 4. Effects of the essential oils on linear incision wound model.

Material	Statistical mean ± SEM	(Tensile strength %)
Vehicle	11.96 ± 2.03	9.3
Negative control	10.94 ± 2.14	–
Anisi aetheroleum	14.30 ± 1.43	19.6
Caryophilli aetheroleum	10.75 ± 2.09	–
Carvi aetheroleum	11.08 ± 2.10	–
Foeniculi aetheroleum	14.10 ± 1.96	17.9
Lauri aetheroleum	15.21 ± 1.29	**27.2***
Lavandulae aetheroleum	15.61 ± 1.21	**30.5****
Melissae aetheroleum	12.85 ± 1.76	7.4
Madecassol®	18.17 ± 0.93	**51.9*****

Notes: Bold values indicate the significant values. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; SEM: Standart error of the mean.Percentage of tensile strength values: vehicle group was compared to negative control group; volatile oils were compared to Vehicle group.

vulgare, *L. nobilis*, *Lavandula angustifolia* ssp. *angustifolia*, and *Melissa officinalis* were investigated in the present study. *In vivo* linear incision and circular excision wound models were used for the evaluation of wound-healing activity. The experimental data were given in the Tables 4–6. Linear incision wound model was employed to measure the breaking strength of the treated tissue, which points out how much the repaired tissue resists to breaking. For this purpose, the newly repaired tissue was removed and tensile strength was measured (15). Topical treatment of the incised wounds with the oils of *L. angustifolia* and *L. nobilis* exerted the best wound tensile strength on day 10, with the values of 30.5 and 27.2%, respectively, while the rest of the essential oils did not display any significant effect in the linear incision wound model. In the present study, we also assessed the wound-healing effects of the essential oils by using circular excision wound model, which enables to evaluate the percentage of the reduction in the wound area (4). The experimental results of the circular excision wound model similarly revealed that the oils of *L. angustifolia* and *L. nobilis* possess remarkable wound-healing effect with the contraction values of 39.05 and 42.22%, respectively (Table 5). The three phases in wound-healing processes, named as inflammation, proliferation, and

Table 5. Effects of the essential oils on circular excision wound model.

	Wound area ± SEM (Contraction %)						
Material	0	2	4	6	8	10	12
Vehicle	19.45 ± 2.06	16.79 ± 2.14 (7.79)	14.16 ± 2.15 (6.78)	12.86 ± 2.10 (12.09)	9.71 ± 1.05 (7.79)	6.47 ± 0.76 (2.41)	3.15 ± 0.29 (7.89)
Negative control	19.78 ± 2.48	18.21 ± 2.54	15.19 ± 2.01	14.63 ± 1.96	10.53 ± 1.83	6.63 ± 1.04	3.42 ± 0.81
Anisi aetheroleum	19.76 ± 1.96	16.40 ± 2.17 (2.32)	13.36 ± 1.95 (5.65)	11.54 ± 2.01 (10.26)	8.51 ± 1.67 (12.36)	5.23 ± 0.67 (19.17)	2.39 ± 0.26 (24.13)
Caryophilli aetheroleum	19.70 ± 2.08	16.03 ± 2.01 (4.53)	14.78 ± 2.10 –	11.55 ± 2.04 (10.19)	8.86 ± 1.12 (8.75)	6.01 ± 0.59 (7.11)	3.55 ± 0.36 –
Carvi aetheroleum	19.88 ± 1.87	16.85 ± 2.23 –	13.61 ± 2.12 (3.88)	12.05 ± 1.86 (6.29)	9.76 ± 1.80 –	6.14 ± 0.60 (5.10)	3.24 ± 0.57 –
Foeniculi aetheroleum	20.49 ± 2.16	16.47 ± 1.96 (1.91)	13.86 ± 1.89 (2.12)	11.96 ± 1.93 (6.99)	9.21 ± 1.25 (5.15)	5.73 ± 1.42 (11.44)	3.87 ± 0.74 –
Lauri aetheroleum	20.71 ± 1.47	15.02 ± 1.69 (10.54)	12.00 ± 1.75 (15.25)	11.24 ± 1.98 (12.59)	7.94 ± 1.68 (18.22)	4.71 ± 0.53 (27.20)	1.92 ± 0.11 **(39.05)***
Lavandulae aetheroleum	19.18 ± 1.97	15.63 ± 2.26 (6.91)	13.07 ± 1.96 (7.69)	10.42 ± 2.24 (18.97)	7.70 ± 1.66 (20.70)	4.17 ± 1.75 **(35.55)***	1.82 ± 0.09 **(42.22)****
Melissae aetheroleum	20.12 ± 2.31	16.81 ± 2.53 –	15.01 ± 2.13 –	11.66 ± 1.87 (9.33)	8.43 ± 1.16 (13.18)	5.18 ± 0.80 (19.94)	2.69 ± 0.49 (14.60)
Madecassol®	19.95 ± 1.91	14.76 ± 1.98 (12.09)	10.88 ± 1.84 (23.16)	7.52 ± 1.13 **(41.52)****	4.06 ± 0.79 **(58.19)****	1.48 ± 0.20 **(77.13)****	0.00 ± 0.00 **(100.00)*****

Notes: Bold values indicate the significant values. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; S.E.M: Standart error of the mean.Percentage of contraction values: Vehicle group was compared to Negative control group; Volatile oils were compared to Vehicle group.

Table 6. Wound-healing processes and healing phases of the experimental groups.

	Wound-healing processes								Healing phases		
Groups	S	U	RE	FP	CD	MNC	PMN	NV	I	P	R
Vehicle	++/+++	++/+++	–	++/+++	++	++	++/+++	++	++	+/++	–
Negative control	++/+++	+++	–	+/++	+	++	++/+++	+	++/+++	+/++	–
Anisi aetheroleum	++/+++	+	+/++	++/+++	++	+/++	+/++	++	++	++	+/++
Caryophilli aetheroleum	++/+++	++	–/+	++	+/++	++	++	++	++	+/++	–/+
Carvi aetheroleum	++	++/+++	–/+	++	+/++	++	++	++	++	+/++	–/+
Foeniculi aetheroleum	++	+	+	++	++	+/++	+/++	++	++	++	+
Lauri aetheroleum	++	–/+	+/++	++	++	+/++	+	++	+/++	++	+/++
Lavandulae aetheroleum	+	–	++	++	+	+	–/+	+/++	+/++	++	++
Melissae aetheroleum	++/+++	+/++	+	++/+++	++	++	+/++	++	++	++	+
Madecassol®	+/++	–	++	++	+	+	–	+	+	++	++

Notes: *HE- and VG-stained sections were scored as mild (+), moderate (++), and severe (+++) for epidermal and/or dermal remodeling. S: Scab, U: Ulcus, RE: Reepithelization, FP: Fibroblast proliferation, CD: Collagen depositions, MNC: Mononuclear cells, PMN: Polymorphonuclear cells, NV: Neovascularization, I: Inflammation phase, P: Proliferation phase, and R: Remodeling phase.

remodeling, were observed within the experimental groups with different degrees (Table 6). The vehicle, negative control, and the groups treated with the oils of *E. caryophyllus* and *C. cyminum* displayed delayed wound healing. Conspicuous fatty changes, localized within the dermis, were recorded in these four groups, predominantly in the oil of *C. cyminum* treated group. In comparison with these groups, faster remodeling was seen in the other groups. In experimental groups, best remodeling, particularly, reepithelization was detected in the reference and then the oils obtained from *L. angustifolia*, *L. nobilis*, *P. anisum*, *F. vulgare*, *M. officinalis* treated groups. On the other hand, pretty much mast cell was detected in the oil of *F. vulgare*-treated group. Histopathological results were supported with figure (Figure 2), which stained with HE and VG.

The normal healing response begins immediately after the tissue is injured. The platelets contact with the exposed collagen which triggers the growth factors and cytokines to be released; after the homeostasis, the

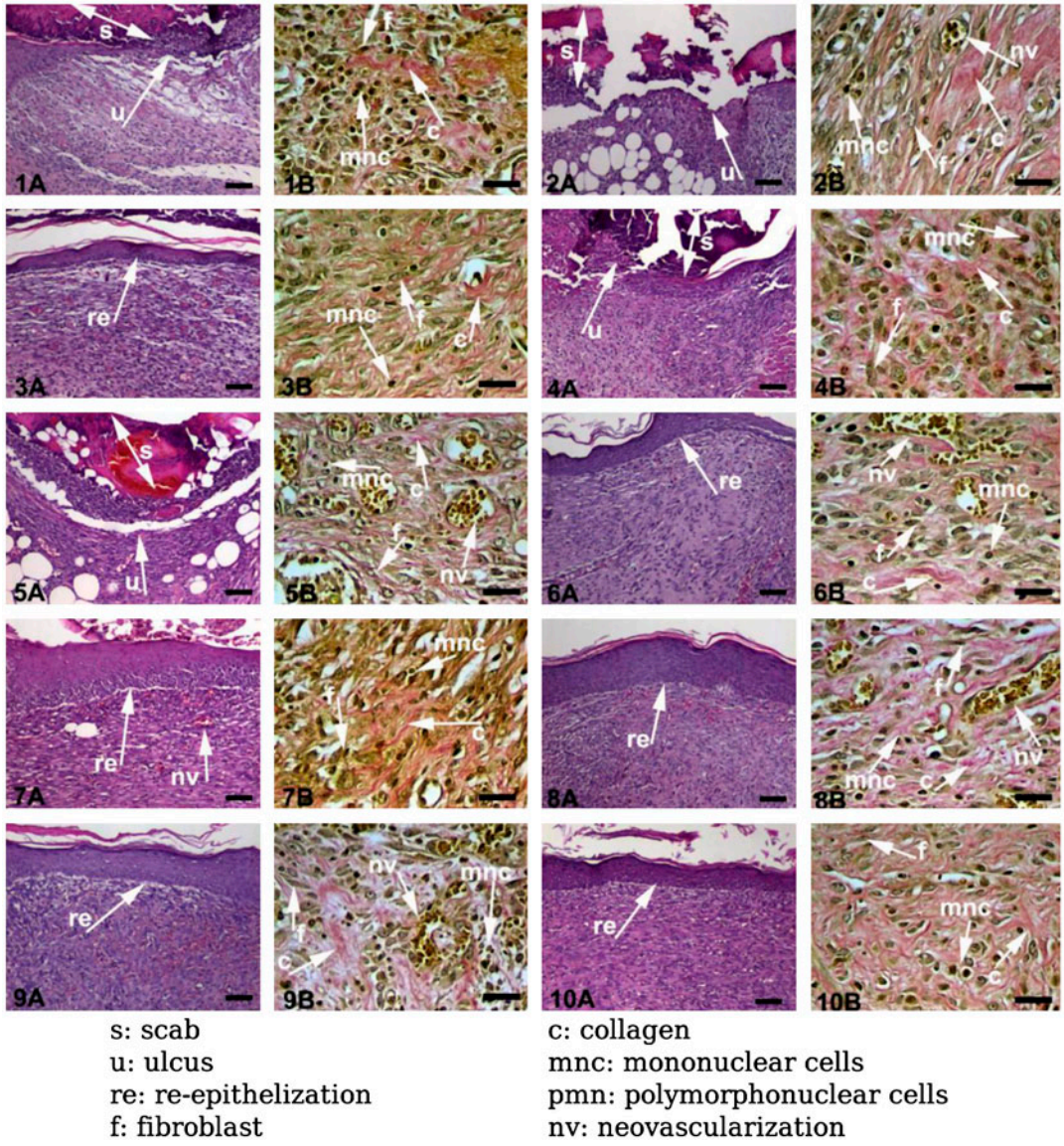

Figure 2. Histopathological view of wound healing and epidermal/dermal remodeling in the experimental groups. Skin sections show the HE-stained epidermis and dermis in A, and the dermis stained with VG in B. The original magnification was × 100 and the scale bars represent 120 μm for figures in A, and the original magnification was × 400 and the scale bars represent 40 μm for B. Data are representative of six animals per group. (1) Vehicle; (2) Negative Control; (3) Anisi aetheroleum; (4) Caryophilli aetheroleum; (5) Carvi aetheroleum; (6) Foeniculi aetheroleum (7) Lauri aetheroleum; (8) Lavandulae aetheroleum; (9) Melissae aetheroleum; and (10) Reference group.

neutrophils attack the wound area and remove the foreign materials, bacteria, and damaged tissue. Following the inflammatory phase, fibroblasts migrate to begin the proliferative phase and deposit new extracellular matrix. Afterwards, the new collagen matrix becomes cross-linked and organized during the final remodeling phase (19). Wound management has a great importance, since the retardation in the wound-healing process and wound infection places a substantial financial burden on health care systems. Despite the major advances in wound management, infection still remains an important factor in wound healing (20). Infection delays healing, causes failure, and increases the depth of wound. Particularly, methicillin-resistant *Staphylococcus aureus* (MRSA) has created major problems for wounds (21). Using the essential oils for the treatment of MRSA could be an alternative therapy due to their antibiotic, antiviral, and antifungal properties (22). Essential oils from several plants – such as lavender oil – have been used for the first aid treatment of wounds, abscess, and burns (23, 24). Indeed, lavender oil has a history of use in wound healing. Although, it was reported to be particularly effective during World War I, there has been no scientific evidence that lavender accelerates wound healing or reduces scarring so far. However, it was reported that lavender essential oils possess a wide range of biological activities that may be beneficial for wound-healing process. For example, lavender essential oils have antimicrobial activity (25), and both anti-inflammatory and analgesic properties (26, 27). Moreover, Bakkali et al. discussed extensively the biological activity of the essential oils and reported on an inhibition of the proliferation of murine leukemia and human mouth epidermal carcinoma cell lines by *L. angustifolia* essential oils (11). *L. angustifolia* is high in alcohols, terpenes, and oxides, and is noted for its effects in the rapid healing of burns, without scarring or infection (10). Lavender has also been promoted as oil that can help relieve the symptoms of other skin conditions such as psoriasis, dermatitis, and eczema (28). In a previous work, the main constituents of lavender oil were found as linalool, linalyl acetate, 1,8-cineole, β-ocimene, terpinen-4-ol, and camphor (25). In the current study, the main constituents of lavender oil (Table 2) are linalool (33.3%), linalool acetate (19.7%), and camphor (9.8%).

On the other hand, the oil of *L. nobilis* also exhibited significant wound-healing activity in the present study. The major components in the essential oil of *L. nobilis* were reported as eugenol and 1,8-cineole according to the previous studies (29, 30). In our study, 1,8 cineole (eucalyptol) (61.6%) and terpinyl acetate (13.8%) were the major components of the oil (Table 3). The wound-healing activity of these two essential oils could be attributed to their phytochemical ingredients. Further biological activity researches should be conducted in order to clarify the mechanism(s) of action.

Conflict of interest statement

No conflict of interest exists.

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