Journal of King Saud University – Science 34 (2022) 102356

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Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Essential oils from wild *Mentha longifolia* subspecies *typhoides* and subspecies *schimperi*: Burn wound healing and antimicrobial candidates

Abdullah Haikal [a,1], Mona El-Neketi [a,1,*], Walaa F. Awadin [b], Madiha A. Hassan [a], Ahmed A. Gohar [a,*]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt
[b] Department of Pathology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

ARTICLE INFO

Article history:
Received 23 February 2022
Revised 26 September 2022
Accepted 1 October 2022
Available online 8 October 2022

Keywords:
Mentha longifolia
Lamiaceae
Essential oils
Burn wound healing activity
Antiquorum-sensing activity
Antimicrobial activity

ABSTRACT

A comparative study was conducted on the essential oils (EOs) chemical composition from two subspecies of wild *Mentha longifolia typhoides* and *schimperi*, growing in Egypt, followed by biological investigation of EOs as antibacterial, antiquorum sensing and burn wound healing agents. Gas chromatography/mass spectrometry analysis of hydro-distillated EOs led to identification of 99 % of oil components. *Schimperi* oil revealed broadspectrum antibacterial activity with MIC values of 156 ~ 625 µg/ml, lower than or close to ampicillin. The oil from *typhoides* exhibited a higher antiquorum-sensing effect. The potential of oils to heal burn injuries was assessed by applying the oils in ointment form to second-degree burn injury in mice for 21 days. Interestingly, skin healing activity in group treated with *typhoides* oil was more effective than that of the positive control (Silver sulfadiazine 1 %). These results suggest a promising candidate in the area of burn wound healing therapy.

1. Introduction

Burn injuries are considered as one of the well-known morbidities worldwide and are associated with serious complications (Tiwari, 2012; He et al., 2017). About 2.4 million burn injuries are recorded annually, of which about 650,000 need treatment (Hashemi et al., 2014). Incidence and mortality rates vary according to different parameters of injuries such as their degree, area affected, depth, level of burns, and depending on treatment. Physiologically, burn wound healing occurs in three phases: inflammation reduction, proliferation and skin regeneration in the wounded area. Any drug that reduces the time for these phases can lead to better wound repair (Sood and Achauer, 2006).

Burn injuries are highly susceptible to infection leading to serious problems and these septic conditions may cause death among burn patients (Mokaddas et al., 1998). The extracts and essential oils of *Mentha* aerial parts were used as an effective disinfectant. They accelerate wound healing and tissue regeneration upon 2nd degree burn therapy in rats i.e. the application of *M. pulegium* extract in the form of dressings or oils (Habibi et al., 2018; Vaghardoost et al., 2019).

M. longifolia (L.) L. family *Lamiaceae* has different accepted synonymous names such as *M. longifolia* subsp. *lavandulacea* (Willd.) Briq.; *M. longifolia* (L.) Huds.; *M. longifolia* var. *microphylla* (Leij & Coutois) Rouy; *M. longifolia* subsp. *schimperi* (Briq.) Briq.; *M. longifolia* subsp. *typhoides* (Briq.) Harley.; and others. The last two names are among the infra-specific taxa in *Mentha* (WCSP, 2021; African Plant Database, 2021). These different names and diversity of *Mentha* varieties or subspecies are attributed to their geographical distribution and habitat (Lawrence 2006; Tucker and Naczi 2007). In Egypt, *M. longifolia* Huds. subsp. *typhoides* is called Habaq El-Mayya, Habaq El-Barr and Felaiyya while, *M. longifolia* Huds. subsp. *schimperi* is called Habaq (Abd El-Maksoud and Azer, 2013). It is noteworthy that these two investigated plants are known worldwide as wild mint or horse mint (Lawrence 2006; Verma et al., 2015).

The two titled plants are among the most promising medicinal plants that show diverse biological activities such as antioxidant,

Abbreviations: LB broth, Luria-Bertani medium (lysogeny broth); MIC, Minimum inhibitory concentration; QS, Quorum-sensing.

* Corresponding authors.
E-mail addresses: melneketi@mans.edu.eg (M. El-Neketi), gohar@mans.edu.eg, ahmedgohar99@yahoo.com, gohar@mans.edu.eg (A.A. Gohar).

[1] These authors contributed equally to this work (Co-first authors in alphabetical order).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2022.102356
1018-3647/

antimicrobial (Habibi et al., 2018; Hajlaoui et al., 2009), cytotoxic (Orhan et al., 2012), anti-diarrheal, antispasmodic and calcium channel blocking (Shah et al., 2010). Mints are used in tradtional medicine for bronchitis, nausea, flatulence, ulcerative colitis, liver complaints and anorexia. The Egyptians are using the wild mint plants to relief pain, fever, various inflammatory disorders, as well as to improve digestion, sedation and wound-healing (Eissa et al., 2014). Worldwide, *M. longifolia* is used traditionally as antiparasitic, anti-inflammatory, antimicrobial and / or antiseptic agents. It is also used for gastrointestinal disorders as peptic ulcer, antiemetic, diarrhea, ulcerative colitis and liver diseases (Verma et al., 2015; Kozan et al., 2006; Aghili, 2009; Darwish and Aburjai, 2010). *M. longifolia* L. has been reported to contain different classes of secondary metabolites mainly flavonoids, essential oil, in addition to many other phenolic constituents (Farzaei et al., 2017). In a previous publication, we reported the presence of triterpenes, steroids, flavonoids, and phenolic acids in the titled plant (Haikal et al., 2021).

These findings along with the growing global need for new candidates in the area of wound healing especially from natural sources, encouraged us to assess the potential role of essential oils from the two wild Egyptian *M. longifolia* subspecies: *typhoides* and *schimperi* as promising wound healing agents and to demonstrate their antibacterial as well as antiqurum-sensing effects.

2. Materials and methods

2.1. Essential oils preparation and analysis

Fresh aerial parts of two subspecies of the wild Egyptian *M. longifolia*: *typhoides* and *schimperi* (250 g, each) were crushed into small pieces and subjected to hydro-distillation to a constant oil volume (8 h) using a Clevenjer-type apparatus to produce yellow-colored oils. Oils were dehydrated using anhydrous sodium sulfate and stored at low temperature for running GC/MS analysis (for the program, please see the supporting information) and biological studies. The oil components were identified by measuremants of component retention indices (Van den Dool and Kratz, 1963), their mass spectral fragmentation patterns (Adams, 2007; Halim et al., 1990) and/or stored data on the mass spectral database NIST/ ChemStation data system.

2.2. Biological activities of essential oils

2.2.1. Antimicrobial assay

The antimicrobial activity of the distilled essential oils was investigated using broth microdilution method. For a detailed procedure, please see the supporting information.

2.2.2. Antiquorum-sensing assay

The anti-quorum sensing activity was assessed for distilled essential oils employing El-Gohary and Shaaban, 2015 procedure. For detailed description, please refere to Haikal et al., 2021.

2.2.3. Burn wound healing activity

2.2.3.1. Preparation of the test samples and burn wound healing bioassay. Essential oils are incorporated into an ointment base formed of glycol stearate: propylene glycol: liquid paraffin (3:6:1); 1 % concentration (De Villiers, 2009; Süntar et al., 2012).

Wister albino mice weighing approximately 25–35 g (40 mice) were used in the experiment. All mice were in good health and had been checked for animal diseases by a veterinarian. Mice were separated in different shelves in sterilized containers in the animal laboratory, Faculty of Veterinary medicine, Mansoura university, at temperature about 22 °C. The experimental procedure were adopted by the Research Ethics Committee, Faculty of Pharmacy, Mansoura University, Egypt (Approval date: November 28th, 2017 to May 25th, 2021 – Approval number: 2021–263).

Forty mice were divided into 4 groups (n = 10). Groups were treated as follow: Group **1**: with plain ointment base, group **2**: with silver sulfadiazine 1 % ointment, group **3**: with *typhoides* subsp. essential oil ointment and group **4**: with *schimperi* subsp. essential oil ointment. Mice were anaesthetized with xylazine (10 mg/kg) and ketamine (60 mg/kg) as I.M injection. Their backs were shaved with a blade. Then, a second-degree burn wound (1.5 cm^2) was generated with a metal cube (2 × 3 × 1 cm) heated to 105 °C for 15 s. Mice were resuscitated with a 1 ml injection of intraperitoneal normal saline solution, and then ointments were applied daily for 21 days to the wound-areas with insufficient amount. All wounds were not dressed in order to increase the visibility of wound conditions (Edraki et al., 2014). A digital camera (Canon power-shot D10) was used to photograph the burned areas on days 3–21.

The wound areas was recorded as 100 % on the first experimental day to which areas on the subsequent days were compared.

2.2.3.2. Histopathological examination. Skin tissue samples were taken for histopathological examination to assess wound re-epithelialization on days 3, 7, 14 and 21 (the end of treatment period). Small excisions containing part of the wound area were done, and then tissues were fixed in 10 % formalin and processed until immersed in paraffin blocks. Paraffin sections with a thickness of 5-µm were cut using a microtome and routinely stained with hematoxylin and eosin. These tissue sections were examined microscopically.

Angiogenesis (neovascularization) and infiltration with inflammatory cells were assessed in tissue sections on the third day after burn injury by giving a score as follows: zero or (-) score was given when vessels and macrophage cells are absent in each high-power field. Mild score 1 or (+) was set when 1–2 vessels or 1–2 macrophages cells were identified. The moderate score was 2 or (++), indicating 3–4 vessels for angiogenesis and 3–4 cells for cell study in each high-power field. The severe score was 3 or (+++) when 5 or more cells or vessels were detected in the high-power field.

A modified scoring system for histopathological criteria to assess surgical wound healing on the 7th, 14th and 21st days was defined according to Edraki et al., 2014; Pereira et al., 2012. The detailed description of histological scoring system are descriped in the supporting information.

3. Results and discussion

3.1. Components of the essential oils

Aerial parts of *M. longifolia* subsp. *typhoides* yielded 0.8 % v/w of a clear faint yellow, lighter than water essential oil. The oil components together with the percentage and retention indices are shown in Table 1. Forty-two compounds (99.865 %) were identified in the oil. Monoterpenes and sesquiterpenes are the main components, accounting for 88.980 % and 10.885 %, respectively. The monoterpenes are represented by piperitenone oxide (55.443 %), piperitone oxide (12.180 %), 1, 8 cineole (6.230 %), D-limonene (3.739 %), α- terpineol (1.858 %), (-) β-pinene (1.737 %) and (+)-α–pinene (1.123 %) as the main constituents. While, sesquiterpenes are represented by caryophyllene (7.127 %) and (+) *epi*-bicyclosesquiphellandrene (1.273 %) as the major constituents. Aerial parts of *M. longifolia* subsp. *schimperi* yielded 0.9 % v/w of

Table 1
Chemical composition of the essential oils of *M. longifolia* subsp. *typhoides* and *M. longifolia* subsp. *schimperi* aerial parts by GC–MS.

No.	Identified compounds	R_t^b	RI	M^+ peak	Base peak	% in subsp. *typhoides*	% in subsp. *schimperi*
1	α- Thujene	5.988	922	136.1	93.0	0.078	0.024
2	(+)-α–pinene	6.194	931	136.1	93.1	1.123	0.485
3	Camphene	6.640	943	136.1	93.0	0.025	0.013
4	Sabinene	7.418	968	136.1	93.1	0.714	0.339
5	(−) β-Pinene	7.527	980	136.1	93.1	1.737	0.599
6	(+)-β-Pinene	7.991	989	136.1	93.0	–	0.391
7	β–Myrcene	8.002	999	136.1	93.1	0.847	–
8	*P*-Mentha- 1(7),8-diene	8.443	1001	136.1	93.0	0.368	0.019
9	(+)-4- Carene	8.912	1007	136.1	93.0	0.021	–
10	Cymene	9.221	1017	134.1	119.0	0.018	0.021
11	D-Limonene	9.387	1029	136.1	68.1	3.739	1.894
12	1, 8 cineole	9.513	1030	154.1	81.1	6.230	1.244
13	Trans- β- Ocimene	9.724	1036	136.0	93.0	0.244	–
14	Cis-β-Ocimene	10.119	1041	136.1	93.0	–	0.075
15	γ- Terpinene	10.531	1051	136.1	93.0	0.041	0.075
16	4- Thujanol	10.943	1075	154.1	93.0	0.070	0.131
17	Terpinolene	11.716	1079	136.1	93.0	0.134	0.193
18	Linalool	12.276	1084	154.0	70.1	0.321	–
19	1,3,8-P-Menthatriene	12.700	1101	134.1	119.0	0.935	–
20	**Menthone**[a]	5.006	156	54.1	12.1	–	**26.593**
21	Isopulegone	15.504	1161	152.1	67.1	–	1.270
22	4- Carvomenthol (Terpinene 4-ol)	15.566	1162	154.1	71.0	0.556	–
23	α- Terpineol	16.202	1172	154.0	59.0	1.858	0.529
24	3-Carene	17.855	1201	136.1	93.0	0.297	–
25	**Pulegone**	18.273	1233	152.1	81.0	0.075	**56.493**
26	(-)-Carvone	18.485	1240	150.0	82.0	0.089	–
27	**Piperitone oxide**	19.022	1266	168.1	69.1	**12.180**	–
28	Bornyl acetate	20.270	1283	196.0	95.0	0.151	–
29	Diosphenol	20.888	1285	168.1	126.0	0.290	–
30	Thymol	21.288	1289	150.1	135.0	0.581	–
31	Eucarvone	22.713	1320	150.1	107.1	0.815	–
32	**Piperitenone oxide**	24.338	1360	166.1	67.1	**55.443**	–
33	Jasmone	25.168	1388	164.1	166.1	–	0.467
	Monoterpenes					88.98	90.855
	Oxygenated monoterpene					78.659	86.727
II. Sesquiterpenes							
34	Caryophyllene	25.952	1399	204.1	93.1	7.127	2.075
35	δ–Cadinene	27.010	1431	204.2	161.1	0.033	–
36	Humulene	27.291	1448	204.2	93.0	0.641	1.099
37	Trans-β- Farnesene	27.480	1452	204.1	69.1	0.143	–
38	(+) *epi*-Bicycloses-quiphell-andrene	27.674	1471	204.2	161.1	1.273	1.081
39	(-)-D-Germacrene	28.407	1483	204.1	161.1	0.639	0.964
40	(+) Valencene	28.658	1495	204.2	161.1	0.014	–
41	Bicyclogermacrene	29.019	1502	204.1	93.1	0.150	–
42	Eremophilene	29.156	1507	204.2	161.1	0.039	–
43	γ- Cadinene	29.728	1512	204.1	161.1	0.335	0.808
44	(-)-Calamenene	30.089	1533	202.2	159.0	0.055	0.062
45	γ- Murolene	30.644	1553	204.1	161.0	0.089	–
46	Caryophyllene oxide	32.418	1588	221.1	79.0	0.258	0.769
47	Cadine-1,4-diene	33.659	1594	204.1	119.1	0.089	0.346
48	Cedrelanol	35.153	1644	222.0	161.1	–	1.498
49	(-)-α-Himachalene	36.314	1650	204.1	93.1	–	0.023
50	Farnesyl acetone	44.136	1820	262.1	69.1	–	0.091
	Sesquiterpene				10.885	8.816	
	Oxygenated sesquiterpenes				0.258	2.358	
	Total oil content				99.865	99.671	

[a]Bold values point out the major components; (–): constituent is absent.
[b]R_t = retention time, RI = retention index.

a clear, faint yellow, lighter than water essential oil. Oil components with percentage and retention indices are shown in Table 1. Thirty compounds (99.671 %) were identified in the oil. Monoterpenes and sesquiterpenes, are the main constituents, accounting for 90.855 % and 8.816 %, respectively. The main constituents of monoterpenes are represented by pulegone (56.493 %), menthone (26.593 %), D-limonene (1.894 %), isopulegone (1.270 %) and 1, 8 cineole (1.244 %), and the main constituents of sesquiterpenes are represented by caryophyllene (2.075 %), humulene (1.099 %), (+)-*epi*-bicyclosesquiphellandrene (1.081 %) and cedrelanol (1.498 %).

3.2. Biological activities of essential oils

3.2.1. Antimicrobial assay

Essential oils isolated from both plants were assessed for their antimicrobial potential using the broth microdilution assay. The results (Table 2) proved that both oils exhibited broad-spectrum antibacterial activity. Oil from subsp. *schimperi* was more active than that from *typhoides*. The former revealed higher antibacterial activities against the tested Gram-positive and Gram-negative bacteria strains with MIC values in the range of 156–625 μg/ml lower than or close to that of ampicillin (MIC 625–2500 μg/ml). Whereas,

Table 2
MIC (μg/mL) of *M. longifolia* subsp. *typhoides* and *M. longifolia* subsp. *schimperi* essential oils.[a]

Micro-organism		Ampicillin	Amphotericin B	*M. longifolia typhoides* oil	*M. longifolia schimperi* oil
Gram-negative	*E. coli*	625	nt	1250	625
	K. pneumoniae	1250	nt	1250	625
Gram-positive	*S. aureus*	625	nt	1250	156.25
	B. cereus	2500	nt	1250	625
Fungus	*C. albicans*	nt	78.125	625	312.5

[a]Sample concentration: 5 mg/ml, Sample volume 100 μL /well, Results are calculated after subtraction of DMSO activity, nt: not tested.

the oil from *typhoides* subsp. exhibited relatively lower antibacterial activity. Both oils are inactive against the fungus *C. albicans*.

High antibacterial activity of *M. longifolia* subsp. *schimperi* oil can be attributed to its content of pulegone as a major oxygenated compound, while the major oxygenated compound of *M. longifolia* subsp. *typhoides* oil is piperitenone oxide that has lower antibacterial activity compared to pulegone. This explains the lower antibacterial potency of *M. longifolia* subsp. *typhoides* oil incomparison to *schimperi* subsp. (Oumzil et al., 2002; Božović et al., 2015).

3.2.2. Antiquorum-sensing activity

Quorum-sensing (QS) in *Ch. violaceum* ATCC 12,472 secretes a purple pigment named violacein in response to the acyl HSLs auto-inducer molecules (Chu et al., 2011; McLean et al., 2004). Consequently, any medicine inhibiting acyl HSL-mediated QS activity in *Ch. violaceum* will stop violacein secretion. The QS activity of both oils was inferred from measurement of the pigment inhibition radius of essential oils. *M. longifolia* subsp. *typhoides* is relatively active (r = 13 mm) than *M. longifolia* subsp. *schimperi* (r = 11 mm) (Fig. 1).

3.2.3. Burn wound healing activity

Macroscopic examination of skin burn wounds at the beginning of therapy (3rd day) and at the end of therapy period (21st day) were demonstrated in (Fig. 1**S&2S**). At the end of this research, the appearance of regenerated skin with the naked eye seems normal and most of the skin appendages are totally repaired. The quality and quantity of burn wound healing and wound infection are directly related to treatment. The average burned area was significantly diminished in group 3 compared to other groups.

Microscopic examination of the skin from all groups on the third post-burn day showed the presence of necrotic epidermal cells, hyaline degeneration of collagenous fibers in the superficial dermis, a loose dermal structure, acute inflammatory reaction characterized by oedema, congested blood vessels and intense inflammatory cells infiltration (mainly neutrophils) with few lymphocyte and macrophages (Fig. 2). Inflammation intensity and prevalence was highest and deepest in group 1, however it was lowest and most superficial in group 3. Whereas, angiogenesis was highest in group 3 and was lowest in group 1 as shown in Table 3. Therefore, inflammation is inhibited due to angiogenesis in the tissues (Ribatti, 2017).

On the 7th post-burn day, microscopic examination of untreated group 1 revealed persistent epidermal and dermal necrosis, neutrophils and sometimes lymphocyte and macrophages infiltration and formation of granulation tissue. The treated groups showed re-epithelialization, fibroblast infiltration, and angiogenesis. The area of granulation tissue formation, maturation, tissue organization and re-epithelialization were significantly higher in group 3 compared to group 1 (Table 3). Intense inflammatory cellular infiltration was detected in untreated group 1. Inflammation was markedly decreased in the treated groups particularly in group 3 (Fig. 3). These microscopic features indicate an early healing process in group 3.

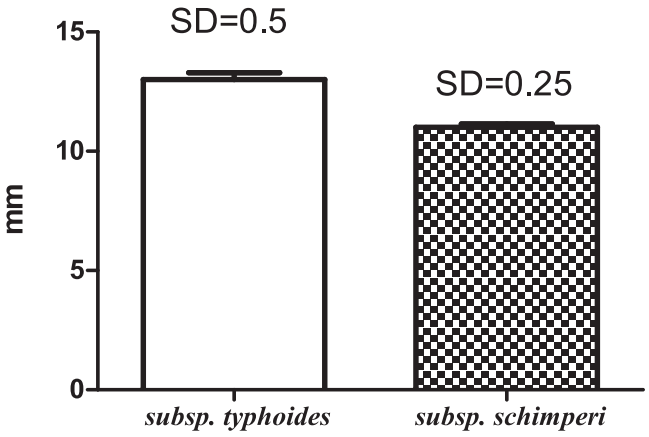

Fig. 1. Antiquorum-sensing activity of *M. longifolia* subspecies essential oils.

On the 14th day post-injury, skin from group 1 showed few squamous and granular epidermal cells with no orthokeratin. In this stage, the inflammatory exudates replaced the necrotic layer showing intense neutrophilic and few macrophages histiocytic infiltrations with few blood vessels. Skin sections of group 2 showed the appearance of a few layers of squamous and granular epidermal cells with parakeratosis. Many macrophages histiocytic infiltration with few blood vessels were noticed. Skin sections from group 3 showed the appearance of several layers of squamous and granular epidermal cells with the presence of orthokeratin many macrophages histiocytic infiltration and few blood vessels. Skin sections from group 4 showed few layers of squamous epidermal cells with parakeratosis, few neutrophilic infiltration, some macrophages histiocytic infiltration and few blood vessels. The appearance of new epithelium was observed in all groups with different thickness. The regenerated "epithelial islands" grow vertically, then migrate towards the surface and cover the wound. Neoformed capillaries are observed and granulation tissues are generated. Group 3 revealed significant extended granulation tissue formation, maturation, tissue organization and re-epithelialization compared to group 1 (Fig. 4) (Table 3).

On the 21st day post-burn injury, skin sections from group 1 showed persistence of inflammatory exudate covering few layers of epidermal cells with an excessive amount of granulation tissue and severe inflammatory cells infiltration (mainly lymphocytes and a few macrophages). Skin sections in group 2 showed normal epidermis with moderate amount of granulation tissue with persistent intense inflammatory reaction. Skin sections from group 3 showed complete healing where the regenerated skin seemed to be normal with new skin appendages formation, increased deposition of well-organized collagen bundles and number of fibroblasts infiltrated with very few inflammatory cells. Skin sections in group 4 showed normal epidermis with early maturation of granulation tissue infiltrated with few inflammatory cells (Fig. 5). Histopathological findings showed that the new dermis

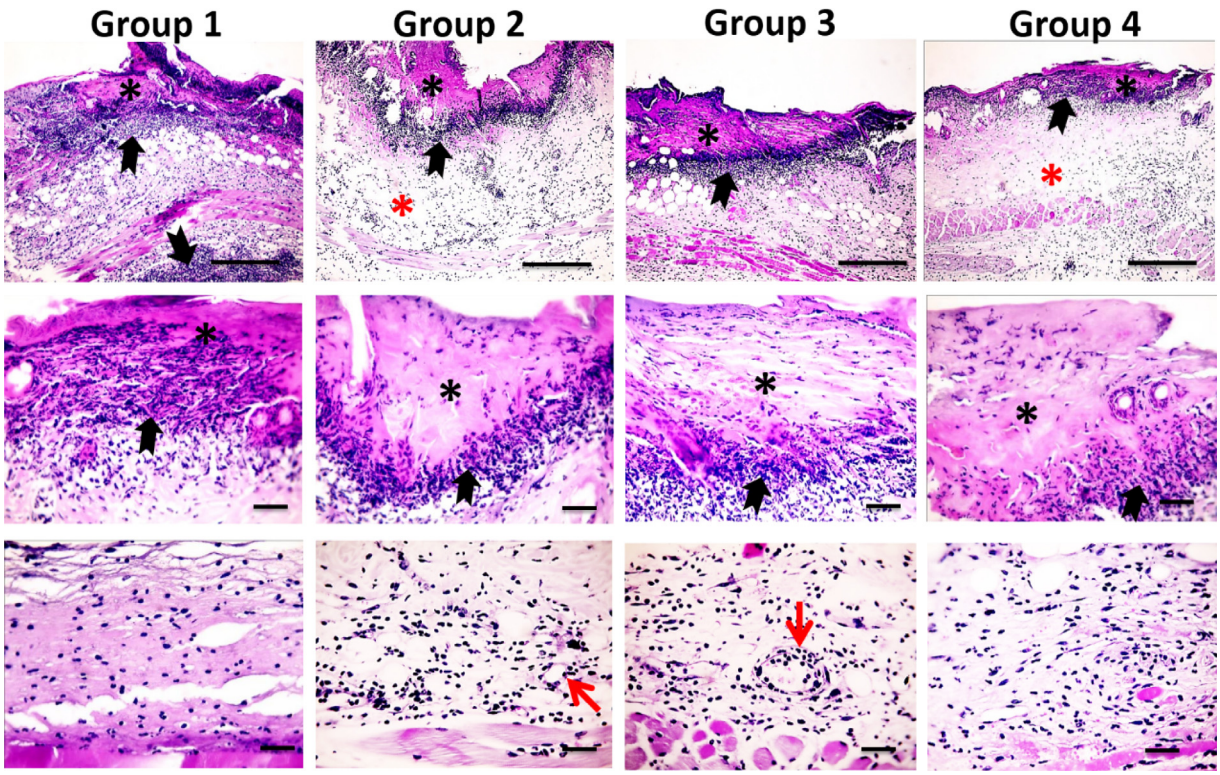

Fig. 2. Microscopic pictures of H&E stained skin sections from four groups on the 3rd day after burn injury showing epiderma necrosis (*) with intense inflammatory reaction (black arrows) in all groups, stronger and deeper inflammation in Group 1, edema (*) in groups 2&4 (X: 100 bar 100 in 1st row). By higher magnification, the inflammatory reaction is mainly neutrophils infiltration (black arrows). Neovascularization (red arrows) appears in Groups 2&3 (X:400 bar 50 in 2nd and 3rd rows).

formation was the best in group 3 followed by group 2 and then group 4 (Table 3).

Sum of the three histopathological component scales of burn wound healing showed that group 3 treated with *M. longifolia* subsp. *typhoides* essential oil had good healing followed by group 2 treated with the positive control (silver sulfadiazine) followed by group 4 treated with *M. longifolia* subsp. *schimperi* essential oil (Table 4).

Histopathologically, the skin of control group 1 had a large region of ulceration, a low degree of scar development, and various new dermis components. When compared to silver sulfadiazine and untreated groups, the results demonstrated that *M. longifolia* subsp. *typhoides* essential oil substantially enhanced various phases of the deep second burn wound and histological components of healing. On day 21, the development of new dermis in group 3 was much higher than in the other groups. The fact that the control groups had a slower rate of wound healing than the other treatments could be due to the presence of bacteria in the wounds or their histopathological abnormalities. (Nasiri et al., 2015).

Wound healing is a complicated process that involves four stages: coagulation, inflammation, debridement, and re-epithelialization. Proliferation, migration, and differentiation of epidermal squamous epithelial cells play critical roles. Collagen deposition and remodelling occur intradermally at the end of the healing phase (Mekonnen et al., 2013).

Essential oil of *M. longifolia* subsp. *typhoides* reduced inflammation, therefore it may successfully prevent edoema, erythema, secretion, and other burn sequelae. On day 7 after the burn, all treated groups began re-epithelialization earlier than group 1 on day 14 after the burn. Previous research has suggested that some natural ingredients can speed up the re-epithelialization of a burn wound (Nasiri et al., 2015). On day 8 following burn injury, the re-epithelialization components of *Malva sylvestris* creams had a good epithelization. However, after 15 days of *Hypericum perforatum* treatment and 16.5 days of *Calendula* (as herbal medicine) treatment, re-epithelialization happened (Sayar et al., 2014). re-epithelialization is influenced by the thickness of the granular cell layer, epidermal thickness, squamous cell maturation and organization, and epithelial cell migration (Sayar et al., 2014). Collagen is the most abundant protein in the extracellular matrix produced by fibroblasts, and it gives the dermis its strength and integrity (Pirbalouti and Koohpyeh, 2011; Razavi et al., 2011). *M. longifolia* subsp. *typhoides* essential oil can increase the amount of well-organized collagen bundles. Similarly, some *Malvacea* and *Boraginaceae* species' anti-inflammatory effects enhanced collagen fibre production, epithelium regeneration, and epithelium thickness (Razavi et al., 2011).

The extent of granulation tissue, re-epithelialization and formation of new dermis, support the idea that topical application of *M. longifolia* subsp. *typhoides* essential oil was more effective in the treatment of second-degree burn wounds than standard common burn wound care. The histologically enhanced and accelerated wound healing may be explained by the anti-inflammatory and antimicrobial properties of *M. longifolia* subsp. *typhoides* essential oil.

4. Conclusion

This study was devoted to evaluate the effect of two subspecies of wild Egyptian *Mentha longifolia* essential oils on burn wound healing in mice. The antimicrobial activity of the *Mentha sp*. oils shown in our results, contributes to the burn wound healing. These findings can be attributed to the chemical composition of the essential oils especially the oxygenated fractions (Habibi et al.,

Table 3
Histopathology of inflammatory degrees, angiogenesis and granulation tissue formation among different groups on the 3rd, 7th, 14th and 21st day after burn injury.

Components/Groups	Group 1	Group 2	Group 3	Group 4	P value
Degrees of inflammation and angiogenesis on the 3rd day after burn					
Macrophage histocytic infiltration	2.5 ± 0.082	1.8 ± 0.013	0.8 ± 0.005	1.5 ± 0.096	0.0026
Angiogenesis	0.3 ± 0.014	1.3 ± 0.018	2.5 ± 0.018	0.8 ± 0.008	0.0027
Degrees of granulation tissue formation and its components on the 7th day after burn					
Macrophage histocytic infiltration (0–3)	8.0 ± 0.07	10.2 ± 0.37	13.0 ± 0.35	8.9 ± 0.14	0.0004
Neovascularization (0–3)					
Fibroblastic proliferation (0–3)					
Matrix mucopolisacharide deposition (0–3)					
Degree of inflammation (0–3)					
Extent of bacterial colonization [(-3)-0]					
Degree of granulation tissue formation (0–3)					
Total (-3–18)					
Degrees of re-epithelialization parameters on the 7th day after burn					
Epidermal thickness (0–3)					
Thickness of granular cell layer (0–3)					
Maturation organization of squamous cells (0–3)					
Extent of keratin layer (0–3)	2.8 ± 0.58	7 ± 0.44	13.20 ± 0.60	5.8 ± 0.37	0.0007
Orthokeratin (0–3)					
Parakeratosis (0–3)					
Total (0–18)					
Degrees of granulation tissue formation and its components on the 14th day after burn					
Macrophage histocytic infiltration (0–3)					
Neovascularization (0–3)					
Fibroblastic proliferation (0–3)					
Matrix mucopolisacharide deposition (0–3)	10 ± 0.70	11 ± 0.54	12.8 ± 0.48	10 ± 0.50	0.03
Degree of inflammation (0–3)					
Extent of bacterial colonization [(-3)-0]					
Degree of granulation tissue formation (0–3)					
Total (-3–18)					
Degrees of re-epithelialization parameters on the 14th day after burn					
Epidermal thickness (0–3)					
Thickness of granular cell layer (0–3)					
Maturation organization of squamous cells (0–3)					
Extent of keratin layer (0–3)	1 ± 0.30	7.8 ± 0.50	13.8 ± 0.24	7.4 ± 0.20	0.0009
Orthokeratin (0–3)					
Parakeratosis (0–3)					
Total (0–18)					
Degrees of new dermis formation parameters on the 21st day after burn					
Degree of scar formation (0–3)					
Organization of collagen formation (0–3)					
Extent of hair follicles (0–3)	5.4 ± 0.74	9.4 ± 0.40	11.4 ± 0.42	7.2 ± 0.37	0.0009
Extent of lymphatic ducts (0–3)					
Degree of innervations (0–3)					
Total (0–15)					

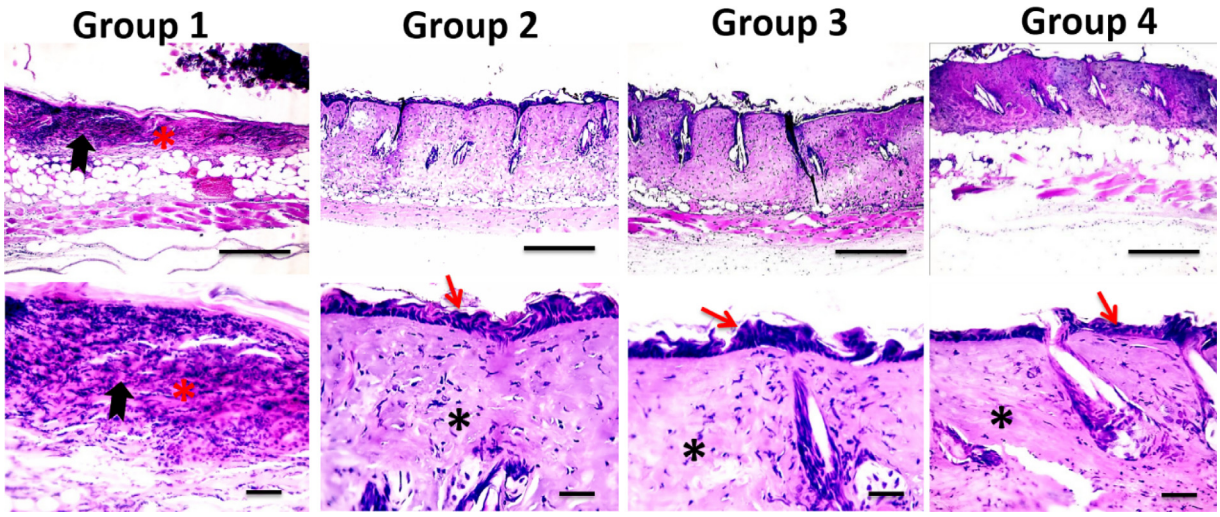

Fig. 3. Microscopic pictures of H&E stained skin sections from four groups on the 7th day after burn injury showing the persistence of epidermal necrosis (*) with intense neutrophilic infiltration (black arrows) in Group 1 (X: 100 bar 100 in 1st row), re-epithelization (red arrows) and deposition of collagen (*) in treated groups 2–3 in higher magnification (X:400 bar 50 in 2nd and 3rd rows).

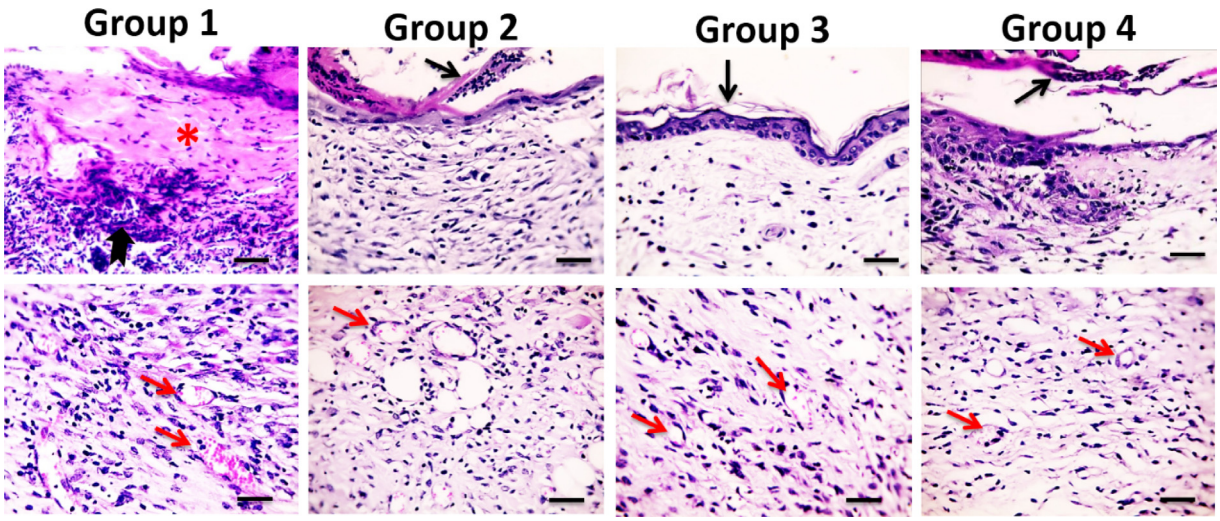

Fig. 4. Microscopic pictures of H&E stained skin sections from four groups on the 14th day after burn injury showing the persistence of epidermal necrosis (*) with intense neutrophilic infiltration (black arrows) in Group 1, parakeratosis (black arrows) in Groups 2&4, orthokeratin in (black arrow) in Group 3, neovascularization (red arrows) in all groups (thick blue arrows) X:400 bar 50.

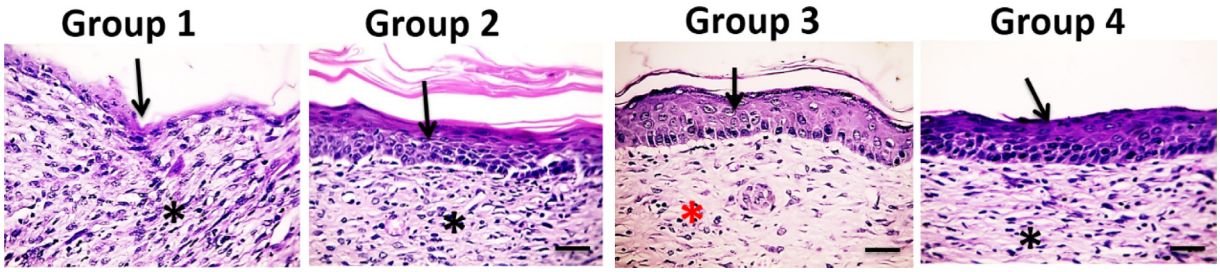

Fig. 5. Microscopic pictures of H&E stained skin sections from four groups on the 21st day after burn injury showing few squamous epidermal cells in Group 1, completed new epithelization in Groups 2–4, inflamed granulation tissue in Groups 1, 2&4 (*), well-organized bands of collagen in Group 3 (*) X:400 bar 50.

Table 4
The sum of the scales of three histopathological components for burn wound healing.[a]

Group	Re-epithelialization [0–15]	Extent of granulation tissue [(-3)-18]	New dermis formation [0–15]	Sum of scales [(-3)-58]
Group 1 B	1 ± 0.30	10 ± 0.70	5.4 ± 0.74	16.40 ± 1.28
Group 2 D	7.8 ± 0.50	11 ± 0.54	9.4 ± 0.40	28.2 ± 0.86
Group 3 O1	13.6 ± 0.24	12.8 ± 0.48	10.40 ± 0.24	38 ± 0.70
Group 4 O2	7.4 ± 0.20	10 ± 0.50	7.2 ± 0.37	25.2 ± 0.80
P value				*0.0006*

[a] Values are means ± SD.

2018; Oumzil et al., 2002; Božović et al., 2015; Shahverdi et al., 2004). However, the healing potential produced by *M. longifolia* subsp. *typhoides* oil is more significant when compared to that produced by *M. longifolia* subsp. *schimperi* oil. This difference is attributed to the high percentage composition of piperitenone oxide (55.443 %) and piperitone oxide (12.180 %) in *M. longifolia* subsp. *typhoides* essential oil, and we suggest them as good candidates for further study in the area of wound healing.

5. Contributors' statement

Abdullah Haikal: Operating the hydrodistillation of plant materials, participated in the identification of the oils components and in the biological part.

Mona El-Neketi: Participated in the identification of the oils components, writing and revision of the manuscript, followed up the biological part and took over the publishing process.

Walaa F. Awadin: Participated in the biological part.

Madiha A. Hassan:Participated in the supervision of the research and revised the manuscript.

Ahmed A. Gohar: Suggested the research point, supervised the progress of the research, wrote and revised the manuscript and took over the publishing process.

Funding

This study did not receive any grant from public, private or non-profit funding agencies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Dr. Soha El-Shaer (Department of Microbiology, Faculty of Pharmacy, Mansoura University) is acknowledged for carrying out the antiquorum–sensing screening assay.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2022.102356.

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