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Effect of *Mentha piperita* L. fatty oil on full thickness excised wound healing in rabbits

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Abstract

The present work aimed to determine the chemical characterization of the aerial partand to assess full thickness excised wound healing activity of the fatty oil of *Mentha piperita* L. (MPFO) grown in Algerian eastern part. The fatty acid composition of *M. piperita* L. leaves hexane extract was analysed by GC-MS. Total thickness excised wound healing activity of hexane extract was carried out on the back of nine adult male New Zealand rabbits; comparing to a reference drug effects (Madecassol®).The treatments were repeated once daily until complete healing. For each four days of post-excision, the percentage of wound contraction was evaluated, and the different healing times were noted. The results showed that *M. piperita* L. leaves contain low levels of oil (1.79%), and GC/MS results of its oil have revealed the presence of different fatty acids among which the main constituents were oleic (43.16 ± 1.01%), palmitic (37.70 ± 0.94%) and linoleic (11.13± 0.45%) acids. The wound healing activity and the level of wound contraction were significantly higher in *M. piperita* L. fatty oil (MPFO) compared to untreated wounds. In addition, both of MPFO and Madecassol® accelerated significantly wound healing activity and their healing times were faster than CONT group. We conclude that *M. piperita* L. fatty oil promotes significantly ($p < 0.05$) wound contraction and reduces epithelization period in rabbit model.

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Introduction

The species *Mentha piperita* L. (Family-Lamiaceae) commonly known as peppermint is a persistent aromatic plant of 80 cm high, generally grown in Europe and North America and dispersed also in the Mediterranean regions (north of Algeria)and Asia, cultivated for the extraction of hydrodistilled oil (McKay and Blumberg, 2006; Sousa *et al.*, 2007).Previous pharmacological studies indicated that *M. piperita* L. had been used extensively as medicinal plant in health care for centuries; for the treatment of nausea, diarrhea and irritable bowel syndrome, as antispasmodic for digestion system, anti-inflammatory, antibacterial, antiseptic treatments and various skin disorders; including wound infections and eczema (Iserin, 2001; WHO, 2002; Priya *et al.*, 2002). Wound healing activity is involved to restore damaged skin, including hemostasis, inflammation, proliferation and maturation (Stadelmann *et al.,* 1998; Enoch and Leaper, 2005; Toporcer *et al.*, 2006; Nguyen *et al.*, 2009; Rieger *et al.*, 2014).

The wounds care prevents infection and enhances wound healing via disinfection; treatment and protection from reinjury (Alizadeh *et al.*, 2009). Several conventional drugs for wound healing take their origin from plants (Habbu *et al.*, 2007). Researches of new plant products that may promote wound healing have recently taken advantages and can serve as new lead compounds for wound healing activities (Wang *et al.*, 2013).I n the majority of our knowledge there are no preceding reports dealing with the wound healing activity of *M. piperita* L. fatty oil (MPFO) grown in Algerian eastern part. The current study was, therefore, conducted to investigate the healing effect of *M. piperita* L. leaves hexane extract on the full thickness excised wounds on rabbits, and the GC/MS composition of fatty acids.

Materials and methods

Plant materials

The medicinal plant of the present study was selected on the basis of an ethnopharmacological survey on the plants traditionally used in Algeria as skin healing. Fresh leaves of *M. piperita* L. (Lamiaceae) were collected from northern east of Algeria, (Constantine) in mid July 2014(just before flowering phase). They were air dried under shadow for three weeks, at room temperature (18±4C°), and kept separately until being used.

Drugs composition

Madecassol® (cream 1%) as commercial product was obtained from the local pharmacy. It contains Hydrocotyle (*Centella asiatica*; reconstituted titrated dry extract containing asiaticoside 40% and madecassic and Asiatic acids 60%). Other ingredients: Ethylene glycol (mono+diester), palmito-stearate, propylene glycol, liquid paraffin, essential oil of lavendar, essential oil of geranium and purified water.

Hexane extraction

A soxhlet apparatus has been used for the hexane extraction of *M. piperita* L. powdered dried leaves. 100g of plant material was placed into an extraction thimble with refluxed hexane (1L) in the extraction flask for 24h (3x8h). Crude extract has been reduced under vacuum at 40° C using a rotary evaporator until fluid hexane extract was obtained. After solvent evaporation, the obtained oil (MPFO) was stored into dark sealed vials at 4 °C prior to the GC-MS analysis and excised wound healing activity.

Esterification of fatty acids and gas chromatography conditions

Esterification

Fatty acids were transmethylated according to the study of Ertas *et al.* (2014). Fatty acid methyl esters (FAME) were analysed by gas chromatography coupled with mass spectrometry and conducted in triplicate; the obtained percentages have been expressed as mean ± SD.

Gas chromatography/Mass Spectroscopy conditions

MPFO oil was analyzed by GC/MS using a *thermo-électron*® (DSQ-MS) mass selective detector coupled with an *Shimadzu*® (TQ8030) gas chromatograph, equipped with silica *Restek*® DB-5 capillary column

(30 m · 0.25 mm · film thickness 0.25 µm). The carrier gas flow was 1mL of N/min. The injector and detector temperatures were kept at 220°C and 290°C respectively. The injection volume was 2,0µl. The ionization energy was 70 eV. The column temperature was held at 60°C for the first5 min, then raised to 240°C at 4°C/min and held there for that last 10 min. The scan interval was 0.5 s (±0.1s).

In vivo full thickness excised wound healing activity

Animals

Nine healthy adult male albinos New-Zealand rabbits (2.15and 2.5kg) were used to test wound healing effect of *M. piperita* L. fatty oil. They were acclimatized in individual stainless steel cages to laboratory conditions 20 days before experiments, in ambient temperature of 19±7°C, and 36±0.9 % of humidity, water and standard diet *ad libitum*. Animal procedure was in accordance with the recommendations of the animal care and laboratory uses guidelines (Lazarus *et al.*, 1994).

Total thickness excision wound model

After shaving animals back hair 24h before excision experiment, rabbits were anesthetized by intramuscular injection of 50 mg/kg ketamine hydrochloride.

Three predetermined circular dorsal surfaces of 2 cm diameter each, have been disinfected by surgical alcohol (ethanol; 70%) and excised by dissecting out total thickness of deep skin using forceps and scalp blade, then kept undressed to the open air and applied treatments.

Treatments

The rabbits were randomly divided into three groups (n=3): (a) control untreated group (CONT), receiving no treatment, (b) Madecassol® treated group as a reference healing drug (MAD), receiving daily 0.5 g cream, (c) *M. piperita* L. fatty oil group (MPFO), receiving daily 0.5 ml of hexane extract. Animals have been daily treated with a topic administration that was applied slowly from the central point extending outside the wound area to ensure inclusion of wound edges.

Measurement of wound area

During 22 days of treatment period, wound sizes were measured every four days, throughout the post surgery period (0, 4, 8, 12, 16, 18 and 20 days) using a software that can trace marginal edges on digital photographed wounds, taken from a constant distance, with a professional camera which has a macro zoom. Percentages of wound contraction have been determined according to the following formula (Boulebda, 2009; Srivasta and Durgaparasad, 2008):

$$\text{Percentage of contraction} = \left[\frac{(\text{size do} - \text{size dn})}{\text{size Do}}\right] \times 100$$

d_0: initial day of experiment

d_n: specific day of measurement

D: Day

Statistical analyses

The results were conducted in triplicate. The values of different parameters were expressed as the mean ± standard deviation of three parallel measurements. Statistical analyses were performed using statistical package SPSS18.0 for windows. Significance was determined by analysis of variance [ANOVA].

The statistical level of significance was defined as $P< 0.05$.

Results and discussion

Fatty acids composition

The results of total oil from *M. piperita* L. leaves showed that hexane extract has low rate of oil (1.79%) (Table1).

Table 1. Oil Contents of *M. Piperita* L. leaves.

Species	Oil (%) hexane extract
M. piperita L.	1.79 ± 0.73

The presented value is the mean of triplicate experiments.

The GC-MS results of MPFO hexane extract indicated three dominant fatty acids: oleic (43,16%), palmitic (37,70%) and linoleic (11,13%) acids, and one moderate fatty acid: stearic acids (4,67%) for MPFO hexane extract, other fatty acids were present with lesser amounts (1.01%), some others remained unidentified (Table2).

Table 2. Fatty acid analysis results of hexane extract from *M. piperita* L. Leaves by GC-MS[2].

Rt (min)[a]	Constituents [b]		Hexane extract[c] %
25.78	Stearicacid, methyl ester	(C18:0)	4.67 ± 0.02
18.60	Myristicacid, methyl ester	(C14:0)	1.01± 0.05
19.83	Pentadecanoicacid, methyl ester	(C15:0)	0.06 ± 0.01
21.94	Palmiticacid, methyl ester	(C16:0)	37.70 ± 0.94
23.86	Heptadecanoicacid, dimethyl ester	(C17:0)	0.11 ± 0.05
29.22	Arachidicacid, methyl ester	(C20:0)	0.32 ± 0.08
	∑SFA		43. 87±0.34
22.57	Palmitoleicacid, methyl ester	(C16:1)	0.89± 0.02
26.38	Oleicacid, methyl ester	(C18:1)	43.16 ± 1.01
	∑ MUFA		44.05±0.22
27.26	Linoleicacid, methyl ester	(C18:2)	11.13± 0.45
28.46	Linolenicacid, methyl ester	(C18:3)	0.41 ± 0.06
	∑ PUFA		11.54±0.03
	SFA/MUFA		0.99
	PUFA/SFA		0.26

[2]Indicates that the values presented are the means of triplicate experiments for each extract.

[a] Retention time (as minute), [b] Compounds listed of elution from a HP-5 MS column, [c] Percentage of relative weight.

Benhammou *et al.* (2008) reported good nutritive quality of the oil is due to its contents in unsaturated fatty acids (Oleic + linoleic = 54.29%) and saturated fatty acids (Palmitic + stearic = 42.37%).In addition, some of the presented fatty acids have been described as bioactive molecules that can solve human health disorders, such as: anti-hyperlipidimic, lowering cholesterol level and blood pressure (Kris-Etherton, 1999; Djerrou, 2014).It was obvious that the prominent class of fatty acid was represented by MUFA at 44.05%, followed by SFA at 43.87%, then PUFA at 11.54%.The SFA/UFA ratio was accounting for0.79;it indicates that *M. piperita* L. fatty oil has much more unsaturated then saturated fatty acids, which attributes dietetic and nutritive properties to this oil. At this stage the results showed that the unsaturated fatty acids (mono- and poly-unsaturated) are leading in the composition of the MPFO (Table 2).

Furthermore, the ratio of MUFA/SFA showed a value up to 1.004, this value indicates its good nutritional quality. According to recognized dietary recommendations, a ratio (PUFA/SFA) around 1.5 and more is an indication of the good nutritional value of the oil (Ribarova *et al.*, 2003).

Excised wound healing

This study investigated the efficiency of *M. piperita* L. hexane extract (MPFO) on total thickness excised wounds healing activity. Throughout the testing stage, no death was noted in the experiment animals. Morphological factor was used to evaluate excised wound healing activity compared to Madecassol®as reference drug, and untreated wounds as control.

The skin of different rabbits has been inspected and the percentage of wound contraction has been recorded every four days. Reading the results presented in Table 3 points out the following observations on the progression of healing: - All wounds had comparable surfaces and morphology, as well as the same signs of inflammation and bleeding; - The first 72h, resorption of inflammatory exudate was initiated; - There was suppuration cases in CONT group around the day 8; - Generally, there has been a gradual reduction in the area of the wound over time in the different treatments (Table 3).

Table 3. Evolution of the healing process (contraction %) of *M. Piperita* L. Oil on full thickness excised wounds.

Treatment	Wound healing (%)				
	Day4	Day8	Day12	Day16	Day 20
CONT (-)	21.33 ± 2.33	39.11 ± 3.65	60.65 ± 1.44	80.83 ± 2.01	85.03 ± 1.01
MAD	28.25 ± 2.81*	45.96 ± 1.66*	66.49 ± 1.29*	87.75 ± 2.46*	93.94 ± 0.66**
MPFO	29.58 ± 2.34*	47.86 ± 1.65*	65.5 8± 1.39*	86.43 ± 2.07*	90.33 ±0.98*
Statistical data					
CONT- MAD	Signif-p≤0.05*	Signif-p≤0.05*	Signif-p≤0.05*	Signif-p≤0.05*	Signif-p≤0.01**
CONT-MPFO	Signif-p≤0.05*	Signif-p≤0.05*	Signif-p≤0.05*	Signif-p≤0.05*	Signif-p≤0.05*
MPFO - MAD	Nonsignif-	Nonsignif-	Nonsignif-	Nonsignif-	Signif-p≤0.05*

The results represent the mean ± S.D. (N=4).

The results showed that both of MPFO and Madecassol®promote significantly (p≤0.05*) wound healing comparing to untreated wounds, during all observations time. However, as there is high significant difference (p≤0.01**) in the last healing phase (day 20) between MAD and CONT groups, it can be concluded that there was reducing in both cell proliferation and maturation healing phases in untreated wounds, and delayingcicatrization effect. Consequently, the period of total healing in CONT group was at least 24 days or more, while in MAD and MPFO groups have been almost completed within 20 days. MPOF showed better contraction percentage than MAD during the first and the second measurement days (day 4 and day 8) which correspond to the cell hemostasis and cell inflammation phases of wound healing process, while MAD produced the highest healing percentage during the last six days of treatment, that were in accordance with the proliferation and maturation phases (Stadelmann, 1998; Enoch, 2005; Toporcer *et al.* 2006; Nguyen *et al.* 2009; Rieger, 2014).

Table 2 showed that MPFO oil has a most interesting polyunsaturated fatty acid composition since it has a worthy ratio (PUFA/SFA 1.004).

It is well established that polyunsaturated fatty acids (n3, n6) not only improve cellular structures, but also normalize the synthesis of eicosanoids, pro-inflammatory mediators (Menvielle-Bourg, 2008). They give the oils that contain it revitalizing properties on the skin. It is involved both in mitotic activity and in the maintenance of the integrity of membranes of epidermal keratinocytes and keratinization (Adlouni, 2010).

It is worth noting that apolar compounds present in the oil could act in synergy with the other constituents by promoting the vascularization at the level of the capillaries and thus enabling the tissues to be nourished and consequently a more rapid regeneration of the injured tissues (Khallouki, 2015).

Due to the lack of work done by other authors, the evolution of the scarring process of PMFO extracted from leaves, or any other part , it is reasonable to suggest that the healing activity traditionally reported for *M. piperita* L. oil, and supported by our experiment, is due to a global and synergistic action of its apolar and also polar constituents, favoring the vascularization in the capillaries, and thus allowing to nourish the tissues and consequently a more rapid regeneration of the injured tissues.

Conclusion

These results are a first indication of the presence of healing properties in the case of *M. piperita* L. fatty oil under the experimental conditions and limitations of the present study. Daily application of *M. piperita* L. fatty oil favors significantly ($p \leq 0.05$ *) the healing (contraction %) process of surgical wounds in the rabbit, with throughout the treatment period, and promotes hemostasis and inflammatory phases of cicatrization. Additionally, effective wound-healing properties of *M. piperita* L. may be attributed to the synergistic effect of its fatty acids; especially oleic, palmitic and linoleic acids and other compounds. Thus, the studied species which is used in traditional therapies needs to be screened well. Moreover, the results of our study can also be useful for nutritional research.

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