

## Antimotility and Antidiarrhoeal Activity of *Myrtus communis* L. Leaves Essential Oil in Mice

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### ABSTRACT

**Objective:** To analyze the myrtle (*Myrtus communis* L.) leaves essential oil (MEO) and to investigate its antimotility and antidiarrhoeal effects in mice. **Methods:** The chemical composition of the volatile fraction of myrtle was studied using GC-MS analysis. The antimotility activity was evaluated using normal gastric emptying and intestinal transit. The antidiarrhoeal and antisecretory activities of the essential oil were tested using castor oil methods in mice. **Results:** The results revealed that MEO characterized by its richness of  $\alpha$ -pinene (54.1%) and 1.8-cineole (26.5%). MEO significantly decreased gastric emptying at the highest dose (500 mg/kg) and the intestinal transit at the three used doses (50, 250 and 500 mg/kg). The essential oil demonstrated also dose dependent antidiarrhoeal and antisecretory activities. **Conclusion:** MEO has antidiarrhoeal and antisecretory activity in mice, which may justify the use of this plant in traditional medicine for treatment of diarrhea and other gastrointestinal motility disorders.

**Keywords:** *Myrtus communis* L., Essential oil, Gastric emptying, Intestinal transit, Diarrhoea, Enteropooling.

### INTRODUCTION

Diarrhoea is a gastrointestinal disorder that can be defined as the passage of three or more watery stools per day. The disease is one of the major causes of childhood morbidity and mortality, the majority of whom are infants and malnourished children under the age of 5 years<sup>1</sup>. Worldwide, it affects about 2.2 million people annually<sup>2</sup>. It is mainly due to increased osmotic load in the intestinal lumen, excessive secretion of electrolytes and water into the intestine, exudation of protein and fluid from the mucosa, and altered gut motility resulting in rapid transit. In most cases, multiple processes are altered simultaneously, leading to a net increase in stool volume and weight accompanied by increased water content<sup>3</sup>. Diarrhoea usually lasts for few days and can result in dehydration due to fluid and electrolyte loss from the body. Treatment of diarrhoea relies on rehydration therapy, antibiotics, and gut motility suppressing agents<sup>4</sup>. Although numerous antidiarrhoeics are available, there is still a need for continuing search for more effective antidiarrhoeal agents with minimal side effects. Medicinal plants play an important role in traditional medicines worldwide; this is due to their economic accessibility and ancestral experiences<sup>5</sup>. Because of this, WHO has encouraged the use of traditional medicine in the prevention and the management of diarrhoea<sup>6</sup>.

*Myrtus communis* L. is one of the important aromatic and medicinal species from the myrtaceae family, which includes 100 genera and about 3000 species<sup>7</sup>. It is a common annual evergreen shrub native to Southern

Europe, North Africa and West Asia. It is distributed in South America, North Western Himalaya and Australia and widespread in the Mediterranean countries including Turkey, Greece, Italy, Algeria, Tunisia and Morocco<sup>7</sup>. In Algeria, *M. communis* is widespread especially in the Tell Atlas and in the coastal regions of Algeria<sup>8</sup>. It is commonly known under the name of El-reihan or El-halmouche.

Various parts of this plant have been used in folk medicine for several centuries. The herb is used traditionally for the treatment of disorders such as diarrhoea, dysentery, peptic ulcer, hemorrhoids, inflammation, pulmonary and skin diseases<sup>9</sup>. In Algeria, the leaves of myrtle are used traditionally in the treatment of respiratory disorders, bronchitis, sinusitis, otitis, diarrhoea and hemorrhoids<sup>10</sup>. However, the scientific basis of this medication is not verified. Thus, the aim of this study was to analyze the chemical composition of the East Northern Algerian myrtle essential oil and to evaluate for the first time its antimotility, antidiarrhoeal and antisecretory activities in mice.

### MATERIELS AND METHODS

#### Chemicals

All chemicals were of analytical grade and purchased from sigma (St Louis, MO, USA) or Fluka Chemical Co. (Buchs, Switzerland).

#### Plant material

The fresh leaves of *M. communis* L. were collected from Jijel (North-East of Algeria) in November, 2014. The taxonomic identity of the plant was performed by

Table 1: Essential oil constituents of *M. communis* L. leaves.

Constituents	LRI	Percentage (%)
(E)-3-Hexen-1-ol	854	Tr
Isopentyl acetate	876	Tr
Propyl butyrate	896	0.3
$\alpha$ -Thujene	931	0.4
$\alpha$ -Pinene	939	<b>54.1</b>
Camphene	954	Tr
Thuja-2,4(10)-diene	958	Tr
Sabinene	977	Tr
$\beta$ -Pinene	980	0.6
Myrcene	991	0.2
$\alpha$ -Phellandrene	1005	Tr
$\delta$ -3-Carene	1011	0.4
Propanoic acid 2-methyl-pentyl ester	1012	0.3
<i>p</i> -Cymene	1026	0.7
Limonene	1031	<b>2.0</b>
1,8-Cineole	1034	<b>26.5</b>
(E)- $\beta$ -Ocimene	1051	0.5
$\gamma$ -Terpinene	1062	0.4
Terpinolene	1089	0.5
Linalool	1099	<b>2.4</b>
Isopentyl isovalerate	1104	0.9
Exo-fenchol	1117	Tr
<i>cis-p</i> -Menth-2-en-1-ol	1122	Tr
$\alpha$ -Campholenal	1126	Tr
<i>cis-p</i> -Mentha-2,8-dien-1-ol	1138	Tr
<i>trans</i> -Pinocarveol	1140	0.2
<i>cis</i> -verbenol	1141	0.1
<i>trans</i> -Pinocamphone	1161	Tr
Pinocarpone	1164	Tr
$\delta$ -Terpineol	1170	Tr
4-Terpineol	1178	0.4
<i>p</i> -Cymene-8-ol	1184	Tr
<i>trans-p</i> -Mentha-1(7),8-dien-2-ol	1190	Tr
$\alpha$ -Terpineol	1192	<b>2.3</b>
Myrtenol	1194	Tr
Estragole	1196	0.4
Verbenone	1205	Tr
<i>trans</i> -Carveol	1218	Tr
3-Methyl-3-hexen-1-yl butanoate	1237	Tr
Geraniol	1256	0.6
Linalyl acetate	1258	0.4
Geranial	1272	Tr
Isobornyl acetate	1286	Tr
<i>trans</i> -Pinocarvyl acetate	1296	Tr
$\alpha$ -Terpinyl acetate	1350	0.4
$\alpha$ -Copaene	1376	Tr
Geranyl acetate	1384	<b>2.3</b>
$\beta$ -Elemene	1391	Tr
Methyl eugenol	1401	1.4
$\beta$ -Caryophyllene	1418	0.4
$\alpha$ -Humulene	1455	0.2

$\beta$ -Chamigrene	1475	Tr
$\beta$ -Selinene	1485	Tr
$\alpha$ -Selinene	1494	0.1
Germacrene A	1503	Tr
$\beta$ -Bisabolene	1509	Tr
Lavandulyl isovalerate	1511	Tr
Germacrene B	1556	Tr
Spathulenol	1576	Tr
Caryophyllene oxide	1581	0.2
Geranyl 2-methylbutyrate	1606	Tr
Humulene oxide II	1608	Tr
Caryophylla-4(14),8(15)-dien-5-ol	1636	Tr
Selin-11-en-4- $\alpha$ -ol	1653	0.2
Monoterpene hydrocarbons		<b>58.8</b>
Oxygenated monoterpenes		<b>35.6</b>
Sesquiterpene hydrocarbons		<b>0.7</b>
Oxygenated sesquiterpenes		<b>0.4</b>
phenylpropanoids		<b>1.8</b>
Non-terpene derivatives		<b>1.5</b>
<b>Total identified</b>		<b>99.8</b>
tr: Trace amounts		

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#### Extraction and GC-MS analysis of essential oil

The volatile components of myrtle were determined and identified using GC/MS analyses. The analyses were carried out with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m 0.25 mm; coating thickness 0.25 mm) and a Varian Saturn 2000 ion trap mass detector. The analytical conditions were as follows: injector and transfer line temperatures 220 and 240C, respectively; oven temperature programmed from 60 to 240 C at 3C/min; carrier gas helium at 1 ml/min; injection of 0.2 ml (10% hexane solution); split ratio 1:30. The identification of the constituents was based on the comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to a series of n-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and constituents of known oils and MS literature data <sup>11,12,13</sup>.

#### Animals

Male Swiss white mice (Pasteur Institute, Algiers, Algeria), weighing between 25 and 35 g, were used in this study. They were initially housed in groups in cages and had free access to water and food ad libitum for a week. In all studies, the animals were fasted for 18-20 h with free access to water until 60 min before the start of the experiment. During the fasting period, the animals were

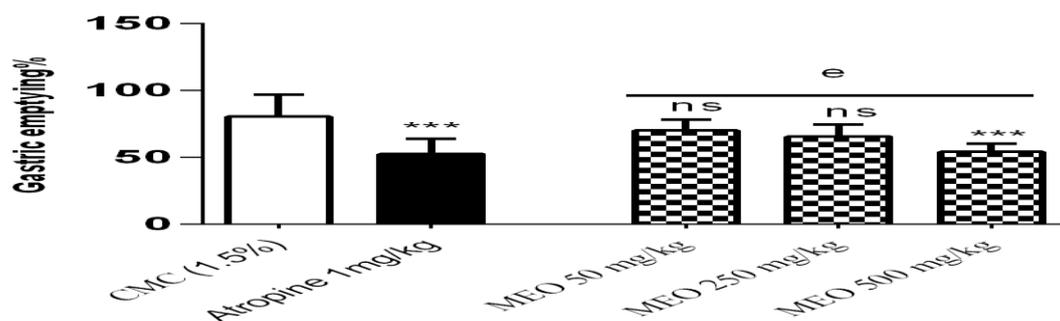


Figure 1: Effect of *M. communis* L. leave essential oil on gastric emptying % in mice. MEO: *Myrtus communis* essential oil. The values of the bars chart are expressed as means  $\pm$  SEM (n=6). (\*\*\* $P \leq 0.001$ ; ns: no significant difference) vs vehicle treated group (CMC 1.5% *p.o.*); <sup>e</sup> $P \geq 0.05$  vs Atropine (1mg/kg *i.p.*).

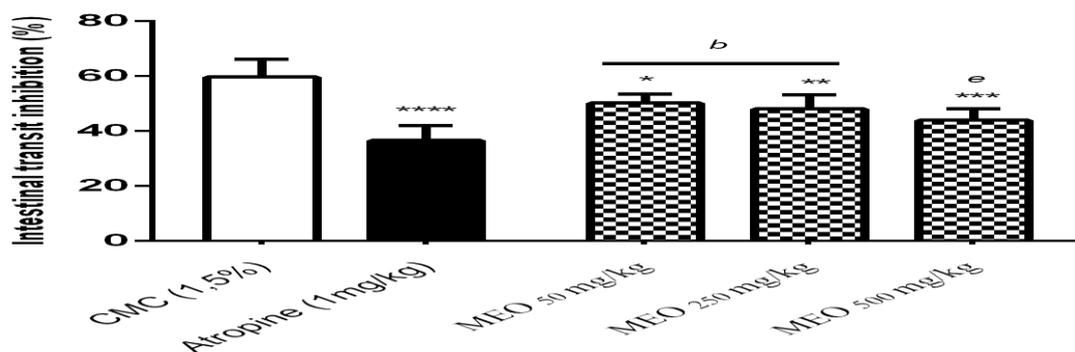


Figure 2: Effect of MEO on intestinal transit. MEO: *Myrtus communis* essential oil. The values of the bars chart are expressed as means  $\pm$  SEM (n=6). (\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ , \*\*\*\* $P \leq 0.0001$ ) vs negative control group (CMC 1.5% *p.o.*), <sup>b</sup> $P \leq 0.001$ , <sup>e</sup> $P \geq 0.05$  vs positive control (Atropine, 1 mg/kg *i.p.*).

placed individually in cages with wide-mesh wire bottoms to prevent coprophagy.

#### Acute oral toxicity

Acute oral toxicity of MEO was performed using few animals according to the limit test recommendation of the Organization of Economic Co-operation and Development (OECD), guideline 423<sup>14</sup>. The oil was administered to the first animal at a single oral dose (2000 mg/kg). The animals were not fed for three hours following administration. Gross behavioral and toxic effects (restlessness, agitation, dullness, writhing etc.) were observed at short intervals for 24 h. As this animal did not die, two more animals were treated in the same way. After 14 days mice were sacrificed and all the organs were removed for gross pathological examination.

#### Gastric emptying and small intestine transit measurements

A test meal made up of 0.1% phenol red (a non-absorbable and easily detectable marker) dissolved in 1.5% of carboxymethyl cellulose (CMC) was used in this study. Gastric emptying was measured according to the method described by Amira *et al.*<sup>15</sup> with slight modifications. After 18-20 h of fasting, mice (n=6) were orally pretreated with MEO (50, 250 and 500 mg/kg) and atropine (1 mg/kg *i.p.*) as positive control. After one hour of the treatment, each animal received orally the test meal (0.2 ml/mouse) and was sacrificed 20 min later. Under laparotomy, the stomach and the small intestine were excised after ligation of the pylorus and the cardia. The stomach was

homogenized with its content in 25 ml 0.1 N NaOH. The homogenate was allowed to settle for 1 h at room temperature and 8 ml of the supernatant were added to 1 ml of 33% trichloroacetic acid to precipitate proteins. After centrifugation (1600 g for 30 min), 2 ml of 2N NaOH were added to the supernatant. The mixture was homogenized and its absorbance was read at 560 nm. On the day of each experiment, 4 animals were sacrificed just after the administration of the test meal and were considered as standards (0% of emptying). The gastric emptying (GE) rate in the 20-min period was calculated according to the following formula:

$$GE (\%) = \frac{Abs_{standard} - Abs_{test}}{Abs_{standard}} * 100.$$

Immediately after the excision of the stomach for gastric emptying test, the whole small intestine of the same animal was removed for the evaluation of the intestinal transit.

The intestine was grossly freed from its mesenteric attachments and its length was measured using a ruler. The intestine was opened at the level of the front of the test meal, which was then exactly localized by a drop of 0.1 N NaOH. The rate of intestinal transit was expressed as the ratio between the distance travelled by the test meal and the total length of the small intestine.

#### Evaluation of the antidiarrhoeal activity

The method described by Awe *et al.*<sup>16</sup> with small modifications was followed for this investigation. Mice were randomly divided into groups of 6 mice each and were treated orally as outlined below:

Table 2: Effect of *M. communis* L. essential oil on castor oil-induced diarrhoea in mice.

Treatment Group	Dose (mg/kg) or (ml/kg)	Onset of diarrhoea (min)	Total number of stool	Number of wet stool	Percentage of wet stool I%	Protection %
Vehicle (CMC 1.5%)	10	59.29 ± 5.54	10.94 ± 1.08	9.17 ± 0.89	85.36±3.08	00
MEO	50	120.87±3.71**** a <sup>λ</sup>	7.5±0.7*** b	4.87±0.44**** d	67.07±5.15	46.88±4.8 <sup>b</sup>
	250	130±3.27**** a	7.71±0.68** b	5±0.57*** d	64.81±4.22	50.96±3.72 <sup>c</sup>
	500	164.28±3.52**** a	6±0.75****	2.85±0.34****	50.68±6.2*	68.86±3.7
Loperamide	5	214.38±8.73****	2.63±0.7****	1.42±0.45****	41.77±09.78****	81.59±5.14****

Group 1: CMC (1.5%), negative control.

Group 2: MEO<sub>50</sub> mg/kg,

Group 3: MEO<sub>250</sub> mg/kg,

Group 4: MEO<sub>500</sub> mg/kg,

Group 5: Loperamide hydrochloride (5 mg/kg), positive control.

One hour after the oral respective treatments (0.125 ml/mouse), acute diarrhoea was induced by oral administration of castor oil (0.3 ml/mouse).

Following the delivery of castor oil, the animals were placed in separate cages over clean white paper that was replaced every hour and inspected for 4 hours for the presence of the typical diarrhoea. The time elapsed between the administration of the cathartic agent, the excretion of the first diarrhoeal faeces, the total number of stools and the total number of wet faeces excreted by the animals in 4 hours were recorded. The percentage of defecation inhibition score was calculated as follows:

$$\% \text{ inhibition of diarrhoea} = \frac{[\text{Mean number of wet defecation (control-test)}]}{\text{Mean wet defecation of control}} \times 100$$

#### Intestinal fluid accumulation (Enteropooling test)

The effect of MEO on castor oil-induced fluid secretion in intestine was studied according to the method described by Awe *et al.*<sup>16</sup>. Animals were randomly divided into 14 groups of six mice per group. Each animal was placed in a single cage the day before the experiment and were fasted for 18-20 h with free access to water until 1 h before the start of the experiment. Group 1 mice were treated with CMC (1.5%) as negative control. Group 2 received 5 mg/kg loperamide (positive control). Groups 3, 4 and 5 were treated with MEO at doses 50, 250 and 500 mg/kg respectively. One hour later, all mice received castor oil (0.3 ml/mouse). The animals were sacrificed 30 min afterwards and the whole length of the small intestine was ligated from the pylorus to the caecum. The weight of the full intestine was recorded and its content was expelled into a graduated measuring cylinder to determine its volume. The weight of the empty intestine was taken, and the difference between the full and empty intestine was thus calculated.

#### Statistical data analysis

Results were expressed as means ± standard error of mean (SEM). Comparison between treatment groups were performed by one way analysis of variance (ANOVA) followed by Tukey's test. The *P* Values of *P* < 0.05 were considered significantly different using Graph Pad Prism Version 6.0 (GraphPad Software, Inc, La Jolla, CA, USA).

## RESULTS

### Essential oil chemical composition

The essential oil volatile compounds, the linear retention index and their percentage are listed in table 1. Beside traces, 30 constituents were identified in MEO and represented 99.8% of the total essential oil of the plant. The essential oil was characterized by a high percentage of monoterpene hydrocarbons (58.8 %) followed by oxygenated monoterpenes (35.6%), phenylpropanoids (1.8%) and non-terpene derivatives (1.5%). Sesquiterpene hydrocarbons and oxygenated sesquiterpene were found in smaller proportions (0.7 and 0.4%) (Table 1). The major components identified were  $\alpha$ -pinene (54.1%), 1,8-cineole (26.5%), linalool (2.4%),  $\alpha$ -terpineol and geranyl acetate (2.3%), limonene (2%) and methyl eugenol (1.4%).

### Acute oral toxicity

In acute toxicity test, no mortality was observed at the test dose (2000 mg/kg) within the 14 days of observation and none of the animals showed any behavioral, neurological or physical changes.

### Gastric emptying

As shown in figure 1, at the lowest doses (50 and 250 mg/kg), MEO did not show any significant decrease in gastric emptying compared to the vehicle treated animals. However, the highest dose (500 mg/kg) showed a very significant (*P* ≤ 0.001) effect. The tested doses did not show any significant (*P* ≤ 0.05) difference against the positive control.

### Intestinal transit

The effect of MEO on intestinal transit is shown in figure 2. Compared with the vehicle treated animals, the MEO dose dependently and significantly (*\*P* < 0.05--*\*\*\*P* < 0.001) decreased the propulsive movement and transit of red phenol through the small intestine (Figure 2). The intestinal transit decreased from 59.67±2.3 as shown in control group to 43.97±.85 at the highest dose (500 mg/kg).

### Castor oil induced diarrhoea

Within the observation period of 4 hours, after castor oil administration, all mice in control group (CMC 1.5%) produced copious diarrhoea. Pretreatment of mice with MEO (50, 250 and 500 mg/kg) caused a dose dependent and significant (*P* ≤ 0.0001) delay of onset of diarrhoea. At the highest dose (500 mg/kg) the onset time increased from 59.29±5.54 in the control group to 164.28±3.52 %, a value less than the positive control drug loperamide (214.38±8.7). In addition, the total number of stool and the

Table 3: Effect of MEO on castor oil-induced intestinal enteropooling and fluid accumulation in mice.

Treatment group	Dose (mg/kg or (ml/kg)	Volume of intestinal fluid (ml)	Mass of intestinal fluid (g)	Inhibition of intestinal fluid volume (ml) %	Inhibition of intestinal mass (g) %
Vehicle (CMC 1.5%)	10	0.72±0.04 <sup>a</sup>	0.86±0.1 <sup>a</sup>	00	00
EO	50	0.48±0.03 <sup>***e</sup>	0.53±0.028 <sup>****c</sup>	32.87±4.26 <sup>a</sup>	38.04±3.3 <sup>b</sup>
	250	0.46±0.04 <sup>***e</sup>	0.49±0.036 <sup>****e</sup>	36.11±5.55 <sup>b</sup>	42.71±4.26 <sup>c</sup>
	500	0.37±0.034 <sup>****e</sup>	0.37±0.02 <sup>****e</sup>	47.91±4.74 <sup>e</sup>	56.58±2.52 <sup>e</sup>
Loperamide	5	0.41±0.03 <sup>****</sup>	0.3±0.030 <sup>****</sup>	40.97±4.29	61.98±2.76

total number of wet stool were significantly and dose dependently reduced. Furthermore, the computed inhibition of defecation increased in a dose dependent manner (with the most remarkable percentage of inhibition at the highest dose (68.86±3.7). At the highest dose, no statistical difference was observed versus the positive control, implying that MEO produced close effect to the positive control group (Table 2).

Animals were pre-treated with various doses of MEO (50, 250 and 500 mg/kg, *p.o.*), reference drug (loperamide, 5 mg/kg, *p.o.*) or vehicle (CMC 1.5%). One hour later, animals received castor oil (0.3 ml/mouse *p.o.*). MEO: *Myrtus communis* essential oil; CMC: carboxy methyl cellulose. \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ; \*\*\*\* $p \leq 0.0001$ ; vs negative control group (CMC). <sup>d</sup> $P \leq 0.05$ ; <sup>c</sup> $P \leq 0.01$ ; <sup>b</sup> $P \leq 0.001$ , <sup>a</sup> $P \leq 0.0001$  vs positive control group. <sup>λ</sup> $P \leq 0.01$  vs MEO<sub>500</sub> mg/kg. (One way ANOVA followed by Tukey's multiple comparison tests).

#### Intestinal fluid accumulation

The effects of MEO on castor oil-induced fluid accumulation are presented in Table 3. Pre-treatment of the test groups of mice with MEO (50, 250 and 500 mg/kg, *p.o.*) dose dependently and significantly inhibited the volume and the mass of intestinal content compared to the vehicle treated group. At the highest dose (500 mg/kg), the volume and the mass of intestinal content significantly ( $P \leq 0.0001$ ) decreased from 0.72±0.04 and 0.86±0.1, respectively (as observed in control group) to 0.37±0.034 and 0.37±0.021, respectively (Table 3). Moreover, the inhibition % of mass intestinal content at the highest dose (500 mg/kg) showed no statistical difference ( $P \geq 0.05$ ) when compared to loperamide group, indicating that MEO produced similar effect as the positive control group (Table 3).

Animals were pre-treated with various doses of MEO (50, 250 and 500 mg/kg, *p.o.*), reference drug (loperamide, 5 mg/kg, *p.o.*) or vehicle (CMC 1.5%). One hour later, animals received castor oil (0.3 ml/mouse). MEO: *Myrtus communis* essential oil; CMC: Carboxymethyl cellulose. \*\*\* $P \leq 0.001$ , \*\*\*\* $P \leq 0.0001$  vs vehicle control group (CMC). <sup>e</sup> $P \geq 0.05$ , <sup>c</sup> $p \leq 0.01$ , <sup>b</sup> $p \leq 0.001$ , <sup>a</sup> $P \leq 0.0001$  vs positive control group (Loperamide). (One way ANOVA followed by Tukey's multiple comparison tests).

## DISCUSSION

The MEO yield in the examined myrtle dried leaves was (0.36%). Compared with oil yields of plants originating from the surrounding Mediterranean areas, like North West of Algeria (0.32%)<sup>17</sup>. The myrtle of the present study

could be characterized as being quite rich in essential oil. Strong variability of the MEO composition could be observed depending on the geographic region of production, the season of harvest and the length of distillation<sup>18,7</sup>. Gardeli *et al.*<sup>19</sup> studied the effect of the seasonal variation on the essential oil content of *M. communis* from a Greek island. The authors observed that, the monoterpenes fraction was the main chemical group in all periods. As reported in the results section, the major components identified in the EO in this study were  $\alpha$ -pinene (54.1%), 1,8-cineole (26.5%), linalool (2.4%),  $\alpha$ -terpineol and geranyl acetate (2.3%), limonene (2%) and methyl eugenol (1.4%). The myrtle EO of the present study is characterized by its richness in  $\alpha$ -pinene (54.1 %) and 1,8-cineole (26.5%), and absence of myrtenyl acetate. Close amounts of  $\alpha$ -pinene were reported for myrtle EO from Northern West of Algeria (50.8%)<sup>20</sup>, Corsica (53.5-56.7)<sup>17</sup> and Tunisia (51.1-52.9)<sup>21,22</sup>. As shown in results, MEO did not show any mortality nor toxic effects at the single tested doses (2 g/kg). So, we conclude, that MEO is safe at 2 g/kg in mice.

Impaired gastrointestinal motility has a well-recognized contribution to some of the pathophysiological conditions of the gastrointestinal tract. The present study reveals that MEO dose dependently decreased gastric emptying, an effect that was significantly different at the highest dose only. The inhibitory effect was very close to that of atropine as a positive control. The MEO also dose dependently and significantly decreased the intestinal transit when compared to control group. However, the percentage of inhibition was lower than that of atropine. The control of gastric emptying is complex and involves the coordination of motor activity of the proximal stomach, the antrum, the pylorus and the duodenum, as well as the passive forces created by intragastric volume and gravity<sup>23</sup>. The tonus of the pyloric sphincter plays a crucial role in the rate of gastric emptying<sup>24</sup>.

Intestinal transit is controlled by neural and myogenic mechanisms that are governed by several neurotransmitters and mediators. The principal excitatory neurotransmitter in the enteric nervous system is acetylcholine, while nitric oxide is the main inhibitory mediator<sup>25</sup>. The delaying effect of the essential oil on gastric emptying can result from the reinforcement of the contraction of the pyloric sphincter, whereas its effect on intestinal transit may be due to its inhibitory effect on the intestinal muscle contraction and /or consolidation of the inhibitory component of the musculature. These effects may be attributed to the peculiar actions of its single

chemical constituents and / or to their synergism. Indeed, Alpha-pinene and 1, 8 -cineole are the two main phytochemical constituents of the MEO. It is believed that these two components are most likely involved in the observed effects of the essential oil. Camara *et al.*<sup>26</sup> found that the essential oil of *Plectranthus barbatus* which contains alpha-pinene as a major constituent as well as pure alpha-pinene in the ratio and amount in the oil had intestinal relaxant and antispasmodic effects in guinea-pig. Similarly, Jalilzadeh-Amin and Mahan<sup>27</sup> found that 1,8-Cineole a major terpene in myrtle essential oil showed a significant decrease in intestinal transit at its highest dose (120 mg/kg). Moreover, the same authors<sup>28</sup> showed that *Mentha longifolia* L. which contains 1,8-Cineole as its major constituent dose-dependently decreased gastrointestinal transit in rats.

Experimental diarrhoea in this study was induced using castor oil. Castor oil provokes diarrhoea through the inflammatory effect of ricinoleic acid, a hydroxylated fatty acid released by lipases in the intestine. Once in the lumen, it induces the secretion of fluid and electrolytes, decreased the absorption and alters the intestinal motility leading to a watery content that is quickly evacuated<sup>29,30</sup>. Ricinoleic acid seems to cause its effect by promoting prostaglandin<sup>31</sup>, platelet-activating factor and nitric oxide formation<sup>32</sup>.

The MEO dose-dependently delayed the onset of diarrhoea, reduced the number of wet stools and thus inhibiting diarrhoea. In the enteropooling test, the essential oil significantly decreases the volume and weight of the intestinal content. Therefore, the antidiarrhoeal effect of MEO seems to occur via the inhibition of gastrointestinal motility, inhibition of intestinal secretion and/or activation of reabsorption. Pure 1, 8 -cineole<sup>27,33</sup>, as well as *Mentha longifolia* L. essential oil containing 1, 8 -cineole as a major constituent<sup>28</sup> delayed intestinal transit and had antidiarrheal activity. Moreover, 1, 8 -cineole and alpha-pinene present as main components in different essential oils had spasmolytic actions in the intestine<sup>34,35,36,37</sup>.

Another mechanism by which monoterpenes can induce the antidiarrhoeal effect is through their anti-inflammatory activity by inhibiting the release of prostaglandins and other autacoids from the intestinal wall. In fact, several studies have demonstrated the anti-inflammatory effects of monoterpenes and among these, the two major constituents of MEO whether as pure compounds or as the main components in other essential oils<sup>38,39,40,28,41</sup>. The precise mechanism(s) of action of MEO and its active components deserve further investigations.

## CONCLUSION

In conclusion, the results of the present study show that the essential oil of myrtle possesses antimotility, antidiarrheal and antisecretory effects, thus supporting the traditional use of this plant in the management of diarrhoea. However, the chemicals involved in this activity still need further characterization.

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