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Evaluation of the antidiarrhoel and antimotility activities of methanolic extract and fractions of *Myrtus communis* L. leaves in mice

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ABSTRACT

Myrtle or Myrtus communis L. *(M. communis L.) belongs to the Myrtaceae family. It is a widespread shrub in the Mediterranean area. The objective of this study is to determine the polyphenol content of the methanol (ME), chloroform (CHE) and ethyl acetate extract (EAE) of myrtle leaves and to investigate their gastrointestinal anti-motility, antidiarrhoeal and intestinal antisecretory effects in mice. The plant powder was extracted with different solvents of increasing polarity to obtain the above different fractions. The extracts were stored at 4 C° until use. The content of total phenols, flavonoids and tannins of the different extracts were determined using spectrophotometric methods. The antimotility activity was evaluated using gastric emptying and intestinal transit phenol red method. Atropine (1mg/kg i.p.) was used as a positive control. The antidiarrhoeal and antisecretory effects were determined using castor oil. Three different doses (50, 250 and 500 mg/kg p.o.) were used with all the tested extracts. Loperamide (5 mg/kg p.o.) was used as a positive control. Finally, the acute toxicity of the plant extracts was performed according to the Organization of Economic Cooperation and Development (OECD) method at 2 single doses (2 and 5 g/kg p.o.). The results revealed that M. communis L. leaves extracts are rich in polyphenols. The different extracts dose dependently decreased the gastric emptying and the intestinal transit. All extracts also showed significant and dose dependent inhibition of diarrhoea and intestinal secretion. M. communis L. extracts showed antimotility, antidiarrhoeal and antisecretory activities in mice, which may provide scientific support of the folkloric medication with this plant against diarrhoea.*

Keywords: *Myrtus communis* L., gastric emptying, intestinal transit, diarrhoea, enteropooling.

INTRODUCTION

Diarrhoea is characterized by a discharge of semisolid or watery fecal matter from the bowel three or more times per day [1]. It is one of the leading causes of morbidity and mortality in developing countries and is responsible for the death of millions of people each year [2]. The use of medicinal plants is widespread among the population of these countries. Some of these plants with medicinal values were found to be a reliable means by herbalists of treating diseases such as diarrhoea and other gastrointestinal disorders [3]. In order to overcome the threatening effects of diarrhoea, the World Health Organization (WHO) [4] encouraged the utilization of traditional herbal medicines as a part of the health managing system mainly because of their accessibility and perceived efficacy.

M. communis L. belongs to the Myrtaceae family and is one of the important aromatic and medicinal species in this family, it is very common in the typical Mediterranean flora. The plant grows abundantly around the Mediterranean sea and Western Asia [5]. In Algeria, *M. communis* L. is widespread especially in the Tell Atlas and in the coastal regions [6]. It is commonly known under the name of El-Reihan or Hlamouche [7].

Since ancient times, different parts of the plant were used for medicinal, food and spices purposes. In Algeria, the leaves of *M. communis* L. are used traditionally in the treatment of respiratory disorders, bronchitis, sinusitis, otitis, diarrhoea and hemorrhoids [7]. A wide range of biologically active compounds are present in this plant [8]. The main goal of the present study was to determine the polyphenolic content of ME, CHE and EAE extracts of *M. communis* L. leaves and to evaluate for the first time their antidiarrhoeal effects.

MATERIALS AND METHODS

2.1. Chemicals

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

2.2. 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 Professor Gonzalez-Tejero and Casares-Porcel Department of Botany, University of Granada, Spain and a voucher number ML 11/14 was deposited at the Laboratory of Phytotherapy Applied to Chronic Diseases, University Setif 1. The collected plant was dried under shade.

2.3. Extraction and fractionation

The extraction procedure was conducted as described by Markham [9] with slight modification. The dried powder of *M. communis* L. leaves was extracted with methanol (85%) at room temperature for 3 days. The resulting suspension was then filtered and concentrated by evaporation at 50 C° and fractioned by successive washing with different solvents of increasing polarity to obtain the following fractions: methanol extract (ME), chloroform extract (CHE) and ethyl acetate extract (EAE).The extracts were stored at 4 °C until use.

2.4. Animals

Male Swiss albino mice (Pasteur Institute, Algiers, Algeria), weighing between 25 and 30 g, were used in this study. Permission for conducting animal in vivo experiments was obtained from the local institutional committee on experimental animals care, and the experiments were carried out according to the ethical principles on experimental animals. 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 1 hour before the start of the experiment. During the fasting period, the animals were placed individually in cages with wide-mesh wire bottoms to prevent coprophagy.

2.5. Determination of total phenolic content

Total phenolic content was assessed by Folin Ciocalteu reagent as described by Li *et al* [10]. A volume of 100 µl of each extract was mixed with 500 µl of Folin Ciocalteu reagent (diluted 10 times). After 4 min, 400 µl of 7.5% of Na_2CO_3 solution was added. The final mixture was shaken and incubated in dark at room temperature for 1 hour and the absorbance of the reaction mixture was measured at 760 nm. The results were expressed as mg of gallic acid equivalent (GAE) per gram of dried plant extract weight (mg GA/g DW) using a calibration curve of gallic acid.

2.6. Determination of total flavonoid content

Total flavonoid content was determined using aluminum chloride assay [11]. Briefly, 1 ml of each tested extract or standard (quercetin) were mixed with 1 ml of $AlCl_3$ (2%). After 10 min of incubation, the absorbance was measured against a prepared blank at 430 nm. The results were expressed as quercetin equivalent per gram of dry plant extract weight (mg QE/g DW) using a calibration curve of quercetin.

2.7. Determination of total tannins

This was achieved by testing the capacity of the different extracts to precipitate haemoglobin from fresh bovine blood according to the method of Bate-smith [12]. Briefly, a volume of each plant extract was mixed with an equal volume of hemolysed bovine blood (absorbance = 1.6). After 20 minutes of incubation at room temperature, the mixture was centrifuged at 4000 rpm, and the absorbance of the supernatant was measured at 765 nm. The results were expressed as mg equivalent tannic acid per gram of extract dry weight (mg TAE/g DW) using a calibration curve of tannic acid.

2.8. Acute oral toxicity

Acute oral toxicity of ME, CHE and EAE was performed using few animals according to the limit test recommendation of the Organization of Economic Cooperation and Development (OECD), guideline 423 [13]. Each extract was administered to the first animal at a single oral dose (2 g/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. The same procedure was repeated in another set of animals using a higher dose (5 g/kg).

2.9. 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% carboxymethyl cellulose (CMC), was used in this study. Gastric emptying was measured according to the method described by Amira *et al*. [14] with slight modifications. After 18-20 h of fasting, mice (n=6) were orally pretreated with ME, CHE and EAE (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 0.2 ml of the test meal and was sacrificed 20 min later. Under a laparatomy, the stomach and the small intestine were excised after ligation of the pylorus and the cardia. The stomach was homogenized with its contents 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 (abs) 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:

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

Immediately after the excision of the stomach of the same rat that was used for gastric emptying, the whole small intestine 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. It was then opened at the level of the front of the test meal, which was 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.

2.10. Evaluation of the antidirrhoeal activity

The method described by Awe *et al* [15]with small modifications was followed for this investigation. Mice randomly divided into groups of 6 mice each were treated orally as outlined below:
Group 1: CMC (1.5%), negative control.
Group 2: ME_{50} mg/kg, Group 3: ME_{250} mg/kg, Group 4: ME_{500} mg/kg.
Group 5: CHE_{50} mg/kg, Group 6: CHE_{250} mg/kg, Group 7: CHE_{500} mg/kg.
Group 8: EAE_{50} mg/kg, Group 9: EAE_{250} mg/kg, Group 10: EAE_{500} mg/kg.
Group11: Loperamide hydrochloride (5 mg/kg), positive control.

One hour after the oral respective treatments (5 ml/kg), acute diarrhoea was induced by oral administration of castor oil (10 ml/kg)

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 signs of diarrhoea. The time elapsed between the administration of the cathartic agent (castor oil), and the excretion of the first diarrhoea faeces 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:

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

2.11. Intestinal fluid accumulation (Enteropooling test)

The effect of *M. communis* L. extracts on castor oil-induced fluid secretion in intestine was studied according to the method described by Awe *et al*. [15]. Animals were randomly divided into 14 groups of six mice per group. Each mouse in each group was subsequently separately placed in a cage. 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 ME at doses 50; 250 and 500 mg/kg. Groups 6, 7 and 8 received 50, 250 and 500 mg/kg of CHE extract. Groups 9, 10 and 11 received 50, 250 and 500 mg/kg of EAE extract. Drugs were suspended in CMC and administered orally (5 ml/kg). One hour later, all mice received castor oil (10 ml/kg). The animals were sacrificed 30 min afterwards and the whole length of the small intestine was legated from the pylorus to the caecum. The weight of the full intestine was determined. The contents of the intestine were then expelled into a graduated measuring cylinder and its volume was determined. The weight of the empty intestine was taken, and the difference between the full and empty intestine was calculated.

2.12. Statistical data analysis

Results were expressed as the 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

3.1. Total phenolics, flavonoids and tannins contents

The total phenolics, flavonoids and tannins contents among the different extracts of *M. communis* L. are presented in Table 1. The total phenolic content in terms of mg GAE/g of dry weight of extract decreased in the following order: ME > EAE > CHE, whereas the highest total flavonoids were found in EAE (38.4±0.9 mg QE/g DW) and tannins contents in ME (83.35±0.36 mg TAE/g DW).

Table 1: Total phenolics, flavonoids and tannins contents of methanolic extract and fractions of M. communis L. leaves

Extracts	Total phenolics (mg GAE/g Dw)	Total flavonoids (mg QE/g DW)	Total tannins (mg TAE/g DW)
ME	149.25 ± 3.11	26.38 ± 0.13	83.35 ± 0.36
CHE	81.0 ± 1.53	28.05 ± 0.15	52.3 ± 0.25
EAE	101.88 ± 1.73	38.4 ± 0.9	49.7 ± 0.98

ME: Methanol extract, CHE: Chloroform extract, EAE: Ethyl acetate extract, DW: Dry weight. Results are expressed as means ±SEM (n=3).

3.2. Acute oral toxicity

In acute toxicity test, no mortality was observed at the test doses for the following 14 days of observation and none of the animals showed any changes in their behavioral, neurological or physical activities at the doses of 2 and 5 g/kg.

3.3. Gastric emptying

All extracts exerted dose dependent reduction in the emptied quantity of the test meal compared to the vehicle (negative control). This effect was significant ($P \leq 0.01$ and $P \leq 0.001$) for the highest doses (250 and 500 mg/kg) and the effect of these doses was not significantly different from that of atropine.

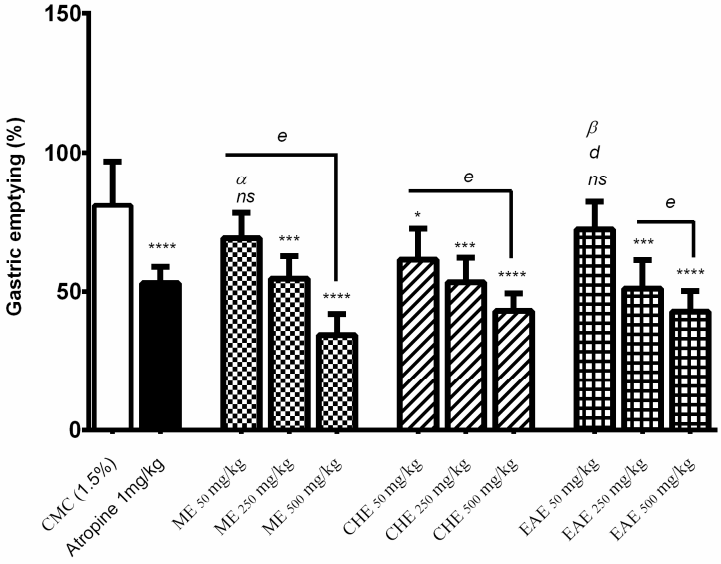

Figure 1: Effect of M. communis L. leave extracts on gastric emptying in mice. ME: Methanol extract, CHE: Chloroform extract, EAE: Ethyl acetate extract. The values of the bars chart are expressed as means ± SEM (n=6). (**P ≤ 0.0001; ***P ≤ 0.001; **P ≤ 0.01) vs vehicle (CMC 1.5% p.o.). (^{a}P ≤ 0.0001, ^{b}P ≤ 0.001, ^{e}P ≥ 0.05) vs positive control group (Atropine sulphate, 1 mg/kg i.p.). $^{\alpha}$P ≤ 0.0001 vs ME $_{500}$ mg/kg p.o.; $^{\beta}$P ≤ 0.05 vs CHE$_{500}$ mg/kg p.o.; $^{\lambda}$ P ≤ 0.01 vs EA$_{250}$ $^{\delta}$P ≤ 0.0001 vs EA$_{500}$ mg/kg p.o.**

3.4. Intestinal transit

The effects of *M. communis* L. extracts on intestinal transit are shown in figure 2. Compared with the vehicle, all tested extracts dose dependently lowered the transit of phenol red through the small intestine. This decrease was significant at the highest dose for all extracts. At this dose, all extracts showed no significant difference in intestinal transit compared to the positive control.

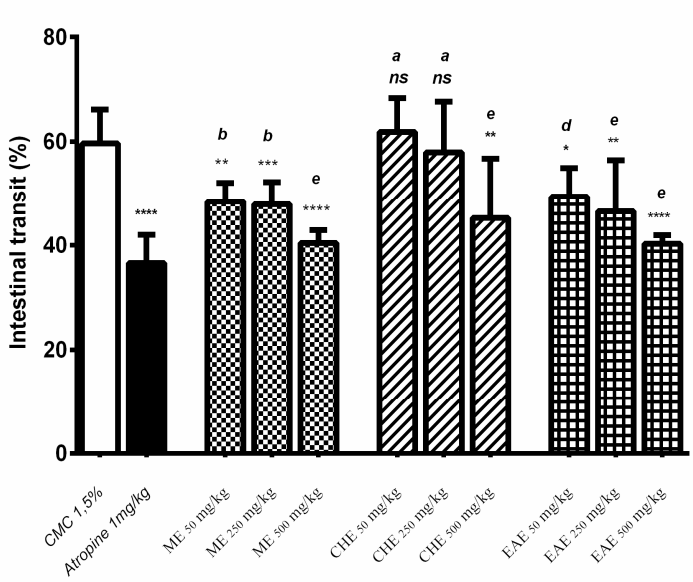

Figure 2: Effect of M. communis L. leave extracts on intestinal transit in mice. ME: Methanol extract, CHE: Chloroform extract, EAE: Ethyl acetate extract. The values of the bars chart are expressed as means ± SEM (n=6). (**P ≤ 0.0001; ***P ≤ 0.001; **P ≤ 0.01, *P ≤ 0.05) vs vehicle (CMC 1.5% p.o.). (^{a}P ≤ 0.0001; ^{d}P ≤ 0.05, ^{e}P ≥ 0.05) vs positive control group (Atropine sulphate, 1 mg/kg i.p.). $^{\alpha}$P ≤ 0.01 vs CHE $_{500}$ mg/kg p.o.)**

3.5. Castor oil induced diarrhoea

Within the observation period of 4 hours, after castor oil administration, all the mice in control group produced copious diarrhoea. Pretreatment of mice with the extracts caused dose dependent and significant delay of onset of diarrhoea. This effect decreased in the following order: ME > CHE > EAE. The most powerful delay was observed for ME extract at 500 mg/kg. At this dose, the onset of diarrhoea increased from 59.29 ± 5.54 min (vehicle group) to 215.71 ± 8.95 min, a value not significantly different from the positive control (214.38 ± 8.7 min). In addition, the total number of stool and the total number of wet stool were reduced significantly and dose dependently. The most significant decrease was noted with the highest dose (Table 3). Furthermore, the inhibition of defecation increased in a dose dependent manner with the most remarkable percentage of inhibition at the highest dose for all extracts: ME (73.53 ± 5.75), CHE (72.53 ± 3.24) and EAE (71.98 ± 4.67). These values were not significantly different from the value of the positive control (81.59 ± 5.14).

Table 3: Effect of methanolic extract and fractions of M. communis L. leaves 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 (%)	Protection (%)
Vehicule (CMC 1.5%)	10	59.29 ± 5.54	10.94 ± 1.08	9.17 ± 0.89	85.36±3.08	00
ME	50	$98.25 \pm 9.03^{**a\,\alpha}$	$7.25\pm0.86^{***b}$	$5.5\pm0.86^{**b}$	73.15 ± 7.86^{d}	$33.06\pm7.3^{a\,\pi}$
	250	$119.28 \pm 8.13^{****a}$	$7\pm1.1^{***c}$	$4.14\pm0.45^{****}$	65.08±9.04	54.85 ± 5^{d}
	500	$215.71 \pm 8.95^{****}$	$5.42\pm0.68^{****}$	$2.42\pm0.52^{****}$	$46.33\pm8.7^{**}$	73.53±5.75
CHE	50	$100.5\pm8.36^{****a\,\beta\,\delta}$	$8.12\pm0.95^{**a}$	$5.87\pm0.58^{**b\,\mu}$	74.19 ± 4.91^{d}	$35.98\pm6.32^{a\,\#\,£}$
	250	$143.28\pm8.93^{****a}$	$4.85\pm0.45^{****}$	$3\pm0.43^{****}$	62.11±6.65	67.31±4.75
	500	$195\pm3.61^{****}$	$4.71\pm0.56^{****}$	$2.42\pm0.29^{****}$	$51.84\pm4.31^{*}$	72.53±3.24
EAE	50	$110 \pm3.13^{****a\alpha}$	$8.12\pm0.51^{**a}$	$5.75\pm0.52^{**b}$	70.56±5.73	37.34 ± 5.73^{a}
	250	$137.14\pm2.64^{***a}$	$5.42\pm1.39^{****}$	$3.57\pm0.89^{****}$	64.46±4.96	$67.31\pm8.89^{\$\,\&}$
	500	$179.28\pm6.21^{****d}$	$4.83\pm0.57^{****}$	$2.57\pm0.42^{****}$	53.27±6.87	71.98±4.67
Loperamide	5	$214.38\pm8.73^{****}$	$2.63\pm0.7^{****}$	$1.42\pm0.45^{****}$	$41.77\pm09.78^{****}$	81.59±5.14

*Animals were pre-treated with various doses of ME, CHE and EAE (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 (10 ml/kg p.o.). *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; **** P ≤ 0.0001; vs negative control group. ^{d}P ≤ 0.05; ^{c} P ≤ 0.01; ^{b}P ≤ 0.001; ^{a} P ≤ 0.0001 vs positive control group ; $^{\alpha}$ P ≤ 0.0001 vs ME $_{500}$ mg/kg); $^{\beta}$P ≤ 0.01 vs CHE $_{250}$ mg/kg; $^{\delta}$P ≤ 0.0001 vs CHE $_{500}$ mg/kg; ^{e}P ≤ 0.0001 vs EA $_{500}$ mg/kg; $^{\mu}$ P ≤ 0.05 vs CHE$_{500}$ mg/kg; $^{\pi}$ P ≤ 0.001 vs ME $_{500}$ mg/kg; ; $^{\#}$ P ≤ 0.05 vs CHE $_{250}$ mg/kg; $^{£}$ P ≤ 0.01 vs CHE $_{500}$ mg/kg; ; $^{\$}$ P ≤ 0.05 vs EAE $_{250}$ mg/kg; (One way ANOVA followed by Tukey's multiple comparison test). ME: Methanol extract, CHE: Chloroform extract, EAE: Ethyl acetate extract.*

3.6. Intestinal fluid accumulation

The enteropooling test was induced by castor oil. Pre-treatment of the test groups dose dependently and significantly inhibited the volume and the mass of intestinal content compared to the vehicle (Table 4). The percent inhibition of

mass intestinal content with both ME and CHE extracts was not significantly different from Loperamide group at the highest dose (Table 3), indicating the efficacy of these extracts.

Table 4: Effect of methanolic extract and fractions of M. communis L. leaves on castor oil-induced intestinal 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%)	5	0.72±0.04^{a}	0.86±0.1^{a}	00	00
ME	50	0.56±0.024^{e}	0.62±0.03^{*a}	22.22±3.4^{a}	27.95±3.8^{a}
	250	0.48±0.02^{***e}	0.56±0.027^{****a}	32.53±3.62^{a}	34.63±3.19^{a}
	500	0.42±0.02^{****e}	0.49±0.06^{****d}	41.66±2.77^{d}	47.71±5.17^{e}
CHE	50	0.55±0.034^{*e}	0.6±0.03^{**a}	23.61±4.74^{a}	29.25±3.5^{a}
	250	0.51±0.03^{d**e}	0.58±0.03^{**a}	25.24±4.26^{a}	32.65±4.39^{a}
	500	0.45±0.03^{****e}	0.45±0.026^{****e}	37.5±4.26^{c}	45.91±2.49^{e}
EAE	50	0.56±0.033^{e}	0.59±0.034^{**a}	21.29±4.62$^{a\,\alpha}$	31.18±4^{a}
	250	0.48±0.016^{***e}	0.49±0.03^{****d}	32.5±2.31^{a}	42.71±4.26^{c}
	500	0.4±0.016^{****e}	0.43±0.02^{****e}	44.44±2.31^{c}	46.91±2.49^{d}
Loperamide	5	0.41±0.03****	0.3±0.030****	40.97±4.29	61.98±2.76

*Animals were pre-treated with various doses of ME, CHE and EAE (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 (10 ml/kg). *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001 vs vehicule group (CMC). ^{e}P ≥ 0.05, ^{d}P ≤ 0.05, ^{c}P ≤ 0.01vs, ^{b}P ≤ 0.001, ^{a}P ≤ 0.0001 vs positive group (Loperamide); $^{\alpha}$P ≤ 0.05 vs EAE$_{500}$ (One way ANOVA followed by Tukey's multiple comparison test). ME: Methanolic extract, CHE: Chloroform extract, EAE: Ethyl acetate extract.*

DISCUSSION

Diarrhoea is a real health problem particularly among people in developing countries representing a prominent cause of morbidity and mortality of millions each year. Although diarrhoea is caused by different factors, at least four major mechanisms are usually involved in its pathophysiology, namely: increased intestinal osmolarity, increased electrolytes secretion, decreased electrolytes absorption and disturbed intestinal motility [16]. Many people use medicinal plants against gastrointestinal disorders without any scientific relevance to this use, thus one of the aims of this study is to provide the scientific bases for the traditional utilization of one of these plants namely *Myrtus communis* L. in the treatment of diarrhoea.

Several reports have described *M. communis* L. As being rich in phenolic acids, Flavonoids, tannins, essential oils and fatty acids [17, 8, 18]. The results of the present study revealed that *M. communis* L. leaves extracts are rich in polyphenols. The highest levels of polyphenols were identified in ME extract. These results were quite close to that found by Kanoun *et al*. [19] and Dahmoune *et al*. [20], but lower than that found by Gardeli *et al*. [21] and Nassar *et al*. [17]. The flavonoids content of this study were in line with those of Romani *et al*. [22], but lower than that of Nassar *et al*. [17] and Tumen *et al*. [23] and higher than those of Dahmoune *et al*. [20], Aidi Wannes *et al*. [24] and Kanoun *et al*. [19]. The highest levels of tannins were detected in the ME extract. These values were lower than those of Amessis-Ouchemoukha *et al*. [25] and higher than those of Dahmoune *et al*., [20]. These discrepancies are probably due to different degrees of polarity of the chemicals used for the methods of extraction and quantification, geographic region, and the season of harvest [21].

Both oral tested doses of *M. communis* L. extracts (2 and 5 g/kg) did not produce any visible signs of toxicity or mortality in the 14 days following treatment. According to this, it was concluded that the tested plant extracts were safe at 5 g/kg.

The present study reveals that *M. communis* L. extracts dose dependently decreased gastric emptying and intestinal transit. This effect is highly significant especially with the highest dose of all extracts (500 mg/kg). At this dose, the extracts showed the most powerful effect; an effect that was close to that of atropine. The control of gastric emptying and intestinal transit is a complex process and involves both neural and myogenic mechanisms that are governed by numerous neurotransmitters and mediators. The main excitatory transmitter is acetylcholine, whereas nitric oxide is the major inhibitory mediator [26]. The delaying effects of the extracts on gastric emptying may result from the relaxation of the stomach musculature and/or from the constriction of the pyloric sphincter, while the delay of intestinal transit may involve the inhibition of muscle contraction and/or consolidation of the inhibitory component of the intestinal muscle.

This inhibitory action of the extracts on gastrointestinal motility will delay the passage of gastrointestinal contents allowing more time for intestinal absorption in a manner similar to atropine[27] and the faeces to become desiccated, thus further retarding movement through the colon [28].

The inhibitory effect of *M. communis* L. extracts on gastrointestinal motility and their antidiarrhoeal activity could be attributed to the polyphenolic compounds; mainly tannins and flavonoids as well as other phytochemicals contained in the extract. The HPLC analysis of polyphenols in the plant methanolic extract revealed the presence of gallic and ellagic acids as major components as well as gentisic acid, hydroxybenzoic acid and quercitin (unpublished results). It is believed that the biological activities of the plants may result from their single chemical constituents or from the synergistic effects of their constituents. According to literature, flavonoids and Tannins have different antidiarrhoeal mechanisms of action and one effect is via the inhibition of the gastrointestinal motility at both gastric and intestinal levels. Indeed several studies have pointed out to the inhibitory effect of the flavonoids whether as pure compounds or as major components of different plant extracts on the motility of the gastrointestinal tract [29-36].

Castor oil from the plant *Recinus communis*, is a well known diarrhoea inducer in rodents. It does so via the release of ricinoleic acid (a hydroxylated fatty acid) in the intestinal lumen under the effect of lipases. Once liberated, it provokes irritation and inflammation of the mucosa leading in this way to increased secretion of fluid and electrolytes, decreased of mucosal absorption, stimulation of intestinal motility and thus inducing a rapid evacuation of the intestinal content [37, 38]. The effect of ricinoleic acid is mediated through several mediators including prostaglandin[32, 39], platelet-activating factor and nitric oxide formation [40].

The plant extracts in the present study dose- dependently delayed the onset time of diarrhoea, reduced the number of wet stools, and decreased the volume and the weight of the intestinal content in the castor oil-treated groups. Thus, the antidiarrhoeal activity of the extracts appears to occur through the inhibition of gastrointestinal motility, inhibition of intestinal water and electrolytes secretion and/or stimulation of reabsorption. These effects are most likely due to the main phytochemicals in the plant. According to previous studies, the antidiarrhoeal activity of many plants has been attributed to the presence of tannins [41-44]. They act mainly through the formation of a precipitated protein coat (protein tannate) that covers the intestinal mucosa and thus reducing hydrolelectolytic secretion [16, 45, 46]. Flavonoids may also inhibit diarrhoea by diminishing the secretion of water and electrolytes from the intestinal mucosa [44, 47-49] or by enhancing their mucosal reabsorption [50].

The induction of secretory diarrhoea by castor oil involves the liberation of several inflammatory mediators including prostaglandins and other autacoids that increase inflammation and motility. The phytochemicals of the plant extracts may exert their antidiarrhoeal effects via the blockade of these mediators, since many studies have pointed out to the anti-inflammatory of these compounds [49, 51-54].

CONCLUSION

The results of the present study show that *M. communis* L. leaves extracts are rich in polyphenols and possess antidiarrhoeal activity in mice via different mechanisms that involves various phytochemicals. Furthermore, the plant extracts are safe up to the dose of 5 g/kg. Together, these findings may provide the scientific basis for the folkloric use of the leaves of this plant in handling diarrhoea. However, further studies including the identification of the exact bioactive principle and the precise mechanism of the extracts action are needed.

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