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# Essential Oil of *Satureja montana* L. from Herzegovina: Assessment of Composition, Antispasmodic, and Antidiarrheal Effects

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**Abstract:** *Satureja montana L.* (SM) has a long traditional use as a spice and a medicine for various gastrointestinal disorders, including painful spasms and diarrhea. Contrary to conventional drugs, administration of SM and its extracts are considered safe. Previous studies have shown that the essential oils (EOs) of *SM* from different areas are rich in monoterpenes, sesquiterpenes, diterpens, and phenolic compounds, including flavonoids, tannins, and acids with great composition variability. Determination of composition of EO from Herzegovinian SM done by gas chromatography-flame ionization detection and gas chromatography mass spectrometry (GC-FID and GC/MS, respectively) revealed carvacrol as a primary substance followed by *γ*-terpinene, *p*-cymene, and *β*-caryophyllene. *Ex vivo* spasmolitic activity caused by EO was evident in different types of isolated rat ileum function with the most potent effect on spontaneous activity followed by electrical field stimulation and KCl- and $CaCl_2$-induced activity. SMEO produced *in vivo* antidiarreal activity on castor oil-induced diarrhea in young rats and showed the potential to cause a decrese water content in the feces of adult Wistar rats.This study indicates that effects of SM on the intestinum could be mediated through combination of Kv channel activation and Ca channel blockade, but additional mechanisms might be involved. The results of this study corroborate the traditional use of SM as antispasmodic, antidiarrheal, and antisecretory agents.

**Keywords:** *Satureja montana*; antispasmodic; antidiarrheal. 

## 1. Introduction

*Satureja montana* L. (SM), commonly called mountain savory or winter savory is a dwarf perennial shrub from the *Lamiaceae* family. The wild plant is a characteristic plant of the Mediterranean, but it can be found in other regions of Europe, Africa, Asia, and America. Because of multipurpose uses, SM has

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been recently cultivated outside of its native regions. Names Montana and mountain are probably connected with rocky and sunny habitats. The names *Satureja* and savory indicate an aromatic spicy taste and have been used for a long time as a spice in cooking. In addition to the intense flavor, the reason for using SM as a spice is based on its food preservation capabilities and supposed positive effects on digestion and the gastrointestinal tract. Various medicinal and cosmetic effects have been attributed to SM. In traditional medicine, SM has been used to treat various gastrointestinal disorders, such as intestinal parasites, liver and bile problems, lack of appetite, indigestion, spasms and diarrhea [1, 2]. Antispasmodic activity and relaxing effect on ileal muscles has been attributed to several plants among which are some species of *Satureja* gender: *Satureja hortensis*, *Satureja obavata*, *Satureja viminea* [3-5].

Diarrhea is a very common disturbance, especially in children, and is characterized by three or more unformed liquid stools a day. Painful intestinal spasms with or without disturbances in defecation constitutes one of the most common symptoms in adults. Conventional drugs used for therapy of diarrhea and spasms in the gastrointestinal tract work mainly by antagonizing muscarinic receptors, but these drugs can cause many side effects. This problem imposes the need to search for appropriate traditional medicines for this condition or more precisely, search for scientific justification of use of traditional medicines in the therapy of intestinal spasms and diarrhea. The aims of this study were to examine chemical composition EO extracted from SM cultivated in Ljubinje, the Mediterranean region of Bosnia and Herzegovina, and to determine whether the EO of SM possesses antispasmodic and antidiarrheal properties.

Although many studies of cultivated and wild SM have been recently published, most of them have focused on the chemical composition of plant extracts, primarily EOs or tinctures [1]. The variability in the SM composition confirms dependence on growing methods, geographical conditions, harvesting times, and extraction methods. However, it is known that EOs of SM are rich in monoterpenes, sesquiterpenes, diterpens, and phenolic compounds, including flavonoids, tannins, and acids [1]. Several studies have scrutinized antimicrobial and antioxidant activities of EO *in vitro*. *In vitro* results confirm the EOs' antimicrobial, antiseptic, and antioxidant effects, which justifies traditional use of this plant in food preparation and medicine for the treatment of infections and inflammation [6-11]. Despite considerable research on SM, data on the effects of this plant and its extracts on the digestive system are still lacking.

## 2. Materials and Methods

### *2.1. Plant Material*

Herbs of SM were harvested in the initial flowering phase in the autumn of 2018 from the plantage in Ljubinje, Eastern Herzegovina (Bosnia and Herzegovina). Dried plants were transported to the Institute for Medicinal Plant Research "Dr Josif Pančić", Belgrade, Serbia, in which the EOs were extracted. Voucher specimen was deposited in the Herbarium of the National Museum of Bosnia and Herzegovina (SARA 53051).

### *2.2. Essential Oil Isolation*

Essential oil (EO) from air-dried and powdered plant material was isolated by hydro-distillation for 4h in a Clevenger-type apparatus using 1 mL of *n*-hexane as a collecting solvent according to the European Pharmacopoeia 7.0 procedure.

### *2.3. Qualiatative and Quantitative Analysis*

EO was chemically analyzed by using gas chromatography flame ionization detection and gas chromatography/mass spectrometry (GC-FID and GC/MS, respectively). The GC analysis was carried out using a gas chromatograph HP 7890 series A, fittedwith an FID and a column HP-5, 30m x 0.32mm, film thickness 0.25 μm, carrier gas $H_2$ flow rate 30 mL/min, airflow 400mL/min, injector temperature 300 ºC (splitratio = 1:15), detector temperature 300 ºC. The temperature program was 55–300 ºC (heating rate 4 ºC/min). The GC/MS analysis was performed using chromatograph HP G1800C, with GCD Series II,

equipped with split/splitless injector using same column and conditions. Aqusition was carried out in a scan mode from 45:450.

Identification of each compound was carried out by comparison of retention times (RTs) with those of standard substances and by matching mass spectral data of oil constiteunts with those in MS libraries (NBS library/Wiley/NIST), and by comparison of the RTs calculated against n-alkanes (C9-C20). The chromatographic analysis was repeated three times. The average peak areas from GC (FID) were used to calculate the percentage of each identified compound in the oil.

*2.4. Animals*

*Wistar* rats were housed under standard laboratory conditions with 3–8 animals per cage at room temperature (21–24ºC) under a natural light–dark cycle with water and food *ad libitum*. For isolated organ studies, adult rats, older than two months and weighing 200–300 g were used in experiments. All procedures conducted in this pharmacological test were approved by the local Ethics Committee (01-1090 01.05.2019) and in compliance with the European Council Directive of November 24, 1986 (86/609/EEC).

*2.5. Ex Vivo Spasmolytic Effect*

Male adult *Wistar* rats (200–250 g) were deprived of food but not water 12 h before experiments. After sacrificing, the ileum segment of intestine was dissected and carefully cleaned of connective tissue. Ileum was cut into 3cm long segments which were vertically mounted in a 10 mL volume organ bath containing Tyrode's solution (mmol/L: NaCl, 136.89; KCl, 2.68; $CaCl_2$, 1.80; $MgSO_4$, 1.05; $NaH_2PO_4$, 0.42; $NaHCO_3$, 11.90; glucose, 5.5) and aerated with mixture of 95% $O_2$ and 5% $CO_2$ at 37°C. The exception was made in a series of experiments with ileum stimulated by $CaCl_2$ when the organ bath was perfused with a modified Tyrode's solution ($CaCl_2$ substituted with KCl in the same molar quantity). After 30 min of equilibration, the ileum was tightened to 1g tension and isotonic contractions were recorded using Ugo Basile transducers and displayed on Ugo Basile ink writer or by LabScribe2 Data Recording software. Contractions of isolated ileum segments arose spontaneously or were induced either by electrical field stimulation (EFS), 60 mM KCl, or by increasing concentrations of $CaCl_2$. Increasing concentrations of SMEO (3.3; 11; 33; 110; 330 μg/mL) and verapamil (0.0033; 0.01; 0.033; 0.1; 0.33 μg/mL) were added in a cumulative manner to stable tonic activity induced by KCl, uniform phasic activity appeared spontaneously or elicited by EFS. In the series of experiments with $CaCl_2$, the ileum contractions were induced by cumulative addition of increasing Ca concentrations (0.4; 0.8; 1.2; 1.6; 2.0; 2.4 and 2.8 mM) every 2 min into depolarizing medium (Tyrode's solution containing KCl instead $CaCl_2$). At least four consecutive induced concentration-response curves of $CaCl_2$ were obtained to confirm the stability of the $CaCl_2$ effect. After that, the same procedures were repeated five times in the presence of five concentrations of SMEO (3.3; 11; 33; 110; 330 μg/mL). Each SMEO concentration was added 15 min before adding the $CaCl_2$.

In a series of experiments with antagonists, the effects of the increasing concentrations of SMEO on EFS and KCl-induced activity of the ileum were studied in the presence of three potassium channel blockers: (1) tetraethylammonium (TEA) $10^{-3}$ M, (2) 4-aminopyridine (4AP) $10^{-3}$M, and (3) glibenclamide (Glb) $10^{-5}$ M, as well as with the inhibitor of NO signal pathway, $N_\omega$-Nitro-L-arginine methyl ester hydrochloride (L-NAME) $10^{-5}$ M, and with the opioid receptor antagonist, naloxone ($10^{-5}$ M).

*2.6. Antidiarrheal Activity of SMEO on Castor-Oil Induced Diarrhea*

In this experiment, 12 male young rats (four weeks old), weighting 50–60 g were used. During the night, animals were without food with free access to water (control group) or 0.1% emulsion of SMEO in water (experimental group). In the morning, castor oil (0.5 mL) was administered to all animals via oral gavage. Time from castor oil application to first diarrhea episode, and the total number of wet defecations on tissue paper was recorded for each animal over a period of 3 h.

*2.7. SMEO Effects on Fecal Water Content*

Adult male Wistar rats weighting approximately 180-220 g were randomly divided into three groups per six animals (control, 0.1% and 0.5% emulsion of SMEO in water). Each animal was treated using an oral gastric tube with 5mL/kg of appropriate solution in the morning and in the evening. The control group received water, whereas experimental groups were treated with 0.1% and 0.5% SMEO emulsions. Rat feces in each group were collected over 24 h. After weighing, the feces were dried at 50°C for 4 h and weighed again. Fecal water content was calculated as percentage as described earlier [12]:
Fecal water content = 100% x (wet weight – dry weight)/wet weight

*2.8. Statistical Analysis*

Statistical analyses were performed using SPSS Statistics 26.0 according to the earlier described protocols [13]. The effects of SMEO on ileal activity were expressed as percentages of maximal contractions. All data are presented as mean ± standard deviation (SD). Two-way analysis of variance (ANOVA) with concentration of SMEO and type of activation or pretreatment as factors and post hoc Tukey's or LSD test were used for comparison of the logarithmically transformed effects of SMEO on the ileal contractility. Mann–Whitney U and Student's t-tests were used for analysis of *in vivo* data, and $p<0.05$ was considered statistically significant.

## 3. Results and Discussion

*3.1. Chemical Composition of EO*

EO extracted by hydro-distillation of dry aerial parts of SM grown in Ljubinjewere light-yellow and had an intense aromatic odor. The oil yield of the Herzegovinian variety of SM was 1.45%. SMEO was analyzed by GC and GC/MS to determine its chemical composition because of previously described discrepancies caused by environmental impact, harvesting time, extraction methods, and other factors [1, 2, 6-10, 14, 15]. The components were identified based on RT and elution order on the HP-5 column. Twenty-nine components, which accounted for 98.5% of total oil content (Tab 1), were identified. Similar to the EOs of the French and Croatian SM variety, primary substances were the phenolic compound, carvacrol (54.9%), followed by monoterpenic hydrocarbons, $\gamma$-terpinene, and *p*-cymene (14.5% and 8.9%, respectively)[14]. Among the other important compounds, the most abundant and probably the most pharmacologically important, was sesquiterpene $\beta$-caryophyllene (3.2%).

The oil yield from the Herzegovinian variety of SM was 1.45%, which is similar to the Croatian and French varieties [10, 11] but much higher than that of the Albanian variety [7]. Based on chemical structure, all components of SMEOs are usually divided into five categories: (1) monoterpene hydrocarbons, (2) oxygenated monoterpenes, (3) sesquiterpene hydrocarbons, (4) oxygenated sesquiterpenes, and (5) others. Although differences in content of those groups of compounds can be found, probably the most important features are the types and quantities of oxygenated monoterpenes in EO from different geographic areas. The most important oxygenated monoterpenes found in different SMEOs are the phenolic monoterpenoid compounds,carvacrol and thymol, and the terpene alcohol, linalool. The literature reportslarge variations in in chemical composition of SMEO, even EO obtained from the plants collected at geographically similar and close regions. The EO profiles of SMs from several parts of Croatia, Bosnia and Herzegovina, Montenegro, Serbia, and Albania have previously been studied [7, 9, 10, 14]. The results have shown great differences in composition and leading substance of EO, varying from monoterpenic phenols to other monoterpenic compounds. Based on the structure of leading substance, EOs of SMs could be divided into phenols (carvacrol type, thymol type, linalool type) and monoterpenic hydrocarbons (*cis*-sabinene-hydrate type and *p*-cymene type) [1]. Unlike the greatest number of SMEOs from the Balkans, SMEOs from Ljubinje belong to the carvacrol type with a surprisingly great amount of this phenolic compound.

**Table 1.** Chemical composition of *Satureja montana* essential oils (SMEOs)

| No | Compound | % | RI[a] | RI[b] | Literature |
|---|---|---|---|---|---|
| 1 | *α*-thujene | 1.3 | 927 | 923-930 | [6, 7, 11, 16, 17] |
| 2 | *α*-pinene | 1.1 | 934 | 930-939 | [6, 7, 11, 18] |
| 3 | camphene | 0.51 | 948 | 946-949 | [7, 11, 19] |
| 4 | *β*-pinene | 1.2 | 975 | 972-979 | [11, 16, 20] |
| 5 | myrcene | 2.1 | 986 | 983-992 | [6, 7, 11, 16] |
| 6 | *α*-phellandrene | 0.3 | 1005 | 998-1006 | [11, 16, 21] |
| 7 | *α*-terpinene | 2.5 | 1015 | 1010-1020 | [7, 11, 16, 22] |
| 8 | *p*-cymene | 8.8 | 1019 | 1014-1027 | [6, 11, 16, 23] |
| 9 | limonene | 0.7 | 1027 | 1023-1030 | [6, 11, 16, 24] |
| 10 | 1,8 cineole | 0.4 | 1030 | 1031-1032 | [7, 16] |
| 11 | *trans*-β-ocimene | 0.1 | 1049 | 1039-1050 | [7, 11, 16, 25] |
| 12 | *γ*-terpinene | 14.5 | 1060 | 1056-1060 | [6, 11, 16, 20] |
| 13 | sabinenehydrate | 0.8 | 1070 | 1068-170 | [11, 16, 26] |
| 14 | *α*-terpinolene | 0.1 | 1088 | 1086-1089 | [7, 16, 27] |
| 15 | linalool | 1.2 | 1097 | 1098-1101 | [7, 11, 16, 28] |
| 16 | borneol | 1.4 | 1170 | 1166-1170 | [7, 11, 16, 29] |
| 17 | terpinene-4-ol | 0.5 | 1175 | 1175-1181 | [6, 7, 11, 16, 30] |
| 18 | *α*-terpineole | 0.2 | 1188 | 1189-1194 | [11, 16, 31] |
| 19 | thymol methyl ether | 0.2 | 1235 | 1232-1235 | [6, 7, 16] |
| 20 | thymol | 0.3 | 1287 | 1290-1298 | [6, 7, 11, 16, 31] |
| 21 | carvacrol | 54.9 | 1297 | 1298-1309 | [6, 7, 11, 16] |
| 22 | *β*-bourbonene | 0.1 | 1380 | 1384-1383 | [6, 16, 25] |
| 23 | *β*-caryophyllene | 3.2 | 1423 | 1419-1426 | [7, 11, 16, 28] |
| 24 | *α*-humulene | 0.2 | 1455 | 1454-1450 | [6, 16] |
| 25 | germacrene D | 0.2 | 1481 | 1478-1488 | [6, 11, 16, 32] |
| 26 | *β*-bisabolene | 1.1 | 1507 | 1506-1511 | [6, 7, 11, 16, 33] |
| 27 | *γ*-cadinene | 0.1 | 1510 | 1511-1521 | [6, 7, 11, 16, 34] |
| 28 | *δ*-cadinene | 0.3 | 1520 | 1523-1529 | [6, 7, 11, 16, 34] |
| 29 | spathulenol | 0.2 | 1566 | 1568-1590 | [7, 11, 16, 30] |
| Grouped components (%) | | | | | |
| Monoterpene hydrocarbons | | | 33.2 | | |
| Oxygenated monocarbones | | | 59.9 | | |
| Sesquiterpene hydrocarbons | | | 5.2 | | |
| Oxygenated sesquiterpene | | | 0.2 | | |
| Unknown | | | 1.5 | | |

RI[a]- retention indices calculated against $C_9$-$C_{20}$ *n*-alkanes mixture on HP-5 column. RI[b]- retention indices of essential oil components for the dimethyl-silicone stationary phase as reported in the literature

*3.2 Spasmolytic Activity: Effects of SMEO on Isolated Rat Ileum*

The ileum is the last section of the small intestine. The lumen of the ileum is lined by the mucosa and below the mucosa, the submucosa, submucosalplexus, circular muscle layer, mienteric plexus, and longitudinal muscle layer are situated. Connections between cells allow quick transfer of electrical signals through the ileum wall. Spasms in the intestine could be a consequence of increased neuronal and neurotransmitter activity; mainly by acetylcholine (Ach) as the major excitatory transmitter in the intestine [35]. Ach induces smooth muscle contraction mainly via activation of $M_1$ and $M_3$ muscarinic receptors and less commonly and importantly, of nicotinic receptors on ileal smooth muscle cells [36]. Stimulation of the muscarinic receptors induces $Ca^{2+}$ influx from extracellular and/or intracellular spaces through $Ca^{2+}$

channels. Interaction of $Ca^{2+}$ with calmodulin is the most important method used by the contracting machinery, but it is not unique. In addition to $Ca^{2+}$ channels, $K^+$ channels can influence both membrane potential and contractile elements in a smooth muscle of intestine. Therefore, ileum smooth muscles could be relaxed by reducing Ach neuronal release, by antagonizing of muscarinic receptors, and/or by blocking the contractile machinery via suppression of $Ca^{2+}$ influx into the cytoplasm or some other route that is distal of the receptors. Some possibilities are via a direct influence on contractile proteins or at some level of their intracellular control [37].

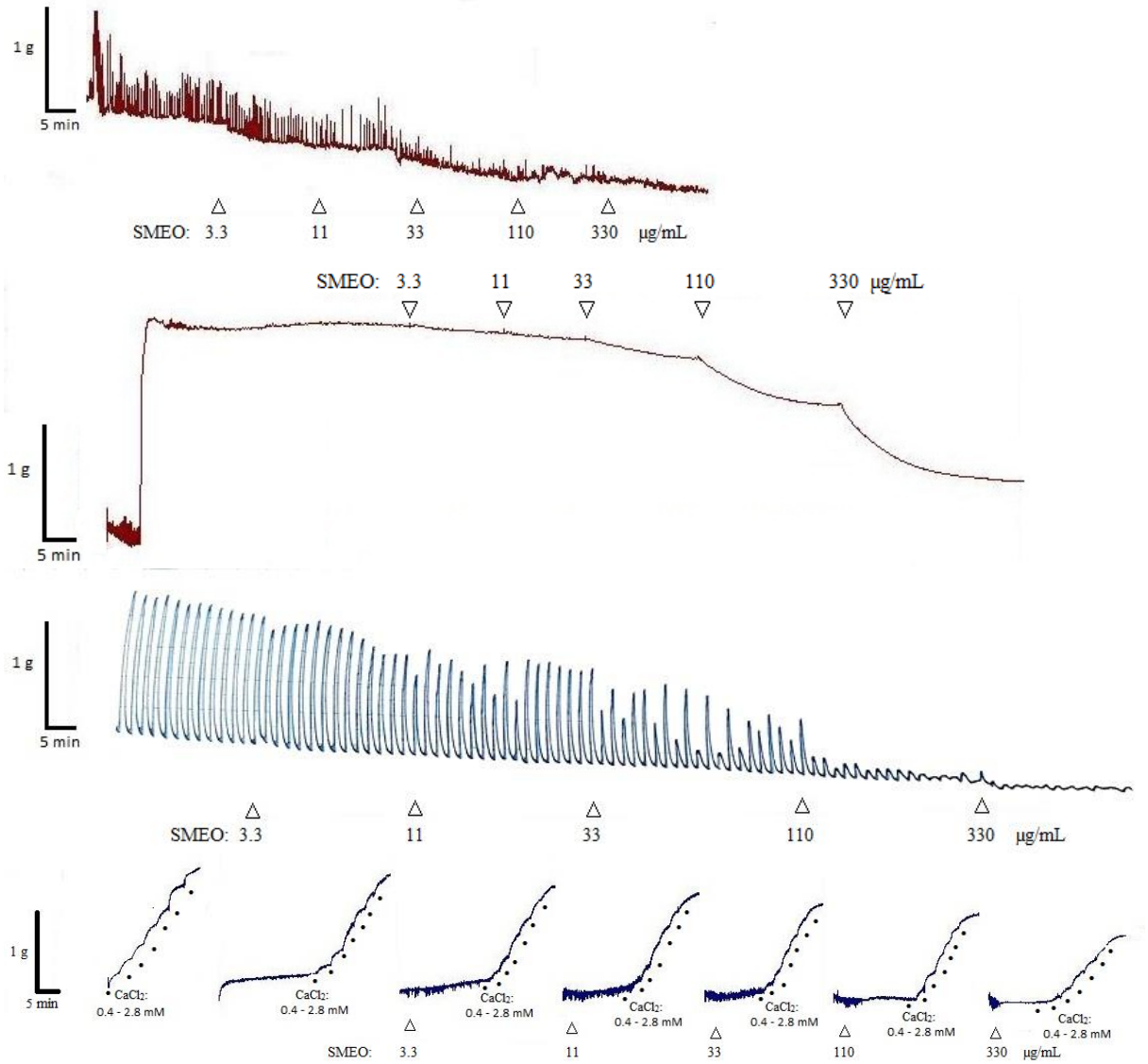


**Figure 1.** Original tracings representing the effect of increasing concentrations of *Satureja Montana* L. essential oils (SMEOs) on spontaneously appearing activity (A), electric field stimulation (EFS)- (B), KCl- (C) and $CaCl_2$- (D) induced rat contractions

Isolated rat ileum after incubation and stretching to basal tonus elicited irregular spontaneous contractile activity which was completely inhibited by increasing concentration of EO (3.3; 11; 33; 110; 330 µg/mL). The same concentrations of SMEO attenuated EFS-, KCl- and $CaCl_2$-induced contractions of isolated rat ileum (Figure 1). Increasing concentrations of SMEO inhibited all studied types of ileum contractions in a concentration-dependent manner but to different extents (Figure 2). SMEO showed the most potent effect on spontaneous activity (max effect = 100% of the maximal amplitude of contractions). The maximal effect of SMEO on EFS-induced activity of ileum was 81.31% ±4.41%, which was not different compared to the maximal effect on KCl-induced activity (maximum effect 80.98% ± 1.81%). Pre-exposure of the isolated ileum to SMEO led to a decrease in $CaCl_2$-induced contractions, but the maximal

inhibitory effect was weaker (58.40% ± 1.21%). The relaxant effect of verapamil was examined on EFS-induced activity of ileum. The concentrations of verapamil were significantly lower than those of SMEO, but the inhibiting effects were comparable (Figure 3).

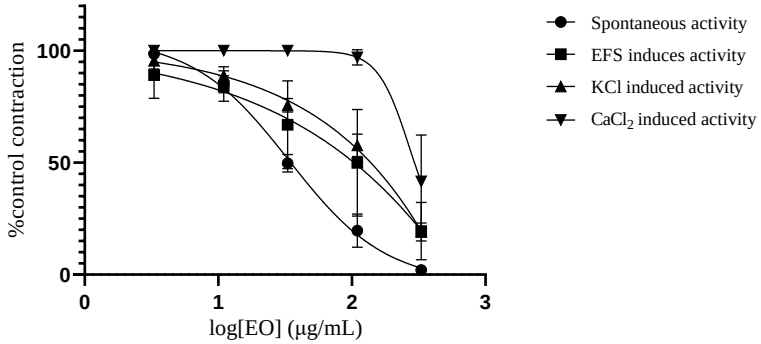


**Figure 2.** Concentration-response curves of the relaxant effects of the SMEO (3.3; 11; 33; 110; 330 µg/mL) on the activity of isolated rat ileum induced spontaneously by EFS, KCl, $CaCl_2$. The data are expressed as the percentage of the maximal stable response achieved before addition of EO. Data are mean ± standard error of the mean (SEM)

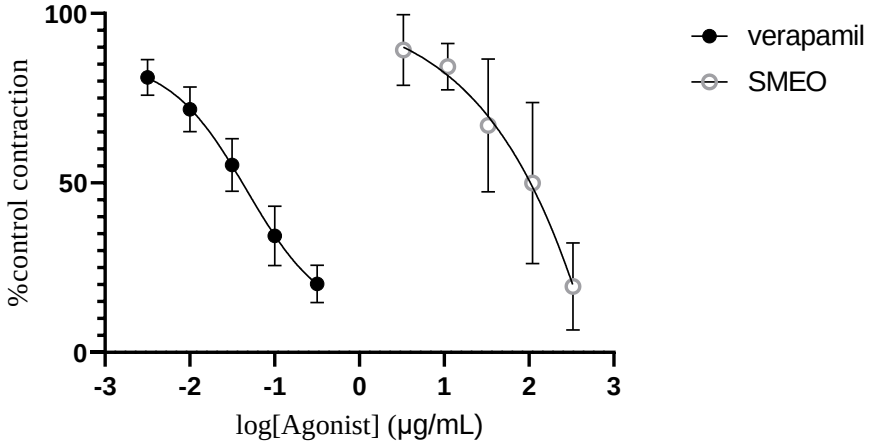


**Figure 3.**Concentration response curves of verapamil (0.0033; 0.01; 0.033; 0.1; 0.33 µg/mL) and SMEO (3.3; 11; 33; 110; 330 µg/mL) on EFS-induced activity of isolated rat ileum. The data are expressed as the percentage of the maximal stable response achieved before adding of the agonist. Data are expressed as mean ± SEM

Statistical significance was tested using a two-way ANOVA with concentration of SMEO and activation type as independent factors on the logarithmically transformed data. The two-way ANOVA confirmed that increasing EO concentrations induced concentration-dependent inhibition of all studied types ofileal contractility (Table 2). Inhibitory effect of EO on ileum contractility was dependent on types of activation (Table 2). A post-hoc Tukey's t-test confirmed difference between all lines except those representing the effects of the increasing concentrations of SMEO on EFS- and KCl- induced activation of isolated ileum.

**Table 2.** Two-way analysis of variance (ANOVA) summary table for the effects of increasing concentrations of SMEOs on different activation types of isolated rat ileum

| Source | df | MS | F | P | Effect size |
|---|---|---|---|---|---|
| Concentration (A) | 4 | 2.929 | 112.770 | <0.001 | 0.849 |
| Activation type (B) | 3 | 1.092 | 42.044 | <0.001 | 0.612 |
| (A)*(B) | 12 | 0.302 | 11.611 | <0.001 | 0.635 |

MS – Mean squares

The relaxing effect of SMEO on EFS-induced activity of rat ileum were partially reduced in the presence of 4-AP (Figure4). Pretreatment of rat ileum with Glb, TEA, L-NAME, and naloxone did not cause a change in the inhibiting effects of SMEO on EFS-induced contractions (Figure 4). Statistical significance was tested by two-way ANOVA, and results are given in Table 3. Based on the ANOVA results, the choice of pretreatment did not influence SMEO-induced effects, but the post hoc least significant difference (LSD) test revealed statistical difference between 4-AP and control (p=0.014).

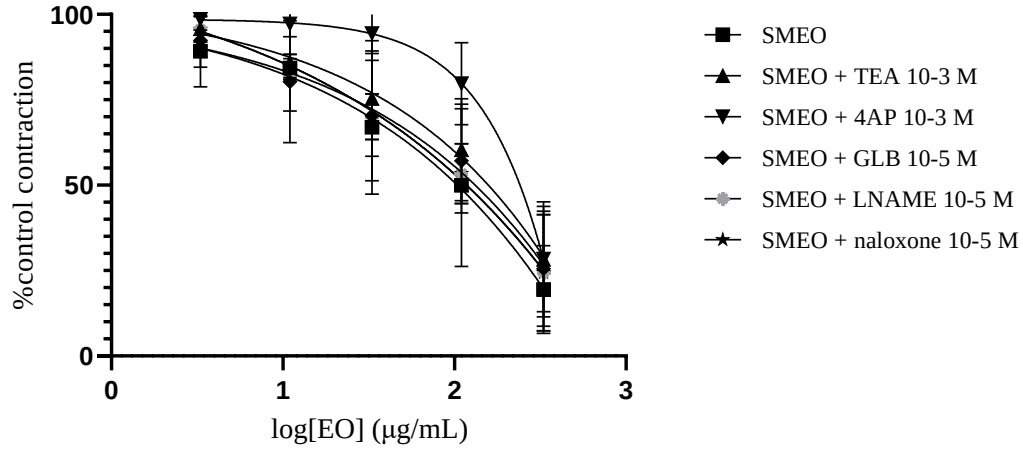


**Figure 4.** Concentration response curves of the relaxant effects of SMEO (3.3; 11; 33; 110; 330 μg/mL) on the activity of isolated rat ileum induced by EFS without antagonists and in the presence of TEA $10^{-3}$ M, 4AP $10^{-3}$M, Glb $10^{-3}$ M, naloxone $10^{-5}$ M, and L-NAME $10^{-5}$ M. Data are expressed as the percentage of the maximal stable response achieved before addition of the EO

**Table 3.** Two-way ANOVA summary table for the effects of increasing concentrations of SMEO on EFS-induced activity in rat ileum without and with pretreatments.

| Source | Df | MS | F | P | Effect size |
|---|---|---|---|---|---|
| Concentration (A) | 4 | 2.469 | 53.956 | <0.001 | 0.633 |
| Pretreatment (B) | 4 | 0.077 | 1.683 | NS | 0.051 |
| (A)*(B) | 12 | 0.014 | 0.299 | NS | 0.037 |

MS – Mean squares

In several studies of traditional anti-spasmogenic remedies, searching for potential effects on muscarinic receptors is the focus [3]. The absence of antimuscarinic side effects of SM extracts indicates that inhibition of muscarinic receptors is probably not the dominant mechanism of its spasmolytic activity. It is consistent with our finding that SMEO inhibits spontaneous contractions of isolated rat ileum. Inhibition of spontaneous ileum activity by SMEO confirms a non-neurogenic mode of action.

Electrically elicited contractions of ileum are neurogenic in their nature, and they can be inhibited by neuronal inhibitors, such as morphine [38]. Stimulation of isolated ileum segment by EFS induces release of several neurotransmitters, and this type of activity is the most similar to the natural activities of the gastrointestinal tract [39]. SMEO was found to inhibit electrically-induced contractions of isolated ileum in a concentration-dependent manner. Pre-treatment with naloxone did not change the effects of SMEO on electrically-induced activity of isolated ileum suggesting a non-neurogenic mechanism of action and non-opioid receptor-dependent mechanism of action. Namely, opioid receptors are found not only on myenteric plexus, but on ileum smooth muscles too. Opioid receptors activation closes voltage gated Ca-channels and leads to relaxation of ileum smooth muscles [40]. 4-AP partially antagonized SMEO effects on EFS-induced ileal activity. This finding suggests that opening of Kv channels play a role in a mechanism of action of SMEO on isolated rat ileum. It is known that nitric oxide (NO) can inhibitileal motility [41], thus providing a reason to check the effect of SMEO in the presence of the NO-synthase inhibitor, L-NAME. Pre-treatment with L-NAME did not lead to changes in the relaxing effects of SMEO, which leads to the conclusion that release of NO is not included in SMEO relaxing effects on isolated ileum.

SMEO caused a reduction in the tonic contractions of isolated ileum induced by high potassium concentrations (60 mMKCl). The contractions of ileum induced by $K^+$ are caused mainly from membrane depolarization and subsequent influx of extracellular $Ca^{2+}$ [42]. Under these circumstances, the plasmalemmal membranes of enteric neurons become depolarized (Em = −20mV) and sodium channels are inactivated, so neuronal transmission is blocked [37]. In addition to this process, Rombolà et al. [43] demonstrated that KCl-evoked contraction of isolated rat ileum did not change in the presence of atropine, suggesting that muscarinic receptors were not involved. On the other hand, nifedipine blocks KCl-induced tonic contraction of ileum [44]. Relaxation of KCl-induced tonus of isolated ileum by SMEO indicates myogenic activity because $K^+$ in high concentration blocks nerve excitability [45]. It is possible that SMEO blocks voltage-dependent $Ca^{2+}$-channels or inhibits the contractile machinery at some other site [39].
To check involvement of $Ca^{2+}$ channel activation in the mechanism of SMEO action, contraction response curves with $Ca^{2+}$ were designed. Contraction induced by increasing concentration of $Ca^{2+}$ in a depolarizing medium depended on activation of $Ca^{2+}$ channels [46]. This result indicates a direct effect of SMEO on smooth muscles via blockade of Ca-channels.

Spasmolytic effects of SMEO could be explained by high content of carvacrol [3], but the presence of β-caryophyllene may also be an important contributor [47, 48]. Despite high contents of carvacrol and monoterpenes with confirmed spasmolytic efficacy, complex composition of SMEO may permit a network of synergistic and/or antagonistic reactions contributing to final observed pharmacological effects [49].

### *3.3. In Vivo Antidiarrheal and Antisecretory Effects of SMEO*

SMEO produced an *in vivo* anti-diarrheal effect in young rats. SMEO-treated groups showed a significant reduction in castor oil-induced diarrhea compared to control group (Figure 5). No difference in fecal weight and time from castor oil application to first diarrhea was found (Table 4). SMEO led to a reduction in fecal water content in adult male rats (Table 5).

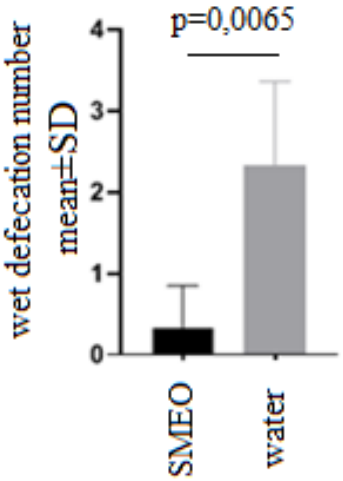


**Figure 5.** Reduced number of castor oil-induced diarrhea episodes in SMEO-treated young rats.

**Table 4.** SMEO effects on number of formed stools and time from castor oil application to first episode of diarrhea

| | **SMEO group** | **Water group (control)** | **Mann-Whitney *p*** |
|---|---|---|---|
| Mean weight of young rats (g) | 56.17±14.20 | 59.17±14.20 | 0.403 |
| Number of formed stools | 0.83±0.75 | 0,00±0,00 | 0.0606 |
| Time to first stool | 59.17±12.56 | 45.33±18.93 | 0.2273 |

**Table 5**. Fecal water content in adult rats after administration of water, 0.1% and 0.5% SMEO

| Group | Fecal water content (%) | Student t-test |
|---|---|---|
| Control | 11.44±0.29 | |
| SMEO 0.1% | 9.09±0.26 | p<0.001 |
| SMEO 0.5% | 8.47±0.17 | p<0.001 |

All values are expressed as mean±SD and p-values compared to the control.

The castor oil-induced model of diarrhea is commonly used model for studying the effects of plant extracts on gastrointestinal motility and secretion [50]. The diarrheal effect originates from ricinoleic acid formed by hydrolysis of the oil in the upper part of small intestine [50]. This acid causes inflammation of the intestinal mucosa and stimulation of prostaglandin release. In the next step, prostaglandins stimulate secretion of water and electrolytes in addition to leading to an increase in gastrointestinal motility. In addition, by inducing edema in the intestinal mucosa, prostaglandins prevent water and electrolyte reabsorption [51]. This effect can be antagonized not only via inhibition of prostanoid biosynthesis or their receptors but also via Ca channel blockade as the downward point in the transition to prostanoid effects [48]. Anti-diarrheal effects may be the consequence of decreased secretion or increase re-absorption of intestinal wall summary manifesting in decreased water content in lumen and finally feces. Decreased water content in the feces after administration of SMEO is additional argument that confirms its antidiarrheal effect.

In conclusion, SMEO acts in a concentration-dependent manner and leads to relaxation of isolated rat ileum contracted via four different mechanisms. These results indicate that effects of SM on intestine could be mediated through combination of the activation of Kv channels and blockade of Ca channels, but additional mechanisms could be involved. In addition to those properties, SMEO possesses antidiarrheal activity against castor oil-induced diarrhea and leads to a reduction of water content in feces. The results of this study are consistent with traditional use of SMs as antispasmodic, antidiarrheal, and antisecretory medicine in intestinal disorders that involve excessive smooth muscle contractility.

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## References

[1] N. Hudz, E. Makowicz, M. Shanaida, M. Białoń, I. Jasicka-Misiak, O. Yezerska, L. Svydenko and P.P. Wieczorek (2020). Phytochemical evaluation of tinctures and essential oil obtained from *Satureja montana* Herb, *Molecules* **25**, 4763.

[2] F. Jafari, F. Ghavidel and M.M. Zarshenas (2016). A critical overview on the pharmacological and clinical aspects of popular *Satureja* species, *J. Acupunct. Meridian. Stud.* **9**, 118-127.

[3] V. Hajhashemi, H. Sadraei, A.R. Ghannadi and M. Mohseni (2000). Antispasmodic and anti-diarrhoeal effect of *Satureja hortensis* L. essential oil, *J. Ethnopharmacol.* **71,** 187-192.

[4] T. Cruz, M.M. Cabo, J.R.P. Jimenez and A. Zarzuelo (1990). Composition and pharmaceutical activity of the essential oil of *Satureja obovata*. II. Spasmolytic activity, *Fitoterapia* **61**, 247-251.

[5] A. Suárez, M.M. Echandi MM, G. Ulate and J.F. Cicció (2003). Pharmacological activity of the essential oil of *Satureja viminea* (Lamiaceae), *Rev. de biol. trop*. **51**, 247–252.

[6] M. Akdeniz, I. Yener, I, A. Ertas, M. Firat, B. Resitoglu, N. Hasimi, S.I. Kandemir, M.A. Yilmaz, A.B. Demirkoz, U. Kolak and S. Oksuz (2021). Biological and chemical comparison of natural and cultivated samples of Satureja macrantha CA Mey, *Rec. Nat. Prod.* **15** (**6**), 568-584.

[7] A. Hajdari, B. Mustafa, A. Kaçiku, X. Mala X, B. Lukas, A. Ibraliu, G. Stefkov and J. Novak (2016). Chemical composition of the essential oil, total phenolics, total flavonoids and antioxidant activity of methanolic extracts of *Satureja montana* L, *Rec. Nat. Prod.* **10**, 750-760.

[8] H. Said-Al Ahl (2014). Antioxidant polyphenolic constituents of *Satureja montana* L. growing in Egypt, *Int. J. Pharm. Pharm. Sci*. **6**, 578-581.

[9] T. Mihajilov-Krstev, D. Radnovic, D. Kitic, V. Stankov-Jovanovic, V. Mitić, Z. Stojanović-Radić and B. Zlatković (2014). Chemical composition, antimicrobial, antioxidative and anticholinesterase activity of *Satureja montana* L. ssp montana essential oil, *Cent. Eur. J. Biol.* **9**, 668-677.

[10] M. Skocibusić M and N. Bezić (2004). Phytochemical analysis and in vitro antimicrobial activity of two *Satureja* species essential oils, *Phytother. Res.* **18**, 967-970.

[11] H. Miladi, R. Slama, D. Mili, S. Zouari, A. Bakhrouf and E. Ammar (2013). Chemical composition and cytotoxic and antioxidant activities of *satureja montana* l. essential oil and its antibacterial potential against *Salmonella* Spp. Strains, *J. Chem.* **2013**, 1-9.

[12] X. Ren, L. Liu, Y. Gamallat, B. Zhang and Y. Xin (2017). Enteromorpha and polysaccharides from enteromorpha ameliorate loperamide-induced constipation in mice, *Biomed. Pharmacother.* **96**, 1075-1081.

[13] D.E. Hinkle, W. Wiersma and S.G. Jurs (2003). Applied statistics for the behavioral sciences, 5th ed. Houghton Mifflin, Boston, MA, USA.

[14] T. Dodoš, S. Janković, P.D. Marin and N. Rajčević (2021). Essential oil composition and micromorphological traits of *Satureja montana* L., *S. subspicata* Bartel ex Vis., and *S. kitaibelii* Wierzb. Ex Heuff. plant organs, *Plants (Basel)* **10**, 511.

[15] A. Ibraliu, B. Dhillon, N. Faslia and B. Stich (2010). Variability of essential oil composition in Albanian accessions of *Satureja montana* L, *J. Med. Plant Res.* **4**, 1359-1364.

[16] V.I. Babushok, P.J. Linstrom and I.G. Zenkevich (2011). Retention indices for frequently reported compounds of plant essential oils, *J. Phys. Chem.Ref. Data* **40**, 043101-043101.

[17] N. Mimica-Dukić, S. Kujundžić, M. Soković and M. Couladis (2003). Essential oil composition and antifungal activity of *Foeniculum vulgare* Mill. obtained by different distillation conditions, *Phytother. Res.* **17**, 368-371.

[18] A. Basta, M. Pavlović, M. Couladis and O. Tzakou (2007). Essential oil composition of the flowerheads of *Chrysanthemum coronarium* L. from Greece, *Flavour Fragr. J.* **22**, 197-200.

[19] R. Baranauskienė, P.R. Venskutonis, P. Viškelis and E. Dambrauskienė (2003). Influence of nitrogen fertilizers on the yield and composition of thyme (*Thymus vulgaris*), *J. Agric. Food Chem*. **51**, 7751-7758.

[20] H. Hajlaoui, H. Mighri, E. Noumi, M. Snoussi, N. Trabelsi, R. Ksouri and A. Bakhrouf (2010). Chemical composition and biological activities of Tunisian *Cuminum cyminum* L. essential oil: A high effectiveness against Vibrio spp. Strains, *Food Chem. Toxicol.* **48**, 2186-2192.

[21] W. Aidi Wannes, B. Mhamdi and B. Marzouk (2009). GC comparative analysis of leaf essential oils from two myrtle varieties at different phenological stages, *Chromatographia* **69**, 145-150.

[22] Y. Yu, T. Huang, B. Yang, X. Liu and G. Duan (2007). Development of gas chromatography–mass spectrometry with microwave distillation and simultaneous solid-phase microextraction for rapid determination of volatile constituents in ginger, *J. Pharm. Biomed. Anal.* **43**, 24-31.

[23] K. Wawrzynczak, B. Sadowska, M. Wieckowska-Szakiel and D. Kalemba, D (2021). Composition and antimicrobial activity of Myrica gale L. leaf and flower essential oils and hydrolates, *Rec. Nat. Prod*. **15** (**1**), 35-45.

[24] C.Y. Du, Y.N. Li, J.T. Fan, R. Tan and H.Z. Jiang (2021). Chemical composition, antioxidant and antimicrobial activities of essential oil from the leaves of Lindera fragrans Oliv., *Rec. Nat. Prod*. **15** (**1**), 65-70.

[25] M. Jalali-Heravi, B. Zekavat and H. Sereshti (2006). Characterization of essential oil components of Iranian geranium oil using gas chromatography–mass spectrometry combined with chemometric resolution techniques, *J. Chromatogr. A.* **1114**, 154-163.

[26] I.A. Ogunwande, G. Flamini, P.L. Cioni, O. Omikorede, R.A. Azeez, A.A. Ayodele and Y.O.Kamil (2010). Aromatic plants growing in Nigeria: Essential oil constituents of *Cassia alata* (Linn.) Roxb. and *Helianthus annuus* L, *Rec. Nat. Prod.* **4**, 211-217.

[27] W. Elghwaji, A.M. El-Sayed, K.S. El-Deeb and A.M. ElSayed (2017). Chemical composition, antimicrobial and antitumor potentiality of essential oil of *Ferula tingitana* L. Apiaceae grow in Libya, *Pharmacogn. Mag.* **13**, S446-S451.

[28] J J.S. Sun, Y.X. Feng, Y. Wang, J.G. Li, K.X. Zou, H. Liu, Y.C. Hu, Y. Xue, L.Y. Yang, S.S. Du and Y. Wu (2020). Investigation of pesticidal effects of Peucedanum terebinthinaceum essential oil on three stored-product insects. *Rec. Nat. Prod*. **14**(**3**), 177-189

[29] K. Zhou, X. Zi, J. Song, Q. Zhao, J. Liu, H. Bao and L. Li (2022). Molecular mechanistic pathways targeted by natural compounds in the prevention and treatment of diabetic kidney disease, *Molecules* **27**, 6221.

[30] M. Mehrabani, A. Asadipour and S.S. Amoli (2004). Chemical constituents of the essential oil of *Nepeta depauperata* Benth. from Iran, *DARU J. Pharm. Sci*. **12**, 98-100.

[31] A. Ghannadi, V. Hajhashemi and H. Jafarabadi (2005). An investigation of the analgesic and anti-inflammatory effects of *Nigella sativa* seed polyphenols, *J. Med. Food* **8**, 488-493.

[32] M. Aćimović, S. Ivanović, K. Simić, L. Pezo, T. Zeremski, J. Ovuka and V. Sikora (2021). Chemical Characterization of *Marrubium vulgare* volatiles from Serbia, *Plants (Basel)* ***10***, 600.

[33] W.A. Asuming, P.S. Beauchamp, J.T. Descalzo, B.C. Dev, V. Dev, S. Frost and C.W. Ma (2005). Essential oil composition of four *Lomatium* Raf. species and their chemotaxonomy, *Biochem. Syst. and Ecol*. **33**, 17-26.

[34] N. Zouari, N. Fakhfakh, S. Zouari, A. Bougatef, A. Karray, M. Neffati and M. Ayadi (2011). Chemical composition, angiotensin I-converting enzyme inhibitory, antioxidant and antimicrobial activities of essential oil of Tunisian *Thymus algeriensis* Boiss. et Reut. (Lamiaceae), *Food Bioprod. Process.* **89**, 257-265.

[35] M. Lu, L. Battinelli, C. Daniele, C. Melchioni, G. Salvatore and G. Mazzanti (2002). Muscle relaxing activity of *Hyssopus officinalis* essential oil on isolated intestinal preparations, *Planta Med*. **68**, 213-216.

[36] A. Chakraborty, V. Sankaran, R. Murugan and D.R. Chellappan (2021). Comparative spasmolytic effect between *Cinnamomum tamala* and *Cinnamomum verum* leaf essential oils and eugenol through in vitro and in silico approaches, *Z. Naturforsch. C. J. Biosci*. **76**, 383-391.

[37] P.J. Magalhães, S. Lahlou and J.H. Leal-Cardoso (2004). Antispasmodic effects of the essential oil of *Croton nepetaefolius* on guinea-pig ileum: a myogenic activity, *Fundam. Clin. Pharmacol.* **18**, 539-546.

[38] F.V. Souza, M.B. da Rocha, D.P. de Souza and R.M. Marçal (2013). (-)-Carvone: antispasmodic effect and mode of action, *Fitoterapia* **85**, 20-24.

[39] H. Sadraei, G. Asghari and A. Naddafi A (2003). Relaxant effect of essential oil and hydro-alcoholic extract of *Pycnocycla spinosa* Decne. ex Boiss. on ileum contractions, *Phytother Res.* **17**, 645-649.

[40] I.J. Reynolds, R.J. Gould and S.H. Snyder (1984). Loperamide: blockade of calcium channels as a mechanism for antidiarrheal effects, *J.Pharmacol. Exp. Ther.* **231**, 628-632.

[41] B.I. Gustafsson and D.S. Delbro DS (1993). Tonic inhibition of small intestinal motility by nitric oxide, *J. Auton. Nerv. Syst.* **44**, 179-187.

[42] J. Yan, J.H. Huang, M. He, H.B. Lu, R. Yang, B. Kong, Q.S. Xu and Y.Z. Liang (2013). Prediction of retention indices for frequently reported compounds of plant essential oils using multiple linear regression, partial least squares, and support vector machine, *J. Sep. Sci.* **36**, 2464-2471.

[43] L. Rombolà, M. Straface, D. Scuteri, T. Sakurada, S. Sakurada, M.T. Corasaniti, G. Bagetta and L.A. Morrone (2022). Antispasmodic effect of bergamot essential oil on rat isolated gut tissues, *Pharmaceutics* **14**, 775.

[44] M. Elorriaga, E. Anselmi, J.M. Hernandez, P. D'Ocon and D. Ivorra (1996). The sources of $Ca^{2+}$ for muscarinic receptor-induced contraction in the rat ileum, *J. Pharm. Pharmacol.* **48**, 817-819.

[45] P. Grafe, C.J. Mayer and J.D. Wood (1980). Synaptic modulation of calcium-dependent potassium conductance in myentericneurones in the guinea-pig, *J. Physiol.* **305**, 235-248.

[46] M.A. Blanco, G.A. Colareda, C. van Baren, A.L. Bandoni, J. Ringuelet and A.E. Consolini (2013). Antispasmodic effects and composition of the essential oils from two South American chemotypes of *Lippia alba*, *J. Ethnopharmacol.***149**, 803-809.

[47] V. Leonhardt, J.H. Leal-Cardoso, S. Lahlou, A.A. Albuquerque, R.S. Porto, N.R. Celedônio, A.C. Oliveira, R.F. Pereira, L.P. Silva, T.M. Garcia-Teófilo, A.P. Silva, P.J. Magalhães, G.P. Duarte and A.N. Coelho-de-Souza (2010). Antispasmodic effects of essential oil of *Pterodon polygalaeflorus* and its main constituent β-caryophyllene on rat isolated ileum, *Fundam.Clin.* **24**, 749-758.

[48] P.M.N. Menezes, H.R. Oliveira, M.C. Brito, G.O. Paiva, L.A.A. Ribeiro, A.M. Lucchese and F.S. Silva (2019). Spasmolytic and antidiarrheal activities of *Lippia thymoides* (Verbenaceae) essential oil, *Nat. Prod. Res.* **33**, 2571-2573.

[49] S.V. Madeira, M. Rabelo, P.M. Soares, E.P. Souza, A.V. Meireles, C. Montenegro, R.F. Lima, A.M. Assreuy and D.N. Criddle (2005). Temporal variation of chemical composition and relaxant action of the essential oil of *Ocimum gratissimum* L. (Labiatae) on guinea-pig ileum, *Phytomedicine* **12**, 506-509.

[50] C.J.E. Niemegeers, F. Awouters and P.A.J. Janssen (1984). The castor oil test in rats: An in vivo method to evaluate antipropulsive and antisecretory activity of antidiarrheals? *Drug Dev. Res.* **4**, 223-227.