

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* spasmolytic 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 CaCl₂-induced activity. SMEO produced *in vivo* antidiarrheal activity on castor oil-induced diarrhea in young rats and showed the potential to cause a decrease 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. © 2022 ACG Publications. All rights reserved.

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. Qualitative 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, fitted with an FID and a column HP-5, 30m x 0.32mm, film thickness 0.25 µm, carrier gas H₂ flow rate 30 mL/min, airflow 400mL/min, injector temperature 300 °C (splitratio = 1:15), detector temperature 300 °C. The temperature program was 55–300 °C (heating rate 4 °C/min). The GC/MS analysis was performed using chromatograph HP G1800C, with GCD Series II,

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equipped with split/splitless injector using same column and conditions. Acquisition 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 constituents 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 (21–24°C) 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 3 cm 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; CaCl₂, 1.80; MgSO₄, 1.05; NaH₂PO₄, 0.42; NaHCO₃, 11.90; glucose, 5.5) and aerated with mixture of 95% O₂ and 5% CO₂ at 37°C. The exception was made in a series of experiments with ileum stimulated by CaCl₂ when the organ bath was perfused with a modified Tyrode's solution (CaCl₂ substituted with KCl in the same molar quantity). After 30 min of equilibration, the ileum was tightened to 1 g 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 CaCl₂. 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 CaCl₂, 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 CaCl₂). At least four consecutive induced concentration-response curves of CaCl₂ were obtained to confirm the stability of the CaCl₂ 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 CaCl₂.

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^ω-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), weighing 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 monoterpene hydrocarbons, γ -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 β -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 monoterpene compounds, carvacrol and thymol, and the terpene alcohol, linalool. The literature reports large variations 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 monoterpene phenols to other monoterpene compounds. Based on the structure of leading substance, EOs of SMs could be divided into phenols (carvacrol type, thymol type, linalool type) and monoterpene 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.

Essential oil of *Satureja montana* L. from Herzegovina**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	<i>p</i> -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	<i>trans</i> - 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 C₉-C₂₀ *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 M₁ and M₃ muscarinic receptors and less commonly and importantly, of nicotinic receptors on ileal smooth muscle cells [36]. Stimulation of the muscarinic receptors induces Ca²⁺ influx from extracellular and/or intracellular spaces through Ca²⁺

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].

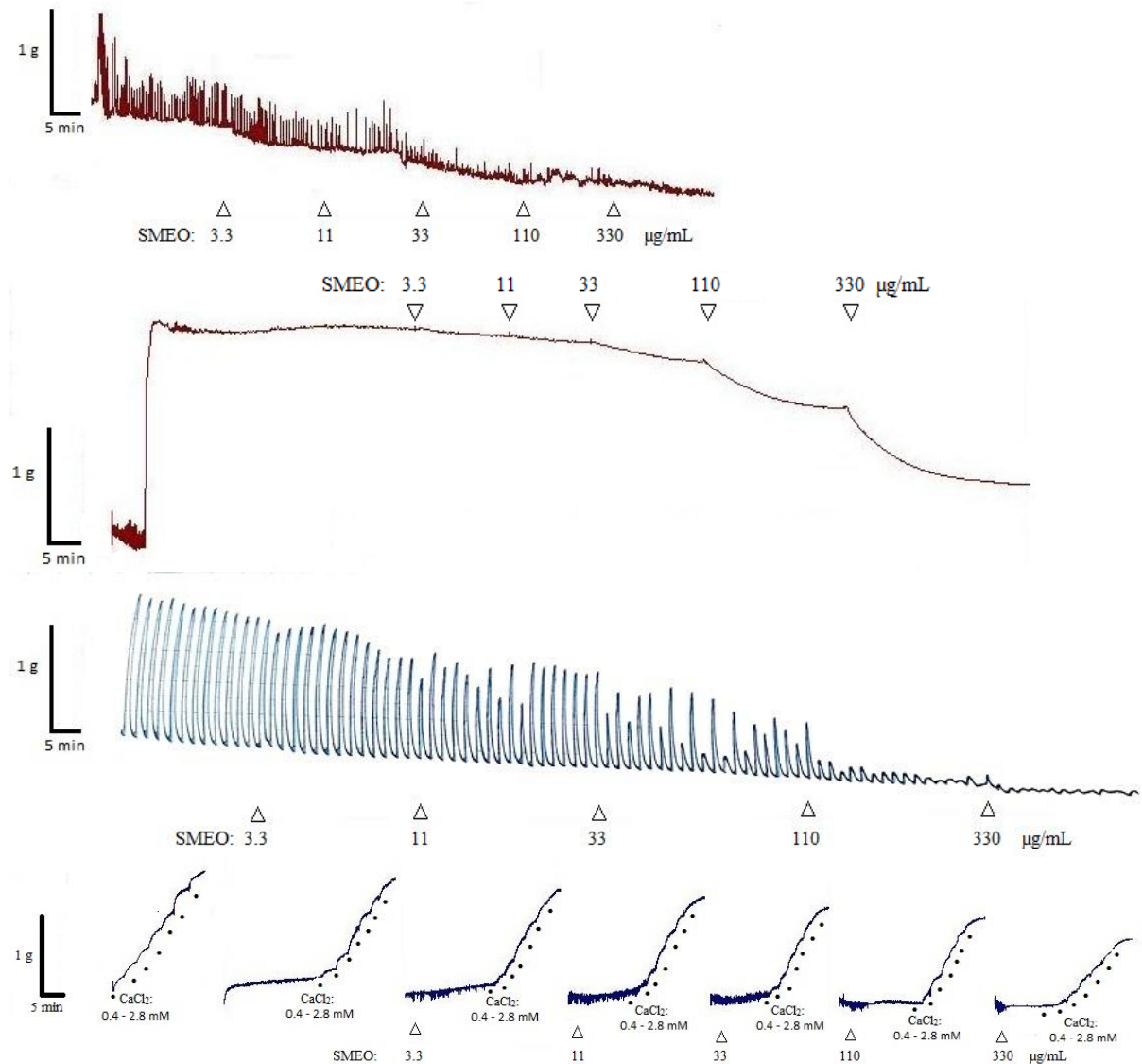


Figure 1. Original tracings representing the effect of increasing concentrations of *Satureja Montana L.* essential oils (SMEOs) on spontaneously appearing activity (A), electric field stimulation (EFS)- (B), KCl- (C) and CaCl_2 - (D) induced rat contractions

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 $81.31\% \pm 4.41\%$, which was not different compared to the maximal effect on KCl-induced activity (maximum effect $80.98\% \pm 1.81\%$). Pre-exposure of the isolated ileum to SMEO led to a decrease in CaCl_2 -induced contractions, but the maximal

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inhibitory effect was weaker ($58.40\% \pm 1.21\%$). 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).

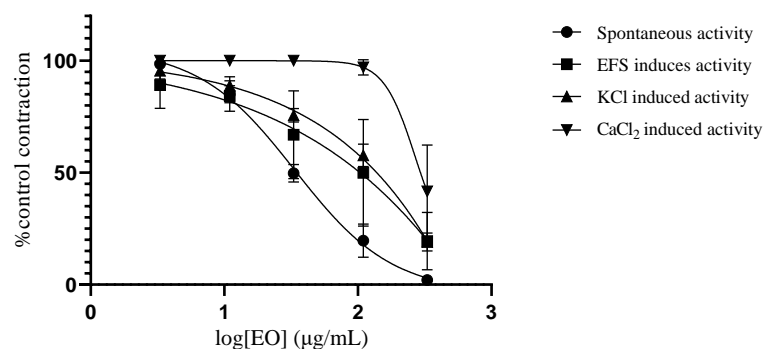


Figure 2. Concentration-response curves of the relaxant effects of the SMEO (3.3; 11; 33; 110; 330 $\mu\text{g/mL}$) on the activity of isolated rat ileum induced spontaneously by EFS, KCl, CaCl_2 . The data are expressed as the percentage of the maximal stable response achieved before addition of EO.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

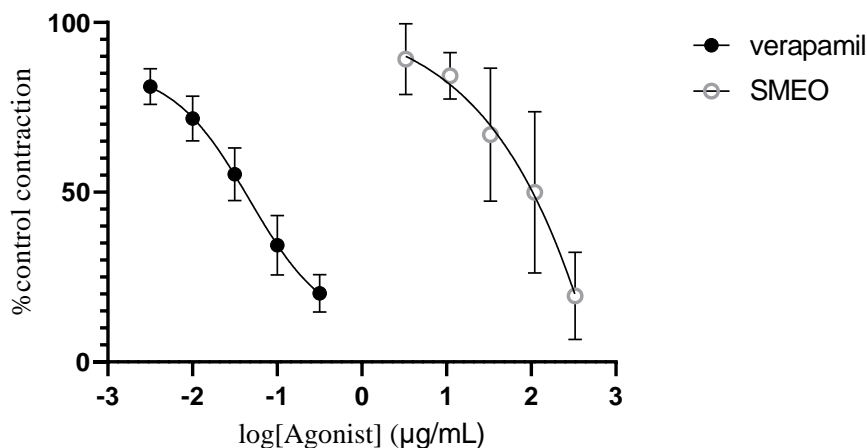


Figure 3. Concentration response curves of verapamil (0.0033; 0.01; 0.033; 0.1; 0.33 $\mu\text{g/mL}$) and SMEO (3.3; 11; 33; 110; 330 $\mu\text{g/mL}$) on EFS-induced activity of isolated rat ileum. The data are expressed as the percentage of the maximal stable response achieved before adding of the agonist. Data are expressed as mean \pm 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 of ileal 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 (Figure 4). 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 ($p=0.014$).

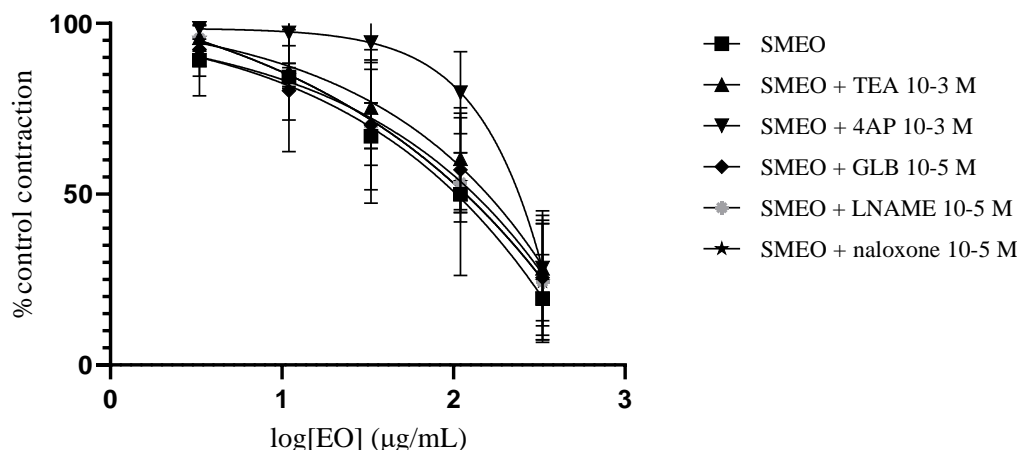


Figure 4. Concentration response curves of the relaxant effects of SMEO (3.3; 11; 33; 110; 330 $\mu\text{g/mL}$) on the activity of isolated rat ileum induced by EFS without antagonists and in the presence of TEA 10^{-3} M, 4AP 10^{-3} M, Glb 10^{-3} M, naloxone 10^{-5} M, and L-NAME 10^{-5} M. Data 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-spasmodic 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 inhibit ileal 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.

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SMEO caused a reduction in the tonic contractions of isolated ileum induced by high potassium concentrations (60 mM KCl). 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 ($E_m = -20\text{mV}$) 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).

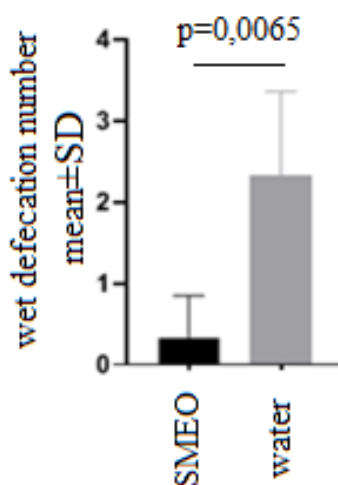


Figure 5. Reduced number of castor oil-induced diarrhea episodes in SMEO-treated young rats.

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 <i>p</i>
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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