

Original Article

Effects of *Bunium persicum* (Boiss.) Essential Oil on the Contractile Responses of Smooth Muscle (An *in vitro* Study)

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Abstract

Bunium persicum (Boiss.) is an economically important medicinal plant growing wild in arid regions in Iran. The essential oil of *B. persicum* (EOBP) was extracted using hydrodistillation. A total of eighteen compounds, representing 96.14 % of the oil was identified by gas chromatography/mass spectrometry (GC/MS). The main compounds were cuminaldehyde (23.04 %), gamma-terpinene (14.48 %), trans-3-Caren-2-ol (12.51 %), acetic acid (10.90 %) and 1,3,8 -p-menthatriene (7.89 %). The effects of 0.1 to 1000 $\mu\text{g mL}^{-1}$ EOBP on ruminal and abomasal smooth muscle of twenty-four healthy sheep and ileum preparations of six rats were assessed *in vitro*. Ruminal preparations showed relaxation ($P < 0.05$) when exposed to 100 - 1000 $\mu\text{g mL}^{-1}$ concentrations of EOBP. In the isolated abomasal preparations, EOBP (0.1 - 1 $\mu\text{g mL}^{-1}$) represented a weak spasmogenic effect followed by relaxation. The spontaneous contraction of abomasal smooth muscles was completely abolished with a high dose (1000 $\mu\text{g mL}^{-1}$) of EOBP. Five-minute incubation with EOBP, significantly ($P < 0.05$) inhibited Ach-induced contraction in higher doses on both tissues. In contrast, rat ileum only showed dose-dependent relaxation effect, and pre-incubated tissues with EOBP, decreased the Ach-induced contraction. The data indicated that the plant contained spasmogenic and spasmolytic constituents. The results also showed that the EOBP profoundly alters gastrointestinal smooth muscle contraction in a dose-dependent and tissue-specific manner.

Key words: *Bunium persicum*, Essential oil, Smooth muscle contraction, Rumen, Abomasum.

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Introduction

Recently, there has been a growing interest in research concerning the possible use of plant extracts as alternatives to growth-promoting antibiotics in animal feeds. Essential oils are among the best-known substances tested for manipulating rumen fermentation.^{1,2} While these properties are increasingly well characterized, there are no data available on the effect of the essential oils on rumen and abomasum motility.

Bunium persicum (Boiss.) seeds locally are called “zireh kuhi”, meaning “wild cumin”, is a native medicinal plant of Iran. It belongs to *Apiaceae* family, growing wild in the arid regions in Iran and its seeds contain high level of essential oils.³ The seeds are consumed widely as a condiment. In traditional medicine, seeds are regarded as stimulants, carminatives and found to be useful in managing diarrhea and dyspepsia.⁴ In addition the plant is used for culinary purposes and flavoring foods and beverages.⁵

In previous studies, antimicrobial^{6,7}, antifungal^{8,9} and antihistaminic¹⁰ effects of this plant have been demonstrated. The aim of this primarily study was to examine the effects of the essential oil on smooth muscle preparations of rumen and abomasum in healthy sheep in vitro. Furthermore, we investigated essential oil effect on contractions of intestinal smooth muscle in rat.

Materials and Methods

Plant material. The air-dried seeds of *B. persicum* (Boiss.) were supplied from the Kerman city of Iran. The plant material was authenticated at Taxonomy Unit, Department of Plant Biology in Faculty of Biological Sciences of Tarbiat Modares University, Tehran, Iran. Voucher specimens have been deposited (#3964) at the Herbarium of the Tarbiat Modares University.

Essential Oil extraction. Dried material (100 g) was soaked in water 24 h before oil extraction before hydrodistillation. Hydrodistillation was done using a Clevenger-type apparatus for 3.5 h. The resulting essential oil of *B. persicum* (EOBP) was separated from water and analyzed by gas chromatography (GC) and mass spectrometry (MS). The essential oil was protected from light in dark glass bottles and kept at 4°C prior to use.

GC-MS analysis of the EOBP. The essential oil was analyzed according to our previous study.¹¹

Preparation of smooth muscle strips. Full-thickness specimens were taken from the medial wall of the cranial dorsal rumen and the mid-region of the abomasal body of clinically healthy sheep (aged 1- 2.5 years) of both sexes, slaughtered at Urmia Industrial Abattoir (West Azerbaijan-Iran). Tissue specimens from different animals were harvested as soon as possible after death of the animal and stomach contents were gently dislodged from each specimen by repeatedly pouring Tyrode's Ringer solution over the tissue. The specimens were then rinsed with Tyrode's Ringer solution (16-20°C) pre-oxygenated for 1.5 h with 95 % O₂ and 5 % CO₂ during transportation (20 min) from the slaughterhouse to the laboratory. The Tyrode's Ringer solution contained (mM): 137 NaCl, 2.7 KCl, 1.8 CaCl₂, 11.9 NaHCO₃, 1.0 MgCl₂, 0.4 Na₂PO₄ and 5.5 glucose. In order to obtain simplified nerve-smooth muscle preparations, the muscular layers were then carefully detached from the mucosa and the submucosa. Thin strips of the double-muscle preparation measuring approximately 20 × 5 mm were cut parallel to the longitudinal rumen¹² and abomasums¹³ smooth muscle, by use of a custom-designed scalpel with two parallel blades to record their activities. Smooth-muscle strips were set up vertically at tensions ranging from 1 to 2 g (according to the marked variation in thickness of the preparations) in a mantled bath of 25 ml

capacity, filled with Tyrode's Ringer solution.

Adult Wistar rats of either sexes weighing 170 – 200 g used in the study were housed in the Laboratory Animal Centre of the College of Veterinary Medicine at Urmia University under standard environmental conditions of temperature, humidity and light. Animal were given tap water *ad libitum* and a standard rodent diet (Livestock feed by Dane Pars Co, Tehran, Iran). All experimental protocols described in this study were approved by the Experimentation Ethics Committee on Animal Use of the Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. Only food (not water) was withdrawn 24 h prior to experiment. Rats were sacrificed by stunning and cervical dislocation and 2-cm pieces of the ileum were dissected from the ileum segment 10 to 20 cm proximal to the ileocecal valve. The intestinal content was removed by flushing with Tyrode's solution. Tissues were mounted for tension recording and allowed to equilibrate for 1.5 h in 25-ml chambers containing Tyrode's solution, under a load of 1g. The Tyrode's solution contained (mM): 136.8 NaCl, 2.7 KCl, 1.3 CaCl₂, 12.0 NaHCO₃, 0.5 MgCl₂, 0.14 Na₂PO₄ and 5.5 glucose.

Smooth muscle recording procedure. Muscle preparations were suspended in tow individual organ bath continuously oxygenated with a mixture of 95 % O₂ + 5 % CO₂ held at 37°C by a circulation thermostat. The distal end of the muscle strip was attached to a hook and the proximal end connected to transducer. The mechanical activity of the preparations was recorded via isometric transducer (MLT0201) coupled with an amplifier (ML110, ADInstruments, Australia) which was continuously displayed and recorded on-line on a personal computer by use of a data acquisition system (ML785 PowerLab, ADInstruments, Australia) using a software (Chart Ver. 5, ADInstruments, Australia). During an

initial 1.5 h equilibration period (until a steady baseline was obtained), the bath buffer solution was changed every 15 min. All specimens were tested for functional viability prior to and after all experiments by the addition of 1×10⁻⁶ M acetylcholine to the tissue bath. Strips which failed to respond to acetylcholine consistently before the start of the experiment were discarded.

A dose-response curve was obtained through exposing the preparation to increasing concentrations added to the bath (1 min to each concentration). The curve was used to determine the effect of acetylcholine. The submaximal (70 % maximal) concentration was determined for each of the strips. The strips which produced three consistent, repeatable responses to submaximal concentration of acetylcholine were used. The intensity of contractions has been presented as a percentage of initial contractions caused only by Ach. Tween 80 facilitates the essential oils' distribution in an aqueous medium¹⁴ therefore the essential oil in this study was initially diluted directly in 1% Tween 80 and added to the bath in amount(s) that reached the final desired concentration. Dilutions were prepared so that the amount added to the bath did not exceed 0.1 ml. Complete inhibitory effects of EOBP could be seen within 5 min of contact with the tissue and were maintained as long as it was present in the bath and persisted 10 –20 min after washing with lower doses.

To assess the effects of EOBP on smooth muscle preparations, two basic protocols were employed. Initially, the effects of EOBP on basal tonus of each preparation were investigated. Therefore, a concentration-effect curve for EOBP was performed by cumulative additions to the bath at increasing concentrations for 5 min contact time necessary to observe the plateau response. In experiments examining the relaxation of the basal tonus of the smooth muscle strips, paired segments were set up; one piece was

exposed to the oil and the other received no treatment. Relaxation due to the EOBP was taken to be the difference between the tonus of control and test segments. Secondly, in an attempt to quantify the effects of EOBP on acetylcholine-induced contractions, the preparations were exposed to the EOBP non-cumulatively for a 5 min period before the addition of sub-maximal concentrations of acetylcholine in the continued presence of EOBP. The tissue was allowed a resting period of 15 min before the next addition but at high concentrations the resting period was 60 min because of tachyphylaxis.

Statistical analysis. The Shapiro-Wilk test was used to determine whether data were normally distributed. When the assumption of normality was not met, the variables were logarithmically transformed to improve the assumptions. When data could not be transformed, Friedman repeated measures for ANOVA on ranks was used to compare the differences among the response of each muscle preparation for various concentrations of essential oil. A repeated ANOVA was used to compare responses when the data were normally distributed. The Dunnett's test was used for comparison of each group versus control group (Tween) if a significance difference was found. Descriptive data are presented as median and interquartile ranges (25th to 75th percentile) for non-normally distributed data. Differences were considered significant at a value of $P < 0.05$. Statistical analyses were carried out using commercial software (Sigma Stat 3 for windows, SPSS Inc, Chicago, IL).

Results

The vehicle (1 % Tween 80) did not show an effect on any of the preparations. When the relaxant effect of the essential oil on the basal tonus was studied at the same time in separate experiments, the basal tonus of the strips did not change during the experimental period in the

absence of EOBP. Any effect induced by EOBP was removed after washout of the tissues and gradually normal contractile response to ACh was re-established in all experiments, showing that the muscle was not damaged by non-specific action. EOBP ($0.1-100 \mu\text{g mL}^{-1}$) on rumen preparations ($n = 6$), resulted in a slight reduction in basal tonus as dose-dependent manner while higher concentration ($100 \mu\text{g mL}^{-1}$) only caused a significant decrease of basal tonus ($P < 0.05$) (Fig. 1A).

In separate experiments, the basal tonus of strips did not change during the experimental period in the absence of EOBP. In isolated sheep abomasums ($n = 6$), the EOBP ($0.1-1 \mu\text{g mL}^{-1}$) induced non-significant dose-dependent spasmogenic effect on the spontaneous tonus of the strips but at higher doses ($10-1000 \mu\text{g mL}^{-1}$) relaxation occurred but this effect in highest dose was significant statistically ($P < 0.05$) (Fig.1B). Normal contractile response to ACh was re-established in all experiments, in 65 ± 7 min. and 79 ± 5 min. after washout of the rumen and abomasum preparations respectively.

Acetylcholine ($1 - 10 \mu\text{M}$) caused a concentration-dependent contraction of the sheep rumen. Pre-exposure of the preparation ($n = 6$) for 5 min to various concentrations of EOBP inhibited the rumen contractions induced by sub-maximal concentrations of ACh in a concentration-dependent mode, also the inhibitory effect at higher concentrations ($100 \mu\text{g mL}^{-1} - 1000 \mu\text{g mL}^{-1}$) was significant (Fig. 2A).

Evaluation of the sheep abomasums ($n = 6$) showed that pre-treatment by lower concentrations ($0.1-10 \mu\text{g mL}^{-1}$) of the EOBP attenuated acetylcholine-induced contractions in a concentration-dependent mode non-significantly. However, EOBP at higher concentrations ($100-1000 \mu\text{g mL}^{-1}$) inhibited the additional ACh effect significantly ($P < 0.05$) (Fig.2B). In comparison with rumen strips, the EOBP have more potent inhibitory effect on abomasum preparations. The EOBP

induced inhibitory effect was cancelled in all strips of rumen and abomasum in 77 ± 10 min. and 83 ± 9 min. after washout of the preparations respectively.

Assessment of EOBP cumulative relaxation effect showed that consecutive additions of various doses ($0.1-1000 \mu\text{g mL}^{-1}$) caused the attenuation of basal tonus significantly ($P < 0.05$) (Fig. 3A). When the tissues was incubated with higher doses the spontaneous motility of smooth muscle, suppressed completely. Acetylcholine ($0.3 \mu\text{M}$) induced reproducible contractions that were 70 % of the maximum response obtainable. The EOBP concentration dependently inhibited these contractions induced by acetylcholine although higher doses ($100-1000 \mu\text{g mL}^{-1}$) cause to complete and significant ($P < 0.05$) inhibition (Fig. 3B) and spontaneous contractions undeveloped. The yield of oil was approximately 4.6 % w/w of dried seeds weight obtained from Zarand city of Kerman province, Iran. The oil was golden yellow in colour, with an odour characteristic to that of the plant.

Eighteen compounds were identified by GC-MS analysis of *B. persicum* [Boiss] representing 99.14 %. The results obtained by this method are presented in Table 1. The major compounds were: cuminaldehyde (23.04 %), gamma-terpinene (14.48 %), acetic acid (10.90 %), terpinolene (8.27%), 1,3,8-p-menthatriene (7.89 %) and trans-3-carene-2-ol (12.51 %).

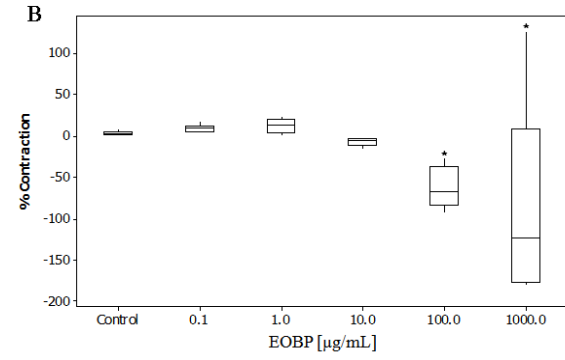
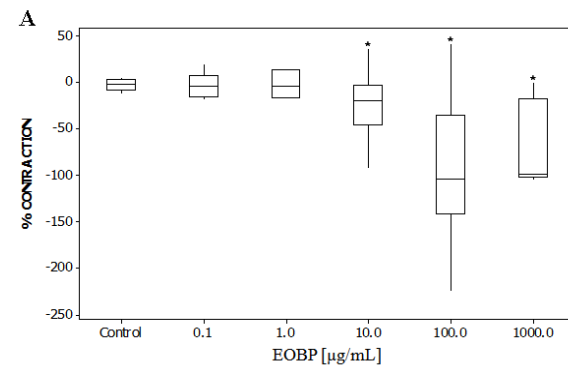


Fig 1. Box plots for relaxant effects of EOBP on basal tonus in the healthy sheep rumen (n = 6) (A) and abomasums (n = 6) (B) preparations with longitudinal orientation. Each box represents the central 50 % of the values, the horizontal line within each box represents the median value, and the whiskers indicate the range of values that are within the inner boundary. Values outside the inner fence are plotted as empty circles. * $P < 0.05$ vs control.

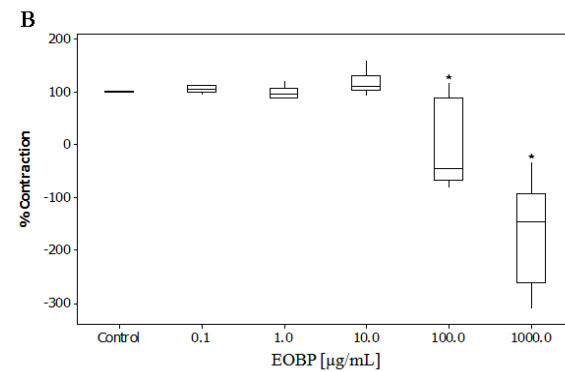
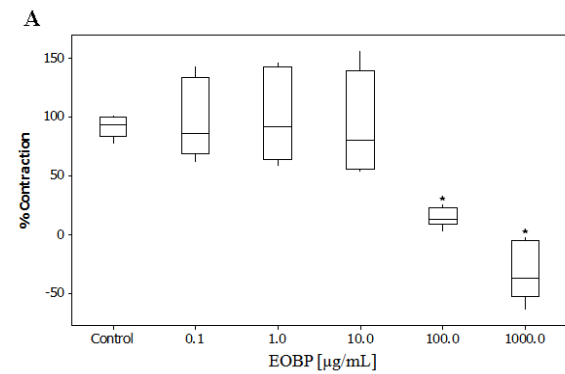


Fig 2. Box plots Inhibitory effects of EOBP on contractions induced by ACh in the healthy sheep rumen (n = 6) (A) and abomasum (n = 6) (B) preparations with longitudinal orientation. Each box represents the central 50 % of the values, the horizontal line within each box represents the median value, and the whiskers indicate the range of values that are within the inner boundary. Values outside the inner fence are plotted as empty circles. * $P < 0.05$ vs control.

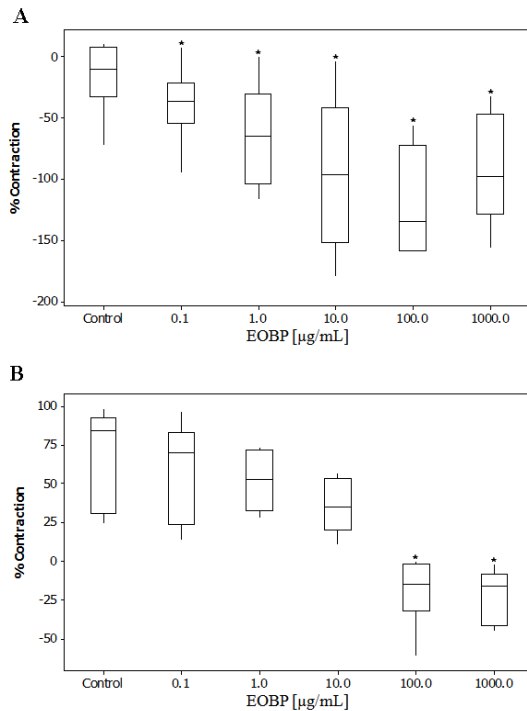


Fig 3. Box plots for relaxant effects of EOBP on basal tonus (A) and inhibitory effects of EOBP on contractions induced by ACh (B) of the rat isolated ileum. Each box represents the central 50 % of the values, the horizontal line within each box represents the median value, and the whiskers indicate the range of values that are within the inner boundary. Values outside the inner fence are plotted as empty circles. Each group with six observations. * $P < 0.05$ vs control.

Discussion

In this study we evaluated the pharmacological activity of EOBP on the isolated tissue preparations from rumen and abomasum of the sheep and ileum of the rat. The present study provides evidence that the EOBP profoundly alters gastrointestinal smooth muscle contraction in a dose-dependent and tissue-specific manner.

Acetylcholine, the main endogenous neurotransmitter in the cholinergic system, causes a contraction of the smooth muscle layers in the forestomach¹⁵ and abomasums¹³ through activation of muscarinic receptors located directly on smooth muscle cells or nerve cells of enteric nervous system¹⁶. The contractile effects of ACh have already been demonstrated in rumen and abomasums

tissues from healthy sheep *in vitro*¹⁷⁻¹⁸ and our findings were in agreement with these reports.

According to the results from our experimental set-up, in all of the evaluated tissues EOBP inhibited the ACh-induced contractions dose-dependently.

When the EOBP was added to the bath cumulatively, it caused relaxation of sheep rumen and rat ileum preparations, while EOBP evoked an immediate but small increase in muscle tone in abomasum tissue preparations followed by a sustained relaxation of muscle strips. However, functions and properties of every organ containing smooth muscles differ depending on the organ in which they occur and on their particular location within the organ.¹⁹ This is another explanation for some paradoxical result observed in rumen, abomasums and ileum in rat. In addition, these findings may reflect differences in specific receptors distribution within the gastrointestinal tract. In the past decade, research has been fuelled by the classification of multiple receptor subtypes and the development of specific agonists and antagonists for many of these receptors. The reported results with specific agonists and antagonists suggest that the serotonergic, adrenergic and dopaminergic control systems markedly differ in ruminants and rodents.²⁰ The observed differences may be due to differences in the structure of receptors (their amino acid sequence) and the distribution of receptor subtypes.²⁰ Overall our results once more confirm the difference in distribution of specific receptors in various regions of the gastrointestinal tract in the mammals.

Table 1. Main constituents (%) of the essential oil of *B. persicum*

No.	Compound	Rt(min) ^a	Relative amount (%)
1	Oxalic acid	1.45	0.62
2	<i>alpha</i> -pinene	2.55	0.90
3	<i>beta</i> -pinene	3.37	2.27
4	1,3,8-p-menthatriene	4.37	7.89
5	Trans-3-Carene-2-ol	5.47	12.51
6	Gamma-terpinene	5.94	14.48
7	Terpinolene	6.03	8.27
8	Isogeraniol	7.21	0.93
9	Cuminaldehyde	8.10	23.04
10	Acetic acid, 3-cyclohex-1-enyl-1-methylprop-2-ynyl ester	10.04	10.90
11	10-(2-oxopropyl) - <i>alpha</i> -Pinene,	10.25	4.41
12	Myristicin	10.61	4.61
13	Apiol	11.17	3.75
14	<i>alpha</i> -Bisabolol	11.51	3.60
15	<i>gamma</i> -Elemene	12.32	0.11
16	Palmitic acid	12.88	0.47
17	2-(7-heptadecyloxy)tetrahydro-2H-Pyran	13.46	0.25
18	8,11,14-Eicosatrienoic acid	13.75	0.13

^aRetention Time

In the present study the action of EOBP on rat's ileal motility interfered with the contractile activity of acetylcholine on the specimens. The inhibitory effect of EOBP on Ach-induced contraction may be due to β -adrenergic stimulatory or cholinergic inhibitory actions of the essential oil. Pinene components reveal relaxant effect²¹⁻²² and since they are major constituents in EOBP, so relaxation effects observed in this study could have been mediated by them. Astudillo *et al.* (2004)²³ showed that monoterpenes such as tymol, camphor and gamma-terpinene induced antispasmodic effect on rabbit jejunum. Thereby high percentage of gamma-terpinene (14.48 %) identified in our EOBP could be responsible for the effect on smooth muscle observed in this study. Cuminaldehyde (23.04 %) as another major compound of EOBP, could be associated with relaxation outcome although it was not investigated yet.

On the other hand, an impaired response of the abomasal muscles to acetylcholine and bethanechol (a muscarinic agonist) has been noticed in patients with abomasal displacement.^{13, 24} The same response (reduced response to ACh) observed in this study may possibly occur *in vivo* when rations are supplemented with these

essential oils, and subsequently predisposes the animals to abomasal displacement, abomasal rotation and or indigestion with induced hypomotility in rumen. Some researchers have evaluated effects of several essential oils on ruminal fermentation at concentration of 300 and 3000 mg/L of ruminal fluid, *in vitro*.²⁵ Subsequently; altered gastrointestinal motility appears to have various effects on animals' performance. A slow passage will improve digestibility of feed. However, an increase in motility will make bacteria to escape faster from the rumen leading to a higher efficiency of nitrogen and energy utilization by decreasing bacterial death and breakdown which may otherwise happen in the rumen.

To the best of our knowledge, this is the first report directly demonstrating an effect of plant essential oil on the isolated tissue preparations of rumen and abomasums in sheep. Recently, relaxant and functional antagonistic effects on muscarinic receptors of isolated guinea pig tracheal chains were demonstrated for an extract of *Carum carvi* a related species of *B. persicum*.²⁶

The yield of oil from our *B. persicum* sample was 4.6 % (v/w) and was among the range that has been reported elsewhere.

Lesser (3.1 %) ²⁷ and more higher (9.1 %) ⁷ levels of the yield was also reported. It should be noted that different extraction compositions could be obtained by different extraction methods applied to natural products. ²⁸

A number of studies on EO content and constituents of *B. persicum* have been performed. Seed oil from India contained cuminaldehyde (27.3 %), gamma-terpinene (25.6-42.9 %), and p-cymene (24- 27.8 %). ²⁹ Sadykov *et al.* (1978) ²⁷ investigated the essential oil constitution and described cuminaldehyde (40.7 %), p-cymene (19.2 %) as the main components, and alpha-terpinene, beta-terpinene and acetic acid were much lower than those reported in this study.

In fruits of Tajikistan origin the main components of *B. persicum* were cuminaldehyde (11.71 %), gamma-terpinene (25.72 %), beta-terpinene (15.62 %) and p-mentha-1,4-dien-7-al (28.98 %). ⁴ We found almost the high amounts of cuminaldehyde and significant lesser amounts of gamma-terpinene in Iranian source of *B. persicum* while beta-terpinene and p-mentha-1,4-dien-7-al were not detected compared to others results. According to Oroojalian *et al.* (2009) ⁷ the main components were cuminaldehyde (16.9 %), gamma-terpinene (44.2 %), gamma-terpinen-7-al (10.5 %), p-cymene (8 %) and. Our results are in agreement with Oroojalian study, although the oil content we found was considerably higher in some cases. In addition, gamma-terpinen-7-al was not detected in the present study. Recently Pourmortazavi *et al.* (2005) ²⁸ investigated the differences in oil constituents obtained by solid phase extraction and hydrodistillation. Remarkable differences were found between the quantitative amounts of the components. Most of the components are present in a higher amount in the hydrodistillation sample that include cuminaldehyde (12.7 %), gamma-terpinene (45.7 %), p-cymene (5.6 %), limonene (10.6 %) and cuminal alcohol (6.4

%) ²⁸ The amount of components was somewhat in agreement with our results.

Generally these results are mainly in agreement with our results. Although there were similarities between these studies however different components was also reported. These chemical differences could be attributed to genetically determined properties, age of the plant and their inhabitate ³⁰ or processing methods for oil extraction ²⁸. Calsamiglia *et al.* (2007) ¹ suggested that researchers should report the concentrations of the main active components in essential oils to prevent inconsistent and confusing results.

The results indicated EOBP is a potential prokinetic and relaxant compound, which may prevent or alleviate dysfunctions of gastrointestinal motility. Further studies are warranted to investigate the effects of various doses and constituents of the EOBP *in vitro* to find out the mechanisms affecting rumen and abomasums motility. Importantly, the antispasmodic action of *B. persicum* would appear to support the popular therapeutic use of the essential oil of this plant in traditional medicine or certain gastrointestinal disorders.

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