Spasmyolytic and Anti-Spasmodic Action of *Trachyspermum ammi* Essence on Rat’s Ileum Contraction

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Abstract

**Background:** *Trachyspermum ammi* is a plant of umbelliferae family and has been traditionally used in the treatment of many gastrointestinal disorders such as indigestion, colic, and diarrhea. Our previous study demonstrated that aqueous extract of *Trachyspermum ammi* reduced the contractile activity of rat’s ileum. **Aims:** This study was designed to examine the effect of *Trachyspermum ammi* essence on acetylcholine-induced contraction in isolated rat’s ileum. **Materials and Methods:** In this experimental procedure, the tissue samples were suspended from the transducer lever and mounted on the organ bath containing Tyrode’s solution. Isotonic responses were recorded by using an isotonic transducer and the amplitude of contractions. This process was induced by cumulative logarithmic concentrations of acetylcholine before and after exposing tissue by saline and different concentrations of essence. **Results:** The chemical constituents of the essence from distillate extract of *Trachyspermum ammi* seeds were studied by gas chromatography mass spectrometry (GC-MS) and the chemical analysis showed many components in which thymol was the main constituent. Our findings showed that the essence derived from the extract in all concentrations used in this study (0.002, 0.005, and 0.01% V/V) significantly reduced acetylcholine-induced contractions (47.34, 60.46, and 86%, respectively, *P* < 0.05). The same concentrations of the essence also exhibit a significant anti-spasmodic action on acetylcholine-induced contractions (0.1, 88.3, and 90.7%, respectively, *P* < 0.05). **Conclusion:** Since thymol was the major constituent of our samples of *Trachyspermum ammi*, the relaxant effect observed in this study is probably due to this agent which can exert an anti-cholinergic property.

Keywords: Acetylcholine, Contraction, Essence, ileum, Smooth muscle, *Trachyspermum ammi*

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Introduction

*Trachyspermum ammi* is a plant of umbelliferae family with a white flower and small brownish seeds. Its major component is essence which is mainly composed of thymol (49.0%), β-terpinene (30.8%), p-cymene (15.7), β-pinene (2.1%), myrcene (0.8%), and limonene (0.7%).[1] *Trachyspermum ammi* has been traditionally used in the treatment of many gastrointestinal disorders such as indigestion, colic, and diarrhea.[2] The extracts obtained from the seeds of carum copticum have several pharmacological effects including anti-cholinergic,[3] analgesic,[4] anti-asthmatic,[5] and anti-tussive[6] activity. Along with the availability of modern medications, the propensity toward the traditional medications is progressively growing throughout the world[7] and promotes the scientific investigators to evaluate the therapeutic effects of medicinal plants and their fractions. In our previous studies, it has been demonstrated that the relaxant effect of carum copticum extract on intestinal motility,[8] and its inhibitory effect on acetylcholine-induced contraction[9] have been demonstrated on isolated rat’s ileum. Since the majority of pharmacologically active constituents of carum copticum have accumulated in its essential oil,[1] this study was conducted to examine the spasmylovic and anti-spasmodic effect of *Trachyspermum ammi* Essence (TAE) on acetylcholine-induced contraction in isolated rat’s ileum.
Materials and Methods

In this study, adult male albino rats weighing 200-250 g, living in the standard environment and feeding conditions, were used for isolation of ileum. All experimental procedures were carried out by permission of the Animal Ethics Committee of Shahid Sadoughi University of Medical Sciences (Yazd, Iran), which was in accordance with the internationally accepted principles for laboratory animal use and care mentioned by the European Community guidelines. *Trachyspermum ammi* seeds were provided by Agricultural Research Center (Yazd, Iran) and identified by a botanist in this center. One hundred grams of air-dried seeds of *Trachyspermum ammi* were gently grounded and mixed with 500 ml of double-distilled water. Then, its essence was prepared by Soxhlet apparatus. The concentration of essence was 1.5% V/V. A sample of the yielded essence was analyzed by Agilent Technologies (Delaware, USA) 6890N network GC system. In this study, 0.002, 0.005, and 0.01% concentrations of essence were examined for their spasmolytic and anti-spasmodic action. Acetylcholine chloride, as a standard stimulant of gastrointestinal smooth muscle was purchased from Sigma Aldrich Chemie Gmbh, Germany. Experiments were performed as described in our previous report.[7,8] Briefly, adult male albino rats were sacrificed by cervical dislocation. Segments of ileum (2 cm in length), were excised, flushed of their contents, and trimmed of their mesentery. The specimens were conserved in Tyrode’s solution until the onset of experimental procedure. The tissue sample was fixed at the bottom of the internal chamber of an organ bath containing 50 ml Tyrode’s solution in the axis of its longitudinal muscle and its opposite end was tightly tied to the isotonic transducer lever with a piece of thread, the chamber was maintained at 37°C and bubbled with 95% O₂ and 5% CO₂. Isotonic responses were recorded by using an isotonic transducer (T2) and an oscillograph recording system (the Bioscience 400 Series Washington Oscillograph). Then, it was allowed to stabilize for 15 min prior to the addition of drug, and washed out in 30-minutes intervals by a fresh Tyrode’s solution. To investigate the spasmolytic action of essence, first we obtain the maximum contraction of ileum using acetylcholine 10⁻⁴ M. Then, we study the effect of different concentrations of essence on maximal contraction. Furthermore, to determine the anti-spasmyotic action of essence, we apply different concentrations of essence to the tissues under study 7 min before the creation of contraction; then, we apply the cumulative concentrations of acetylcholine (10⁻¹² up to 10⁻² M) on tissues to see the effect of the essence on the induced contraction.

Statistical Analysis: The effect of different experimental solutions were expressed as Mean ± standard deviation (SD) of percentage inhibition of contraction amplitude and compared to maximum effect induced by acetylcholine. All statistical analyses and comparisons were made by means of the analysis of variance (ANOVA), followed by Tukey’s test. The statistical significance was considered as \( P < 0.05 \).

Results

The chemical constituents of the essence from distillate extract of *Trachyspermum ammi* seeds were studied by GC-MS. The results showed that there are numerous ingredients in the sample, and thymol was its main constituent [Table 1]. Our findings showed that the essence derived from *Trachyspermum ammi* seeds in concentrations of 0.002, 0.005, and 0.01% reduced acetylcholine (10⁻⁴M)-induced contractions significantly by 52.66 ± 4.12, 39.44 ± 4.46, and 14 ± 2.4, respectively vs. 100 ± 0.00 \( (P < 0.05 \) [Figure 1]. The inhibitory effect of TAE (0.01 %) on acetylcholine-induced contraction was immediately initiated upon its addition to the organ bath, when it reached to its maximum within 0.5 min and persisted at least for 5 min [Figure 2]. On the other hand, the same concentrations of *Trachyspermum ammi* (0.002, 0.005, and 0.01%) showed a significant anti-spasmodic action against cumulative concentrations of 10⁻⁸ up to 10⁻² M acetylcholine \( (P < 0.05 \) [Figure 3]. In the presence of 10⁻³ M acetylcholine, the maximum (96.16%) and minimum (88.17%) inhibition of contraction were induced by 0.01 and 0.002% of *Trachyspermum ammi*, respectively, and the difference between the

| Compound                  | Composition (%) | Retention index |
|---------------------------|-----------------|-----------------|
| Thymol                    | 86.265          | 416             |
| Carvacrol                 | 0.470           | 416             |
| α-terpinyl propionate     | 0.051           | 416             |
| Piperitenone               | 0.071           | 416             |
| Dimethyl phthalate         | 0.699           | 811             |
| 2,4-Di-tert-butylphenol    | 0.610           | 811             |
| Dillapirole               | 0.022           | 342             |
| α-thujene                 | 0.009           | 769             |
| α-pinene                  | 0.020           | 619             |
| β-pinene                  | 0.078           | 619             |
| β-myrcene                 | 0.010           | 662             |
| α-terpinene               | 0.013           | 662             |
| α-Cymene                  | 1.370           | 662             |
| Eucalyptol                | 0.024           | 662             |
| Limonene                  | 0.005           | 662             |
| γ-terpinene               | 0.526           | 662             |
| 4-terpineol               | 0.041           | 684             |
| α-terpineol               | 0.032           | 678             |
anti-spasmodic effects of these two concentrations was statistically significant \( P < 0.05 \), Figure 4. Due to the high concentration of thymol in TAE, the anti-spasmodic effect of TAE and thymol on isolated rat’s ileum was compared [Figure 5]. Each point indicates the mean of six experiments, the vertical bars represent the SD, and *indicates the significant difference between the acetylcholine-induced contractions in the presence of different concentrations of thymol and TAE \( (P < 0.05) \).

**Discussion**

The results of the present study showed that different concentrations of TAE can inhibit a potent spasmolytic and anti-spasmodic effect on isolated rat’s ileum. This finding was consistent with our previous studies in which the aqueous and ethanolic extracts showed a significant relaxant effect on ileal smooth muscle.\[8,9\] However, TAE had more potent inhibitory effect on Ach-induced contractions as compared to aqueous extracts. According to our previous work, only distillate concentrations of more than 1% could inhibit the maximum contractile response of the effective concentration \( (10^{-4} \text{ M}) \) of acetylcholine by about 60%,\[9\] whereas in the present study, the minimum concentration of TAE \( (0.002\%) \) suppressed the contractile activity of \( 10^{-3}\text{M} \) acetylcholine up to 88.17%. The time course for essence in exerting its anti-spasmodic action was also shorter than that of aqueous extracts. The concentration of 0.01% essence exerted its inhibitory effect immediately and reached to its maximum rate within 30 seconds, whereas its aqueous extracts \( (1\%) \) initiated the inhibitory action on acetylcholine-induced contraction after one minute and completed its action within 2 min.\[9\]

The essence of *Trachyspermum ammi* in our sample mainly consisted of thymol, cymene, gamma-terpinene,
The comparison between anti-spasmodic effect of Trachyspermum ammi Essence (TAE) and thymol on isolated rat’s ileum. Each point indicates the mean of six experiments and the vertical bars represent the standard deviation (SD). *indicates the significant difference between the acetylcholine-induced contractions in the presence of different concentrations of thymol and TAE (P < 0.05).

![Graph showing comparison between anti-spasmodic effect of TAE and thymol on isolated rat's ileum.](image)

**Figure 5:** The comparison between anti-spasmodic effect of Trachyspermum ammi Essence (TAE) and thymol on isolated rat’s ileum. Each point indicates the mean of six experiments and the vertical bars represent the standard deviation (SD). *indicates the significant difference between the acetylcholine-induced contractions in the presence of different concentrations of thymol and TAE (P < 0.05).

carvacrol, and pinenes. These compounds have also been previously reported as the major constituents of carum copticum extracts,[1,10] however, the percentage of thymol extracted in our sample was much higher than the previous reports. All of the above-mentioned components have been reported to have potent relaxant effect on smooth muscle in various organs.[11-14]

Various mechanisms are involved in gastrointestinal smooth muscle relaxation. These include the blocking action on excitatory pathways, such as cholinergic[15] and histaminergic[16] or via agonistic action on inhibitory modulators such as adrenergic,[17] purinergic,[18] GABAergic,[19] and/or nitric oxide.[20] Of the components of the extract and its essence, the anti-cholinergic activity of thymol has been reported.[21] On the basis of our previous studies in which the extract showed a significant muscle relaxant effect and also regarding the present study in which the essence has had more potent anti-spasmodic effect, it could be concluded that the observed inhibitory effect of essence on smooth muscle contraction may be through the thymol action. There have also been reported that carum copticum ingredients influence the tracheal smooth muscle via their anti-cholinergic,[22] anti-histaminic,[23] or calcium channel blocking activity.[24] These reports have mentioned that the functional anti-cholinergic effect of its extract and essence could be indirectly related to β-adrenergic stimulatory action or may be due to their direct inhibitory effect on cholinergic receptors.[23]

The inhibitory effect of extracts of this plant on isolated ileum preparations which were pre-treated and post-treated with Ach, indicates their functional antagonistic effect on cholinergic receptors in rat’s ileum. As a rule of thumb, smooth muscle contraction is a calcium- and adenosine triphosphate (ATP)-dependent process, and thymol exerted its relaxant effect through opposing this process.[25] It can also block the Ca$^{2+}$ influxes through the cell membrane[26] and reduction of the calcium content of the sarcoplasmic reticulum.[27] On the other hand, carvacrol which is another pharmacological active constituent of the essence has been shown to possess a significant relaxant effect on smooth muscles through its blocking action on muscarinic receptors and/or β-adrenergic stimulation.[21] It has been demonstrated that Pinene compounds which are present in essence, exhibit a relaxant effect on the tonic contractions induced by either KCl or Ach.[28] Since these inhibitory compounds are mainly deposited in the essence, the more potent spasmylytic effect of essence as compared to extract may be related to the possible mechanisms of actions ascribed to these constituents. Finally, more details of the issue demands further investigation.

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**References**

1. Khajeh M, Yamini Y, Sefidkon F, Bahramifar N. Comparison of essential oil of Carum Copticum obtained by supercritical carbon dioxide extraction and hydrodistillation methods. Food Chem 2004;86:587-91.
2. Avesina A, Sharatkandi A. Law in Medicine. Vol. 2. Tehran: Soroush Press; 1985. p. 187.
3. Devasankaraiah G, Hanin J, Haranath PS, Ramanamurthy PS. Cholinomimetic effects of aqueous extracts from Carum copticum seeds. Br J Pharmacol 1974;52:613-4.
4. Dashti-Rahmatabadi MH, Hejazian SH, Morshedhi A, Rafati A. The analgesic effect of Carum copticum extract and morphine on phasic pain in mice. J Ethnopharmacol 2007;109:226-8.
5. Boskabady MH, Alizadeh M, Jahanbin B. Bronchodilatory effect of Carum copticum in airways of asthmatic patients. Therapie 2007;62:23-9.
6. Boskabady MH, Jandaghi P, Kiani S, Hasanzadeh L. Antitussive effect of Carum copticum in guinea pigs. J Ethnopharmacol 2005;97:79-82.
7. O’Malley P, Trimble N, Browning M. Are herbal therapies worth the risks? Nurse Pract 2004;29:71-5.
8. Hejazian H, Morowatisharifabad M, Mahdavi SM. Relaxant effect of carum copticum on intestinal motility in ileum of rat. World Journal of Zoology 2007;2:15-8.
9. Hejazian-Y SH, Dashiti-R MH, Mahdavi SM, Qureshi MA. The effect of Carum Copticum extract on acetylcholine induced contraction in isolated rat’s ileum. J Acupunct Meridian Stud 2009;2:75-8.
10. Sahib BY, Moharramipou S, Meshkatalsadat MH. Chemical constituents and fumigant toxicity of essential oil from Carum Copticum against two stored product beetles. Insect Sci 2007;14:213-8.
11. Boskabady MH, Ramazani M, Tabei T. Relaxant effects of different fractions of essential oil from Carum copticum on guinea pig tracheal chains. Phytother Res 2003;17:1145-9.
12. Aydin S, Seker E. Effect of an aqueous distillate of Origanum onites L. on isolated rat fundus, duodenum and ileum: Evidence for the role of oxygenated monoterpens. Pharmazie 2005;60:147-50.
13. Prakash O, Kasana VK, Pant AK, Zafar A, Hore SK. Phytochemical composition of essential oil from seeds of Zingiber Roscum Rosc. and its antispasmodic activity in rat duodenum. J Ethnopharmacol 2006;106:344-7.
14. Beer AM, Lukanov J, Sagarchev P. Effect of Thymol on the spontaneous contractile activity of the smooth muscles. Phytotherapy 2007;14:65-9.
15. Unno T, Matsuyma H, Izumi Y, Yamada M, Wess J, Komori S. Roles of M2 and M3 muscarinic receptors in cholinergic nerve-induced contractions in mouse ileum studied with receptor knockout mice. Br J Pharmacol 2006;149:1022-30.
16. Sá-Nunes A, Corrado AP, Barufi MD, Faccioli LH. Disodium cromoglycate prevents ileum hyperreactivity to histamine in Toxocara canis-infected guinea pigs. Pharmacol Res 2003;48:451-5.
17. Roberts SJ, Papaiouannou M, Evans BA, Summers RJ. Characterization of beta-adrenoceptor mediated smooth muscle relaxation and the detection of mRNA for beta1-, beta2- and beta3-adrenoceptors in rat ileum. Br J Pharmacol 1999;127:949-61.
18. Van Crombruggen K, Van Nassauw L, Timmermans JP, Lefebvre RA. Inhibitory purinergic P2 receptor characterisation in rat distal colon. Neuropharmacology 2007;53:287-71.
19. Zizzo MG, Mule F, Serio R. Functional evidence for GABA as modulator of the contractility of the longitudinal muscle in mouse duodenum: Role of GABA (A) and GABA(C) receptors. Neuropharmacology 2007;52:1685-90.
20. Kito Y, Suzuki H. Effects of Dai-kenchu-To on spontaneous activity in the mouse small intestine. J Smooth Muscle Res 2006;42:189-201.
21. Hisayama T, Takayanagi I. Increased 45Ca-efflux from smooth muscle microsomes by a rise in an extra microsomal Ca ion concentration, and the effect of thymol. J Pharm Pharmacol 1983;35:532-3.
22. Boskabady MH, Moetamedshariati V. Bronchodilatory and anti-cholinergic effects of Carum Copticum on isolated guinea-pig tracheal chain. Eur Respir J 1996;23:28s.
23. Boskabady MH, Shaikh J. Inhibitory effect of Carum Copticum on histamine (H1) receptors of isolated guinea-pig tracheal chain. J Ethnopharmacol 2000;69:217-27.
24. Gilani AH, Jabeen Q, Ghayur MN, Janbaz KH, Akhtar MS. Studies on the antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities of the Carum copticum seed extract. J Ethnopharmacol 2005;98:127-35.
25. Tamura T, Iwamoto H. Thymol: A classical small molecule compound that has a dual effect (potentiating and inhibitory) on myosin. Biochem Biophys Res Commun 2004;318:786-91.
26. Peixoto-Neves D, Silva-Alves KS, Gomes MD, Lima FC, Lahlou S, Magalhães PJ, et al. Vasorelaxant effect of the monoterpenic phenol isomers, carvacrol and thymol, on rat isolated aorta. Fundam Clin Pharmacol 2009;24:341-50.
27. Szentandrássy N, Szigeti G, Szegedi C, Sárközi S, Magyar J, Bányaśz T, et al. Effect of thymol on calcium handling in mammalian ventricular Myocardium. Life Sci 2004;74:909-21.
28. Hajhashemi V, Sadraei H, Ghannadi AR, Mohseni M. Antispasmodic and anti-diarrheal effect of Satureja hortensis L. essential oil. J Ethnopharmacol 2000;71:187-92.

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