Spasmolytic Action of the Essential Oil from *Hyssopus officinalis* L. var. *decumbens* and Its Major Components

G. Mazzanti, 1 *M.* Lu 1 and G. Salvatore 2

1 Institute of Pharmacology and Pharmacognosy, University ‘La Sapienza’, P.le Aldo Moro, 5, 00185 Rome Italy
2 Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy

The spasmolytic activity of the essential oil from *Hyssopus officinalis* L. var. *decumbens* (HOD) and its major pure components, linalool, 1,8-cineole and limonene was studied on isolated guinea-pig ileum contracted by acetylcholine and BaCl 2 . HOD and linalool inhibited the acetylcholine- and BaCl 2 -induced contractions in a concentration-dependent manner (IC 50 values: HOD, 37 \mu g/mL and 60 \mu g/mL; linalool 10 \mu g/mL and 51 \mu g/mL). The antagonism appeared non-competitive. In contrast, 1,8-cineole and limonene showed only a weak spasmogen action.

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**Keywords:** *Hyssopus officinalis* L. var. *decumbens*; essential oil; linalool; spasmolytic activity.

**INTRODUCTION**

*Hyssopus officinalis* L. var. *decumbens* (Lamiaceae) is a plant growing in Provence, France. Our recent studies show that the essential oil obtained from its aerial parts has in vitro antimicrobial activity, due mainly to one of its chemical components, linalool (Mazzanti et al., in press). Several essential oils, including those obtained from some Lamiaceae (*Mentha piperita* L., *Salvia officinalis* L. and *Rosmarinus officinalis* L.) possess spasmolytic action (Cabo et al., 1986; Taddei et al., 1988; De la Puerta and Herrera, 1995), which accounts for their use in relieving gastrointestinal spasms (Rees et al., 1979; Leicester and Hunt, 1982). In this study we used an in vitro test to evaluate the spasmolytic activity of an essential oil prepared from *Hyssopus officinalis* L. var. *decumbens* (HOD). In a parallel bioassay we also investigated the major pure components of the oil: linalool; 1,8-cineole and limonene.

**MATERIALS AND METHODS**

**Plant material and corresponding essential oil.** Aerial parts from *H. officinalis* L. var. *decumbens*, were collected in Banon, France, identified and stored in bags under vacuum. Fresh material was steam distilled by SCEA Petit-chêne (Banon) to obtain the HOD. The oil was kept in a refrigerator at 4°C–6°C. Determination of the chemical composition by gas chromatography, confirmed by gas chromatography/mass-spectrometry, identified the following components: linalool (49.0%); 1,8-cineole (14.9%); limonene (5.0%); \(\alpha\)-pinene (2.3%); \(\beta\)-pinene (2.9%); \(\beta\)-caryophyllene (2.7%); caryophyllene oxide (1.7%); camphene (1.7%); sabinene (0.9%); myrcene (1.3%); pinocamphone (0.6%); isopinocamphone (1.4%); \(\beta\)-bourbounene (0.9%) and other unknown components (1.4%) (Salvatore et al., in press).

**Substances.** Acetylcholine chloride, BaCl 2 , linalool (97% pure), 1,8-cineole (99% pure) and limonene (97% pure) were obtained from Sigma-Aldrich. For use in biological tests, acetylcholine and BaCl 2 were dissolved in distilled water then diluted in Tyrode solution; the acetylcholine concentration is expressed as free base. HOD, linalool, 1,8-cineole and limonene were dissolved in dimethyl sulphoxide (DMSO) at a ratio 1:9 (w/w). Preliminary experiments showed that DMSO, at the maximum volume used (10 \mu L), left the response to acetylcholine and BaCl 2 unchanged.

**Spasmolytic activity.** Biological activity was evaluated on isolated guinea-pig ileum preparations. Male Hartley guinea-pigs, weighing 300–350 g, deprived of food intake for 24 h before the experiments, were used. Guinea-pigs were killed by cervical dislocation and the abdominal cavity was opened by a midline incision. Segments of guinea-pig ileum 2–3 cm long were removed after thorough cleaning of the surrounding peritoneal tissues. The preparations were suspended in a 10 mL organ bath containing Tyrode solution at 37°C, bubbled with 95% O 2 and 5% CO 2 gas mixture, and were attached to an isotonic force displacement transducer (Ugo Basile - Varese, Italy - model 7006). Responses were recorded with a microdynamometer (Basile model 7050). A resting tension of 1 g was applied to each organ.
and the tissues were allowed to stabilize for 30 min before the experiments. The spasmolytic activity of HOD was evaluated using the cumulative dose-response procedure described by Van Rossum (1963); Ach ($1.7 \times 10^{-7} - 1.7 \times 10^{-4} M$) and BaCl$_2$ ($3.0 \times 10^{-5} - 1.0 \times 10^{-2} M$) were used as contracting agents. The potency of the agonists was evaluated by determining pD$_2$ values; pD$_2$ is the negative logarithm of $A_{50}$ (the agonist concentration inducing 50% of the maximal response). The antagonistic activity of HOD and its components was evaluated by constructing two agonist concentration-response curves and re-determining the concentration-response curve after incubation of the organ for 5 min with the antagonist. The potency of HOD and its pure compounds as relaxant agents is expressed as IC$_{50}$ (the antagonist concentration that reduces by 50% the maximal response induced by the contracting agent).

$A_{50}$ and IC$_{50}$ were calculated by analysing the regression line obtained with the minimum squares method; to compare the regression lines we used the parallel lines test (Tallarida and Murray, 1981).

RESULTS AND DISCUSSION

As expected, the contracting agent acetylcholine had higher agonist potency than BaCl$_2$, (pD$_2$ values, 5.1 ± 0.22 vs 3.6 ± 0.03).

The essential oil prepared from Hyssopus officinalis L. var. decumbens (HOD) showed a spasmolytic activity on isolated guinea-pig ileum. It inhibited acetylcholine- and BaCl$_2$-induced contractions in a concentration-dependent manner. At a concentration of 42 µg/mL it inhibited the maximal response to acetylcholine by about 80%, and the maximal response to BaCl$_2$ by about 40% (Fig. 1A and B). Washing reversed the antagonism. Analysis of the
data showed a linear relationship; the values of correlation coefficients (r) were 0.997 for acetylcholine and 0.992 for BaCl₂. The corresponding IC₅₀ values were 37 μg/mL for acetylcholine and 60 μg/mL for BaCl₂. The dose-response curves for acetylcholine with and without HOD (17 μg/mL) had significantly different slopes (37.8 ± 4.66 vs 21.5 ± 2.97). The dose-response curves of BaCl₂ with and without HOD (42 μg/mL) also had significantly different slopes (40.7 ± 6.21 vs 23.4 ± 2.35). These results and the shape of the agonist concentration-response curves in the presence of HOD—showing that the agonists no longer evoked the maximum tissue contraction—suggest non-competitive antagonism.

Linalool, the main component of HOD, showed a similar spasmolytic action on guinea-pig ileum (Fig. 2A and B). It inhibited the acetylcholine- and BaCl₂-induced contractions in a dose-dependent manner (IC₅₀ values, 10 μg/mL for acetylcholine and 51 μg/mL for BaCl₂). Again the antagonism appeared non-competitive.

Conversely, neither 1,8-cineole nor limonene showed spasmolytic activity on guinea-pig ileum, indeed they slightly contracted the tissues. These results show that HOD has a spasmolytic action on isolated guinea-pig ileum. In doing so it acts on receptor-stimulated and on ion-stimulated contractions. The concentration-response curves indicate a non-specific mechanism of action. Our studies on the major pure components of the essential oil show that the constituent mostly responsible for the spasmolytic activity is linalool. This substance, which constitutes 49% of the essential oil prepared from *Hyssopus officinalis* L. var. *decumbens* is a more potent relaxant agent than the oil itself. Whether other minor components also contribute to its relaxant action remains to be investigated.

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**REFERENCES**


