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Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

LOCAL ANAESTHETIC AVTIVITY OF ESSENTIAL OIL OF LAVANDULA ANGUSTIFOLIA / C. GHELARDINI; N. GALEOTTI; G. SALVATORE; G. MAZZANTI. - In: PLANTA MEDICA. - ISSN 0032-0943. - STAMPA. - 65:(1999), pp. 700-703. [10.1055/s-1999-14045]

Availability:

The webpage <https://hdl.handle.net/2158/15805> of the repository was last updated on 2017-10-31T12:00:35Z

Published version:

DOI: 10.1055/s-1999-14045

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Local Anaesthetic Activity of the Essential Oil of *Lavandula angustifolia*

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Received: January 7, 1999; Accepted: June 12, 1999

Abstract: In this work we studied the local anaesthetic activity of the essential oil obtained from *Lavandula angustifolia* Mill., a medicinal plant traditionally used as an antispasmodic. We compared its activity to the essential oils obtained from two citrus fruits, *Citrus reticulata* Blanco and *Citrus limon* (L.) Burm. f., which have no medical uses. Biological tests were also performed on the major pure components of *L. angustifolia* Mill. essential oil: linalol and linalyl acetate as determined by GC and confirmed by GC-MS. Anaesthetic activity was evaluated *in vivo* in the rabbit conjunctival reflex test, and *in vitro* in a rat phrenic nerve-hemidiaphragm preparation. The essential oil of *L. angustifolia*, linalyl acetate and linalol (0.01–10 µg/ml) but not the oils of *Citrus reticulata* and *Citrus limon* were able to drastically reduce, in a dose-dependent manner, the electrically evoked contractions of rat phrenic-hemidiaphragm. In the rabbit conjunctival reflex test treatment with a solution of essential oil of *L. angustifolia*, as well as linalyl acetate and linalol (30–2500 µg/ml administered in the conjunctival sac) allow a dose-dependent increase in the number of stimuli necessary to provoke the reflex, thus confirming *in vivo* the local anaesthetic activity observed *in vitro*.

Key words: *Lavandula angustifolia*, Lamiaceae, essential oil, linalol, linalyl acetate, local anaesthetic activity.

Introduction

Several medicinal plants, including *Lavandula angustifolia* Mill., *Mentha piperita* L., *Salvia officinalis* L. (Lamiaceae), *Foeniculum vulgare* Mill. and *Carum carvi* L. (Umbelliferae), possess antispasmodic and/or spasmolytic properties that justify their traditional use for digestive problems (1), (2), (3). The chemical components responsible for this biological activity are the essential oils, some of which have been used to relieve gastrointestinal spasms (4), (5). Studies performed *in vitro* on antispasmodic action of essential oils extracted from the above mentioned plants show that they are capable of blocking the contractions induced by various spasmogens, acting by means of a pharmacological mechanism, which points to an unspecific antagonism (6), (7). It has been suggested that, due to

their high lipid solubility, the essential oils may interact with the lipid bilayer of the plasma membrane, inhibiting Ca⁺⁺ influx or preventing the increase in Na⁺ permeability, and thereby blocking the neurotransmission (8).

Furthermore, it is known that eugenol is a membrane stabilizing drug with local anaesthetic activity (9), (10), (11) and that menthol is one component of analgesic and local anaesthetic preparations (12), (13).

In this work we studied *L. angustifolia* essential oil in order to evaluate its possible local anaesthetic activity. The main components of this essential oil are linalol and linalyl acetate. In order to establish a ratio between the potential local anaesthetic activity of *L. angustifolia* essential oil and its chemical composition we parallelly examined two essential oils of *Citrus* fruits which contain the cyclic monoterpene limonene as major component. The investigated compounds were obtained from *L. angustifolia* Mill. (Lamiaceae), a medicinal plant traditionally used as an antispasmodic, sedative and antiseptic drug (14), (15) and from *Citrus reticulata* Blanco and *Citrus limon* (L.) Burm. f. (Rutaceae), that have no medical uses. Our previous study showed that linalol has a noteworthy antimicrobial activity *in vitro* while limonene is poorly active (16); in this context it appeared of interest to study comparatively the *L. angustifolia* and the *Citrus* oils.

Materials and Methods

Animals

Male Wistar rats (150–200 g), male albino guinea-pigs (300–400 g) and male New Zealand rabbits (2000–3000 mg) from Morini (San Polo d'Enza, Italy) breeding farms were used. The cages were placed in the experimental room 24 h before the test for acclimatization. The animals were kept at 23 ± 1 °C with a 12 h light/dark cycle, light at 7 a.m., with food and water *ad libitum*. All experiments were carried out according to the guidelines of the European Community Council for experimental animal care.

Rat phrenic nerve-hemidiaphragm preparation

Experiments were performed according to the method described by Bülbring (17), and modified by Wessler and Kilbinger (18). The effect of drugs in the presence of electrical

stimulation (0.2 Hz, 0.5 msec, double threshold voltage) was calculated as the percentage variation of electrically-evoked contractions in the presence of the drug versus pre-drug evoked efflux.

Rabbit conjunctival reflex

The test was performed according to the method described by Donatelli and Buffoni (19). Briefly the external side of the rabbit eye was stimulated with a cat whisker to induce a conjunctival reflex. The local anaesthetic activity of the drug dropped in the rabbit conjunctival sac is evidenced by the necessity of a higher number of stimuli to provoke the palpebral closure.

Essential oils and reference substances

L. angustifolia essential oil was provided by Janousec Industriale (Muggia, TS, Italy) while *Citrus reticulata* and *Citrus limon* essential oils were produced by Simone Gatto factory (S. Pier Miceto, ME, Italy). The chemical composition of the oils was determined at the Department of Comparative Toxicology and Ecotoxicology, (Istituto Superiore di Sanità, Viale Regina Elena 299, Rome, Italy). The pure reference substances, (\pm)-linalol and linalyl acetate (both 97% pure) were purchased from Sigma-Aldrich; procaine hydrochloride and lidocaine hydrochloride were purchased from RBI (Milan, Italy). For biological tests the oils and pure substances were solubilized in a vehicle of 0.5% solution of DMSO in H₂O. Procaine hydrochloride and lidocaine hydrochloride were purchased from Sigma (Milan, Italy).

Gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS)

Gas chromatography equipment used included: a Perkin Elmer AutoSystem equipped with two fused-silica SPB 5 columns (60 m \times 0.25 mm i.d., film thickness 0.25 μ m), mounted in parallel in the same oven, with two detectors: FID and Q-Mass 910 (electron ionization 70 eV electron energy, transfer line 220 °C). Carrier gas was oxygen and moisture-free helium obtained from SUPELCO™ High capacity Heated Carrier Gas Purifier, provided with OMI-2 indicating tube, at the average flow rate of 1 ml/min. Oven temperature programme was 60 °C for 4 min, then 2 °C/min to 180 °C, then 3 °C/min to 250 °C. Detector temperature was 280 °C; injector temperature: 280 °C. The volume of injected essential oil or pure substance was 0.1 μ l and split ratio was 1:50. Two distinct data systems are connected to the GC-FID or GC-MS: Turbochrom and Q-Mass Analytical Workstation Software with NIST/EPA/MSDC Mass Spectral database, respectively.

Chemical identification and quantitative estimation

Chemical components were identified by co-gas chromatography (taking in account their retention times) and the known retention indices according to Adams (20) and the GC/MS with authentic substances as well as comparison with spectra of the NIST/EPA/MSDC Mass Spectral Database. Quantitative data were based on peak area normalization without use of correction factors.

Statistical analysis

All experimental results are given as the mean \pm S.E.M. Analysis of variance (ANOVA), followed by Fisher's Protected Least Significant Difference (PLSD) procedure for post-hoc comparison, was used to verify significance between two means. Data were analysed with the StatView software for the Macintosh (1992). P values of less than 0.05 were considered significant.

Results

The main components of the tested essential oils are reported in Table 1. The components are listed in order of their elution on the SPB5 column with their percentage. Regarding the *L. angustifolia* oil the main components, accounting for 86.5%, were identified.

Table 1 Main constituents of the essential oil of *Lavandula angustifolia* Mill., *Citrus reticulata* Blanco and *Citrus limon* (L.) Burm. f.

Components %	Retention indices*	<i>Lavandula angustifolia</i> Mill.*	<i>Citrus reticulata</i> Blanco	<i>Citrus limon</i> (L.) Burm. f.
α -Tujene	931		1.1	0.6
α -Pinene	939		2.9	2.6
Sabinene	976		0.4	2.7
β -Pinene	980		2.6	15.7
Myrcene	991	0.7	2.2	2.0
Limonene	1031		61.1	55.2
1,8-Cineole	1033	0.9		
β -Ocimene	1040	1.8		0.2
γ -Terpinene	1062		25.1	12.7
Terpinolene	1088		1.1	0.5
Linalol	1098	31.5		
Unidentified	**	3.0		
Neral	1240			1.4
Linalyl acetate	1257	43.6		
Geranial	1270			2.3
N-Methylmethyl-anthranylate	1402		0.5	
β -Caryophyllene	1418	5.0		
Bergamottene	1436			0.4
β -Bisabolene	1504			0.7

* Retention indices on apolar column as reported by Adams (1995).

** This component showed a spectrum [MS: m/z (%) 154 (M^+ 19.6), 136 (80.3), 121 (90.7), 119 (16.8), 111 (34.0), 105 (15.7), 93 (100), 92 (21.2), 91 (60.4), 79 (21.0), 77 (30.0), 71 (22.9)] with the higher SI (Similarity Index) for the γ -terpineol, as reported in NIST/EPA/MSDC Mass Spectral Database.

The essential oil of *L. angustifolia*, as well as linalyl acetate and linalol, in the concentration range of 0.1–1000 μ g/ml were able to reduce, in a dose-dependent manner, the electrically evoked contractions of rat phrenic-hemidiaphragm *in vitro*, up to complete abolishment of contractions for *L. angustifolia* oil (Fig. 1) and linalyl acetate (Fig. 2) and up to 25% by linalol (Fig. 3). In the same experimental conditions a similar profile to that shown by the essential oil of *L. angustifolia*, linalyl acetate and linalol, was exhibited by two classical local anaesthetics: procaine (Fig. 1) and lidocaine (Fig. 2) used as reference drugs (12). In contrast, the oils of *C. reticulata* and *C. li-*

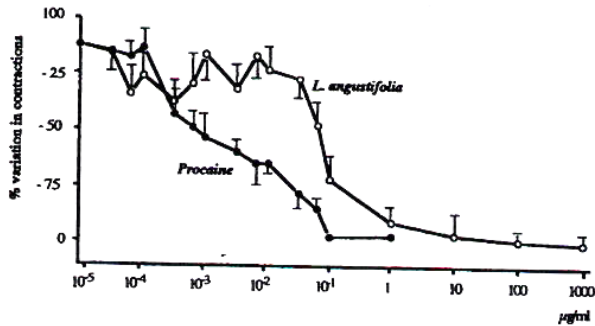


Fig. 1 Dose-response curve of *Lavandula angustifolia* essential oil in comparison with procaine on electrically-evoked contractions of phrenic nerve-hemidiaphragm. Each point represents the mean of 4 experiments. Vertical lines give s.e.m.

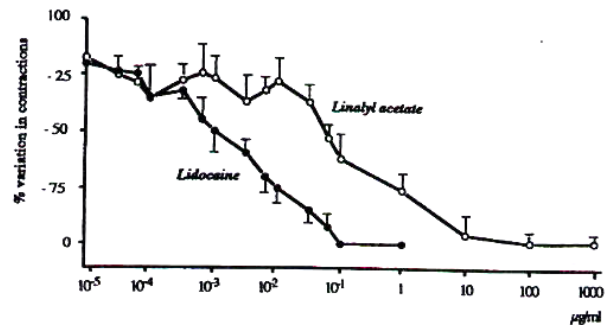


Fig. 2 Dose-response curve of linalyl acetate in comparison with lidocaine on electrically-evoked contractions of phrenic nerve-hemidiaphragm. Each point represents the mean of 5 experiments. Vertical lines give s.e.m.

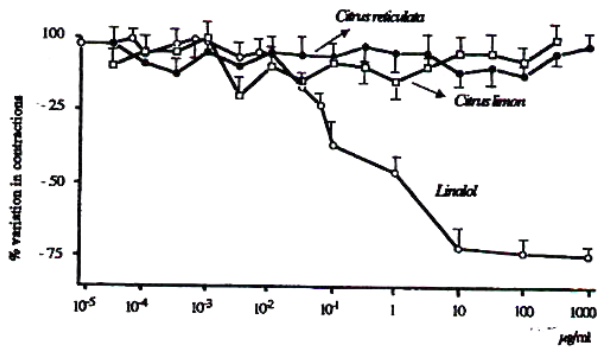


Fig. 3 Dose-response curve of linalol and of essential oil of *Citrus reticulata* and *Citrus limon* on electrically-evoked contractions of phrenic nerve-hemidiaphragm. Each point represents the mean of 5 experiments. Vertical lines give s.e.m.

mon were devoid of the ability to reduce electrically-evoked contractions (Fig. 3). The essential oil of *L. angustifolia*, as well as linalyl acetate and linalol, did not modify the contractions evoked through direct stimulation of the diaphragm muscle (data not shown).

In vivo the local anaesthetic activity of the above mentioned essential oil and of pure components was confirmed in the conjunctival reflex test in the rabbit. Treatment with a solution of essential oil of *L. angustifolia* (30–2500 µg/ml administered in the conjunctival sac) permitted a dose-dependent increase in the number of stimuli necessary to provoke the reflex (Fig. 4). Figure 4 shows that linalyl acetate and linalol, at the same doses as *L. angustifolia* oil, also allow an increase in the number of stimuli necessary to induce conjunctival reflex. On the contrary, in the same experimental conditions, the *C. reticulata* and *C. limon* oils, up to a dose of 2500 µg/ml administered in the conjunctival sac, and the vehicle constituted by a 0.5% solution of DMSO in H₂O, were devoid of any effect (Fig. 4). The local anaesthetic activity exhibited by essential oil of *L. angustifolia*, linalyl acetate and linalol was observed starting 5 min after administration, then quickly diminished and disappeared within 15 min (Fig. 5).

Linalyl acetate and linalol injected subcutaneously at concentrations of 10–2500 µg/ml, also inhibited cutaneous muscle reflex in guinea-pig dorsal skin (data not shown).

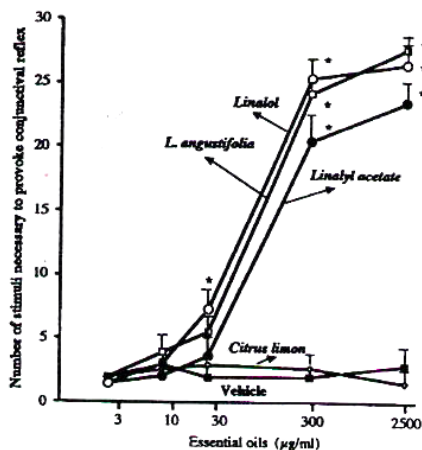


Fig. 4 Dose-response curves of linalyl acetate, linalol and of the essential oil of *L. angustifolia*, and *Citrus limon* in the rabbit conjunctival reflex test evaluated 5 min after administration. $P < 0.01$. Each value represents the mean of 4 independent experiments. Vertical lines give s.e.m.

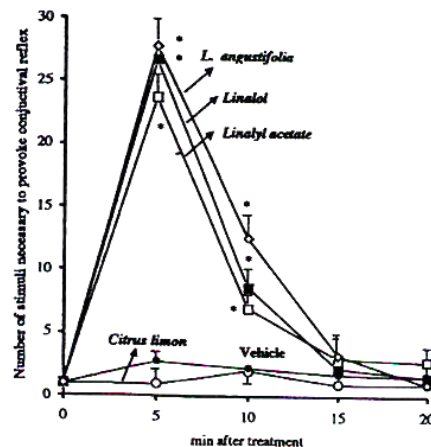


Fig. 5 Effect of linalyl acetate, linalol and the essential oil of *L. angustifolia* and *Citrus limon* in the rabbit conjunctival reflex test. All drugs were administered at the dose of 2500 µg/ml. $P < 0.01$. Each value represents the mean of 4 independent experiments. Vertical lines give s.e.m.

Discussion

Our results show that the essential oil from *L. angustifolia* possesses local anaesthetic activity which could be responsible, at least in part, for its muscle relaxing properties. Linalyl acetate exhibits a very similar profile both *in vitro* and *in vivo* to linalol, the other major component of *L. angustifolia* oil. The local anaesthetic activity appears to be strictly dependent on its chemical composition since *Citrus* oils are devoid of it. The major components of *L. angustifolia* oil are linalol and linalyl acetate whereas those of *Citrus* oils are limonene, β -pinene and γ -terpinene. All these substances are terpenic compounds and possess lipophilic characteristics, but linalol and linalyl acetate also contain a hydrophilic unit that is not present in the *Citrus* oil components; this unit may be important for biological activity.

Regarding the mechanism of action we can exclude that the essential oil from *L. angustifolia* is endowed with antimuscarinic activity since in the rat hemi-diaphragm test the antagonists of muscarinic receptors increase the electrically-evoked contractions starting at 10^{-6} M (21).

It has been observed that linalol inhibits the acetylcholine release and reduces the channel open time in the mouse neuromuscular junction (22). According these data the local anaesthetic activity of *L. angustifolia* essential oil could be due to the ability of its components to block Na^+ and/or Ca^{++} channels.

Acknowledgements

The authors wish to thank Mary Forrest for linguistic revision of the manuscript. This work was supported by a grant from MURST.

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