Linalool: a review on a key odorant molecule with valuable biological properties

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ABSTRACT: This paper reports on the occurrence, biosynthesis, metabolism, biological and toxicological profile, and assessment of the authenticity of linalool. The main biological properties of linalool – sedative, anxiolytic, analgesic, anticonvulsant, anti-inflammatory, local anaesthetic – are discussed in terms of the molecule's chirality influence, the mechanisms of activity and type of study (*in vitro*, *in vivo*, clinical studies). Also, there is a discussion of the recent data on the skin sensitizing potential of linalool based on numerous scientific studies which have been performed in the last few years. Comments of the authenticity assessment of linalool are made considering the limitations imposed by the chemical structure, vegetal matrix or processing methods, but also from the perspective of the powerful and sophisticated analytical techniques available today (GC-C-IRMS, enantio-MDGC coupled to GC-C-IRMS, SNIF-NMR). Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: linalool; fragrance; biological activities; authenticity; review

Introduction

Linalool (3,7-dimethyl-1,6-octadien-3-ol) is an acyclic monoterpene tertiary alcohol and also one of the major floral scents in nature. About 70% of the terpenoids of floral scents are represented by linalool.[1] Because of the chiral properties of its hydroxylated third carbon, two linalool enantiomers occur in plants: (3S)-(+)-linalool (coriandrol) and (3R)-(−)-linalool (licareol) (Figure 1). These enantiomers have different fragrance profiles: coriandrol is perceived as sweet, floral, herbaceous and petitgrain-like with citrus and fruity notes while licareol has a woody, lavender-like aroma. The odour threshold value of the (R)-enantiomer is about nine times smaller than that of (S)-linalool (0.8 vs. 7.4 ppb).[2–4] In some vegetal products (passion fruit, apricots), linalool is found as racemate; the racemate is also a product of the fermentation processes that occur in foodstuff manufacture and during isolation of the essential oils depending on the extraction technique.[5]

In perfumery, linalool is a very much used fragrant ingredient being a component of the perfumes' top notes. Therefore, linalool, mainly of synthetic origin, is found in 60–90% of cosmetic products (body lotions, shampoos, shower gels, soaps, hairsprays, creams, antiperspirants).[6] It is also added to household detergents, furniture care products, waxes, as well as to processed food and beverages, as a fragrance and flavour agent. The International Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an Acceptable Daily Intake (ADI) of 0–0.5 mg/kg body weight/day for linalool. In industry, it is an important intermediate in the vitamin E synthesis, but it is also used for the production of vitamin A, farnesol, ionones and citronellol. Besides, linalool is used as insecticide for pet ectoparasite control. The annual worldwide consumption of linalool exceeds 1000 metric tons.[6,7]

Occurrence in Nature

Linalool is found in the essential oils of over 200 monocotyledonous and dicotyledonous plant species, belonging to different families (over 50% of plant families)[1] widely spread from tropical areas to boreal regions. In particular, many plants of Lamiaceae, Lauraceae and Rutaceae families produce (R)-(−)- or (S)-(+)-linalool in significant amounts.[8] Rosewood oil and Ho oil are the main sources of (R)-(−)-linalool (90% and more),[9] while coriander oil and orthodon oil contain important levels of (S)-(+)-linalool (over 80%) (Table 1). Also, some groups of fungi produce this monoterpene alcohol.[8] 3(R)-(−)-linalool is more common in nature.

Synthetic Linalool

In addition to natural sources, linalool is obtained by semisynthesis from α, β-pinene or other terpenes (geraniol, nerol, myrcene) and also by organic synthesis via 2-methyl-2-hepten-6-one.[8,10] Synthetic linalool contains traces of dihydrolinalool and dehydrolinalool.[11] Also, there might be present chlorinated impurities that confer a metallic character to linalool odour.[12]

Biosynthesis

Linalool is biosynthetisized in floral and non-floral tissues from isopentenyl pyrophosphate (IPP) and its isomer, dimethylallyl diphosphate (DMAPP). The two C5 units, IPP and DMAPP, are generated in the 2-methylerythritol-4-phosphate (MEP) pathway in plastids, starting from pyruvate and glyceraldehyde 3-phosphate (GA-3P), via deoxy-D-xylose 5-phosphate (DOXP).[36,37] Condensation of one molecule of each compound, IPP and DMAPP, leads

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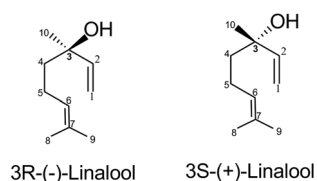
Figure 1. The linalool enantiomers

to geranyl diphosphate (GPP), the universal precursor of monoterpenes.[38] GPP is a substrate for linalool synthases (LIS and R-LIS), enantioselective membrane-bound monoterpene synthases (Figure 2). At the same time, linalool, as many other terpenoids, may be produced in minor biosynthetic pathways as by-products of other processes such as geraniol and nerol biosynthesis. In plants, linalool accumulates in compartmentalized secretory structures of glandular trichomes or is emitted into the environment.[39]

Metabolism

Experimental studies on rats, performed with ^{14}C-labelled linalool (500 mg/kg bw), showed that it is rapidly absorbed from the intestinal tract after oral or gavage administration. In humans and animals, after absorption, most of linalool is quickly metabolized in the liver to polar compounds that are mainly

excreted in the urine as free form or conjugates; to a lesser extent, the metabolites are excreted in the faeces. The main metabolic pathway of linalool involves conjugation with glucuronic acid. In the case of repeated administration, allylic oxidation becomes an important metabolic pathway, being mediated by cytocrome P-450 system. 8-Hydroxy- and 8-carboxy-linalool were detected as major metabolites after 20 days administration of 800 mg linalool/kg bw/day. A minor route of linalool metabolism consists in its partial ring closure to α-terpineol, with the generation of small amounts of geraniol and nerol. These metabolites are also excreted in urine as free forms or conjugates. Products of linalool reduction (dihydro-, tetrahydrolinalool) were also identified in rodent urine.[8,40] A significant proportion of oral linalool follows intermediary metabolism pathways and is eliminated in exhaled air as CO_2 (Figure 3).[8]

Biological Activities

Linalool is one of the most investigated odorant molecules. Numerous studies have been published reporting different biological activities for linalool. Most of the researches were performed *in vitro* or in animals using various routes of administration, but there is still a lack of consistent clinical studies on linalool. The chirality of linalool determines not only the sensory character, but also the biological and pharmacokinetic

Table 1. Main plant essential oil sources of linalool

| Essential oil	Botanical source	Linalool content (%); characteristic enantiomer	Ref.
Rosewood, bois de rose	Wood of *Aniba rosaeodora* Ducke, Lauraceae	about 100; *R*-(−)	13
Ho leaf	Leaf of *Cinnamomum camphora* Nees & Eberm var. *linaloolifera*, Lauraceae	66–95; *R*-(−)	14
Ho (China) = Shiu (Japan, Taiwan)	Wood of *Cinnamomum camphora* Nees & Eberm var. *linaloolifera*, Lauraceae	90; *R*-(−)	15
Orthodon oil	Aerial parts of *Orthodon linalooliferum* Fujita, Lamiaceae	82; *S*-(+)	16
Coriander	Fruits of *Coriandrum sativum* L., Apiaceae	45–85; *S*-(+)	17,18
Lavender	Flowering tops of *Lavandula officinalis* Chaix sin. *L. angustifolia* Mill., Lamiaceae	25–38; *R*-(−)	18,19
Spike lavender	Flowering tops of *Lavandula latifolia* (DC) Vill., Lamiaceae	19–48; *R*-(−)	19
Linaloe (Mexican lavender, or Indian lavender)	Wood of *Bursera delpechiana* Poiss ex.Engl, *Bursera* spp., Burseraceae	30; *R*-(−)	20,21
Bushy lippia	Leaves of *Lippia alba* (Mill.) N.E.Br. ex Britton & P.Wilson, Verbenaceae	65; *S*-(+)	3,22
Winged prickly-ash	Seeds of *Zanthoxylum alatum* Roxb., Rutaceae	71; ND	23
Sweet basil	Leaves of *Ocimum basilicum* L., Lamiaceae	26–50; *R*-(−)	22,24,25
Holy basil (tulsi)	Leaves of *Ocimum sanctum* L., Lamiaceae	26; *S*-(+)	25,26
Hoary basil	Leaves of *Ocimum canum* Sims, Lamiaceae	25; *S*-(+)	16
Ylang-ylang	Flowers of *Cananga odorata* (Lam.) Hook f. & Thomson *forma genuina*, Annonaceae	15–24; *R*-(−)	27,28
Neroli	Blossom of *Citrus aurantium* L., Rutaceae, bitter orange	28–40; *R*-(−)	3,29
Sweet orange	Flowers of *Citrus sinensis* Osbeck, Rutaceae	15–32; *S*-(+)	3,30
Sweet marjoram	Aerial parts of *Origanum majorana* L., Lamiaceae	30–40; *S*-(+)	31
Bitter orange petitgrain oils	Leaves and twigs of *Citrus aurantium* L., Rutaceae	> 27; *S*-(+)	32,33
Clary sage	Aerial parts of *Salvia sclarea* L., Lamiaceae	10–21; racemate	34
Laurel	Leaves of *Laurus nobilis* L., Lamiaceae	16; *R*-(−)	35

ND, not determined
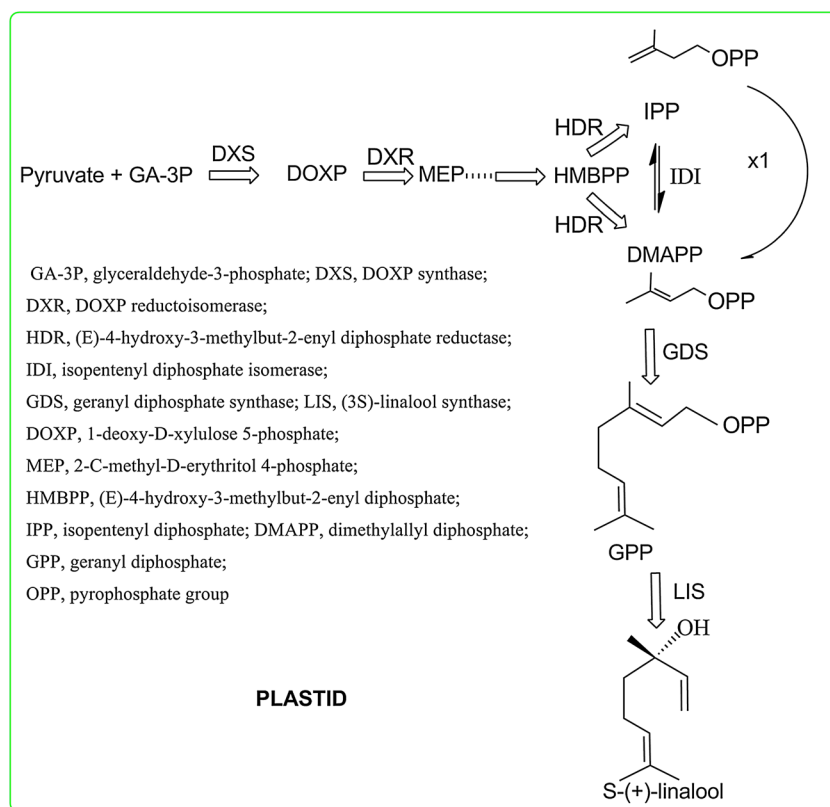
Figure 2. Biosynthesis of (S)-(+)-linalool[37]

properties. The question that arises is to what extent chirality affects the pharmacological activity. What are the differences between the two optical antipodes of linalool? Or what are racemate activities? Is one enantiomer more potent than the other? The most important biological activities demonstrated for linalool correlated with the mechanism of activity and chirality involvement are presented below.

Sedative Activity

Lavender is one of the oldest remedies that man has used in various forms (massage, aromatherapy, medicinal baths) for the calming, relaxing effect, nervous tension abolishing and sleep inducing properties. The sedative activity of lavender essential oil and linalool (R)-, (S)-enantiomers, racemate) has been assessed in different experimental models and clinical trials (Table 2). In animals, pretreated with caffeine administered intraperitoneally (i.p.), the sedation produced by inhaling lavender essential oil was even more pronounced than that of the controls.[41] The sedative activity of linalool (racemate or unspecified enantiomer) is dose dependent and includes hypnotic and hypothermal effects, an increase in sleep time induced by pentobarbital and a reduction of spontaneous locomotion, without affecting motor skills.[42–44] In addition, linalool racemate and lavender essential oil exhibit behavioural sedative-like effects by decreasing renal sympathetic nerve activity and increasing parasympathetic nerve activity.[45,46] In humans, inhalation of lavender essential oil and (R)-(−)-linalool causes sedation, relaxation, reduced aggressiveness and hostility.[47] The effect seems to be not only dependent on the chirality of the molecule, but also on the tasks assigned to the subjects. The inhalation of (R)-(−)-linalool

after hearing environmental sounds produces a sedative effect associated with a noticeable decrease of beta waves. After mental work, inhalation of (R)-(−)-linalool leads rather to a state of agitation and alertness as well as to an increase of beta waves. The racemate showed similar effects, although not very consistent in the case of mental work, while (S)-(+)-linalool behaved differently, causing reversal effects.[48,49] In humans exposed to experimentally induced stress, inhalation of linalool causes specific physiological responses depending on the molecule chirality. (S)-(+)-Linalool acts as an activator agent in terms of blood pressure and heart rate, while (R)-(−)-linalool has an opposite effect on heart rate, functioning as a stress-relieving agent.[50,51] Lavender essential oil behaves similarly, although relaxation may be influenced by the previous association with its characteristic aroma.[52,53]

The experimental findings support the hypothesis that the sedative effect of linalool can be accomplished by modulation of glutamatergic neurotransmission. In vitro and in vivo studies showed that linalool racemate acts as a competitive antagonist of the excitatory neurotransmitter glutamate binding to glutamatergic N-methyl-D-aspartate (NMDA) receptors.[54,55] In addition, linalool racemate reduces the potassium stimulated-release of glutamate.[56] There is still little evidence to explain these mechanisms and the full picture of this compound's influence on the cognitive functions. Due to the involvement of NMDA receptors in brain plasticity, future studies should elucidate to what extent their chronic blockade by linalool could impair memory and learning processes. Besides, given that in studies on human subjects, (R)-(−)-linalool proved to have the best well-defined sedative activity, it would be recommended to use it in pharmacological tests. The interaction with biological receptors involves a specific configuration of the ligand
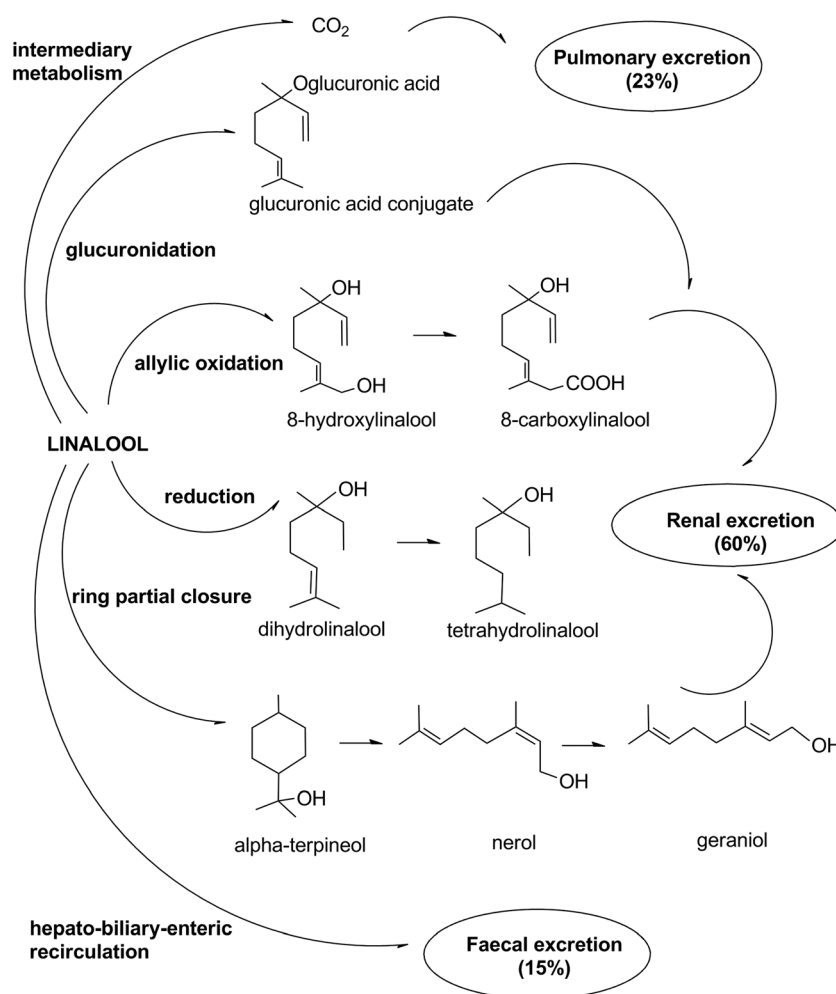
Figure 3. Main pathway of linalool metabolism in mammals[8,40]

molecule. In the case of optically active compounds, the affinity for the receptors, the interaction with the receptors, as well as the intensity of the effect are essentially determined by the type of enantiomer. Studies should also include positive controls and, in the case of lavender essential oil, it is necessary to specify not only the linalool content but also the enantiomeric purity of linalool.

Anxiolytic Activity

Anxiety disorders are the most prevalent mental issues and commonly encountered in modern societies. They affect about 16.6% of the world's population[57] and 14% of that of Europe.[58] The clinical picture is complex and is characterized mainly by exacerbated worry, concern and fear, alongside other emotional, cognitive, behavioural and somatic symptoms. Anxiety illnesses may acquire severe and debilitating touches, interfering with the normal daily life of the subject, leading to altered mental status and quality of life and assuming large social and economic costs. In classical medicine, anxiety is often treated with benzodiazepines which show a wide range of undesirable side effects including sedation, fatigue, impaired cognitive and motor functions, depression and, in the long term, development of tolerance, drug dependence and addiction. The identification of drugs having the ability to diminish anxiety disorder without

causing serious side effects, but especially without harming cognitive and motor functions, is the biggest challenge in the treatment of this disease. In folk medicine, lavender and its essential oil are some of the most popular anxiolytic phytotherapeutics. Various preclinical studies such as Geller and Vogel anticonflict tests in mice,[59] open field test in rats[60] and elevated plus-maze test in Mongolian gerbils[61] have demonstrated anxiolytic effects for lavender oil (Table 3). The activity profile of lavender oil, particularly in chronic administration, is similar to that of diazepam or close to that of chlordiazepoxide, well-known anxiolytic drugs.[59,62] The anxiolytic effects are dose-dependent and also exposure time-dependent (in case of inhalation). High doses of lavender essential oil and a prolonged exposure time may cause a sedative activity and a possible impairment of the locomotor activity. In mice, inhalation of 3% linalool (unspecified enantiomer) for one hour increases social interaction and decreases aggressive behavior in the light/dark tests, but it adversely affects memory in high concentrations.[63] In addition, administration of (*R*)-(−)-linalool (50, 100 mg/kg, i.p.) to rats influences different types of memory, the most affected being long-term memory in the majority of tests and in both doses.[64]

There are clinical studies on the anxiolytic activity of lavender oil, but not on linalool itself. Positive outcomes were obtained in healthy subjects[65] and in different special groups such as geriatric patients with severe dementia,[66] dental patients in

Table 2. Overview of the main studies on the sedative effects of linalool/linalool-containing essential oils

Study, subjects	Compound	Administration, dosage	Tests/investigated parameters	Outcome	Ref.
Mice	LIN-rac	Inhalation 60 min; 1%, 3%	Locomotion; Body temperature; BST, RRT	Reduced locomotion; Decreased body temperature; Increased pentobarbital-induced sleeping time; Unaffected locomotor coordination	42
Female juvenile outbred Swiss mice	LIN[a]	Inhalation: 0.1 ng/ml blood serum	Behavioural and motility assessment	Sedation even after agitating stimulus; Decrease of motility	43
Adult male ICR mice	LIN[a]	i.p.: 100 mg/kg	OFT, EC, RRT, TT	Decreased spontaneous locomotor activity; Decreased exploratory activity; Sedative-like activity	44
Urethane-anesthetized male Wistar rats	(a) LAVO (45.18% LIN); (b) LIN-rac	Inhalation 10 min, dilutions: (a) 1/100, 1/1000; (b) 1/30, 1/300	RSNA; BP; GVNA	Decreased RSNA, BP; elevated GVNA; mechanism via central histaminergic nerves and hypothalamic suprachiasmatic nucleus	45
Male Wistar rats	LAVO (45.18% LIN); LIN-rac	Inhalation 10 min, dilutions: (a) 1/10 000, 1/1000, 1/100 (LAVO); 1/10 000 (LIN-rac); (b) 1/100 000 (LAVO); 1/5000 (LIN-rac); (c) 1/100 000, 15 min/day, 33 days, LAVO; 1/5000 (LIN-rac)	(a) Activity of adrenal sympathetic nerves; (b) Determination of plasma glycerol; (c) Effects on food intake	Both LAVO and LIN-rac similarly inhibited sympathetic adrenal nerve activity; enhanced parasympathetic nerve activity; Enhanced appetite	46
Human adult subjects	(R)-(−)-LIN; (S)-(+)-LIN LIN-rac	Inhalation, 20 mg/ml	Sensory tests; PFSE	(R)-(−)-LIN and LIN-rac decreased the beta wave after environmental sounds; (S)-(+)-LIN exhibited an opposite tendency	48
Human volunteers (n = 24)	(R)-(−)-LIN; (S)-(+)-LIN	2.7 mg/m³: (R)-(−)-LIN; 9.8 mg/m³: (S)-(+)-LIN	Standardized stress task; Physiological parameters: HA, BP, EA, SC	(R)-(−)-LIN exhibited sedative effects; (S)-(+)-LIN exhibited activating effects; (R)-(−)-LIN is a potential stress-relieving agent	50
Healthy normotensive human volunteers (n = 24)	(R)-(−)-LIN; (S)-(+)-LIN; jasmine tea [(R)-(−)-LIN: 530.6 ppb]; LAVO [40.9% LIN; 88% ee for (R)-(−)-LIN]	Inhalation, 0.03 ppm	Evaluation of ANS activity; Evaluation of mood states	(R)-(−)-LIN decreased HA, increased parasympathetic nerve activity and increased the positive mood scale; (S)-(+)-linalool showed opposite effects on autonomic nervous system activity and mood states. The low intensity of jasmine tea and LAVO odour have sedative effects on ANS activity and mood states	51

(Continues)

Table 2. *(Continued)*

Study, subjects	Compound	Administration, dosage	Tests/investigated parameters	Outcome	Ref.
Double-blind, aroma × instruction × time mixed factorial placebo-controlled trial (n = 96)	LA	Inhalation, 10 min	Galvanic skin response; Instructional priming procedure	Previous associations of LA with assisted relaxation may have been influenced by expectancy biases	52
Human volunteers (n = 30)	LAVO	Inhalation, 10 min, 150 µl/filter paper	SC, cA	Stress-relieving effect	53

aenantiomeric identity is not specified.
ANS, autonomic nervous system; BST, barbital-sleeping time; BP, blood pressure; cA, chromogranin A; EA, electrodermal activity; EC, exploratory cylinder; ee, enantiomeric excess; HA, heart rate; GVNA, gastric vagal nerve activity; LA, lavender aroma; LAVO, lavender essential oil; LIN, linalool, LIN-rac, linalool racemate; OFT, open field test; PFSE, portable forehead surface electroencephalography; RRT, rota-rod; RSNA, renal sympathetic nerve activity; SC, salivary cortisol; TT, traction test.

ambulatory practice[67] or hospitalized patients[68] (Table 3). However, most randomized clinical trials targeting the anxiolytic effect of lavender present a poor methodology which limits its assessment.[69] The study groups are small and might be too diverse. Only two studies[70,71] that evaluated the efficacy of an oral lavender essential oil preparation (Silexan) meet methodological criteria and can be taken into consideration. Silexan is an essential oil of high selected quality obtained by steam-distillation of lavender flowers (*Lavandula angustifolia* Mill. sin. *L. officinalis* Chaix, Lamiaceae). This oil complies with the requirements of the *European Pharmacopoeia* (20–45% linalool and 25–46% linalyl acetate)[72] (even exceeds them) and was approved in Germany in 2009 for the treatment of restlessness related to anxiety. The efficacy and safety of Silexan in anxiety disorders as well as in generalized anxiety disorder (GAD) have been demonstrated in several clinical randomized controlled trials and in an open pilot study.[73] Therapeutic benefit appears also in patients who suffer from neurasthenia, post-traumatic stress disorder or somatization disorder.[74] Silexan (80 mg/day) has a comparable efficacy with lorazepam (0.5 mg/day) in adults with GAD.[71] The sleep disturbances related to anxiety are clearly improved and at a similar level in both Silexan and lorazepam groups; the clinical global impression and effectiveness are even better in the Silexan group. In addition, the anxiolytic effect of lavender oil is maintained while reducing the dose. The adverse effects of oral lavender oil preparation are minor and related to gastrointestinal reactions. Compared to benzodiazepines, lavender oil shows a favourable safety profile at the recommended doses and more important, it does not cause drug dependence and hang-over effects,[71] as proven in discrimination procedures in animals.[73] Future clinical trials that target the anxiolytic effects of orally administered lavender oil should have an improved design, adopt an appropriate procedure of randomization and describe it to allow independent replication, to be triple-blind (if possible) and include indistinguishable placebos. The use of other administration routes is not supported by convincing efficacy. The most difficult to manage are the studies based on inhalation. The characteristic odour of lavender oil and linalool excludes the labelling as blind study if administration is by inhalation and lavender is compared with an indoor compound. Because of its specific scent, lavender itself may participate in accomplishment of the calming effect via evoking associative memory through action on the limbic system, amygdale and hippocampus. It would be more appropriate, as many authors affirm, to compare lavender with a similar smell. Studies should provide information on the side effects and report dropouts. The adopted methodology should be circumscribed CONSORT and PRISMA guidelines.[69] Therefore, future studies should also investigate the effects of lavender oil chronic use on memory. The same issues should be considered when linalool is clinically tested.

The mechanism of linalool and lavender essential oil anxiolytic activity is not completely known and understood. Previously, it was considered that the anxiolytic activity of lavender essential oil is mainly determined by the interactions with the olfactory system and its central projections; today, it is estimated that this mechanism is secondary. Induced anosmia in animals has revealed that the intact olfactory perception is not necessary for the anxiolytic effect of lavender essential oil.[75] *In vitro* tests have shown that lavender oil would act like benzodiazepines on GABA$_A$ receptors but the researches using GABA$_A$ agonists radioligands (flunitrazepam, muscimol) and electrophysiological

Table 3. Overview of studies regarding anxiolytic effects of linalool/ lavender essential oil

Study, subjects	Compound	Administration, dosage	Tests/investigated parameters	Outcome	Ref.
Adult male ICR mice	LIN[a]	i.p.; 100 mg/kg	EPMT	Anxiolytic-like activity	44
Male ICR mice	LIN[a], LAVO (26.12% LIN)	i.p., 800 mg/kg; 1600 mg/kg (LIN); 200–1600 mg/kg (LAVO)	Geller, Vogel tests	Dose-dependent anticonflict effects similar to those exhibited by diazepam	59
Adult male Sprague–Dawley albino rats	LAVO (25% LIN)	Inhalation, 0.1–2 ml; 30 min, 1 h; positive control: chlordiazepoxide i.p.; 1 ml/kg	OFT	Anxiolytic activity, but sedative effect at the highest dose and longer exposure time	60
Mongolian male, female gerbils	LAVO (38.47% LIN)	LAVO acute exposure: 24 h; LAVO chronic exposure: 24 h, 14 days; olfactive delivery	EPMT	Mild anxiolysis in acute exposure; Pronounced anxiolysis in chronic exposure	61
Adult male Sprague–Dawley albino rats	LAVO	Inhalation (0.5 ml); positive control: chlordiazepoxide; i.p.; 10 mg/kg	OFT	LAVO showed anxiolytic behavioural effects, but they were weaker than the action of chlordiazepoxide	62
Adult male albino mice	LIN[a]	Inhalation 60 min; 3% LIN; positive control: diazepam; i.p.; 0.5 mg, 1 mg/kg	LDT; SDIA; SIT; Aggressive behaviour	Anxiolytic activity; Increases social interaction; Decreases aggressive behaviour	63
Male Wistar rats	(R)-(−)-LIN	i.p.; 50, 100 mg/kg	ROT; IAT; OFT	Linalool was able to impair the memory acquisition	64
Double-blind randomized study: anxiety-provoking film-clips (n = 96)	LAVO	Oral, 1 capsule/day; 100, 200 µl/capsule	STAI; PANAS; Physiological parameters: heart rate, respiration rate; galvanic skin response	Anxiolytic effects in humans under low anxiety conditions	65
Placebo-controlled study, patients with severe dementia and agitated behaviour (n = 15)	LAVO	2% aromatherapy; 2 h alternated with placebo every other day, ten treatment sessions	PAS	Modest efficacy in the treatment of agitated behaviour from severe dementia	66
Cluster randomized-controlled trial, healthy adult dental patients (n = 343)	LAVO	Five drops in 10 ml diffused by candle	STAI; MDAS	Decreases anxiety in dental patients	67
Double-blind, randomized, placebo-controlled multi-centre trial: Anxiety disorder (n = 107)	Silexan	Oral, 80 mg/day, 10 weeks	HAS, ZSA; PSQI; SF-36 HSQ; CGI	Effectiveness in the relief of anxiety disorder	70
Double-blind, double-dummy randomized reference, controlled multicentre	Silexan	Oral, 80 mg/day, 6 weeks; positive control: lorazepam, 5 mg/day	HAS, ZSA; SF-36 HSQ; CGI	The anxiolytic activity of Silexan was comparable to that of lorazepam	71

(Continues)

Table 3. *(Continued)*

Study, subjects	Compound	Administration, dosage	Tests/investigated parameters	Outcome	Ref.
trial: generalized anxiety disorder (n = 40) Male Sprague–Dawley rats	Silexan	i.p.; 3,10, 30 mg/kg; diazepam, i.p.; 0.3, 1, 2 mg/kg	Drug discrimination training phase; Silexan substitution testing phase	No diazepam-like interoceptive properties for Silexan	73
Open, non-comparative, monocentre pilot study: neurasthenia, post-traumatic stress disorder, somatization disorder (n = 50)	Silexan	Oral, 80 mg/day, 6 weeks	SC-90-R; STAI; ZDS; MBI; SF-36 HSQ	Significant and clinically improvements of symptoms, such as restlessness, sleep disturbances, sub-threshold anxiety	74
Adult male outbred Swiss Webster albino mice	LAVO (46.5% LIN)	Inhalation, 15 min; 0.5%, 2.5%, 5%	OD; Locomotor activity evaluation; MBT	Anxiolytic activity; Zn-induced anosmia does not affect anxiolytic effect of inhaled lavender	75
Adult male Swiss albino mice	LAVO (46.5% LIN)	Inhalation, 15 min; 1%, 2.5%, 5%	MBT; EPMT; Serotonin-induced syndrome	Anxiolytic-like activity unlikely mediated by the GABA$_A$/BDZ complex; The locomotor activity was not affected at anxiolytic doses; The attenuation of serotonin syndrome	79

[a]enantiomeric identity is not specified

CGI, Clinical Global Impressions; EPMT, elevated plus-maze test; HAS, Hamilton Anxiety Scale; IAT, inhibitory avoidance test; i.p., intraperitoneal; LAVO, lavender essential oil; LDT, light/dark test; LIN, linalool; MBI, Maslach Burnout Inventory; MBT, marble-burying test; MDAS, Modified Dental Anxiety Scale; OD, olfactory discrimination; OFT, open field test; PANAS, mood, positive and negative affect scale; PAS, Pittsburgh Agitation Scale; PSQI, Pittsburgh Sleep Quality Index; ROT, recognition object task; SC-90-R, Symptom Checklist-90-Revised; SDIA, step down inhibitory avoidance; SF-36 HSQ, SF-36 Health Survey Questionnaire; SIT, social interaction test; STAI, State Trait Anxiety Inventory; ZDS, von Zerssn's Depression Scale; ZSA, Zung Self-rating Anxiety Scale.

investigations have denied this hypothesis.[76,77] Lavender oil interacts with the channel pore of the receptors, but not with the specific benzodiazepine binding site, so that the inhibition of the neuronal activity does not involve an interaction with GABA$_A$ receptors.[73] This could be a possible explanation for the different behaviour in acute exposure compared to the one of diazepam. On the basis of a large number of animal experiments and electrophysiological tests, Leal-Cardoso's group[78] concluded that the inhibition of voltage-gated sodium channels is probably the major mechanism by which linalool affects neuronal excitability. Blockage of voltage-gated sodium channels causes premature finalization of action potential generation *per se* which would lead to decrease of the neuro-transmitters release by exocytosis. The serotoninergic system via 5-HT1A receptors plays also an important role in expression of lavender oil anxiolytic action.[79] Further studies are needed to investigate the detailed mechanism of action.

On the basis of existing studies it is impossible to assess clearly the influence of chirality on linalool anxiolytic activity. The studies were performed predominantly with lavender essential oil or linalool, whose enantiomeric identity was not specified. In the case of lavender essential oil, the linalool content is variable (where it is mentioned), and the enantiomeric purity is not indicated. However, considering that (R)-(−)-linalool is the main component of lavender oil, it appears to be plausible to consider the enantiomer with the most important anxiolytic activity. The future studies on linalool should take into consideration the definite identity of enantiomer, dose extrapolation from preclinical testing in clinical trials, establishing anxiolytic doses and demonstrating the dose–response relationship.

Anticonvulsant Activity

The epileptic disorders are chronic, recurrent affections arising from an excessive neuronal activity. It is estimated that approximately 1% of the world's population is affected by this group of complaints.[80] Although there is an important therapeutic intervention arsenal, paroxystic convulsive episodes that characterize the disease are resistant to current medication in 30% of cases.[81] The potential of neuronal excitability inhibition by linalool or lavender oil was an important premise for the research on their anticonvulsant activity. Lavender acts in a multifaceted manner in the control of seizures: inhibiting their onset and duration and diminishing the intensity of the convulsive crisis. Linalool, tested mainly as racemate, has proven to be effective in various experimental models of seizures induced in animals (Table 4). The administration of linalool protects against seizures induced by picrotoxine, quinolinic acid, pentylenetetrazol, transcorneal electroshock and delays the onset of convulsions induced by NMDA.[55,82] In mice, lavender essential oil increases the latency of seizures induced by pentylenetetrazol as well as the percentage of survival.[83] The mechanism of action, demonstrated *in vitro* and *in vivo*, involves mainly an antagonist dose-dependent interaction with glutamatergic NMDA receptors, which play an important role in the genesis and propagation of convulsions. Although initial studies in mice have suggested a direct agonist interaction with GABA$_A$ receptors, Silva Brum et al.[76] showed that linalool racemate has no effect on binding of GABA$_A$ agonists to mouse cortical membranes. However, the authors do not exclude another mechanism of linalool intervention in GABAergic transmission. Related to the fact that cellular cyclic adenosine monophosphate (cAMP)

accumulation is involved in the pathophysiology of epilepsy, Sampaio et al.[84] showed that (R)-(−)-linalool and its racemate inhibit adenylate cyclase activity in chick retinas, the most effective being the levorotatory isomer. Other mechanisms that might be involved in the anticonvulsant effect of linalool are changes of the nicotinic receptor-ion channel kinetics, calcium channel blocking and inhibition of the release of acetylcho-line.[82] De Sousa et al.[85] showed that although linalool enantiomers and racemate were effective in preventing tonic seizures, their potencies are different. (R)-(−)-linalool and racemate form are more active than (S)-(+)-enantiomer, the effects being comparable to those of known anticonvulsant agents (diazepam, phenytoine).

Local Anaesthetic Activity

Both *in vitro*[86] and *in vivo*,[86–88] linalool as well as lavender essential oil, exhibit local anaesthetic activity on different neuronal groups (Table 5). Linalool blocks, in a reversible and concentration-dependent way, the excitability and conductivity of all types of myelinated fibres of the sciatic nerve; the pharmacological potency is higher for fibres with low speed conduction.[78] The local anaesthetic properties of linalool are related to its effects on the nicotinic receptors, mainly to its ability to modify their kinetics, to inhibit acetylcholine release but also to block the action potential. Linalool inhibits nerve excitability by affecting voltage-dependent sodium conductance. The inhibition of Na$^+$ current is probably the prominent mechanism of action through which linalool blocks the action potential.[78] Besides, Buchbauer and Jirovetz[89] suggested that a change in the nerve cells membrane properties by some volatile constituents could be due to their membrane accumulation and sterically blocking of ion channels. Since the enantiomeric identity of linalool is not specified and there are no comparative researches to investigate the two enantiomers of linalool, we cannot say which form is more active as a local anaesthetic. (R)-(−)-linalool appears to have better abilities to inhibit neuronal activity so that it could be the most potent enantiomer as a local anaesthetic.

Analgesic Activity

Pain is a symptom considered to be a defensive reaction of the body, signaling potentially a disease, but when it is chronic and persistent, it becomes a condition that prejudices health and quality of people life. Worldwide it is estimated that there is still a poor management of pain. The most effective analgesics are the opioids, but they are classified as controlled agents because of their huge abusive potential. Opioids have a strict regime of prescribing and administration. So, the identification of new potent analgesic agents deprived of major side effects or drug dependence/addiction potential is very important. From a clinical point of view, pain is nociceptive if it is considered that it starts through the continuous activation of the nociceptor system by damaging stimuli. The physiology of nociception involves a complex interaction of the central and peripheral structures of the central nervous system, which leads to the release of numerous proinflammatory and pain mediators, accompanied by an increase in the sensitivity of the peripheral and central sensory pathways. The neuronal sensitization process occurs through the activation of intracellular mediators and induction of nitric oxide (NO), bradykinin and prostaglandin E$_2$ (PGE$_2$). Inflammatory nociception is mediated by cytokines,

Table 4. Overview of the studies on anticonvulsant effects of linalool/linalool-containing essential oils

Study, subjects	Compound	Administration, dosage	Tests/investigated parameters	Outcome	Ref.
Adult male Wistar rats; rats cerebral cortex	LIN-rac	Incubation, 25°C/30°C; 0–6.5 mM	Adenylate cyclase activity; ³H-glutamate binding	Inhibition of adenylate cyclase basal activity; Inhibition of glutamate binding	54
Male adult Wistar rats; male albino mice CF-1	LIN-rac	(a) Incubation, 30°C, 15 min; 0.1, 0.3, 3, 5 mM; (b) i.p., 350 mg/kg; diazepam 30 mg/kg, s.c.; (c) i.c.v.; 15, 30, 45 mM; (d) oral, every third day, 6 treatments; 2.2. g/kg; 2.5 g/kg; phenobarbital 15 mg/kg	(a) ³H-glutamate binding; (b) NMDA-induced convulsions; (c) Quinolinic-acid induced convulsions; (d) PTZ-induced kindling	(a) Dose-dependent inhibition of glutamate binding; (b) Delay of NMDA-induced convulsions; (c) Blockage of quinolinic-acid induced convulsions; (d) Partial inhibition and significant delay of the PTZ-kindling expression	55
Male adult albino mice CF1; cortical synaptosomes	LIN-rac	Incubation, 1 min, 37°C; 1; 3 mM; 0.1–3 mM	Radiolabelled glutamate uptake–scintillation counter	Decrease in potassium-stimulated glutamate release; Decrease in glutamate uptake.	56
Mouse cortical membranes	LIN-rac	(a) Incubation, 1 h, 25°C; 0.1; 0.3; 1; 3; 5 mM; (b) incubation, 30 min, 4°C; 0.3; 1; 3 mM	(a) ³H-MK-801 (NMDA antagonist) binding; (b) ³H-muscimol (GABA$_A$ agonist) binding	(a) Dose-dependent non-competitive inhibition of ³H-MK-801 binding; (b) No effect on ³H-muscimol binding	76
Adult female Balb-c mice	LAVO (27.2% LIN)	i.p.; 1.6 ml/kg	PTZ-mouse model of generalized myoclonic seizures	Increased seizure latency; Decreased seizure intensity; Increased survival percentage	83
Chick retinas	Rosewood oil (*Aniba rosaeodora* Ducke): LIN 84.8%: (*R*)-(−)-LIN, 50.62%; (*S*)-(+)-LIN, 49.38%; (*R*)-(−)-LIN; LIN-rac	Incubation, 40 min, 37°C; 40 µM to 17.5 mM	cAMP assays	Inhibition of cAMP accumulation; (*R*)-(−)-LIN was the most effective compound	84
Adult male albino Swiss mice	(*R*)-(−)-LIN; (*S*)-(+)-LIN (*Coriandrum sativum*); LIN-rac	i.p.; 100, 200, 300 mg/kg; positive controls: (a) diazepam, i.p., 4 mg/kg; (b) diazepam, i.p., 8 mg/kg; (c) phenytoin, i.p., 25 mg/kg	(a) PTZ-induced convulsions; (b) picrotoxin-induced convulsions; (c) maximal electroshock-induced convulsions	Anticonvulsant effects (200 mg; 300 mg/kg)	85

cAMP, cyclic adenosine monophosphate; i.c.v., intracerebroventricular; i.p., intraperitoneal; LAVO, lavender essential oil; LIN, linalool; LIN-rac, linalool racemate; NMDA, *N*-methyl-D-aspartate; PTZ, pentylentetrazol; s.c., subcutaneous.

Table 5. Overview of the studies regarding local anaesthetic effects of linalool/lavender essential oil

Study	Compound	Administration, dosage	Tests/investigated parameters	Outcome	Ref.
Wistar rats; sciatic nerve; dorsal root ganglia	LIN[a]	Extracellular: 0.3; 0.6; 0.8;1; 2 mM; intracellular: 0.1; 0.6; 2; 4 mM	Patch-clamp; Electrophysiological parameters	Anaesthetic effect on peripheral somatosensory neurons by acting on voltage-dependent Na$^+$ channels	78
In vitro: rat phrenic nerve–hemidiaphragm preparation; *in vivo*: rabbit conjunctival reflex test	LAVO; LIN[a]	*In vitro*: 0.01–10 µg/ml *in vivo*: 30–2500 µg/ml	Electrically evoked contractions; Number of stimuli necessary to provoke conjunctival reflex	Local anaesthetic effect	86
Mouse phrenic nerve–hemidiaphragm preparation	LIN[a]	2 µg/ml; 20 µg/ml; 80 µg/ml	Spontaneous and evoked end-plate currents; resting membrane potential	Inhibitory effect on the acetylcholine release and channel open time in the mouse neuromuscular junction	87
Olfactory receptors cells, retinal neurons from the olfactory epithelium or retina of *Cynops pyrrhogaster*; rat cerebellar Purkinje cells	LIN[a]	Bath application; 3 mM	Whole-cell patch clamp; Ca^{2+} imaging techniques	Anaesthetic effect on olfactory receptors cells and central nervous system cells by suppressing the voltage-gated currents	88

[a]Enantiomeric identity is not specified.
LAVO, lavender essential oil; LIN, linalool.

glutamate, prostaglandins, histamine, serotonin, NO and immunological factors.[90]

(*R*)-(−)-Linalool is one of the most studied monoterpenoids regarding analgesic activity. The inhibitory effect on neuronal activity and stress-calming effect facilitate the expression of analgesic properties. Besides, linalool develops anti-allodynic effects. Most data on the analgesic properties of linalool were obtained from different experimental nociceptive models (Table 6) using the (*R*)-enantiomer or racemate; to our knowledge, there no clinical trials have been performed to evaluate this effect. Various administration routes (systemic, oral, intraplantar, intrathecal) and different doses of (*R*)-(−)-linalool (25–200 mg/kg) in rats or mice, reduce the painful response to diverse noxious stimuli (acetic acid, high temperatures, formaldehyde) and inhibits the carrageenan-induced edema; it also relieves hyperalgesia induced by L-glutamate, PGE$_2$, carrageenan and pro-inflammatory cytokines.[91–93] (*R*)-(−)-Linalool (50–200 mg/kg, i.p.) decreases mechanical hypersensitivity induced in a model of neuropathic pain, as well as mechanical and low temperature hypersensitivity in a model of chronic inflammation without causing tolerance.[93] An anti-allodynic activity has also been demonstrated for linalool racemate (intraplantar, 10 µg/paw) in mechanical induced neuropathic pain by partial sciatic nerve ligation. Besides, linalool improves the morphine anti-allodynic activity, which is a hopeful fact because neuropathic pain is often resistant to opioids.[94]

It seems that at least 10 different systems involved in pain are influenced by linalool, which illustrates an amazing versatility of analgesic activity for this compound.[95] Mainly, the antinociceptive properties of (*R*)-(−)-linalool were assigned to the positive interference of opioid, dopaminergic and muscarinic transmission as well as to the negative modulation of glutamatergic transmission.[96,97] These effects do not exclude the involvement of adenosine-5′-triphosphate (ATP)-sensitive potassium channels, because glibenclamide, an inhibitor of these channels, cancels the antinociceptive effect of linalool. Opening of K$^+$ channels in the cell membrane plays an important role in the positive modulation of the analgesic effect of opioid and muscarinic agonists.[96] The antagonist effect of linalool on the NMDA receptors causes supraspinal analgesia mediated by the stimulation of the central opioid receptors and the D$_1$ and D$_2$ dopaminergic receptors. The systemic administration of local anaesthetics induces antinociception dependent on a central cholinergic mechanism while the pretreatment with muscarinic antagonists reduces this effect.[96] Given this, the local anaesthetic properties of linalool might be involved in the antinociceptive activity.

The inhibition of NO synthesis/release,[91] positive modulation of adenosine release and its uptake inhibition[98] by (*R*)-(−)-linalool could be other mechanisms involved in the antinociceptive activity. Experimental researches on animals have shown that adenosine agonists are effective in the treatment of neuropathic pain, suggesting a possible use of linalool in this type of pain.[99] Furthermore, (*R*)-(−)-linalool inhibits transient receptor potential A1 (TRPA1), a sensor for noxious cold temperatures and a wide category of chemical compounds including capsaicin; in addition, (*R*)-(−)-linalool activates transient receptor potential cation channel 8 (TRPM8), a thermosensitive receptor, which is involved in the control of neuropathic pain.[95]

The mechanisms of the anti-allodynic activity, although still unclear, involve the inhibition of glutamatergic transmission, but also a local anti-inflammatory activity. The inhibition of spinal extracellular signal-regulated protein kinase (ERK) phosphorylation by linalool racemate could be involved in the expression of anti-allodynic effects and also in the synergism with morphine as the occurrence of somatic hyperalgesia following the induction of peripheral inflammation process is associated with spinal activation of ERK.[94]

(*R*)-(−)-Linalool is the enantiomer with the most well-defined analgesic activity. To our knowledge, there are no studies on dextrorotatory isomer activity as a single compound. A pharmacological profile which has many similarities with that of morphine as well as the involvement of opioid and muscarinic neurochemical systems, raise the question whether in the case of this compound may arise characteristic tolerance and drug dependence. Indeed, a development of tolerance to antinociceptive effects was registered after chronic administration of (*R*)-linalool, but the antinociceptive doses did not cause conditioned reinforcement, a critical factor for induction of addictive properties.[99]

Anti-inflammatory Activity

If acute inflammation is an organic reaction representing a protective mechanism of the body against various stimuli, chronic inflammation is harmful and must be combated. Chronic inflammation is involved in the aetiology of different diseases: cancer, asthma, rheumatism, atopic dermatitis, depression and gastrointestinal disorders. Many anti-inflammatory agents used in therapeutics have unwanted side effects mainly ulcerogenic properties, so the identification of new candidates that provide a superior safety profile from this point of view is always a topic of interest.

An overview on the major studies concerning the anti-inflammatory activity of linalool is presented in Table 6. In various experimental models of inflammation, linalool [(*R*)-enantiomer, racemate or undetermined enantiomer] acts anti-oedematously, limiting the inflammatory response and its related histological changes.[100] *In vitro*, (*R*)-(−)-linalool inhibits significantly and dose-dependently the lipopolysaccharide-induced production of TNF-α and IL-6 cytokines.[100] Also, in animal studies it has been shown that (*R*)-(−)-linalool and the racemate cause significant inhibition of carrageenan-induced edema, but the kinetics of the effect is different depending on the dose and enantiomer. High doses of (*R*)-(−)-linalool (50 or 75 mg/kg, subcutaneous) act immediately while low doses (25 mg/kg) have a considerable delayed effect. In the case of the racemate, the behaviour is opposite.[101]

The mechanisms suggested by *in vitro* and *in vivo* studies show that (*R*)-(−)-linalool may act by inhibiting the production of inflammatory cytokines via blocking NF-κB and MAPK pathways, antagonizing the NMDA effects[101] and reducing the synthesis or release of NO. The fact that linalool, as well as linalyl acetate, significantly diminishes (over 50%) the areas of ethanol-induced gastric erosion indicates a possible anti-inflammatory effect without specific ulcerogenic adverse disadvantage of most NSAIDs.[95,102] However, the complex pharmacological profile of (*R*)-(−)-linalool requires detailed further investigations to probe the anti-inflammatory potency compared to synthetic agents, safety at prolonged use and specificity of action depending on the location of the inflammatory process. Besides, the gastro-protective effect should be proved also for (*R*)-(−)-linalool, since the tested compound is not enantiomerically defined.

Table 6. Overview of studies on antinociceptive and anti-inflammatory effects of linalool/linalool-containing essential oils

Study, subjects	Compound	Administration, dosage	Tests/investigated parameters	Outcome	Ref.
LPS-stimulated macrophage cell line J774. A1	(R)-(−)-LIN	$10^{-7}, 10^{-5}, 10^{-3}$ M	Nitrite in the culture medium; PGE$_2$ production; iNOS and COX-2 expression	Diminuation of nitric oxide production/release	91
Male Swiss mice	(R)-(−)-LIN	(a) i.p.; 10–200 mg/kg oral; 5–100 mg/kg; i.t., 0.1–3 µg/site; (b) i.pl.; 910–300 ng/paw, i.p.; 200 mg/kg; (c) i.p.;10–200 mg/kg, i.t.; 03–3 µg/site	(a) Glutamate-induced licking in the mouse paw; glutamate-induced nociception in the mouse paw; (b) Nociception induced by ionotropic and metabotropic agonists of glutamatergic receptors; (c) RRT	Significant inhibition of glutamate-induced nociceptive response; Unaltered locomotor activity	92
Adult female Swiss mice	(R)-(−)-LIN	i.p; 50, 200 mg/kg	Paw oedema induced by i.pl. injection of complete Freund's adjuvant; PSNL-induced neuropathic hypersensitivity	Anti-allodynic activity; anti-inflammatory activity	93
Male mice of ddY strain	LIN-rac	i.pl.; 10 µg/paw, 7 days following PSNL	PSNL-induced neuropathic hypersensitivity; ERK-spinal expression	Anti-allodynic effect; Decreased spinal ERK-activation	94
CD1 male mice Male ddY (SD) mice	(R)-(−)-LIN LIN-rac	s.c.; 50, 100, 150 mg/kg i.pl; 2.5, 5, 10 µg/paw	HPT; FT CT	Antinociceptive activity Reduction of capsaicin-induced nociceptive response; Combination with morphine enhanced antinociceptive effect; Opioid mechanism is involved in antinociceptive activity	96 97
CD male mice	(R)-(−)-LIN	s.c.; 25, 50, 100 mg/kg	HPT	Antinociceptive activity partially mediated by the activity of adenosine A$_1$ and A$_{2A}$ receptors	98
In vitro: RAW 264.7 LPS-stimulated mouse macrophage cell	LIN[a]	i.p.; 25 mg/kg	In vitro: MTT, cytokine assay (TNF-α, IL-6); in vivo: acute lung injury induction; measurement of	Decreased LPS-induced production of TNF-α and IL-6 cytokines; Blocking of NF-κB and	100

(Continues)

Table 6. *(Continued)*

Study, subjects	Compound	Administration, dosage	Tests/investigated parameters	Outcome	Ref.
line; *in vivo*: male BALB/c mice			cytokines, inflammatory cell counts of bronchoalveolar lavage fluid; histo-pathologic evaluation of lung tissue	MAPK activation; Mitigation of lung histopathological changes	
Male albino Wistar rats	(*R*)-(−)-LIN; LIN-rac	s.c.;12.5; 25; 50, 75 mg/kg (LIN-rac); 25; 50, 75 mg/kg (*R*)-LIN); positive control: aspirin (150 mg/kg, s.c.).	CHPE	Anti-inflammatory activity	101
Adult male Swiss mice; female Wistar rats	LHVO (33.44% LIN); LIN[a]	Gavage; 100 mg/kg; gavage; 33 mg/kg; inhalation, 60 min; 2.4 μl/l	AAWT; HPT; Locomotor activity; Indometacin and ethanol-induced lesions	Antinociceptive effect of LHVO mainly after inhalation; Significant reduction of ethanol-induced gastric injury at oral administration of LHVO and linalool; Unaltered locomotor activity	102

[a]Enantiomeric identity is not specified.

AAWT, acetic acid writhing test; CHPE, carrageenan-induced hind paw oedema; COX-2, cyclooxygenase 2; CT, capsaicin test; ERK, extracellular signal-regulated protein kinase; HPT, hot-plate test; FT, formalin test; iNOS, inducible nitric oxide synthase; IL-6, interleukin-6; i.p, intraperitoneal; i.pl, intraplantar; i.t, intrathecal; MAPK, mitogen-activated protein kinase; MTT, 3,5-diphenyl-1-(4,5-dimethyl-2-thiazolyl) formazan; LHVO, *Lavandula hybrida* essential oil; LIN, linalool; LIN-rac, linalool racemate; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; PGE$_2$, prostaglandin E2; PSNL, partial sciatic nerve ligation; RRT, rota rod task; s.c., subcutaneous; TNF-α, tumor necrosis factor alpha.

Miscellaneous

Other biological effects of linalool are presented in Table 7. With one exception, the enantiomeric identity of linalool is not specified in these studies.

Antitumoral activity

Linalool exhibits high cytotoxicity on hematologic malignant cell lines[103] as well as antiproliferative activity against a broad spectrum of carcinoma cells[104,105] including multidrug resistant human breast adenocarcinoma cells.[106] In haematopoietic malignancies, linalool does not affect the development of normal haematopoietic cells when exposed to cytotoxic concentrations (130 μM), and even to higher concentrations (650 μM). As an antitumor agent, linalool activates the tumor suppressor protein p53 and some cyclin-dependent kinase inhibitors (CDKI). p53 is a prominent regulator of the cellular cycle and it plays a key role in preventing inappropriate cell proliferation and maintaining genomic integrity following the exposure to genotoxic stress.[103] The mitochondria can be another possible target of linalool. In addition, linalool may enhance doxorobicin's antitumoral activity, probably by promoting doxorubicin's influx in the cells.[106–108] However, the antitumoral potential of linalool is limited because of the high doses required *in vivo*; its chemical structure can nevertheless be modified and optimized, so that linalool may serve as parent molecule for the development of new antitumor agents.[103] In this respect, it is necessary to mention the identity of the enantiomer in antitumor tests.

Cholesterol-lowering activity

In mice, oral administration of linalool reduces total cholesterol, LDL-cholesterol and triglyceride plasmatic levels. In human hepatoma HepG2 cells, linalool decreases dose-dependently the cellular cholesterol and triglyceride concentration. Molecular mechanisms of cholesterol-lowering effects involve both the suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) transcription via reducing the expression of sterol regulatory element binding proteins and the acceleration of HMGCR ubiquitin-dependent degradation. The ability to determine enzyme proteolysis is related to the non-sterol isoprene structure of linalool that basically mimics the effects of some intermediary products in sterol biosynthesis (geranylgeranyl, lanosterol or oxysterols), but which may also regulate the ubiquitination process.[109]

Antimicrobial activity

In concentrations of 0.1% (v/v), linalool exhibits antimicrobial activity against different microorganisms (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pasteurella multocida*) being more active against Gram-positive bacteria compared to Gram-negative bacteria. Its antibacterial activity was attributed to functional destabilization of bacterial membrane and to an increase of bacterial strains susceptibility to classical antimicrobial agents.[110] A remarkable activity has been shown for linalool against periodontopathic and cariogenic bacteria, minimum inhibitory concentration and minimum bactericidal concentration values ranging from 0.1 to 1.6 mg/ml. However, due to the cytotoxic effects on oral epithelial carcinoma cell line (KB cells), linalool incorporation in oral hygiene products should be done in concentrations lower than 0.4 mg/ml.[111] Linalool isolated from coriander acts antifungally on oral *Candida* isolates in patiens with dental

problems,[112] as well as *Candida albicans* and non-*Candida albicans* clinical isolates with differential sensitivity to fluconazol. At very low concentrations (0.08%, v/v), linalool presents an excellent synergistic activity in combination with fluconazole by increasing the antimycotic transmembrane flux.[113]

Antioxidant activity

Linalool acts mainly as an anti-lipoperoxidant agent. Antioxidant activity is more significant in case of linalool-containing essential oils, most probably due to a synergy between components. Thus, lavender essential oil exhibits potent antioxidant activity expressed especially in free-radical scavenging abilities.[114,115] At relatively high concentrations (120 mg/kg) and long-time exposure, linalool protects guinea-pig brain tissue against hydrogen peroxide oxidative stress, its effects being similar to those exhibited by lipoic acid and vitamin E.[116] However, in thiobarbituric acid reactive species assay, linalool behaves as a pro-oxidant; in micellar system with linoleic acid, its antioxidant activity has not been demonstrated.[117] Linalool is an alcohol containing an unsatured allyl moiety that predisposes to auto-oxidation, favored by atmospheric exposure. The concentration of linalool, the test system, the initiator of oxidative reactions, the auto-oxidation potential of linalool are some of the variables that influence the final outcome. In order to assess the antioxidant effectiveness of linalool, a dose-dependent study is required.

Spasmolytic effect

Linalool acts as a spasmolytic agent on the intestinal and tracheal smooth muscles. The mechanism, likely mediated by an increase of cAMP, is a result of adenylate cyclase enzyme stimulation. The intestinal muscles are more sensitive to linalool.[118]

Enhancement of the percutaneous penetration of drugs

Linalool promotes percutaneous penetration of other therapeutic agents by increasing the permeability of the skin and mucous membranes, so it can be used as an absorption promoter in topical preparations.[119] Meanwhile, it seems that the absorption of linalool in stratum corneum is higher from hydrogel-type formulations than from emulsions or oily solutions.[6]

Toxicological Data

Repeated dose dermal toxicity studies led to the determination of a NOAEL value (No Observed Adverse Effect Level) for linalool of 250 mg/kg bw/day, while LOAEL (Lowest Observed Adverse Effect Level) is 1000 mg/kg bw/day. After oral exposure, NOAEL value is 50 mg/kg bw/day.[40] Linalool showed no mutagenicity and expressed no genotoxicity. There are no long-term studies related to carcinogenicity of linalool. Given the lack of genotoxic effect, the absence of chemical structural elements to confer a carcinogenic risk, as well as the high NOAEL values in toxicity studies, it is reasonable to consider that linalool does not cause any carcinogenicity in current conditions of use as fragrance and flavour agent. Data regarding reproductive and developmental toxicity are limited and do not give any indication of relevant adverse effects on reproductive function or human body growth. In addition, NOAEL values for maternal and developmental toxicity are too high compared to current levels in case of human exposure and do not raise safety concerns (NOAEL maternal = 500 mg/kg bw/day; NOAEL developmental = 1000 mg/kg bw/day).[40,120]

Table 7. Overview of studies on other biological effects of linalool/lavender essential oil

Study, subjects	Compound	Administration, dosage	Tests/investigated parameters	Outcome	Ref.
Haematological malignant cell lines	LIN[a]	Treatment 48 h; 20–640 μM	Apoptosis assessment; cancer signal transduction pathway cDNA microarray; qPCR; MTT; cell cycle analysis	Inhibition of leukaemia cell growth; Decrease of G_0/G_1 phase cells proportion	103
Carcinoma cells of the bladder (J82), stomach (AGS), lung (H 520; H 661), bone (V2OS); cervix (HeLa); Carcinoma cells of the skin (BCC1), kidney (RTCC1), mouth (OSCC-1)	LIN[a]	Incubation, 48 h, 37°C; different concentrations	XTT	Antiproliferative activity	104
Cell line human breast carcinoma BT-20 ATCC HTB 19; Human epithelioid cervix carcinoma cell hela ATCC CCL 219	LIN[a]	Incubation 72 h; different concentrations	MTT	Weak cytotoxic activity	105
Human breast adenocarcinoma MCF 7 WT and multidrug resistant MCF 7 Adr[R] cells	LIN[a]	Exposure 72 h; 10, 50 μM; DOX: 0.05–10 μM (MCF 7 WT cells); 1–250 μM (MCF 7 Adr[R] cells)	MTT; flow cytometry; immunodetection of p53, p21, Bcl2, Bax, Bcl-x_L, surviving	Moderate inhibition of cell proliferation; Potentiates DOX-induced cytotoxicity	106
Cell lines: Hepg2, Caco2, NIH3t3, MCF7, Hek293; Hepg2 isolated mitochondria	LIN[a]	Incubation 24 h; 1–200 μM	Cell viability tests; respiratory chain complexes I and II; intracellular GSH, ATP, reactive oxygen species levels	100% decrease in the viability of HepG2 cells (2 μM LIN); Concentration-dependent inhibition of respiratory chain complexes I and II activities	107
In vitro, P388 leukaemia cells; *in vivo*, male BDF1 mice with P388 leukaemia cells	LIN[a]	*In vitro*: incubation 37°C, 48 h; 0.01, 0.1, 1 μM; 17 nM DOX. *in vivo*: oral; 1 mg/kg/day, 4 days; DOX, ip.; 2 mg/kg/day, at 3, 5, 7 and 9 days after the inoculation; incubation 20°C, 60 min; 0.1 M; 9 μM DOX	LIN effects on DOX cytotoxicity in P388 leukemia cells, DOX antitumor activity and permeability	Promotes DOX influx in tumour cells; enhances DOX antitumor activity	108
High-fat fed male C57 BL/67 mice; Hepg2 cells	LIN[a]	0.57 mg; 120 mg/mouse/day, 6 weeks; incubation 24 h, 0, 0.1, 0.5 mM	Plasma and cellular lipid determination; qPCR; immunoblotting,	Lipid-lowering effects; decrease of HMG-CoA reductase decrease of SREBP-2 expression	109

Sample	Compound	Concentration	Assay	Effect	Ref.
Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Fusobacterium nucleatum, Aggregatibacter actinomycetemcomitans, Streptococcus mutans, Streptococcus sobrinus	LIN[a]	0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 mg/ml; positive control: ampicillin (100 mg/ml)	nuclear and membrane protein isolation Microdilution assay (NCCLS standard)	Antibacterial activity against periodontopathic and cariogenic bacteria	111
Candida isolates from patients with denture stomatitis: C. albicans, C. glabrata, C. dubliniensis, C. guilliermondii, C. krusei, C. parapsilosis, C. tropicalis (n = 38)	LIN (Coriandrum sativum)	0.01–8% (v/v); positive control: fluconazole 0.12–64 mg/ml	Broth microdilution	Strong fungicidal activity (MFC_{90} = 0.5%)[b]	112
Candida albicans clinical isolates (n = 36), standard strains (n = 3); non-Candida albicans ssp. (n = 9)	LIN[a]	(a) 0.5–0.004% (v/v) (b) 0.048% (c) 0.5–0.08% (v/v)	(a) Broth microdilution; (b) time-dependent kill curve; (c) microtitre plate base morphological assay	Fungicidal effect	113
In vitro, antioxidant; Hep G2; NC-NC human B lymphoblastoid cell line	LIN[a]	(a) 0.058–2.9 mm; (b) 032, 0.64, 324, 648, 9.72 mm, 48 h; (c) 0.01, 0.1, 1, 10 µg/ml, 20 h; (d) 6.48 µM, 20 h	(a) Fe^{2+}/ascorbate induced lipid peroxidation; (b) bacterial mutagenicity/antimutagenicity assay; (c) MTT; (d) comet assay	Antiliperoxidative properties; Protective effect against genotoxicity induced by oxidizing agents	114
Healthy volunteers (n = 22)	LAVO	Aroma natural breathing, 5 min; dilutions in propyleneglycol (× 10;×1000)	FRSA, SC, SS IgA; α-amylase activity	Enhances FRSA; Decreases the stress hormone level	115
Guinea-pig brain tissue	LIN[a]	i.p., 120 mg/kg; 12 mg hydrogen peroxide/kg; 4 weeks, every other day; positive control: vitamin E (24 mg/kg), lipoic acid (3 mg/kg)	Total lipids; Fatty acid analysis	Protective effects against the hydrogen peroxide-induced stress; Antioxidant properties the same as vitamin E and lipoic acid	116

(Continues)

Table 7. *(Continued)*

Study, subjects	Compound	Administration, dosage	Tests/investigated parameters	Outcome	Ref.
In vitro, antioxidant	LIN[a]	(a) 1000 ppm, 500 ppm, 1000 ppm; (b) 10^{-2} to 10^{-4} M	(a) TBARS; (b) quantification of hydroperoxydienes in micellar system	(a) Pro-oxidant effect; (b) The lack of antioxidant activity	117

[a]Enantiomeric identity is not specified.
[b]The minimum concentration of drug that is fungicidal to 90% of the isolates.
DOX, doxorubicin; FRSA, salivary free radical scavenging activity; HMG-CoA reductase, methylglutaryl-coenzyme A reductase; i.p, intraperitoneal; LAVO, lavender essential oil; LIN, linalool; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; OFT, open field test; qPCR, quantitative real-time polymerase chain reaction; SC, salivary cortisol; SREBP-2, sterol regulatory element binding protein-2; SS IgA, salivary secretory IgA; TBARS, thiobarbituric acid reactive species; TFT, tail-flick test; XTT, 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl-2*H*-tetrazolium-5-carboxanilide salt).

Skin Sensitization Potential

Besides the metal salts (especially nickel) and preservative agents, fragrances are among the most common triggers of allergic contact dermatitis[121] also known as contact allergy or delayed contact hypersensitivity. Irritations and contact dermatitis represent over 90% of the skin occupational diseases[122] and fragrance allergy has a prevalence of about 1–4.2% in the general population of the industrialized world.[123]

In general, skin sensitization potential is related to the ability of odorous compounds to cross the stratum corneum and reach the derma and haptenate skin macromolecules.[121] Thus, the allergen must have an electrophilic structure or be a reactive radical for interacting with macromolecules in the skin and trigger the immune reaction. Linalool itself does not have such a structure, so it is not an allergenic compound *per se* or it has only a weak allergenic potential. After atmospheric exposure, the skin sensitization capacity increases dramatically due to linalool auto-oxidation products. The unsaturated structure of linalool with allylic hydrogen atoms is suitable for auto-oxidation by exposure to air at room temperature.[124–126] By auto-oxidation linalool generates a complex mixture including hydroperoxides, furan oxides, pyranoxides, alcohols and linalyl aldehyde.[127,128] The most potent skin sensitizers proved to be hydroperoxides according to the local lymph node assay (LLNA).[129]

After 10 week air-exposure, linalool concentration drops to about 80% and after 45 weeks of exposure to air, oxidation mixture contains only about 30% unoxidized linalool.[124] The same oxidation products are generated in the case of lavender essential oil by exposure to air. Although we would expect that in case of essential oils there is a *per se* protection against auto-oxidation, studies have shown that terpenes in lavender oil and mainly linalool undergo auto-oxidation at about the same rate in oil as pure compounds. Basically auto-oxidation follows the same mechanism in both cases, essential oil and pure terpenes. The sensitization ability of oxidized oil detected by LLNA is similar to oxidized linalool.[125] Linalool hydroperoxides are important haptens. Skin sensitization mechanism triggered by these compounds could involve radical reactions that take place either by forming covalent bonds with proteins or by an intramolecular rearrangement with generation of epoxides that act like electrophilic haptens.[121]

Autooxidation process is influenced by the purity of the compound and storage temperature, but it can be prevented by the addition of antioxidants (α-tocopherol or butylhydroxytoluene).[130] IFRA standard regulates that linalool may be used as fragrance material only when the peroxide level is very low (≤ 20 mmol/l).[40,120]

According to the EU Cosmetic Directive 76/768,[131] due to skin sensitization effects, linalool, as well as limonene, belong to the group of odorous chemicals which must be labelled as cosmetics when they are used in concentrations higher than 10 ppm (in rinse-off products) and above 100 ppm (in rinse-on products). However, it is questionable to label linalool as a common allergen odorant because patch testing with the pure compound does not cause or leads to very few positive reactions (0.3%). On the other hand, mixtures of linalool oxidation products cause positive patch test reactions in the same degree as standard allergens. The use of standardized and stable oxidation mixtures could be the most effective way to detect contact allergy in dermatitis patients. The patch concentration must be safe regarding sensitization with a low incidence of doubtful

reactions and irritancy. A recent international multicentric study conducted on 2900 dermatitis patients showed that oxidized linalool 6% in petrolatum (pet) can be successfully used in future screening of contact allergy. The standardized preparation of oxidized linalool contains 1% total hydroperoxides, causes only 6.9% positive patch reactions and exhibits a good stability for 3 months.[128]

Assessment of the Authenticity of Linalool: Current State of the Art

Highlighting the genuineness of flavours and fragrances has been and continues to be a challenge due to the complex matrices represented by the products where they are found. It requires extremely powerful and sophisticated analytical tools that lead to reliable results regarding the differention of natural sources from non-natural compounds. The enantioselectivity of odorant molecules, but also isotope discrimination related to biosynthesis origin, were introduced as new and substantial indicators for assessment of fragrance and flavour authenticity.[132] The enantiomer ratios of chiral constituents of essential oils or fragrances represent precious indicators in authentication of these products; they also serve to assess the biosynthetic pathways leading to a particular compound, geographical origin of vegetable matrix, applied technological treatments, influence of storage and aging process on molecule chirality but also to correlate chemical composition with organoleptic properties. In this respect, linalool chirality is extremely useful to determine the authenticity of the products.[133–135]

The characteristic enantiomer purity and the ratio of linalool enantiomers can function as a genuine fingerprint, their assessment allowing the identification of a possible addition of a synthetic compound, source non-authenticity or potential contamination of lavender, bergamot or sweet orange essential oils. The essential oils of Sicilian and Spanish sweet oranges contain mainly (S)-(+)-linalool, while in bitter orange oil (R)-(−)-linalool prevails. Evaluation of the enantiomeric ratio allows detection of bitter orange oil adulteration with cheaper sweet orange oil.[136]

Reliable analysis using linalool as an authentication marker requires a good knowledge on the chirality changes that may affect this pH-sensitive compound, depending on the extraction technique, time of extraction or processed product. Limitations in the use of linalool as an indicator of authenticity refers to the susceptibility of the 1-alken-3-ole structure to degrade in acidic medium. Linalool undergoes total or partial racemization depending on the temperature and pH of the distillation medium with a change in the enantiomeric ratios. One of the most used raw materials in perfumery for its floral, soft and fresh notes is genuine lavender oil which is characterized by an enantiomeric excess of (R)-(−)-linalool (95.1–98.2%) and (R)-(−)-linalyl acetate (>99%). The prolonged hydrodistillation (more than 1.5 h) of the lavender flowers and a low pH (about 5.5) of the extraction medium leads to a partial racemization of linalool. The classic enantiomeric excess in favour of (R)-(−)-linalool decreases with the generation of up to 8% (S)-(+)-linalool.[11] However, respecting the rules of good practice in lavender oil processing, namely steam distillation of fresh flowers in a short time (30 min), the genuine chirality of linalool does not change and the molecule can be used as an indicator for authenticity assessment. As enantiomeric purity of (R)-(−)-linalyl acetate is

preserved regardless of species, operating conditions or storage, the assessment of its chirality is a more reliable indicator for assessing lavender oil authenticity. Practically, both molecules are analysed. The presence of a quantity of (S)-(+)-linalool higher than 15% in lavender essential oil could indicate the addition of synthetic linalool (racemate).[137] The *European Pharmacopoeia*, 6th edition, requires a lower value of (S)-(+)-linalool in natural oil, namely 12%.[72] Bergamot essential oil is another example in which the processing conditions influence linalool enantiomers ratio. Authentic product obtained by standard technology, namely cold-pressing of the *Citrus aurantium* L. subsp. *bergamia* (Rutaceae) peels, contains exclusively (R)-(−)-enantiomers of linalool (about 10%) and linalyl acetate (25–30%). Subsequent processing of the bergamot essential oil for perfumery use by removing phototoxic furanocoumarins does not lead to a change in the enantiomeric purity of linalool and linalyl acetate. But methods of oil isolation by hydrodistillation and plant material influence the enantiomers distribution. Prolonged hydrodistillation (more than 2 h) of fresh bergamot peels favours linalool racemization leading to the (S)-(+)-enantiomer in concentrations higher than 30%. If bergamot oil is isolated by steam distillation of the residues after cold pressing of the peels (feccia oil), its linalyl acetate content decreases dramatically and the linalool racemization rate is lower [(S)-(+)-linalool content is about 8.5%].[11]

Another precious ingredient in expensive perfumes, jasmine concrete or absolute contains (S)-(+)-linalool as major constituent (more than 85%). This enantiomer prevails regardless the geographic origin or harvest year. The extraction with hexane of fresh jasmine flowers (*Jasminum grandiflorum*, Oleaceae) leads to an excess of (S)-(+)-enantiomer (86–88%). In the case of supercritical extraction, linalool excess reaches 96% which would suggest a possible racemization during classical extraction with hexane.[11]

Not only does the extraction process influence the enantiomer ratio and purity, but also the vegetable matrix. Rosewood (*Aniba rosaeodora*, Lauraceae) always shows a racemic distribution of linalool isomers. The racemization does not occur during steam distillation, but it occurs during plant growth because of the acid matrix where the compound is biosynthesized. In the case of *Geranium* oils (*Pelargonium graveolens* L. Herit ex Ait, Geraniaceae), due to the acidic matrix of the leaves (pH = 3), the enantiomeric excess is in favour of the (S)-(+)-isomer regardless of the method of extraction.[11]

The method, pH and time of distillation are critical parameters in assessing linalool chirality. Although hydrodistillation provides a more representative composition of essential oils, it does not reproduce the native purity of enantiomers. Other techniques of extraction can improve this aspect. Enantiomeric excess (ee %) of linalool in lavender volatile fraction obtained by microwave-assisted hydrodistillation or supercritical fluid extraction is higher than in regular lavender essential oil (72% and 80%, respectively, in comparison to 68%).[138] However, in assessing the purity of native enantiomers, the headspace sampling (HS) of plants is recommended to avoid racemization and generation of artefacts during processing.[139] Different HS techniques were used in the analysis of linalool-containing plants and food matrices: HS-solid-phase microextraction (HS-SPME), HS-solid-phase dynamic extraction (HS-SPDE), HS-liquid-phase microextraction (HS-LPME) and dynamic headspace extraction based on purge and trapp approach (D-HS, P&T).[133] Besides the fact that these techniques avoid the interferences arising from the matrix, they

are versatile, easily automated, time-saving and can be used in combination with advanced GC methods. Such approach allows the integration of sample preparation and its analysis in a single phase. A detailed review of this is presented by Bicchi *et al.*[140]

There are the current hyphenated analytical methods to investigate the linalool enantiomeric composition in various essential oils or fragrances and to identify sophisticated adulterations. Nowadays, the latter does not involve the use of synthetic derivatives, but dilution with cheaper volatile fractions from other natural sources. Genuine lavender oil (*Lavandula angustifolia*) can be adulterated by mixing with cheaper oils from other lavender species (*Lavandula x intermedia*, *Lavandula latifolia*) or addition of linalool and linalyl acetate racemates. Counterfeiting with synthetic linalool may be accompanied by the existence of dihydrolinalool and dehydrolinalool traces in essential oil composition as by-products from synthesis process.[11,141] Adulteration of bergamot oil can be achieved by addition of synthetic racemate or natural cheaper material, but not enantiomerically pure. Reconstituted bergamot essential oils on the market are obtained most commonly by addition of up to 8% linalool of natural or synthetic origin.[142] Neroli oil, another expensive ingredient in fragrance and flavour industry, is isolated by hydrodistillation of bitter orange blossoms. It contains (R)-$(-)$-linalool as characteristic enantiomer. It is often falsified with petitgrain oil obtained from leaves and branches of bitter orange which contains (S)-$(+)$-linalool as specific isomer.[11]

The main techniques used in analysis of linalool enantiomeric composition in various plant and food matrices are: enantioselective capillary gas chromatography (enantio-cGC), enantio-multidimensional GC analysis (enantio-MDGC), capillary gas-chromatography coupled with IRMS via a combustion interface (cGC-C-IRMS), enantio-MDGC coupled with GC-C-IRMS and site specific nuclear isotopic fractionation studied by nuclear magnetic resonance (SNIF-NMR). Table 8 lists the most important applications of those techniques. As the main analytical technique used in the analyis of volatile chiral molecules, enantio-cGC allows the discrimination, separation and determination of linalool enantiomers distribution, the presence of synthetic racemate in the case of complex matrices such as essential oils. This technique uses capillary columns containing cyclodextrin derivatives as chiral selectors diluted in a suitable polymeric stationary phase, apolar or moderately polar polysiloxane.[135,143]

Different stationary phases were used for linalool stereodifferentiation.[135,144–148,150–154] Oktakis(3-*O*-butyryl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) can be used to determine the adulteration of bergamot oil if the racemate addition is less than 5%.[141] Heptakis(2,3-di-*O*-acetyl-6-*O*-*t*-butyldimethylsilyl)-β-cyclo-dextrin also allows separation of linalyl acetate enantiomers, another major marker for authentication of essential oils.[141,144] In the case of heptakis(2,3-di-*O*-methyl-6-*O*-*t*-butyldimethylsilyl)-β-cyclodextrin (12.5%) and heptakis(2,3-di-*O*-acetyl-6-*O*-*t*-butyldimethylsilyl)-β-cyclodextrin (37.5%) mixture, the enantioselectivity is significantly influenced by the ratio of the chiral phase constituents as even minor deviations can cause total loss of enantioselectivity or affect the separation.[151]

When it comes to detect the counterfeiting that occurs by the addition of a non-racemic material, for example due to natural variability in the case of bergamot oil constituents, enantio-cGC is insufficient.[141] Also, enantio-cGC method cannot be used if the essential oil adulteration was produced solely with natural racemates, when racemization is a consequence of natural product processing and/or storage. In addition, enantio-cGC cannot

be used to analyse mixtures of natural and synthetic chiral compounds or non-chiral molecules[132,133,139]

The most indicated technique to eliminate matrix interferences and to achieve samples clean-up is the enantio-multidimensional GC analysis (enantio-MDGC) with flame ionization (FID) or mass-selective (MS) detectors. Two main approaches of MDGC: heart-cut MDGC and two-dimensional comprehensive GC (GCxGC) were used for linalool analysis in vegetable matrices or complex mixtures. Heart-cut MDGC uses a combination of conventional capillary column and an enantioselective column with different polarities. By column-switching techniques, unresolved compounds selected in first column (achiral) are diverted in the second column (chiral) to ensure complete separation and their subsequent quantitative determination. It is a powerful tool for the systematic assessment of origin-specific enantiomeric ratios and differentiation between synthetic and natural odorant compounds. Besides, enantio-MDGC-MS coupling with head sampling techniques and *in vivo* head sampling allows reliable determination of genuine enantiomeric ratios.[139] Using heart-cut MDGC different authors have determined an enantiomeric excess of linalool (above 94%) in favour of the (R)-$(-)$-isomer in genuine lavender essential oils.[137,155] The enantiomeric distribution identified in commercial lavender essential oils by enantio-MDGC suggests an elevated addition of linalool racemate, even higher than 40%.[155]

In GCxGC, each peak that eluted from the first column is cut into thin slices within a few seconds by cryogenic focusing which serves as a peak modulator. Each slice is then injected on-line in the second column. The combination with MS is one of the most powerful current available separation systems. Using comprehensive GC-enantio-GC, Shellie *et al.*[143] have determined an enantiomeric excess of 52–99% in favour of (S)-$(+)$-linalool in most *Melaleuca* sp. essential oils (Myrtaceae).

Another valuable, fast and useful tool to assess linalool or essential oils authenticity is isotope discrimination. Two special techniques of mass spectrometry and nuclear magnetic resonance (NMR) are used for isotope discrimination assessment, namely: isotope ratio mass spectrometry (IRMS) which evaluates the ratios between stable isotopes ($^{13}C/^{12}C$, $^2H/^1H$, $^{18}O/^{16}O$, $^{15}N/^{14}N$) and site-specific nuclear isotopic fractionation studied by nuclear magnetic resonance (SNIF-NMR); the latter determines site-specific isotope ratios. Online coupling capillary gas-chromatography with IRMS via a combustion interface (GC-C-IRMS) is the important technique used to evaluate authenticity of linalool and other chiral molecules. The method can also provide information on geographical origin, biogenesis, prohibited agrotechniques or extraction process.[156] The determination of isotope ratios $\delta^{13}C$ values is a reliable indicator because enantiomers from the same natural source are expected to have similar $\delta^{13}C$, even in the case of partial racemization of chiral molecules; in the identical body, racemic compounds are generally formed by the same biochemical pathways.[157] Using a cGC-IRMS technique, Frank *et al.*[157] have established a ratio of linalool enantiomers of 13% (R): 87% (S) for genuine coriander oils. This finding is in disagreement with those mentioned by Cassabianca *et al.*[11] showing that the presence of 81% enantiomeric excess of the (S)-$(+)$-linalool in coriander oil reveals an abnormal enantiomeric distribution and should be considered as a potential indicator of oil adulteration. Respecting the findings of each author, use of reliable techniques such as GC-IRMS could serve as a strong counter-argument to Cassabianca's conclusions. We must say that the choice of analysis method is crucial to

Table 8. Applications of the main analytical techniques used in assessing the genuineness of linalool

Essential oil/matrix	Extraction	ee (%) (R)-$(-)$-LIN	ee (%) (S)-$(+)$-LIN	Technique, column, detection	Ref.
Lavandula angustifolia EO	SD SFE C	95.5–98.2 96 96–100	4.5–1.85 4 4–0	Enantio-MDGC; 2,6-di-methyl-3-pentyl-β-cyclodextrin; 2,3-diethyl-6-t-butyl-dimethylsilyl-β-cyclodextrin; MS; FID	11
Citrus aurantium var. *bergamia* EO	CP SD	99.9–100 74–79	0.1–0 26–21	Enantio-MDGC; 2,6-di-methyl-3-pentyl-β-cyclodextrin; 2,3-diethyl-6-t-butyl-dimethylsilyl-β-cyclodextrin; MS; FID	11
Petitgrain EO	SD	72.2	27.8	Enantio-MDGC; 2,6-di-methyl-3-pentyl-β-cyclodextrin; 2,3-diethyl-6-t-butyl-dimethylsilyl-β-cyclodextrin; MS; FID	11
Ocimum basilicum EO	SD SFE	99–100 99	1–0 1	Enantio-MDGC; 2,6-di-methyl-3-pentyl-β-cyclodextrin; 2,3-diethyl-6-t-butyl-dimethylsilyl-β-cyclodextrin; MS; FID	11
Coriandrum sativum EO	SD SE	10 13	90 87	Enantio-MDGC; 2,6-di-methyl-3-pentyl-β-cyclodextrin; 2,3-diethyl-6-t-butyl-dimethylsilyl-β-cyclodextrin; MS; FID	11
Lavandula angustifolia EO	SD	min. 88	max. 12	Enantio-cGC-MS, 2,3-di-O-ethyl-6-O-t-butyldimethylsilyl-β-cyclodextrin; FID	72
	C	94.5–98.2	5.5–1.8	Enantio-MDGC; Carbowax 20 M/heptakis (2,3-di-O-acetyl-6-O-t-butyldimethylsilyl)-β-cyclodextrin in OV-1701-vinyl; FID	137
Melaleuca alternifolia EO	SD	63–70	37–30	SBSE-enantio-MDGC; heptakis (2,3-di-O-methyl-6-O-t-	139

(Continues)

Table 8. *(Continued)*

Essential oil/matrix	Extraction	ee (%) (R)-(−)-LIN	ee (%) (S)-(+)-LIN	Technique, column, detection	Ref.
				butyldimethyl-silyl)-β-cyclodextrin; FID	
Melaleuca alternifolia	HSSE	67–71	37–29	Enantio-MDGC; heptakis (2,3-di-O-methyl-6-O-t-butyldimethyl-silyl)-β-cyclodextrin; FID	139
	In vivo HSSE	91–95	9–5		
Thymus vulgaris	*In vivo* HSSE	> 99	<1	Enantio-MDGC; heptakis (2,3-di-O-methyl-6-O-t-butyldimethyl-silyl)-β-cyclodextrin; FID	139
Citrus aurantium var. *bergamia* EO	CP	100	0	Enantio-GC; Oktakis (3-O-butyryl-2,6-di-O-pentyl)-γ-cyclodextrin (Lipodex E) in OV-1701; FID	141
	C	20–95	5–80		
	SD	68.8	31.2	Enantio-GC; Oktakis (3-O-butyryl-2,6-di-O-pentyl)-γ-cyclodextrin (Lipodex E) in OV-1701; FID	141
	CP	99.5–99.7	0.3–0.5	Enantio-GC; GC-C-IRMS; Megadex DETTBS-diethyl-t-butylsilyl-)-β-cyclodextrin; FID	142
	PD	97.8–98.9	1.1–2.2		
Melaleuca alternifolia EO	C	32–48	68–52	Enantio GCxGC; diethyl-t-butylsilyl-β-cyclodextrin; Rtx5-MS low polarity stationary phase column; FID	143
Oenothera odorata	HS-LPME	Qualitative data	Qualitative data	Enantio-cGC-MS; heptakis 30% (2,3-di-O-methyl-6-O-t-butyldimethyl-silyl)-)-β-cyclodextrin; MS	146
Ocimum basilicum EO	HD	92–100	8–0	Enantio-GC, heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin; FID	147
Ocimum gratissimum EO	HD	100	0		147
Ocimum sanctum EO	HD	5	95	Enantio-GC, heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin; FID	147
Ocimum canum EO	HD	6.5	93.5	Enantio-GC, heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin; FID	147

Sample	Extraction	(%)	(%)	Analytical method / column	Ref.
Ocimum kilimandscharicum EO	HD	29.7	70.3	Enantio GC; enantio GC-qMS; Restek Rt™-β-DEXse (6-t-butyldimethylsilyl-2,3-diethyl-β-cyclodextrin); FID	148
Raw and roasted cocoa beans	C	8–2.5	92–97.5	Enantio-MDCG, heptakis (2,3-di-O-methyl-6-O-t-butyldimethylsilyl)-β-cyclodextrin; FID	149
Cocoa products (cocoa powders, chocolates)	C	5–13	95–87		
White wines	HS-SPME	47–100	53–0	MDCG, permethylated β-cyclodextrin (chirasil-β-Dex); FID	150
Citrus limonum EO	CP	50.19–59.58	49.81–40.22	Enantio-cGC, Megadex DETTBS-β-(diethyl-t-butylsilyl-β-cyclodextrin); FID	152
Citrus reticulata EO	Sfumatura	45.6	54.4	Enantio-MDGC-MS, Megadex diethyl-t-butylsilyl-β-cyclodextrin; FID	153
	Brown oil extractor	30.6	69.4	Enantio-MDGC-MS, Megadex diethyl-t-butylsilyl-β-cyclodextrin; FID	153
Flavoured strawberry foods	HS-SPME	59.75	40.25	GC-C-IRMS	154
Lavandula angustifolia EO	C	49.5–78	50.5–22	Enantio-MDGC; Superox heptakis (2,3,6-tri-O-ethyl)-β-cyclodextrin) in OV-1701-vinyl; FID	155
	BP	98	2	Enantio-MDGC; Superox heptakis (2,3,6-tri-O-ethyl)-β-cyclodextrin) in OV-1701-vinyl; FID	155
Lavandula latifolia EO	C	64	36	Enantio-MDGC; Superox heptakis (2,3,6-tri-O-ethyl)-β-cyclodextrin) in OV-1701-vinyl; FID	155
Lavandin EO	C	> 97	<3	Enantio-MDGC; Superox heptakis (2,3,6-tri-O-	155

(Continues)

Table 8. *(Continued)*

Essential oil/matrix	Extraction	ee (%)		Technique, column, detection	Ref.
		(R)-(−)-LIN	(S)-(+)-LIN		
				ethyl)-β-cyclodextrin) in OV-1701-vinyl; FID	157
Coriandrum sativum EO	SD, C	13, 27–34	87, 73–66	cGC-IRMS; Stabilwax (2,3-di-O-methyl-6-O-t-butyldimethylsilyl)-β-cyclodextrin; FID	
Citrus aurantifolia EO	CP	12–34	88–66	Enantio-MDGC, GC-C-IRMS; Megadex diethyl-t-butylsilyl-β-cyclodextrin; MS; FID	158

BP, biological production; C, commercial; CP, cold-pressing; ee, enantiomeric excess; EO, essential oil; HSSE, headspace sorptive extraction; HS-LPME, HS-liquid-phase microextraction; HS-SPME, HS-solid-phase microextraction; HD, hydrodistillation; LIN, linalool; PD, Peratoner distillation; SBSE, Stir bar sorptive extraction; SD, steam distillation; SE, solvent extraction; SFE, supercritical fluid extraction.

highlight the authenticity of a product. For pharmaceutical coriander oil, the *European Pharmacopoeia* 6th edition,[72] admits a maximum level of 14% (S)-(+)-linalool. A more efficient tool is enantioselective MDGC coupled with GC-C-IRMS. The method is highly sophisticated and IRMS enhances data accuracy, allowing to detect subtle adulterations (addition of suitable synthetic products or racemates to essential oils containing optically active components) or even to distinguish counterfeit oils from contamined ones.[133,158] Applying this method, Mosandl *et al.* established a range of authenticity for petitgrain oils, correlating isotopic data and ratios of linalool enantiomers along with other chiral compounds characteristics.[132,159] However it seems that the ratio of deuterium/hydrogen (D/H) allows a better appreciation of the linalool authenticity because its value differs significantly in case of natural and synthetic linalool (123.7 compared to 130.1). To characterize the natural or synthetic status of a sample, isotopic fingerprint provided by SNIF-NMR is considerably more powerful than IRMS. The method performs easily a qualitative differentiation between natural, semisynthetic and synthetic linalool. NMR determination of 10 site-specific hydrogen isotope ratios in the case of unnatural and natural linalool reveals important differences between the values obtained for each type of linalool. Ethylene groups play a central role in the isotopic fingerprint. The natural compound is characterized by a strong depletion in the heavy isotope on site 1 and a relative enrichment in site 6; methyl isotopomers 7 and 8 are less abundant than isotopomer 10. In contrast, the synthetic molecule is characterized rather by a plate distribution at sites 3 and 6–10 on the one hand, and on the other hand, at sites 1, 2 and 4. In the case of linalool obtained from pinene by semi-synthesis, the molecular site 3 is more enriched in deuterium than natural linalool.[160]

Conclusion

The extraordinary ability to combine both versatility of the floral scent and the multifaceted biological profile grafted on a low toxicity background, indicates linalool as a valuable fragrant molecule with significant therapeutic potential. Additionally, linalool can be a precious marker in assessment of flavours, fragrances and essential oils authenticity but its use is significantly conditioned by the chirality changes induced by the vegetal matrix and processing methods.

The most important biological properties are central nervous system depressant effects, analgesic and anti-inflammatory activities. Linalool is capable of interacting with various brain neurotransmitters (glutamic acid, GABA, acetylcholine, dopamine, serotonin) and ionic channels but the mechanisms of activity are still poorly understood. It is difficult to assess the difference in activity between (R)- and (S)-linalool. Most studies have been conducted on (R)-(−)-linalool, linalool racemate or the compound without any specified enantiomeric identity. The mechanisms of activity have been demonstrated predominantly for linalool racemate and (R)-(−)-linalool. They have a similar profile of activity but the effects of (R)-(−)-linalool are more intense. For an accurate assessment of the biological activity, future studies should also include the influence of enantiomers biotransformation in the human body.

Many questions remain to be answered, and not just to clarify and highlight certain mechanisms of activity, but also with regard to adopting consistent methodological criteria for clinical research. Another significant issue to be determined is

to what extent linalool achieves and maintains concentrations required for affecting brain neurotransmitters. To what extent the effect of linalool is as intense and durable as those of conventional medicines?

Skin sensitization potential is the most important side effect of linalool. While it is absolutely true only for oxidized linalool, pure compound is labelled as a common allergen odorant, strict standards of use being specified. The sensitizing routine tests should include oxidized linalool. However, pharmacological and toxicological substantiation of a pharmaceutical product containing linalool should take into account the skin sensitization potential, particularly in combination with other similar medicines.

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