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Abstract

When we take a look at the scientific literature we can see that there are sufficient studies that investigated properties of essential oils or analysed the composition of chiral constituents. But just a small amount of them investigated properties and biological activities of these chiral compounds. This review gives an overview over studies published within the past 20 years concerning properties of α-bisabolol, borneol, camphor, carvone, citronellal, citronellol, curcumene, limonene, linalool, menthol, α- and β-pinene, α-santalol and terpinen-4-ol enantiomers.

In conclusion this review indicates that

(1) there cannot be a common sense statement about the activity of enantiomers, because all possible situations are common: The enantiomers possess qualitatively similar activities but different potencies, they can have a complete different or even opposite biological activity or all stereoisomers exert almost the same activity. After considerations, a small trend to the (+)-enantiomer being the more potent enantiomer of discussed monoterpenes is visible,

(2) some chiral constituents display remarkable differences in their biological activity,

(3) referring to antimicrobial activity the question arises whether it is worthwhile to take the single enantiomer or better to work with the whole essential oil to probably benefit from synergistic effects. Even here a common sense statement is not possible, because single monoterpenes could as well display a higher antimicrobial activity,

(4) after all, analysing enantiomeric excess plays an important role in quality and authenticity control of essential oils.

Zusammenfassung

Bei näherer Betrachtung der wissenschaftlichen Literatur erschließt sich eine Fülle von Studien, die die Eigenschaften ätherischer Öle erforschen oder ihre chemische Zusammensetzung hinsichtlich chiraler Inhaltsstoffe untersuchen. Jedoch nur ein kleiner Teil dieser Studien beschäftigt sich mit der biologischen Aktivität und den Eigenschaften jener chiraler Einzelkomponenten. Dieser Review wirft einen Blick auf die Studienlage der letzten 20 Jahre und fasst jene Arbeiten zusammen, welche die Eigenschaften von α-Bisabolol, Borneol, Campher, Carvon, Citronellal, Citronellol, Curcumen, Limonen, Linalool, Menthol, α- und β-Pinen, α-Santalol und Terpinen-4-ol in ihren enantiomeren Formen untersuchen.

Die vorliegende Arbeit kommt zu den Schlussfolgerungen, dass

(1) keine allgemein gültige Aussage bezüglich der biologischen Aktivität von chiralen Monoterpenen getroffen werden kann, denn qualitativ ähnliche Aktivitäten (jedoch in unterschiedlicher Ausprägung) sind genauso möglich wie ganz andere bzw. sogar entgegengesetzte Eigenschaften. Es konnte jedoch ein leichter Trend sichtbar gemacht werden, der aufzeigt, dass das jeweilige (+)-Enantiomer der besprochenen Monoterpene eine höhere biologische Aktivität zeigt,

(2) einige chirale Inhaltsstoffe bemerkenswerte Unterschiede in ihrer biologischen Aktivität aufweisen,

(3) sich die Frage stellt, ob es bezüglich der antimikrobiellen Aktivität überhaupt lohnenswert ist, das einzelne Enantiomer zu isolieren oder besser mit dem kompletten ätherischen Öl zu arbeiten, um so Synergieeffekte zu nutzen. Aber auch hier kann keine allgemein gültige Aussage getroffen werden, da eine höhere antimikrobielle Aktivität einzelner Monoterpene auch möglich ist,

(4) schlussendlich die Bestimmung des enantiomeren Überschusses eine wichtige Rolle in Qualitäts- und Authentizitätskontrollen von ätherischen Ölen spielt.

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1 INTRODUCTION

Over the years many studies were carried out in order to investigate essential oils for their antimicrobial, anticancer, antiviral and anti-phlogistic activities and insect repellent effects. We live in a chiral environment and nowadays it is well known that chirality has a deep impact on the activity of molecules. Here the question arises how important this fact is when we take a closer examination on chiral constituents.

A short literature research suffices to see that there are several recent reviews [1-3] that dealt with bioactivity of essential oils, but there is a limited number of studies that investigated effects of single chiral constituents. Therefore, the aim of this review is to give an overview of the scientific literature published within the past 20 years.

2 CHIRAL CONSTITUENTS

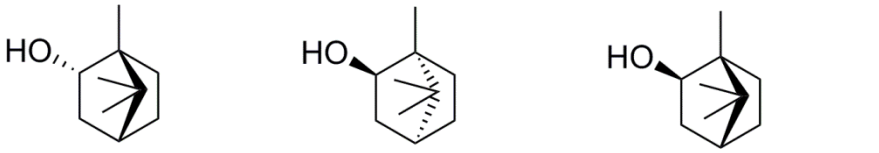
The intention of this review was to compare chirality pairs of mono- and sesquiterpenes. As a matter of fact, for some terpenes only data from one single enantiomeric form is available and the vast majority of researched literature concentrates on monoterpenes. In the following the results of studies on chiral constituents are presented, first dealing with chirality pairs and then reviewing single enantiomers.

2.1 Monoterpenic chirality pairs

In this part the most prominent monoterpenes are listed in alphabetical order. After a short introduction about common facts each chapter deals with the results from reviewed studies since 1995 in chronological order.

In this subject area most efforts have been done so far to elucidate the topic of different biological activities comparing enantiomers with each other. This chapter presents studies on borneol, camphor, carvone, citronellal, citronellol, limonene, linalool, menthol and pinene enantiomers.

2.1.1 Borneol



(1R,2S,4R)-(+)-Borneol (1S,2R,4S)-(-)-Borneol (1R,2R,4R)-Isoborneol

Structure 1 Borneol stereoisomers

Borneol is a bicyclic monoterpene alcohol and exists in three enantiomeric forms. Isoborneol is the so called exo isomer [4] because the asymmetric hydroxyl group is furthest away from the longest bridge [5]. Borneol is a structural analogue to camphor. (+)-Borneol expires a slightly sharp camphoraceous flavour with earthy-peppery note and often occurs in camphor essential oil [4] or in *Rosmarinus* and *Lavandula* species (Lamiaceae) [6]. (-)-Borneol is less sharp and earthy-peppery and is often found in *Pinus* and *Abies* species (Pinaceae) [4].

Only few studies examined differences in biological activity regarding chirality.
Tabanca et al. (2001) [6] examined antimicrobial activity of essential oil from *Micromeria cristata* subsp. *phrygia* (Lamiaceae) grown in three different locations and compared its effect with both borneol enantiomers. Essential oil was obtained by water-distillation and analysed by a multidimensional gas chromatography-mass spectrometry system. All samples contained pure (-)-borneol with an enantiomeric excess (ee) of 100% (and 99% (-)-camphor). Quantities of (-)-borneol in extracted essential oil varied between 29.9% and 39.3%. Minimal inhibitory concentration (MIC) was determined by microdilution broth susceptibility assay and Gram-positive, Gram-negative baceteria as well as yeast were examined (for detailed information see Table 1). All tested substances displayed antimicrobial activity and mostly the whole essential oil was more active than the single enantiomer. Remarkably, activity of tested substances remained below standard activity except for the pathogens *Pseudomonas aeruginosa, Enterobacter aerogenes* and *Candida albicans*. Here the activity was equipotent or even higher. The authors pointed out, that they have previously shown that (-)-camphor had a comparatively higher activity than borneol against micro-organisms [7].

Pathogen	Minimal inhibitory concentration (mg/ml)					
	Sample 1	**Sample 2**	**Sample 3**	**(-)-Borneol**	**(+)-Borneol**	**Standard**
Escherichia coli	62.5	250	250	125	250	62.5*
Staphylococcus aureus	250	250	125	250	125	7.81*
Pseudomonas aeruginosa	250	250	125	250	125	250*
Enterobacter aerogenes	125	125	125	125	125	125*
Proteus vulgaris	125	62.5	62.5	125	125	31.25*
Salmonella typhimurium	62.5	31.25	62.5	125	125	62.5*
Candida albicans	62.5	125	31.25	125	250	125**
* Chloramphenicol ** Ketoconazole						

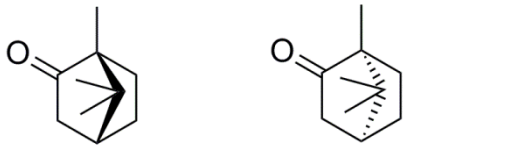
Table 1 MIC (mg/ml) for examined micro-organisms (newly drawn and adapted from Tabanca et al. [6], p. 4302)

In Chinese medicine borneol is used as an analgesic and anaesthetic drug [8]. Hall et al. (2004) investigated effects on $GABA_A$ receptor and glycine receptor of several monoterpenes ((-)-borneol, camphor enantiomers, carvone enantiomers, menthol enantiomers, (-)-menthone α-thujone and α/β-thujone) [20]. The $GABA_A$ receptor is a ligand gated chloride ion channel. Its ligand GABA (gamma-aminobutyric acid) acts anxiolytic, sedative and analgesic, so the receptor is an important target for sedatives, such as benzodiazepines and barbiturates (those are allosteric modulators and amplify the effect of GABA). The glycine receptor is also an inhibitory receptor and belongs also to the family of ligand-gated ion channels. Oocytes from *Xenopus laevis* (the African clawed frog) were used to express human recombinant $\alpha_1\beta_2\gamma_{2s}$ $GABA_A$ receptors and homomers of the α1 subunits from glycine receptors. At concentrations between 10 and 300 µM (-)-borneol expressed positive modulatory effects on both receptors (not as high as menthol enantiomers, but higher than camphor and carvone enantiomers) [20]. Unfortunately (+)-borneol was not tested. Another study on $GABA_A$ receptors was implemented by Granger et al. (2005) [8]. Again, oocytes from *Xenopus laevis* were used to express human recombinant $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors. All three stereoisomers of borneol, (-)-bornyl acetate and camphor racemate were tested on both, their effects on GABA activity as well as on direct receptor activity. At very low GABA concentrations (+)-borneol produced the highest responses with over 90% enhancement of maximal

GABA response, followed by (-)-borneol. This effect did not endure at higher GABA concentrations. The other monoterpenes only produced marginal elevation of GABA response. At maximal GABA concentration, dose-response relationships were tried to create with (+)-borneol (at concentration of 500 µM) being the most promising monoterpene causing a 19% potentiation. In contrast to this (-)-borneol and isoborneol generated a clear decrease in GABA response (21% and 44%). At high concentrations (1.5 mM and higher) borneol enantiomers directly activated $GABA_A$ receptor ((+)-borneol to a greater extent than (-)-borneol), but the authors emphasised that it would be improbable for those concentrations to reach the brain. So this study came to the conclusion that (+)-borneol and (-)-borneol act as divergent modulators on GABA response (at maximal GABA concentrations) [8].

These findings are supported by a most recent study from Jiang et al. (2015) [9]. The authors pointed out, that other papers previously proved that borneol exerts neuroprotective and anti-inflammatory effects [10-12] and that it could be helpful in treating neuropathic pain. To investigate influence on nociceptive threshold a segmental spinal nerve ligation, complete Freund's adjuvant test (causes inflammatory pain) and tests with mechanical hypersensitivity on mice were conducted. (+)-Borneol was either given perorally (between 125 and 500 mg/kg) or intrathecally (between 15 and 60 µg). In all models (+)-borneol was able to significantly elevate pain threshold and on this effect $GABA_A$ receptor is involved because bicuculline (a selective $GABA_A$ receptor antagonist) abolished the (+)-borneol effect [9].

2.1.2 Camphor



(1R,4R)-(+)-Camphor (1S,4S)-(-)-Camphor

Structure 2 Camphor enantiomers

Camphor is a bicyclic monoterpene ketone and the major compound in camphor essential oil, with a mean value of 75% [13]. The enantiomers can be found quite often in nature, they occur e.g. in essential oils of *Ocimum* species (Lamiaceae; (+)-camphor, which is

the more widespread enantiomer) and *Tanacetum* species (Asteraceae; (-)-camphor). Both have similar slightly minty and camphoraceous odours [4].

There are only few studies that dealt with isolated camphor enantiomers. As mentioned before some papers investigated effects of camphor enantiomers [20] and camphor racemate [8] on $GABA_A$ receptor. In both studies camphor only produced marginal impact on positive modulation on $GABA_A$ receptor. Furthermore there were no differences in biological activity of camphor enantiomers, but they displayed a higher activity than carvone enantiomers [20].

Tabanca et al. (2007) tested antimicrobial activity of essential oil from *Tanacetum argenteum* subsp. *flabellifolium,* (Asteraceae; the oil contains high amouts of (-)-camphor) against *Candida albicans* (yeast), *Staphylococcus aureus* (Gram-positive bacteria)*, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Enterobacter aerogenes and Salmonella typhimurium* (Gram-negative bacteria) with various success [14]. It showed good growth inhibition against *C. albicans*, *E. aerogenes* and *P. aeruginosa* with minimal inhibitory concentration of 125 µg/ ml. Enantiomeric distribution was examined by using γ-cyclodextrine capillary column. The main compounds were α-pinene with 29% (72% (-)-α-pinene enantiomeric excess (ee)) and camphor with 14% (100% (-)-camphor ee) [14]. The authors emphasised that it can be worthwhile testing single major chiral constituents on their antimicrobial activity. Also Viljoen et al. showed that (-)-camphor exposed good antimicrobial activity (even higher activity in combination with 1,8-cineole) [7,15].

Other studies investigated the enantiomer-specific antifungal activity of camphor.

Pragadheesha et al. (2013) tested *Cinnamomum camphora* (Lauraceae) essential oil (which main component is (+)-camphor) as well as some chiral constituents on their activity against a special phytopathogenic fungus (*Choanephora cucurbitarum*) [13]. The oil was obtained from the leaves of *C. camphora* and the enantiomeric excess was determined by using cyclodextrin coated β-DEX 110 and Restek Rt^{TM}-β-DEXse fused silica capillary columns. Diffusion assay and poisoned food technique were used to test growth inhibition of the fungus by different amounts of *C. camphora* essential oil. In addition to that, standard (+)-camphor, (-)-β-pinene, (+)-limonene and (-)-limonene were tested the same way. Control plates were utilised to measure growth inhibition. Chiral (+)-camphor exposed good antifungal activity (70-80%, see Figure 1). Nevertheless, it revealed that the whole essential oil is most potent (approximately 90%) in its antifungal activity [13].

Furthermore, (+)-camphor can be used to prove the authenticity of *C. camphora* essential oil, because in all tested samples an enantiomeric excess of over 99% of (+)-camphor was observed, whereas (-)-camphor was below 1% ee [13].

The same authors [16] investigated the effects of essential oil from two *Ocimum* species (*O. canum* and *O. kilimandscharicum,* both Lamiaceae) on the fungi *Rhizoctonia solani* and *Choanephora cucurbitarum*. Both essential oils contain an enantiomeric excess of over 99% (+)-camphor and in *O. kilimandscharicum* essential oil it is the most exclusive constituent with 66.5%. Micro-organisms were completely inhibited by the essential oil of *O. kilimandscharicum* (whereas essential oil from *O. canum* only inhibited *R. solani*, which can be due to the smaller amount of (+)-camphor with 33.2%) [16].

After all, studies usually use the whole essential oil to study antimicrobial activity and not its single constituents [13,14,16].

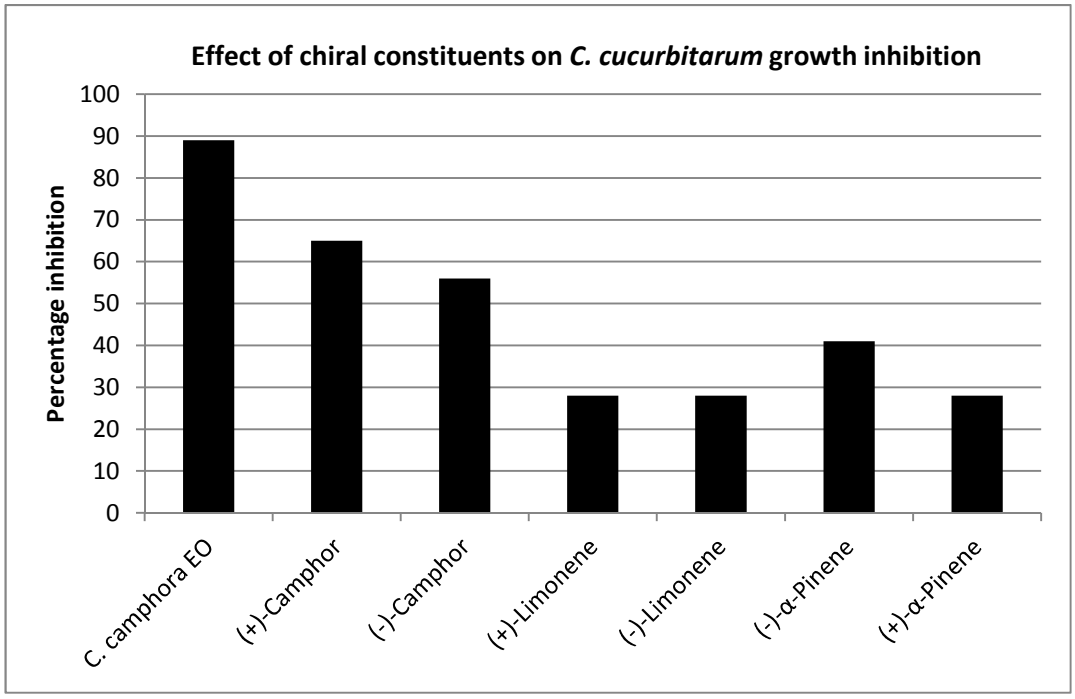
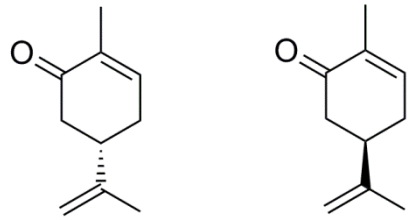


Figure 1 Growth inhibition in percent of *Choanephora cucurbitarum (*phytopathogenic fungus) of major chiral monoterpenes and *Cinnamomum camphora* essential oil measured 24 hours after treatment; mean value of three independent experiments (newly drawn and adapted from Pragadheesha et al.[13] p. 631)

2.1.3 Carvone



Structure 3 Carvone enantiomers

Carvone is a cyclic monoterpene ketone. Probably, it is one of most astonishing chiral monoterpenes, because its enantiomers show rather different odours and properties. (-)-Carvone is a major constituent in spearmint (*Mentha spicata*, Lamiaceae), whereas (+)-carvone is the main aroma in caraway (*Carum carvi*, Apiaceae) [4].

Many facts about their antifungal, antibacterial and insecticidal activity are known yet.
Hartmans et al. (1995) tested (+)-carvone in agriculture against silver scurf (a common disease of potatoes caused by the fungus *Helminthosporium solani*) and other fungi as well as sprout suppression of potatoes, with promising results [17]. Over three different storage seasons potatoes were treated with two different concentrations of (+)-carvone and a special sprout inhibitor for ware potatoes, whose active component is propham/chloropropham (IPC/CIPC). Sprout suppression was equal or better than standard treatment with IPC/CIPC and silver scurf showed a significantly reduced amount of infections (silver scurf index of 33 with IPC/CIPC versus 6 with carvone; this number is composed of percentage of surface affected, multiplied by special factors and divided by the total number of examined potatoes) [17].
(-)-Carvone naturally occurs together with (-)-limonene in spearmint oil (*Mentha spicata*, Lamiaceae; they make up to 83.9% of the oil) and (+)-carvone arises with (+)-limonene in Indian dill oil (*Anethum sowa*, Apiaceae; together up to 76.9% of the oil) [18].
Aggarwal et al. (2001) tested these two essential oils and their major chiral compounds on their biological activity against 12 human pathogen bacteria strains (see Table 2) and 7 fungi (see Table 3) by use of broth dilution assay to ascertain minimal inhibitory dilution [18] (lowest dilution of test substance that inhibits growth).
Results for limonene are discussed separately in chapter 2.1.6 Limonene.

Regarding bacterial growth inhibition, (+)-carvone revealed to be the most potent substance followed by spearmint oil and (-)-carvone. Indian dill oil displayed the lowest activity. Both essential oils showed similar activity against the tested fungal strains, and diverse results in comparison with carvone enantiomers (Table 3).

Summing up, (+)-carvone was to some extent more effective (30-33%) than (-)-carvone in inhibiting bacterial and fungal growth [18].

Human pathogen	**Zone of microbial growth inhibition (mm) caused by:**				
	M. spicata oil	**(-)-Carvone**	**A. sowa oil**	**(+)-Carvone**	**Kanamycin (30 µg/disc)**
Escherichia coli	4	- [a]	2	-	11
Enterococcus faecalis	5	9	3	12	15
Enterobacter aerogenes	4	9	1	10	10
Pseudomonas aeruginosa	-	6	-	11	10
Salmonella typhimurium	4	9	2	10	14
Salmonella typhi	-	9	-	9	NT [b]
Staphylococcus epidermidis	11	-	5	10	14
Streptococcus mutans	8	10	5	13	12
Mycobacterium smegmatis	8	9	5	18	15
Staphylococcus aureus	10	-	6	12	10
Yersinia enterocolitica	6	12	2	11	15
Klebsiella pneumoniae	4	10	1	10	10
Bacillus subtilis	9	9	7	9	17

[a].....Zone of inhinition not realized

[b].....NT, not tested

Table 2 Antibacterial activity of essential oil from *Mentha spicata* and *Anethum sowa* and carvone entantiomers (newly drawn and adapted from Aggarwal et al. [18] p. 61)

Fungus	Zone of microbial growth inhibition (mm) caused by:			
	M. spicata oil	**(-)-Carvone**	**A. sowa oil**	**(+)-Carvone**
Aspergillus niger	6	12 (1/400) [a]	7	17
Aspergillus flavus	5	- [b]	9	-
Candida albicans AI	8	29 (1/800)	8	25 (1/800)
Candida albicans	11	30 (1/800)	8	29 (1/800)
Microsporum gypseum	36	20 (1/400)	34	24 (1/600)
Sporothrix schenckii	10	23 (1/400)	15	31 (1/400)
Trichophyton rubrum	16	13 (1/400)	13	31 (1/400)

[a].....Value in bracket is the corresponding minimal inhibitory concentration (MIC) as estimated by broth dilution method

[b].....Zone of inhibition not realized

Table 3 Antifungal activity of essential oil from *Mentha spicata* and *Anethum sowa* and carvone entantiomers (newly drawn and adapted from Aggarwal et al. [18], p. 62)

Many studies concentrate on physiological effects of carvone enantiomers [19-23].

Carvone and limonene enantiomers were often examined together (maybe because of their frequent co-appearance in nature). Heuberger et al. (2001) carried out studies on human volunteers [19]. Effects on autonomic nervous system (alteration in pulse rate, breathing rate, blood pressure, skin temperature, etc.) accompanied by subjective evaluation (calmness, pleasantness, mood, etc.) were tested by inhalation in three trials using an A-A-B design. Results for limonene can be found in chapter 2.1.6 Limonene. (-)-Carvone led to increase in subjective restlessness, puls rate and diastolic blood pressure while (+)-carvone only increased systolic and diastolic blood pressure. After all, the authors pointed out that chirality does have an influence on the mode of action, but also psychological mechanisms contribute to these effects (a subjective evaluation of the odour is always given in in-vivo studies) [19].

As previously discussed, Hall et al. (2004) investigated several monoterpenes on their interaction with $GABA_A$ and glycine receptor [20]. As mentioned before the $GABA_A$ receptor is a ligand gated chloride ion channel (see chapter 2.1.1 Borneol). The authors used oocytes from *Xenopus laevis* to investigate modulation on the receptors. Only the (+)-enantiomer was tested and expressed just a minimal positive modulation at the receptors (small increase of GABA effects) [20].

Buchbauer et al. (2005) observed locomotion activity in mice which were pre-treated with analeptics or sedatives (intraperitoneal) and received either (-)-carvone or (+)-carvone by inhalation of the vapour [21]. Locomotion activity was measured by light barrier in a cage and compared to a control group. The study showed various outcomes: (-)-carvone counteracts the stimulating effects of analeptics such as caffeine and amphetamine by about 50%, whereas it increases the effects of methamphetamine. (+)-Carvone led to more locomotion activity in phenobarbital (sedative) and methamphetamine (analeptic) treated mice, but also decreases activity of caffeine and amphetamine treated mice. Remarkably, both enantiomers did not alter effects of diazepam [21], which supports the findings of the previously spoken study [20] in which (+)-carvone displayed only poor impact on $GABA_A$ receptor.

Sousa et al. (2007) studied effects on central nervous system (CNS) of mice by injecting either (-)-carvone or (+)-carvone [22]. Parameters such as LD_{50}, locomotion activity, pentobarbital-induced sleeping time, pentylenetetrazole- and picrotoxin-induced convulsions were determined. Both enantiomers showed significantly depressing effects see Figure 2). Only (+)-carvone displayed considerably anticonvulsive effects by prolonging latency time of convulsions. Because of the fact that picrotoxin is a known $GABA_A$ antagonist the authors suggest that (+)-carvone must be able to bind at the picrotoxin binding site to decrease its effects and therefore can be useful in epilepsy treatment [22].

Also Sanchez-Borzone et al. (2014) tested (+)-carvone, (-)-carvone and thujone on $GABA_A$ receptor [23]. Generally, all drugs that increase effects of GABA are analgesic, anxiolytic and sedative substances. Beyond that, the $GABA_A$-receptor is a major target for insecticides, they act as non-competitive antagonists and stop $GABA_A$ induced chloride influx, thus there is a permanent irritation of neurons. Primary cell cultures of cortical neurons from the cerebral cortices of rat foetuses were used to study receptor binding. The authors suggest that (+)-carvone and (-)-carvone (as well as thujone) are negative allosteric modulators because all three monoterpenes were able to inhibit benzodiazepine ([^{3}H]flunitrazepam) binding. This mechanism contributes to the insecticidal properties of carvone, with (+)-carvone as the more potent enantiomer [23].

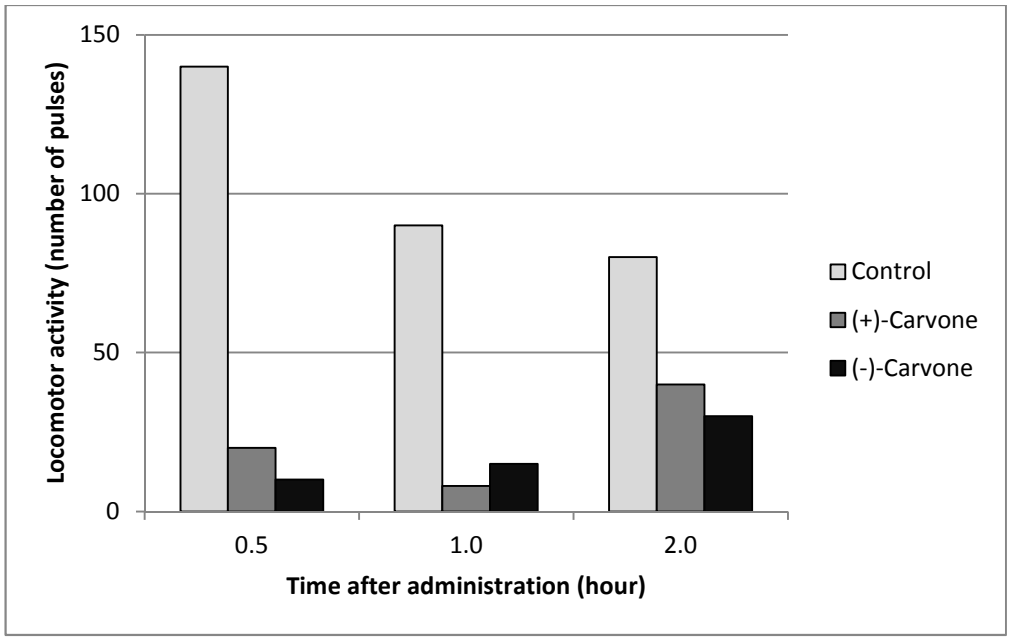
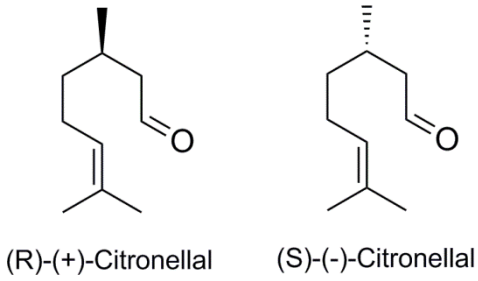


Figure 2 Effect of carvone enantiomers on the locomotion activity on mice; measured in activity cage (total number of pulses); mean values for 6 SEM from eight mice (newly drawn and adapted from Sousa et al. [22] p. 265)

After all, the findings from Sanchez-Borzone et al. [23] stand in contrast to previously discussed studies [20,21] where the enantiomers did not show any significant effect or only displayed a low effect on GABA binding on the receptor. On the contrary it supports the finding from Sousa et al. [22] that carvone enantiomers might be negative allosteric modulators.

2.1.4 Citronellal



Structure 4 Citronellal enantiomers

Citronellal is an acyclic monoterpene aldehyde and occurs in many essential oils in both enantiomeric forms or in a racemic mixture. (+)-Citronellal derives from citronella oil

(*Cymbopogon nardus and winterianus*, Poaceae) whereas *Backhousia citriodora* (Myrtaceae) contains high amounts of (-)-citronellal. Citronellal is a very important flavour and fragrance chemical and is used for citrus flavours [4].

There are just a few studies where the influence of chirality on their biological activity was investigated.

Nhu-Trang et al. (2006) [24] emphasized that chirality of citronellal plays an important role in proving authenticity of essential oils from *Melissa officinalis* (Lamiaceae; lemon balm oil), *Citrus limonum* (Rutaceae, lemon oil) and *Cymbopogon nardus* as well as *Cymbopogon winterianus* (Poaceae, citronella oil). Especially lemon balm oil is a very precious and quite expensive oil so the danger of adulteration exists. For chiral gas chromatography the authors used fused silica column coated with heptakis-(2,3-di-O-acetyl-6-O-tert-butyldimethylsilyl)-β-cyclodextrin SPB20poly (20% diphenyl/80% dimethylsiloxane) with MS and FID detectors. A higher amount of (+)-citronellal was found in *Melissa officinalis* (between 73.5% and 98.8%) and in the oil from *Cymbopogon nardus and winterianus* (between 81.0% and 91.8%) whereas amounts from (-)-citronellal in *Citrus limonum* oil ranged between 82.2% and 91.5% [24].

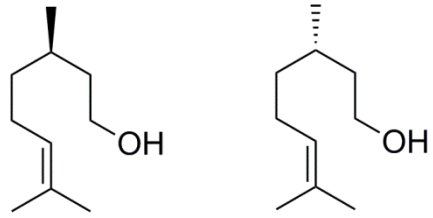
Sousa et al. (2011) examined the spasmolytic activity of chiral citronellyl acetate and chiral linalyl acetate on guinea pig isolated ileum [25]. The paper showed that the racemate displayed higher spasmolytic activity than the single enantiomers. Because of the fact that linalyl acetate was the more effective monoterpene the study is described in chapter 2.1.7 Linalool.

A study from Altshuler et al. (2013) dealt with the effect on cell division of animal and plant cells [26]. (-)-Citronellal and (+)-citronellal were tested for their potency to interfere either with the actin cytoskeleton or microtubules in cells. Microtubules play an important role in mitosis and therefore are targets in anti-cancer therapy. The authors chose two different cell lines: HeLa (most commonly used human cell line; derives from cancer cells [27]) and Ref52 cells (rat cell line [28]). Colchicine, vinblastine and paclitaxel (three well known mitosis-inhibitors) were used as controls. After dissolving the two enantiomers in DMSO they were diluted in the cell growth medium. Then cells were treated with the substances, fixed, stained and quantitatively analysed by the software FiberScore (analyses total fluorescence associated with fibers). Several tests in vitro and with plant cells were done, and all came to the same conclusion: Only (+)-citronellal showed significant activity in disrupting microtubules whereas (-)-citronellal was inactive. The values of total fluorescence associated with fibers were comparable to those

of colchicine and vinblastine treatment, but it should be mentioned that the effect was time and dose-dependent. (+)-Citronellal definitely acts as weak mitosis inhibitor (active concentration was 27.5 µM versus 0.1 µM of colchicine or vinblastine) and therefore possesses anti-cancer activity [26].

Also a most recent paper from Maßberg et al. (2015) dealt with anti-cancer properties by investigating cellular signal transduction by calcium release and over cAMP dependent pathway as well as MAP-kinase pathway [29]. The authors mentioned that olfactory receptors (ORs; there are more than 350 of these G-Protein coupled receptors in humans [29]) influence cancer cell invasiveness and metastasis. Furthermore, they assumed that carcinoma cells might express ectopically ORs. Huh7, a cell line for hepatocellular carcinoma, was used for investigating intracellular calcium levels in a cell culture. Calcium influx was measured by fluorometric calcium imaging. (-)-Menthol, carvone, linalool, limonene, citronellol and some other monoterpenes were tested as well, but (-)-citronellal was the most promising substance, so for further experiments only (-)-citronellal was used (unfortunately (+)-citronellal was not in focus of investigation). A range of RNA experiments were performed with the outcome that (-)-citronellal induced intracellular calcium release via OR1A2, activated MAP-kinase pathway over phosphorylation of p38 and therefore influences cell proliferation [29].

2.1.5 Citronellol



Structure 5 Citronellol enantiomers

Citronellol is an acyclic monoterpene alcohol. The dominance of one form is rarely found in nature. An example for this is *Boronia citriodora* (Rutaceae) which contains high amounts of (+)-citronellol whereas (-)-citronellol is a major compound in geranium

oils [4]. Both enantiomers exert a sweet roselike fragrance and it is said that (-)-citronellol is more delicate than its (+)-enantiomer [4].

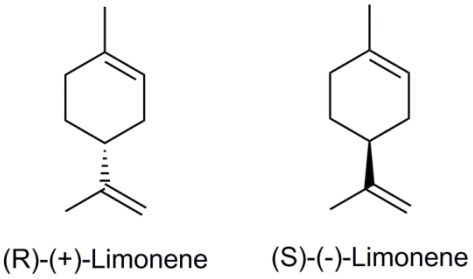
Usually a racemic mixture of both enantiomers is found in essential oils and possibly this explains the fact why there are hardly any studies on citronellol enantiomers. Both enantiomers are a common fragrance in cosmetic products, skin care products and non-cosmetic products (detergents and household cleaners) [30,31].

In 2008 two reviews [30,31] from Lapcynzki et al. about toxicological and dermatological properties of (-)-citronellol and (+)-citronellol were conducted with only moderate findings. For (+)-citronellol not a single paper on skin irritation, toxicity, mutagenicity, genotoxicity and carcinogenicity was available [31].

For the (-)-enantiomer some animal studies on skin sensitization and studies on humans with sensitive skin were conducted with only small reactions or no reactions to (-)-citronellol application. Two animal studies on skin irritation showed that (-)-citronellol applied in different concentrations and dilutions can act as a mild irritant and can cause moderate erythema [30].

For the period between 2008 and 2015 it was not possible to find any studies on biological activities, except a paper [25] on chiral citronellyl acetate (tested together with chiral linalyl acetate) which showed that the racemate displayed higher spasmolytic activity than the single enantiomers. For further information see chapter 2.1.7 Linalool.

2.1.6 Limonene



Structure 6 Limonene enantiomers

Limonene is an unsaturated cyclic monoterpene hydrocarbon and both enantiomers plentifully occur in essential oils. (+)-Limonene exerts an orange flavour and makes up to 90% in citrus peel oils, whereas (-)-limonene smells turpentine-like and is a constituent in essential oil of conifers and in *Mentha* species (Lamiaceae) [4]. Especially

(+)-limonene is often used as aromatic substance in soft drinks, fruit juices, ice cream, etc. [32].

Studies about limonene enantiomers came to considerably different results regarding their anti-microbiological activity. In most studies [18,33,34] (+)-limonene revealed to be the more potent enantiomer.

Lis-Balchin et al. (1996) for instance tested limonene enantiomers against a wide range of bacteria and some fungal strains [34]. (+)-Limonene was highly active against most of bacteria and fungi. Different varieties of *Listeria monocytogenes* (a Gram-positive bacteria; especially dairy products and meat often contain this potential highly pathogenic bacterium that is likely to cause listeriosis) were tested separately with the result that (+)-limonene caused higher growth inhibition (13 out of 20 strains) [34].

As mentioned before, Aggarwal et al. [18] (2001) tested spearmint oil (*Mentha spicata*, Lamiaceae; major chiral compounds are (-)-carvone and (-)-limonene) and Indian dill oil (*Anethum sowa*, Apiaceae; major chiral compounds are (+)-limonene and (+)-carvone) on their biological activity against 12 pathogen bacteria strains (*Bacillus subtilis, Enterobacter aerogenes*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae, Mycobacterium smegmatis, Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Staphylococcus aureus*, *Yersinia enterocolitica*), 5 fungi (*Aspergillus flavus, Aspergillus niger, Microsporum gypseum, Sporothrix schenckii, Trichophyton rubrum)* and the two yeasts (*Candida albicans, Candida albicans AI*) by use of broth dilution assay to ascertain the minimal inhibitory dilution. Single carvone and limonene enantiomers were tested as well. Results for carvone were previously discussed at chapter 2.1.3 Carvone. (-)-Limonene showed just marginal antimicrobial activity because it was only active against the fungus *Microsporum gypseum* (causes dermatophytosis [35]). (+)-Limonene possessed remarkable antibacterial and antifungal activity and was able to inhibit growth of all tested bacterial and fungal strains with fold dilutions from 1/200 to 1/3000. It exhibited highest activity against the dental caries bacterium *Streptococcus mutan*s and the fungus *Microsporum gypseum.* Notably, both isolated (+)-enantiomers are present in the Indian dill oil, but spearmint oil revealed to be the more potent essential oil, which indicates that there are minor compounds that have synergistic effects [18].

Also previously discussed, Heuberger et al. (2001) studied reactions of the autonomic nervous system and impact on the propands' feelings (human volunteers) of carvone and limonene enantiomers [19]. (+)-Limonene caused increased levels of systolic blood

pressure, restlessness and subjective alertness while (-)-limonene only caused an increase in systolic blood pressure. After all, the authors emphasised that chirality does have an influence on the mode of action, but also psychological mechanisms contribute to these effects (a subjective evaluation of the odour is always given in in-vivo studies) [19]. For results for carvone see chapter 2.1.3 Carvone.

Antimicrobial activity of limonene enantiomers was as well determined by van Vuuren and Viljoen (2007) regarding following microorganisms [36]: *Staphylococcus aureus, Enterococcus faecalis* and *Bacillus cereus* (Gram-positive bacteria), *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Moraxella catarrhalis* (Gram-negative bacteria) and *Cryptococcus neoformans* (yeast). 1,8-cineole and its synergistic effects was also in the centre of interest (both occur naturally in essential oils of e.g. *Thymus vulgaris* (Lamiaceae), *Coriandrum sativum* (Apiaceae) or *Zingiber officinale* (Zingiberaceae). Microtitre plate method was used to determine minimal inhibitory concentrations. In this study (-)-limonene clearly revealed to be the more potent enantiomer (see Table 4), as an example it is three times more effective against *S. aureus* than (+)-limonene. These findings are confirmed by a French study [37], but also are in conflict with some other papers [18,33,34]. Interestingly, combinations of limonene and 1,8-cineole showed reduced activity when combined with single enantiomers, but mostly synergistic effects when mixed with the racemate (for exceptions compare Table 4). The authors pointed out that antimicrobial activity is influenced by chirality and emphasized that this activity is highly a pathogen-specific matter. It is suggested that it can be worthwhile to generally use the whole essential oil for antimicrobial treatment to benefit from synergistic effects [36].

A review on (+)-limonene from J. Sun (2007) [38] pointed out its low toxicity, mutagenicity and carcinogenicity in humans and its application in cholesterol-containing gallstone treatment. Furthermore, studies in the 1980s and early 1990s proved good anti-cancer activity against various carcinomas such as mammary cancer, pulmonary adenoma, liver cancer and forestomach tumors [39-44].

Micro-organism	Minimal inhibitory concentration of constituent (mg/ml)						
	(+)-L.	(-)-L.	L. rac. (1:1)	(+)-L.+1,8-Cin.(1:1)	(-)-L.+1,8-Cin.(1:1)	L.rac.+1,8-Cin.	Control
Staphylococcus aureus	13	4	8	16	8	8	$0.3x10^{-3}$
Bacillus cereus	3	3	4	8	4	4	$0.3x10^{-3}$
Enterococcus faecalis	27	27	8	16	8	8	$1.25x10^{-3}$
Escherichia coli	11	8	8	32	27	16	$0.4x10^{-4}$
Pseudomonas aeruginosa	4	4	4	8	8	8	$0.3x10^{-3}$
Klebsiella pneumonia	12	6	8	16	16	16	$0.6x10^{-3}$
Moraxella catarrhalis	8	4	1	16	16	16	$0.3x10^{-3}$
Cryptococcus neoformans	3	2	4	3	2	2	$1.6x10^{-3}$

(+)-L. ..... (+)-Limonene
(-)-L. ..... (-)-Limonene
L. rac. (1:1) ..... Limonene racemate
(+)-L.+1,8-Cin.(1:1) ..... (+)-Limonene and 1,8-Cineole
(-)-L.+1,8-Cin.(1:1) ..... (-)-Limonene and 1,8-Cineole
L.rac.+1,8-Cin. ..... Limonene racemate and 1,8-Cineole
Controls ..... amphotericin B for the yeast *Cryptococcus neoformans*; ciprofloxacin for bacteria

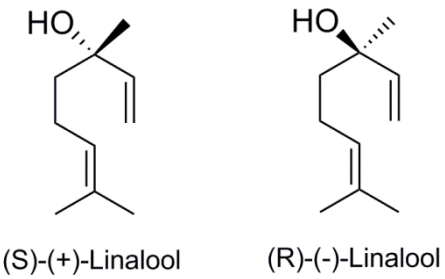
Table 4 Average MIC (mg/ml) of limonene enantiomers and racemate, combinations with 1,8-cineol and controls for selected pathogens (newly drawn and adapted from van Vuuren et al. [36] p. 541)

Moraes et al. (2009) laid the focus on effects on the gastrointestinal tract, because the essential oil from *Citrus aurantium* (Rutaceae; its dominant constituent is (+)-limonene) is often used to treat indigestions [45]. A chromatographic analysis of applied essential oil revealed that limonene was the major component with 97.83%. To test gastric mucus secretion rodents were pre-treated (p.o.) with essential oil (up to 250 mg/kg), limonene (245 mg/kg) or carbenoxolone (100 mg/kg). Afterwards indomethacin (100 mg/kg) or absolute ethanol was administered to cause gastric ulcera and stomachs were examined later on. It could be proved that *Citrus aurantium* essential oil and limonene have a

significant positive impact on the gastrointestinal tract because H^+ concentration in gastric juice was decreased compared to the control group, the mucus production was elevated, and compared to cimetidine treatment no increase of serum gastrin levels (caused by antisecretory acid activity) could be observed [45].

Another study from Moraes et al. (2013) confirmed these findings [46]. The authors compared healing activity of essential oil extracted from *C. aurantium* (Rutaceae) with (+)-limonene by treating acetic acid-induced gastric ulcers in rats. An immunohistochemical analysis was made to measure proliferation of cell nuclear antigen in mucosa cells, VEGF (vascular endothelial growth factor) which mediates blood vessels genesis) and COX-2 (cyclooxygenase 2) expression. Both substances increased gastric mucosa production. It should be emphasised that a lower concentration of (+)-limonene (245 mg/kg) was a bit more effective in epithelial healing than whole essential oil at a concentration of 250 mg/kg (56% versus 44%) [46].

2.1.7 Linalool



Structure 7 Linalool enantiomers

Linalool is an acyclic monoterpenoid alcohol and a very common constituent in essential oils in nature (e.g. *Achillea* (Asteraceae), *Thymus* (Lamiaceae), *Ocimum* (Lamiaceae), *Tanacetum* (Asteraceae), *Coriandrum* (Apiaceae) species). Ho leaf essential oil which derives from *Cinnamomum camphora* (Lauraceae) contains more than 90% linalool, its acetate is very widespread in plant life (e.g. *Citrus*, *Lavandula* and *Thymus* species) and a significant fragrance in lavender [47]. (-)-Linalool has a woody, lavender odour, while (+)-linalool smells sweet and like petitgrain (bitter orange essential oil).[48]

Sugawara et al. (1998) [49] examined sedative effects of linalool enantiomers and its racemate on volunteers by using a sensory test with impression scale and additionally a conventional forehead surface electroencephalography (a physiological measurement to

determine alpha and beta waves; alpha waves signal relaxed awakeness, and normal attentive awakeness is attributed to beta waves which have a higher frequency [58]). After inhalation, odour impressions were recorded before and after doing mental and physical work and hearing environmental noise. The Student's t-test was used to determine statistical significant changes on impression scale. A tendency to a decrease of beta waves (which means a trend to relaxation) by (-)-linalool and a tendency to an increase of beta waves by (+)-linalool (which means a trend to activation) was observed [49].

As linalool is a competitive antagonist of NMDA receptor (N-methyl-D-aspartate) it causes antinociception [50,51] so it is worthwhile to examine whether there are enantiomer-specific differences. Peana et al. carried out several studies [52-55] about antinociceptive and anti-inflammatory effects of linalool enantiomers, especially (-)-linalool. In an anti-inflammatory study [52] (2002) rat paw edema were treated with either linalool racemate, (-)-linalool and linalyl acetate (systemically application). Both, the racemate and the pure enantiomer caused edema reduction at a dose of 25 mg/kg body weight. Racemic linalool did not show effects at doses of 12.5 mg/kg body weight. Furthermore, at higher doses (-)-linalool led to a prolonged anti-inflammatory effect and even caused maximum inhibition of edema. Linalyl acetate was least potent [52].

In another study from Peana et al. (2003) only (-)-linalool was tested in two different experimental models of pain in mice (hot plate test to investigate supraspinal analgesia and acetic acid-induced writhing response for anti-inflammatory effects), because of its widespread occurrence in essential oils [53]. Significant effects were achieved at doses between 25 and 75 mg/kg body weight in acetic acid-induced writhing response test. In the hot plate test the enantiomer was less potent and only showed analgesic effects at concentrations of 100 mg/kg body weight. This study also confirms that (-)-linalool definitely displays anti-inflammatory effects [53].

Two other studies explored sedative properties of linalool. Kuroda et al. (2005) tested the effect of linalool enantiomers (as well as jasmine tea) on 24 volunteers in two intervention groups and one control group [56]. Their heart rate was measured by electrocardiography while inhaling chiral linalool under standardised conditions. The POMS test (Profile of Mood States) was performed before and after inhaling. All odours remained beyond their threshold. Results clearly revealed that chirality does have an influence on sedative properties. With (+)-linalool there was a significant elevation of heart rate whereas (-)-linalool decreased it. Evaluating the mood scales it turned out that

(-)-linalool decreased negative mood scales while (+)-linalool increased negative mood scales and decreased the positive scales [56].

Höferl et al (2006) implemented a study on 24 volunteers in which physiological parameters such as blood pressure, heart rate and electrodermal activity, as well as salivary cortisol were determined before, during and after performing mental stress tests [57]. Results were compared with a control group. Linalool enantiomers were atomized in two different experimental rooms and participants were not told about being exposed to the odour to avoid being influenced by expectation. Also in this study (-)-linalool indicated a significant decrease in heart rate whereas (+)-linalool showed the opposite effects on blood pressure and heart rate (incidentally both enantiomers displayed relaxing effects by decreasing salivary cortisol levels). These results go in accordance with Sugawara et al [57].

Özek et al. (2010) examined 42 different essential oils (including various species of *Thymus, Salvia, Ocimum, Lavandula, Nepeta, Xanthoxylum, Satureja, Origanum, Achillea, Cinnamomum* and *Micromeria*) for their content of linalool enantiomers [59]. In some plant families it was possible to find pure enantiomers: (-)-Linalool showed 100% enantiomeric excess (ee) in *Achillea grandifolia* (Asteraceae), *Ocimum basilicum* (Lamiaceae), *Origanum floribundum, Origanum majorana* (Lamiaceae), various *Thymus* species (Lamiaceae) and *Xanthoxylum armatum* (Rutaceae). 100% ee of (+)-linalool was found in five different *Nepeta* species (Lamiaceae), *Satureja spinosa* (Lamiaceae) and *Tanacetum chiliophyllum* (Asteraceae) (see Table 5). To analyse the accurate composition of chiral constituents can be a very useful tool in the quality control of essential oils.

Biological activities of linalool enantiomers were examined with regard to their antimicrobial and antimalarial properties [59]. Antimicrobial assay was performed with some bacterial strains, yeasts and several fungal species and compared to commercial fungicides. Both enantiomers revealed similar results. Enantiomers were inactive against *Candida albicans, Candida glabrata, Candida krusei, Cryptococcus neoformans* (yeasts), methicillin-resistant *Staphylococcus aureus, Mycobacterium intracellulare* (Gram-positive bacteria) at their highest test concentration (200 µg/ml). Only with micro-dilution broth assay against *Botrytis cinerea* (a mould which infects many different plants) an approx. growth inhibition of 50% could be attained. Activity against other fungi (A*spergillus fumigatus*, *Colletotrichum* species, *Phomopsis* species and *Fusarium*

oxysporum) was not observed. Antimalarial activity was examined against *Plasmodium D6* and *W2* clones with negative results (highest concentration was 4.76 µg/ml) [59].

Plant name	Linalool in the oil (%)	(+)-Linalool (%)	(-)-Linalool (%)
Achillea grandifolia Friv.	18.1	-	100
Nepeta italica L.	39.9	100	-
Lavandula angustifolia Mill.	30.4	5.9	94.1
Ocimum basilicum L.	43.1	-	100
Origanum floribundum Munby	16.1	-	100
Origanum majorana L.	12.1 41.2	- -	100 100
Origanum onites L.	90.3 79.8	1.6 -	98.4 100
Salvia palaestina Benth.	70.6	17.9	82.8
Satureja spinosa L.	61.5	100	-
Tanacetum chiliophyllum Sch. Bip. var. *monochephalum* Grierson	10.3	100	-
Thymus revolutus Celak.	15.5	-	100
Thymus sibthorpii Benth.	20.4	-	100
Thymus zygioides Griseb. var. *zygioides*	33.7	-	100
Xantoxylum armatum D.C.	46.1	-	100

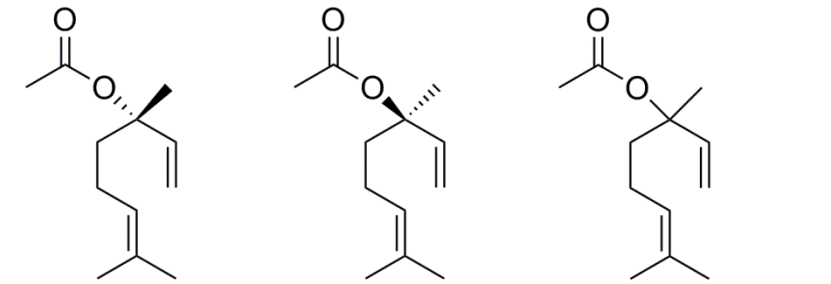
Table 5 Content of linalool essential oil and enantiomeric excess in selected plants (newly drawn and adapted from Özek et al.[59] p. 187f)

As previously mentioned, a study from Sousa et al. (2011) examined spasmolytic activity of (+)-linalyl acetate and (-)-linalyl acetate as well as their racemate [25]. Their spasmolytic effect was tested on ileum smooth muscle cells of guinea-pig by evaluating a concentration-response curve and determining the EC_{50} values. Linalyl acetates enantiomers and linalyl racemate all were equipotent. Same tests were done with citronellyl acetate, with slightly different results. Citronellyl acetate enantiomers were equipotent, their racemate displayed higher activity. The authors assumed that the position of the functional groups on the acyclic esters is decisive for their activity and in citronellyl acetate racemate they might have synergistic effects. Linalyl acetate generally showed higher activity than citronellyl acetate (see Table 6).

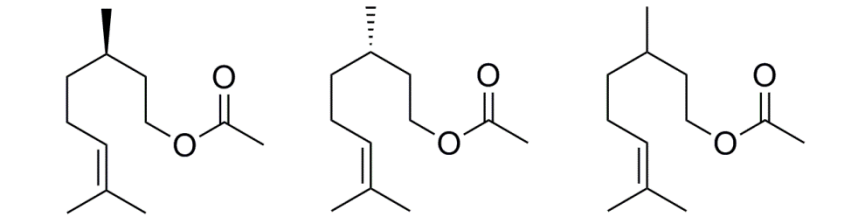
Compound	EC_{50}	Maximum effect (% of tonus reduction of BetOH)
(-)-Citronellyl acetate	3.3×10^{-4}	100
(+)-Citronellyl acetate	3.4×10^{-4}	100
Citronellyl acetate racemate	6.6×10^{-5}	100
(-)-Linalyl acetate	1.7×10^{-5}	100
(+)-Linalyl acetate	2.3×10^{-5}	100
Linalyl acetate racemate	2.1×10^{-5}	100

Table 6 EC_{50} values from concentration-response curves for citronellyl and linalyl enantiomers and racemates obtained from examinations of isolated guinea pig ileum; tonus of ileum was elevated by bethanechol (a muscarine receptor agonist); newly drawn and adapted from Sousa et al.[25] p. 121)

Besides, (-)-linalyl acetate is one of the major components of lavender oil. Other studies [60] found out that this enantiomer is a good marker for the quality control of essential oils from *Lavandula* species (Lamiaceae), because it remains stable under various workup and storage conditions.



Structure 8 Linalyl acetate enantiomers and racemate

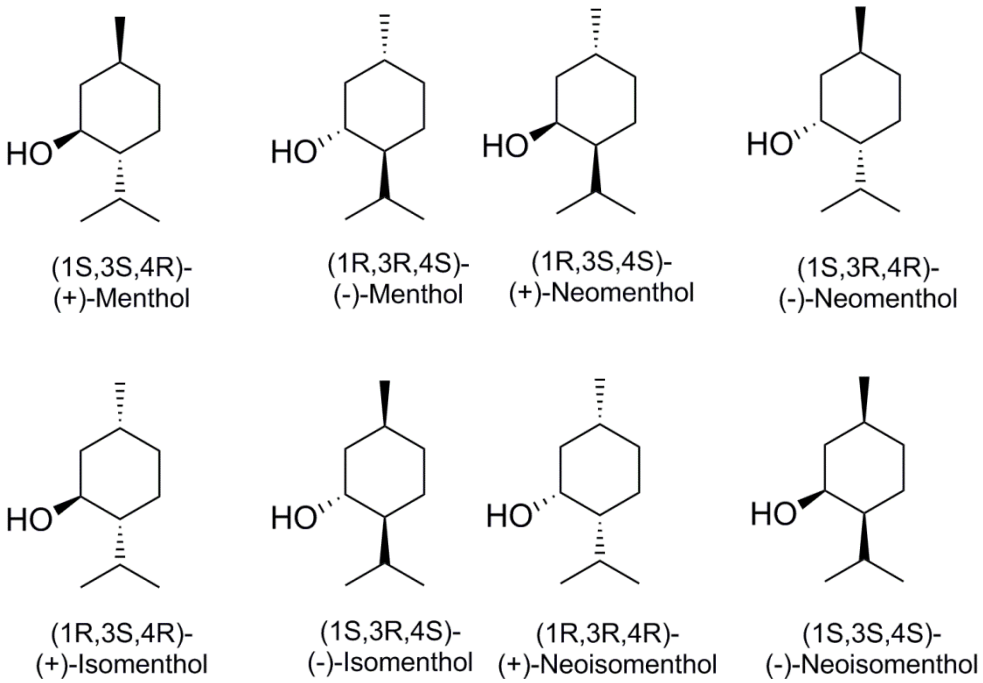


Structure 9 Citronellyl acetate enantiomers and racemate

Effects on nervous systems of mice were examined by Venancio et al. (2011). The authors compared antinociceptive effects of *Ocimum basilicum* (Lamiaceae) essential oil and pure (-)-linalool [61]. Mice were treated with formalin, capsaicin and glutamate injection (into upper lip) after receiving different doses of either (-)-linalool or leaf essential oil (50, 100 and 200 mg/kg intraperitoneal) as well as morphine. (-)-Linalool turned out to be the more effective substance and was able to reduce significantly face rubbing of mice at all three concentrations in the formaline group. Five minutes after application, (-)-linalool in its highest concentration was even more potent than morphine (99.1% versus 97.9% inhibition of face rubbing). In glutamate group and capsaicin group results for all concentrations were comparable, except leaf essential oil, which showed a remarkable inhibition of 95.7% in glutamate group with a dose of 200 mg/kg (all were less potent than morphine) [61].

A recent paper from Verzera et al. (2014) pointed out that linalool enantiomers are helpful in identifying authenticity of unifloral honeys [62]. They chose orange honey for their investigations and found out that the content of (+)-linalool remained stable and was almost independent of storage, packing or production conditions. This is in contrast to other volatile compounds, which varied over a wide range in the tested samples [62].

2.1.8 Menthol



Structure 10 Menthol stereoisomers

Menthol is a cyclic monoterpene alcohol. With tree chiral centres it can express eight different stereoisomers, but only (-)-menthol is the most dominant form in nature [63] and is naturally produced in Lamiaceae, especially in several *Mentha* species. (-)-Menthol is well known for its cooling effect and its minty taste, whereas (+)-menthol possesses a less minty, dusty and vegetable flavour [48]. Due to their diverse odorant qualities the question arises whether there are more differences. Dambolena et al. [64] emphasises that the position of the substitutes in the chiral centres exerts influences on the stability of the molecule. (+)-Menthol and (-)-menthol have all their substitutes in equatorial position and therefore are most stable.

In 2013 Kamatou et al. [63] wrote a review on biological properties of menthol and emphasised that this monoterpene shows remarkable antifungal, antibacterial, analgesic, anticancer, antipruritic effects, but also added that only few studies explored different effects of menthol stereoisomers.

Galeotti et al. (2002) investigated analgesic properties of menthol with special focus on enantiomer activity [65]. The hot plate test and the abdominal constriction test on mice were used to compare antinociceptive potency of (-)-menthol and (+)-menthol. As

control substances nor-binaltorphimine dihydrochloride (nor-NBI; a selective κ–opioid receptor antagonist), naloxone hydrochloride (an unselective opioid receptor antagonist), naltriben methanesulfonate (a δ_2-opioid receptor antagonist), d-phe-cys-tyr-d-trp-orn-thr-pen-thr-amide (CTOP; a μ-opioid receptor antagonist) and 7-benzylidenenaltrexone maleate (a δ_1-opioid receptor antagonist) were administered either i.p., p.o. or intracerebroventricularly. (-)-Menthol was able to elevate pain threshold at a concentration of 3-10 mg/kg. (+)-Menthol did not show this effect. Furthermore, this impact was reversed by naloxone and nor-NBI but not by naltriben, CTOP or 7-benzylidenenaltrexon. As a conclusion it can be said that (-)-menthol is a weak κ-opioid receptor agonist [65].

In a study from Trombetta et al. (2005) (+)-menthol was tested for its antibacterial activity [66] (together with thymol, a non-chiral monoterpene and racemic linalyl acetate) against two important bacteria strains: *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). Central aim was to understand the mechanisms of interaction with cell membranes. The authors used model membranes for their studies. It was discovered that all tree monoterpenes interact with plasma membrane by disturbance of the lipid fraction. (+)-Menthol displayed highest toxicity against *E. coli* (with a MIC of 2.5 mg/ml) and second highest activity against *S. aureus* (MIC was 0.62 mg/ml). Beside, (-)-menthol showed similar effects, which was only mentioned but detailed information was not available in that paper [66]. It is emphasised that activity highly depends on the structure and composition of bacteria membrane. *E. coli* exhibited rather lower sensitivity to the substances, because the outer layer of Gram-negative bacteria membrane consists of a thick layer of lipopolysaccharide that acts like a hydrophilic barrier against lipophilic monoterpenes [67,68].

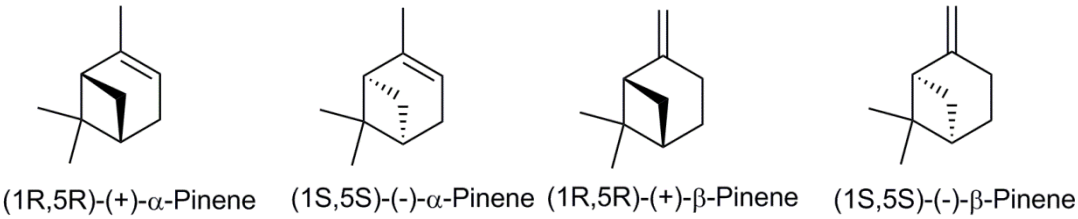
There are some papers [20,69] that put their focus on menthol isomers interacting with the $GABA_A$ receptor. As mentioned before, $GABA_A$ is a ligand gated chloride ion channel which is an important target for sedatives, such as benzodiazepines and barbiturates (allosteric modulators; amplify the effect of GABA). In a previously discussed study from Hall et al. [20] (2004) about the impact of various monoterpenes on $GABA_A$ and glycine receptors (derived from oocytes of *Xenopus laevis*, the African clawed frog) both, (+)-menthol and (-)-menthol showed positive modulation on $GABA_A$ receptor (concentrations ranged between 10 und 100 μM). In other words, both have the potency to act analgesic, sedative and anxiolytic. On glycine receptor the enantiomers also acted equipotent. Remarkably, (+)-menthol and (-)-menthol exert the highest activity of tested

monoterpenes ((-)-borneol, camphor enantiomers, carvone enantiomers, menthol enantiomers, (-)-menthone α-thujone and α/β-thujone) [20]. Corvalan et al. (2009) used chick forebrain membranes to study [^{3}H]-flunitrazepam binding on $GABA_A$ [69]. Membranes were exhibited to different concentrations of five menthol stereoisomers ((+)-menthol, (-)-menthol, (+)-neomenthol, (-)-neomenthol and (+)-isomenthol) to measure concentration-response. Only (+)-menthol showed a significant increase in [^{3}H]-flunitrazepam binding. (-)-Menthol (and (+)-isomenthol) did not exhibit substantial effects which stands in contrast to Hall et al.[20] where both enantiomers displayed activity. This can be explained due to the different subunits of $GABA_A$ receptors that were used. At their highest concentration (1000 µM) (-)-neomenthol and (+)-neomenthol were indeed able to increase binding, but without any significant values (matching data with a sigmoid concentration-response curve was not possible). The authors assume that the special configuration (all substituents in equatorial position, and the nearness and free rotation possibility of a non-polar group (isopropyl group) to a polar group (hydroxyl group)) is deciding for allosteric modulation on $GABA_A$ receptor [69].

A paper from Dambolena et al. (2010) dealt with antifungal activity of menthol stereoisomers ((-)-menthol, (+)-menthol, (-)-neomenthol, (+)-neomenthol, (+)-isomenthol) [64]. For the investigation the authors chose *Fusarium verticillioides*, a mould which causes fungal infestation on cereals. Good results were obtained with minimum inhibitory concentration (MIC) values of 1.50 mM for (-)-menthol and (+)-menthol and 2.00 mM for (+)-neomenthol. The constituents were not able to stop fungal growth completely, but growth was only 25% compared to control group. Furthermore, sporulation, mycelial growth and inhibition of fumonisin B_1 biosynthesis (a mycotoxin which exerts hepatotoxic and nephrotoxic properties) were examined. The test of radial growth inhibition on petri plates revealed unsatisfactory results. (-)-Menthol and (+)-menthol achieved the best results with 30 cm^2 versus 40 cm^2 in control group. Additionally only those two enantiomers showed significant inhibition of fungal sporulation. Most interesting was the inhibition of fumonisin B1 biosynthesis: (-)-menthol was the most active inhibitor. The authors assume that because of the substituents order of (-)-menthol (all equatorial position), there is an influence on the fungal membrane and is able to affect Ca^{2+}-influx so that mycotoxin FB_1 production is irritated [64]. Moreover, menthol was tested for its acaricidal properties against *Tyrophagus putrescentiae* (a cosmopolitan mite species; infests food which is rich in protein and fat [70]) by Park et al. (2014) [71]. Peppermint oil and single menthol

stereoisomers were examined by fumigant bioassay and filter paper bioassay using benzyl benzoate (a synthetic acaricide) as control substance. In both assays racemic menthol revealed to be most effective against *Tyrophagus putrescentiae*, beyond that even more than 10 times more effective than benzyl benzoate. The stereoisomers showed slightly different acaricidal activity when comparing the assays outcome. In fumigant bioassay (+)-neomenthol displayed second highest activity, followed by (-)-menthol and (+)-menthol. In filter paper bioassay it was (-)-menthol, followed by (+)-menthol and (+)-neomenthol. Remarkably, (+)-isomenthol was completely inactive [71].

2.1.9 α- and β-Pinene



Structure 11 α- and β-Pinene enantiomers

Pinenes are bicyclic monoterpene hydrocarbons with two constitutional isomers: α-pinene and β-pinene. They are the main components of *Pinaceae* species essential oil [4], especially turpentine. (-)-α-Pinene is the most widespread enantiomer, holds a coniferous odour and is more common in *Picea* species in Europe whereas (+)-α-pinene smells slightly minty and occurs often in North American *Picea* species [14,74] . The two enantiomers of β-pinene are also major compounds in pines and especially (-)-β-pinene is a constituent in turpentine [72].

Some papers [73-75] examined the whole essential oil for their biological activity or looked at single constituents with diverse results.

Lis-Balchin et al. (1999) studied the bioactivity of α-pinene enantiomers [73]. Growth inhibition of 25 different bacterial strains (common human pathogens; special regard was put on the different activity against Gram-negative and Gram-positive strains) and three different fungal strains (*Aspergillus niger, Apergillus ochraceus* and *Fusarium culmorum*) was measured by treating agar gel petri-plates with either (-)-α-pinene or (+)-α-pinene. Separately, 20 different *Listeria monocytogenes* strains from different sources (raw chicken, milk product, vegetables, margarine, etc.) were tested. Greater

inhibition was obtained by (-)-α-pinene (18 bacteria; round 67% of Gram-positive and round 69% of Gram-negative strains were inhibited, largest zone of inhibition was found in the test on *Clostridium sporogenes* (41.8 mm; it is worthwhile noting that (+)-α-pinene also exhibited its highest activity with 35.9 mm inhibition zone). Greatest differences in antibacterial activity were shown with *Streptococcus faecalis* (Gram-positive; 12.0 mm with (-)-α-pinene versus no inhibition by (+)-α-pinene), *Micrococcus luteus* (Gram-positive; 16.7 mm versus 7.5 mm) and *Yersinia enterocolitica* (Gram-negative; 10.2 mm versus 6.9 mm inhibition zone). No activity of both enantiomers against *Pseudomonas aeruginosa* and *Serratia marcescens* (both Gram-negative) was detected. Growth inhibition of *Listeria monocytogenes* showed the opposite: (+)-α-Pinene proved to be a bit more effective in 19 of 20 strains. In most cases the zone of inhibition was about 6 mm with the (-)-enantiomer and about 7.5 mm with the (+)-enantiomer. The only exception was a *Listeria monocytogenes* strain from vegetables where (+)-α-pinene turned out to be distinctly more effective (21.6 mm versus 11.8 mm zone inhibition). Pharmacological studies on isolated guinea-pig ileum to examine effects on smooth muscles revealed that (-)-α-pinene exhibited a higher contractility and caused a higher muscle tonus than the (+)-enantiomer [73].

The aim of Rivas da Silva et al. (2012) was to test the antimicrobial activity of the enantiomers (+)-α-pinene, (-)-α-pinene, (+)-β-pinene and (-)-β-pinene [74]. Minimal inhibitory concentration (MIC), time-kill curves and inhibition of microbial enzymes were determined regarding the fungi *Candida albicans*, *Cryptococcus neoformans* and *Rhizopus oryzae* as well as methicillin-resistant *Staphylococcus aureus* bacteria. (+)-α-Pinene and (+)-β-pinene showed good activity against all fungi and bacteria, whereas (-)-α-pinene and (-)-β-pinene were ineffective (Table 7).

Furthermore, the effectiveness of the positive isomers was determined by time-kill-curves. Both were toxic for *C. albicans* and were able to erase the fungus within 60 minutes whereas it took 6 hours of incubation to kill *S. aureus*. Regarding the inhibition of microbial phospholipase and esterase statistically significant results were attained with (+)-α-pinene (with 50% phospholipase activity inhibition of *C. neoformans*), and (+)-β-pinene (with 72% esterase activity inhibition of *C. neoformans).*The negative enantiomers did not show any significant effects [74].

Micro-organisms	Minimal inhibitory concentrations (µg/ml)*					
	(+)-α-Pinene	**(-)-α-Pinene**	**(+)-β-Pinene**	**(-)-β-Pinene**	**AMB**	**CIP**
Candida albicans	3.125	na	187	na	0.125	-
Cryptococcus neoformans	117	na	234	na	0.125	-
Rhizopus oryzae	390	na	780	na	0.48	-
Staphylococcus aureus	4.150	na	6.25	na	-	0.5
na - no activity, AMB – amphotericin B; CIP – ciprofloxacin; *all MICs were microbicidal						

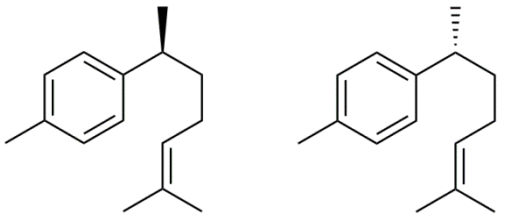
Table 7 MICs of α- and β-pinene enantiomers and two antimicrobial drugs regarding three fungi and one bacteria strain (newly drawn and adapted from Rivas da Silva et al. [74] p. 6307)

Rufino et al. (2014) examined the anti-inflammatory effects and chondroprotective activity of chiral pinene isomers [75]. The authors pointed out that information about single enantiomer activities is quite rare and is only limited to antifungal, antibacterial and insecticide effects. Screening methods of human chondrocytes were used to determine IL-1β inhibition (this cytokine is responsible for activation of inflammatory pathways over NF-κB and JNK); α- and β-pinene were tested together with other pinane derivates by evaluating cell viability. It turned out that (+)-α-pinene was the most promising enantiomer with the highest inhibition activity (and therefore with the highest anti-inflammatory potential) followed by (-)-α-pinene. The β-enantiomers showed no activity [75].

2.2 Sesquiterpenic chirality pairs

Studies concentrating on chirality of sesquiterpenes are very rare; a comparative study was only available on curcumene enantiomers.

2.2.1 Curcumene



Structure 12 Curcumene enantiomers

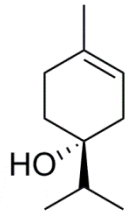
Curcumene is an unsaturated monocyclic sesquiterpene. (+)-Curcumene can be seen as a dehydrogenated zingiberen derivate and occurs together with it in ginger oil from *Zingiber officinale* (Zingiberaceae) [76]. Ginger oil has an aromatic, persistent fragrance of ginger [4]. It was found out that (-)-curcumene is a constituent in wild tomato species (*Solanum habrochaites*, Solanaceae) [76].

A remarkable study from Bleeker et al. (2011) demonstrated that (-)-curcumene (as well as 7-epizingiberene) possesses repellent effects to whiteflies (*Bemisia tabaci*) whereas (+)-curcumene (and zingiberene) did not express this quality [76]. In a prior study [77] the authors found that whiteflies avoided contact to wild tomatoes and that sesquiterpenes such as zingiberene and curcumene are responsible for that. In the study from 2011 NMR and x-ray analyses were conducted to determine the different stereoisomers. The experiments included free choice assay between cultivated tomatoe (*Solanum lycopersicum*, Solanaceae) and wild tomatoe (*Solanum habrochaites*, Solanaceae). Over 90% of the whiteflies avoided landing on wild tomatoes. When they were given no choice, all whiteflies died within 48 hours after landing on wild tomatoe plants. Beside these toxic effects, 7-epizingiberene and (-)-curcumene showed significantly repellent activity when cultivated tomatoe plants where pre-treated with either zingiberene, (+)-curcumene, 7-epizingiberene or (-)-curcumene. Via electroantennographic response it was possible to find out that whiteflies could detect these sesquiterpenes with their antennae and that the highest response was registered to (-)-curcumene. Obviously, (-)-curcumene (and 7-epizingiberene) are toxic plant compounds and act as semiochemicals; therefore they made cultivated tomatoe plants less attractive. The authors emphasised that whiteflies were only clearly repelled by (-)-curcumene and not by (+)-curcumene [76].

2.3 Monoterpenic single enantiomers

For some constituents only studies on single enantiomers are available. Nevertheless, it is worthwhile to review these papers as well. Comparative studies between monoterpene enantiomers are more common, but in some cases it happens to be that one predominant enantiomer is in the focus of interest, like (+)-terpinen-4-ol.

2.3.1 (+)-Terpinen-4-ol



(S)-(+)-Terpinen-4-ol

Structure 13 (+)-Terpinen-4-ol

Terpinen-4-ol is an unsaturated cyclic monoterpene alcohol. Both enantiomeric forms as well as its racemate occur in many essential oils from *Pinus* (Pinaceae) and *Eucalyptus* species (Myrtaceae). (+)-Terpinen-4-ol is the main constituent in *Melaleuca alternifolia* (Myrtaceae) essential oil with an ee of over 60% [78]. Both, (+)-terpinen-4-ol and (-)-terpinen-4-ol exert a spicy, woody-earthy, nutmeg-like smell with a liliac-like flavour [4].

The cited studies only concentrate on the (+)-enantiomer. As mentioned before, it is the main component of tea tree oil which became increasingly popular within the past 15 years. It contains (+)-terpinen-4-ol at an amount of 40% of essential oil [79] and possesses remarkable antimicrobial and anti-inflammatory activity (see a review from Carson et al. [79]).

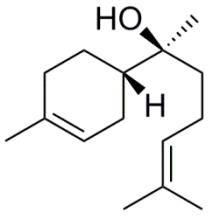
A study from Hart et al. (2000) definitely found out that terpinen-4-ol is the active constituent in tea tree oil concerning anti-inflammatory activity [80]. From tested known compounds only terpinen-4-ol was able to reduce production of inflammatory agents like PGE2 (prostaglandin E2), IL-1β, IL-8, IL-10 (interleukine) and TNFα (tumour necrosis factor-alpha) in monocytes. Furthermore, it even could be proved that the single

constituent displayed a higher antimicrobial activity than tea tree oil [80]. Cox et al. (2001) determined MIC (v/v) and time-kill curves against four common pathogens (*S. aureus, E. coli, P. aeruginosa* and *C. albicans*) [81]. In a prior study [82] it was elucidated that the mechanism of action functions by disrupting cell membrane structures (compare also Trombetta et al. 2005 [66]). Cox et al. [81] revealed that aqueous compounds such as terpinen-4-ol show higher membrane-damaging effects because of its better aqueous solubility. In in-vitro-studies non-oxygenated monoterpenes even lower its activity [81], which means that a complex mixture of monoterpenes – as it occurs in essential oils – exerts a lower antimicrobial activity. Another in-vitro-study on RC-37 cells (a cultured cell line derived from kidneys of the African green monkey [83]) from Schnitzler et al. (2001) [84] showed that tea tree oil is an active agent against HSV-1 and HSV-2 (Herpes Simplex Virus). It was emphasised that its antiviral constituent is not known yet and that the oil exerts its highest activity when cell cultures were pre-treated with essential oil, but not when cells were already infected with the virus [84]. In vivo-studies mainly dealt with acne therapy and dental applications respectively oral bacteria treatment [79].
Wang et al. (2015) emphasised that the determined amount of (+)-terpinen-4-ol plays an important role in the quality evaluation of tea tree oil [85].

2.4 Sesquiterpenic single enantiomers

As mentioned before, studies on chirality play a minor part at the topic of sesquiterpenes. However, some studies on single chiral constituents were conducted. This chapter contains studies on (-)-α-bisabolol and (+)-α-santalol.

2.4.1 (-)-α-Bisabolol



(6S,7S)-(-)-α-Bisabolol

Structure 14 (-)-α-Bisabolol

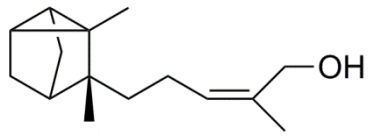
(-)-α-Bisabolol is an unsaturated monocyclic sesquiterpenes alcohol and very well known for its anti-inflammatory activity [72]. Together with chamazulene it is the main constituent in blue chamomile oil derived from *Matricaria chamomilla* (Asteraceae), which exerts a bitter-aromatic taste with a strong, characteristic odour [4]. Even though there is an (+)-enantiomer, (-)-α-bisabolol has gained great attention because the well-known healing effects of chamomile oil are attributed to the (-)-enantiomer [91].

Several studies [86,87,91,92] showed that (-)-α-bisabolol exerts remarkable physiological activities. Rocha et al. (2011) investigated anti-inflammatory and anti-nociceptive effects on mice [86]. For anti-inflammatory evaluation paw oedema and peritonitis were induced by carrageenan, dextran or 5-HT. After administration of 200 mg/kg (-)-α-bisabolol (p.o.) a significant reduction of oedema and migration of leukocytes to peritoneum was observed. There was no reduction when oedema where caused by histamine. With models of pain such as the hot plate test, the formalin test and the abdominal writhing test anti-nociception was investigated. No differences to control group were observed with the hot plate test and the formalin test ((-)-α-bisabolol concentrations of 25 and 50 mg/kg, p.o. were used). When mice were pre-treated with just-mentioned concentrations of (-)-α-bisabolol it was possible to significantly reduce numbers of writhing (induced by acetic acid). These results indicate that (-)-α-bisabolol may act as an anti-nociceptive over a peripheral mechanism and not over central nervous system [86]. In a follow-up study from Rocha et al. [87] investigations were extended to inflammatory events in gastric mucosa. Animals with ethanol-induced gastric ulcera were pre-treated with (-)-α-bisabolol with promising results. It is known that ethanol induces oedema, haemorrhage and loss of epithelial cells in mucosa. Tissue inflammation leads to the increased formation of Reactive Oxygen Species (ROS) which can cause cell damage and other degenerative changes. (-)-α-Bisabolol (at doses of 100 and 200 mg/kg, p.o.) was able to reduce migration of neutrophil granulocytes. Furthermore, it interferes with the antioxidant defense system by means of increasing activity of superoxide dismutase (SOD). Therefore it reduces oxidative stress and shows gastro-protective effects [87].

Based on prior studies [88-90] Siqueira et al. (2014) explored effects on voltage-dependent calcium channels in smooth muscle [91]. In silico analysis and experiments with rat aortic rings were done to find out whether (-)-α-bisabolol may act as calcium channel inhibitor. In smooth muscle preparations (-)-α-bisabolol only reduced contractions induced by $CaCl_2$ in KCl. In silico models revealed a putative docking site on the $Ca_v\beta_{2a}$ subunit. The results indicate that (-)-α-bisabolol has an allosteric influence on calcium channel by

reducing calcium influx (and does not block the channel pore) and therefore possesses vasodilatory effects [91]. Corpas-Lopez et al. (2015) carried out in-vitro tests on macrophages and in-vivo tests on mice to evaluate effectiveness of (-)-α-bisabolol against *Leishmania infantum* and *Leishmania donovani* [92]. It displayed good activity in treating visceral leishmaniosis and was as effective as the antimonial therapy.
Moreover, α-bisabolol exerts anti-cancer activity by means of inducing apoptosis in carcinoma cells [93-95]. Unfortunately, these studies did neither determine enantiomeric excess nor sense of rotary, so it is unknown whether they used racemic or enantiomeric α-bisabolol.

2.4.2 (+)-α-Santalol



(7R,10Z)-(+)-α-Santalol

Structure 15 (+)-α-Santalol

α-Santalol is a tricyclic sesquiterpene alcohol and - beside β-santalol - it is the main compound of sandalwood oil derived from *Santalum album* (Santalaceae) [4]. Both isomers make up to 90% of essential oil and are mainly responsible for its odour. The scent of the oil is described as sweet and woody with a long-lasting fragrance [4]. Even though there is more than one chiral centre, only the one on position 7 is decisive for its sense of rotary. The double bound in Z position is essential for its odour [96].

The essential oil of sandalwood is extensively used in perfumery and was often tested in regard to its effects on central nervous system [97,98] and anti-cancer activity [99-103]. After determining enantiomeric excess Satou et al. (2014) found out that sandalwood essential oil contains about 50% (+)-α-santalol [104]. In a following study the authors examined effects of the single enantiomer on anxiolytic behaviour of mice [105]. Behavioural tests such as the elevated-plus maze test and the water immersions test were performed after inhalation or intraperitoneally injection of (+)-α-santalol. The behaviour of mice did not alter compared to control group. After injection, a decrease of locomotor activity was observable, which indicates a sedative effect. After the tests mice were analysed to

determine the concentration of (+)-α-santalol in the brain. Interestingly, (+)-α-santalol was only detectable after injection but not after inhalation, which indicates that there must be a pharmacological transfer route [105]. The authors emphasised that their previous study [104] proved that sandalwood essential oil induced anxiolytic-like activity whereas (+)-α-santalol did not, which leads to the conclusion that (+)-α-santalol is not responsible for this effect.

It should be pointed out, that many studies use α-santalol extracted from sandalwood oil without analysing its enantiomeric excess and without declaring origine plant of essential oil [99,106,107]. A study from Lawrence (1991) showed that there is a great variability of α-santalol and β-santalol quantities in different *Santalum* species [108].

3 CONCLUSION

Several studies which dealt with the topic of chirality came to the point that there cannot be a common sense statement regarding the activity of enantiomers. Tombo and Blaser [109] (who work on the field of agrochemistry and pesticides) pointed out, that all possible situations are common: The enantiomers have qualitatively similar activities but different potencies, they can have a complete different or even opposite biological activity or all stereoisomers exert almost the same properties. When we look at chiral monoterpenes we often discern that they have similar antimicrobial properties (e.g. citronellal and limonene enantiomers). But it should be pointed out that some exert quite different physiological effects (e.g. linalool and carvone enantiomers).

Producers of phytomedicinal drugs should be aware of this fact because only then a constant quality and the same effects on health can be achieved. A paper from Sybilska and Asztemborska (2002) [110] studied the enantiomeric composition of various drugs (e.g. Uroterp® and Terpinex® for the treatment of urethral stones or Vick Vapo Rub® and Rub arom® for the treatment of respiratory diseases) that contain monoterpenes (borneol, camphor, fenchone, menthol and menthone enantiomers). The enantiomeric excess from borneol enantiomers and fenchone enantiomers varied over a wide range between different products while it remained stable for (-)-menthol and (-)-menthone. Camphor was only detected as racemic mixture which indicates its synthetic origin [110].

However, the biological activity of discussed chiral terpenes is often dependent on micro-organisms, cells or types of receptors that were used for the investigations (e.g.

different composition of subunits from $GABA_A$ receptors led to slightly different results in activity, compare [20,69]).

Comparing the effectiveness of (+)- and (-)-enantiomer, a clear generally valid statement is not possible in most cases, even though that there seems to be a small trend to the (+)-enantiomer of discussed monoterpenes displaying higher biological activity. Finally, it is obvious that the receptor system of all living beings must express stereo selectivity because it is composed of chiral amino acids and other chiral biopolymers [18]. Studies on physiological activity have shown that there are remarkable differences between (+)- and (-)-enantiomers. Some achievements to elicit this topic have been done so far, but further investigations would be welcome. The following (see Table 8 and Table 9) roughly sums up the results from the discussed studies.

Chiral substituent	**Activity**
Monoterpenes	
(1R,2S,4R)-(+)-Borneol	Moderate antimicrobial activity, equipotent with its (-)-enantiomer, but slightly lower than whole essential oil Physiological effects: increase of GABA activity at $GABA_A$ receptor; significantly elevates pain threshold
(1S,2R,4S)-(-)-Borneol	Moderate antimicrobial activity, equipotent with its (+)-enantiomer, but slightly lower than whole essential oil Physiological effects: decrease of GABA activity at $GABA_A$ receptor
(1R,4R)-(+)-Camphor	Good antifungal activity (but even less than essential oil), higher than its (-)-enantiomer Physiological effects: (+)-camphor and camphor racemate only displayed marginal effects on GABA response at $GABA_A$ receptor (enantiomers were equipotent)

(1S,4S)-(-)-Camphor	Antifungal activity, lower than its (+)-enantiomer Moderate antibacterial activity (tested as a component in whole essential oil) Physiological effects: (-)-camphor and camphor racemate only displayed marginal effects on GABA response at $GABA_A$ receptor (enantiomers were equipotent)
(S)-(+)-Carvone	Good sprout suppression (potatoes) Good antifungal and antibacterial activity, sometimes equipotent with essential oils or higher potency Insecticide Physiological effects: rather negative allosteric modulator on $GABA_A$ receptor, results inconsistent, tendential depressing, more potent than its (-)-enantiomer, anticonvulsive
(R)-(-)-Carvone	Lower antifungal and antibacterial activity than its (+)-enantiomer, sometimes equipotent with essential oil or lower potency Physiological effects: rather negative allosteric modulator on $GABA_A$ receptor, results inconsistent, tendential depressing, less potent than its (+)-enantiomer
(R)-(+)-Citronellal	Acetate: Spasmolytic activity on smooth muscle, equipotent with (-)-enantiomer, lower activity than racemate Physiological effects: exhibits anti-cancer activity by mitosis-inhibition by means of disrupting microtubules
(S)-(-)-Citronellal	Acetate: Spasmolytic activity on smooth muscle, equipotent with (+)-enantiomer, lower activity than racemate Physiological effects: no mitosis-inhibition at all; reduces cell proliferation in hepatocellular carcinoma via olfactory receptor

(R)-(+)-Citronellol	No studies available
(S)-(-)-Citronellol	Topical applications: may act as mild irritant at higher concentrations
(R)-(+)-Limonene	Good antimicrobial activity, potency depending on study, varies between higher and lower activity compared to (-)-enantiomer Physiological effects: stimulating on autonomic nervous system (increase in systolic blood pressure, subjective alertness) Gastro protective (by means of increased mucus production and lower H^+ secretion), higher activity than essential oil (from *C. aurantium*)
(S)-(-)-Limonene	Good antimicrobial activity, potency depending on study, varies between higher and lower activity compared to (+)-enantiomer Physiological effects: less stimulating on autonomic nervous system Gastro-protective effect not tested
(S)-(+)-Linalool	Low antifungal activity, equipotent with (-)-enantiomer No antimicrobial activity No antimalarial activity Acetate: Spasmolytic activity on smooth muscle, equipotent with (-)-enantiomer and racemate Physiological effects: slightly stimulating on autonomic nervous system (increase in heart rate and blood pressure), tendency to activation Antinociceptive activity not tested Anti-inflammatory activity not tested

(R)-(-)-Linalool	Low antifungal activity, equipotent with (+)-enantiomer No antimicrobial activity No antimalarial activity Acetate: Spasmolytic activity on smooth muscle, equipotent with (+)-enantiomer and racemate High antinociceptive activity in in vivo-tests, mostly more potent than essential oil Physiological effects: clearly depressing on autonomic nervous system (decrease in heart rate, sedative) Good anti-inflammatory activity in in vivo-tests, better than racemate
(1S,3S,4R)-(+)-Menthol	Good antibacterial activity, similar to (-)-enantiomer Good antifungal activity Acaricide; (+)-neomenthol showed highest activity (all stereoisomers were less potent than essential oil) Physiological effects: positive allosteric modulator on $GABA_A$ receptor (increases effect of benzothiazepines and therefore GABA) Because of low stability all other stereoisomers mostly showed – if at all - marginal effects
(1R,3R,4S)-(-)-Menthol	Good antibacterial activity, similar to (+)-enantiomer Good antifungal activity, very active in inhibition of mycotoxin production Acaricide; (-)-menthol displayed higher potency than (+)-menthol but lower than (+)-neomenthol (all stereoisomers were less potent than essential oil) Physiological effects: results inconsistent, rather positive allosteric modulator on $GABA_A$ receptor, rather less potent than its (+)-enantiomer κ-opioid receptor agonist, therefore antinociceptive Because of low stability all other stereoisomers mostly showed – if at all - marginal effects

(1R,5R)-(+)-Pinene	Good antifungal and antibacterial activity; α-pinene showed higher activity than β-pinene; in total more potent than (-)-enantiomer Physiological effects: good anti-inflammatory activity (better than (-)-enantiomer); α-pinene displayed highest activity
(1S,5S)-(-)-Pinene	Antifungal and antibacterial activity with diverse results; rather lower activity than (+)-enantiomer (or even no activity) Physiological effects: (-)-α-pinene less anti-inflammatory activity than (+)-enantiomer; β-pinene expressed no activity
(S)-(+)-Terpinen-4-ol	Racemic terpinen-4-ol exerts higher antimicrobial activity than essential oil Physiological effects: good anti-inflammatory activity

Table 8 Summary of biological activities of chiral essential oil monoterpenes

Regarding the antimicrobial activity the question arises whether it is worthwhile to take the single enantiomer or better to work with the whole essential oil, because one can benefit from synergistic effects (see camphor essential oil). Even here one meets the fact that the single chiral monoterpene can be more effective than the essential oil (see terpinen-4-ol or limonene). After all it certainly depends on the amount and the enantiomeric excess that is expressed in essential oil (e.g. (-)-linalool makes up to 79.8% of essential oil from *Origanum onites* (Lamiaceae) with 100% ee versus 30.4% in essential oil of *Lavandula angustifolia* (Lamiaceae) with 91.4% ee).

Table 10 gives examples for plants which exhibit a high enantiomeric excess of one enantiomer in their essential oil.

Chiral constituent	Activity
Sesquiterpenes	
(6S,7S)-(-)-α-Bisabolol	Good anti-inflammatory activity in animal studies; anti-nociceptive effect may act over a peripheral mechanism Gastro-protective effects Induces relaxation on vascular smooth muscle by means of putative allosteric influence on voltage-dependent calcium channels Effective in treating visceral leishmaniosis
(S)-(+)-Curcumene	No repellent effects to whiteflies (an agricultural pest)
(R)-(-)-Curcumene	Good repellent effects to whiteflies (an agricultural pest)
(7R,10Z)-(+)-α-Santalol	Physiological effects: sedative effects in animal studies; no anxiolytic activity (in contrast to sandalwood essential oil)

Table 9 Summary of biological activities of chiral essential oil sesquiterpenes

Last enlightenment was that analysing enantiomeric composition of single constituents is very valuable for quality controls, because it is an important fact for proving authenticity of an essential oil. Quality control is also a point when it comes to consumer protection. Analysing enantiomeric excess of phytomedicinal products could contribute to a consistent activity profile.

dominant enantiomer	plant containing essential oil
(-)-α-Bisabolol [87]	*Matricaria chamomilla* (Asteraceae)
(+)-Borneol [6]	*Salvia tomentosa* (Lamiaceae)
(-)-Borneol [6]	*Micromeria cristata subsp. Phrygia* (Lamiaceae)
(+)-Camphor [4]	*Cinnamomum camphora* (Lauraceae)
(-)-Camphor [14]	*Tanacetum parthenium* (Asteraceae)
(+)-Carvone [4]	*Carum carvi* (Apiaceae)
(-)-Carvone [4]	*Mentha spicata* (Lamiaceae)
(+)-Citronellal [24]	*Melissa officinalis* (Lamiaceae)
(-)-Citronellal [24]	*Citrus limonum* (Rutaceae)
(+)-Citronellol [4]	*Boronia citriodora* (Rutaceae)
(-)-Citronellol [4]	*Geranium* species (Geraniaceae)
S(+)-Curcumene [76]	*Zingiber officinalis* (Zingiberaceae)
R(-)-Curcumene [76]	*Solanum habrochaites* (Solanaceae)
(+)-Limonene [111]	*Citrus aurantium* (Rutaceae)
(-)-Limonene [112]	*Mentha spicata L* (Lamiaceae)
(+)-Linalool [59]	*Ocimum basilicum* (Lamiaceae)
(-)-Linalool [59]	*Satureja spinosa* (Lamiaceae)
(+)-Menthol	no natural source found
(-)-Menthol [4]	*Mentha* x *piperita* (Lamiaceae)
(+)-α-Pinene [14]	*Pinus sylvestris* (Pinaceae)
(-)-α-Pinene [72]	*Pinus caribaea* (Pinaceae)
(+)-β-Pinene [113]	often produced synthetically
(-)-β-Pinene [72]	*Pinus* species (especially turpentine; Pinaceae)
(+)-α-Santalol [114]	*Santalum album* (Santalaceae)
(+)-Terpinen-4-ol [78]	*Melaleuca alternifolia* (Myrtaceae)
(-)-Terpinen-4-ol [72]	*Xanthoxylum rhetsa* (Rutaceae)

Table 10 Examples for dominant monoterpene and sesquiterpene enantiomers in selected plants

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