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Enantiomeric Distribution of Some Linalool Containing Essential Oils and Their Biological Activities

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Abstract: The enantiomeric composition of linalool was determined in 42 essential oils using chiral columns. Essential oils were analyzed by multidimentional gas chromatography-mass spectrometry using a non-chiral and chiral FSC columns combination with modified γ -cyclodextrine (Lipodex E) as the chiral stationary phase without previous isolation of the compound from the mixture. The essential oils of *Achillea, Ballota, Calamintha, Micromeria, Hedychium, Tanacetum, Coriandrum, Xanthoxylum, Ocimum, Thymus, Lavandula, Elettaria, Cinnamomum, Salvia, Origanum, Satureja, Nepeta, Stachys were used as source material for enantiomeric separation of linalool. Enantiomeric distribution of linalool showed (-)-linalool was much more common than the (+)-linalool in the essential oils in this study. (-)- and (+)-linalool enantiomers were evaluated for antimicrobial, antifungal and antimalarial activities. Both enantiomers demonstrated approximately 50% growth inhibition of <i>Botrytis cinerea* at 48 hrs.

Keywords: Linalool; Chiral separation; Enantiomer; MDGC-MS; Lipodex E; *Colletotrichum*; Biological activity; Essential oil

1. Introduction

Chiral compounds frequently occur in plant extracts such as essential oils and flavoring agents. These compounds exist as optical isomers, or enantiomers, which are non-superimpossible mirror images of one another and differ only in their interaction with polarized light [1,2]. Enantiomers have identical physical properties such as boiling point, melting point and spectroscopic features [2]. However, enantiomers may possess different aroma and flavor characteristics that can affect quality of essential oils and flavor extracts. Chiral compounds may also exhibit differences in toxicity and biological activity as seen in the greater activity of (-)-ephedrine as compared to low activity in (+)-ephedrine.

Compounds occurring in nature typically exist as one predominant optical form dextrorotatoryisomer (*d*) that rotates the plane to the right (*clockwise*) is designated (+) and levorotatory-isomer (*l*) that rotates the plane to the left (*counterclockwise*) is designated (-) [1,2].

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Linalool (*syn : 3,7-dimethyl-1,6-octadien-3-ol*) is one of the most important compounds to the perfume and flavor industries and is found in large amounts in several plant species. Since linalool is an important intermediate in the manufacture of vitamin E, large-scale processes have been developed for its production. Because of its high volatility industry, it gives naturalness to top notes to the oil. It is used frequently in perfumery and many flowery fragrance compositions for fruity-fresh odors like neroli, lavender, lily of the valley [3-7].

Linalool occurs naturally as two isomeric forms. The levorotatory isomer is 3R-(-)-linalool called licareol that has a lavender or lily-of-the-valley odor. Licareol is a colourless liquid and practically insoluble in water, miscible with alcohol and ether [3,6]. The dextrorotatory isomer is 3S-(+)-linalool called coriandrol, has a herbaceous and musty green smell, and is often the main component in a number of essential oils [4]. Coriandrol is also a colourless liquid soluble in alcohol [3,6]. The specific rotation of linalool reported in the literature as $[\alpha]_D = -20$ to + 19 [3].

In this study, essential oils obtained by hydrodistillation from different plant species were analyzed by GC/MS and then enantiomeric separations were determined by MDGC/MS. To determine if any difference in biological activities existed for each enantiomer of linalool, chromatographic and biological assays were carried out. For this purpose, we evaluated linalool enantiomers for antimalarial and antimicrobial activity against human pathogenic bacteria and fungi. Linalool enantiomers were also evaluated for activity against three important plant pathogenic fungi *Colletotrichum* species using direct-bioautography. Licareol and coriandrol were subsequently tested for activity using a dose-response format in micro-dilution broth assays against *Colletotrichum acutatum, C. fragariae, C. gloeosporioides, Fusarium oxysporum, Botrytis cinerea, Phomopsis obscurans* and *P.viticola*.

2. Materials and Methods

2.1. Preparation of essential oils

Essential oils of 42 different plant materials used in this investigation were hydro-distilled in our laboratory using a Clevenger apparatus or purchased from commercial sources. Sources of plant materials and essential oil percent yields, calculated on moisture-free basis are shown in Table 1.

2.2. Isolation of (-) and (+)-linalool

Origanum majorana and Satureja spinosa essential oils were used to obtain (-) and (+)-linalool. Origanum majorana oil (100 mg) was subjected to High Performance Flash Chromatography system (HPFC, Biotage, Inc., HorizonTM Pump, Charlottesville, Virginia) using a Biotage SI 12M column (150 x 12 mm i.d.; 9 g KP-SilTM silica; 40-63 μ m particle size; flow rate: 5.0 mL/min) and eluted with n-hexane containing increasing amounts of Et₂O (to 5%) to give 15 fractions (F₁-F₁₆). Fraction 6 (hexane: Et₂O, 97:3, v/v) yielded 5.0 mg of (-)-linalool. Satureja spinosa oil (80 mg) was subjected to HPFC system and same separation conditions were as described above to yield 3.0 mg of (+)-linalool. Both enantiomers were then re-analyzed by GC/MS to confirm their identity.

Essential Oil	Family	Source	Type of Obtaining Method	Oil Yield (%)
Achillea grandifolia Friv.	Asteraceae	Turkey	Hydro-distillation	-
Achillea millefolium L.	Asteraceae	Turkey	Hydro-distillation	0.27
Ballota sechmenii Gemici & Leblebici	Lamiaceae	Turkey	Hydro-distillation	0.02
Calamintha tauricola P.H. Davis	Lamiaceae	Turkey	Hydro-distillation	0.02
<i>Cinnamomum tamala</i> (BuchHam.) T.Nees & C.H.Eberm.	Lauraceae	Pakistan	Hydro-distillation	-
Coriandrum sativum L.	Apiaceae	Pakistan	Hydro-distillation	2.22
Elettaria cardamomum Maton	Zingiberaceae	Pakistan	Hydro-distillation	-
Hedychium flavum Roxb.	Zingiberaceae	Madagascar	Commercial sources	-
Lavandula angustifolia Mill.	Lamiaceae	Turkey	Commercial sources	-
<i>Micromeria juliana</i> (L.) Bentham ex Reichb.	Lamiaceae	Turkey	Hydro-distillation	0.05
Nepeta cadmea Boiss.	Lamiaceae	Turkey	Hydro-distillation	0.41
Nepeta cataria L.	Lamiaceae	Turkey	Hydro-distillation	0.65
<i>Nepeta conferta</i> I.C. Hedge & J.M. Lamond	Lamiaceae	Turkey	Hydro-distillation	0.10
Nepeta flavida A. Huber-Morath	Lamiaceae	Turkey	Hydro-distillation	1.35
Nepeta italica L.	Lamiaceae	Turkey	Hydro-distillation	1.43
Ocimum basilicum L.	Lamiaceae	Nepal	Commercial sources	-
		Turkey	Hydro-distillation	0.29
		Turkey	Hydro-distillation	-
Origanum floribundum Munby	Lamiaceae	Turkey	Hydro-distillation	-
Origanum majorana L.	Lamiaceae	Turkey	Commercial sources	-
			Hydro-distillation	5.20
<i>Origanum micranthum</i> Hort. ex Colla	Lamiaceae	Turkey	Hydro-distillation	0.38
Origanum onites L.	Lamiaceae	Turkey	Hydro-distillation	- 2.80
Salvia microstegia Boiss. & Bal.	Lamiaceae	Turkey	Hydro-distillation	0.05
Salvia multicaulis Vahl	Lamiaceae	Turkey	Hydro-distillation	0.13
Salvia palaestina Benth.	Lamiaceae	Turkey	Hydro-distillation	0.27
Salvia sclarea L.	Lamiaceae	Turkey	Hydro-distillation	1.15
Salvia trichoclada Benth.	Lamiaceae	Turkey	Hydro-distillation	0.27
Salvia viridis L.	Lamiaceae	Turkey	Hydro-distillation	0.27
Satureja pilosa Velen.	Lamiaceae	Turkey	Hydro-distillation	-

Table 1. The sources of plant materials and oil yields of the essential oil samples.

Essential Oil	Family	Source	Type of Obtaining Method	Oil Yield (%)
Satureja spinosa L.	Lamiaceae	Turkey	Hydro-distillation	0.47
<i>Stachys antalyensis</i> Y. Ayasligil & P.H. Davis	Lamiaceae	Turkey	Hydro-distillation	0.18
Tanacetum chiliophyllum Sch. Bip. var. monocephalum Grierson	Asteraceae	Turkey	Hydro-distillation	0.17
Thymus cherlerioides Vis.	Lamiaceae	Turkey	Hydro-distillation	0.06
Thymus fedtschenkoi Ronniger var. handelii (Ronniger) Jalas	Lamiaceae	Turkey	Hydro-distillation	0.23
<i>Thymus leucostomus</i> Hausskn. & Velen. var. <i>argillaceus</i> Jalas	Lamiaceae	Turkey	Hydro-distillation	0.92
<i>Thymus leucostomus</i> Hausskn. & Velen. var. <i>leucostomus</i>	Lamiaceae	Turkey	Hydro-distillation	0.41
Thymus longicaulis C. Presl ssp. longicaulis var. subisophyllus	Lamiaceae	Turkey	Hydro-distillation	1.70
<i>Thymus migricus</i> Klokov & Des Shost.	Lamiaceae	Turkey	Hydro-distillation	-
<i>Thymus praecox</i> Opiz var. <i>arcticus</i> (Durand) T. Karlsson	Lamiaceae	Germany	Hydro-distillation	-
<i>Thymus pseudopulegioides</i> Klokov & DesShost.	Lamiaceae	Turkey	Hydro-distillation	0.60
Thymus revolutus Celak	Lamiaceae	Turkey	Hydro-distillation	0.38
Thymus sibthorpii Benth.	Lamiaceae	Turkey	Hydro-distillation	1.30
Thymus zygioides Griseb. var. zygioides	Lamiaceae	Turkey	Hydro-distillation	1.12
Xanthoxylum armatum D.C.	Rutaceae	Nepal	Commercial sources	-

2.3. GC/MS Conditions

Oils were analyzed by GC/MS using a Hewlett Packard GCD System (SEM Ltd., Istanbul, Turkey). The identification of the separated volatile organic compounds was achieved through retention indices and mass spectrometry by the comparing mass spectra of the unknown peaks with those stored in the Wiley GC/MS Library, MassFinder and the in-house "Baser Library of Essential Oil Constituents" which includes over 3200 genuine compounds with MS and retention data. n-Alkanes (C₉-C₂₀) were used as reference points in the calculation of retention indices. HP-Innowax FSC column (60 m x 0.25 mm, 0.25 μ m film thickness) was used with helium as carrier gas (1.0 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 50:1. The injector temperature was 250°C. Mass spectra were taken at 70 eV. Mass range was from *m/z* 35 to 450.

Linalool containing essential oils

2.4. MD-GC/MS Conditions

Chiral separations were performed on a multidimensional gas chromatography-mass spectrometry (MDGC/MS) system (SEM Ltd., Istanbul, Turkey). The system was equipped with two oven gas chromatographs, two independent temperature controls and connected with Cooled Injection System (CIS), Cryo-Trap System (CTS) and a Multi-Column Switching (MCS) system. The precolumn was equipped with a FID detector (250°C) and the main column was connected with MS detector. Relative percentage amounts were calculated from FID by computer. Identification of compounds was carried out using the Baser Library of Essential Oil Constituents, Wiley, Adams, NIH and NIST libraries.

2.5. Cooled Injection System (CIS) Parameteres

HP-Innowax silica capillary column (60 m x 0.25 mm i.d., with 0.25 μ m film thickness) was used as pre-column. Injection in to the system was carried out at 40°C by using Cooled Injection System (CIS). After 0.5 min splitless and 0.5 min equilibrium time, split was applied to the system with 10:1 split ratio. Temperature was programmed to 300°C at a rate of 10°C/s and kept at this temperature for 2 min. GC oven temperature was kept at 60°C for 10 min and programmed rise to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed rise to 240°C at a rate of 1°C/min, and then kept constant at 220°C for 40 min.

2.6. Multi Column Switching (MCS) Peneumatic Parameters

Helium was used as a carrier gas. Make-up, split and desorption flows were 20 mL/min. Control flows 1 and 2 were 10 mL/min and 20 mL/min respectively.

2.6.1. CUT Parameters

Both of Cut 1 and Cut 2 were on while Cut 2 was with 20 kPa pressure in the beginning. In 30 min Cut 1 was off until 32.5 min.

Main column was Lipodex E, [=Octakis (3-O-butyryl-2,6-di-O-pentyl)- γ -cyclodextrin], (70% in OV1701), (25 m x 0.25 mm i.d., 0.25 µm film thickness) (Prof. W. König, Hamburg, Germany). GC oven temperature was kept at 40°C for 34 min and programmed to 120°C at a rate of 1°C/min, and kept constant at 120°C for 6 min. MS were taken at 70 eV. Mass range was from m/z 35 to 450.

2.7. Cryo Trap System Parameters

Cryo trap was liquid nitrogen and transfer line and initial temperature was 250°C. Initial time was 29.5 min then it was cooled down to -100° C at a rate of 20°C/s. After 3.5 min then heated up to 240°C at a rate of 20°C/s. and kept at this temperature for 3 min.

2.8. Assays for Biological Activities

Antimicrobial and in vitro antimalarial assay procedures were utilized at the NCNPR, University of Mississippi. Antimicrobial activity was determined against *Candida albicans* (ATCC 90028), *Cryptococcus neoformans* (ATCC 90113), *Aspergillus fumigatus* (ATCC 90906), *Staphylococcus aureus* (ATCC 29213), methicillin-resistant *S. aureus* (ATCC 43300), *Pseudomonas aeruginosa* (ATCC 27853), and *Mycobacterium intracellulare* (ATCC 23068) using a modified version of the CLSI (Clinical and Laboratory Standards Institute) formerly NCCLS (National Committe for Clinical Laboratory Standards) methods [8-11] as reported previously [12]. Antimicrobial standards ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi were included as positive control in each assay. In vitro antimalarial activity was determined against D6 (chloroquine sensitive) and W2 (chloroquine resistant) clones of

Plasmodium falciparum. The assay was based on the determination of parasite lactic acid dehydrogenase (LDH) activity using Malstat reagent and was performed as described previsously by Tabanca et al.[12]. Chloroquine (Aldrich-Sigma, ST, Louis, MO) and artemisinin (Aldrich-Sigma, ST, Louis, MO) standards were included in each assay. Antifungal activity was evaluated against fungal plant pathogens at the USDA-ARS, NPURU, University of Mississippi. Azoxystrobin, benomyl, captan, and cyprodinil (Chem Service, Inc. West Chester, PA) were used as commercial fungicide standards. Each test fungicide and standard was run in duplicate at each concentration and the experiment was performed independently three times. 1D-bioautography (*Colletotrichum acutatum, C. fragariae* and *C. gloeosporioides*) and microtiter assays (*C. acutatum, C. fragariae, C. gloeosporioides, F. oxysporum, B. cinerea, P. obscurans,* and *P. viticola*) have been described in our previous papers [8-11]. Antimicrobial standards ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi were included as positive control in each assay.

2.8.1. Direct Bioautography Assay for Activity Against Plant Pathogenic Fungi

Pathogen production and bioautography procedures of Tabanca et al. [12], Meazza et al. [13] and Fokialakis et al. [14] were used to evaluate antifungal activity against three fungal plant pathogenic *Colletotrichum* species. Sensitivity of each fungal species to each test compound was determined 4 day after treatment by comparing size of inhibitory zones, affording means and standard deviations of inhibitory zone size were used to evaluate antifungal activity of test compounds. Bioautography experiments were performed multiple times using both dose- and non-dose-response formats. Fungicide technical grade standards benomyl, cyprodinil, azoxystrobin, and captan (Chem Service, Inc. West Chester, PA) were used as controls at 2 mM in 2 μ L of 95% ethanol. Linalool enantiomers were applied at 4 and 8 μ L of 2 mM concentration in *n*-hexane.

2.8.2. Microdilution Broth Assay Against Plant Pathogenic Fungi

A standardized 96-well microtiter plate assay developed by Wedge and Kuhajek [15] and Tabanca et al [12] was used to evaluate antifungal activity of isolated compounds against *Colletotrichum acutatum, C. fragariae, C. gloeosporioides, Fusarium oxysporum, Botrytis cinerea,Phomopsis obscurans* and *P.viticola*. Azoxystrobin, benomyl, captan, and cyprodinil were used as commercial fungicide standards. Each fungus was challenged in a dose-response format using test compounds where the preliminary treatment concentrations were 0.3, 3.0 and 30.0 μ M Microtiter plates (Nunc MicroWell, untreated; Roskilde, Denmark) were covered with a plastic lid and incubated in a growth chamber as described previously. Fungal growth was then evaluated by measuring absorbance of each well at 620 nm using a microplate photometer (Packard Spectra Count, Packard Instrument Co., Downers Grove, IL). Mean growth inhibition and standard errors for each fungus at each dose of test compound were used to evaluate fungal growth. Each test fungicide and standard was run in duplicate at each concentration and the experiment was performed independently three times.

3. Results and Discussion

3.1. Enantiomeric distribution of linalool

The enantiometric distribution of linalool in essential oils from Apiaceae, Asteraceae, Lamiaceae, Lauraceae, Rutaceae and Zingiberaceae was determined on a Lipodex E fused silica chiral

capillary column using enantio-MDGC/MS, which allows a selective and sensitive analysis of the chiral compounds.

Relative percentages of linalool in essential oil showed a wide variation between genera. The highest content of linalool (90.3 %) was found in the linalool-chemotype *Origanum onites* oil while *Micromeria juliana* contained the lowest linalool (4.5 %) concentration among the essential oils studied (Table 2).

The non and chiral columns combination of the MDGC/MS system allowed a fast and exact determination of enantiomeric distribution of linalool (Figure 1). The average analysis time of linalool separation was about 2 hours including sample preparation. However, isolation of each enantiomer individually by column chromatography or TLC applications was more time consuming.

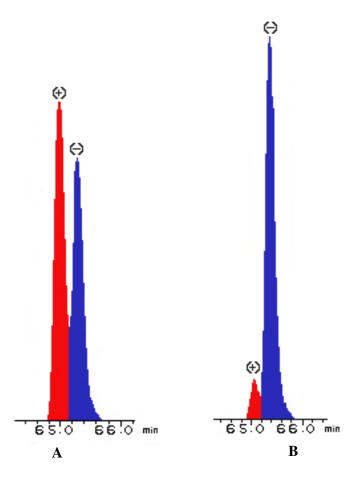


Figure 1. Enantiomeric separation of linalool (A) from *Salvia microstegia* (B) from *Lavandula angustifolia* oil.

Enantiomerically pure (+)-linalool was found in the essential oils of *Nepeta cadmea*, *Nepeta cataria*, *Nepeta conferta*, *Nepeta flavida*, *Nepeta italica*, *Satureja spinosa* and *Tanacetum chiliophyllum* var. *monocephalum*, while it was the predominant enantiomer in the essential oils of Coriandrum sativum (83.9 %) and *Elettaria cardamonum* (76.9 %) (Table 2).

Plant name	Linalool in the oil (%)	(+)-Linalool (%)	(-)-Linalool (%)
Achillea grandifolia Friv.	18.1	-	100
Achillea millefolium L.	5.5	3.3	96.7
Ballota sechmenii Gemici & Leblebici	5.0	26.9	73.1
Calamintha tauricola P.H. Davis	5.1	17.3	82.7
<i>Cinnamomum tamala</i> (BuchHam.) T.Nees & C.H.Eberm.	13.4	9.0	91.0
Coriandrum sativum L.	73.7	83.9	16.1
Elettaria cardamomum Maton	8.7	76.9	23.1
Hedychium flavum Roxb.	19.2	3.4	96.6
Lavandula angustifolia Mill.	30.4	5.9	94.1
Micromeria juliana (L.) Bentham ex Reichb.	4.5	3.3	96.7
Nepeta cadmea Boiss.	13.5	100	-
Nepeta cataria L.	20.5	100	-
Nepeta conferta I.C. Hedge & J.M. Lamond	11.4	100	-
Nepeta flavida A. Huber-Morath.	37.7	100	-
Nepeta italica L.	39.9	100	-
Ocimum basilicum L.	43.1	-	100
	29.0	-	100
	18.5	-	100
Origanum floribundum Munby	16.1	-	100
Origanum majorana L.	12.1	-	100
	41.2	-	100
Origanum micranthum Hort. ex Colla	10.9	14.0	86.0
Origanum onites L.	90.3	1.6	98.4
	79.8	-	100
Salvia microstegia Boiss. & Bal	9.0	52.4	47.6
Salvia multicaulis Vahl	11.9	17.2	82.8
Salvia palaestina Benth.	70.6	17.8	82.2
Salvia sclarea L.	13.3	23.7	76.3
Salvia trichoclada Benth.	25.6	12.4	87.6
Salvia viridis L.	12.7	32.4	67.6
Satureja pilosa Velen.	35.7	41.0	59.0
~			

61.5

100

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Table 2. The Relative quantity (%) and enantiomeric composition (%) of linalool in labdistilled and commercial essential oil samples

Satureja spinosa L.

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Linalool	containing	essential	OILS
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Plant name	Linalool in the oil (%)	(+)-Linalool (%)	(-)-Linalool (%)
Stachys antalyensis Y. Ayasligil & P.H. Davis	24.4	59.9	40.1
<i>Tanacetum chiliophyllum</i> Sch. Bip. var. <i>monocephalum</i> Grierson	10.3	100	-
Thymus cherlerioides Vis.	12.5	0.1	99.9
Thymus fedtschenkoi Ronniger var. handelii (Ronniger) Jalas	12.9	-	100
<i>Thymus leucostomus</i> Hausskn. & Velen. var. <i>argillaceus</i> Jalas	18.1	0.6	99.4
Thymus leucostomus Hausskn. & Velen. var. leucostomus	12.3	0.1	99.9
Thymus longicaulis C. Presl ssp. longicaulis var. subisophyllus	18.2	30.3	69.7
Thymus migricus Klokov & DesShost.	9.7	-	100
<i>Thymus praecox</i> Opiz var. <i>arcticus</i> (Durand) T. Karlsson	18.4	21.0	79.0
Thymus pseudopulegioides Klakov & DesShost.	21.6	6.7	93.3
Thymus revolutus Celak.	15.5	-	100
Thymus sibthorpii Benth.	20.4	-	100
Thymus zygioides Griseb. var. zygioides	33.7	-	100
Xanthoxylum armatum D.C.	46.1	-	100

Enantiomerically pure (-)-linalool was found in the essential oils of Achillea grandifolia, Ocimum basilicum, Origanum floribundum, Origanum majorana, Origanum onites, Thymus fedtschenkoi var. handelii, Thymus migricus, Thymus revolutus, Thymus sibthorpii, Thymus zygioides var. zygioides and Xanthoxylum armatum, while (-)-linalool was the predominant enantiomer in the essential oils of Thymus leucostomus var. leucostomus (99.9 %), Thymus cherlerioides (99.9 %) and Thymus leucostomus var. argillaceus (99.4 %) (Table 2).

(-)-Linalool occurred in the range of 67.6% to 98.4% in the oils of Achillea millefolium, Ballota sechmenii, Calamintha tauricola, Cinnamomum tamala, Hedychium flavum, Lavandula x intermedia, Micromeria juliana, Origanum micranthum, Origanum onites, Salvia multicaulis, Salvia palaestina, Salvia sclarea, Salvia trichoclada, Salvia viridis, Thymus longicaulis ssp. longicaulis, Thymus praecox var. arcticus and Thymus pseudopulegioides.

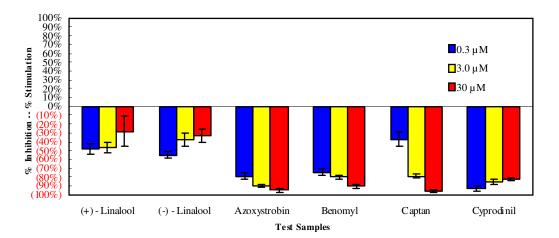
Linalool was almost racemic in the oils of *Salvia microstegia* [52.4 % -(+)], *Satureja pilosa* [59.0 % -(-)] and *Stachys antalyensis* [59.9 % -(+)].

The enantiomeric distribution of linalool showed that both enantiomers are found in the nature but (-)-linalool was more common than (+)-linalool in the essential oils in the study samples. In the literature there are enantiomeric distribution of linalool from different sources. [16-40]. Although, there is no exact rule for purity of enantiomers in any family members, some of the genus like *Nepeta*, *Origanum*, *Thymus* etc. were rich in only one enantiomer in our study. The enantiomeric distributions of some enantiomers were found to be useful in the quality assessment of the essential oils. Linalool is one of the good criteria to make a decision about the origin of the oil (natural or synthetic) [40].

3.2. Biological activity of (-) and (+)-linalool enantiomers

Pure enantiomers can have different activity or odour properties from each other. Literature citation shows that there are some studies on the biological activities of linalool enantiomers [41-49]. One of the literature is reviewing the biological activity of linalool as a commercially important compound [43]. The essential oils which contain only pure enantiomer can be used for isolation purposes. In this case, these properties can be evaluated separately by using single pure enantiomer.

As part of our effort to investigate the biological activity of natural products and their pure compounds, we evaluated (-) and (+)-linalool enantiomers for their antimalarial and antimicrobial activities against human and plant pathogenic microorganisms. Antifungal activity against strawberry anthracnose disease (Colletotrichum spp.) was evaluated using direct-bioautography assay. Antifungal activity was evident by the presence of clear zones with a dark background where fungal mycelia or reproductive stroma was not present on the TLC plate. No activity was observed at 20 mg/mL, using a 4 and 8 µL test volume, against Colletotrichum acutatum, C. fragariae, C. gloeosporioides, as compared with the commercial fungicide standards. We also subsequently evaluated both linalool enantiomers in our secondary assay (96-well micro-dilution broth assay) against seven fungal species. The 96-well micro-dilution assay was used to determine sensitivity of C. acutatum, C. fragariae, C. gloeosporioides, F. oxysporum, B. cinerea, P. obscurans, and P. viticola to the various antifungal agents in comparison with the commercial fungicides. In the micro-dilution broth assay caused 0.3 µM (+)-linalool and (-)-linalool demonstrated approximately 50% growth inhibition of Botrytis cinerea at 48 hrs (Figure 2). Both enantiomers showed no activity against any of the three Collectorichum species, *Phomopsis* species, and *Fusarium oxysporum*. However, the upward trending growth curve of micro-dilution broth assay indicated that both enantiomers may be insoluble in the aqueous based environment above 0.3 μ M and as the concentration increases to 3.0 μ M and then 30.0 μ M the spectrophotometer can not differentiate fungal growth from chemical precipitation. This in vitro assay anomaly is also seen with the cyprodinil control. No antimicrobial activity was observed at the highest test concentration of 200 µg/mL against Candida albicans, C. glabrata, C. krusei, Cryptococcus neoformans, methicillin-resistant Staphylococcus aureus, Mycobacterium intracellulare and Aspergillus fumigatus. Both enantiomers were inactive up to 4.76 µg/mL against Plasmodium falciparum D6 (chloroquine sensitive) and W2 (chloroquine resistant) clones.



B. cinerea Growth Response to Samples at 48 hrs.

Figure 2. Percent mean growth inhibition of Linalool enantiomers against *Botrytis cinerea* at 48 hrs.

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