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Characterization of volatile constituents of *Scaligeria tripartita* and studies on the antifungal activity against phytopathogenic fungi

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Abstract

The chemical composition of the essential oils obtained from stems and leaves, fruits and roots of *Scaligeria tripartita* oil was analyzed by gas chromatography–mass spectrometry. A total of 38 compounds were identified ranging 89–94% of the oil samples. Geijerene was found as a main compound in the oils of the stems and leaves (37%) and fruits (55%), whereas epoxypseudoisoeugenol angelate (37%) was found as a main compound in the root oil. Oils were subsequently evaluated for their antimalarial, antimicrobial against human pathogenic bacteria or fungi and antifungal activities against plant pathogens. Antifungal activity of *Scaligeria* oils was observed against the strawberry anthracnose-causing fungal plant pathogens *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides* using the direct overlay bioautography assay. Chemotaxonomically important pure compounds indicated in the bioautography assay were subsequently evaluated in a 96-well microdilution broth assay. The performance of overpressured layer chromatography (OPLC) and TLC for the analysis of *Scaligeria* essential oils was also compared.

Keywords: *Scaligeria tripartita*; *Pimpinella* species; Overpressured layer chromatography (OPLC); Essential oil; GC/MS; Apiaceae; Phenylpropanoids; Antimalarial activity; Antimicrobial activity

1. Introduction

The family Apiaceae Lindl. (Umbelliferae) comprises 300–455 genera with 3000–3750 species distributed in the northern hemisphere [1,2]. Apiaceae is one of the best known families of flowering plants, because of its characteristic inflorescences and fruits and the distinctive chemistry reflected in the odor and flavor [3]. This family includes many familiar edible plants (e.g., carrot, parsley, celery, fennel, dill, cumin, anise), as well as several deadly poisons (e.g., poison-hemlock, water-hemlock, fool's-parsley) [1–3]. Apiaceae species are rich sources of essential oils. They may contain essential oils originating in any organ including fruits, leaves, roots or whole plant [3].

The family Apiaceae is represented by 100 genera belonging to 430 species of which 130 are endemic species in Turkey [3]. *Scaligeria* DC. is represented in Turkey by seven species of which two are endemic [4,5] and very little work has been carried out on this genus. Davis reported botanical identification and a key to the following species; *Scaligeria napiformis* (Sprengel) Grande, *S. tripartita* (Kalen.) Tamamsch, *S. lazica* Boiss. (endemic), *S. meifolia* (Fenzl) Boiss., *S. glaucescens* (DC.) Boiss., *S. hermonis* Post, *S. capillifolia* Post (endemic) [4,5]. *S. napiformis* is known as "peynir cicegi" in Mugla, Southern Turkey [6]. *S. tripartita* has four different taxonomic synonyms: *Albovia tripartita* (Kalen.) Schischkin, *Pimpinella rotundifolia* Bieb., *P. tripartita* Kalen., *S. rotundifolia* (Bieb.) Boiss. [4] and it was therefore interesting to see, whether this species contains any trinorsesquiterpenes and pseudoisoeugenol derivatives that have been found up till now only in the species of the genus *Pimpinella* species and so far unknown in other

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umbellifers. Herbal parts of *S. lazica* have a strong smell characteristic of anise due to the presence of *ca*. 50% *trans*-anethol in its oil [7]. Fruit oil of the same species was found as a rich source of (*Z*)-β-Farnesene (89%) [8]. After removal of the oil, the aqueous distillate of the herb was extracted with hexane. It contained 2-hydroxy-5-methoxy-benzaldehyde (22%) and phenylacetaldehyde (14%) as main constituents [7].

The present study of *S. tripartita* was carried out to determine the essential oil composition and the presence of pseudoisoeugenol derivatives and trinorsesquiterpenoids. We also evaluated essential oils for antimalarial and antimicrobial activities against human pathogenic bacteria and fungi. Furthermore, activities of essential oils and characteristic important compounds were evaluated for activity against three important plant pathogenic fungi *Colletotrichum* species using direct bioautography. Those compounds were subsequently evaluated for activity in micro-dilution broth assays against *Colletotricum acutatum*, *C. fragariae*, *C. gloeosporioides*, *Fusarium oxysporum*, *Botrytis cinerea*, and *Phomopsis obscurans*. To the best of our knowledge, we report for the first time the composition and biological activity of the essential oils of *S. tripartita*.

In this work we also report the overpressured layer chromatography (OPLC) and thin layer chromatography (TLC) analysis of *Scaligeria* essential oils. OPLC is a unique liquid chromatography technology system using an on-line or off-line high performance TLC development system. OPLC was first described as one of several planar chromatography techniques by Tyihak et al. [9]. Nyiredy [10] described OPLC as a bridge between thin layer chromatography and high performance liquid chromatography. The planar adsorbent bed and forced flow allows OPLC to combine the benefits of HPLC and TLC to provide rapid and efficient separations [10–16]. OPLC is also suitable for direct bioautography of a variety of biologically active compounds [11–12].

2. Experimental

2.1. Plant material

Plant material was collected in June 2001 from Ordu, Mesudiye in Northern Turkey. Voucher specimens were placed at the Herbarium of the Faculty of Pharmacy (ESSE 13951), Anadolu University, Eskisehir, Turkey.

2.2. Isolation of essential oils

Dried stems (S_{SL}), roots (S_R), and fruits (S_F) of *S. tripartita* were hydrodistilled for 3 h using a Clevenger apparatus to obtain essential oils [17]. *Scaligeria* fruit oil yield was calculated at 2.24% on moisture-free basis, and trace amounts of oils were obtained from stem, leaves and roots. In order to recover geijerene and 1,4-dimethyl azulene, *Pimpinella tragium* ssp. *lithophila* stem and leaf essential oil was purified. *P. tragium* ssp. *lithophila* oil (0.2 g) was subjected to High Performance Flash Chromatography system (Biotage Inc., A Dynax Corp. Company, USA) using a Biotage SI 12 M column (150 mm × 12 mm i.d.; 9 g KP-Sil^{TM} silica; 40–63 μm particle size; flow rate: 2.0 mL/min) and eluted with *n*-hexane (50 mL and 3.0 mL each eluent), *n*-hexane–diethylether mixtures (99:1 → 90:10, 90:10 → 80:20, 80:20 → 70:30 each 30 mL and 3.0 mL each eluent) and finally washing with *n*-hexane–diethylether (60:40, 60 mL and 3.0 mL each eluent). Similar fractions according to TLC profiles (*n*-hexane–diethylether (95:5, 90:10, 85:15, 80:20, 70:30 v/v) were combined to give 14 pooled samples (fractions A–N, each 3 mL). Fraction A gave compound **4** (geijerene, 3.0 mg). Fraction C afforded compound **33** (1,4-dimethyl azulene, 5.0 mg). Compounds geijerene and 1,4-dimethyl azulene were identified by their identical spectra. Compounds **24** (traginone), **31** (dictamnol), **35** (epoxypseudoisoeugenol angelate) and **37** (epoxypseudoisouegenol tiglate) were chromatographically purified from our previously reported studies [18,19]. All these compounds are reported in Table 1.

2.3. GC/MS analysis of the essential oil of S. tripartita

GC/MS analysis was performed with a Hewlett-Packard GCD, system (SEM Ltd., Istanbul, Turkey) and an Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) was used with helium as a carrier gas. Oven temperature program was set as follows: 60 °C for 10 min at 4 °C/min to 220 °C held 10 min, at 1 °C/min to 240 °C. Split flow was adjusted at 50 mL/min, the injector temperature was 250 °C, and mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 425. *n*-Alkanes (C9–C20) were used as reference points in the calculation of retention indices (RRI) [20–22]. Identification of essential oil components was carried out by comparison of their relative retention times to those of authentic samples or by comparison of their relative retention indices (RRI) to *n*-alkanes series. Computer matching against commercial libraries (Wiley and MassFinder 2.1) [23,24], "Baser Library of Essential Oil Constituents" built from genuine compounds and components of known oils, and reported MS literature data [25–27] were used for final identification. Relative percentages of the characterized components are as cited in Table 1.

2.4. OPLC and TLC analyses

A Personal-OPLC instrument (OPLC-NIT, Budapest, Hungary) was used for the development of chromatograms at 5.0 MPa external pressure. Plates used were fine particle silica gel 60 F_{254} 20 cm × 20 cm on aluminium sheet (LA 001, OPLC-NIT Ltd., Budapest Hungary) with 200 μm adsorbent thickness. Off-line sample application was carried out onto the dry adsorbent layer by means of LINOMAT III sample applicator (Camag, Muttenz, Switzerland). Two and four microliters of 16, 18, and 16 mg/mL samples in hexane with 10 mm band size of S_{SL}, S_F and S_R oils were applied at 27 mm measured from the bottom edge of the adsorbent layer. Double infusion developments [28] were applied with a two elution protocol. The first elution was conducted with *n*-hexane–Et_2O (95:5, v/v) with 4400 μL of solvent with start flash volume 300 μL, flow rate 500 μL/min and total elution time 534 s. The second elution was conduced using pure 4350 μL *n*-hexane using conditions described above. The plate was inspected under UV light (254 nm) and also by visu-

Table 1
The composition of the essential oils of *Scaligeria tripartita*

No.	Compound	RRI	S_{SL}	S_F	S_R
1	γ-Terpinene	1255	–	–	0.4
2	*p*-Cymene	1280	–	–	1.9
3	Geijerene isomer[a]	1311	4.9	12.0	2.9
4	Geijerene	1338	36.8	54.6	28.5
5	Isogeijerene C	1490	0.5	0.7	–
6	Linalool	1553	1.1	–	–
7	Pregeijerene	1594	4.5	6.4	–
8	*trans*-β-Bergamotene	1594	0.1	–	–
9	Isothymol methyl ether	1595	–	–	2.9
10	β-Elemene	1600	–	0.1	–
11	Thymol methyl ether (=methyl thymol)	1604	–		1.8
12	β-Caryophyllene	1612	–	1.8	–
13	Lavandulyl acetate	1617	6.4	–	–
14	(*Z*)-β-Farnesene	1668	9.4	–	–
15	Lavandulol	1686	0.3	–	–
16	α-Humulene	1687	–	0.2	–
17	α-Terpinyl acetate	1709	0.4	–	–
18	Germacrene D	1726	2.2	0.1	–
19	β-Bisabolene	1741	9.0	7.1	1.3
20	Bicyclogermacrene	1755	0.2	–	–
21	δ-Cadinene	1773	–	tr	–
22	(*E*)-Anethol	1845		0.7	–
23	*p*-Cymen-8-ol	1864	0.1	–	–
24	Traginone	1881	6.4	1.6	2.4
25	Shyobunol	1953	0.1		–
26	Caryophyllene oxide	2008	0.9	0.1	–
27	Perilla alcohol	2029	0.8		–
28	Humulene epoxide-III	2081	–	0.1	–
29	Elemol	2096	–	0.4	–
30	Spathulenol	2144	0.5	–	–
31	Dictamnol	2170	8.7	5.2	9.4
32	Carvacrol	2239	0.2	–	–
33	1,4-Dimethyl azulene	2291	0.3	tr	–
34	Phytol	2622	0.1	–	–
35	4-Methoxy-2-(3-methyloxiranyl)phenyl angelate (=epoxypseudoisoeugenyl angelate)	2825	0.4	–	36.9
36	Unknown	2917	–	–	11.1
37	4-Methoxy-2-(3-methyloxiranyl)phenyl tiglate (=epoxypseudoisoeugenyl tiglate)	2926	–	0.8	0.6
38	Osthol	2942	–	1.4	–
	Total of identified compounds		94.3	93.3	89.0
	Unidentified compound				11.1

S_{SL}: *S. tripartita* stems and leaves oil; S_F: *S. tripartita* fruit oil; S_R: *S. tripartita* root oil; RRI: Relative retention indices calculated against *n*-alkanes on the HP Innowax column. Unknown: EIMS, 70 eV, *m/z* (rel. int.): 180(50), 138(19), 137(100), 123(7), 109(8), 94(6), 77(10), 66(6), 59(3), 43(41). % calculated from TIC data. tr: Trace (<0.1%).

[a] Correct isomer not identified.

alization with vanillin–H_2SO_4 reagent (0.1 g vanillin in 100 mL EtOH and 2 mL H_2SO_4) and then the plate was heated at 110 °C for 3 min after double developments. Photography was accomplished by using simple digital camera under UV_{254} or visible light.

Classical TLC analysis was conducted by using precoated silica gel 60 F_{254} (Merck, Suwanee, GA). Four microliters of 20 mg/mL samples in hexane were applied to plate using AS-30 sample applicator (Desaga, Wiesloch, Germany). The TLC plate was developed in a presaturated solvent chamber (*n*-hexane–Et_2O, 95:5 v/v). After solvent migration of 8 cm, the TLC plate was air dried and inspected under UV light (254 nm) and visualization made with vanillin–H_2SO_4 (1 g vanillin in 100 mL of 20% H_2SO_4 in EtOH) reagent and the plate was heated.

2.5. Assay for antimalarial activity

The *in vitro* antimalarial activity was determined against the D6 (chloroquine sensitive) and W2 (chloroquine resistant) clones of *Plasmodium falciparum*. The assay was based on the determination of parasite LDH activity using Malstat reagent and was performed as described earlier [19]. Chloroquine (Aldrich–Sigma, St. Louis, MO) and artemisinin (Aldrich–Sigma, St. Louis, MO) were included as control drugs in each assay [19].

2.6. Assay for antimicrobial activity

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 (formerly NCCLS methods) [29–32] as reported previously [19,33]. Antimicrobial standards ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi were included as control drugs in each assay.

2.7. Direct bioautography assay for activity against plant pathogenic fungi

Bioautography procedures of Meazza et al. [34] and Tabanca et al. [19] for detection of naturally occurring antifungal agents were used to evaluate antifungal activity against fungal plant pathogens. 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 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 μL of 95% ethanol. Compounds **4**, **24**, **31**, **33**, **35** and **37** were applied at 4 μL of 2 mM in hexane. *Scaligeria* essential oils were applied at 16 to 18 mg/mL in 2 and 4 μL of hexane onto a silica OPLC plate.

2.8. Microdilution broth assay for activity against plant pathogenic fungi

A standardized 96-well microtiter plate assay developed by Wedge and Kuhajek [35] was used to evaluate antifungal activity of isolated compounds against *C. acutatum*, *C. fragariae*, *C. gloeosporioides*, *F. oxysporum*, *B. cinerea*, and *P. obscurans*. Azoxystrobin was used as commercial fungicide standards. Each fungus was challenged in a dose-response format using test compounds where the final treatment concentrations were 0.3, 3.0 and 30.0 μM. 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).

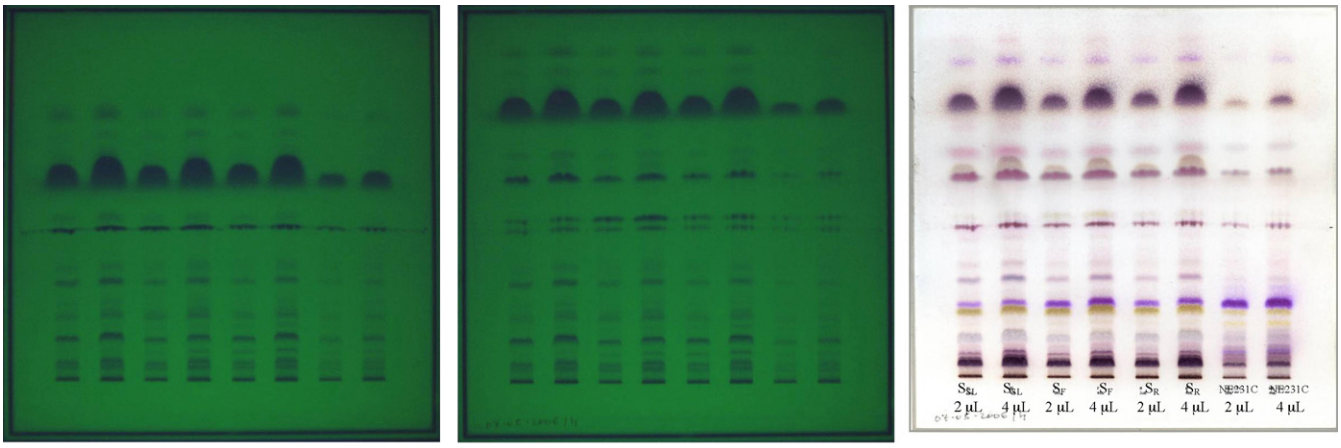
3. Results and discussion

Water distilled essential oils from stems and leaves, fruits and roots of *S. tripatita* were analyzed by GC–MS system. The compounds characterized and reported with their relative percentages are listed in Table 1. A total of 38 compounds were identified ranging 89–94% of the oil composition. Essential oils obtained from stems and leaves (SL) and fruits (F) contained considerable amounts of C_{12} hydrocarbons of which geijerene predominated (SL: 36.8%; F: 54.6%). A geijerene isomer (SL: 4.9%; F: 12.0%), pregeijerene (SL: 4.5%; F: 6.4%), isogeijerene C (SL: 0.55%; F: 0.7%) and 1,4-dimethylazulene (SL: 0.3%) were also detected. In addition, oxygenated derivatives of C_{12}-constituents such as traginone (SL: 6.4%; F: 1.6%), dictamnol (SL: 8.7%; F: 5.2%) were found in stems and leaves and fruit oils. Traginone and dictamnol were previously isolated and identified originally from *P. tragium* ssp. *lithophila* oil [18] and we were the first to show the occurrence of traginone and dictamnol in *Pimpinella*. Kiran et al. recently reported that geijerene and pregeijerene displayed oviposition deterrence effects on the tobacco cutworm, *Spodoptera litura*, at low concentration [36]. The quantity of geijerene and pregeijerene present make *S. tripartita* a good source of these compounds as potential natural pesticides. Geijerenes were recently reported in the Asteraceae (*Chromolaena odorata*), Lamiaceae (*Wiedemanniana orientalis*, *Nepeta govaniana*, *Lallemantia peltata*), Rosaceae (*Rubus rosifolius*), Rutaceae (*Geijera parviflora*, *Ruta graveolens*, *Amyris diatrypa*, *Vepris heterophylla*, *Boenninghausenia albiflora*, *Acronychia* species, *Flindersia* species), Leguminosae (*Tephrosia egregia*), Apiaceae (*Pimpinella* species) [37–38].

Kubeczka reported [39] that *A. tripartita* root oil contained *ca*. 50% epoxypseudoisoeugenol angelate (EPA) and corroborated with our results that showed *S. tripartita* root oil contained 37% EPA (Table 1). *Scaligeria* root oil also contained one unknown constituent (11%) and a minor amount of epoxypseudoisoeugenol tiglate (EPT) (0.6%). The unknown constituent seems to be an oxygen containing C_{12}-derivative with the formula $C_{12}H_{20}O$. Because of limited sample availability, it has not been possible yet to isolate a consistent amount of compound for structure elucidation studies.

Pseudoisoeugenol type phenylpropanoids and C_{12}-compounds are important chemosytematic characters in the genus *Pimpinella*. The 2-hydroxy-5-methoxy-1-(*E*)-propenylbenzene skeleton of the pseudoisoeugenols are unique to *Pimpinella*. The isoeugenol derivatives are related to 2-methoxy-4-(1-propenyl)-phenol, while the pseudoisoeugenols are related to 4-methoxy-2-(1-propenyl)-phenol [40]. Kubecka et al. [39] showed that isolated phenylpropanoids from *Pimpinella* had pseudoisoeugenol skeletons and not the original isoeugenol structures as reported. Some studies reported that pseudoisoeugenols had also been found in another genus of Apiaceae namely *Ligusticum mucronatum* and misinterpreted the isoeugenol and pseudoisoeugenol differences in the *L. mucronatum* report [41,42]. Bohlmann [43] found isoeugenol-based phenylpropanoids in *L. mucronatum*. However, there has been no subsequent confirmation of phenylpropanoids in this species and only pseudoisoeugenol type phenylpropanoids were found in *Pimpinella* so far.

Differences in off-line OPLC and TLC separation can be seen in Figs. 1 and 2. Fig. 1a shows the single developed OPLC



(a) 1st run with hexane-Et_2O (95:5, v/v) UV_{245} nm

(b) 2nd run with hexane UV_{245} nm

(c) 2nd run with hexane vanillin-sulfuric acid + heating = visible

Fig. 1. Chromatogram of off-line OPLC separation of *Scaligeria* oils under UV and visible with (a) hexane-Et_2O, (b) hexane, and (c) visualized by vanillin-sulfuric acid reagent. Two and 4 μL of 16, 18, and 16 mg/mL with 10 mm band size of S_{SL}, S_F and S_R oils were applied at 27 mm measured from the bottom edge of the adsorbent layer. S_{SL}: *Scaligeria tripartita* stems and leaves oil; S_F: *S. tripartita* fruits oil; S_R: *S. tripartita* roots oil; NE231C: Reference oil of *Salvia recognita*.

chromatogram using infusion operation (the chamber outlet is closed during development) and hexane/diethyl ether (95:5, v/v) of *Scaligeria* oils obtained from stems, fruits and roots. Fig. 1b shows a double developed OPLC chromatogram made by infusion operation using hexane/diethyl ether (95:5, v/v) followed by a second development with hexane. Double development applied provides better separation of fractions and resolution of co-migrating compounds of the essential oils. Fig. 1c shows the reference double developed chromatogram visualized by vanillin-sulfuric acid reagent. Fig. 2 shows the chromatogram of the same *Scaligeria* oils obtained from stems and leaves, fruit and root using classical TLC. Nonpolar compounds co-migrated together in the top of the classical TLC chromatogram while those compounds were well separated by OPLC. As can be seen

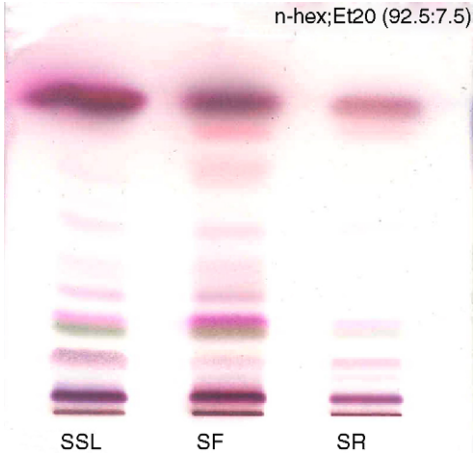


Fig. 2. Chromatogram of classical TLC separation of *Scaligeria* oils using hexane and Et_2O (95:5, v/v). Oils were applied as a 20 mg/ml in 4 μL of hexane onto a silica TLC plate. The plate was visualized by vanillin-sulfuric acid reagent. S_{SL}: *Scaligeria tripartita* stems and leaves oil; S_F: *S. tripartita* fruit oil; S_R: *S. tripartita* root oil.

OPLC gave better overall separation of *Scaligeria* oils than did classical TLC.

OPLC system uses a programmable pump to deliver the mobile phase to the "flat column" and the resulting forced flow leads to a faster separation and improved efficiency than capillary flow. Also, the use of longer separation distances increases zone capacity compared to classical TLC. OPLC applications result in possibility of less time and less solvent. One clear advantage of OPLC is the ability to stop the elution process and remove the plate/column and visualize the separation of compounds under UV. Then re-insert the plate/column and continue the elution and/or change the elution solvent or utilize a double-development method. Rapid speed of elution of OPLC columns limits the diffusion effects, making the separation of closely related compounds cleaner than classical TLC, thereby concentrating bioactive compounds into a smaller more compact zone or band and thereby increasing compound/mm^2 and enhancing direct bioautography detection. The other advantage of OPLC is that the eluent flow rate is adjustable and A, B, and C solvent systems can be chosen for isocratic or step-wise gradient runs [13–16].

We found that OPLC is especially well suited for the separation of essential oils. OPLC coupled with direct bioautography is an especially powerful tool to separate and determine the number of bioactive compounds in a lipophilic, non-polar and even complex mixture. Direct bioautography coupled with OPLC offers numerous advantages for the discovery of natural product for pest management. Evaluation of interaction zones between fungi or bacteria and biologically active natural products directly on the adsorbent bed of the OPLC plate can greatly help with the identification of potential plant protectants. OPLC instrumentation produced better banding due to increased migration velocity and decreased diffusion effects of the migrating compounds in this present study.

Table 2
Antifungal activity of *Scaligeria tripartita* essential oils and pure compounds against *Colletotrichum* species using direct bioautography

Sample	Mean fungal growth inhibition (mm) ± SEM		
	C. acutatum	*C. fragariae*	*C. gloeosporoides*
S_{SL} oil	3.5 ± 0.71	4.5 ± 0.71	3.5 ± 0.71
S_F oil	3.5 ± 0.71	5.5 ± 0.71	3.5 ± 0.71
S_R oil	8.5 ± 0.71	12.5 ± 0.71	6.5 ± 0.71
Geijerene (**4**)	NA	NA	NA
1,4-Dimethyl azulene (**33**)	NA	NA	NA
Traginone (**24**)	NA	NA	NA
Dictamnol (**31**)	NA	NA	NA
Epoxypseudoisoeugenyl angelate (**35**)	11 ± 0	13.5 ± 0.71	9.5 ± 0.71
Epoxypseudoisoeugenyl tiglate (**37**)	9.5 ± 0.71	13 ± 1.41	8.5 ± 0.71
Benomyl[a]	Dfs	24 ± 0.71	Dfs
Captan[a]	15 ± 1.41	15.5 ± 0.71	14.5 ± 0.71
Cyprodinil[a]	Dfs	14 ± 1.41	Dfs
Azoxystobin[a]	24 ± 1.41	Dfs	24.5 ± 0.71

S_{SL}: *S. tripartita* stems and leaves oil; S_F: *S. tripartita* fruit oil; S_R: *S. tripartita* root oil; *Scaligeria* essential oils were applied as a 20 mg/ml in 4 μL of hexane onto a silica TLC plate. NA: not active; Dfs: diffuse inhibitory zone. The bold numbers represent the compounds in Table 1 and pure compounds were applied 4 μl of 2 mM in hexane. Mean inhibitory clear zones and standard errors were used to determine the level of antifungal activity against each fungal species.

[a] Technical grade agrochemical fungicides (without formulation) with different modes of action were used as internal standards. Chemical standards were applied 2 μl of 2 mM in EtOH.

Essential oils from different plant parts were also evaluated for further biological activities. Antimalarial and antimicrobial activity was tested against human pathogenic bacteria, filamentous fungi, and yeasts. Samples were also evaluated against three plant fungal pathogens of *Colletotrichum* using direct-bioautography. *S. tripartita* essential oils up to 4.76 μg/mL showed no antimalarial activity against *P. falciparum* D6 and W2 clones. *Scaligeria* oils demonstrated good antimycobac-

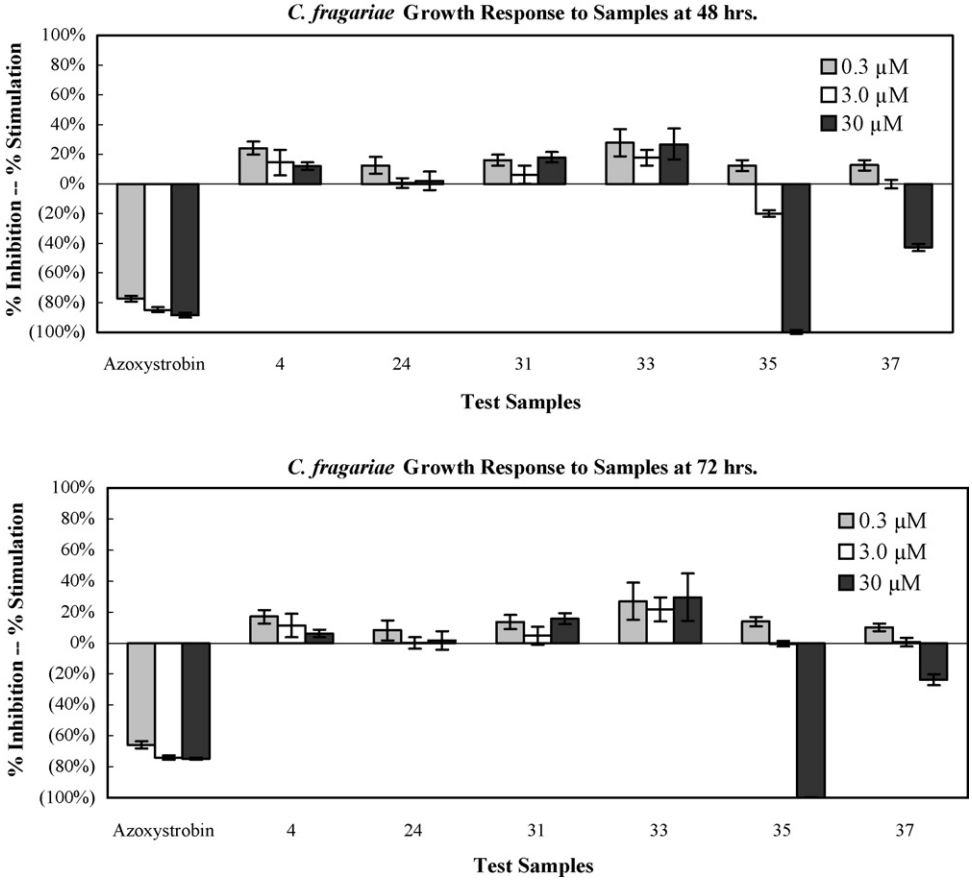


Fig. 3. Percent mean growth inhibition of compounds **4**, **24**, **31**, **33**, **35** and **37** against *Colletotrichum fragariae* at 48 and 72 h. The bold numbers represent the compounds in Table 1.

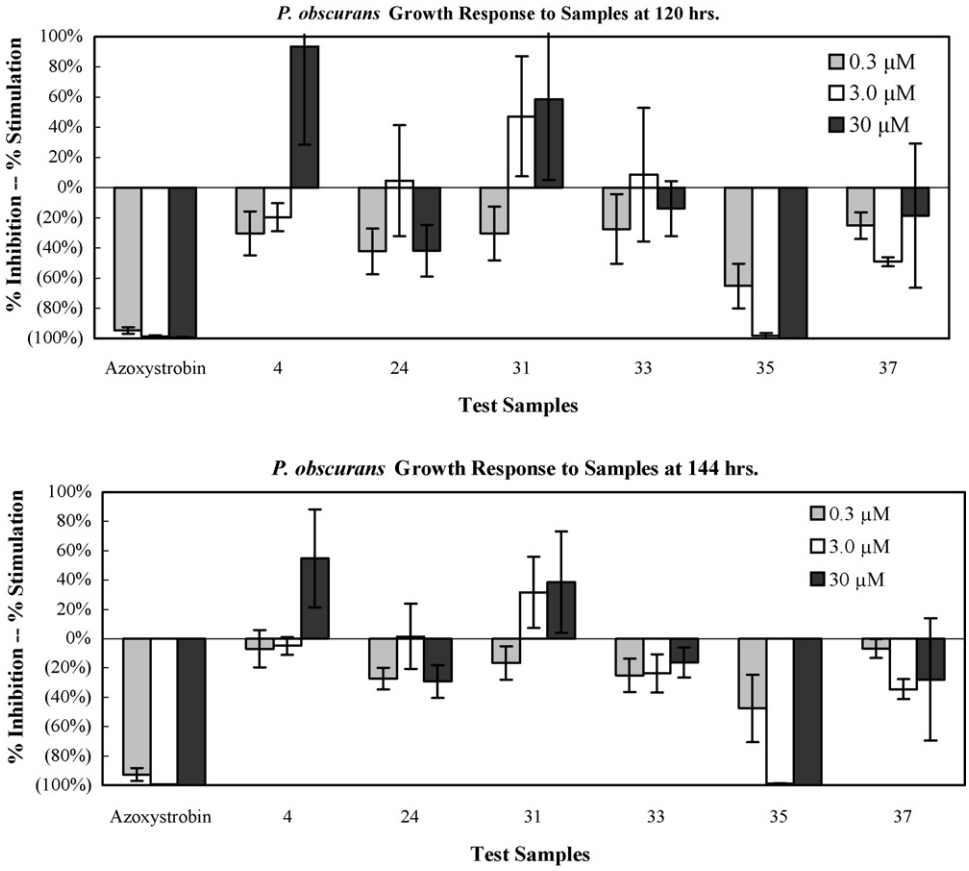


Fig. 4. Percent mean growth inhibition of compounds **4**, **24**, **31**, **33**, **35** and **37** against *Phomopsis obscurans* at 120 and 144 h. The bold numbers represent the compounds in Table 1.

terial activity against *M. intracellulare* with IC_{50} values 15, 8.5 and 10 μg/mL for the S_{SL}, S_F, and S_R samples, respectively. Oils showed mild activity against *C. neoformans* with IC_{50} value of S_{SL}: 55, S_F and S_R: 40 μg/mL. The positive controls amphotericin B and ciprofloxacin had IC_{50} values of 0.70 and 0.15 μg/mL for *C. neoformans* and *M. intracellulare*, respectively. However, no antimicrobial activity was observed against *C. albicans*, *S. aureus*, methicillin-resistant *S. aureus*, *P. aeruginosa* and *A. fumigatus* at concentrations up to 200 μg/mL.

Essential oils and characteristic important compounds were evaluated as concerns their antifungal activities against three plant pathogenic *Colletotrichum* species using direct-bioautography. TLC bioautography revealed that the essential oil obtained from roots appeared more active against *C. acutatum*, *C. fragariae*, and *C. gloeosporioides* than stems and leaves and fruits (Table 2). Geijerene (**4**), traginone (**24**), dictamnol (**31**), 1,4-dimethylazulene (**33**), epoxypseudoisoegenol angelate (**35**), and tiglate (**37**) were subsequently evaluated for antifungal activity. Geijerene, 1,4-dimethylazulene, traginone, and dictamnol were determined to possess insignificant activity at 4 μL of 2 mM against all three *Colletotrichum* species. Epoxypseudoisoeugenol angelate and epoxypseudoisoeugenol tiglate showed 87% and 84%, 73% and 63%, and 66% and 59% of the activity of the captan standard against *C. fragariae*, *C. acutatum* and *C. gloeosporioides*, respectively. Captan is a multi-site inhibitor with no systemic activity and is used as a protectant fungicide to prevent anthracnose diseases in many fruits and ornamentals [34,35]. Epoxypseudoisoeugenol angelate and tiglate demonstrated non-selective activity against each of three *Colletotrichum* species (Table 2). Subsequently compounds (**4**, **24**, **31**, **33**, **35** and **37**) were also evaluated in a 96-well microdilution broth assay against *P. obscurans*, *F. oxysporum*, *B. cinerea* and three *Colletotrichum* species. EPA at 30 μM showed 99.9% growth inhibition of *C. fragariae* at 48 h whereas EPT showed 42.9% growth inhibition (Fig. 3). EPA was more effective at reducing growth in *C. fragariae* than the commercial fungicide azoxystrobin. At 3.0 and 30 μM, EPA was the most active compound and caused 98.2% and 100% growth inhibition of *P. obscurans* at 120 h (Fig. 4). All pure compounds at 30 μM showed weak antifungal activity with 14.0–45.78% growth inhibition of *B. cinerea* at 96 h (Fig. 5).

S. tripartita appears to be related to the genus *Pimpinella* due to the presence of characteristic pseudoisoeugenol-type phenylpropanoids and trinorsesquiterpenes in the essential oil. Future research to focus on genetic analysis and comparison of *Scaligeria* molecular markers with *Pimpinella* species is warranted. Our future research goals are to evaluate the remaining five *Scaligeria* species as they become available. As a result, phenylpropanoids and trinorsesquiterpenes may have potential applications as novel pharmaceutical and agrochemical agents in agriculture and medicine.

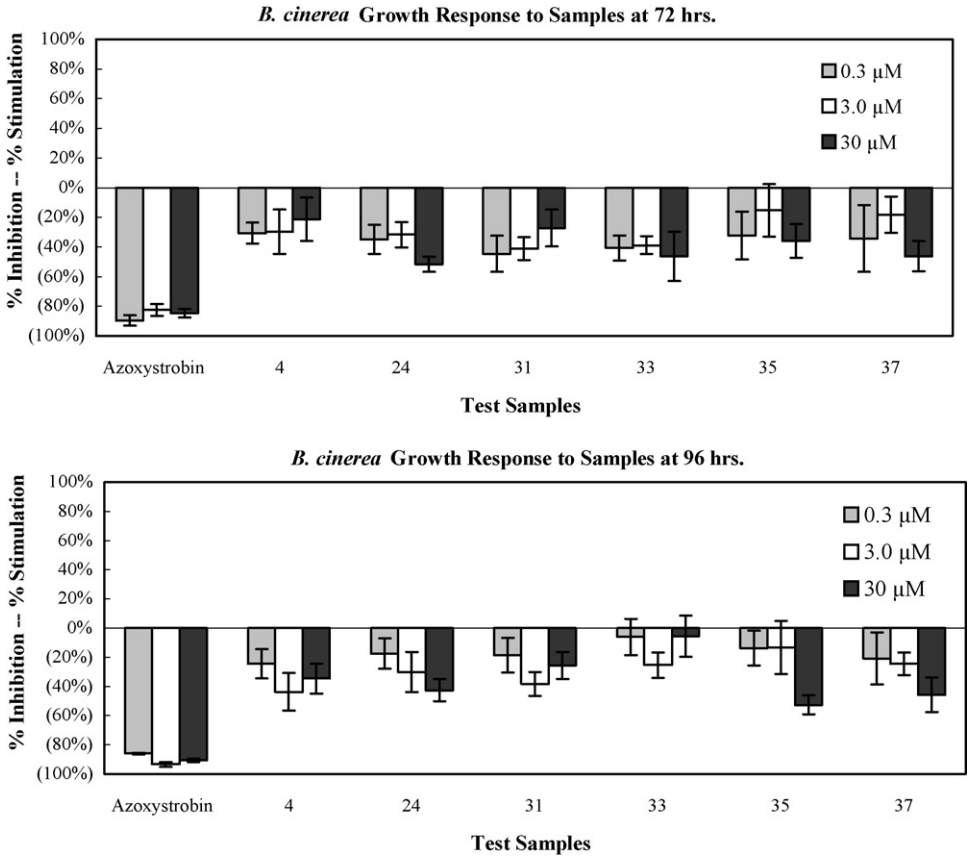


Fig. 5. Percent mean growth inhibition of compounds **4**, **24**, **31**, **33**, **35** and **37** against *Botrytis cinerea* at 72 and 96 h. The bold numbers represent the compounds in Table 1.

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