

Chemical Composition and Antifungal Activity of *Salvia macrochlamys* and *Salvia recognita* Essential Oils

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Essential oils of *Salvia macrochlamys* and *Salvia recognita* were obtained by hydrodistillation of dried aerial parts and characterized by gas chromatography and gas chromatography–mass spectrometry. One hundred and twenty identified constituents representing 97.7% in *S. macrochlamys* and 96.4% in *S. recognita* were characterized, and 1,8-cineole, borneol, and camphor were identified as major components of the essential oils. The oils were evaluated for their antimalarial, antimicrobial, and antifungal activities. Antifungal activity of the essential oils from both *Salvia* species was nonselective at inhibiting growth and development of reproductive stroma of the plant pathogens *Colletotrichum acutatum*, *Colletotrichum fragariae*, and *Colletotrichum gloeosporioides*. *S. macrochlamys* oil had good antimycobacterial activity against *Mycobacterium intracellulare*; however, the oils showed no antimicrobial activity against human pathogenic bacteria or fungi up to a concentration of 200 µg/mL. *S. recognita* oil exhibited a weak antimalarial activity against *Plasmodium falciparum*.

KEYWORDS: *Salvia macrochlamys*; *Salvia recognita*; essential oil; GC-MS; 1,8-cineole; borneol; camphor; antimalarial activity; antimicrobial activity; *Colletotrichum acutatum*; *Colletotrichum fragariae*; *Colletotrichum gloeosporioides*

INTRODUCTION

The United States is the world's largest producer of strawberries, accounting for over a fourth of the total world production annually over the past 15 years. Strawberries rank as the fifth most consumed fresh fruit in the United States after bananas, apples, oranges, and grapes (1). Strawberry anthracnose, caused by the plant pathogens *Colletotrichum* sp., is one of the most important diseases affecting strawberries worldwide (2). Lesions can occur on all parts of the plant, including the crown, fruit, stolon, petiole, leaf, flower, and root. Losses caused by fruit spot are particularly damaging economically. New approaches to anthracnose disease control are necessary as the efficacy and availability of commercial fungicides decrease. Therefore, discovering new natural product based fungicides with low environmental and mammalian toxicity is especially important. Several sensitive detection systems are used for the evaluation of natural product based antifungal agents. Bioautography assays

using *Colletotrichum* as the indicator species are used routinely to identify antifungal components from plant extracts, essential oils, and other natural sources. Their activity and selectivity toward three agronomically important *Colletotrichum* species are reported (2–6).

The genus *Salvia* (Lamiaceae) is represented in Turkey by 89 species, 45 of which are endemic (7). Several species of *Salvia* have been used in Turkish folk medicine as antiseptic, antibacterial, diuretic, spasmolytic, stomachic, and carminative agents (7–9). *Salvia* plants and their essential oils are of economical importance worldwide in food, flavoring agents, perfumery, and cosmetics (9–11). Several *Salvia* species are known locally as “adaçayi” where they grow in southern and western Turkey and are consumed as hot teas due to their unique flavor, pleasant aroma, and medicinal properties (7–9). The oil of *Salvia fruticosa* Miller is traditionally used as a carminative, stomachic, diaphoretic, and diuretic by mixing 3–5 drops with 75 mL of water per day internally and by applying on the abdomen externally (8). As previous work, five known triterpenoids from the aerial parts and seven diterpenoids, three being new, from the roots of *Salvia recognita* were isolated (9, 12).

In our continuing research with Turkish medicinal and aromatic plants, we investigated the essential oils of *Salvia*

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macrochlamys Boiss. & Kotschy ex Boiss and *S. recognita* Fisch. & Mey. and their biological activities. To the best of our knowledge, we report for the first time the composition and biological activity of the essential oils of *S. macrochlamys* and *S. recognita*.

MATERIALS AND METHODS

General. Pure essential oil compounds (borneol, camphor, and 1,8-cineole) (>98%, Aldrich-Sigma, St. Louis, MO) and fungicide technical grade standards benomyl, cyprodinil, azoxystrobin, and captan (Chem Service, Inc., West Chester, PA) were purchased from commercial sources.

Plant Material. The aerial plant parts of *S. macrochlamys* (Sm) were collected in July 2001 from Sirnak, Senova, and Hakkari in southeastern Turkey along roadsides with rocky soil formations at 1400 m elevation. *S. recognita* (Sr) was collected from Alidag mountain at 1700 m in Kayseri, central Turkey. Voucher specimens of *S. macrochlamys* (GAZI 8172) were deposited at GAZI, Gazi University, Ankara, Turkey, and *S. recognita* (ESSE 13964) was deposited at the Faculty of Pharmacy Herbarium, Anadolu University, Eskisehir, Turkey.

Chromatographic Conditions. Two-dimensional thin-layer chromatography (2D-TLC) analysis was conducted by using precoated silica gel 60 F₂₅₄ (Merck, Suwanee, GA). Four microliters of 20 mg/mL essential oil was applied to form a single small spot in the left corner 1 cm from either of two sides of the TLC plate. TLC plates were placed in a presaturated solvent chamber (*n*-hexane/EtOAc, 95:5), and the spot was allowed to develop along the left-hand side of the plate. After solvent migration, the TLC plates were air-dried and subsequently placed at 90° (horizontally) to the first direction in a second presaturated solvent chamber containing (*n*-hexane/Et₂O, 9:1) and allowed to develop. Each plate was allowed to develop until the solvent front reached 1 cm from the top of the plate. All plates were inspected under UV light visualization with vanillin/H₂SO₄ (1 g of vanillin in 100 mL of 20% H₂SO₄ in ETOH). One plate was heated and prepared as a reference plate, and the other plates were subjected to 2D-TLC (4).

Optimum performance laminar chromatography (OPLC), using silica gel 60 F₂₅₄ 10 × 20 cm on an aluminum sheet (BSLA 012, Bionisis Inc., Le Plessis-Robinson, France) overpressured layer chromatography (Personal OPLC; OPLC-NIT Ltd, Budapest, Hungary), using silica gel 60 F₂₅₄ 10 × 20 cm on aluminium sheet (LG011, OPLC-NIT Ltd, Budapest, Hungary). Four microliters of 20 mg/mL essential oils and 4 μL of 2 mM carvacrol and thymol standards were applied to form a single small spot 2 cm from the bottom corner of the plate. Double developments were applied with a two-elution protocol. The first elution was conducted with *n*-hexane/diethyl ether (92.5:7.5) with 1800 μL of solvent with an external pressure of 50 bar, start flash volume of 150 L, flow rate of 200 μL/min, and total elution time of 547 s. The second elution was conducted using 1800 μL of pure *n*-hexane using conditions described above. Visualization was achieved by vanillin/sulfuric acid reagent and heat. Multiple plates for direct bioautography were developed sequentially using the protocol described above. Both 2D-TLC and double-developed OPLC plates were subjected to our bioautography methods.

Isolation of the Essential Oils. The aerial parts of each plant were water distilled for 3 h using a Clevenger-type apparatus to obtain essential oils (13). *Salvia* oils were then calculated on a moisture-free basis at 0.15% for *S. macrochlamys* and 0.33% for *S. recognita*.

Gas Chromatography (GC) Analysis Conditions. Oils were analyzed by GC using a Hewlett-Packard (SEM Ltd., Istanbul, Turkey) 6890 system equipped with a flame ionization detector (FID). An HP Innovax FSC column (60 m × 0.25 mm i.d., 0.25 μm film thickness) was used with nitrogen at 1 mL/min. The oven temperature was 60 °C for 10 min, increased to 220 °C at 4 °C/min, then held at 220 °C for 10 min, and increased to 240 °C at 1 °C/min. The injector temperature was 250 °C. Percentage composition of individual components was obtained from electronic integration using flame ionization detection (FID, 250 °C). *n*-Alkanes (C₉–C₂₀) were used as reference points in the calculation of retention indices (RI) (14–16). Relative percentages of the characterized *Salvia* components are cited in **Table 1**.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis Conditions. GC-MS analysis was performed with a Hewlett-Packard GCD system (SEM Ltd.), and an Innovax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) was used with helium. GC oven temperature conditions were as described above, split flow was adjusted at 50 mL/min, and the injector temperature was at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 425.

Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RI) to a series of *n*-alkanes. Computer matching against commercial (Wiley and MassFinder 2.1) (17, 18) and in-house (Baser Library of Essential Oil Constituents) libraries, built by genuine compounds and components of known oils, as well as MS literature data (19–22), was also used for the identification.

Antimalarial Assay. The in vitro antimalarial activity was determined against D6 (chloroquine sensitive) and W2 (chloroquine resistant) strains of *Plasmodium falciparum*. The assay was based on the determination of parasite LDH activity using Malstat reagent (23). Chloroquine (Aldrich-Sigma) and artemisinin (Aldrich-Sigma) are included as control drugs in each assay.

Antimicrobial Assay. All organisms were obtained from the American Type Culture Collection (Manassas, VA) and include *Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 90113, *Aspergillus fumigatus* ATCC 90906, *Staphylococcus aureus* ATCC 29213, methicillin-resistant *S. aureus* ATCC 43300 (MRS), *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068. Susceptibility testing was performed using a modified version of the NCCLS methods (24–27) and modified Alamar Blue procedure for *M. intracellulare* (28). Assay was performed according to a previous procedure (29).

Bioautography. The bioautography procedures of Meeza et al. (2) and Tabanca et al. (30) for the detection of naturally occurring antifungal agents were used to evaluate antifungal activity of *Salvia* essential oils to *Colletotrichum fragariae*, *Colletotrichum acutatum*, and *Colletotrichum gloeosporioides*. The sensitivity of each fungal species to each test compound was determined by comparing the sizes of the inhibitory zones. Means and standard deviations of inhibitory zone size were used to evaluate antifungal activity of essential oils, solvent fractions, and pure 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.) were used as controls at 2 mM in 2 μL of EtOH. Technical grade borneol, camphor, and 1,8-cineole (Aldrich-Sigma) standards were used at 2 mM in 4 μL of acetone for comparison.

RESULTS AND DISCUSSION

Water-distilled essential oils from aerial parts of *S. macrochlamys* and *S. recognita* were analyzed by GC and GC-MS systems. The components identified are given in **Table 1** with their relative percentages. Eighty compounds were characterized in the oil of *S. macrochlamys*, making up of 97.7% of the oil. Eighty-two compounds were identified in the oil of *S. recognita*, representing 96.4% of the oil. The oil of *S. macrochlamys* was characterized with 1,8-cineole (27%), borneol (13%), and camphor (11%) as major constituents. Major components described in the oil of *S. recognita* were camphor (42%) and 1,8-cineole (12%). Together with 1,8-cineole, borneol and camphor have been reported in many *Salvia* oils, for instance in *S. fruticosa* Miller, *S. tomentosa* Miller, *S. pomifera* L., *S. willeana* (Holmboe) Hedge, and *S. officinalis* L. (31). Essential oils of the *Salvia* species of Turkey have been reviewed (32). According to a listing given in that paper, *S. recognita* oil falls into the camphor/1,8-cineole (CaCi) type, whereas that of *S. macrochlamys* can be classified as 1,8-cineole/camphor type.

Borneol has been reported to have antibacterial, antifungal, and antispasmodic effects (33). 1,8-Cineole, the major constituent of *Eucalyptus* essential oils, is used as a fragrance in

Table 1. Composition of Essential Oils of *S. macrochlamys* (Sm) and *S. recognita* (Sr)

RI ^a	compound	Sm % ^b	Sr % ^b	ID method ^c	RI ^a	compound	Sm % ^b	Sr % ^b	ID method ^c
1014	tricyclene	0.3	0.3	b	1719	borneol	13.0	4.6	a, b
1032	α -pinene	2.9	4.4	a, b	1726	germacrene D	0.1	0.2	b
1035	α -thujene	tr		b	1737	(<i>Z,E</i>)- α -farnesene	0.1		b
1076	camphene	6.6	6.6	a, b	1740	α -muurolene		0.3	b
1118	β -pinene	3.5	1.4	a, b	1740	valencene		0.2	b
1132	sabinene	2.1		a, b	1741	β -bisabolene	0.1		b
1174	myrcene	0.6	0.4	a, b	1748	piperitone		tr	a, b
1188	α -terpinene	0.1		a, b	1755	bicyclogermacrene	0.2	0.3	b
1194	heptanal	0.1		b	1758	(<i>E,E</i>)- α -farnesene	0.2		b
1195	dehydro-1,8-cineole		0.1	b	1766	decanol	0.1	0.6	b
1203	limonene	1.5	2.1	a, b	1773	δ -cadinene		0.2	b
1213	1,8-cineole	26.9	11.5	a, b	1776	γ -cadinene		0.1	b
1225	(<i>Z</i>)-3-hexenal		0.1	b	1783	β -sesquiphellandrene	0.1		b
1255	γ -terpinene	0.4		a, b	1804	myrtenol	0.1	0.2	b
1280	<i>p</i> -cymene	2.5	1.0	a, b	1805	α -campholene alcohol	0.1		b
1290	terpinolene	0.1		a, b	1845	<i>trans</i> -carveol	0.1		a, b
1304	1-octen-3-one		0.1	b	1849	calamenene ^d		0.1	b
1360	hexanol	0.1		b	1854	germacrene-B	tr		b
1384	α -pinene oxide		0.1	a, b	1864	<i>p</i> -cymen-8-ol	0.2	0.2	a, b
1391	(<i>Z</i>)-3-hexenol	0.2		b	1941	α -calacorene		0.1	b
1393	3-octanol		0.1	b	1969	<i>cis</i> -jasmone	tr		b
1400	nonanal	tr	tr	b	1984	(<i>E</i>)-12-norcaryophyll-5-en-2-one		0.1	b
1450	<i>trans</i> -linalool oxide (furanoid)		tr	b	2001	isocaryophyllene oxide	1.0		a, b
1451	β -thujone		1.7	a, b	2008	caryophyllene oxide	8.0	1.6	a, b
1452	α , <i>p</i> -dimethylstyrene	tr		b	2030	methyleugenol	0.1	0.4	a, b
1452	1-octen-3-ol	0.1	tr	b	2071	humulene epoxide II	0.3	0.1	b
1474	<i>trans</i> -sabinene hydrate	1.0	tr	b	2074	caryophylla-2(12),6(13)-dien-5-one	tr		b
1478	<i>cis</i> -linalool oxide (furanoid)		0.1	b	2080	cubanol	tr	0.1	b
1479	δ -elemene	0.4		b	2088	1- <i>epi</i> -cubanol		0.1	b
1483	octyl acetate		0.2	b	2098	globulol		0.3	b
1493	α -ylangene		0.2	b	2103	guaiol	tr		b
1497	α -copaene		0.2	b	2104	viridiflorol		0.1	b
1499	α -campholene aldehyde	0.1		b	2113	cumin alcohol	tr		a, b
1532	camphor	10.9	42.0	a, b	2131	hexahydrofarnesyl acetone	0.1		b
1541	benzaldehyde	0.1		a, b	2144	rosifolol		0.1	b
1547	octyl isobutyrate		0.1	b	2144	spathulenol	0.4	0.7	b
1553	linalool	0.1	0.5	a, b	2148	(<i>Z</i>)-3-hexen-1-yl benzoate		0.1	b
1556	<i>cis</i> -sabinene hydrate	0.9	0.1	b	2173	6- <i>epi</i> -cubanol		0.6	b
1562	octanol	0.1	0.1	b	2174	cinnamyl acetate		0.1	b
1571	<i>trans</i> - <i>p</i> -menth-2-en-1-ol	0.1	0.1	b	2187	T-cadinol	0.1	0.1	b
1586	pinocarvone	0.1		a, b	2192	nonanoic acid	0.1		a, b
1588	bornyl formate	tr		b	2198	thymol	0.1	tr	a, b
1590	bornyl acetate	0.4	1.5	b	2239	carvacrol	0.2	0.8	a, b
1594	<i>trans</i> - β -bergamotene	0.1		b	2245	elemicine		0.1	b
1611	terpinen-4-ol		1.8	a, b	2250	α -eudesmol	tr		b
1612	β -caryophyllene	7.2	1.7	a, b	2255	α -cadinol		0.1	b
1628	aromadendrene		0.2	b	2256	cadalene		0.4	b
1638	<i>cis</i> - <i>p</i> -menth-2-en-1-ol	0.1	0.2	b	2257	β -eudesmol	0.1		b
1648	myrtenal	0.2	0.1	b	2260	alismol	0.7	tr	b
1650	γ -elemene	tr		b	2298	decanoic acid		tr	a, b
1661	alloaromadendrene		0.1	b	2300	γ -undecalactone	0.1		b
1663	phenylacetaldehyde	tr		b	2316	caryophylla-2(12),6(13)-dien-5 β -ol (= caryophylladienol I)	0.1	0.2	b
1668	(<i>Z</i>)- β -farnesene	tr	0.3	b					
1670	<i>trans</i> -pinocarveol	0.3	tr	a, b	2324	caryophylla-2(12),6(13)-dien-5 α -ol (= caryophylladienol II)	0.4	0.4	b
1682	δ -terpineol		0.1	b					
1683	<i>trans</i> -verbenol	0.2	0.3	b	2389	caryophylla-2(12),6-dien-5 α -ol (= caryophyllenol I)		0.2	b
1686	lavandulol			b					
1687	decyl acetate		0.5	b	2392	caryophylla-2(12),6-dien-5 β -ol (= caryophyllenol II)	0.4	0.2	b
1687	α -humulene	0.4		a, b					
1700	<i>p</i> -mentha-1,8-dien-4-ol (= limonen-4-ol)	0.2	0.2	b	2607	1-octadecanol	0.1		b
1704	γ -muurolene		0.8	b	2670	tetradecanoic acid	tr	0.1	a, b
1706	α -terpineol	0.1	0.8	a, b					
1709	α -terpinyl acetate			a, b		total %	97.7	96.4	

^a Retention indices calculated against *n*-alkanes (C₉–C₂₀). ^b Calculated from flame ionization detector data; tr, trace (<0.1%). ^c a, identification based on retention times of genuine compounds on the HP Innovax column; b, tentatively identified on the basis of computer matching of the mass spectra of peaks with the Wiley and MassFinder libraries. ^d Correct isomer not identified.

perfumes and flavoring in food (34). Viljoen et al (35) have demonstrated that 1,8-cineole and (–)-camphor have a higher antimicrobial activity against *Candida albicans* in combination than when used independently. Camphor is widely used in medicinal preparations as a local anesthetic and as a remedy

for rheumatic conditions, muscular strain, and similar inflammations (7). Camphor is a characteristic component in the essential oils of *S. officinalis* and *S. fruticosa* (31). Sage oil (*S. officinalis*) is used in food, flavoring, and pharmaceutical preparations (7, 31). Leaves of *S. fruticosa* are used in Turkey

Table 2. Antifungal Activity of *Salvia* Essential Oils Using Direct Bioautography with Three *Colletotrichum* Test Species^a

sample	mean fungal growth inhibition (mm) ± SEM		
	<i>C. acutatum</i>	<i>C. fragariae</i>	<i>C. gloeosporioides</i>
<i>S. macrochlamys</i>	9.67 ± 0.33	9.67 ± 0.33	9.33 ± 0.33
<i>S. recognita</i>	13.00 ± 0.58	13.00 ± 0.58	12.33 ± 0.33
(-)-borneol ^b	0 ± 0	0 ± 0	0 ± 0
(+)-borneol ^b	0 ± 0	0 ± 0	0 ± 0
(-)-camphor ^b	0 ± 0	0 ± 0	0 ± 0
(+)-camphor ^b	0 ± 0	0 ± 0	0 ± 0
1,8-cineole ^b	0 ± 0	0 ± 0	0 ± 0
thymol ^b	0 ± 0	0 ± 0	0 ± 0
carvacrol ^b	4.5 ± 0.35	3.4 ± 0.21	3.5 ± 0.71
benomyl ^c	18.00 ± 0.58	18.33 ± 0.33	19.33 ± 0.33
captan ^c	14.67 ± 0.033	15.00 ± 0	10.33 ± 0.33
cyprodinil ^c	29.33 ± 0.33	30.33 ± 0.33	29.33 ± 0.33
azoxystrobin ^c	24.33 ± 0.033	19.33 ± 0.33	28.33 ± 0.33

^a *Salvia* essential oils were applied as a 20 mg/mL solution in 4 μ L of sample onto a silica TLC plate. Mean inhibitory clear zones and standard errors were used to determine the level of antifungal activity against each fungal species.

^b Standards were applied at 4 μ L of 2 mM of acetone. ^c Technical grade agrochemical fungicides (without formulation) with different modes of action were used as internal standards at 2 μ L of 2 mM of ETOH.

because it is a native plant rather than the cultured *S. officinalis* (7).

Essential oils from both plants were evaluated for their antimalarial, antimicrobial, and antifungal activities. No antimicrobial activity at the highest test concentration of 200 μ g/mL was observed against *C. albicans*, *C. glabrata*, *C. krusei*, *Cryptococcus neoformans*, methicillin-resistant *Staphylococcus aureus*, *Mycobacterium intracellulare*, and *Aspergillus fumigatus* using the method previously described (29). Essential oil from *S. recognita* showed antimalarial activity against D6 (chloroquine sensitive) and W2 (chloroquine resistant) strains of *P. falciparum* with IC₅₀ values of 17 and 12 μ g/mL, respectively; however, *S. macrochlamys* did not show antimalarial activity.

Essential oils are hydrophobic and insoluble in aqueous-based antimicrobial disc diffusion and microtiter bioassays. Direct bioautography on silica gel is our preferred primary screening bioassay for lipophilic compounds as fungicides for agricultural use, and more closely mimics a leaf surface than aqueous-based assays. Direct bioautography on silica gel TLC revealed moderate antifungal activity of *S. macrochlamys* and *S. recognita* essential oils against the plant pathogens *C. acutatum*, *C. fragariae*, and *C. gloeosporioides*. Antifungal activity was evident by the presence of clear zones with a dark background where fungal mycelia or reproductive stroma were not present on the TLC plate. *Salvia* essential oils showed activity against all three *Colletotrichum* species (Table 2). This is the first report of the antifungal activity of *Salvia* oils against *C. acutatum*, *C. fragariae*, and *C. gloeosporioides*. Antifungal activity of the essential oils from both *Salvia* species was nonselective at inhibiting growth and development of reproductive stroma of *C. acutatum*, *C. fragariae*, and *C. gloeosporioides*; however, *S. recognita* was more active against all *Colletotrichum* species and demonstrated 34% more antifungal activity than *S. macrochlamys*. Essential oils from *Salvia* were less active than the systemic fungicide standards benomyl, cyprodinil, and azoxystrobin and were in general less active than the protectant fungicide captan.

Commercial standards of (+)- and (-)-borneol, (+)- and (-)-camphor, and 1,8-cineole were purchased (Aldrich-Sigma) and evaluated for activity and were determined to possess insignificant activity against all three *Colletotrichum* species.

TLC profiles of *S. recognita* and *S. macrochlamys* in *n*-hexane/diethyl ether (9:1, 8:2) were subsequently tested against *Colletotrichum* spp., and minor polar compounds appear to be responsible for antifungal activity. Antifungal essential oils were evaluated according to the 2D direct bioautography method of Wedge and Nagle (4). 2D bioautography demonstrated the presence of an antifungal inhibitory zone with nonselective antifungal activity against each of the three *Colletotrichum* species. *R_f* values of the two different *Salvia* essential oil (Sm, Sr) constituents with antifungal activity were 0.31 and 0.30, respectively. Subsequent one-dimensional OPLC of the *Salvia* oils and carvacrol and thymol standards demonstrated nonselective activity with an *R_f* value of 0.29. 2D bioautography revealed a single *R_f* value of 0.29 in both *Salvia* oils that appeared to correspond with the carvacrol and thymol *R_f* values. However, thymol was not active at the 2 mM concentration used in this assay. We determined that thymol demonstrated nonselective activity with a 3 mm inhibitory zone in each of the three *Colletotrichum* species at 4 μ L of 50 mM. Demirci et al. (36) recently reported that carvacrol had an antifungal activity against all three *Colletotrichum* species. We hypothesize that both carvacrol and thymol are active in the bioautography assay and may possess synergistic antifungal activity in the unpurified essential oil. However, when separated, the pure carvacrol and thymol demonstrate concentration-dependent antifungal activity. Discovery of new lead chemistries from essential oils from medicinal plants and aromatic herbs has revealed a number of compounds such as carvacrol and others. Because of the volatile and broad-spectrum antifungal nature, essential oils may possess novel applications as plant protectants for postharvest storage of fruits, nuts, vegetables, and other plant products.

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