Pure Appl. Chem., Vol. 79, No. 4, pp. 539–556, 2007.
doi:10.1351/pac200779040539

Recent advances in the chemistry and biological activities of the *Pimpinella* species of Turkey*

K. Husnu Can Baser[1,‡], Nurhayat Tabanca[1,3,5], Nese Kirimer[1], Erdal Bedir[2], Ikhlas A. Khan[3,4], and David E. Wedge[5]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470, Eskisehir, Turkey; [2]Department of Bioengineering, Faculty of Engineering, Ege University, Bornova, Izmir 35100, Turkey; [3]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi, University, MS 38677, USA; [4]Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677, USA; [5]USDA-ARS-NPURU, The University of Mississippi, University, MS 38677, USA

Abstract: Two new natural products incorporating a phenylpropyl subunit ("phenylpropanoids"), [4-(prop-2-enyl)phenyl angelate and 4-(3-methyloxiranyl)phenyl 2-methylbutyrate], one new bisabolene-type sesquiterpenoid [1-methyl-4-(6-methylhepta-1,5-dien-2-yl)-7-oxabicyclo[4.1.0]heptane ("aureane")], and one new trinorsesquiterpene [4-(6-methylbicyclo[4.1.0]hept-2-en-7yl)butan-2-one ("traginone")] have been isolated from the essential oils of *Pimpinella* species occurring in Turkey, together with nine known phenylpropanoids and other natural products. Six of the known compounds are newly reported for Umbelliferae, whilst one is newly reported for *Pimpinella* species. Compound structures were determined by 1D and 2D NMR analysis. Isolated compounds were evaluated for antimalarial, antimicrobial, estrogenic, and aphidicidal activities. Pure compounds were also tested for antifungal activity against strawberry anthracnose-causing fungal plant pathogens *Colletotrichum acutatum*, *C. fragariae*, and *C. gloeosporioides* using direct bioautography and microdilution broth assays.

Keywords: *Pimpinella*; essential oils; GC; GC/MS; phenylpropanoids; sesquiterpenes; monoterpenes.

INTRODUCTION

Pimpinella is a member of the Apiaceae that comprises approximately 150 species distributed in the northern hemisphere [1]. *Pimpinella* is represented in Turkey by 23 spp. (5 endemic), 2 subspecies, and 2 varieties, representing a total of 27 [2]. *Pimpinella anisum* L. (anise) fruits (aniseeds) are used for their expectorant, antispasmodic, carminative, and diuretic properties as well as a broncho-dilator in chronic bronchitis [3,4]. Aniseed is an important agricultural crop in Turkey. Turkey produces ca. 10 000 000 kg/yr of aniseed of which ca. 7 000 000 kg are used in the manufacturing of the famous

Pure Appl. Chem.* **79, 467–823 (2007). An issue of reviews and research papers based on lectures presented at the 25th International Symposium on Chemistry of Natural Products (ISCNP-25) and 5th International Conference on Biodiversity (ICOB-5), held jointly in Kyoto, Japan, 23–28 July 2006, on the theme of natural products.
‡Corresponding author: Tel.: +90-222-3350580-3631; Fax: +90-222-3350750; E-mail: khcbaser@anadolu.edu.tr

Turkish alcoholic beverage "raki" and 3 000 000 kg are exported around the world. Aniseed contains over 90 % (*E*)-anethole and is used in the production of alcoholic beverages like raki, uzo, pernod, anisette, ricard, and granier, etc. in various countries, as well as in the food and pharmaceutical industries. However, in Turkey, its use in the food industry is confined solely to raki [5]. The roots of *P. major* and *P. saxifraga* are registered in the German Pharmacopoeia as expectorant and broncho-secretory [5]. In Austria, the roots of *P. major* are sold for its antibacterial virtues [4]. *Pimpinella saxifraga* is known in Turkey as "Teke maydonozu" (goat parsley) or "tas maydonozu" (rock parsley), and its roots are used as a demulcent, stomachic, expectorant, and tonic [6]. Fresh leaves of the endemic species *P. anisetum* known as "Ezeltere" are used in Turkey locally in salads. Its fruits are used in pickling [3]. This species is locally cultivated. *Pimpinella isaurica*, *P. aurea*, and *P. corymbosa* are used as animal feed to increase milk secretion [3]. In our earlier studies of isolated compounds from several *Pimpinella* species, we reported 4 new and 18 known compounds, their chemical compositions, the genetic diversity of 26 *Pimpinella* species, and their estrogenic, antimalarial, antimicrobial, and antifungal activities [3,4,7,8]. Recently, we reported on the detailed analysis of essential oils from different plant parts of *Pimpinella* species by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) [6,9].

EXPERIMENTAL

Plant material

The samples of each *Pimpinella* species were collected from eastern and southern Turkey in June/July 2001. Collection localities are given in Table 1. Voucher specimens were placed at the Herbarium of the Faculty of Pharmacy, Anadolu University in Eskisehir, Turkey (ESSE). Botanical identifications were carried out by Prof. Dr. Zeki Aytac, Gazi University, Ankara, Turkey.

Isolation of essential oils

Air-dried fruits, stems and leaves, and roots were crushed separately using a mortar followed by water distillation for 3 h using a modified Clevenger-type apparatus to obtain essential oils [10]. The percent yields of oils calculated on a moisture-free basis are shown in Table 1. Our results were compared to previous studies [11,12].

Table 1 Essential oil yields (%) of *Pimpinella*.

Species	Fruits [11,12][a]	Ground fruit	Fruitless aerial parts	Roots
P. cretica var. *arabica*	10.0	–	–	–
P. anisetum	8.3–8.7	5.05	1.06	3.20
P. flabellifolia	7.5–8.0	1.88	0.85	1.43
P. anisum	7.0–7.5	2.49	–	–
P. aurea	4.2	5.05	0.30	0.13
P. peregrina	3.2	1.08	0.08	1.14
P. kotschyana	0.5–3.0	1.02	0.06	0.10
P. eriocarpa	3.0	–	–	–
P. corymbosa	2.8	0.26	0.15	0.23
P. rhodantha	2.0–2.6	0.41	0.06	0.10
P. tragium subsp. *pseudotragium*	2.3	1.87	0.08	0.11
P. olivieroides	2.3	0.20	0.27	0.06
P. cappadocica var. *cappadocica*	2.2	2.06	0.14	0.18
P. nudicaulis	2.0	5.10	1.29	0.31
P. affinis	1.9	2.12	0.09	0.23
P. tragium subsp. *polyclada*	1.8	0.92	0.10	0.22
P. saxifraga	–	1.67	0.32	0.17
P. peucedanifolia	1.5	0.23	0.01	0.13
P. tragium subsp. *lithophila*	1.1	0.73	0.22	0.17
P. isaurica	0.3–1.0	1.43	0.29	0.31
P. anthriscoides var. *cruciata*	0.7	–	–	–
P. puberula	–	0.32	0.23	–

[a]Reference numbers in the text.

Gas chromatography

Essential oils were analyzed by GC using a Hewlett Packard 6890 system. An HP-Innowax FSC (60 m × 0.25 mm i.d., with 0.25 μm film thickness) was used with nitrogen as the carrier gas (1 mL/min). The oven temperature was kept at 60 °C for 10 min, programmed to reach 220 °C at a rate of 4 °C/min, then kept constant at 220 °C for 10 min before proceeding to 240 °C at a rate of 1 °C/min. The split ratio was adjusted to 50:1. Flame ionization detection and injector temperature were performed at 250 °C .

Gas chromatography/mass spectrometry

Essential oils were analyzed by GC/MS using a Hewlett-Packard GCD system. An Innowax FSC column (60 m × 0.25 i.d., 0.25 μm film thickness) was used with helium as the carrier gas (1 mL/min). GC oven temperature and conditions were as described above. The injector temperature was at 250 °C. Mass spectra were recorded at 70 eV. The mass range was from *m/z* 35 to 425.

Identification of essential oil constituents

Identification of the essential oil components was carried out by a comparison of their relative retention times with those of authentic samples or by a comparison of their relative retention index (RRI) to the series of *n*-alkanes. Computer matching against commercial (Wiley and MassFinder 2.1) [13,14] libraries and in-house Baser Library of Essential Oil Constituents built up by genuine compounds and components of known oils, as well as MS literature data [15–18], was also used for the identification.

After the GC/MS analysis, some compounds were not identifiable in the Wiley GC/MS Library and the Baser Library of Essential Oil Constituents. Separation of these compounds was therefore necessary. *Pimpinella* essential oils were subjected to column chromatography (silica gel) using *n*-hexane and diethyl ether according to our published procedures [3,7,8]. Structure elucidation of the isolated compounds was achieved by a combination of 1D and 2D NMR techniques using Bruker Avance DRX 500 at 500 (^{1}H) and 125 MHz (^{13}C), Bruker DRX 400 at 400 (^{1}H) and 100 MHz (^{13}C), and Bruker DRX 300 at 300 (^{1}H) and 75 MHz (^{13}C) instruments, electrospray ionization (ESI)/MS, and the known compounds were compared with literature values [19–30].

Yeast estrogen screen

The assay was performed on 96-well plates as previously described [7].

Assay for antimalarial activity

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 lactate dehydrogenase (LDH) activity using Malstat reagent [3]. Chloroquine (Aldrich-Sigma, St. Louis, MO) and artemisinin (Aldrich-Sigma, St. Louis, MO) were included as control drugs in each assay.

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 NCCLS methods as reported previously [3]. Antimicrobial standards ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi were included as control drugs in each assay.

Direct bioautography assay

The bioautography procedures of Meazza et al. (2003) [31] and Tabanca et al. (2003) [3] 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 four days after treatment by comparing the size of inhibitory zones. Means and standard deviations of the inhibitory zone size were used to evaluate the 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.

Microdilution broth assay

A standardized 96-well microtiter plate assay developed by Wedge and Kuhajek [32] was used to evaluate the antifungal activity of isolated compounds toward *Colletotricum acutatum*, *C. fragariae*, *C. gloeosporioides*, *Fusarium oxysporum*, *Botrytis cinerea*, and *Phomopsis obscurans.* Azoxystrobin was used as a commercial fungicide standard. 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 [32]. Fungal growth was then evaluated by measuring the absorbance of each well at 620 nm using a microplate photometer (Packard Spectra Count, Packard Instrument Co., Downers Grove, IL).

RESULTS AND DISCUSSION

Essential oil yields from different parts of *Pimpinella* were variable and ranged from 0.2 to 5.1 % (ground fruits), 0.01 to 1.1 % (fruitless aerial parts), and 0.1 to 3.2 % (roots) (Table 1). Essential oils were analyzed by GC and GC/MS systems using a polar column and the reliable in-house Baser Library of Essential Oil Constituents and other libraries.

Pimpinella oils are characterized by high contents of phenylpropanoid derivatives. Kubeczka [29] has classified phenylpropanoids as two specific types, a propenylphenol-type (4-monosubstituted phenylpropanoid) and a pseudoisoeugenol-type (2,5-disubstituted phenylpropanoid). The 2-hydroxy-5-methoxy-1-(*E*)-propenylbenzene skeleton of these compounds, known as pseudoisoeugenol, is unique to *Pimpinella* [29]. Trinorsesquiterpenes (geijerenes and azulenes) were also found to be characteristic constituents in most *Pimpinella* oils.

Of the 22 isolated compounds during this investigation, two new phenylpropanoids, 4-(prop-2-enyl)phenyl angelate **1**, and 4-(3-methyloxiranyl)phenyl 2-methylbutyrate **3**, one new bisabolene-type sesquiterpenoid, 1-methyl-4-(6-methylhepta-1,5-dien-2-yl)-7-oxabicyclo[4.1.0] heptane (“aureane”) **2**, and one new trinorsesquiterpene, 4-(6-methylbicyclo[4.1.0]hept-2-en-7yl)butan-2-one (“traginone”) **4**, were identified and characterized by spectral techniques [3,8]. Six further compounds **5–10** were identified for the first time as constituents of Umbelliferae, whilst *trans*-isoosmorhizole **11**, was identified for the first time as a constituent of *Pimpinella* species. Nine known phenylpropanoids **12–20**, as well as (*E*)-anethole **21** and eugenol methyl ether **22**, were isolated and identified from different *Pimpinella* oils (Table 2, Fig. 1). The properties of known compounds were compared with previously published data [19–29], and after identification were registered in the Baser Library of Essential Oil Constituents. *Pimpinella* oils were reanalyzed by GC/MS, and those isolated compounds were detected in other *Pimpinella* oils (Table 2). Oils from four *Pimpinella* taxa (Table 2) were rich in (*E*)-anethole. *Pimpinella anisetum* and *P. anisum* fruit oils were characterized by higher contents of (*E*)-anethole (81–94 %) than the other species.

Table 2 Isolated compounds from *Pimpinella* species.

#	Compound name	Species	Fruitless aerial parts	Fruits	Roots
1	4-(Prop-2-enyl)phenyl angelate[a] $C_{14}H_{16}O_2$, RRI_{polar} 2252	*isaurica*	43.3	13.7	10.8
2	(1*R*,4*R*,6*S*)-1-Methyl-4-(6-methylhepta-1,5-dien-2-yl))-7-oxabicyclo [4.1.0] heptane = aureane[a] $C_{15}H_{24}O$, RRI_{polar} 2038 $[\alpha]_D^{25}$ +43.5 (*c* 2.7 $CHCl_3$)	*aurea* *peregrina* *tragium* ssp. *lithophila* *nudicaulis*	19.8 2.7 0.2 0.1	33.5 1.2 0.1 0.02	9.7 0.2 0.2 0.1
3	4-[(2*R*,3*R*)-3-Methyloxiranyl]phenyl (2*S*)-methylbutyrate[a] $C_{14}H_{18}O_3$, RRI_{polar} 2506 $[\alpha]_D^{25}$ +26.0 (*c* 1.0 $CHCl_3$)	*saxifraga* *aurea* *peregrina* *peucedanifolia*	0.3 0.4 – –	0.7 1.2 – –	3.7 1.9 0.3 0.9
4	4-[(1*S*,6*R*,7*S*)-6-Methyl-bicyclo[4.1.0]hept-2-en-7-yl] butan-2-one = traginone[a] $C_{12}H_{18}O$, RRI_{polar} 1881	*tragium* ssp. *lithophila* *affinis* *puberula* *tragium* ssp. *pseudotragium* *cappadocica* var. *cappadocica* *rhodantha*	4.9 4.5 0.2 – – –	1.4 0.8 – – – –	0.8 0.6 1.2 0.9 0.1 0.1
5	4-(1-Prop-(1*E*)-enyl)phenyl (2*S*)-methylbutyrate = anethol 2-methylbutyrate[b] $C_{14}H_{18}O_2$, RRI_{polar} 2284	*corymbosa* *olivieroides* *kotschyana* *peucadanifolia* *saxifraga* *peregrina* *tragium* ssp. *lithophila* *tragium* ssp. *pseudotragium*	0.4 – 0.3 3.1 1.3 2.1 – 0.2	– 0.1 0.1 2.1 1.5 0.3 0.2 0.2	33.4 39.0 34.3 5.6 3.3 0.1 – –
6	Dictamnol[b] $C_{12}H_{18}O$, RRI_{polar} 2170 $[\alpha]_D^{25}$ +53.3 (*c* 0.3 $CHCl_3$)	*tragium* ssp. *lithophila* *affinis* *puberula* *anisetum* *kotschyana* *rhodantha* *peucadanifolia* *tragium* ssp. *pseudotragium* *saxifraga* *cappadocica* var. *cappadocica*	6.1 4.2 0.6 0.2 0.1 0.4 0.3 – – –	– 1.8 – – 0.04 – – 0.6 0.2 0.1	– 1.2 2.8 0.5 0.3 0.2 – 0.6 0.2 0.1
7	4,6-Guaiadiene[b] $C_{15}H_{24}$, RRI_{polar} 1711 $[\alpha]_D^{25}$ –46.6 (*c* 0.6 $CHCl_3$)	*tragium* ssp. *lithophila* *kotschyana* *corymbosa* *peregrina*	0.3 2.4 – 0.1	– 2.1 0.6 –	7.2 0.6 – –
8	4-(Prop-(1*E*)-enyl)phenyl isobutyrate[b] $C_{13}H_{16}O_2$, RRI_{polar} 2182	*peucadanifolia* *kotschyana* *corymbosa*	0.1 0.3 –	1.8 0.3 –	– 0.8 0.5
9	Alismol[b] $C_{15}H_{24}O$, RRI_{polar} 2272 $[\alpha]_D^{25}$ +8.0 (*c* 0.5 $CHCl_3$)	*peucadanifolia* *rhodantha* *isaurica*	– 1.4 –	0.3 – 0.4	– 0.7 –
10	12-Hydroxy-β-caryophyllene acetate[b] $C_{17}H_{26}O_2$, RRI_{polar} 2331 $[\alpha]_D^{25}$ –30.0 (*c* 0.8 $CHCl_3$)	*kotschyana* *corymbosa*	4.6 3.1	11.5 4.9	0.03 0.04

(*continues on next page*)

Table 2 (*Continued*).

#	Compound name	Species	Fruitless aerial parts	Fruits	Roots
11	*trans*-Isoosmorhizole[c] =	*nudicaulis*	12.2	20.6	78.9
	2,4-dimethoxy propenylbenzene	*flabellifolia*	0.1	–	2.0
	$C_{11}H_{14}O_2$, RRI_{polar} 2212	*tragium* ssp. *pseudotragium*	0.3	0.6	0.8
		peregrina	0.3	–	–
		saxifraga	0.1	–	–
12	4-(Prop-(1*E*)-enyl)phenyl tiglate[d] = anol tiglate	*isaurica*	12.6	1.4	16.1
	$C_{14}H_{16}O_2$, RRI_{polar} 2406	*aurea*	1.2	2.5	1.6
		tragium ssp. *pseudotragium*	0.4	0.8	0.5
		cappadocica var. *cappadocica*	–	–	0.1
13	4-[(2*R*,3*R*)-3-Methyloxiranyl]phenyl tiglate[d]	*aurea*	–	0.3	–
	$C_{14}H_{16}O_3$, RRI_{polar} 2642				
	$[\alpha]_D^{25}$ +16.6 (*c* 0.5 $CHCl_3$)				
14	4-Methoxy-2-(prop-(1*E*)-enyl)phenyl angelate[d]	*isaurica*	0.5	–	0.2
	$C_{15}H_{18}O_3$, RRI_{polar} 2658	*anisetum*	0.2	0.2	–
15	4-Methoxy-2-[(2*R*,3*R*)-3-methyloxiranyl]phenyl	*isaurica*	0.3	–	2.0
	tiglate[d]	*tragium* ssp. *polyclada*	4.7	5.7	12.1
	$C_{15}H_{18}O_4$, RRI_{polar} 2926	*tragium* ssp. *pseudotragium*	–	–	0.3
	$[\alpha]_D^{25}$ +29.4 (*c* 0.2 $CHCl_3$)	*peregrina*	0.6	–	0.04
		anisetum	0.3	–	0.1
		affinis	–	–	0.1
		aurea	–	–	0.2
		cappadocica var. *cappadocica*	–	–	1.3
		olivieroides	–	–	0.5
16	4-Methoxy-2-[(2*R*,3*R*)-3-methyloxiranyl]phenyl	*corymbosa*	0.1	–	42.8
	(2*S*)-methylbutyrate[d]	*anisetum*	23.6	–	56.4
	= epoxypseudoisoeugenyl 2-methylbutyrate	*aurea*	–	–	39.0
	$C_{15}H_{20}O_4$, RRI_{polar} 2698	*kotschyana*	–	–	35.5
	$[\alpha]_D^{25}$ +26.0 (*c* 1.0 $CHCl_3$)	*olivieroides*	0.5	0.03	32.6
		peucedanifolia	0.8	–	82.6
		saxifraga	0.6	0.2	66.6
		cappadocica var. *cappadocica*	0.6	0.1	43.0
		tragium ssp. *polyclada*	21.7	20.0	16.0
		tragium ssp. *pseudotragium*	5.7	10.0	18.6
		tragium ssp. *lithophila*	3.8	2.5	1.5
		affinis	0.2	–	2.3
		rhodantha	0.5	–	0.2
		puberula	0.1	–	–
17	4-Methoxy-2-[(2*R*,3*S*)-3-methyloxiranyl]phenyl	*peregrina*	5.5	3.7	44.8
	isobutyrate[d]	*peucedanifolia*	–	–	2.4
	$C_{14}H_{18}O_4$, RRI_{polar} 2613	*kotschaya*	–	–	0.7
	$[\alpha]_D^{25}$ +29.4 (*c* 1.0 $CHCl_3$)				
18	4-Methoxy-2-(prop (1*E*)-enyl)phenyl tiglate	*rhodantha*	0.5	0.2	1.6
	$C_{15}H_{18}O_3$, RRI_{polar} 2766	*tragium* ssp. *polyclada*	0.5	–	0.4

(*continues on next page*)

Table 2 (*Continued*).

#	Compound name	Species	Fruitless aerial parts	Fruits	Roots
19	4-Methoxy-2-(prop-(1*E*)-enyl)phenyl (2*S*)-methylbutyrate[d] = pseudoisoeugenyl 2-methylbutyrate $C_{15}H_{20}O_3$, RRI_{polar} 2567 $[\alpha]_D^{25}$ +22.0 (*c* 1.0 $CHCl_3$)	*anisum*	–	0.7	–
		aurea	0.1	–	4.4
		saxifraga	–	–	1.8
		peregrina	–	–	0.8
		cappadocica var. *cappadocica*	–	–	0.5
		olivieroides	–	–	0.2
		kotschyana	–	–	0.1
		tragium ssp. *lithophila*	–	0.1	0.6
		tragium ssp. *polyclada*	1.2	0.7	0.01
		tragium ssp. *pseudotragium*	0.3	0.5	1.0
20	4-Methoxy-2-[(2*R*,3*R*)-3-methyloxiranyl]phenyl angelate[d] $C_{15}H_{18}O_4$, RRI_{polar} 2825 $[\alpha]_D^{25}$ +22.0 (*c* 0.5 $CHCl_3$)	*isaurica*	3.4	0.1	6.8
		tragium ssp. *polyclada*	1.5	0.5	39.9
		tragium ssp. *pseudotragium*	0.2	0.8	30.7
		rhodantha	2.5	0.8	29.1
		affinis	0.2	–	11.2
		anisetum	5.1	–	8.0
		cappadocica var. *cappadocica*	–	–	2.9
		peregrina	8.1	–	0.1
21	(*E*)-anethole $C_{10}H_{12}O$, RRI_{polar} 1845	*affinis*	<0.1	–	<0.1
		anisum	N	94.2	N
		anisetum	54.2	80.7	5.1
		aurea	0.2	0.1	0.2
		cappadocica var. *cappadocica*	1.2	0.2	1.3
		corymbosa	0.1	0.1	0.1
		flabellifolia	41.0	63.6	67.9
		isaurica	0.2	–	0.1
		kotschyana	0.1	–	0.7
		nudicaulis	27.8	63.5	12.5
		tragium ssp. *lithophila*	0.1	0.1	–
		tragium ssp. *polyclada*	0.1	<0.1	–
		tragium ssp. *pseudotragium*	0.3	–	0.2
		peucedanifolia	0.7	–	0.1
		peregrina	0.4	0.3	0.3
		rhodantha	0.2	0.1	0.4
		saxifraga	<0.1	–	–
22	Eugenol methyl ether $C_{11}H_{14}O_2$, RRI_{polar} 2030	*corymbosa*	0.1	–	–
		olivieroides	51.6	70.6	1.2
		puberula	23.1	29.6	N
		rhodantha	–	0.2	–
		saxifraga	<0.1	<0.1	–

[a]New compounds for nature.
[b]New compounds for Umbelliferae.
[c]New compounds for *Pimpinella.*
[d]Known compounds for *Pimpinella.*
RRI: retention indices on an innowax column calculated against *n*-alkanes (C9–C20).
1–22 represent the compounds in the text.
N: Not collected.

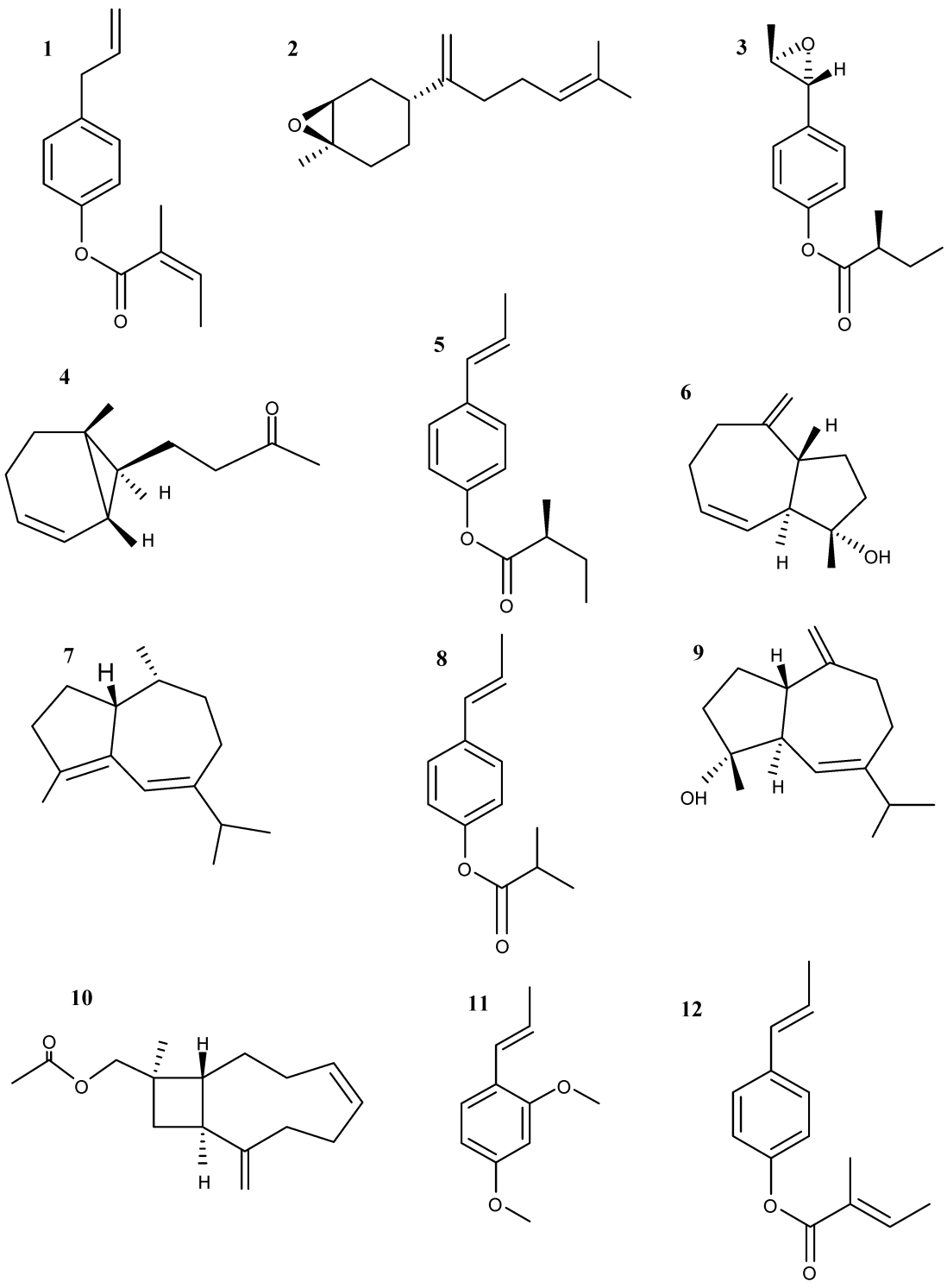


Fig. 1 Isolated compounds from *Pimpinella*. Bold numbers represent the compounds in Table 2.

(*continues on next page*)

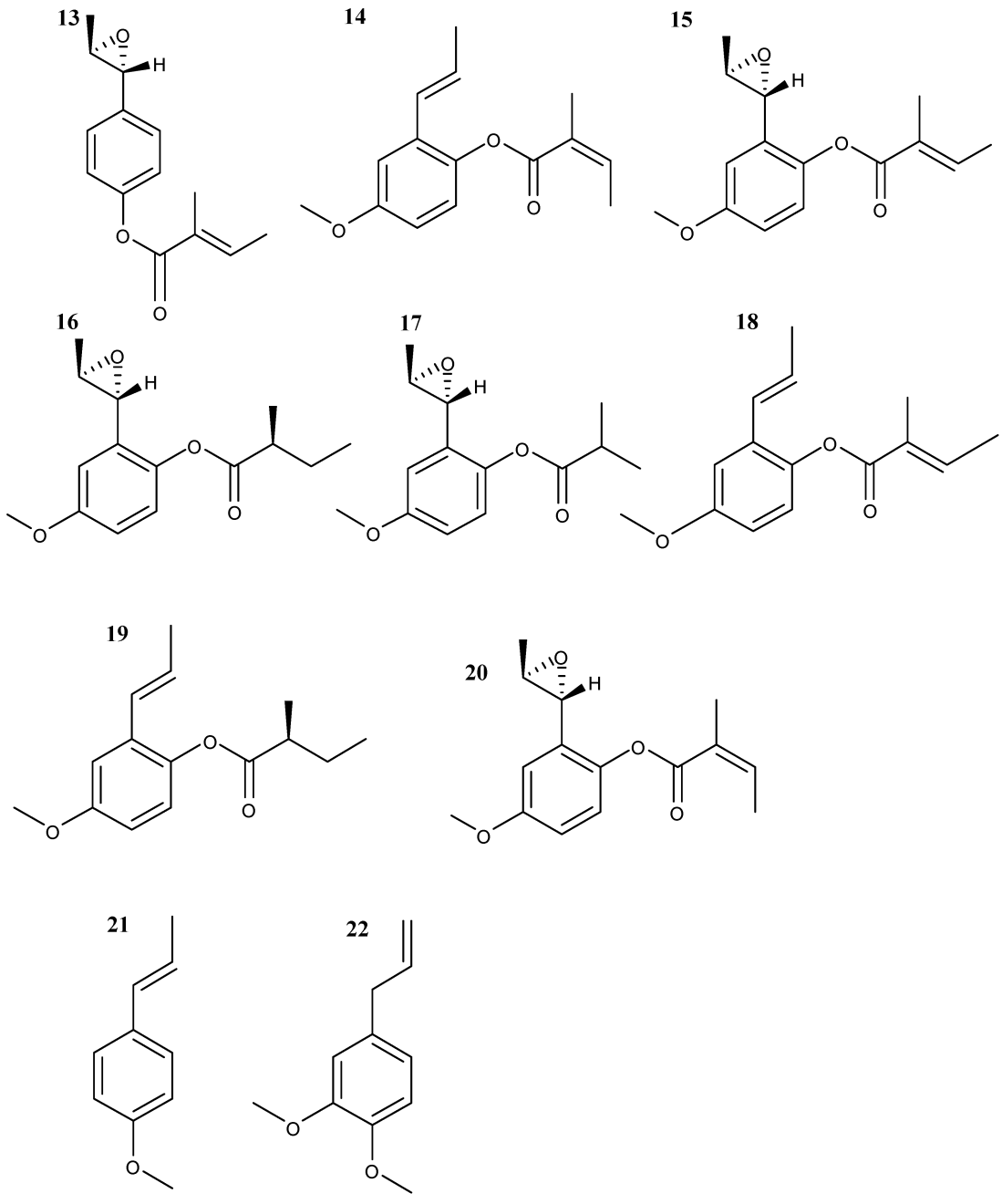


Fig. 1 (*Continued*).

Numerous compounds and 30 essential oils were evaluated for estrogenic activity using the YES assay. Of the pure compounds, only (*E*)-anethole **21** showed estrogenic activity with an EC_{50} of 625 μg/mL, relative estrogenic potency of 8.6×10^{-8} compared to 17β-estradiol. Dose–response curves of representative oils and anethole are shown in Fig. 2 along with the dose curve of 17β-estradiol. The highest activity among the oils was observed with fruitless aerial parts of *P. peucedanifolia* (EC_{50} =

45 μg/mL) followed by the root of *P. nudicaulis* (EC_{50} = 80 μg/mL) and fruit of *P. peucedanifolia* (EC_{50} = 130 μg/mL) (Table 3). It was noteworthy that fruit of *P. isaurica* and *P. peucedanifolia* have none or trace amounts of anethole, but they were estrogenic. The study indicates that the estrogenic activity of *Pimpinella* oils is not solely due to the presence of anethole. Components other than anethole may be responsible for contributing toward estrogenic activity.

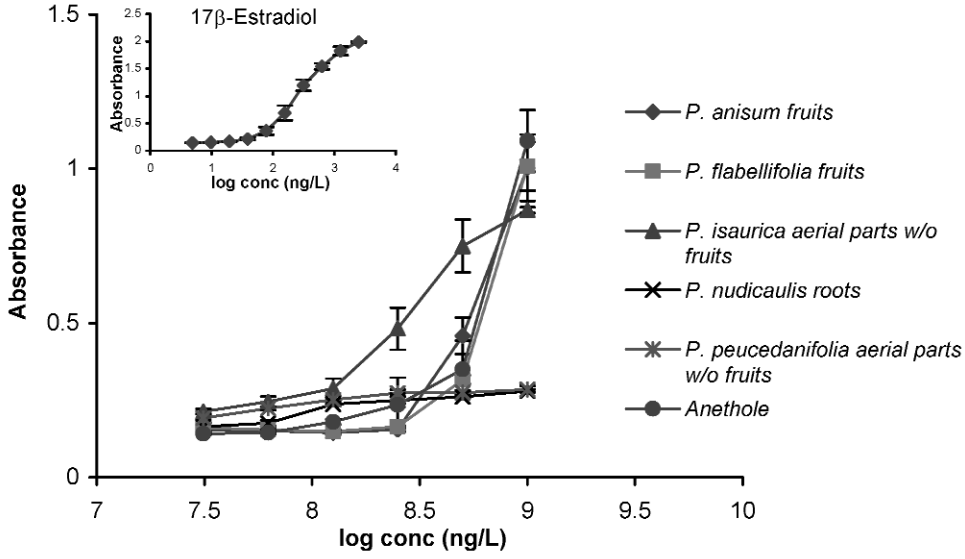


Fig. 2 Dose–response curves of representative oils and the standard curve for 17β-estradiol.

Table 3 Estrogenic activity of essential oils of *Pimpinella* species.

Species	Plant part	EC_{50} (μg/mL)	Relative potency	Percent maximal response
P. anisetum	fruits	600	9.0×10^{-8}	31.2
P. anisum	fruits	570	9.47×10^{-8}	50.1
P. flabellifolia	fruits	650	8.3×10^{-8}	50.4
	roots	425	1.27×10^{-7}	30.7
P. isaurica	fruits	250	2.16×10^{-7}	33.3
	fruitless aerial part	250	2.08×10^{-7}	43.5
P. nudicaulis	fruits	250	2.16×10^{-7}	44.0
	fruitless aerial part	310	1.74×10^{-7}	18.7
	roots	80	6.75×10^{-7}	14.4
P. peucedanifolia	fruits	130	4.15×10^{-7}	12.6
	fruitless aerial part	45	1.2×10^{-6}	13.8
Anethole		625	8.6×10^{-8}	54.5
17β-estradiol		5.44×10^{-5}	1	100

EC_{50} = The concentration that produces 50 % maximal response.
Relative potency = EC_{50} of estradiol/EC_{50} of sample.
Percent maximal response = 100 (maximum absorbance of sample/maximum absorbance of estradiol).

Compounds **1–22** have been evaluated for their antimicrobial and antimalarial activities. The antimycobacterial activity results indicated that compounds **1–3**, **12**, **13**, **16**, and **20** showed growth inhibition activity against *M. intracellulare* with IC_{50} values of 7.0, 10.0, 0.65, 15.0, 0.30, 1.5, and 2.5 μg/mL, respectively. The MIC values ranged from 1.25 to 20 μg/mL as shown in Table 4. Compound **16** showed mild activity against *A. fumigatus* with an active concentration of 50.0 μg/mL. Compounds **1** and **12** showed mild antifungal activity against *C. neoformans* (IC_{50} = 40 and 25 μg/mL, respectively). Since compounds **3**, **13**, and **16** showed potent activity against *M. intracellulare*, we also tested them against other species of *Mycobacteria* (Table 4). The results of the antimalarial screening showed that only compounds **1** and **16** demonstrated moderate activity against *P. falciparum* D6 (IC_{50} = 2.2 and 3.0 μg/mL) and W2 clone (IC_{50} = 1.8 and 1.3 μg/mL) (Table 5).

Table 4 Antimycobacterial activity.

Compound	IC_{50} (μg/mL)	MIC
4-Methoxy-2-(3-methyloxiranyl)phenyl 2-methylbutyrate[a] (**16**)	1.5	3.1
4-Methoxy-2-(3-methyloxiranyl)phenyl angelate (**20**)	2.5	5.0
4-(Prop-2-enyl)phenyl angelate (**1**)	7.0	10.0
4-(Prop-1-enyl)phenyl tiglate (**12**)	15.0	20.0
Aureane (**2**)	10.0	20.0
4-(3-Methyloxiranyl)phenyl 2-methylbutyrate (**3**)	0.65	2.5
4-(3-Methyloxiranyl)phenyl tiglate (**13**)	0.30	1.25
Ciprofloxacin[b]	0.25	1.25
Streptomycin[b]	1.5	5.0

[a]Also active against *M. fortuitum* (IC_{50}: 3.0, MIC: 6.25); *M. aurum* (IC_{50}: 1.5, MIC: 3.1); *M. phlei* (IC_{50}: 0.85, MIC: 1.56). IC_{50} = The concentration that affords 50 % growth inhibition. MIC = minimum inhibitory concentration.
[b]Positive controls.
The numbers represent the compounds in Table 2.

Table 5 Antimalarial activity (*Plasmodium falciparum*).

Compound	D6 clone IC_{50} (μg/mL)	W2 clone IC_{50} (μg/mL)
4-Methoxy-2-(3-methyloxiranyl)phenyl 2-methylbutyrate (**16**)	3.0	1.3
4-(Prop-2-enyl)phenylangelate (**1**)	2.2	1.8
Chloroquine[a]	0.020	–
Artemisinin[a]	0.006	0.007

[a]Positive controls
The numbers represent the compounds in Table 2.

Isolated compounds were also evaluated for their antifungal activities against three plant pathogenic *Colletotrichum* species using direct bioautography. Compounds **3** and **16** showed activity against *C. acutatum*, *C. fragariae*, and *C. gloesporioides* (Table 6). Compounds **3**, **4**, **6**, and **16** were subsequently evaluated in a 96-well microdilution broth assay against *P. obscurans*, *F. oxysporum*, *B. cinerea*, and the three *Colletotrichum* species. Compound **16** appeared to be the most active antifungal compound across species with a therapeutic threshold occurring about at 3.0 μM for *P. obscurans* and *C. fragariae* (Figs. 4 and 5). At 30.0 μM, **16** showed weak antifungal activity and produced 40.0 % growth inhibition in *B. cinerea*, 54.1 % in *C. acutatum*, and 73.7 % in *C. gloeosporioides* at 48 h

(Figs. 3–5). At 30.0 μM, **3** showed almost 100 % growth inhibition of all test organisms except *F. oxysporium* (65.1 %).

Table 6 Antifungal activity of isolated compounds using direct bioautography with three *Colletotrichum* test species.

Compound	Mean fungal growth inhibition (mm)					
	C. acutatum		*C. fragariae*		*C. gloeosporoides*	
	2 μg	4 μg	2 μg	4 μg	2 μg	4 μg
4-Methoxy-2-(3-methyloxiranyl)phenyl 2-methylbutyrate (**16**)	8	11	8	15.5	8	11.5
4-(3-Methyloxiranyl)phenyl 2-methylbutyrate (**3**)	8	11	10	18.5	7	10
Benomyl[a]	19.7	NT	19.7	NT	20.2	NT
Captan[a]	14.7	NT	14.7	NT	9.6	NT
Cyprodinil[a]	30.3	NT	30.8	NT	30.3	NT
Azoxystrobin[a]	24.8	NT	27.7	NT	30.3	NT

[a]Technical-grade agrochemical fungicides (without formulation) with different modes of action were used as internal standards.
NT: not tested.
The numbers represent the compounds in Table 2.

Pimpinella anisum and *P. isaurica* oils rich in phenylpropanoids were tested for aphidicidal activity and showed moderate activity against turnip aphids (*Lipaphis pseudobrassicae*) [33]. The fruitless aerial parts of *P. isaurica* demonstrated better activity than the isolated phenylpropanoids [4-(2-propenyl)phenylangelate **1**, 4-(1-propenyl)phenyltiglate **12**, and 4-methoxy-2-(1-propenyl)phenylangelate **14**] and the combination of phenylpropanoids was more active than the individual compounds. It implies that we still need to find an even rarer, but critical initiator or activator compound.

Our results indicate that phenylpropanoids and C_{12}-compounds are useful chemotaxonomic markers in separating closely related species in the genus *Pimpinella.* Since phenylpropanoids have a unique structure and biological activity, they may have potential applications as novel pharmaceutical and agrochemical agents in agriculture and medicine.

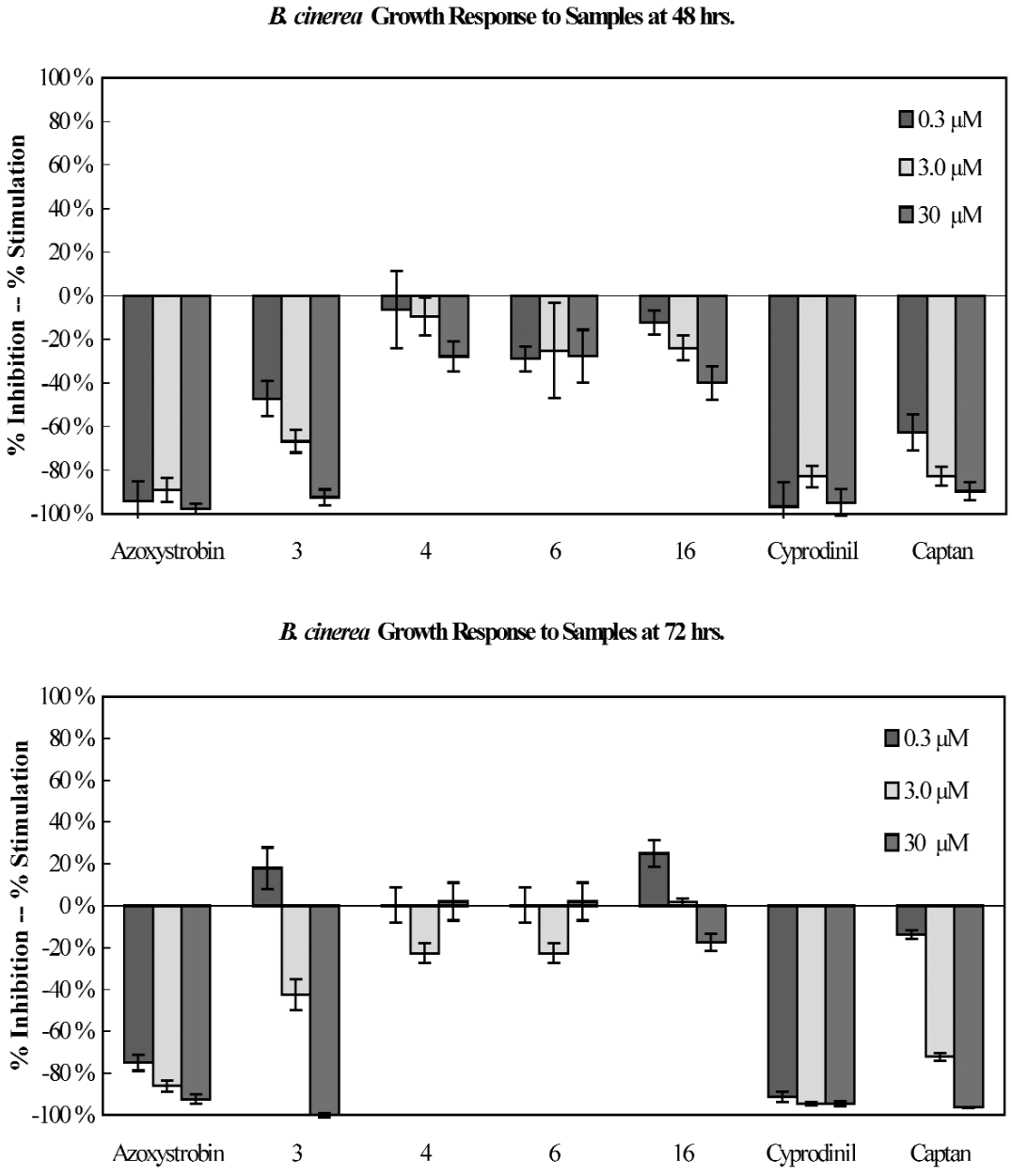
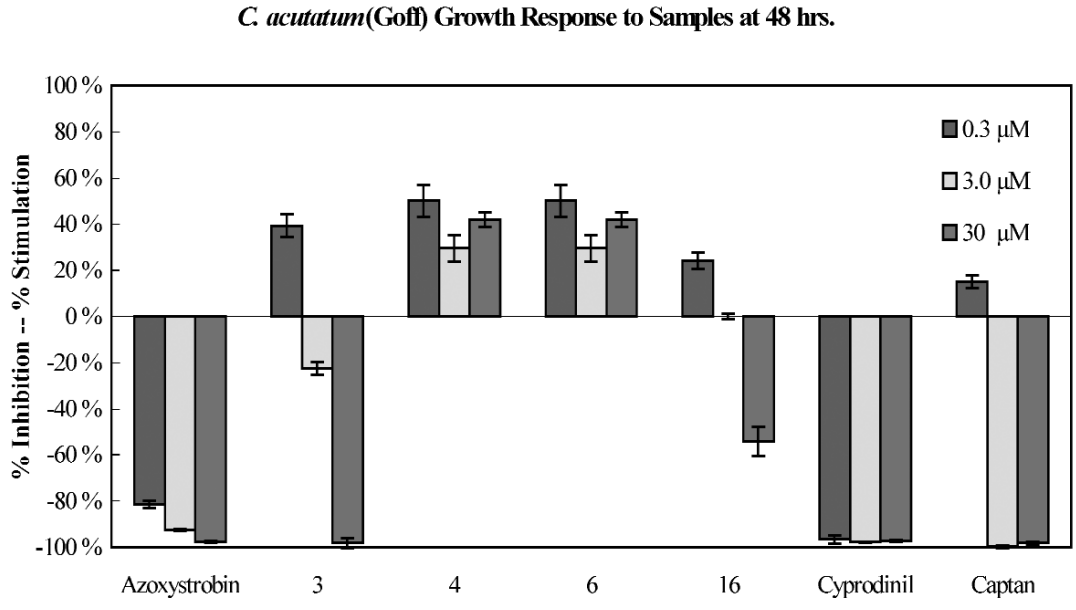


Fig. 3 Percent mean growth inhibition of compounds **3**, **4**, **6**, and **16** against *B. cinerea* at 48 and 72 h. The numbers represent the compounds in Table 2.



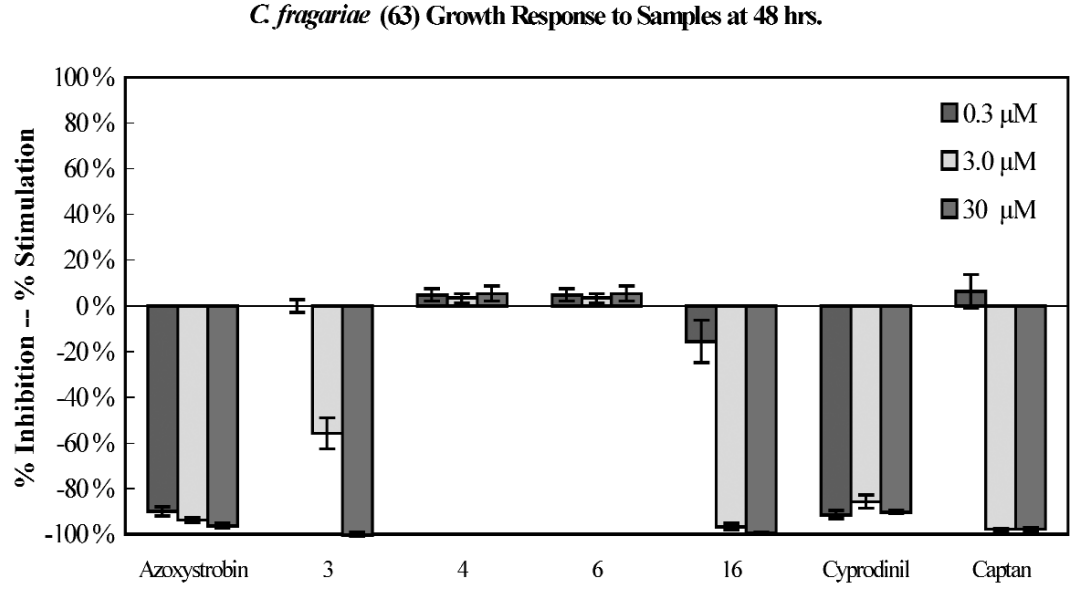


Fig. 4 Percent mean growth inhibition of compounds **3**, **4**, **6**, and **16** against *C. acutatum* and *C. fragariae* at 48 h. The numbers represent the compounds in Table 2.

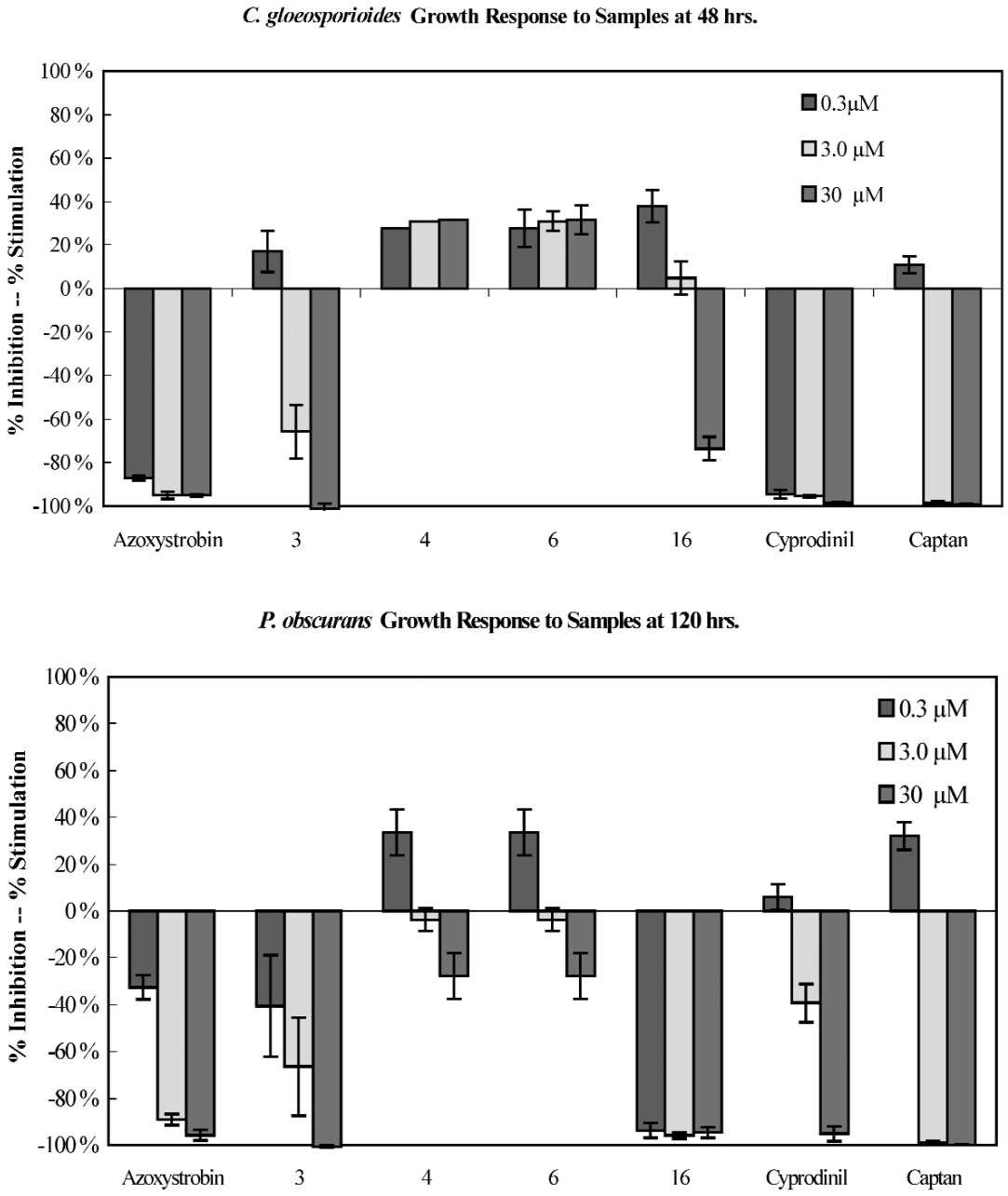


Fig. 5 Percent mean growth inhibition of compounds **3**, **4**, **6**, and **16** against *C. gloeosporioides* at 48 h and *P. obscurans* at 120 h. The numbers represent the compounds in Table 2.

ACKNOWLEDGMENTS

This research was supported by Anadolu University Research Fund, Eskisehir, Turkey (AUAF 00 03 34) and in part by the U.S. Department of Agriculture, Agricultural Research Service Specific Cooperative Agreement No. 58-6408-7-012. The authors are grateful to Prof. Dr. Zeki Aytac of Gazi University, Ankara, Turkey for identification of the plant materials. We thank Drs. B. Demirci and T. Ozek for GC/MS analysis of the essential oils. We thank also J. Linda Robertson, M. Dewayne Harries, John Trott, and Marsha Wright for their contributions in performing biological assays.

REFERENCES

1. V. H. Heywood. *The Biology and Chemistry of the Apiaceae*, p. 232, The Linnean Society of London, London (1971).
2. V. A. Mathew. "*Pimpinella* L.", in *Flora of Turkey and East Aegean Islands*, Vol. 4, P. H. Davis (Ed.), p. 352, Edinburgh University Press, Edinburgh (1972).
3. N. Tabanca, E. Bedir, N. Kirimer, K. H. C. Baser, S. I. Khan, M. R. Jacob, I. A. Khan. *Planta Med*. **69**, 933 (2003).
4. N. Tabanca, A. W. Douglas, E. Bedir, F. E. Dayan, N. Kirimer, K. H. C. Baser, Z. Aytac, I. A. Khan, B. E. Scheffler. *Plant Genet. Resour.* **3**, 149 (2005).
5. K. H. C. Baser. In *Recent Advances on the Umbelliferae Essential Oils of Turkey*, Proceedings of the 8th International Symposium on Natural Product Chemistry, Atta-ur-Rahman, M. I. Choudhary, K. M. Khan (Eds.), p. 271, Print Arts, Karachi, Pakistan (2002).
6. N. Tabanca, B. Demirci, T. Ozek, N. Kirimer, K. H. C. Baser, E. Bedir, I. A. Khan, D. E. Wedge. *J. Chromatogr., A* **1117**, 194 (2006).
7. N. Tabanca, S. I. Khan, E. Bedir, S. Annavarapu, K. Willet, I. A. Khan, N. Kirimer, K. H. C. Baser. *Planta Med*. **70**, 728 (2004).
8. N. Tabanca, E. Bedir, D. Ferreira, D. Slade, D. E. Wedge, M. R. Jacob, S. I Khan, I. A. Khan, N. Kirimer, K. H. C Baser. *Chem. Biodiv.* **2**, 221 (2005).
9. N. Tabanca, B. Demirci, N. Kirimer, K. H. C. Baser, E. Bedir, I. A. Khan, D. E. Wedge. *J. Chromatogr., A* **1097**, 192 (2005).
10. *European Pharmacopoeia*, 5th ed., Vol. 1 (1/2005), p. 217, Council of Europe, Strasbourg (2004).
11. N. Tanker, F. Izgu. *J. Ankara Faculty Pharmacy* **18**, 57 (1988).
12. O. Ozbay. "Studies on *Pimpinella flabellifolia* (Boiss.) Benth. Et Hook ex Drude (Umbelliferae)", M.Sc. dissertation, Ankara (1999).
13. F. W. McLafferty, D. B. Stauffer. *The Wiley/NBS Registry of Mass Spectral Data*, John Wiley, New York (1989).
14. D. Joulain, W. A. König, D. H. Hochmuth. *Terpenoids and Related Constituents of Essential Oils. Library of MassFinder 2.1*, Hamburg, Germany (2001).
15. D. Joulain, W. A. König. *The Atlas of Spectra Data of Sesquiterpene Hydrocarbons*, E.B.-Verlag, Hamburg (1998).
16. ESO 2000. *The Complete Database of Essential Oils, Boelens Aroma Chemical Information Service*, The Netherlands (1999).
17. W. G. Jennings, T. Shibamoto. *Quantitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary GC*, Academic Press, New York (1980).
18. Y. Yukawa, S. Ito. *Spectral Atlas of Terpenes and the Related Compounds*, Hirokawa Publishing, Tokyo (1973).
19. M. J. Macias, V. Martin, M. Grande, K. H. Kubeczka. *Phytochemistry* **37**, 539 (1994).
20. R. Kleiman, D. R. Plattner, D. Weisleder. *J. Nat. Prod.* **52**, 249 (1988).
21. R. Martin, J. Reichling, H. Becker. *Planta Med.* **3**, 198 (1985).
22. M. Ahmed, A. A. Leslie Gunatilaka. *Pharmazie* **43**, 372 (1988).

23. K. H. Kubeczka, A. Bartsch, I. Ullmann. "Recent investigations of essential oils of Apiaceae", in *Aetherische Oele*, K. H. Kubeczka (Ed.), pp. 158–187, George Thieme Verlag, Stuttgart (1982).
24. N. Takeuchi, T. Fujita, K. Goto, N. Morisaki, N. Osone, S. Tobinaga. *Chem. Pharm. Bull.* **41**, 923 (1993).
25. H. D. Friedel, R. Matusch. *Helv. Chim. Acta* **70**, 1616 (1987).
26. V. Dev, A. T. Bottini. *J. Nat. Prod.* **50**, 968 (1987).
27. W. A. Koning, N. Bulow, C. Fricke, S. Melching, A. Rieck, H. Muhle. *Phytochemistry* **43**, 629 (1996).
28. G. P. Peng, G. Tian, X. F. Huang, F. C. Lou. *Phytochemistry* **63**, 877 (2003).
29. H. J. Williams, G. Moyna, S. B. Vinson, A. I. Scott, A. A. Bell, R. B. Stipanovic. *Nat. Prod. Lett.* **11**, 25 (1997).
30. K. H. Kubeczka. In *Proceedings of the 28th International Symposium on Essential Oils*, K. H. C. Baser, N. Kirimer (Eds.), 1–3 September, Anadolu University Press, Eskisehir, Turkey, p. 35 (1997).
31. G. Meazza, F. E. Dayan, D. E. Wedge. *J. Agric. Food Chem.* **51**, 3824 (2003).
32. D. E. Wedge, J. M. Kuhajek. *SAAS Bulletin Biochem. Biotechnol*. **11**, 1 (1998).
33. B. J. Sampson, N. Tabanca, N. Kirimer, B. Demirci, K. H. C. Baser, I. A. Khan, J. M. Spiers, D. E. Wedge. *Pest Manag. Sci*. **61**, 1122 (2005).