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Chemical Composition and Antifungal Activity of Essential Oil of *Chrysactinia mexicana* Gray

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The chemical composition of the essential oil of *Chysactinia mexicana* was analyzed by gas chromatography−mass spectrometry. Seventeen compounds were characterized; eucalyptol (41.3%), piperitone (37.7%), and linalyl acetate (9.1%) were found as the major components. The essential oil of leaves and piperitone completely inhibited *Aspergillus flavus* growth at relatively low concentrations (1.25 and 0.6 mg/mL, respectively).

KEYWORDS: ***Chrysactinia mexicana*; piperitone; essential oil composition; antifungal activity; *Aspergillus flavus***

INTRODUCTION

Specific conditions of temperature and humidity during storage may contribute to promoting fungal growth in grains and feeds (*1*). Fungi may discolor grains and change their chemical and nutritional characteristics, and, most importantly, some species also generate mycotoxins such as aflatoxins, which may produce carcinomas, mainly in the liver and lung (*2*, *3*).

Chemicals are one of the main methods used to control phytopathogenic fungi; however, synthetic fungicides may produce resistant plagues and might be carcinogenic and teratogenic (*4*). Pesticides derived from plants have been found to be nontoxic with mammals and easily biodegradable (*5*, *6*). Many essential oils from plants have shown to be effective against fungi contamination (*7*−*9*). The essential oil isolated from *Calamintha nepeta* has activity against *Aspergillus niger* (*10*) the same as the essential oil isolated from *Nepeta cataria* (*11*); in both, one of the main constituents is piperitone. However, no reports about the effect of this compound against fungi were found.

Chrysactinia mexicana Gray (Astereaceae), commonly known as St. Nicholas's herb or "false Damiana", is a small shrub distributed throughout central and northern Mexico (*12*). This plant is used in folk medicine for treatment of respiratory ailments and skin infections (*13*). Previous studies on the chemical constituents of *C. mexicana* reported flavonoid glycosides (*14*), thiophenes (*15*), and terpenes (*16*−*18*). However, a survey of the literature showed no studies done with regard to either chemical composition or antifungal activity of the essential oil.

In an earlier work, we found that the hexane extract of this plant has antifungal activity on *Aspergillus flavus* Link (*19*). On the basis of these antecedents, our objectives in the present work consisted first of determining the chemical composition of the essential oil by GC-MS analysis and second, of evaluating its activity on *A. flavus* growth.

MATERIALS AND METHODS

Plant Material. *C. mexicana* leaves were collected in September 2003, 2 km from the crossroads of Highway 57 to Guadalcázar in the central Mexican state of San Luis Potosí. Material was authenticated by taxonomist José García-Pérez, and a voucher specimen (SLPM 37571) was deposited at the Isidro Palacios Herbolarium of the Universidad Autónoma de San Luis Potosí's (UASLP) Desert Zone Research Institute.

Isolation and Analysis of Essential Oil. Leaves of fresh plants were used, and essential oil was extracted by steam distillation. The oil and steam were condensed in a refrigerant system followed by extraction with ethyl ether. We obtained a yellow oil: $\eta_D^{20\times bb} = 1.4790$; $p^{20\times bb} = 0.9290$ g/cm^3; yield = 0.94% v/w. Analysis of the essential oil was performed using a Hewlett-Packard 6890 GC equipped with DB-Wax (30 m, 0.25 mm i.d., 0.25-μm film thickness) and a JEOL GC-mate as detector in EI mode at 70 eV. The carrier gas was helium, at a flow rate of 1 mL/min; split ratio was 1:10. Column temperature was initially 50 °C and gradually increased to 85 °C at 4 °C/min; subsequently, it was increased to 130 °C at 1 °C/min and finally increased at 4 °C/min to 220 °C for 10 min; 0.1 μL was injected. The relative retention times

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obtained in gas chromatography for the components of essential oil were compared with those obtained at the same conditions with commercial compounds, and their mass spectra were compared with those of pure samples and with mass spectra from the U.S. National Institute of Standards and Technology/National Bureau of Standards (NIST/NBS) Library.

Antifungic Screening. Antifungic screening was performed using Czapek agar (Difco). Nutrient medium was prepared according to manufacturer-recommended procedures and sterilized for 15 min at 120 °C. *A. flavus* Link SRRC 1273 from The National Center for Agricultural Utilization (Peoria, IL) was used for the determination of antifungic activity; the diffusion method consisted of well plates on agar (*20*).

Each culture was prepared to turbidity equivalent to a Wikerham scale cross and spread on the test plate. A total of 1.25, 2.5, 5.0, 10, or 20 mg of essential oil in DMSO was placed in a well 6 mm in diameter made on the test plate and cultured for 72 h at 28 ± 1 °C; all determinations were repeated five times. Five micrograms of daconil (tetrachloroisophthalonitrile) (Coraza) was used as positive control and DMSO (50 μL) as negative control. Inhibition diameters were determined after incubation.

Minimum Inhibitory Concentration (MIC). MIC determination was made using a dilution technique (*21*). Tests were performed in dextrose Sabouraud broth. Different concentrations of essential oil or one of its compounds were prepared in 4 mL of broth to obtain final concentrations of 0.125−1.5 mg/mL. These solutions were placed in culture tubes and then inoculated with ∼1000 spores per 0.1 mL of suspension of *A. flavus*. Tubes were incubated at 28 ± 1 °C for 72 h. MIC was determined when no evident growth of the microorganism was demonstrated. When it was considered to be necessary, concentrations were increased or decreased accordingly.

Fungicidal or Fungistatic Activity. A 0.1 mL suspension with the microorganism was inoculated to a tube of dextrose Sabouraud broth containing the same concentration of the essential oil or one of its compounds at which growth was not detected in the MIC and incubated for 72 h at 28 ± 1 °C. After this time, a sample (0.1 mL) was dispersed on an agar Czapek plate and incubated for 72 h at the same conditions. The concentration that showed growth inhibition of *A. flavus* in the dextrose Sabouraud broth with growth on Czapek agar was considered to have fungistatic activity. When there was no growth in both (dextrose Sabouraud broth and Czapek agar), a fungicidal activity was considered (minimum fungicidal concentration, MFC).

RESULTS AND DISCUSSION

Leaves of *C. mexicana* (1.6 kg) were extracted, and 15 mL of crude essential oil was obtained. The composition of *C. mexicana* essential oil was determined by comparing relative retention times and mass spectra of oil components with those of authentic samples and mass spectra from the data library. Seventeen components of *C. mexicana* essential oil were identified; nonetheless, it is characterized by the dominant presence of two substances—eucalyptol (41.3%) and piperitone (37.7%)—but analysis revealed an additional 30 constituents (**Table 1**). Among these the following were detected: linalyl acetate (9.1%); α-myrcene (1.2%); α-thujone (1.2%); and 3,7-dimethyl-1,6-octadien-3-ol (1.4%). The remaining 24 compounds detected by GC-MS were present in very low proportions (<1%).

The antifungal screening showed that the essential oil of *C. mexicana* caused inhibition of *A. flavus* growth. Inhibition diameters were 17.8 ± 0.017 and 25.2 ± 0.022 mm with 1.25 and 2.5 mg of the oil, respectively. When we used 5.0, 10.0, and 20.0 mg, no growth of fungi was observed.

The essential oil of *C. mexicana* (**Table 2**) was fungistatic and fungicidal against *A. flavus* at 1.0 and 1.25 mg/mL concentrations, respectively, in liquid culture media. Several reports are available on the effect of essential oils on growth and aflatoxin formation of *A. flavus* (*22*, *23*). It was found that lemongrass essential oil was fungistatic and fungicidal against this fungus at 0.6 and 1.0 mg/mL concentrations, respectively (*7*). Daferera and co-workers (*24*) reported the effect of oregano, thyme, dictamus, and marjoram essential oils, which completely inhibited conidial germination of *Penicillium digitatum* at a concentration of ∼250 μg/mL. A study carried out by Bankole (*25*) revealed complete inhibition on growth and aflatoxin production of *A. flavus* at 1000 ppm of *Morinda lucida* and at 500 ppm of *Azadirachta indica* seed oil.

Pure commercial eucalyptol, linalyl acetate, and piperitone, the major constituents of *C. mexicana* oil, were tested against *A. flavus* (**Table 3**) under identical conditions to compare its activity with that of the investigated oil. Only piperitone effectively inhibited mycelial growth, and complete inhibition was observed at a concentration of 0.6 mg/mL. Pitarokili (*26*) found that linalyl acetate presented inhibitory action on *Sclerotinia sclerotiorum* at an EC_{50} (concentration causing 50% inhibition of mycelial growth on control media) of 569.62 μL/L, whereas with other soilborne pathogen fungi the EC_{50} values exceeded 1500 μL/L. However, at a similar concentration (600 mg/mL), this compound had no effect on *A. flavus*.

On the other hand, it was found that piperitone possesses electrophysiological and behavioral responses of female *Helicoverpa armigera* (*27*) that could have affected the survival of

Table 1. Composition of the Essential Oil Isolated from *C. mexicana*

retention time (min)	compound	composition (%)
11.58	α-phellandrene	0.25
13.07	α-myrcene	1.20
13.46	α-thujone	1.17
15.22	1-methyl-4-(1-methylethenyl)cyclohexanol acetate	0.63
17.51	eucalyptol	41.30
19.34	2-ethyl-6-methylbenzenamine	0.47
43.02	3,7-dimethyl-1,6-octadien-3-ol	1.39
46.11	*trans*-limonene	0.69
49.55	4-methyl-1-(1-methylethyl)-3-cyclo-hexen-1-ol	0.10
52.58	*cis*-limonene	0.44
57.05	*S*-(tetrahydro-2*H*-pyran-3-yl)ethanethioic ester	0.04
59.23	linalyl acetate	9.08
62.06	piperitone	37.74
63.43	*exo*-2-hydroxy cineole acetate	0.11
67.14	*exo*-2-hydroxy cineole	0.87
71.59	phenol	0.06
75.37	3-phenyl-2-propenyl ethyl ester	0.29

Table 2. Determination of MIC and MFC with Essential Oil[a]

	concentration (mg/mL)						
essential oil	0.125	0.25	0.5	0.75	1.0	1.25	1.5
DSB	+	+	+	+	−	−	−
Czapek	+	+	+	+	+	−	−

[a] DSB, dextrose Sabouraud broth; +, growth; –, no growth.

Table 3. Antifungic Activity of Eucalyptol, Linalyl Acetate, and Piperitone (Milligrams per Milliliter) on *A. flavus*[a]

compound	0.1	0.2	0.3	0.4	0.5	0.6
eucalyptol	+/+	+/+	+/+	+/+	+/+	+/+
linalyl acetate	+/+	+/+	+/+	+/+	+/+	+/+
piperitone	+/+	+/+	+/+	+/+	+/+	–/–

[a] +/+, growth in DSB/growth in Czapek agar; –/+, no growth in DSB/growth in Czapek agar; –/–, no growth in DSB/no growth in Czapek agar.

two insect species (*28*) and also can have antimicrobial activity (*29*) and antispasmodic action (*30*); in addition, piperitone is used as a flavoring agent. Nevertheless, this is the first time that the fungicidal activity of this compound has been reported; its efficacy against *A. flavus* is evident from the current study. Therefore, the use of piperitone could be recommended for protecting grains with further investigations in field trials.

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