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The essential oil of *Coleus amboinicus* Loureiro chemical composition and evaluation of insect anti-feedant effects

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Abstract

The chemical composition of the essential oil of *Coleus amboinicus* Loureiro, an aromatic plant with ethnobotanical uses, has been analyzed by gas chromatography coupled with mass spectrometry and its insect antifeedant properties examined using *Sitophilus oryzae* and *Tribolium castaneum*, two important stored grain insect pests. Two samples were collected, one at Rancherías (1.100 meters above sea level) and at the Medicinal Plants Garden, Faculty of Pharmacy, Mérida (1400 m.a.s.l.). Carvacrol and p-cymene were found to compose most of the volatile material, 74.5% (Mérida) and 74.1% (Rancherías). The oil from Mérida, was found to contain a larger proportion of carvacrol (64.7%) and sesquiterpenes (17.4%) than the oil from Rancherias (55.3% and 10.5%). On the other hand the oil from Rancherías was richer in p-cymene (18.8% against 9.8%) and monoterpenes. The essential oil in large dosage (1.25%) on hardened flour feeding disks was found unable to induce any negative response in the feeding behavior of the test insects, nor was any mortality recorded within the 60 h of exposure of the bioassays.

Key word: *Coleus amboinicus* Loureiro; essential oil; carvacrol; p-cymene, *Tribolium Castaneum; Sitophilus oryzae.*

El aceite esencial del *Coleus amboinicus* Loureiro composición química y evaluación de sus efectos anti-alimentarios en insectos

Resumen

La composición química del aceite esencial de *Coleus amboinicus* Loureiro, una planta aromática con usos etnobotánicos, ha sido analizada mediante cromatografía de gases acoplada con espectrometría de masas, así como sus propiedades antialimentarias contra los coleópteros *Sitophilus oryzae* y *Tribolium castaneum*, dos insectos plaga de importancia en los cereales. Se colectaron dos muestras, una en Rancherías (1100 m.s.n.m.) y otra en el Jardín de Plantas Medicinales de la Facultad de Farmacia en Mérida (1400 m.s.n.m.). Se encontró que el Carvacrol y el p-cymene constituían la porción mayoritaria del aceite esencial, 74,5% (Mérida) y 74,1% (Rancherías). El aceite proveniente de Mérida posee una mayor proporción de carvacrol

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(64,7%) y sesquiterpenos (17,4%) que el aceite de Rancherías (55,3% y 10,5%). Por otra parte el aceite proveniente de Rancherías es más rico en p-cimeno (18,8% vs 9,8%) y monoterpenos. Este aceite esencial en altas dosis (1,25%) sobre discos endurecidos de harina no fue capaz de inducir respuesta negativa alguna en el comportamiento alimentario de los insectos de prueba, así como tampoco hubo mortalidad durante las 60 horas de exposición que duraron los bioensayos.

Palabras clave: Aceite esencial*; carvacrol; Coleus amboinicus* Loureiro; p-cymene; Sitophilus oryzae; *Tribolium castaneum*.

Introduction

Coleus amboinicus Lour (Lamiaceae), popularly known as "Orégano orejón", is an aromatic shrub widely distributed in Venezuela. It is used to treat colds and cough as well as arthritic inflammations (1). According to the Missouri Botanical Gardens the name *Plectranthus amboinicus* is a synonym of *C. amboinicus*. Its insect repellent properties have been tested (2) and at least another member of the *Coleus* genus, *C. aromaticus*, has been found to cause reduction in egg laying capacity, retard in adult emergence and weight loss in the pulse beetle *Callosobruchus maculatus* F (3). A moderate allelopahic effect of the powdered leaves of *C. amboinicus* against the water hyacinth is also on record (4). Studies performed in India demonstrated the "fungistatic" properties of the essential oil of this plant (5), but studies on its essential oil composition have not been reported. Brieskorn and Riedel (6) isolated eight triterpenic acids from the leaves of *C. amboinicus* and later they reported the isolation of six flavones, one flavanone and one flavonolol (7). No further phytochemical studies of this plant have been reported but a review (8) describes the components and medicinal importance of plants belonging to different *Coleus* species. The essential oil of *C. aromaticus* has been studied by Baslas *et al* (9) and plectrin, an aphid antifeedant diterpene has been isolated from *Plectranthus barbatus* (10). Considering the record of some *Coleus* species, it was decided to study the composition of the essential oil of *C. amboinicus* and to test its anti-feedant properties on *Tribolium castaneum* and *Sitophilus oryzae* two important stored grain insect pests.

Materials and Methods

Plant Material

Leaves of *C. amboinicus* were collected at Rancherías, Campo Elías Municipality, Mérida State, at 1.200 meters above sea level and at the Medicinal Plants Garden (1400 m. asl), Faculty of Pharmacy, University of Los Andes, Merida. Voucher specimens (A.Usubillaga 24a and 24b) have been deposited at the MERF Herbarium, Faculty of Pharmacy; University of Los Andes.

Insects

Malathion-resistant strain of red flour beetles *Tribolium castaneum* (Herbst) from a 13-year old collection at Grupo de Química Ecológica, was cultivated in ½ liter glass jars using a mixture of whole wheat flour and wheat germ (5%) as growth medium at 28 ± 1°C. Rice weevils *Sitophilus oryzae* (L.) from a 15 year old collection of the same laboratory were grown in ½ liter glass jars in whole wheat, barley or maize grains from local suppliers.

Extraction of the oil and measurement of its physical constants

The plant material (1.35 Kg and 1.1 Kg respectively) was cut and hydrodistilled in a Clevenger-type apparatus for 3 h yielding a colorless oil. The oil was dried over anhydrous sodium sulfate and stored under nitrogen at 4°- 6°C. Optical rotations were measured in a Jasco DIP 370 electropolarimeter at 23°C; density with a picnometer and refractive index with an Abbe refractometer at 25°C.

Analysis

GC analyses were performed using a Perkin-Elmer AutoSystem gas chromatograph equipped with FID detector and data handling system. A 5% phenylmethyl polysiloxane fused-silica column (HP-5, 30 m, 0.25 mm i.d., film thickness 0.25 µm) was used. Column oven temperature was programmed from 60 to 200°C at 4°C/min, subsequently at 8°C/min up to 280°C. The injector and detector temperatures were 200°C and 280°C, respectively. The carrier gas was helium at 0.8 mL/min. The samples (1.0 µL) were injected using a split ratio of 1:100. Retention indices were calculated relative to C_8-C_{24} alkanes. The percentage composition of the oils were calculated by the normalization method from the GC peak areas.

GC-MS analysis was carried out on a Model 5973 Hewlett Packard GC-MS system fitted with a HP-5MS fused-silica column (30 m x 0.25 mm i.d., film thickness 0.25 µm). Oven temperature program was the same used for the HP-5 column for GC analysis; the transfer line temperature was held at 280°C; source temperature, 230°C; quadrupole temperature, 150°C; carrier gas, helium, adjusted to a linear velocity of 34 m/s; ionization energy, 70 eV; scan range, 10–500 amu; 2.2 scans/s. Samples (1.0 µL) were injected as 2% solutions of the oils in n-heptane. A Hewlett Packard ALS injector was used with split ratio 1:100. The identity of the oil components was established from their GC retention indices (11, 12), by comparison of their MS spectra with those of standard compounds available in the laboratory, and by library search (Wiley 6th ed.).

Bioassays

Thin 0.5 cm^2 hardened flour disks were prepared from a mixture of wheat flour and water with 2% butter added to prevent cracking followed by mild heating (40°C) for 1 h in a ventilated oven. Treatment disks were dosaged with 20 µL solutions of essential oil of *C. amboinicus* in acetone with the aid of a microsyringe to yield 500 µg of solute per cm^2 of flour disk (1.25% w/w). Control disks were dosaged with pure acetone. Treatment and control feed were ventilated under mild heating until all traces of solvent had been removed. Disks and insects were weighted before and after bioassays. For one way assay, ten unsexed 7 -12 day old adult insects were placed in a 8 cm Petri dish containing one treated disk and placed in the dark for 60 h, after which the remaining food was removed and weighted. There were five replicates per experiment. From the weight change in insect and disk masses the following parameters were calculated (13): feeding preference index Fi, amount of disk and compound devoured, insect mass gained or lost during feeding, and feed conversion efficiency into biomass (ECI). For comparison of the means the Kurkal-Wallis and Tuckey non parametric tests (P= 0.001) were employed using Statistix V 4.0 from Analytical Software Inc., Minneapolis, Minesota.

Results and Discussion

Table 1 presents density, optical rotation, refractive indices, and essential oil yields obtained from leaves of *C. amboinicus* collected at Rancherías and at the Faculty of Pharmacy, Mérida.

The results of the analyses of the volatile components from the leaves of *C. amboinicus* are listed in Table 2 in order of elution on the HP-5 column. The identified components represented 99.6% of the oil from Rancherías and 99.8% of the oil from the Faculty of Pharmacy Garden. The most abundant constituent in both cases was carvacrol (55.3% and 64.7%), followed by p-

Table 1
Yields and physical properties of essential oil obtained from leaves of *Coleus amboinicus* collected at Rancherías and at the Faculty of Pharmacy, University of Los Andes, Merida

Site of Collection	Oil yield %v/w	Density g/mL	Optical Rotation	Refractive Index
Rancherías	0.22	0.9241	-2.4°	1.5075
Faculty of Pharmacy	0.20	0.8894	-1.8°	1.4903

cymene (18.8% and 9.8%) and γ-terpinene (7.2% and 4.7%). The sesquiterpene fraction made up 10.5% of the oil from Rancherías, where t-caryophyllene (5.4%) and α-bergamotene (3.3%) were the most abundant compounds while the oil from the Faculty of Pharmacy contained a larger proportion of sesquiterpenes (17.4%) but t-caryophyllene (9.1%) and a-bergamotene (4.6%) were also the most abundant constituents of that fraction. It is interesting to point out that the sum of the aromatic monoterpenes carvacrol and p-cymene was practically the same (74.1% and 74.5%) for both oils. Rancherías is located at an altitude of 1100 m while the Faculty of Pharmacy of the University of Los Andes is at 1400 m, and both places are not more than 10 Km apart but the weather is quite different in both locations. Rancherias is a rather dry place while at the Garden of the Faculty of Pharmacy the weather is humid most of the time. This difference in weather conditions would explain the differences in oil composition.

Sitophilus oryzae and *Tribolium castaneum* provided two feeding strategies among the stored cereal insect pests. The former is a primary seed predator as it bores the kernell shell with powerful mandibles located at the tip of the elongated feeding apparatus. Only when the pericarp and mesocarp have been perforated the insect begins feeding. By contrast, *T. castaneum* is a secondary grain pest that profits from bores practised by primary seed predators, flour and powdered meal derived therefrom. Therefore, the two insects provide a wide spectrum of feeding strategies used by the many cereal grain insect species. In addition, their expo-

Table 2
Percentage composition of the essential oil from the leaves of *Coleus amboinicus* Loureiro collected at Rancherías and at the Faculty of Pharmacy, Merida

KI	Compound	% oil from Rancherias	% oil from Mérida
920	α-thujene	0.4	–
927	α-pinene	0.2	–
949	camphene	1.0	–
981	1-octen-3-ol	1.0	0.8
993	β-myrcene	1.1	0.3
1001	α-felandrene	0.1	–
1009	δ3-carene	0.1	–
1015	α-terpinene	1.3	0.6
1022	p-cymene	18.8	9.8
1038	β-felandrene	0.6	–
1053	γ-terpinene	7.2	4.7
1064	c-sabinene hydrate	0.2	–
1080	terpinolene	0.2	–
1095	t-sabinene hydrate	0.1	–
1161	borneol	0.1	–
1172	terpin-4-ol	0.9	1.0
1300	thymol	0.5	0.5
1310	carvacrol	55.3	64.7
1421	t-caryophyllene	5.4	9.1
1433	α-bergamotene	3.3	4.6
1453	α-humulene	1.3	2.6
1582	caryophyllene oxide	0.4	1.1
1625	di-elapiol	0.1	–

sure to hardened flour disks whose surface have been treated with purportedly obnoxious chemicals from *Coleus amboinicus* and other plant derived essential oils allows to test the repellency as well as feeding inhibition properties of the material.

Tables 3 and 4 collect the effects of *C. amboinicus* oil from two sources in Mérida State on the feeding behavior of the two insect species. In the large doses employed (1.25% v/w) there was no difference in the feeding response of *S. oryzae* relative to controls and no noticeable after-effect upon the ingestion of as much as 10 µg of the oil per insect. On its part, *T. castaneum* showed a slight increase in the phagoactivity of the treated disk relative to the control although the body weight did not show any added increment from the excess feed ingested. This is reflected in the conservative values of the ECI or efficiency of conversion of ingested food. Hence, no adverse physiological reaction with influence on body weight gain could be detected. It may be concluded, therefore that carvacrol and p-cymene, the major components of *C. amboinicus* oil, are innocuous to these insects at the large dosage presented. The repellency effects recorded earlier (2) do not apply to the stored grain insects tested here.

Conclusions

Carvacrol and p-cymene were found to compose about 74% of the essential oil of *Coleus amboinicus*. This oil did not induce

Table 3

One way assay feeding parameters of *Sitophilus oryzae* exposed to feed treated with 1.25% (w / w) of *C. amboinicus* essential oil from plants collected in two localities of Mérida State; (a): Medicinal Plants, garden of the Faculty of Pharmacy, Universidad de Los Andes (1400 m asl) and (b) Campo Elías Municipality, 1200 m asl. Equal letters denote statistical undifferentiation (N = 5, 10 individuals per replicate)

Sample	Consumption/insect (mg) ±SD	Consumption relative to control (%)	Ingested ess. Oil (µg/insect) ±SD	Mortality (%)
Oil (a)	0.744 ± 0.043 (c)	96.9	10615 ± 1172 (c)	0
Oil (b)	0.669 ± 0.132 (c)	87.0	8001 ± 1809 (c)	0
Control	0.768 ± 0.032 (c)	100.0	0	0

Table 4

One way assay feeding parameters of *Tribolium castaneum* obtained under the same conditions as described in the legend of Table 2. DW represents the insect body weight change relative to the unfed individual during the same time period, determined in a parallel assay. Equal letters denote statistical undifferentiation (N = 5, 10 individuals per replicate)

Sample	Consumption/insect (mg) ± SD	Consumption relative to control (%)	Ingested ess. Oil (µg/insect) ± SD	DW/insect (mg) ± SD	% weight change	ECI ± SD
Oil (a)	0.428 ± 0.046 [c]	118.2	6339 ± 1158 [e]	0.04 ± 0.009 [f]	2	0.321 ± 0.027 [g]
Oil (b)	0.452 ± 0.059 [c,d]	124.9	6435 ± 0.788 [e]	0.04 ± 0.01 [f]	2	0.315 ± 0.044 [g]
Control	0.362 ± 0.085 [c]	100	0	0.04 ± 0.01 [f]	2	0.397 ± 0.067 [g]

any negative response in the feeding behavior of *Tribolium castaneum* or *Sitophilus oryzae*.

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