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Eugenol and Methyl Eugenol Chemotypes of Essential Oil of Species *Ocimum gratissimum* L. and *Ocimum campechianum* Mill. from Colombia

Nayive Pino Benitez[1], Erika M. Meléndez León[1], and Elena E. Stashenko[2,*]

[1]Universidad Tecnológica del Chocó, bloque 6 laboratorio 316, Grupo de Productos Naturales, Quibdó- Chocó- Colombia and
[2]Research Center of Excellence CENIVAM, Chromatography Laboratory, CIBIMOL, Universidad Industrial de Santander. Carrera 27 calle 9. Bucaramanga, Colombia.

Abstract

Essential oils chemical constituents of leaves of *O. gratissimum* and *O. campechianum* of the Lamiaceae family, collected in Chocó of northwest Colombian, were obtained by microwave-assisted hydrodistillation and analyzed by gas chromatography coupled with mass spectrometry. A total of 33 and 37 compounds were identified in the essential oil of *O. gratissimum* and *O. campechianum*, respectively. *O. gratissimum*'s main essential oils were eugenol (43.2%), 1,8-cineole (12.8%) and β-selinene (9.0%); in the *O. campechianum* essential oil, the main components were methyl eugenol (12.0%), germacrene D (10.1%), and eugenol (9.0%). Main distribution of compounds in these essential oils are 25.0% monoterpenes hydrocarbons, 15.0% monoterpenes oxygenated, 35.0% sesquiterpenes hydrocarbons, 7.5% other oxygenated components for *O. gratissimum*, 33.9% sesquiterpenes hydrocarbons, and 10.7% their respective oxygenated derivates; for *O. campechianum,* the distribution was 10.7% monoterpenes hydrocarbons and 7.1% their respective oxygenated derivates and 3.6% phenylpropanes. According to the essential oils chemical composition of *Ocimum gratissimum* and *O. campechianum*, they are classified as eugenol and methyl eugenol chemotype, respectively.

Introduction

The family Lamiaceae comprises about 220 genera and about 4000 species, almost cosmopolitan, as reported by Hedge (1). In Colombia, this family has about 23 genera and 205 species as described by Fernandez (2). Within the genera of this family is the genus *Ocimum*, whose species are composed of up to 1.5 million herbs. They are characterized as aromatic flowers arranged in pedicels on terminal clusters, and can be white or purple with white bilabiada basis, according to Cronquist (3). This genus is made up between 50–150 species, as noted by Hiltunen and Holm (4), which is characterized by high variability and morphological chemotype (5). The species *Ocimum*, commonly known as basils, have a pleasant aroma and can grow in different regions of the world. There are several utilities that have been given to these species, the main one being its culinary use as a condiment; equally essential oil extracted from these species is used in the preparation of foodstuffs such as tomato paste, sauces, pickles, and vinegar. It is also used for medicinal, magical, and religious purposes and as ornamental leaves (5–7).

According to Ozcan et al. (6), depending on the type of chemical compounds that form the essential oil of these plants, several features are present in the aroma. Essential oils of species of the genus *Ocimum* have marked differences in their chemical composition (8); Some chemotype have been classified according to their geographical origin, among which are: European chemotype, which is considered to have a very sharp smell due to linalool and estragole; Reunion chemotype, which is characterized as having high concentrations of estragole; Tropical chemotype, which is rich in methyl cinnamate; and another chemotype of northern Africa and the former Soviet Union, which is rich in eugenol.

The classification of chemical composition of *Ocimum* has been developed to study chemical essential oils of the species *O. gratissimum* to determine their chemotype. Pressoa et al. (9) classified chemotype eugenol, the essential oil extracted from fresh leaves of *O. gratissimum* harvested in Brazil, while essential oil of that species collected in India was classified as ethyl cinnamate chemotype according to Dubey et al (10). Change in the essential oil extracted from plants of Cameroon was classified as the thymol chemotype (11).

Experimental

Plant material

O. gratissimum and *O. campechianum* plant material was collected by a research group at the Natural Products Technological University of Chocó in the settlement of Pacurita (Municipality of Quibdó, Chocó).

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*Author to whom correspondence should be sent: email: elena@tucan.uis.edu.co.

The taxonomic identification of the plants was carried out in the Colombian National Herbarium (COL) of the Institute of Natural Sciences (Bogotá). The witnessed papers of each plant were stored as a permanent display in COL as follows: *Ocimum gratissimum* L. No. 520292 and *Ocimum campechianum* Mill No. 520281.

Table I. Comparison of Chemical Composition Percentage of Essential Oils Extracted By MWHD of the Leaves of *O. gratissimum* and *O. campechianum*

		Relative amount (%)			
Compound*	I_k†	*O. gratissimum* Chocó	*O. gratissimum* Brazil	*O. campechianum* Chocó	*O. campechianum* Ecuador
cis-3-Hexenol	857	0.3		–	
Camphene	937	1.2		0.4	0.07
α-Pinene	954	–	0.95	0.2	0.24
Sabinene	977	1.2		0.1	
β-Pinene	983	2.4	3.02	0.8	0.75
β-Myrcene	991	0.9	0.65	0.2	0.26
Limonene	1034	0.3		0.3	
1.8-Cineole	1039	12.8	32.7	3.3	5.35
trans-β-Ocimene	1048	0.6		–	0.35
Linalool	1101	0.9	0.48	2.9	1.49
allo-Ocimene	1127	0.5		–	2.42
δ-Terpineole	1171	0.6	0.58	–	
Borneol	1175	–		0.5	0.14
Terpineno 4-ol	1181	0.3		–	0.45
α-Terpineol	1194	1.7		0.3	
Bicicloelemene	1335	–		0.2	
γ-Elemene	1338	–		0.6	
Eugenol	1363	43.2	43.7	9.0	46.55
α-Copaene	1384	0.6		1.9	
β-Bourbunene	1393	0.2		9.5	
β-Elemene	1398	1.6	0.53	–	9.06
Methyl eugenol	1409	–		12.0	
trans-β-Caryophyllene	1436	6.4	4.05	7.8	11.94
trans-α-Bergamotene	1438	0.6		–	0.13
α-Guaiene	1445	0.1		5.6	
cis-β-Farnesene	1457	–		0.5	
Sesquisabinene	1459	–		0.2	
α-Humulene	1469	1.7		2.8	2.4
α-Aromadendrene	1473	0.4		–	
γ-Muurolene	1483			0.3	
Germacrene D	1496	3.9	1.27	10.1	0.13
β-Selinene	1506	9.0	4.04	1.4	0.86
Biciclogermacrene	1510	–		3.4	2.9
α-Selinene	1511	4.5	1.34	–	
α-Bulnesene	1517	–		7.1	
γ-Cadinene	1527	–		0.5	
δ-Cadinene	1528	0.4		2.0	
β-Sesquifelandrene	1531	–		0.4	
3.7-(11)-Eudesmadiene	1534	1.6		–	
Spathulenol	1591	–		0.4	1.15
Caryophyllene oxide	1595	–		0.4	1.23
epi-α-Muurolol	1655	–		2.0	
T-Muurolol	1669	–		0.7	
11- Selinen-4-α-ol	1676	–		1.1	0.21

* Striped compounds in elution order

† Retention index in a column DB-5MS

Extraction of essential oil

Essential oils are extracted from *O. gratissimum* and *O. campechianum* leaves, respectively. Microwave-assisted hydrodistillation (MWHD) was used as an extracting method as described by Stashenko et al (12). A sort of Clevenger-type distillation device with *Dean-Stark* distillation reservoir was used. Adaptation to global warming was carried out by microwave radiation through a conventional LG microwave, MS-1242 ZK model, set at 2450 MHz, 800 W. Two hundred fifty milliliters of distilled water were added to the plant material. The extraction was conducted for 10 intervals of 10 min each (with 5 min rest time between intervals).

Chromatographic analysis

A portion of each essential oil (20 µL) was dissolved in dichloromethane to a final volume of 1 mL, transferring this volume into vials for gas chromatography (GC). Essential oils chromatographic analysis of was performed using an Agilent 6890 Plus GC (HP, Palo Alto, CA) coupled to an Agilent MSD 5975 selective mass detector equipped with a split/splitless injector port (1:50 split ratio), an automatic injector Agilent 7863, and a data system HP ChemStation. One microliter solving the essential oil was injected into the GC–mass spectrometer (MS) equipment for the corresponding chromatographic analysis. A 60 m × 0.25 mm, i.d. × 0.25 µm column was used with 5%-phenyl poly(methylsiloxane) stationary phase. The oven temperature was programmed at 45°C for 5 min, then increased 4°C/ min up to 150°C (2min), then to 5°C/ min up to 250°C (5 min), and finally at 10°C/min up to 275°C. Helium was used as carrier gas with 16.47 psi column head pressure and 1 mL/min linear velocity. The retention indices (I_R) values were calculated through a series of homologues aliphatic saturated hydrocarbons (C_8–C_{25}) analyzed in the same column with the same chromatographic conditions that were used for the essential oils analysis. The components identification were based on Kováts indices (I_k) and by comparison of the mass spectra fragmentation patterns with those found in databases or libraries (NIST02, Adams, Wiley7n) (13,14).

Results and Discussion

Table I shows the results for the chemical composition of the *O. gratissimum* and *O. Campechianum* essential oils. The components are listed according to their order of elution in column DB-5MS and were relatively greater than 0.2%. Thirty-three compounds were identified for *O. gratissimum*, constituting 82.6% of the essential oil. The major compound was phenylpropanoid eugenol (43.2%). Ten compounds are given as hydrocarbon monoterpenes (25%) with β-pinene (2.4%), α-pinene (1.2%), and sabinene (1.2%) as the main components. Six compounds are reported as oxygenated monoterpenes, (15%), whose major component is α-terpineole (1.7%). Fourteen sesquiterpenes hydrocarbons represent 35.0% of the essential oil, whose main components are β-selinene (9.0%), α-selinene (4.5%), and *trans*-β-caryophyllene (6.4%). Three components are presented as another type of oxygenated compound, which represent 7.5% (Figure 1).

Thirty-seven compounds were identified for *O. campechianum,* constituting 66.1% of the essential oil. Nineteen of these compounds are sesquiterpenes hydrocarbons (33.9%), whose main components are: germacrene D (10.1%), β-bourbonene (9.5%), *trans*-â-caryophyllene (7.8%), α-bulnesene (7.1), and α-guaiene (5.6%). Six compounds are presented as oxygenated sesquiterpenes (10.7%), where *epi*-α-murolol (2.0%) and 11-selinene-4-α-ol (1.1%) were the major components. Six compounds were monoterpenes hydrocarbons (10.7%), 4 as oxygenated monoterpenes (7.1%) with 1,8-cineole (3.3%) and linalool (2.9%) as major components. Methyl eugenol (12.0%) and eugenol (9.0%), among other oxygenated compounds, constitute 3.6% of the essential oil (Figure 2).

Within the chemical constituents of the essential oil of *O. gratissimum*, eugenol (43.2%) is the main compound followed by 1.8-cineol (12.8%). This result matches different studies made by Pressoa et al. (9) in Brazil, Nakamura et al. (15), Vasconcelos et al. (16) and Murbach et al. (17), all who report eugenol and 1.8-cineol as major components of the essential oil of *O. gratissimum* and classify it as eugenol chemotype. The chemotype classification system, proposed by Grayer et al. (18), is based on the chemicals combination of its major components rather than the sole dominant compound or a major component as one with content close to 20%.

The essential oils from the specie *O. gratissimum* mainly extracted in Europe are characterized by containing high amounts of phenolic compounds, as described by Guenther (19). In accordance with Hiltunen and Holm (4), three types of chemotypes can be found from *O. gratissimum* including chemotype thymol, which is characterized for having high concentrations of thymol but no presence of eugenol. The second chemotype reported for this specie is the chemotype citral, whose major components are citral followed by geraniol. The third chemotype is the eugenol, which is characterized for being rich in eugenol but also for the absence of thymol. According to the chemical composition of essential oil of *O. gratissimum* in this study, *O. gratissimum* is classified as chemotype eugenol.

The potential of these species from the Colombian Pacific relies on the presence of eugenol, which is an aromatic agent in the food industry and a fragrance agent in the cosmetics industry (15). It is also used in endodontics as a sedative in dental surgery. This compound induces effects on the central nervous system in mammals (hypothermia, decreased in motor activity, anticonvulsant, and a general anaesthetic). It also has antibacterial, antiviral, and antifungal activity (20, 21) and also antioxidant and prooxidant activity (22,23).

Referring to the chemical composition of the essential oil of the *O.campechianum* collected in northwest Colombia, methyl eugenol (12%) is the major component followed by germacrene D (10.1%), β-bourbonene (9.5%), eugenol (9%), *trans*-β-caryophyllene (7.8%), and α-bulnesene (7.1%). Methyl eugenol has been reported as major component in other species of *Ocimum*, [*O. sanctum* (20%), *O. basilicum var. minimum* of Taiwanese origin (42.9–64.3%), *O. basilicum* at the root of the species of the same origin (53.1%)], whereas this compound has been found in traces or very low rates in *O. canum, O. basilicum var. Canum, O. gratissimum,* and *O. rubrum*. As it was said before, species *Ocimum* are characterized by containing various chemotypes, which differ in the chemical composition of the essential oil. This variation is mainly due to chemical polymorphism, which according to Hiltunen and Holm (4), Marotti (8) and McGimpsey et al. (24), is caused by the intraspecific hybridization. These differences are also attributed to genetics and other type of factors, such as climatic conditions, geographical origins, and development phase of the plant. In this order of ideas, there are authors who refer to eugenol as the major component of the essential oil of *O.campechianum* (25–27). Maia et al. (28) in Brazil reports 1,8-Cineol as the major compound followed by α-Humuleno, *trans*-Ocimene, Aromadendrene, and

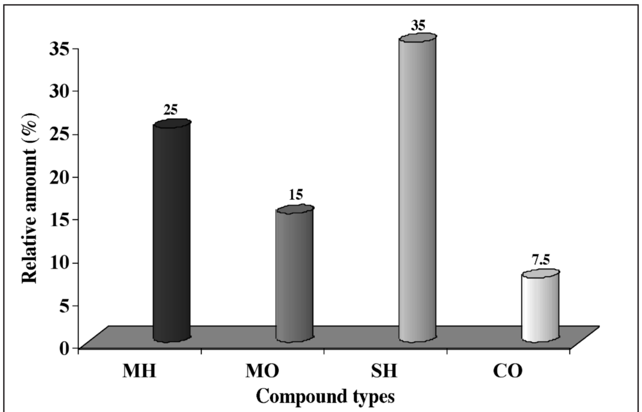

Figure 1. Distribution of the main families of compounds in the essential oil of *O. gratissimum*. MH: monoterpenes hydrocarbons, MO: monoterpenes oxygenated, SH: sesquiterpenes hydrocarbons, CO: others oxygenated components.

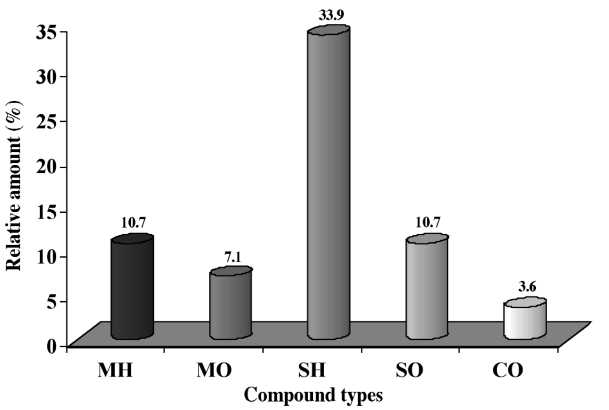

Figure 2. Distribution of the main families of compounds in the essential oil of *O. campechianum*. MH: monoterpenes hydrocarbons, MO: monoterpenes oxygenated, SH: sesquiterpenes hydrocarbons, SO: sesquiterpenes oxygenated, CO: others oxygenated components.

β-Caryophillene; Viña and Murillo (29) in the Department of Tolima in Colombia reports *trans*-methyl cinnamate (74.52%) followed by methyl chavicol (12.33%) and *cis*-methyl cinnamate (5.27%), classifying this oil as methyl cinnamate chemotype.

Regarding the study of essential oils, GC–MS technique represents an important tool in determining the chemical composition in a reliable way, which is very important in order to identify biological resources potentially useful for humanity. Both eugenol and its analogue methyl eugenol have a powerful antidepressant activity of the central nervous system (15,30). This compound is also used as a flavoring agent in the food and perfume industry (31).

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