

Essential Oil Composition from Two Species of Piperaceae Family Grown in Colombia

Nayive Pino Benitez^{1,*}, Erika M. Meléndez León¹, and Elena E. Stashenko²

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

Abstract

Essential oil compositions of aerial parts from two species in the *Piper* (*Piperaceae* family) genera: *Piper lanceaeifolium* Kunth and *Piper hispidum* Sw., frequently called deflated (for the anti-inflammatory activity) or cord. *Piperaceae* leaves were collected in different regions of the Chocó department in northwestern Colombia and identified by botanists from Colombian National Herbarium, where a voucher of each specimen were deposited (No- COL 519993 and No- COL 519969, respectively). The essential oils were obtained by microwave-assisted hydrodistillation (MWH) and analyzed by gas chromatography-mass spectrometry (GC-MS). The *P. lanceaeifolium* essential oil was sesquiterpenoid type (71.7%). This composition was represented by sesquiterpenes hydrocarbons (58.5%) and by their oxygenated derivatives (13.2%); the main compounds were, trans- β -caryophyllene (11.6%) and germacrene D (10.7%) followed by α -selinene (7.8%), β -pinene (5.4%), β -selinene (4.8%), and α -cubebene (4.3%). The *Piper hispidum* essential oil also was sesquiterpene type (74.4%) and oxygenated sesquiterpenes (46.4%) followed by sesquiterpenes hydrocarbons (28.0%). The main compounds were trans-nerolidol (23.6%) and caryophyllene oxide (5.4%) followed by β -elemene (5.1%), trans- β -caryophyllene (5.1%), curzerene (4.9%), and germacrene B (4.5%). Trans- β -caryophyllene presents the higher percentage of the common compounds in the two species' essential oil (11.6% and 5.1% in *P. lanceaeifolium* and *P. hispidum*, respectively).

Introduction

Piperaceae family has four genera and more than 2000 species, many of which are used in traditional medicine in the treatment of diverse illnesses like rheumatic, molars and stomach pains. It has an antinfectious and healing nature as Pino et al. described it (1).

These plants are in a great variety of habitats. They can be bushes, frutices, or herbs. They grow in humid and shady places,

and they can grow in warm, mild, or cold climates, according to García et al (2). Forero and Gentry (3) reports Piperaceae family is one of the dicotyledoneae better represented in the chochoan floristic with four genera and 140 species. The species used in this study, *P. lanceaeifolium* and *P. hispidum*, are also part of the Peperomia genera. The analysis of the volatile constituents of some Piperaceae family species has been the subject of diverse studies, which have revealed the presence of monoterpenes, sesquiterpenes, and arylpropanes (apiol, dillapiol, miristicin, safrol, and sarisan). These show important biological properties, according to Dias Do Santos et al (4). The essential oil chemical composition of *P. fulvescens*, recollected in Paraguay and analyzed by gas chromatography (GC) and GC-mass spectrometry (MS), was reported to have these main components: anetol (26.4%) followed by ishwarano (12.1%), according to that described by Vila et al (5).

In the study developed by Martins et al. (6), the essential oils of *P. capense*, *P. guineense*, *P. umbellatum* aerial parts, and *P. nigrum* fruit were collected in the west of Africa. The *P. capense* main components were β -pinene (32.5%) and trans- β -caryophyllene (12.6%). The *P. nigrum* major essential oil constituents were limonene (18.8%), trans- β -caryophyllene (15.4%), sabinene (16.5%), and β -pinene (15.4%). *P. umbellatum* essential oil was characterized by high content of β -pinene (26.8%), α -pinene (17.6%), and trans-nerolidol (12.4%). On the other hand, the most important group of compounds in the *P. guineense* essential oil was phenylpropanes derived from dill apiol (44.8%) followed by myristicin (9.8%).

Essential oils extracted from *P. aduncum*, *P. tuberculatum*, and *P. arboreum* collected in Brazil presented a high percentage of monoterpenes type compounds; in the fruits and roots of *P. aduncum* and *P. tuberculatum* contained (85.1% / 66.9%) and (82.3% / 62.1%), respectively. In the *P. arboreum* essential oil, the sesquiterpenes content was higher (94.4% / 72.2%) than the monoterpenes (22.6% / 25.6%) for both parts of the plants' fruits and roots. In the leaves, the sesquiterpenes presence was more abundant, and β -caryophyllene made up 9.1%, 25.1%, and 40.2% in the *P. aduncum*, *P. arboreum*, and *P. tuberculatum* species, respectively, according to that described by Debonisi et al (7).

*Author to whom correspondence should be sent: email nayivepino@yahoo.com.

Experimental

Plant material

All plant material of the *Piper* species was collected by research group Natural Products of Technological University of Chocó, located in the settlement of Pacurita, Municipality of Quibdó (Chocó). The taxonomic identification of the plants was carried out in the Colombian National Herbarium (COL) of the Institute of Natural Sciences, Faculty of Sciences of the National University of Colombia (Bogotá). The witnesses sheets of each plant were stored as permanent displays as follows: *Piper lanceaefolium* Kunth. COL No. 519993 and *Piper hispidum* Sw., COL No. 519969.

Extraction of essential oil

Essential oils are extracted from *P. lanceaefolium* and *P. hispidum* leaves, respectively. Microwave-assisted hydrodistillation (MWHHD) was used as an extracting method as Stashenko et al. (8) describes it, using a sort of Clevenger-type distillation device with a Dean-Stark distillation reservoir. This adapted to global warming by microwave radiation through a conventional LG microwave MS-1242 ZK model set at 2450 MHz, 800 W. Two hundred milliliters of distilled water were added to the plant material. The extraction was conducted in 10 intervals; 10 min for each one with 5 min rest time each one.

Sample preparation

A portion of each essential oil (20 μ L) was dissolved in dichloromethane to a final volume of 1 mL, and then transferred to vials in this volume for GC-MS.

GC-MS

The chromatographic analysis was performed using an Agilent Technologies 6890 Plus (Palo Alto, CA) GC coupled to an Agilent Technologies MSD 5975 selective detector mass equipped with a split/splitless injector port (1:50 split ratio), an automatic injector Agilent 7863, and a data system HP ChemStation. One microliter of solvent was injected into the GC-MS equipment for the corresponding chromatographic analysis. A 60 m \times 0.25 mm i.d. \times 0.25 μ m with 5% phenyl poly(methylsiloxane) stationary phase. The oven temperature was set at 45°C for 5 min, then increased 4°C/min up to 150°C for 2 min, then to 5°C/min up to 250°C for 5 min, and finally at 10°C/min up to 275°C. Helium was used as a carrier gas with 16.47 psi column head pressure and 1 mL/min linear velocity.

Retention indices

The retention indices (IR) were calculated through a series of homologues aliphatic saturated hydrocarbons between C8-C25, analyzed in the same column with the same chromatographic conditions which were used for the essential oils analysis.

Compounds identification

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) (9,10).

Results and Discussion

The chemical composition results found for the essential oils from two Piperaceae family species are presented in Table I. The *P. lanceaefolium* specie essential oil, characterized by high sesquiterpenes content (71.7%), is comprised of sesquiterpenes hydrocarbons (58.5%) and oxygenated derivatives (13.2%); the main components are trans- β -caryophyllene (11.6%) and germacrene D (10.7%) followed by α -selinene (7.8%), β -pinene (5.4%), β -selinene (4.8%), and α -cubebene (4.3%). The main distribution compound in the *P. lanceaefolium* essential oil is given in Figure 1. The *P. hispidum* essential oil was characterized to have higher sesquiterpenes compound content (74.4%), which is represented by sesquiterpenes hydrocarbons (28.0%) and their oxygenated derivatives (46.4%). Transnerolidol and caryophyllene oxide (23.6% and 5.4%, respectively) were the main components, followed by β -elemene (5.1%), trans- β -caryophyllene (5.1%), curzerene (4.9%), and germacrene B (4.5%). The main distribution compound in the *P. hispidum* essential oil is given in Figure 2.

Table I. Composition of Essential Oils Determined in Two Species of Piper Collected in Chocó, Colombia

Compound*	I_k^\dagger	Relative amount (%)	
		<i>P. lanceaefolium</i>	<i>P. hispidum</i>
α -Pinene	936	3.6	3.4
Canfene	954	0.2	–
β -Pinene	983	5.4	3.6
β -Myrcene	990	0.3	0.4
p-Cymene	1026	0.8	–
Limonene	1031	0.3	0.6
cis- β -Ocimene	1034	0.2	0.4
trans- β -Ocimene	1044	–	3.1
γ -Terpinene	1057	0.2	–
Linalool	1093	0.5	1.9
Perilene	1106	–	0.3
α -Terpineole	1196	0.3	0.3
Linalyl Acetate	1244	–	0.7
Bicycloelemene	1336	0.1	–
α -Cubebene	1353	4.3	0.2
α -Ylangene	1377	0.2	1.4
Sesquiterpene C ₁₅ H ₂₄	1385	3.8	0.3
β -Elemene	1394	2.5	5.1
α -Gurjuneno	1417	0.2	–
Sesquiterpene C ₁₅ H ₂₄	1428	–	0.2
trans- β -Caryophyllene	1433	11.6	5.1
γ -Elemene	1435	–	2.8
β -Copaene	1440	0.8	–
Sesquiterpene C ₁₅ H ₂₄	1446	–	0.4
Aromadendrene	1449	0.4	–
trans- β -Farnesene	1456	0.4	–
trans-Muurolo-3,5-diene	1459	0.2	–
α -Humulene	1467	2.6	0.7
allo-Aromadendrene	1471	–	0.3
cis-Cadina-1(6),4-diene	1472	0.3	–
γ -Muuroloene	1483	4.0	1.2
Sesquiterpene C ₁₅ H ₂₄	1487	0.5	0.3
trans-Muurolo-4(14)-5-diene	1491	–	2.9
Germacrene D	1493	10.7	–
Pentadecane	1497	2.5	–
Curzerene	1500	–	4.9
β -Selinene	1501	4.8	–
Sesquiterpenol	1506	0.9	–
Sesquiterpenol	1507	–	2.9

* Compounds listed in elution order;

† Retention Index in DB-5MS column.

The compounds, α -pinene, β -pinene, limonene, linalool, α -cubebene, β -elemene, trans- β -caryophyllene, α -humulene, γ -cadinene, trans-nerolidol, spathulenol, and caryophyllene oxide were found in the two species' oils, presenting a proportion variable in their percentages among these common compounds. The trans- β -caryophyllene presented the higher percentage (11.6% and 5.1% in *P. lanceifolium* and *P. hispidum*, respectively), according to that described by Fernandes et al (11). The sesquiterpenes β -humulene and trans- β -caryophyllene have anti-inflammatory activity.

The chemical composition of the essential oil of the specie *P. lanceaefolium*, collected both in Colombia and Costa Rica, shows high levels of the sesquiterpenes hydrocarbons, β -caryophyllene and germacrene D, with 58.0% and 42.8%, respectively. However, there is a marked difference regarding the presence of phenylpropanoid (36.3%), elemicin (16.4%), parsley, and apiol (9.8%) in the essential oil from Costa Rica, as referred to by Mundina et al. (12) that were not found in the oil collected in Colombia. *P. lanceaefolium* also presents chemical polymorphism, which is corroborated by literature (12). In addition, after analyzing the results of the chemical composition, it

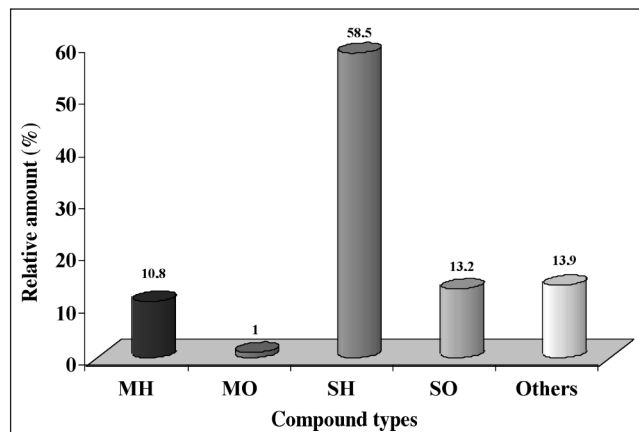


Figure 1. Distribution of the compound in *P. lanceifolium* essential oil: MH, hydrocarbons monoterpenes; MO, oxygenated monoterpenes; SH, hydrocarbons sesquiterpenes; SO, oxygenated sesquiterpenes; CO, others components oxygenated.

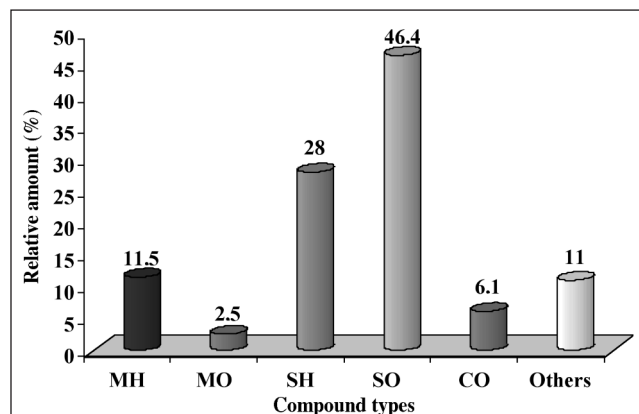


Figure 2. Distribution of the compound in *P. hispidum* essential oil: MH, hydrocarbons monoterpenes; MO, oxygenated monoterpenes; SH, hydrocarbons sesquiterpenes; SO, oxygenated sesquiterpenes; CO, others components oxygenated.

was found that the trans-nerolidol (23.6%) is the major component of the essential oil of the *P. hispidum* that grows in Chocó-Colombia contrary to the results obtained by Pino et al. (13) in Cuba. The chemical composition of the essential oil of the *P. hispidum* leaves presented great variability, and the β -eudesmol (17.5%) is the major component. Since both essential oils were obtained by using similar methods, MWHD in a Clevenger device. Differences in the composition of their essential oils could be due to natural chemical variations called chemotype, which occur in the secondary metabolism of plants and could possibly be induced by environmental factors such as soil type, altitude, sun exposure, rain, gather or seasonal variation, and in accordance with McGimpsey et al. (14), can also be influenced by genetic factors.

The difference between the chemical composition of the fruits' essential oil [limonene (16.3%), β -pinene (14.5%), linalool (9.6%), α -terpineol (8.5%)] (15) and the chemical composition of the leaves of the *P. hispidum* of this work [trans-nerolidol (23.6%) followed by caryophyllene oxide (5.4%), β -elemene (5.1%), trans- β -caryophyllene (5.1%), curzerene (4.9%), and germacrene B (4.5%)] can be due to the fact that the chemical composition of the essential oil varies according to the part of the plant that contains it.

Table I. (Continued) Composition of Essential Oils Determined in Two Species of Piper Collected in Chocó, Colombia

Compound*	I _R [†]	Relative amount (%)	
		<i>P. lanceaefolium</i>	<i>P. hispidum</i>
α -Selinene	1508	7.8	–
Sesquiterpene C ₁₅ H ₂₄	1511	–	1.4
γ -Cadinene	1523	1.2	0.4
Sesquiterpenol	1526	–	1.2
trans-Calamenene	1532	2.1	–
trans-Cadina-1,4-diene	1541	0.3	–
Sesquiterpene C ₁₅ H ₂₄	1545	–	0.2
Elemicin	1548	0.8	–
Sesquiterpene C ₁₅ H ₂₄	1550	–	0.6
Sesquiterpenol	1552	–	0.7
trans-nerolidol	1555	0.3	23.6
Elemol	1557	–	3.6
β -Calacorene	1573	0.1	–
Germacrene B	1574	–	4.5
Davanona B	1582	–	0.3
Spathulenol	1592	2.4	0.8
Caryophyllene oxide	1599	3.4	5.4
Humulene epoxide II	1625	0.4	–
Humulene oxide	1626	–	0.4
Sesquiterpenol	1632	0.8	0.5
Oxygenated compound C ₁₅ H ₂₆ O	1640	1.8	–
Sesquiterpenol	1640	0.4	–
γ -Eudesmol	1645	–	1.1
α -Eudesmol	1672	–	2.4
Sesquiterpenol	1675	2.6	–
11-Selinene-4- α -ol	1676	–	1.4
Sesquiterpenol	1697	–	0.3
Sesquiterpenol	1725	–	0.5
Monoterpene hydrocarbons		10.8	11.5
Oxygenated monoterpenes		1.0	2.5
Sesquiterpene hydrocarbons		58.5	28.0
Oxygenated sesquiterpenes		13.2	46.4
Oxygenated compounds		–	6.1
N.I.		13.9	4.9
Others		–	6.1
Total		97.4	99.4

* Compounds listed in elution order; [†] Retention Index in DB-5MS column.

Studies have been made about the composition of several essential oils of Piper genera plants (16). Phenylpropanes, monoterpenes, and sesquiterpenes were established as the main constituents in this study. It was observed that the two analyzed species are of the sesquiterpene type as well.

In *P. lanceaefolium* essential oil, *trans*- β -caryophyllene and sesquiterpene are also present in natural products, such as the oil of cloves, cinnamon leaves, and copaiba balsam, all of which have been used as natural remedies and as fragrances. The odor of *trans*- β -caryophyllene is described as woody and spicy (17,18). It also showed anti-inflammatory activity in several animal models (19,20). Due to the presence of transnerolidol compound, a major component in *P. hispidum* essential oil, this species has potential use (21–23) and can act as a food-flavoring agent and exhibits antineoplastic against the malaria parasite and leishmanicidal activity.

Acknowledgements

Financial support from CENIVAM and COLCIENCIAS (Colombia) are gratefully acknowledged.

References

1. N. Pino Benítez, T. Yurgaky, and J. Cuesta. Aspectos botánicos y química preliminar de seis especies del género Piper usadas como medicinales en el Municipio de Quibdó-Chocó. Universidad Tecnológica del Chocó. "Diego Luis Córdoba". Rev. Institucional (23). Investigación Biodiversidad y Desarrollo. (2005).
2. H. García. Flora Medicinal de Colombia, Botánica Médica. Tomo I. 2nd Ed., Tercer Mundo Ed. Colombia, 1992, p. 222.
3. E. Forero and A. Gentry. Lista de las Plantas del Departamento del Chocó. Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Guadalupe Ltda Ed., Bogotá-Colombia, 1989, p. 11, 121.
4. P.R. Dias, D. de Lima, E.F. Guimara, and M.A. Coelho. Essential oil analysis of 10 Piperaceae species from the Brazilian Atlantic forest. *Phytochem.* **58**: 547–551 (2001).
5. R. Vila, B. Milo, F. Tomi, J. Casanova, E.A. Ferro, and S. Cañigueral. Chemical composition of the essential oil from the leaves of *Piper fulvescens*, a plant traditionally used in Paraguay. *J. Ethnopharmacol.* **76**: 105–107 (2001).
6. P. Martins, L. Salgueiro, R. Vila, F. Tomi, S. Cañigueral, J. Casanova, A. Proença, and T. Adzet. Essential oils from four Piper species. *Phytochem.* **49**(7): 2019–2023 (1998).
7. H.M. Debonis, A. Morandim, A.C. Alécio, L.O. Regasini, D.C.B. Bergamo, M. Telascra, A.J. Cavalheiro, M.N. Lopes, V.da S. Bolzani, M. Furlan, M.O.M. Marques, M.C.M.Young, and M. J. Kato. Composition and antifungal activity of essential oils from *Piper aduncum*, *Piper arboreum* and *Piper tuberculatum*. *Quím. Nova* **29**(3): (2006).
8. E.E. Stashenko, B.E. Jaramillo, and J.R. Martinez. Comparison of different extraction methods for the analysis of volatile secondary metabolites of *Lippia alba* (Mill.) N.E. Brown, grown in Colombia, and evaluation of its in vitro antioxidant activity. *J. Chromatogr. A* **1025**: 93–103 (2004).
9. R.P. Adams and R.P. Adams. Identification of essential oil components by gas chromatography/quadrupole mass spectrometry, 4th ed., Allured Publishing Corporation. Carol Stream, Illinois. (2004).
10. D. Joulan and W. Kong. The atlas of spectral data of sesquiterpene hydrocarbons, E.B. Verlag Hamburg, Germany. (1998).
11. E.S. Fernandes, G.F. Passos, R. Medeiros, F.M. da Cunha, J. Ferreira, M.M. Campos, L.F. Pianowski, and J.B. Calixto. Anti-inflammatory effects of compounds alpha-humulene and (-)-*trans*-caryophyllene isolated from the essential oil of *Cordia verbenacea*. *Eur. J. Pharmacol.* **569**: 228–236 (2007).
12. M. Mundina, R. Vila, F. Tomi, X. Tomàs, J.F. Ciccio, T. Adzet, J. Casanova, and S. Cañigueral. Composition and chemical polymorphism of the essential oils from *Piper lanceaefolium*. *Biochem. Syst. Ecol.* **29**: 739–748 (2001).
13. J.A. Pino, R. Marbot, A. Bello, and A. Urquiola. Composition of the essential oil of *Piper hispidum* Sw. from Cuba. *J. Essent. Oil Res.* **16**: 459–460 (2004).
14. J.A. McGimpsey, M.H. Douglas, J.W. van Klink, D.A. Beavregard, and N.B. Perry. Seasonal variation in essential oil yield and composition from naturalized *Thymus vulgaris* L., in New Zealand. *Flavor Fragr. J.* **9**: 347–352 (1994).
15. W.A. Delgado and L.E. Cuca. Composición química del aceite esencial de los frutos de *P. hispidum* Kunth. *Revista de Productos Naturales. Artículos de Investigación* **1**(1): 5–8 (2007).
16. J.F. Ciccio Constituyentes del aceite esencial de las hojas de *Piper terrabanum* (Piperaceae). *Revista de Biología Tropical* **44**: 507–511 (1996).
17. M. Sköld, A.T. Karlberg, M. Matura, and A. Börje. The fragrance chemical β -caryophyllene-air oxidation and skin sensitization. *Food Chem. Toxicol.* **44**: 538–545 (2006).
18. J. Verghese. Fragrances from caryophyllene, the sesquiterpene constituent of clove oil. *J. Pafai.* **16**: 21–25 (1994).
19. S. Martin, E. Padilla, M.A. Ocete, J. Galvez, Jiménez and J.A. Zarzuelo. Anti-inflammatory activity of the essential oil of *Bupleurum frutescens*. *Planta Med.* **59**: 533–536. (1993).
20. C. Ghelardini, N. Galeotti, L. Mannelli, G. Mazzanti, and A. Bartolini. Local anaesthetic activity of β -caryophyllene. *Il Farmaco* **56**: 387–389 (2001).
21. L.W. Wattenberg. Inhibition of azoxymethane-induced neoplasia of the large bowel by 3-hydroxy-3,7,11-trimethyl-1,6,10-dodecatriene (nerolidol). *Carcinogenesis* **12**: 151–152. (1991).
22. N.P. Lopes, M.J. Kato, E.H. Andrade, J.G. Maia, M. Yoshida, A.R. Planchart, and A.M. Katzin. Antimalarial use of volatile oil from leaves of *Virola surinamensis* (Rol.) Warb. by Waiapi Amazon Indians. *J. Ethnopharmacol.* **67**: 313–319. (1999).
23. D.C. Arruda, F.L. D'Alexandri, M. Katzin, and S.R. Uliana. Antileishmanial Activity of the Terpene Nerolidol Antimicrob. Agents Chemother. 1679–1687 (2005).

Manuscript received November 5, 2008;
Revision received April 11, 2009.