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Comparative study on the Chemical composition and antibacterial activity of the essential oil of *Chromolaena laevigata* (Lam.) R.M. King & H. Rob. collected from Mérida-Venezuela

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Abstract

Fresh leaves of *C. laevigata* collected from the same location in January (**Jan**), July (**Jul**) and December (**Dec**) 2010 were analyzed by **GC/MS**. The main compounds of the **Jan** sample were laevigatine (59.60%), germacrene-*D* (7.75%) and β-sesquiphellandrene (6.56%), for the **Jul** oil laevigatine (62.61%), germacrene-*D* (7.37%), β-sesquiphellandrene (6.67%) and β-caryophyllene (5.39%), while **Dec** sample showed laevigatine (44.05%), β-sesquiphellandrene (8.59%) and β-caryophyllene (5.96%) as major components. Antibacterial assays showed activity against *E.faecalis* (1000 μg/mL), *S. aureus* (25 μg/mL) and *S. aureus* methicilline resistant (1000 μg/mL).

Keywords: *Chromolaena laevigata*, essential oil, laevigatine, antibacterial activity.

Estudio comparativo de la composición química y actividad antibacteriana del aceite esencial de *Chromolaena laevigata* (Lam.) R. M. King & H. Rob. colectada en Mérida-Venezuela

Resumen

Las hojas frescas de *C. laevigata* recolectadas en la misma localidad en enero (**Jan**), julio (**Jul**) y diciembre (**Dec**) 2010 fueron analizados por **CG/EM**. Los componentes principales de la muestra (**Jan)** fueron laevigatina (59,60%), germacreno-*D* (7,75%) y β-sesquifelandreno (6,56%), para la muestra (**Jul)** fueron laevigatina (62,61%), germacreno-*D* (7,37%), β-sesquifelandreno (6,67%) y β-cariofileno (5,39%), mientras que la muestra (**Dec)** mostró como mayoritarios: laevigatina (44,05%), β-sesquifelandreno (8,59%) y β-cariofileno (5,96%). Los ensayos realizados mostraron actividad antibacteriana contra *E. faecalis* (1000 μg/mL), *S. aureus* (25 μg/mL) y *S. aureus* resistente a la meticilina (1000 μg/mL).

Palabras clave: *Chromolaena laevigata*, aceite esencial, laevigatina, actividad antibacteriana.

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Introduction

Chromolaena laevigata (Lam.) R. M. King & H. Rob. (syn. *Eupatorium laevigata*) belongs to Asteraceae family. Comprises approximately 165 species distributed in the tropical and warm temperate regions of the Americas, ranging from Mexico to Argentina (1, 2). Traditionally has been used to treat inflammations (3), malaria (4), coughs, colds, skin diseases (2), as insect repellent (5, 6) and for its antibacterial activities (7, 8); moreover, this plant exhibits allelopathic effects and has been reported to cause livestock death (9).

Phytochemical studies have revealed diterpene glucosides (10) and flavones (11) as main compounds in different *Chromolaena* species. Otherwise, the essential oil composition of several *Chromolaena* and *Eupatorium* species have also been reported: for *E. cannabinum* germacrene-*B*, germacrene-*D* and β-caryophyllene were the main components, *E. odoratum* from Cameroon and Congo showed α-pinene and *p*-cymene being the most dominant, while *E. coelestinum* collected from Vietnam revealed methyl chavicol in stems and leaves (12). The present investigation aims to reveal the composition of the essential oil of *C. laevigata* collected from Mérida and evaluate its antibacterial activity. According to the references consulted this is the first report in Venezuela regarding the volatile compounds of this species as well as its antibacterial potential.

Materials and methods

Plant material: fresh leaves of *C. laevigata* were collected in three different seasons of the year 2010, **Jan** (2930 g), **Jul** (2569 g) and **Dec** (1224 g) from La Hechicera, Municipio Libertador, Mérida state, located at 1950 m above sea level. A voucher specimen (**MERF**-001) was deposited in the Herbarium of the Faculty of Pharmacy and Bioanalysis, University of Los Andes.

Isolation of essential oil: fresh leaves were cut into small pieces and subjected to hydrodistillation for 4 h, using a Clevenger-type apparatus. The oil was dried over anhydrous sodium sulfate, to avoid water traces remained on the oil, and then it was stored at 4ºC. The yields were as follow: **Jan** (0.8 mL/0.02%), **Jul** (0.6 mL/0.02%) and **Dec** (0.3 mL/0.03%).

Gas chromatography (GC): **GC** analyses were performed on a Perkin-Elmer AutoSystem gas chromatograph equipped with flame ionization detectors. A 5% phenylmethyl polysiloxane fused-silica capillary column (AT-5, Alltech Associates Inc., Deerfield, IL), 60 m x 0.25 mm, film thickness 0.25 μm was used for the **GC** analysis. The initial oven temperature was 60°C; this was then raised to 260°C at 4°C/min, and the final temperature maintained for 20 min. The injector and detector temperatures were 200°C and 250°C, respectively. The carrier gas was helium at 1.0 mL/min. The sample was injected using a split ratio of 1:100. Retention indices were calculated relative to C_8-C_{24} n-alkanes, and compared with values reported in the literature (13, 14).

Gas chromatography-mass spectrometry (GC-MS): The **GC-MS** analyses were carried out on a Hewlett Packard **GC-MS** system, Model 5973, fitted with a 30 m long, cross-linked 5% phenylmethyl siloxane (HP-5MS, Hewlett Packard, USA) fused-silica column (0.25 mm, film thickness 0.25 μm). 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 40-500 amu; 3.9 scans/s. The injected volume was 1.0 μL of a 2% dilution of oil in n-heptane. A Hewlett-Packard ALS injector was used with split ratio 1:100. The identification of the oil components was based on a Wiley **MS** Data Library (6th edn), followed by comparisons of **MS** data with published literature (13).

Bacterial strains: Five strains from the *American Type Culture Collection (**ATCC**) were used in the present investigation: Staphylococcus aureus* (25923), *Enterococcus faecalis* (29212), *Escherichia coli* (25992), *Pseudomonas aeruginosa* (27853) and *Klebsiella pneumoniae* (23357). Furthermore, five multirresistant bacterial strains of nosocomial origin were also included in this study: *Acinetobacter baumanii*, *Klebsiella pneumoniae* producing Extended Spectrum β-lactamases (**ESBL**), *Klebsiella pneumoniae* non producing **ESBL**, *Pseudomonas aeruginosa* and *Staphylococcus aureus* methicillin resistant.

Antimicrobial method: The antimicrobial assay was carried out according to the disc diffusion method described by Rondón *et al.*, (15). The strains were maintained in agar conservation at room temperature. Every bacterial inoculum (2.5 mL) was incubated in Mueller-Hinton broth at 37ºC for 18 h. The bacterial inoculum was diluted in sterile 0.85% saline to obtain turbidity visually comparable to a McFarland Nº 0.5 standard (106-8 CFU/mL). Every inoculum was spread over plates containing Mueller-Hinton agar and a filter paper disc (6 mm in diameter) saturated with 10 μL of essential oil. The plates were left for 30 min at room temperature and then incubated at 37ºC for 24 h. The inhibitory zone around the disc was measured and expressed in mm. A positive control was also used to check the sensitivity of the tested organisms using: Ciprofloxacin® (5μg), Imipenem® (10μg), Piperacillin-tazobactam® (100μg/10μg), Vancomycin® (30 μg), Ampicillin-sulbactam® (10μg/10μg) and Polymyxin B® (300 units), that are reference antibiotics commonly used to treat this kind of bacteria. The minimal inhibitory concentration (**MIC**) was determined only with microorganisms that displayed inhibitory zones. **MIC** was determined by dilution of the essential oil in dimethylsulfoxide (**DMSO**) and pipetting 10 μL of each dilution onto a filter paper disc. Dilutions of the oil within a concentration range of 25-1000 μg/mL were utilized. **MIC** was defined as the lowest concentration that inhibited the visible bacterial growth (16).

A negative control was also included using a filter paper disc saturated with **DMSO** to check possible activity of this solvent against the bacteria assayed. The experiments were repeated three times.

Statistical analysis: All the experiments were conducted by triplicate and statistical analysis of the data was performed by analysis of variance (**ANOVA**) using STARGRAPHICS plus 5.1 (17). Differences with p values < 0.05 were considered statistically significant. Data is presented as mean values ± standard.

Results and Discussion

Fresh leaves of *C. laevigata* collected from the same location in **Jan**, **Jul** and **Dec** 2010 were analyzed by **GC/MS** detecting the presence of 17, 14 and 13 components respectively. A list of identified components, along with their percentages of the total oil is given in table 1. The main compounds of the **Jan** sample were laevigatine (59.60%), germacrene-*D* (7.75%) and β-sesquiphellandrene (6.56%), for the **Jul** oil laevigatine (62.61%), germacrene-*D* (7.37%), β-sesquiphellandrene (6.67%) and β-caryophyllene (5.39%), while **Dec** sample showed laevigatine (44.05%), an unknown component showing the following mass spectrum [m/z (rel int.): M^+210(100);195(25);165(25)] (15.20%), β-sesquiphellandrene (8.59%) and β-caryophyllene (5.96%) as major components.

Several differences might be observed in the three oil samples, limonene and β-chamigrene were detected only in the **Jan** sample, while 3-hexenol and apofarnesol-*(Z)*-dehydro were only observed in the **Jan** and **Jul** samples. *Z*-α-bisabolene was absent in the **Jul** sample but present in the other two.

Table 1
Essential oil composition of fresh leaves of *Chromolaena laevigata* collected in three different seasons of the year

Compounds	**Jan** (%)	**Jul** (%)	**Dec** (%)	**RI**
3-hexenol	0.24	0.19	–	851
limonene	0.15	–	–	1030
β-caryophyllene	0.52	5.39	5.96	1428
β-farnesene	**4.03**	**3.90**	**4.39**	**1468**
β-*chamigrene*	0.6	–	–	1472
τ-muurolene	0.25	0.24	0.29	1489
germacrene-*D*	**7.75**	**7.37**	**5.07**	**1494**
β-bisabolene	**2.81**	**2.74**	**2.96**	**1497**
Z-α-bisabolene	0.35	–	0.46	1514
β-sesquiphellandrene	**6.56**	**6.67**	**8.59**	**1521**
τ-γ-bisabolene	1.28	1.32	1.35	1527
apofarnesol-(*Z*)-dehydro	0.50	0.42	–	1568
caryophyllene oxide	0.46	0.43	1.00	1588
α-cadinol	0.39	0.35	0.54	1630
laevigatine	**59.60**	**62.61**	**44.05**	**1731**
210(100%); 195(25%) 165(25%)	1.75	1.75	15.20	1920
kaur-16-ene	0.58	0.56	1.03	2040

The composition of the essential oil was determined by comparison of the **MS** of each component with Wiley **GC/MS** library data and also from its retention index (**RI**).

Previous investigations in several *Eupatorium* species have reported the presence of germacrene-*D*, germacrene-*B*, caryophyllene, caryophyllene oxide, valencene, methyl chavicol among theirs components (18-20). However valencene, α-pinene, *p*-cymene, -pinene, pregeijerene and zingiberene have also been reported (21, 22).

Laevigatine was present in considerable amounts in the three collections of *C. laevigata* achieved in different seasons in the present investigation, however, the same component has also been reported in high concentrations previously, for example, *E. laevigata* (23, 24) showed 59.63% of laevigatine alone with 23.63% of aristolone; another study carried out with flowers, leaves and aerial parts of *E. laevigatum* collected from Brazil, reported around 30% of laevigatine, being the main component in the three samples analyzed (25).

On the other hand, antibacterial activity was tested with five **ATCC** strains and five multirresistant bacterial strains of nosocomial origin, showing activity against *E. faecalis* (1000 μg/mL), *S. aureus* (25 μg/mL) and *S. aureus* methicilline resistant (1000 μg/mL); the complete results of this assay are detailed in table 2. For this analysis a mixture of the three oil samples was used;

Table 2
Antibacterial activity of the essential oil of *Chromolaena laevigata* in ATCC and multirresistant bacterial strains of nosocomial origin

		***Inhibition zone (mm)**						**CIM (µg/mL)**
		Essential oil	**Positive control**					
			Cpr	**Imp**	**P/t**	**Va**	**A/S**	**Po**	
Microorganisms	*Enterococcus faecalis* **ATCC 29212**	7	24						1000
	Klebsiella pneumoniae **ATCC 23357**	NA		25					NT
	Pseudomonas aeruginosa **ATCC 27853**	NA			25				NT
	Staphylococcus aureus **ATCC 25923**	10				17			25
	Escherichia coli **ATCC 25922**	NA					33		NT
Multi resistant bacterial strains of nosocomial origen	*Acinetobacter baumanii*	NA						15	NT
	Klebsiella pneumoniae producing **ESBL**	NA		28					NT
	Klebsiella pneumoniae non producing **ESBL**	NA		18					NT
	Pseudomonas aeruginosa	NA			26				NT
	Staphylococcus aureus methicillin resistant	8				16			1000

Cpr: Ciprofloxacin® (5µg), **Imp**: Imipenem® (10µg), **P/t**: Piperacillin-tazobactam® (100µg/10µg), **Va**: Vancomycin® (30µg), **A/s**: Ampicillin-sulbactam® (10µg/10µg), **Po**: Polymyxin B® (300 units), **ESBL**: Extended Spectrum β-*Lactamases*;***Inhibition zone**, diameter measured in mm, disc diameter 6 mm, values are means ± standard deviation of three separated experiments. **MIC**: Minimal Inhibitory Concentration (concentration range: 25-1000µg/mL), **NA**: None active, **NT**: None tested.

this combination was likely due to the very low amounts obtained for each one and also considering that major compounds were similar in the three collections.

Antimicrobial activity has also been performed previously for several *Chromolaena* species. Essential oil obtained from aerial parts of *C. laevigata* showed activity against *C. albicans* and *S. aureus* (26). In addition *C. odorata* from Nigeria (27) showed activity against *Bacillus cereus* (39 μg/mL) and antifungal activity against *Aspergillus niger* (78 μg/mL), while the methanol, dichloromethane and hexane extracts of *C. laevigata (28)* leaves revealed the inhibition of *Staphylococcus aureus* and *Bacillus cereus at the* concentration of 40 mg/mL.

Conclusions

The **GC/MS** analysis of the essential oil of *C. laevigata* collected in three different times of the year (January, July and December) showed almost same yielding for the major compound, laevigatine in the **Jan** and **Jul** collections, but this amount decreased in the **Dec** sample, possibly due to environmental factors and plant growth stage. For β-sesquiphellandrene, germacrene-D, β-bisabolene and β-farnesene the yielding values were very similar between the three collections. On the other hand, antibacterial activity was observed against *E. faecalis* (1000 μg/mL), *S. aureus* (25 μg/mL) and *S. aureus* methicillin resistant (1000 μg/mL) in the oil analized.

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Bibliographic references

1. MABBERLEY D. ***The plant book***. Cambridge University Press, 2nd Ed., Cambridge (UK). p 155. 1997.
2. MORTON J. ***Atlas of medicinal plants of middle america, Vol. II***. Charles C. Thomas, Publisher,Illinois (USA). pp 932-933. 1981.
3. BEDI G., TONZIBO Z., OUSSOU K., CHOPPARD C., MAHY J., N'GUESSAN T. ***Afr J Pharm Pharacol*** 4(8): 535-538. 2010.
4. BEDI G., TONZIBO Z., N'GUESSAN T. ***J Soc Ouest-Afr Chim*** 11: 29-37. 2001.
5. BOUDA H., TAPONDJOU LA., FONTEM DA., GUMEDZOE MY. ***J Stored Prod Res*** 37: 103-109. 2001.
6. CUI S., TAN S., OUYANG G., JIANG S., PAWLISZYN J. ***J Chromatogr B*** 877: 1901-1906. 2009.
7. INYA-AGHA SI., OGUNTIMEIN BO., SOFOWORA A., BENJAMIN TV. ***Int J Crude Drug Res*** 25: 49-52. 1987.
8. BAMBAD., BESSIÈRE JM., MARION C., PÉLISSIER Y., FOURASTÉ I. ***Planta Med*** 59:184-185. 1993.
9. MUNIAPPAN R., REDDY G., RAMAN A. ***Biological Control of Tropical Weeds Using Arthropods.*** Cambridge University Press, Cambridge (UK). pp 130-160. 2009.
10. EL-SEEDI H., SATA N., TORSSELL K., NISHIYAMA S. ***J Nat Prod*** 65: 728-729. 2002.
11. FLORES M., D' ARMAS H., ESTABA A., MÉNDEZ B. ***Ciencia*** 14(2): 244-252. 2006.
12. MIRZA M., NAVAEI M., DINI M. ***Iran J Pharm Res*** 2: 149-152. 2006.
13. ADAMS R. ***Identification of essential oil components by GC/MS.*** Allured Publishing Corporation, Carol Stream IL, (USA), 2007.
14. DAVIES N. ***J Chromatogr A*** 503: 1-24, 1990.

15. RONDÓN M., VELASCO J., MORALES A., ROJAS J., CARMONA J., GUALTIERI M., HERNÁNDEZ V. ***Rev Latinoamer Quím*** 33: 55-59. 2005.

16. Clinical and Laboratory Standards Institute. ***CLSI*** 35(15): 1-17. 2010.

17. STARGRAPHICS. ***Statistics program plus 5.1***. StatPoint Technologies Inc., Warrenton (USA). 2001.

18. MIRZA M., NAVAEI M., DINI M. ***Iran J Pharm Res*** 2: 149-152. 2006.

19. NGUYEN X., LUU D., PHAM V., MUSELLI A., CASANOVA J., BARTHEL A., LECLERCQ P. ***J Essent Oil Res*** 10: 478-482. 1998.

20. PINO J., ROSADO A., FUENTES V. ***J Essent Oil Res*** 10: 79-80.1998.

21. BAMBA D., BESSIERE J., MARION C., PELISSIER Y., FOURASTE I. ***Planta Med*** 59: 184-185. 1993.

22. LAMATY G., MENUT C., ZOLLO P., KUIATE J., BESSIERE J., OUAMBA J. SILOU T. ***J Essent Oil Res*** 4: 101-105. 1992.

23. MAIA J., ZOGHBI M., ANDRADE E., DA SILVA M., LUZ A., DA-SILVA J. ***Biochem Syst Ecol*** 30(11): 1071-1077. 2002.

24. OLIVEIRA A.B., CARAZZA F., RAMOS L.S., MAIA J.G. ***J Essent Oil Res*** 2: 49-50. 1990.

25. MURAKAMI C., BRUMATI F., MORENO P.R., MARX M.C**.** ***30ª Reunião Anual da Sociedade Brasileira de Química*** 204. Águas de Lindóia (Brasil). 2007**.**

26. MURAKAMI C., LIMA M.L., MORENO P.R., MARX M.C. ***32ª Reunião Anual da Sociedade Brasileira de Química.*** 1780-1781. Fortaleza (Brasil). 2009.

27. OWOLABI M.S., KAMIL A.O., LABUNMI Y., VILLANUEVA H.E., TUTEN J.A., SETZER W.N. ***Rec Nat Prod*** 4 (1): 72-78. 2010.

28. FLORES M., D'ARMAS H., HERRERA H**.** ***Ciencia*** 15(3), 421-432. 2007.