CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF *ASTRONIUM GRAVEOLENS* JACQ ESSENTIAL OIL

Vanessa Hernández[a]*, Flor Mora[a], María Araque[b], Soumi De Montijo[b], Luis Rojas[c], Pablo Meléndez[a] and Nunziatina De Tommasi[d]

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ABSTRACT

In this paper is presented a study on the chemical characterization of *Astronium graveolens* Jacq essential oil, obtained by hydrodistillation of its aerial parts. The separation of the components was performed by GC-MS. Twenty-six compounds (99.3% of the sample) were identified of which, the major ones (84.8%). Were *trans*-β-ocimene (23.9%), α-pinene (19.8%), δ-3-carene (15.5%), α-phellandrene (12.7%), *cis*-ocimene (6.4%), α-terpineol (3.3%) and *p*-cimene (3.2%). The essential oil showed a strong antibacterial activity, regardless of bacterial species or resistance mechanism. *www.relaquim.com*

KEYWORDS: *Astronium graveolens* Jacq, Anacardiaceae, *trans*-β-ocimene, essential oil, antibacterial activity, nosocomial infections.

RESUMEN

En este trabajo se presenta un estudio sobre la caracterización química del aceite esencial de *Astronium graveolens* Jacq, obtenido por hidrodestilación de sus partes aéreas. La separación de los componentes se realizó por CG-MS. Veintiséis compuestos (99,3%) fueron identificados, de los cuales los más importantes (84,8%) fueron *trans*-β-ocimeno (23,9%), α-pineno (19,8%), δ-3-careno (15,5%), α-felandreno (12,7%), *cis*-ocimeno (6,4%), α-terpineol (3,3%) y *p*-cimeno (3,2%). El aceite esencial mostró una fuerte actividad antibacteriana, independientemente de la especie bacteriana o mecanismo de resistencia. *www.relaquim.com*

PALABRAS CLAVES: *Astronium graveolens* Jacq, Anacardiaceae, *trans*-β-ocimeno, aceite esencial, actividad antibacteriana, infección hospitalaria.

[a] Department of Pharmacognosy and Organic Medicaments, Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida, Venezuela, 5101.
[b] Department of Microbiology and Parasitology, Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida, Venezuela, 5101.
[c] Research Institute, Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida, Venezuela, 5101.
[d] Department of Pharmaceutical and Biomedical Sciences, University of Salerno, Fisciano, Italia, 84084.
* Sector Campo de Oro. Facultad de Farmacia y Bioanálisis Universidad de Los Andes. Mérida. Venezuela. TFL. 00582742403486 vanessah@ula.ve

INTRODUCTION

The Anacardiaceae L. family includes more than 82 genera and 700 species, mainly distributed in the tropics (Pell, 2004). From the 32 Anacardiaceae genera 82 species are known to cause contact dermatitis because of the presence of phenolic compounds (Aguilar and Sosa, 2004). They are probably a defense against pests or insects and vertebrates (Aguilar and Sosa, 2004). *Astronium* genus (synonym: *Myracrodruon M.* Allemão), comprises 23 species of native trees from Brazil, Suriname, Colombia and Venezuela rainforests. From this genus *Astronium urundeuva* is a widely used medicinal plant in northeastern Brazil, being the aqueous extract of the bark of branches used in gynecology as an anti-inflammatory of the female genital tract, in the treatment of respiratory and urinary infections, hemoptysis, metrorrhagia and diarrhea; while as an infusion or decoction it is used in the skin wounds treatment (Viana, *et al.*, 2003).

The essential oils of few *Astronium* species have been studied. Their main components are: (Z)-β-ocimene, bicyclogermacrene, limonene, (E)-β-ocimene and δ-carene (Alencar, *et al.*, 1996; Maia, *et al.*, 2002).

Astronium graveolens Jacq commonly known as "Gateado" or "Gateado-Galán," is a tree of more 25 m tall, which blooms from March to May. It presents a clear, sticky resin that smells like turpentine. It is mainly used for its high quality wood, grained and highly regarded for making fine furniture (Mendoza, 2008). From its fresh leaves it has been reported the presence of *trans*-ocimene as main volatile compound showing repellent activity against *Acromyrnex lundi* (Chen and Wiemer, 1984). The essential oil composition of *A. graveolens* leaves from Colombia, showed the presence of (*E*)-β-ocimene (40.1%) and myrcene (17.0%) as the major constituents (Rodriguez-Burbano, *et al.*, 2010). In this study we aim to chemically characterize the essential oil of *A. graveolens* Jacq, from the Caparo Forest Reserve (Barinas Province, Venezuela). And to evaluate its antibacterial activity against reference bacteria.

MATERIAL AND METHODS

Plant material: The aerial parts of *Astronium graveolens* were collected (November 2009) at the Experimental Station "Caparo", Barinas Province, Venezuela. A sample was deposited in the Faculty of Pharmacy and Bioanalysis Herbarium (FM031), Universidad de Los Andes, Merida, Venezuela (MERF Herbarium).

Isolation of the essential oil: Fresh leaves (1000 g) were cut into small pieces and subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The oil (1.5 % v/w) was dried over anhydrous sodium sulphate and stored at 4°C (Adams, 2007).

Gas chromatography: GC analyses were performed using a Perkin-Elmer AutoSystem gas chromatograph equipped with a flame ionization detector and a data handling system. A 5% phenylmethyl polysiloxane fused-silica column (AT-5, Alltech Associates Inc., Deerfield, IL), 60 m x 0.25 mm, 0.25 μm film thickness, was used. The temperature program was as follows 60-260°C at 4°C/min, 260°C (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 (1μL) was injected using a Hewlett-Packard ALS injector with a split ratio of 50:1. Retention indices were calculated relative to C_8-C_{24} *n*-alkanes, and compared with values reported in the literature (Sandra, 1987).

Gas chromatography-mass spectrometry: GC-MS analyses were 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, Hewlett-Packard). The oven

Table 1: Chemical composition of the essential oil of *Astronium graveolens**

Compound	Peak Area (%)	LRI
trans-2-hexenal	0.4	851
α-pinene	19.1	939
camphene	0.3	953
β-pinene	0.2	980
myrcene	0.8	990
α-phellandrene	12.7	1006
δ-3-carene	0.5	1012
α-terpinene	1.0	1019
p-cymene	3.1	1027
δ-3-carene	15.5	1032
cis-ocymene	6.4	1040
trans-β-ocymene	23.9	1052
α-terpinolene	3.2	1091
2-cyclohexen-1-ol	0.3	1127
α-terpineol	2.0	1197
4.5-Epoxy-1-isopropyl-4-methyl-cyclohexene	0.6	1215
2-propyl-thiophene	0.4	1261
α-copaene	0.8	1383
β-caryophyllene	2.4	1429
α-humulene	0.6	1467
α-amorphene	0.6	1490
β-selinene	1.1	1500
α-selinene	1.2	1509
δ-cadinene	1.0	1536
Total	99.3	

***** Chemical composition was determined by comparison of the mass spectrum of each component with Wiley GC/MS library data base and from retention index (RI) data. KI: Kóvats index

temperature program was the same used for the GC analysis; the source and quadrupole temperatures were 230 °C and 150 °C respectively; carrier gas, helium, (34 cm/s); ionization energy, 70 eV; scan range, 40:500 amu; 3.9 scans/s. The sample was diluted with diethyl ether (20µL in 1 mL) and 1µL was injected using a Hewlett-Packard ALS injector with a split ratio of 50:1. The identity of the oil components was established from their GC retention indices, by comparison of their MS spectra with those of standard compounds available in the laboratory, and by a library search (Nist, 05) (Sandra, 1987; Adams, 2007).

Bacterial strains and antibacterial assays: The inhibitory activity of *A. graveolens* essential oil was tested against eight selective resistant strains bacteria from nosocomial origin. Two Gram-positive: *Enterococcus faecalis* LMM904 (resistant to vancomicyn [VAM B]); *Staphylococcus aureus* LMM906 (resistant to oxacillin [*mec*A]); and six Gram-negative bacteria:

Salmonella Heidelberg LMM175 (CTX-M-2 and SHV-12 β–lactamases producer); *Klebsiella pneumoniae* LMM28 (CTX-M-1 β–lactamase producer and resistant aminoglucosides); *Escherichia coli* LMM913 (AmpC β–lactamase producer); *Pseudomonas aeruginosa* LMM85 (VIM-2 metallo-β–lactamase producer); *Pseudomonas aeruginosa* LMM299 (mutant OPR resistant all β–lactamic antibiotics); *Pseudomonas aeruginosa* LMM301 (KPC β–lactamase producer). *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were used as control strains for susceptibility testing. Overnight cultures (37 ^{0}C) in Brain Heart Infusion (BHI) were adjusted to 0.5 Mac Farland standard (10^{5-6} cfu/ml). Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by the dilution-in-broth method (Mueller Hinton) according to the Clinical Laboratory Standard Institute (CLSI, 2011). MIC was determined in the range of 0.012 – 8 µg/ml. The essential oil was dissolved in DMSO and MICs was carried out twice for all the strains tested (CLSI, 2011). Tigecycline was used as positive control.

RESULTS AND DISCUSSION

The essential oil of *A. graveolens*, was obtained with 1.5 % v/w yield. Analysis of the essential oil by GC-MS allowed the identification of twenty-six compounds (99.3% of the sample). The monoterpenes were the predominant ones in the sample (59 % of the sample): *trans-β*-ocimene (23.9%), α-pinene (19.8%), δ-3-carene (15.5%), α-phellandrene (12.7%).

The chemical composition of the essential oil of *A. graveolens* collected at the forest reserve "Caparo" (Venezuela), was comparable with the one reported for *A. graveolens* collected in Colombia, and other *Astronium* species from Brasil. The major component, *trans*-β-ocimene, seems to be the common and most abundant component for all the species of the genus studied (Alencar, *et al.*, 1996; Maia, *et al.*, 2002; Rodriguez-Burbano, *et al.*, 2010).

Regarding the antibacterial activity was evaluated, the "Caparo" essential oil showed strong inhibitory properties against all resistant bacteria strains tested (Table 2). Bacterial growth was considerably in-

Table 2: Antimicrobial activity of the essential oil of *Astronium graveolens*.

Bacteria	Essential oil of leaves Range 0.012 – 0.8 µg/mL		Tigecycline* Range 0.025 -16 µg/mL	
	MIC	MBC	MIC	MBC
Gram positive				
S. aureus ATCC 29213	0.2	0.5	0.1	0.2
E. faecalis LMM904	0.5	1.0	0.2	0.5
S. aureus LMM906	0.2	0.2	0.1	0.3
Gram negative				
E. coli ATCC 25922	1.0	2.0	0.25	0.5
S. Heidelberg LMM175	0.5	2.0	1.0	0.5
K. pneumoniae LMM28	1.0	2.0	1.0	4.0
E. coli LMM913	2.0	4.0	0.5	2.0
P. aeruginosa LMM85	2.0	8.0	0.5	4.0
P. aeruginosa LMM299	1.0	4.0	0.25	1.0
P. aeruginosa LMM301	0.5	2.0	1.0	4.0

MIC: Mininal Inhibitory Concentration; MBC Minimal Bactericidal Concentration; ATCC: American Type Culture Collection; LMM: Laboratorio Microbiología Molecular
* Positive control

hibited with concentrations between 0.25 to 2 μg/mL. Regardless of bacterial species or resistance mechanism, the MIC values were very near to MBC (0.25 to 8 μg/mL). Hence, MICs are almost at biocidal levels. These results were comparable with the excellent inhibitory activity of tigecycline.

The results obtained suggest that the *A. graveolens* leaves essential oil had inhibitory activity against resistant nosocomial pathogens. The correlation composition/activity leads us to suppose that the antibacterial properties of the essential oil could be attributable to its main monoterpene constituents: *trans*-β-ocimene (23.9%), α-pinene (19.1%) and δ-3-carene (15.5%). Further investigations are necessary to determine the antibacterial activity of these compounds, either pure or combined, to establish the possible mechanism of action of the most active compound to combat resistant human pathogens.

CONCLUSIONS

This report represents a new contribution to the phytochemical and biological study of on *A. graveolens* essential oil. Most important, considering *A. graveolens* is a timber species, the results obtained suggest the use of their leaves to prepare medicinal essential oils should be a good way to take advantage of the leaves that are throwing aside in the wood industry.

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REFERENCES

Adams, R. (2007) Identification of essential oils components by gas chromatography/mass spectroscopy. 4th Edition. Allured Publishing. Corporation, Carol Stream IL, USA.

Aguilar, C., Sosa V. (2004) The evolution of toxic phenolic compounds in a group of Anacardiaceae genera. *Taxon* **53** : 357-364.

Alencar, J., Matos F., Machado M., Craveiro A. (1996) Essential oil from *Astronium fraxinifolium* Schott (Anacardiaceae) in different growth stages. *Journal of essential oil research* **8** : 177-178.

Chen, T., Wiemer D. (1984) A volatile leafcutter ant repellent from *Astronium graveolens*. *Naturwissenschaften* **71** : 97-98.

Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing; Twentieth Informational supplement.* CLSI (2011) Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania, USA.

Davies, N. (1990) Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20 M phases. *Journal of Chromatography A* **503** : 1-24.

Maia, J., Da Silva M., Andrade E., Zoghbi M., Carreira L. (2002) Essential oils from *Astronium urundeuva* (Allemao) Engl. and *A. fraxinifolium* Schott ex Spreng. *Flavour and Fragrance Journal* **17** : 72–74.

Mendoza, A. (2008) *Astronium graveolens* Jacq. Universidad Nacional Autónoma de México. Instituto de Biología. México.

Pell, S. (2004) Molecular systematics of the cashew family (Anacardiaceae). Louisiana State University and Agricultural and Mechanical College. Louisiana, USA.

Rodriguez-Burbano, D., Quijano-Celis C., Pino J. (2010) Composition of the essential oil from leaves of *Astronium graveolens* Jacq grown in Colombia. *Journal of Essential Oil Research* **22** : 488-489.

Sandra, P., Bicchi, C. (1987) Capillary gas chromatography in essential oil analysis. Huethig. Heidelberg.

Viana, G., Bandeira M., Matos F. (2003) Analgesic and antiinflammatory effects of chalcones isolated from *Myracrodruon urundeuva* Allemão. *Phytomedicine* **10** : 189–195.