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‖ RESEARCH NOTE ‖

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# Chemical composition and antimicrobial activity of the hexanic extract from the leaves and stems of *Croton micradenus* (Euphorbiaceae)

Composición química y actividad antimicrobiana del extracto hexánico de las hojas y tallos de *Croton micradenus* (Euphorbiaceae)

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## ABSTRACT

The chemical composition was determined for the first time of the hexanic extract from the leaves and stems of the endemic Cuban species potentially medicinal *Croton micradenus* Urb. by means of Gas Chromatography-Mass Spectra (GC-MS). Antimicrobial activity against of the references strains *Escherichia coli* (Migula) Castellani & Chalmers (American Type Culture Collection - ATCC 10536), *Staphylococcus aureus* Rosenbach (ATCC 6538), *Candida albicans* (Robin) Berkhout (ATCC 10231) and *Pseudomonas aeruginosa* (Schoroeter) Migula (ATCC 9027), was reported.

**Keywords:** endemic, GC-MS, *Croton*, potentially medicinal plant

## RESUMEN

Se determinó por primera vez la composición química del extracto hexánico de las hojas y tallos de la especie endémica cubana potencialmente medicinal *Croton micradenus* Urb. mediante Cromatografía Gaseosa-Espectrometría de Masas (CG-EM). Se reportó la actividad antimicrobiana frente a cepas de referencia de *Escherichia coli* (Migula) Castellani & Chalmers (ATCC 10536), *Staphylococcus aureus* Rosenbach (ATCC 6538), *Candida albicans* (Robin) Berkhout (ATCC 10231) and *Pseudomonas aeruginosa* (Schoroeter) Migula (ATCC 9027).

**Palabras clave:** endémica, CG-EM, *Croton*, planta potencialmente medicinal

## INTRODUCTION

The Cuban flora have a wealth and diversity of the native species (around 7500) and a high percentage of the endemism (51.4%) (CITMA, 2014). Inside this high vegetable diversity, it stands out the family Euphorbiaceae, no only to be one the most numerous, but also for the quantity and diversity of the chemical elements and the applications it possesses (Jimenez, 1992). In Cuba, the mentioned family is integrated by 37 genera, among which *Croton* stands out so much for the number of species that compose it (54), of them 38 endemics that represent the 70.4% (Greuter y Rankin, 2022), like for the reports as for the uses in traditional medicine and other fines (Greinge, 1988; Jimenez, 1992; Napralert Database, 2013). To mention some examples, they can be related its effectiveness like diuretic, diaphoretic, detersive, spasmolytic, antimalarial, antimicrobial, insecticide, molusquicide and nematicide.

The alkaloids and essential oils constitute important groups of the secondary metabolites of the plants for the varied physiologic activity that unchain, but less interesting has been composed of the hexanics extracts of several species of the family, being included of the genus *Croton*, among them *Croton campestres* A.St-Hil. (Matias, 2010) and *Croton scabiosus* Bebb. (Sarojini, 2011) to exhibit antimicrobial activity in front of *Staphylococcus aereus* Rosenbach, *Pseudomonas aeruginosa* (Schoroeter) Migula and *Escherichia coli* (Migula) Castellani & Chalmers, among other pathogenic microorganisms. Herein, we report for the first time the composition of the hexanic extract from the leaves and stems of the Cuban endemic species potentially medicinal *Croton micradenus* Urb. and its antimicrobial activity in front of the references strains *S. aureus, E. coli, P. aeuroginosa* and *Candida albicans* (Robin) Berkhout.

## MATERIAL AND METHODS

### *General Procedures*

Gas Chromatography-Mass Spectra (GC-MS), Gas Chromatograph 6890N to a selective detector mass 5975 B inert (Agilent, USA) with a computation system and a capillary column HP-5 Ms (30 m x 0.25 mm Inner Diameter and 0.25 m thickness, Agilent, USA). The identification was carried out by comparison of the spectra obtained with the libraries NIST 2011 and Wiley-275, as well as with substances commercial references available and the literature. The retention times (Rt) were also compared with substances commercial of reference. The determination of the relative percentage was based on the internal normalization method ($n$=3).

### *Plant material*

The leaves and stems of individual adults sterile of the specie *C. micradenus*, were recollected by Ricardo Rosa Angulo in May 2012 in Mine Yeso, Baitiquirí, San Antonio del Sur, Guantánamo, Cuba, and identified by Ramona Oviedo Prieto. A voucher specimen (number HAC 41947) is retained in the Herbarium of the Institute, CITMA, Havana, Cuba.

### *Extraction and obtaining of chromatograms*

The dried (40°C) and ground leaves and stems of *C. micradenus* (100 g) were extracted with n-hexane to reflux at 100°C (4 hours) of residue upon evaporation in vacuum. For GC-MS analysis, 4 mg of the hexanic extract from the leaves and stems was dissolved in 0.2 mL of chloroform and derivatized with 100 µL of N-metil-N-trimetilsilil trifluoro acetamida (MSTFA) to 70ºC during 30 min in a dry thermostat.

### *Biological assay*

The antimicrobial activity of the hexanic extract from the leaves and stems was determinate for microdilution plating method (Wayne, 2000) for determining the Minimum Inhibitory Concentration (MIC) according the National Committee for Clinical Laboratory Standard. The microorganisms *S. aereus* (ATCC 6538), *P. aeruginosa* (ATCC 9027), *E. coli* (ATCC 10536) and *C. albicans* (ATCC 10231) were adjusted with the turbidity standard McFarland 0.5. It was used as control the antibiotic chloramphenicol to a concentration of 5 mg/mL. The MIC is the lowest concentration capable of inhibiting bacterial growth.

## RESULTS AND DISCUSSION

Through GC-MS analysis, we have identified around 62 compounds in hexanic extract from the leaves and stems of *C. micradenus*. These compounds were identified for the first time in this specie, representing an important contribution to the chemo-taxonomical characterization of the same one. Alcohols (32.1%) and aldehyde (21.9%) are the main constituents of this organic fraction. Some of these compounds were also found in the other specie of the same genus (Mukherjee, 1984; Peres, 1997; 1998; Maciel, 1998; Santos, 1999; Maciel, 2000; Murillo, 2001; Dos Santos, 2002; Catalán, 2003; Barbosa, 2003; 2004).

In the Figure 1 is shown the chromatogram of the extract. The identification of the compounds is given in Appendix 1, and it was corroborated when comparing with the libraries spectra of the equipment, available patterns and for that described in the literature. Of the 65 compounds found in the extract, 62 were identified what represents 95.4%. The majority components were 1-octacosanol (C28OH) (12.5%), triacontanal (C30Al) (9.11%), octacosanal (C28Al) (7.67%), heptacosanal (C27Al) (6.58%), 1-hexacosanol (C26OH) (6.58%), 5,5-dimetil-1-etil 1,3-ciclopentadiene (5.70%), triacontanol (C30OH) (4.62%), nonacosane (C29) (3.46%), triacontanoic acid (C30:0) (3.44%), palmitic acid (C16:0) (3.36%), 1-tetracosanol (C24OH) (2.90%) and 1-nonacosanol (C29OH) (2.24%), which represent 68.2% of the total.

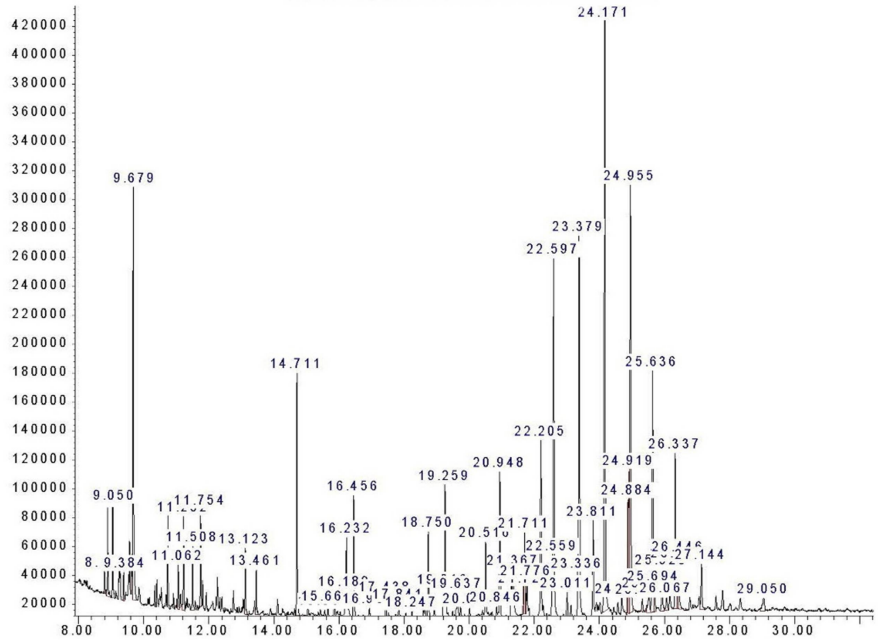


**Figure 1.** Chromatogram (abundance vs. time) obtained of the hexanic extract from the leaves and stems of *Croton micradenus.*

**Figura 1.** Cromatograma (abundancia vs. tiempo) obtenido del extracto hexánico de las hojas y tallos de *Croton micradenus.*

The hexanic extract from the leaves and stems of *C. micradenus* showed antimicrobial activity of 3.1 mg/mL MIC in front of *S. aureus* and *E. coli* with regard to the control Chloramphenicol (5 mg/mL), not being this way in front of the bacteria *P. aeruginosa* and to the mushroom *C. albicans* that turn out to be negative. Despite being of another species of another family and genus, similar result to those reported by Heng *et al.* (2020) for the hexanic, extract from the rhizome hairs of *Cibotium barometz* (L.) J. Sm. (Cibotiaceae) which shows antimicrobial activity of 2.50 mg/mL MIC in from of *S. aureus*.

Sengupta *et al.* (2018) reported that the octacosanol also showed anti-bacterial activities against *E. coli* to a concentrations of 1 mg/mL and 2 mg/mL. At 2 mg/mL, octacosanol was observed to inhibit microbial activity by gram-negative bacteria *E. coli*. They were compared with the activity of a standard antibiotic, tetracycline 500 using an isopropanol control. According to Mustapha *et al.* (2016), the preliminary antimicrobial assay of the hexane extract from the heartwood of *Albizia adianthifolia* (Schumach) (Fabaceae), showed activity against *E. coli* with MIC of 1 µg. Among of constituents identified and characterized include palmitic acid, oleic acid, stigmasterol and 1-octacosanol present in the hexanic extract of *C. micradenus*, some majority. On the other hand, Dambatta *et al.* (2017) reported that the hexanic extract of root part of *Cola millenii* K. Schum (Malvaceae) showed antimicrobial activity of 7.5 mg/mL MIC in from of *E. coli*. Taking into account the presence of octacosanol (12.5%) (Marrero, 2013), palmitic acid (3.36%), oleic acid (1.48%) (Saxton, 1979) and estigmasterol (0.65%) (Marrero, 2013) in the hexanic extract of leaves and stems of *C. micradenus*, they confer him responsibility on the antimicrobial activity expressed by the sample.

## CONCLUSIONS

The chemical composition and antimicrobial activity against *E. coli* and *S. aureus* was determined for the first time in the hexanic extract from the leaves and stems of Cuban endemism *C. micradenus*. This, is one of the species that characterizes to the phytogeographic district of Coastal Area Maisí-Guantánamo for its representativeness in the most part of the xerophytic vegetation the south of the oriental counties mainly, compare these results whit those of others of endemics species that integrate the main populations in this Oriental region of Cuba, will be interesting.

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## Appendix

**Appendix 1.** Composition of the hexanic extract from the leaves and stems of *Croton micradenus*.

**Anexo 1.** Composición del extracto hexánico de las hojas y los tallos de *Croton micradenus*.

| Compound | Retention time | Percentage |
|---|---|---|
| *cis*-verbenone | 8.800 | 0.26 |
| borneol | 8.899 | 1.08 |
| benzoic acid | 9.050 | 1.16 |
| glycerin | 9.264 | 0.10 |
| bornil acetate | 9.384 | 0.29 |
| 5,5-dimethyl-1-ethyl-1,3-ciclopentadiene | 9.679 | 5.70 |
| No Identified | 10.407 | 0.03 |
| *cis*-calamenene | 11.062 | 0.55 |
| phytol | 11.232 | 1.32 |
| No Identified | 11.508 | 0.88 |
| lauric acid (C12:0) | 11.754 | 1.29 |
| azelaic acid | 12.758 | 0.10 |
| miristic acid (C14:0) | 13.123 | 1.05 |
| No Identified | 13.462 | 0.68 |
| pentadecanoic acid (C15:0) | 13.883 | 0.05 |
| palmitic acid (C16:0) | 14.711 | 3.36 |
| margaric acid (C17:0) | 15.564 | 0.11 |
| 1-octadecanol (C18OH) | 15.663 | 0.18 |
| linoleic acid (C18:2) | 16.189 | 0.34 |
| oleic acid (C18:1) | 16.232 | 1.48 |
| stearic acid (C18:0)+ NI | 16.456 | 1.91 |
| tricosane (C23) | 16.937 | 0.18 |
| 1-eicosanol (C20OH) | 17.438 | 0.36 |
| tetracosane (C24) | 17.841 | 0.24 |
| eicosanoic acid (C20:0) | 18.247 | 0.14 |
| pentacosane (C25) | 18.750 | 1.62 |
| 1-docosanol (C22OH) | 19.216 | 0.32 |
| hexacosane (C26) | 19.637 | 0.69 |
| docosanoic acid (C22:0) | 20.015 | 0.16 |

| Compound | Retention time | Percentage |
|---|---|---|
| heptacosane (C27) | 20.516 | 1.46 |
| pentacosanal (C25Al) | 20.846 | 0.22 |
| tricosanoicacid (C23:0) | 20.881 | 0.03 |
| 1-tetracosanol (C24OH) | 20.948 | 2.90 |
| octacosane (C28) | 21.367 | 1.75 |
| hexacosanal (C26Al) | 21.711 | 1.43 |
| tetracosanoic acid (C24:0) | 21.726 | 0.30 |
| 1-pentacosanol (C25OH) | 21.776 | 0.67 |
| nonacosane (C29) | 22.205 | 3.46 |
| pentacosanoic acid (C25:0) | 22.539 | 0.03 |
| heptacosanal (C27Al) | 22.559 | 1.59 |
| 1-hexacosanol (C26OH) | 22.597 | 6.58 |
| triacontane (C30) | 23.011 | 0.57 |
| hexacosanoic acid (C26:0) | 23.336 | 0.57 |
| octacosanal (C28Al) | 23.379 | 7.67 |
| 1-heptacosanol (C27OH) | 23.394 | 0.10 |
| hentriacontane (C31) | 23.811 | 1.84 |
| heptacosanoic acid (C27:0) | 24.116 | 0.20 |
| 1-octacosanol (C28OH) | 24.171 | 12.50 |
| dotriacontane (C32) | 24.692 | 0.43 |
| octacosanoic acid (C28:0) | 24.884 | 1.78 |
| 1-nonacosanol (C29OH) | 24.930 | 2.24 |
| triacontanal (C30Al) | 24.955 | 9.11 |
| tritriacontane (C33) | 25.321 | 0.24 |
| stigmasterol | 25.525 | 0.65 |
| nonacosanoic acid (C29:0) | 25.592 | 0.20 |
| triacontanol (C30OH) | 25.636 | 4.62 |
| hentriacontanal (C31Al) | 25.694 | 0.61 |
| *β*-sitosterol | 25.928 | 0.97 |
| *β*-amirine | 26.067 | 0.33 |
| triacontanoic acid (C30:0) | 26.337 | 3.44 |
| lupeol | 26.406 | 0.88 |
| dotriacontanal (C32Al) | 26.446 | 1.23 |
| dotriacontanol (C32OH) | 27.144 | 1.08 |
| dotriacontanoic acid (C32:0) | 28.017 | 0.05 |
| tetratriacontanol (C34OH) | 29.050 | 0.57 |