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# Chemical Composition of the Essential Oil from the Fresh Fruits of *Xylopia laevigata* and its Cytotoxic Evaluation

**Emmanoel Vilaça Costa[a], Thanany Brasil da Silva[b], Cinara Oliveira D'Souza Costa[c], Milena Botelho Pereira Soares[c,d] and Daniel Pereira Bezerra[c]**

[a]*Department of Chemistry*, *Amazonas Federal University*, *Manaus*, *AM, Brazil*, *69077-000*

[b]*Department of Chemistry*, *Sergipe Federal University*, *São Cristóvão*, *SE*, *Brazil*, *49100-000*

[c]*Gonçalo Moniz Research Center*, *Oswaldo Cruz Foundation (CPqGM-FIOCRUZ)*, *Salvador*, *Bahia*, *Brazil*, *40296-710*

[d]*Center of Biotechnology and Cell Therapy*, *Hospital São Rafael*, *Salvador*, *Bahia*, *Brazil*, *41253-190*

*emmanoelvc@gmail.com*

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The essential oil obtained by hydrodistillation from the fresh fruits of *Xylopia laevigata* was analyzed by gas chromatography using a flame ionization detector (GC–FID) coupled to a mass spectrometer (GC–MS). Monoterpenes predominated, forming 95.0% of the total essential oil. The major constituents identified were limonene (56.2%), α-pinene (28.0%), and β-pinene (5.5%). Cytotoxic activity against tumor cell lines and non-tumor cells was also investigated; however, neither the essential oil nor its major constituents evaluated presented any cytotoxic activity ($IC_{50}$ > 25.0 μg $mL^{-1}$).

**Keywords:** *Xylopia laevigata*, Fresh fruits, Essential oil, Cytotoxicity activity.

*Xylopia laevigata* (Mart.) R.E. Fries, a medicinal plant belonging to the family Annonaceae, is found in the tropical American forest. In Brazil, it is popularly known as "meiú" and "pindaíba" and is distributed in the States of Piauí, Paraiba, Sergipe, Rio de Janeiro, and São Paulo. Although popular uses of this plant are almost unknown, we found that a decoction of its leaves and flowers is used in the folk medicine of the Brazilian Northeast for several purposes, including painful disorders, heart disease, treating tumors, and inflammatory conditions [1]. Previous phytochemical investigations of this species reported the presence of terpenes [2], essential oils [1,3,4,5], and alkaloids [6], which presented larvicidal [2], antimicrobial [2], antioxidant [3], antitumor [1], tripanocidal [3,4], anti-inflammatory [5], and antinociceptive properties [5]. Herein, the chemical composition and cytotoxicity of the essential oil obtained from the fresh fruits of *X. laevigata* were investigated for the first time.

Hydrodistillation of the fresh fruits of *X. laevigata* produced a colorless crude essential oil, with a yield of 0.4±0.1%, in relation to the dry weight of the plant material. Ten compounds were identified in the essential oil, with monoterpenes forming 95.0±1.5% (Table 1).

The major compounds identified in the essential oil of *X. laevigata* were limonene (56.2±1.4%), α-pinene (28.0±1.5%), and β-pinene (5.5±0.1%) (Table 1). These compounds have been observed in the essential oil from the leaves of this species, but as minor components [1,3,5]. In the leaves, the major compounds are the sesquiterpenes, γ-muurolene, δ-cadinene, germacrene B, α-copaene, germacrene D, bicyclogermacrene, and (*E*)-caryophyllene [1,3,5], indicating a significant variation in the chemical composition of the essential oil obtained from the leaves and fruits. According to Quintans *et al.* [1], Costa *et al.* [3], and Queiroz *et al*. [5] the leaf essential oil of this species presents a chemical composition varying from 33 to 44 components more than were observed here. These variations in the composition of the major constituents, as well as the contents of all components, could be related to soil, climatic conditions, water stress, collection place, nutrition, and other abiotic factors. On the other hand, sabinene, myrcene, *p*-metha-1(7),8-diene, and 1,8-cineole are described for the first time in *X. laevigata*, and contribute to the chemotaxonomic knowledge of this species, as well as for the genus *Xylopia*. In fact, most of the studies on essential oils from fresh fruits of *Xylopia* species report high concentrations of α- or β-pinene or both, which was observed in this work, contributing to chemotaxonomic knowledge of *Xylopia* spp.

**Table 1:** Chemical composition of the essential oil from the fresh fruits of *Xylopia laevigata*.

| | Compound | RI[a] | RI[b] | Peak area % |
|---|---|---|---|---|
| 1 | α-Pinene | 927 | 932 | 28.0±1.5 |
| 2 | Camphene | 941 | 946 | 3.0±0.0 |
| 3 | Sabinene | 966 | 969 | 0.4±0.0 |
| 4 | β-Pinene | 969 | 974 | 5.5±0.1 |
| 5 | Myrcene | 985 | 988 | 1.3±0.2 |
| 6 | *p*-Mentha-1(7),8-diene | 997 | 1003 | 0.2±0.0 |
| 7 | Limonene | 1025 | 1024 | 56.2±1.4 |
| 8 | 1,8-Cineole | 1027 | 1026 | 0.4±0.1 |
| 9 | (*Z*)-Caryophyllene | 1411 | 1408 | 0.7±0.2 |
| 10 | Germacrene D | 1474 | 1484 | 3.9±0.7 |
| | Monoterpenes | | | 95.0±1.5 |
| | Sesquiterpenes | | | 4.6±1.0 |
| | Total Identified | | | 99.6±0.4 |

Data are expressed as mean ± SD of three analyses. RI (retention indices): [a]calculated on RTx®-5SilMS column according to Van Den Dool and Kratz [7a] based on a homologous series of *n*-alkanes; [b]according to Adams [7b].

*In vitro* cytotoxic activity of the essential oil from the fresh fruits of *X. laevigata* and its major constituents (limonene, α-pinene, and β-pinene) was evaluated against four tumor cell lines, B16-F10 (mouse melanoma), HepG2 (human hepatocellular carcinoma), HL-60 (human promyelocytic leukemia), and K562 (human chronic myelocytic leukemia), and one non-tumor cells (PBMC; human peripheral blood mononuclear cells activated with concanavalin A – human lymphoblast) using the Alamar blue assay after 72 hours of incubation. However, neither essential oil nor its major constituents, presented cytotoxic activity ($IC_{50}$ > 25.0 μg $mL^{-1}$).

In conclusion, the essential oil from the fresh fruits of *X. laevigata* contained limonene, α-pinene, and β-pinene as major compounds. Neither the essential oil nor its major constituents evaluated presented cytotoxic activity at the concentration tested.

## Experimental

***Cells:*** Tumor cells lines B16-F10, HepG2, K562 and HL-60, donated by the Hospital A.C. Camargo, São Paulo, SP, Brazil, were used. All cell lines were tested for mycoplasma using Mycoplasma Stain kit (Sigma-Aldrich), and all cells were free from contamination. To obtain non-tumor cells, heparinized blood (from healthy, 20-35 year-old, non-smoking donors who had not taken any drugs for at least 15 days prior to sampling) was collected, and peripheral blood mononuclear cells (PBMCs) were isolated by a standard protocol using a Ficoll density gradient in a GE Ficoll-Paque Plus. ConA (10 μg $mL^{-1}$) was added at the beginning of culture, and cells were treated with the test drugs after 24 h. The Research Ethics Committee of the Oswaldo Cruz Foundation (Salvador, Bahia, Brazil) approved the experimental protocol (number 031019/2013).

***Plant material:*** Fresh fruits of *X. laevigata* were collected in November 2012 in the Caju Farm Municipality of Itaporanga D'juda, Sergipe State, Brazil, coordinates: [11°10'04" S and 37°11'32" W]. The identity of the plant was confirmed by Dr Ana Paula do Nascimento Prata from Department of Biology of Sergipe Federal University (DBI/UFS), Brazil. A voucher specimen (number 26593) was deposited in the ASE herbarium of Sergipe Federal University, Brazil.

***Hydrodistillation of the essential oil:*** The essential oil from the fresh fruits of *X. laevigata* (180 g) was obtained by hydrodistillation for 3 h using a Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulfate and the content percentage was calculated in relation to the dry weight of plant material used. It was then stored in a freezer until analysis. Hydrodistillation was performed in triplicate.

***GC–FID and CG–MS analysis of the essential oil:*** GC–FID analyses were performed on a Shimadzu GC-2010 Plus GCMS-QP2010 Ultra GC–FID, equipped with a Shimadzu AOC-20i auto-injector. Separation of the compounds was achieved on a RTx®-5MS fused capillary chromatography column (30 m x 0.25 mm x 0.25 μm film thickness) coated with 5%-diphenyl-95%-dimethylpolysiloxane. Helium was the carrier gas at 1.2 mL $mL^{-1}$ flow rate. The column temperature program was: 40ºC/4 min, a rate of 4ºC/min to 240ºC, then a rate of 10ºC/min to 280ºC, and then 280ºC/2min. The injector and detector temperatures were 250ºC and 280ºC, respectively. A sample of 10 mg $mL^{-1}$ in $CH_2Cl_2$ was injected with a 1:50 split ratio. Retention indices were generated with a standard solution of *n*-alkanes ($C_8$-$C_{20}$). The relative amounts of individual compounds were computed from GC peak areas without FID response factor correction. GC-MS analyses were performed on a Shimadzu QP5050A GC-MS system equipped with a Shimadzu AOC-20i auto-injector. In this system, the separation of the compounds was achieved employing a RTx®-5SilMS fused capillary chromatography column (30 m x 0.25 mm x 0.25 μm film thickness) coated with 5%-diphenyl-95%-dimethylpolysiloxane. All other conditions were similar to the GC analysis. Mass spectra were obtained at 70 eV with a scan interval of 0.5 s and fragments from 40-500 Da. All analysis (GC–FID and GC–MS) were performed in triplicate.

***Identification of constituents:*** The compounds in the essential oil were identified by comparison of their retention times ($t_R$) with those of standard compounds (α- and β-pinene, and limonene) under identical conditions, as well as by the retention indices of a series of *n*-alkanes ($C_8$-$C_{20}$), according to van Den Dool and Kratz [7a], and their mass spectra with those in the NIST (05, 05s, 21 and 107) and Wiley 8 mass spectral libraries, and published data in the literature [7b].

***In vitro cytotoxic activity assay:*** Cell viability was quantified using the alamar blue assay, as previously described [8], with minor modifications, according to Quintans *et al*. [1] and Rodrigues *et al*. [9] The cytotoxic activity assay was performed according to Quintans *et al.* [1] and Rodrigues *et al.* [9].

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