Abstract: *Ocotea porosa* (Nees & Mart.) Barroso, commonly known as “imbuia”, “canela-imbuia” or “imbuia-amarela” in Brazil, is a tree of the Southern Atlantic Forest. The present study investigates the anatomy of leaf and stem, volatile oil chemistry, as well as cytotoxicity and insecticidal activities of the essential oil of *O. porosa*. Species identification was achieved by anatomy features, mainly due to paracytic and anomocytic stomata; non-glandular trichomes; biconvex midrib and petiole with a collateral open arc vascular bundle; presence of a sclerenchymatous layer, starch grains and crystal sand in the stem; and the presence of
phenolic compounds in the epidermis, phloem and xylem of the midrib, petiole and stem. The main volatile components of the essential oil were α-pinene (19.71%), β-pinene (13.86%) and bicyclogermacrene (24.62%). Cytotoxicity against human cancer cell (MCF-7), mouse cancer cell (B16F10) and mouse non-tumoral cell (McCoy) was observed as well as insecticidal activity of the essential oil against susceptible ‘Ft. Dix’ bed bugs (*Cimex lectularius* L.) by topical application.

**Keywords:** anticancer; bed bugs; *Cimex lectularius*; cytotoxic effect; imbuia; light and scanning electron microscopy.

**INTRODUCTION**

*Ocotea* Aubl. is one of the most representative genus of Lauraceae comprising 428 species [1], with 168 of these species occurring in Brazil [2]. *Ocotea porosa* (Nees & Mart.) Barroso and *O. odorifera* (Vell.) Rohwer are the most important Brazilian representatives of this genus [3]. The taxonomy and delimitation of *Ocotea* are problematic due to similar features with *Cinnamomum* Schaeff. and *Nectandra* Rol. ex Rottb. [4].

Several species of *Ocotea* have been reported to have important biological activities, such as antitherperetic [5], antimycobacterial [6], antibacterial [7], antinociceptive [8], antiplatelet and antithrombotic [9], acaricidal [10,11], anti-inflammatory [12], antiprotozoal activity against *Trypanosoma cruzi* and *Leishmania* [13], cytotoxic [14], antioxidant and antimutagenic activities [15]. The essential oils of *Ocotea* species have also exhibited promising antimicrobial activities against *Escherichia coli* and showed cytotoxicity against MCF-7 cells [9].

Monoterpenoids, sesquiterpenoids and phenylpropanoids were found in their essential oils [16,17]. Other metabolites such as benzylisoquinolinic and aporphinic alkaloids [18,19], lignans [20] were also described from the genus.

*Ocotea porosa*, usually called “imbuia”, “imbuia-amarela”, “imbuia-zebrina” in Brazil, is a typical tree species from Southern Atlantic Forest that reaches up to 15 m high. In spite of legal instruments that prevent the species exploitation, its wood is still considered as one of the most valuable for furniture and construction industry due to its moderately density and resistance to fungal infection [21]. This species is in danger of extinction due to overexploitation for wood extraction [2].

Considering the wide number of biological activities for essential oils from *Ocotea*, and the fact that no previous work was devoted to investigate the chemical composition of leaf essential oil and the anatomy of *O. porosa*, the present study aims to investigate the chemical profile of the volatile oil and its cytotoxic and insecticidal activities, as well as the anatomy of the leaf and stem of this species in order to provide accurate information to support the identification of plant materials.

**MATERIAL AND METHODS**

**Plant Material**

Fresh samples of leaves and stems of *Ocotea porosa* were collected from plants growing in open and sunny areas of União da Vitória, Paraná, Brazil (26º13'48” S and 51º05'11” W) in July 2017. The voucher specimen was identified and deposited in the herbarium of State University of Ponta Grossa under number HUPG 22243. The access to botanical materials was authorized and licensed by the Genetic Heritage Administration Council (CGEN/SISGEN) according to code AD3F256.

At least six samples of mature leaves (cut from median, intercostal and margin regions) were obtained from the sixth node and below, as well as stem fragments 5 to 10 cm from the shoot were collected for anatomical analysis.

For the extraction of essential oil, the plant material was selected and standardized in order to acquire leaves and stems in the same pattern. The plant material was then dried in shade and cut into small pieces (~1 cm).

**Microscopic procedure**

Freshly collected leaves and stems of *O. porosa* were fixed in formalin–acetic acid–alcohol (FAA) solution [22], for three days, washed in distilled water and then stored in 70% ethanol (v/v) [23] until use. Cross and longitudinal sections were freehand prepared using razor blades, placed on glass slides, hydrated, and stained with toluidine blue [24] or Astra blue and basic fuchsine combination [25]. Kraus and Arduin [26] methods were used to analyze epidermal features.
Quantitative studies of stomata were performed by taking ten measurements from multiple leaf specimens. The length and width of stomata were measured from ten stomata at different places on the leaf blade for each species to determine the average stomatal size.

For the analysis by field emission scanning electron microscopy (FEG-SEM), the samples fixed in FAA were passed through a series of ethanol solutions of increasing concentrations and then dried in a critical point dryer (Balzers CPD-030) using liquid CO₂. The dried samples were mounted on aluminum stubs using glued carbon tapes and then coated with gold using a Quorum SC7620 sputter coater [27]. Photomicrographs were prepared and examined using a Mira 3 Tescan FEG-SEM equipment located at the State University of Ponta Grossa (UEPG).

For histochemical tests, the following standard solutions were used: potassium dichromate (10%) [28] and ferric chloride (2%) [22] for phenolic compounds, phloroglucinol/HCl for lignin [29], Sudan III for lipophilic components [30], and iodine (1%) for starch [23]. Controls were prepared in parallel with the tests and were carried out as described above. Photomicrographs were prepared using digital camera (C7070) attached to the light microscope (Olympus CX 31) at UEPG.

**Extraction of essential oil (EO) and GC-MS analysis**

Dried plant material (300 g) was subjected to hydrodistillation for 4 h in triplicate using a modified Clevenger-type apparatus for EO extraction. The obtained oil was dried using anhydrous Na₂SO₄ and kept in glass vials with Teflon-sealed caps at 4 ± 0.5°C in absence of light.

The EO was analyzed using a Shimadzu GC-2010 Plus GC-MS/MS apparatus coupled to a TQ8040 triple quadrupole type tandem mass detector and AOC-5000 Plus automatic injector for analysis of liquid samples (headspace) and solid phase microextraction (SPME). The samples were diluted at 1% (v/v) in methylene chloride and characterized using the following analytical conditions: Rtx-5MS fused silica capillary column (5% diphenyl + 95% dimethylpolysiloxane (30 m x 0.25 mm x 0.25 μm). The carrier gas used was helium at a flow rate of 1.02 mL/min, in 1:90 split mode. The injector was set at 250°C and the ionization system at 70 eV. 1 μL of sample was injected into the following heating ramp: initial temperature 60 °C to 250 °C with heating rate at 3 °C/min.

A homologous series of linear saturated hydrocarbons, C₈ to C₁₉ was used to calculate the retention index. The experimental retention index was obtained using the following Van den Dool and Kratz equation:

\[
\text{Retention index} = 100 \times \left( \frac{C_n - C_{n-1}}{T_x - T_{n-1}} \right) \times \left( \frac{T_x - T_{n-1}}{T_{n} - T_{n-1}} \right) + 100 \times C_{n-1}
\]

In the above equation, \(C_n\) is the number of carbon atoms of the n-alkane whose retention time is immediately greater than the retention time of the analyte; \(C_{n-1}\) is the number of carbon atoms of the n-alkane whose retention time is immediately less than the retention time of the analyte; \(T_x\) is the retention time of the analyte; \(T_n\) is the retention time of the \(C_n\) alkane; \(T_{n-1}\) is the retention time of the \(C_{n-1}\) alkane.

The EO volatile components were identified by comparing retention indices and mass spectra with literature [31] and mass spectra were also compared with the NIST 02 mass library (NIST, Gaithersburg, MD, USA). The relative quantification was determined from the normalization of each peak area with the total chromatogram, with no use of any correction factor. This experimental was carried out at the Federal University of Paraná.

**Cell culture**

The human cancer cell (MCF-7, breast cancer, Rio de Janeiro cell bank n. 0162), mouse cancer cell (B16F10, melanoma, Rio de Janeiro cell bank n. 0342) and mouse non-tumoral cell (McCoy, fibroblast, Rio de Janeiro cell bank n. 0160) were maintained in RPMI 1640 medium (pH 7.4) supplemented with 10% fetal bovine serum (FBS), 24 mmol/L of sodium bicarbonate and 1% penicillin and streptomycin under controlled temperature (37 °C) and humidified atmosphere (5% CO₂). To cell and subcultures expansion, the same conditions were used.

**Cytotoxic assay**

This assay relies on the ability of viable cells to metabolically reduce a yellow tetrazolium salt (MTT) to a purple formazan product. This reaction takes place when mitochondrial reductases are active [32]. Cells were placed in 96 wells plates (4 x10³ cell/well). After 24 h, aliquots of \(O.\ porosa\) EO ranging from 0.77 μg/mL to 77 mg/mL dissolved in RPMI were added upon each cell and incubated for 72 h. The medium was...
removed, replaced with 100 µL of MTT solution (0.5 mg/mL) and incubated at 37 °C for 2 h. After this period of incubation, the resulting formazan crystals were solubilized in isopropyl alcohol acid and the optical density was read at 550 nm using an Elisa plate reader. The negative control was prepared as described above, but each cell was only incubated using RPMI medium. Tests were performed in quadruplicate and repeated three times.

IC$_{50}$ and selectivity index

IC$_{50}$ were determined using logarithmic dose-response sigmoidal curves based on nonlinear regression (using GraphPad Prism software). The selectivity index (SI) was calculated as the ratio IC$_{50}$ (control cells – McCoy)/IC$_{50}$ (tumor cell lines – B6F10 or MCF-7). A selectivity index > 1 indicates that the cytotoxicity on cancer cells surpassed the one on the healthy non-tumor cells.

Morphological study

Cells (3 x 10$^3$ cell/well) were seeded in 24-well plates containing a coverslip on the bottom, and incubated at 37 °C and 5% CO$_2$ for 24 h. After this time, the cells were treated with O. porosa EO (77 µg/mL), and incubated for 24 h. After that the culture medium was removed, washed with PBS, fixed with 2% formaldehyde for 2 min and stained with May-Grünwald stain. Coverslips were mounted and observed under a microscope coupled to a digital camera.

Insecticidal activity studies

Strains of Cimex lectularius, Bayonne (insecticide resistant) and Ft. Dix (susceptible) were provided by Dr. Changlu Wang, Department of Entomology, Rutgers University, New Brunswick, NJ, and their colony was raised using blood feeders (CG-1836-75 ChemGlass). The insecticidal activity of O. porosa EO against bed bugs was assessed by fumigation, topical application, and residual studies.

For fumigation study, bed bugs were exposed to vapor toxicity in 125 mL clear glass jars. A small piece of paper was placed in the jar's bottom to deliver a substrate for the bed bugs to rest during the tests. Bed bugs were introduced in the jars 2–4 h before treatment to acclimatize. A treatment solution or acetone aliquot of 2 µL was located directly onto the internal surface of the bottle side wall ~4 cm from bottle bottom using a 50 µL gas-tight syringe (Hamilton Company, Reno, NV) attached to a PB600 (Hamilton Company, Reno, NV) repeating dispense. Five concentrations viz., 15.6, 31.25, 62.5, 125 and 250 µg EO/125 sq.cm were tested against the bed bugs. The jars were placed in the growth chamber and data for mortality were verified 24 h after the treatment. The 2,2-dichlrorovinil-dimetylphosphate (DDVP) was used as the standard.

Studies using O. porosa EO in topical application were carried out with adult insects, which were separated in the Petri dishes and anesthetized with CO$_2$. 1 µL of treatment solution (50 µg/bug) in acetone was carried onto the dorsal surface of the abdomen, using a hand-held repeating dispenser. Control bugs received 1 µL of acetone alone. Data for the mortality of the bed bugs were verified for seven days after treatment. There were three replicates with ten bugs (mixed sex)/replicate. The standard was deltamethrin (2.4 ng/bug).

For residual studies, an aliquot of 100 µL of treatment (diluted in acetone) was applied on 20 cm$^2$ Whatman #1 filter paper achieving 100 µg/cm$^2$ of residues. The treated filter papers were then placed in the Petri dish. Only acetone was used in control tests. Ten adult bed bugs were released on the filter paper and mortality was recorded as mentioned in topical application. Deltamethrin was used as standard insecticide.

Statistical analysis

Cytotoxicity assays were analyzed by the difference of experimental statistical significance using analysis of variance (ANOVA) followed by Tukey's test. The experimental values were expressed as the mean ± standard error of the mean. The data were analyzed using Graph Pad Prism 7.0 software. The level of p<0.05 was used to determine statistical significance.

Treatment means of vapor toxicity were compared using two factor analysis of variance (ANOVA) and separated by Tukey's honestly significant difference (HSD). Analysis were performed in SAS® 9.3 and JMP®10.0.
RESULTS AND DISCUSSION

Microscopical analysis

_Ocotea porosa_ (Figure 1 A, B) leaves have domatia in the nerve axils beneath (Figure 1 D). This feature has also been observed in other species, such as _O. urbaniana_ Mez, _O. pulchella_ (Nees & Mart.) Mez, _O. tristis_ (Nees & Mart.) Mez and _O. catharinensis_ Mez [33].

From the surface view, the leaf possesses straight anticlinal cell walls on adaxial side and wavy on abaxial epidermis (Figure 1 C, E). This feature is also found in _O. puberula_ (Rich.) Nees [34]. However, _O. indecora_ (Schott) Mez had sinuous anticlinal walls on both sides [35] whereas _O. gardneri_ (Meisn.) Mez had both epidermises with straight anticlinal cell walls [36]. This is a significant feature in distinguishing various species of _Ocotea_.

Epicuticular waxes are found on the epidermis, especially on the stomata (Figure 1 G, H). This characteristic was not mentioned for other species of _Ocotea_. The stomata are of paracytic or anomocytic types (Figure 1 E) and the leaves are hipostomatic as also reported for several other _Ocotea_ species [7,35–39]. The average size of stomata is 29 × 23 µm. The stomatal index calculated for _O. porosa_ is 16.43 per unit area (1 sq. mm) on the abaxial side.

_Ocotea porosa_ evidences simple unicellular non-glandular trichomes on both surfaces although rarely on the adaxial side (Figure 1 F, G). Similar trichomes have also been reported for _O. puberula_ [34] and _O. indecora_ [35]. Even though this feature is common in the taxa of Lauraceae [35], _O. gardneri_ had glabrous leaves [36].

![Figure 1. Morpho-anatomy of Ocotea porosa [c, e: Light microscopy; d, f, g, h: FEG-SEM]. a- Plant in habit. b- Leaves. c-h- Leaf in surface view (c- adaxial, e-h- abaxial). [gl-domatia of glands, st- stomata, nt- non-glandular trichome, wa-waxes]. Scale bar: a = 70 cm; b = 4 cm; d = 1 mm; f = 100 µm; c, e = 50 µm; g = 20 µm; h= 5µm.](image-url)
vascular bundles surrounded by sclerenchymatous sheath with extensions that reach both faces of the epidermis (Figure 2B). Dorsiventral mesophyll is frequent in the family Lauraceae [40]. However, *O. gardneri* evidenced isobilateral mesophyll [36].

The edge of the lamina is slightly curved downwards. The epidermis possesses cells with irregular shape and covered by thick cuticle. Underneath the epidermis many layers of sclerenchyma cells are found (Figure 2A). These characteristics have also been observed in *O. gardneri* [36].

Secretory cells with spherical to oblong shape (Figure 2B) and with light yellow lipophilic contents reacted with Sudan III in the histochemical test (Figure 3A) are found in the lamina, especially in the adaxial side as well as in the midrib regions. Secretory cells are widely reported in the species of *Ocotea* [34–39].

In transverse section, the midrib is biconvex in outline (Figure 2C). This characteristic has also been observed in *O. odorifera* (Vell.) Rohwer [37], *O. puberula* [34], *O. indecora* [35] and *O. gardneri* [36]. The uniseriate epidermis is formed by cells with different shapes and sizes containing phenolic compounds which reacted with ferric chloride (Figure 3D). The epidermis is covered by a thick cuticle evidenced by Sudan III in the histochemical tests (Figure 3A). Beneath the epidermis several layers of annular collenchyma are found. Annular collenchyma was also found in *O. odorifera* [37] whereas the angular type was observed in *O. puberula* [34].

The vascular system of the midrib is represented by one collateral vascular bundle in open arc that is surrounded by a continuous sheath of sclerenchymatous fibers (Figures 2C, 3A-D) which reacted with phloroglucinol/HCl (Figure 3B). Phenolic compounds are found in some cells of xylem and in several cells of phloem. These compounds are evidenced in the histochemical tests using potassium dichromate 10% (Figure 3C). Idioblasts containing phenolic compounds were also found in the midrib of *O. odorifera* [37] and *O. puberula* [34].

The petiole, in cross-section, varies from biconvex shape with two lateral extensions in the distal region (Figure 2D), flat-convex in the medial region (Figure 3E) to cylindrical in the proximal region. This pattern has also been observed in *O. diospyrifolia* (Meisn.) Mez, *O. pulchella* (Nees & Mart.) Mez and *O. tristis* (Ness & Mart.) Mez in the proximal region. However, biconvex shape with two lateral extensions occurred in the median and distal regions of *O. pulchella*, whereas flat-convex shape was found in the same regions in *O.
Ocotea porosa: Anatomy, Chemistry and biological activities

The petiole shape can help the *Ocotea* species identification as observed in *Passiflora* L. [41] and *Mikania* Wild. [42].

The epidermis is unilayered and covered by thick cuticle (Figure 3 E) and has numerous non-glandular trichomes. Underneath the epidermis, several layers of annular collenchyma are observed on both sides (Figure 3 E). Some secretory cells are distributed in the petiole (Figure 3 F). *Ocotea gardneri* showed similar non-glandular trichomes only in the petiole [36].

![Figure 3. Histochemistry of Ocotea porosa](image)

In an incipient secondary structure, the stem is circular in shape (Figure 2 F). The epidermis is uniseriate and covered by a thin cuticle. Beneath the epidermis, a layer of sclerenchymatous cells (Figure 2 G) and a layer of cells containing phenolic compounds are found (Figure 2 G, H). The cortical parenchyma presents 10-12 layers (Figure 2 G, H). Secretory idioblasts are also present in the cortex (Figure 2 H). The vascular system has phloem towards the periphery, and xylem facing the pith, separated by a cambium (Figure 2 I). Perivascular fiber patches are adjoined to the phloem (Figure 2G-I). The fibers and xylem are reacted with phloroglucinol/HCl and stained pink evidencing lignification in the walls (Figure 3 K). The pith is made up of thin-walled parenchymatous cells. Starch grains (Figure 2 J) and sand crystals are found (Figure 2 K) in the pith region.

**Yield and chemical composition of essential oil (EO)**

The EO of *O. porosa* presents a light-yellow color and a strong and characteristic aroma. The light-yellow coloration is common to several *Ocotea* species [17]. The yield of *O. porosa* EO is 1.03%. The species *O. caudata* (Nees) Mez, *O. cujumary* Mart. and *O. canaliculata* (Rich.) Mez, which were collected in the National Forest of Caxiuana, Amazonas, Brazil, presented an average yield of 0.8% [7]. In the present study, leaves were used for EO extraction. The anatomical study evidenced several secretory cavities (Figures 2 B, 3 A, D, F) that store EO in the leaf blade and petiole.

The chemical composition of the EO extracted from *O. porosa* leaves was analyzed by GC-MS and is summarized in Table 1. Comparing the groups, *O. porosa* EO has 38.19% of monoterpenoid hydrocarbons, 1.02% of oxygenated monoterpenoids, 35.21% of sesquiterpenoid hydrocarbons and 18.20% of oxygenated...
sesquiterpenoids. High concentrations of sesquiterpenoids were also found in other species of *Ocotea*, such as *O. gomezii* W.C. Burger and *O. morae* Gómez-Laur [43].

Table 1. Chemical composition of *Ocotea porosa* essential oil.

| #  | Rt   | Al¹ | Al² | Chemical compound        | %    |
|----|------|-----|-----|--------------------------|------|
| 1  | 6.080| 933 | 932 | α-Pinene                 | 19.71|
| 2  | 6.544| 947 | 946 | Camphene                 | 0.48 |
| 3  | 7.354| 973 | 969 | Sabinene                 | 0.60 |
| 4  | 7.464| 976 | 974 | β-Pinene                 | 13.86|
| 5  | 7.954| 991 | 988 | Mircene                  | 2.07 |
| 6  | 8.908| 1017| 1014| α-Terpinene              | 0.25 |
| 7  | 9.368| 1028| 1024| Limonene                 | 0.78 |
| 8  | 9.459| 1031| 1026| 1,8-Cineole              | 0.24 |
| 9  | 10.581| 1059| 1054| γ-Terpinene              | 0.36 |
| 10 | 15.640| 1177| 1174| Terpinen-4-ol            | 0.43 |
| 11 | 16.248| 1191| 1186| α-Terpineol              | 0.35 |
| 12 | 24.286| 1376| 1374| α-Copaene                | 0.34 |
| 13 | 24.661| 1385| 1387| β-Bourbonol              | 0.53 |
| 14 | 24.994| 1392| 1389| β-Elemene                | 0.51 |
| 15 | 26.100| 1419| 1417| (E)-Caryophyllene        | 1.70 |
| 16 | 26.905| 1439| 1440| Aromadendrene            | 0.86 |
| 17 | 27.491| 1453| 1452| α-Humulene               | 0.57 |
| 18 | 27.799| 1461| 1458| Allo-aromadendrene       | 1.98 |
| 19 | 28.626| 1481| 1484| Germacrene D             | 1.88 |
| 20 | 29.266| 1497| 1500| **Bicyclogermacrene**    | 24.62|
| 21 | 29.959| 1515| 1513| γ-Cadinene               | 1.17 |
| 22 | 30.346| 1525| 1522| δ-Cadinene               | 0.66 |
| 23 | 31.331| 1550| 1548| Elemol                   | 0.74 |
| 24 | 31.620| 1558| 1559| Germacrene B             | 0.39 |
| 25 | 32.415| 1578| 1577| Spathulenol              | 5.34 |
| 26 | 32.650| 1584| 1590| Globulol                 | 2.09 |
| 27 | 32.951| 1592| 1592| Viridiflorol             | 0.99 |
| 28 | 33.207| 1599| 1600| Guaiol                   | 1.95 |
| 29 | 33.846| 1616| 1618| 1,10-di-epi-cubenol      | 0.95 |
| 30 | 34.470| 1633| 1630| γ-Eudesmol               | 0.42 |
| 31 | 34.716| 1640| 1639| alloaromadendrene epoxide| 0.72 |
| 32 | 35.136| 1651| 1649| β-Eudesmol               | 1.77 |
| 33 | 35.251| 1654| 1652| α-Eudesmol               | 1.09 |
| 34 | 35.418| 1659| 1656| Valeranol                | 1.40 |
| 35 | 35.797| 1669| 1670| Bulnesol                 | 0.75 |

Classes of compounds

- Monoterpenoids hydrocarbons 38.12%
- Oxygenated Monoterpenoids 1.02%
- Sesquiterpenoids hydrocarbons 35.21%
- Oxygenated Sesquiterpenoids 18.20%
- **Total** 92.54%

Rt: Retention time of calculated compounds compared to n-alkanes in HP-5MS column. %: abundance of essential oil components. ¹ Calculated retention index. ² Literature retention index [31]. Compounds of concentration > 0.2% were identified. The major compounds are highlighted in bold.

Thirty-five volatile compounds (92.54%) of EO of *O. porosa* were identified. The major compounds were bicyclogermacrene (24.62%), α-pinene (19.71%) and β-pinene (13.86%). Weyerstahl and coworkers [44] verified a distinct chemical composition for the EO of *O. porosa* extracted from the wood. These authors found carquejila acetate (2.1%), α-copaene (5.6%), γ-copaene (3.5%), δ-cadinene (3.1%), cremoligenol (8.4%), β-eudesmol (8.4%), valerianol (5%), α-bisabolol (3.6%) and β-bisabolol (2.9%) as the major compounds of EO derived from the wood. Reynolds and coworkers [45] analyzed EO extracted from *O. porosa* stem barks and found as major components α-copaene (6.25%), δ-cadinene (3.28%), β-eudesmol (6.86%), valerianol (7.55%) and α-bisabolol (3.33%).

In the present study, the differences found in the chemical composition of *O. porosa* in relation to the literature data occurred due to the fact that EO was obtained from leaves and not from wood or stem barks. In addition, not only the composition of EO, but also the concentrations of the compounds vary depending on
the age of the plant as well as other factors, such as circadian rhythms, seasonal conditions and environmental influences [46]. Takaku and coauthors [17] analyzed the volatile components of leaves of several Ocotea species from Costa Rica, namely O. floribunda (Sw.) Mez, O. holdridgeana W.C.Burger, O. meziana C.K.Allen, O. sinuata (Mez) Rohwer, O. toduzi Standl, O. valeroana (Standl.) W.C.Burger, O. veraguensis (Meisn.) Mez, and O. whitei Woodson. The most common volatile compounds among these species were α-pinene, β-pinene, β-caryophyllene and germacrene D. In the present study, α-pinene and β-pinene were present in high concentrations.

Considering the biological activities, the chemical composition of EO is extremely important and should be evaluated[10]. In the present study, bicyclogermacrene, the most abundant compound in EO of O. porosa, showed a larvicidal action on the vectors of malaria, dengue, Japanese encephalitis [47] and fungicidal activities [9].

The volatile compounds α-pinene and β-pinene were also found in high concentrations in the present study and have been evaluated for biological activities. Both compounds presented antibacterial, antiviral, antifungal and hypotensive activities [48], α-pinene presented anti-inflammatory, hypoglycemic [49], and gastroprotective activities [50] whereas β-pinene showed antidepressant [51] and antiviral [52] properties.

**Insecticidal activities**

Ocotea porosa EO was exposed to toxicity test against two strains of Cimex lectularius (Insecticide resistant 'Bayonne' and susceptible 'Ft. Dix') using three delivery methods i.e topical, residual and fumigation. The EO of O. porosa (100 µg/bug) produced 13.3% mortality in Ft. Dix strain that could reach to 23.3% 7 days after treatment, whereas no mortality was recorded in Bayonne strain. EO of O. porosa was not toxic to bed bugs in fumigation (250 µg/125 mL of air) and residual (100 µg/cm sq.) assays. Using the same methods, EO of Baccharis sphenophylla Dusén ex Malme produced 66.67 ± 3.33% mortality in the insecticide-resistant strain 'Bayonne', while producing 83.33 ± 3.33% mortality in the susceptible strain 'Ft. Dix', 24 h after treatment [53]. The EO of Schinus molle L. produced 100.0 ± 0.00% (Ft. Dix) and 90.0 ± 5.77% (Bayonne) of mortality 24 h after the treatment [54]. In that sense, O. porosa EO cannot be considered as an effective insecticide against bed bugs.

**Cytotoxicity activities**

There are no reports of the activities of O. porosa EO against melanoma and breast cancer cell lines or the possible mechanisms related to these activities. Thus, an initial evaluation of the cytotoxic effect of O. porosa EO against MCF-7 and B16F10 cells lines was performed (Figure 4) by an MTT reduction assay, and the IC<sub>50</sub> and SI (selectivity index) values are presented in Table 2. Ocotea porosa EO showed cytotoxic effects against all cell lines tested at different concentrations with the lowest IC<sub>50</sub> value achieved after 72 h of treatment.

Statistically significant results were obtained for MCF-7 and B16F10 cells up to the concentration of 7 µg/mL. McCoy cells presented cytotoxicity with statistical difference to the control until 77 µg/mL. Essential oils of O. caudata, O. cujumary and O. caniculata displayed promising cytotoxic activities against MCF-7 cells showing median inhibitory concentration (IC<sub>50</sub>) ∼ 65.0 µg·mL<sup>−1</sup> [9]. The major compounds found in the present study, α-pinene, β-pinene and bicyclogermacrene also showed cytotoxic activities against MCF-7 cells in a study by Grecco and coworkers [55]. Taking all these into account, EO of O. porosa can be further investigated regarding both selectivity and cytotoxic mechanisms.

However, an ideal anticancer drug must produce a cytotoxic effect for cancer cells in low concentrations without affecting normal cells. Ashley and coworkers [56] suggested that for a compound to be considered of low toxicity and has good chances of became a new anticancer drug, it should present an SI higher than 2. The results showed in Table 2 presented an SI of 1.05 and 0.05 for B16F10 and MCF-7 when compared to fibroblast normal cells (McCoyline), respectively. These data restrict possible use of EO from O. porosa as novel anticancer product. However, these values may be improved after EO fractionation and isolation of more suitable compounds.
Figure 4. Cell cytotoxicity was determined using MTT assay. A – MCF-7, B – B16F10 and C – McCoy cells. **p < 0.01 and *** p < 0.001 compared to control. One-way ANOVA with Tukey's post hoc test. Three independent experiments were performed.

Table 2. IC_{50} values of *O. porosa* oil for different cell lines and selectivity index (SI). IC_{50} data were expressed as mean and ± standard error of the mean of three independent experiments.

| Cell lineage | IC_{50} (mg/mL) | SI  |
|--------------|-----------------|-----|
| McCoy        | 9.23 ± 7.04     | -   |
| B16F10       | 8.82 ± 6.27     | 1.05|
| MCF-7        | 185.4 ± 10.91   | 0.05|

The morphological features of MCF-7 and B16F10 cell lines were also investigated by studying the effects of *O. porosa* EO on cells (77 µg/mL, for 24 h). EO of *O. porosa* induced cell death with apoptotic characteristics as cell rounding, membrane blebbing and chromatin condensation (Figure 5). Apoptosis and necrosis are the two major processes leading to cell death. Apoptosis occurs under normal physiological conditions and the cell is an active participant in its own demise. Due to this efficient mechanism for the removal of apoptotic cells, no inflammatory response is elicited [57]. These results suggest that EO of *O. porosa* provides a more suitable cell death mechanism than other essential oils as EO of *Baccharis milleflora* (Less.) DC. [57] and *Lavandula dentata* L. [58] which promoted necrotic and apoptotic processes, simultaneously.

Figure 5. Morphology of MCF-7 and B16F10 cells after 24 h of treatment with *O. porosa* EO. A and C - Control cells incubated with RPMI only. B and D - cells treated with 77 µg/mL of *O. porosa* EO. 1: cell rounding, -: bleb formation, ▲: chromatin condensation. Magnification = 1000x, bar = 20 µm.

CONCLUSION

In the present work, the chemical profiles of *Ocotea porosa* EO were analyzed. The volatile compositions of EO of the leaves were reported for the first time. The major volatile compounds were α-pinene, β-pinene...
and bicyclogermacrene. EO of \textit{O. porosa} demonstrated 13.3\% mortality in Ft. Dix strain of bed bugs that could reach to 23.3\% 7 days after treatment, while no mortality was recorded in Bayonne strain. EO of \textit{O. porosa} was not toxic to bed bugs in fumigation and residual assays. The EO of \textit{O. porosa} was cytotoxic to murine fibroblast cell lines (McCoy), murine melanoma (B16F10) and human breast adenocarcinoma (MCF7) probably by apoptosis. However, there was no evidence of selectivity against the tumor cells under study. The anatomical characteristics that were observed in this study may help in the correct identification of \textit{O. porosa}. Noteworthy anatomical features include the hypostomatic leaves with paracytic and anomocytic stomata; epicuticular wax, especially on the stomata; non-glandular trichomes; biconvex midrib and petiole with a collateral open arc vascular bundle; presence of a sclerenchymatous layer, starch grains and crystal sand in the stem; and the presence of phenolic compounds in the epidermis, phloem and xylem of the midrib, petiole and stem.

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