Check for updates

NPC Natural Product Communications

2017
Vol. 12
No. 1
131 - 134

# Chemical Profile and *in vitro* Biological Activities of Essential Oils of *Nectandra puberula* and *N. cuspidata* from the Amazon

**Joyce Kelly R. da Silva[a,d]*, Eloísa Helena A. Andrade[b], Rosa Helena V. Mourão[c], José Guilherme S. Maia[c], Noura S. Dosoky[d] and William N. Setzer[d]**

[a]*Programa de Pós-Graduação em Biotecnologia, Universidade Federal do Pará, Belém, PA 66075-900, Brazil*
[b]*Coordenação de Botânica, Museu Paraense Emilio Goeldi, Belém, PA 66077-530, Brazil*
[c]*Programa de Pós-Graduação em Recursos Naturais da Amazônia, Universidade Federal do Oeste do Pará, Santarém, PA 68035-110, Brazil*
[d]*Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA*

*joycekellys@ufpa.br*

**Received: July 5th, 2016; Accepted: September 8th, 2016**

Essential oils (EO) from leaves and branches of *Nectandra puberula* Schott (Nees) and from leaves of *N. cuspidata* Nees & Mart. were obtained by hydrodistillation and their chemical compositions determined by gas chromatography - mass spectrometry (GC-MS). The main compounds identified from *N. puberula* EO were apiole (22.2%), β-caryophyllene (15.1%), β-pinene (13.3%), germacrene D (8.3%), pogostol (6.6%) and bicyclogermacrene (6.4%) in the leaves; and apiole (28.1%), pogostol (19.8%) and guaiol (11.2%) in the branches. The EO of *N. cuspidata* leaves showed β-caryophyllene (26.9%), bicyclogermacrene (16.0%) and spathulenol (5.2%) as the main compounds. The EOs were subjected to antibacterial screening and displayed promising activity against *Escherichia coli* (MIC = 19.5 μg.mL$^{-1}$). In addition, the EOs were tested for cytotoxic activity against MCF-7 breast tumor cells and the $IC_{50}$ values were 64.5 ± 1.6 and 117.1 ± 11.9 μg.mL$^{-1}$ for the leaf EOs of *N. puberula* and *N. cuspidata*, respectively.

**Keywords:** Lauraceae, Sesquiterpenes, Phenylpropanoids, Breast cancer, *Escherichia coli*.

The Lauraceae is a pantropic family, containing about 50 genera and probably 2500-3500 species, including trees and shrubs. Southeastern Asia and tropical America are the main centers for diversity of the Lauraceae. These species frequently occur in lowland rain forests to tropical montane forests with an altitude up to ca. 4000 m [1, 2]. Many species are highlighted due to their economic importance in cooking, in carpentry and construction, papermaking, in the perfume industry, the chemical industry, and folk medicine [3]. The genus *Nectandra* belongs to the *Ocotea* complex and comprises about 114 species. It is endemic to the Neotropics with restricted distribution in tropical and subtropical Americas, with an estimated 43 species occurring in Brazil [4, 5, 6]. This genus has reported folk medicinal uses as an antifungal, anti-inflammatory, antimalarial, anticancer, analgesic, and febrifuge, as well as being used for the treatment of ulcers, among others [5].

*N. puberula* Schott (Nees) is commonly known in the Amazon region as “louro amarelo” and “yellow cinnamon” and is presently restricted in distribution to the north, southeast, and southern regions of Brazil [7]. It is a tree up to 20 m in height. Neolignans have been found in the extract of the trunk wood [8]. *N. cuspidata* is a shrub or tree, 5–30 m in height. The plant is not endemic to Brazil, but occurs in most states [2, 7]. A phytochemical investigation of the leaf extract resulted in the isolation and characterization of megastigmane, aporphinoid alkaloids, sesquiterpenes, polyprenols and sterols [9].

Despite the high aromatic potential, there have been few studies on the chemical composition of essential oils (EOs) of *Nectandra* species. To our knowledge, there are no previous reports on either the phytochemistry or the biological activities of the EOs of these two *Nectandra* species.

Fifty-nine volatile components were identified in the EOs from *Nectandra* species, comprising approximately 97.7% of the total composition of the oils (Table 1). The most representative class of compounds in the leaf EOs of both species was oxygenated terpenoids; about 42.3% in *N. puberula* and 76.2% in *N. cuspidata*. Comparison of the chemical profiles of the samples is shown in Figure 1. *N. puberula* oils showed a significant difference in terpene accumulation between leaves and branches. The branch oil displayed a higher concentration of oxygenated sesquiterpenoids (44.7%). The main compounds identified in the EO from *N. puberula* were apiole (22.2%), β-caryophyllene (15.1%), β-pinene (13.3%), germacrene D (8.3%), pogostol (6.6%) and bicyclogermacrene (6.4%) in the leaves, and apiole (28.1%), pogostol (19.8%) and guaiol (11.2%) in the branches. The EO of *N. cuspidata* leaves showed β-caryophyllene (26.9%), bicyclogermacrene (16.0%) and spathulenol (5.2%) as the main compounds.

The occurrence of sesquiterpene hydrocarbons and oxygenated sesquiterpenoids in *Nectandra* species has been reported. The main compounds of the EO from *N. megapotamica* leaves were α-bisabolol (62.3–69.4%) and δ-elemene (8.2–22.6%) [10]. The leaf oil of *N. salicina* growing wild in Costa Rica was dominated by atractylone (14.6%), viridiflorene (10.1%), α-pinene (9.4%), β-caryophyllene (7.2%), α-humulene (7.0%), δ-cadinene (6.1%), β-pinene (6.0%) and germacrene D (5.8%) [11]. Similarly, the leaf oil of *N. membranacea* from Costa Rica was rich in δ-cadinene (14.1%), α-copaene (12.5%), germacrene D (6.9%), α-humulene (5.6%), β-caryophyllene (5.5%), and bicyclogermacrene (4.8%), as well as the monoterpenes α-pinene (22.4%) and β-pinene (12.6%) [12]. The EO of *N. coriacea* showed valerianol (16.7%) and γ-eudesmol (12.3%) [13]. In addition, the occurrence of monoterpenoids in *Nectandra* oils was reported such as mentha-

1(7),8-diene, α-terpinolene, α-pinene, β-pinene and α-terpineol in *N. falcifolia* and *N. elaiophora* [14, 15]. However, the occurrence of phenylpropanoid derivatives such as dillapiole has not been previously reported in volatile oils from *Nectandra* species.

The antiproliferative effect of metabolites isolated from *Nectandra* species against tumor cell lines have been reported [16]. The neolignans from *N. megapotamica* displayed cytotoxic activity and induced apoptosis in leukemia cells (HL-60) [17]. The crude extract of *N. rigida* was rich in dehydrodiisoeugenol, which is reported to be a cytotoxic agent [18]. In this study, the EOs showed good activity against the MCF-7 breast tumor cell line. The $IC_{50}$ values were 64.5 ± 1.6 and 117.1 ± 11.9 $\mu g.mL^{-1}$ for EOs from leaves of *N. puberula* and *N. cuspidata*, respectively.

The most activity was observed for *N. puberula* oil, which is characterized by high contents of apiole (22.2%), a phenylpropanoid, followed by β-caryophyllene (15.1%) and β-pinene (13.3%). The cytotoxic effects against MCF-7 cell lines have been observed for phenylpropanoids such as anethole, cinnamaldehyde and eugenol [19]. Apiole was administered to mice at 1-30 mg/kg body weight through intraperitoneal injection and displayed a promising antitumor effect against colon tumors (COLO 205) in an *in vivo* xenograft model [20]. The $IC_{50}$ values for α-pinene and β-caryophyllene have been found to be 20.6 and 19.7 $\mu g.mL^{-1}$, respectively, against MCF-7 cells [21]. In addition, β-caryophyllene potentiated the anticancer activity of paclitaxel on MCF-7 at a concentration of 10 $\mu g.mL^{-1}$ [22]. The main compounds in *N. cuspidata* oil were β-caryophyllene (26.9%), bicyclogermacrene (16.0%) and spathulenol (5.2%). The EO from *N. leucantha*, rich in bicyclogermacrene (28.4%) and spathulenol (5.8%), displayed significant cytotoxic activity against murine melanoma (B16F10-Nex2), human glioblastoma (U-87) and human cervical carcinoma (HeLa) cell lines. However, the $IC_{50}$ values against MCF-7 were 193.8 $\mu g.mL^{-1}$ and 19.0 $\mu g.mL^{-1}$ for the EO and bicyclogermacrene, respectively [23].

The antimicrobial property is of great importance in the applications of EO against certain human or animal pathogens [24]. In bacteria, the permeabilization of the membranes is associated with loss of ions and reduction of membrane potential, collapse of the proton pump and depletion of the ATP pool [25]. *Nectandra* leaf oils showed notable antibacterial activity against *E. coli* (MIC = 19.5 $\mu g.mL^{-1}$) (Table 2).

The antibacterial activities of the main compounds, apiole, β-caryophyllene, β-pinene, germacrene D, bicyclogermacrene and spathulenol have been reported previously [26]. The EO from *Psammogeton canescens*, rich in α-pinene (20.0%) and apiole (15.3%), exhibited strong antibacterial activity against *Escherichia coli* (MIC, 14.0 ± 0.8 $\mu g.mL^{-1}$) [27]. *Eugenia uniflora* oil, rich in apiole (11.1%) and oxygenated sesquiterpenes, was active towards two Gram-positive bacteria, *Streptococcus equi* and *Staphylococcus epidermis* [28]. The oil of the trunk bark of *Onychopetalum amazonicum*, rich in β-caryophyllene and spathulenol, exhibited good activity against *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 10538) and *Kocuria rhizophila* (ATCC 9341), with MIC values of 62.5 $\mu g.mL^{-1}$ [29]. In addition, β-caryophyllene exhibited strong antibacterial effect against *E. coli* (MTCC 732), with a MIC value of 9.0 ± 2.2 μM [30].

In summary, our results indicate that *Nectrandra* oils could be used as natural antimicrobial agents against *E. coli* and tested in future studies for the treatment of breast cancer.

**Table 1:** Chemical composition of EOs from *Nectandra* species from the Amazon.

| Constituents | $RI^{calc.}$ | $RI^{Lit.}$ | *N. puberula* | | *N. cuspidata* |
|---|---|---|---|---|---|
| | | | Leaves | Branches | Leaves |
| α-Pinene | 934 | 932 | 5.0 | | |
| β-Pinene | 978 | 974 | **13.3** | 1.4 | |
| Myrcene | 992 | 988 | 0.8 | | |
| Limonene | 1028 | 1024 | 2.7 | 3.0 | |
| Linalool | 1102 | 1095 | | 0.8 | |
| δ-Elemene | 1336 | 1335 | | | 4.4 |
| α-Copaene | 1374 | 1374 | | | 4.6 |
| β-Bourbonene | 1386 | 1387 | 0.5 | | |
| β-Elemene | 1391 | 1389 | 2.0 | 2.1 | 0.8 |
| β-Caryophyllene | 1421 | 1417 | **15.1** | 2.8 | **26.9** |
| β-Copaene | 1427 | 1430 | | | 0.5 |
| β-Gurjunene | 1432 | 1431 | | | 0.4 |
| α-*trans*-Bergamotene | 1435 | 1432 | | | 3.1 |
| γ-Elemene | 1435 | 1434 | | 0.9 | |
| α-Guaiene | 1440 | 1437 | 1.3 | 0.8 | |
| Aromadendrene | 1443 | 1439 | | | 0.4 |
| α-Humulene | 1452 | 1452 | 1.8 | 1.2 | 3.2 |
| (*E*)-β-Farnesene | 1458 | 1454 | | | 1.5 |
| γ-Muurolene | 1476 | 1478 | | | 0.5 |
| Amorpha-4,7(11)-diene | 1480 | 1479 | | | 4.5 |
| Germacrene D | 1483 | 1484 | **8.3** | 4.5 | |
| γ-Himachalene | 1484 | 1481 | | | 2.5 |
| *cis*-Eudesma-6,11-diene | 1488 | 1489 | 2.3 | 1.7 | |
| unidentified | 1490 | | 1.1 | | |
| *epi*-Cubebol | 1496 | 1493 | | 1.5 | |
| Bicyclogermacrene | 1498 | 1500 | **6.4** | | **16.0** |
| 4-*epi*-*cis*-Dihydroagarofuran | 1501 | 1499 | | 1.0 | |
| α-Bulnesene | 1508 | 1509 | 2.3 | 1.7 | |
| β-Bisabolene | 1509 | 1505 | | | 1.4 |
| γ-Cadinene | 1512 | 1513 | | 0.8 | 1.2 |
| 7-*epi*-α-Selinene | 1516 | 1520 | | | 0.3 |
| δ-Cadinene | 1523 | 1522 | 1.1 | 3.0 | 3.5 |
| *trans*-Cadina-1,4-diene | 1531 | 1533 | | | 0.5 |
| Elemol | 1551 | 1548 | | 0.9 | |
| Germacrene B | 1558 | 1559 | 1.3 | | |
| Muurol-5-en-4-α-ol | 1558 | 1559 | | 1.2 | |
| Maaliol | 1564 | 1566 | | | 0.6 |
| Caryophyllenyl alcohol | 1567 | 1570 | | | 0.3 |
| Spathulenol | 1578 | 1577 | 1.7 | | **5.2** |
| Unidentified | 1583 | | | | 4.7 |
| Caryophyllene oxide | 1584 | 1582 | 2.1 | 1.3 | |
| Viridiflorol | 1590 | 1592 | | | 1.6 |
| Guaiol | 1597 | 1600 | | **11.2** | 0.8 |
| Rosifoliol | 1600 | 1600 | | | 0.9 |
| 6-Methoxy-elemicin | 1601 | 1595 | 1.7 | | |
| Rosifoliol | 1610 | 1600 | | 0.7 | |
| unidentified | 1621 | | | | 0.6 |
| 1-*epi*-Cubenol | 1626 | 1627 | | | 0.7 |
| Muurola-4,10(14)-dien-1-β-ol | 1630 | 1630 | | 1.1 | |
| Camphoric acid | 1633 | 1634 | | | 0.6 |
| *allo*-Aromadendrene epoxide | 1637 | 1639 | | | 1.6 |
| *epi*-α-Muurolol (=τ-Muurolol) | 1640 | 1640 | | 4.4 | 1.5 |
| α-Muurolol (=Torreyol) | 1644 | 1644 | | 1.3 | 0.3 |
| α-Eudesmol | 1652 | 1652 | | 1.3 | |
| α-Cadinol | 1653 | 1652 | | | 1.7 |
| Selin-11-en-4-α-ol | 1655 | 1658 | | | 0.4 |
| Pogostol | 1656 | 1651 | **6.6** | **19.8** | |
| Unidentified | 1660 | | 0.7 | | |
| Bulnesol | 1669 | 1670 | | 1.4 | |
| *epi*-β-Bisabolol | 1670 | 1670 | | | 1.4 |
| α-Bisabolol | 1682 | 1685 | | | 1.0 |
| Apiole | 1687 | 1677 | **22.2** | **28.1** | |
| Monoterpene hydrocarbons | | | 21.7 | 4.4 | |
| Oxygenated monoterpenoids | | | | 0.8 | |
| Sesquiterpene hydrocarbons | | | 42.3 | 19.5 | 76.2 |
| Oxygenated sesquiterpenoids | | | 10.3 | 44.7 | 18.0 |
| Phenylpropanoids | | | 23.9 | 28.1 | |
| Others | | | | 0.6 | |
| **Total identified** | | | **98.3** | **100.0** | **94.8** |

$RI^{Calc.}$ = based on DB-5ms capillary column and alkane standards (C8-C32).
$RI^{Lit.}$ = based on Adams [31].

**Table 2:** Antimicrobial activity of *Nectandra* essential oils from the Amazon.

| | Minimum inhibitory concentration ($\mu g \cdot mL^{-1}$) | | |
|---|---|---|---|
| | Gentamicin (positive control) | *N. puberula* | *N. cuspidata* |
| *P. aeruginosa* | <19.5 | 1250 | 1250 |
| *E. coli* | <19.5 | 19.5 | 19.5 |
| *S. epidermidis* | <19.5 | 1250 | 1250 |
| *S. aureus* | <19.5 | 625 | 625 |
| *B.cereus* | <19.5 | 625 | 312.5 |

## Experimental

***Plant material:*** *Nectandra puberula* Nees was collected in Santarém (S 02° 25.0′ 10.2″ W 54° 44′ 26.9″) and a voucher (HSTM000092) was deposited in the herbarium of Universidade Federal do Oeste do Pará, Santarém, Pará State, Brazil. *N. cuspidata* was collected in Caxiuanã National Forest, Marajó Island (S 01º 44′ 18.8″ W 51º 27′ 27.4″), and a voucher (MG 104948) was deposited in the Herbarium of Museum Paraense Emílio Goeldi, Belém, Pará state, Brazil.

Leaves and branches from several mature plants were air-dried, pulverized, and subjected to hydrodistillation using a Clevenger-type apparatus (100 g, 3 h). The essential oils were dried over anhydrous sodium sulfate, and their percentage contents were calculated on the basis of the dry weight of plant material. The moisture contents of the samples were calculated after phase separation using a Dean–Stark trap (5 g, 60 min) using toluene as the solvent phase.

***Gas chromatographic – mass spectral analysis:*** The essential oils were analyzed by GC-MS using an Agilent 6890 GC with an Agilent 5973 mass selective detector (MSD) [operated in the EI mode (electron energy = 70 eV), scan range = 40-400 amu, and scan rate = 3.99 scans/sec], and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, film thickness of 0.25 µm, length of 30 m, and internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. Injector temperature was 200ºC and detector temperature was 280ºC. The GC oven temperature program was used as follows: 40ºC initial temperature, held for 10 min; increased at 3ºC/min to 200ºC; increased at 2ºC/min to 220°C. A 0.2%, w/v, solution of the sample in $CH_2Cl_2$ was prepared and 1 µL was injected using a splitless injection technique. The percentages of each component are reported as raw percentages based on total ion current without standardization. Individual components were identified by comparison of both mass spectrum and GC retention data with authentic compounds present in commercial libraries [31].

***Antibacterial assay:*** The essential oils were screened for antimicrobial activity against *Escherichia coli* (ATCC No. 10798), *Pseudomonas aeruginosa* (ATCC No. 27853), *Bacillus cereus* (ATCC No. 14579), *Staphylococcus aureus* (ATCC No. 29213), and *Staphylococcus epidermidis* (ATCC No. 12228), using the microbroth dilution technique, as previously reported [32]. Thus, 50 µL of 1%, w/v, solution of the samples in DMSO was placed in a well of 96 well plates and 50 µL of cation-adjusted Mueller Hinton broth (CAMHB) was added. The sample solutions were then serially diluted (1:1) by transferring 50 µL of sample-CAMHB mixture to the next lane and adding 50 µL fresh CAMHB to obtain concentration from 2500 $\mu g.mL^{-1}$ to 12.5 $\mu g.mL^{-1}$. The bacteria were harvested from a fresh culture and added to each well at a concentration of approximately $1.5 \times 10^8$ colony forming units (CFU)/mL. The plates were incubated at 37°C for 24 h and the final minimum inhibitory concentration (MIC) was determined as the lowest concentration with no turbidity. Gentamicin was used as positive antibiotic control and DMSO was used as negative control.

***Cytotoxic assay:*** MCF-7 human breast adenocarcinoma cells (ATCC No. HTB-22) were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 30 mM HEPES, $NaHCO_3$, and penicillin streptomycin. *In vitro* cytotoxic activity of essential oils on MCF-7 cells was performed using the 96-well MTT assay, as previously reported [33]. Cells were plated into 96-well cell culture plates at a concentration of $1.2 \times 10^4$ cells/well and a volume of 100 µL in each well. The plate was then labeled and incubated at 37°C and 5% $CO_2$ for 48 h. By then, the cells had reached 70-80% confluent growth. The supernatant fluid was gently aspirated (without touching the bottom of the well to avoid removing cells) and replaced with 100 µL growth medium containing 1.0 µL and 0.5 µL of essential oil (1% in DMSO), giving a final concentration of 100 and 50 $\mu g.mL^{-1}$. The plate was then incubated at 37°C and 5% $CO_2$ for 48 h. Then, the liquid was gently aspirated from each well. In a tube, 10 mL feeding medium was mixed with 2 mL of MTT stock solution (and was protected from light). Into each well, 100 µL of the MTT solution was added and the pre-read absorbance was immediately measured spectrophotometrically at 570 nm (using a Molecular Devices SpectraMax Plus 384 microplate reader. Formazan crystals were formed over the course of 4 h at 37°C and 5% $CO_2$. After 4h, DMSO was used to dissolve the purple crystals. The amount of MTT-formazan produced was determined spectrophotometrically at 570 nm. Growing medium, DMSO, and tingenone (100 $\mu g.mL^{-1}$) served as negative, compound, and positive controls, respectively. Solutions were added to wells in 8 replicates. Average absorbances, standard deviations, and percent kill ratios (% $kill_{oil}$ / % $kill_{control}$) were calculated. Median inhibitory concentrations ($IC_{50}$) were determined using the Reed-Muench method [34].

**Acknowledgments** – The authors are grateful to CNPq - Programa "Ciências sem fronteiras" for financial support.

## References


[1] Shi G-L, Xie Z-M, Li H-M. (**2014**). High diversity of Lauraceae from the Oligocene of Ningming, South China. *Palaeoworld*, ***23***, 336-356.

[2] Rohwer JG. Lauraceae. K Kubitzki, Rohwer JG., Bittrich V. (Eds.) (**1993**) *The families and genera of vascular plants II Flowering plants, dicotyledons, Magnoliid, Hamamelid and Caryophyllid families*. Springer-Verlag, Berlin, Heidelberg, New York, pp. 366-391.

[3] Marques CA. (**2001**) Importância econômica da família Lauraceae Lindl. Floresta e Meio Ambiente, ***8***,195-206.

[4] Chanderbali AS, Werff H, Renner SS. (**2001**) Phylogeny and historical biogeography of Lauraceae: evidence from the chloroplast and nuclear genomes. *Annals of the Missouri Botanical Garden*, ***88***,104-134.

[5] Macías-Villamizar VE, Cuca-Suárez LE, Coy-Barrera ED. (**2015**) Genus *Nectandra*: Phytochemistry and biological activity. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, ***14***, 317-342.

[6] Baitello JB, Lorea-Hernández FG, Moraes PLR, Esteves R, Marcovino JR. (**2003**) Lauraceae. In *Flora Fanerogâmica do Estado de São Paulo*. Vol. 3. Wanderley MGL, Shepherd GJ, Giulietti AM, Melhem T. (Eds.). Fapesp-Rilla, São Paulo, 149-223

[7] Zanon MMF, Goldenberg R, De Moraes PLR. (**2009**) O gênero *Nectandra* Rol. ex Rottb. (Lauraceae) no Estado do Paraná, Brasil. *Acta Botanica Brasilica*, ***23***, 22-35.

[8] Moro JC, Fernandes JB, Vieira PC, Yoshida M, Gottlieb OR, Gottlieb HE. (**1986**) Neolignans from *Nectandra puberula*. *Phytochemistry*, ***26***, 269-272.

[9] Batista AL, Yoshida NC, Garcez FR, Garcez WS. (**2015**) Chemical constituents from *Nectandra cuspidata* Nees with Lauraceae. *Biochemical Systematics and Ecology*, ***61***, 229-231.

[10] Romoff P, Ferreira MJP, Padilla R, Toyama DO, Fávero OA, Lago JHG. (**2010**) Chemical composition of volatile oils from leaves of *Nectandra megapotamica* Spreng. (Lauraceae). *Química Nova*, ***33***, 1119-1121.

[11] Cicció JF, Chaverri C, Díaz C. (**2009**) Volatile compounds of *Nectandra salicina* (Lauraceae) from Costa Rica and their cytotoxic activity on cell lines. *Química Nova*, ***32***, 417-420.

[12] Wu X, Vogler B, Haber WA, Setzer WN. (**2006**) A phytochemical investigation of *Nectandra membranacea* from Monteverde, Costa Rica. *Natural Products Communications*, ***1***, 465-468.

[13] Pino JA, Marbot R, Paya A, Chao D, Herrera D. (**2005**) Aromatic plants from western Cuba. III. Composition of the leaf oils of *Lippia strigulosa* (Mart. et Gal.) Mold. and *Nectandra coriacea* (Sw.) Griseb. *Journal of Essential Oil Research*, ***17,*** 648-650.

[14] Torres AM, Ricciardi GAL, Agrelo de Nassif AE, Ricciardi AIA, Dellacassa E. (**2005**) Examen del aceite esencial de *Nectandra angustifolia* (Schrad.) Nees & Mart. ex Nees. Comunicaciones Científicas y Tecnológicas. Universidad Nacional del Nordeste, Argentina, Resumen E-013, http://www.unne.edu.ar/unnevieja/Web/cyt/com2005/index.htm, accessed on April 2016.

[15] Naves YR. (**1951**) Etudes sur les matieres vegetales volatiles-CXVIII-Sur l'huile essentielle du Louro inhamuy (*Nectandra elaiphora* Barb Rodr) de l'Amazonie Bresilienne. *Bulletin de la Société Chimique de France*, ***18***, 987-991.

[16] Cuca LE, Coy ED, Alarcón MA, Fernández A, Aristizábal FA. (**2011**) Cytotoxic effect of some natural compounds isolated from Lauraceae plants and synthetic derivatives. *Biomedica*, ***31***, 335-343.

[17] Ponci V, Figueiredo CR, Massaoka MH, de Farias CF, Matsuo AL, Sartorelli P, Lago JH. (**2015**) Neolignans from *Nectandra megapotamica* (Lauraceae) display *in vitro* cytotoxic activity and induce apoptosis in leukemia cells. *Molecules*, ***20***, 12757-12768.

[18] Le Quesne PW, Larrahondo JE, Raffauf RF. (**1980**) Antitumor plants. X. Constituents of *Nectandra rigida*. *Journal of Natural Products*, ***43***, 353-359.

[19] (a) Nakagawa Y, Suzuki T. (**2003**) Cytotoxic and xenoestrogenic effects via biotransformation of *trans*-anethole on isolated rat hepatocytes and cultured MCF-7 human breast cancer cells. *Biochemical Pharmacology*, ***66***, 63-73; (b) Liao J-F, Huang S-Y, Jan Y-M, Yu L-L, Chen C-F. (**1998**) Central inhibitory effects of water extract of *Acori graminei* rhizoma in mice. *Journal of Ethnopharmacology*, ***61***, 185-193; (c) Jaganathan SK, Supriyanto E. (**2012**) Antiproliferative and molecular mechanism of eugenol-induced apoptosis in cancer cells. *Molecules*, ***17***, 6290-6304; (d) Carvalho AA, Andrade LN, Sousa EBV, De Sousa, DP. (**2015**) Antitumor phenylpropanoids found in essential oils. *BioMed Research International* 2015:392674.

[20] Wei PL, Tu SH, Lien HM, Chen LC, Chen CS, Wu CH, Huang CS, Chang HW, Chang CH, Tseng H, Ho YS. (**2012**) The *in vivo* antitumor effects on human COLO 205 cancer cells of the 4,7-dimethoxy-5-(2-propen-1-yl)-1,3-benzodioxole (apiole) derivative of 5-substituted 4,7-dimethoxy-5-methyl-l,3-benzodioxole (SY-1) isolated from the fruiting body of *Antrodia camphorata. Journal of Cancer Research and Therapeutics,* ***8***, 532-536.

[21] Cole RA, Bansal A, Moriarity DM, Haber WA, Setzer WN. (**2007**) Chemical composition and cytotoxic activity of the leaf essential oil of *Eugenia zuchowskiae* from Monteverde, Costa Rica. *Journal of Natural Medicines*, ***61***, 414-417.

[22] Legault J, Pichette A. (**2007**) Potentiating effect of beta-caryophyllene on anticancer activity of alpha-humulene, isocaryophyllene and paclitaxel. *Journal of Pharmacy and Pharmacology*, ***59***, 643-647.

[23] Grecco SS, Martins EG, Girola N, de Figueiredo CR, Matsuo AL, Soares MG, Bertoldo Bde C, Sartorelli P, Lago JH. (**2015**) Chemical composition and *in vitro* cytotoxic effects of the essential oil from *Nectandra leucantha* leaves. *Pharmaceutical Biology*, ***53***, 133-137.

[24] Bakkali F, Averbeck S, Averbeck D, Idaomar M. (**2008**) Biological effects of essential oils - A review. *Food and Chemical Toxicology*, ***46***, 446-475.

[25] Knobloch K, Pauli A, Iberl B, Weigand H, Weis N. (**1989**) Antibacterial and antifungal properties of essential oil components. *Journal of Essential Oil Research*, ***1***, 119-128.

[26] Schmidt JM, Noletto JA, Vogler V, Setzer WN. (**2007**) Abaco bush medicine: Chemical composition of essential oils of four aromatic medicinal plants from Abaco Island, Bahamas. *Journal of Herbs, Spices & Medicinal Plants*, ***12***, 43-65.

[27] Kazemi M, Rostami H. (**2015**) Chemical composition, antimicrobial and antioxidant activities of the essential oil of *Psammogeton canescens*. *Natural Product Research*, ***29***, 277-280.

[28] Lago JH, Souza ED, Mariane B, Pascon R, Vallim MA, Martins RC, Baroli AA, Carvalho BA, Soares MG, dos Santos RT, Sartorelli P. (**2011**) Chemical and biological evaluation of essential oils from two species of Myrtaceae - *Eugenia uniflora* L. and *Plinia trunciflora* (O. Berg) Kausel. *Molecules*, ***16***, 9827-9837.

[29] de Lima BR, da Silva FM, Soares ER, de Almeida RA, da Silva Filho FA, Pereira Junior RC, Hernandez Tasco ÁJ, Salvador MJ, Koolen HH, de Souza AD, Pinheiro ML. (**2016**) Chemical composition and antimicrobial activity of the essential oils of *Onychopetalum amazonicum* R.E.Fr. *Natural Product Research*, ***31***, 1-4.

[30] Dahham SS, Tabana YM, Iqbal MA, Ahamed MB, Ezzat MO, Majid AS, Majid AM. (**2015**) The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of *Aquilaria crassna*. *Molecules*, ***20***, 11808-11829.

[31] Adams RP. (**2007**) *Identification of essential oil components by gas chromatography / mass spectrometry*, 4th Ed. Allured Publishing, Carol Stream, Illinois, USA.

[32] Setzer MC, Moriarity DM, Lawton RO, Setzer WN, Gentry GA, Haber WA. (**2003**) Phytomedicinal potential of tropical cloudforest plants from Monteverde, Costa Rica. *Revista de Biología Tropical*, ***51***, 647-674.

[33] Palazzo MC, Wright HL, Agius BR, Wright BS, Moriarity DM, Haber WA, Setzer WN. (**2009**) Chemical compositions and biological activities of leaf essential oils of six species of Annonaceae from Monteverde, Costa Rica. *Records of Natural Products*, ***3***, 153-160.

[34] Reed LJ, Muench H.. (**1938**) A simple method of estimating fifty per cent endpoints. *American Journal of Hygiene*, ***27***, 493-497.