ISSN 0974 – 5211

Journal of Natural Products

Volume 6 (2013)

www.JournalofNaturalProducts.Com

Research Paper

Anti-proliferative activity of four aromatic plants of Benin

J.P. Noudogbessi[1], L. Delort[2, 3], P. Chalard[4, 5], H. Billard[2, 3], G. Figueredo[6], N. Ruiz[2, 3], J.C. Chalchat[7], D. Sohounhloue[1*], F.C. Chézet[2, 3]

[1]Laboratoire d'Etude et de Recherche en Chimie Appliquée (LERCA), Ecole Polytechnique d'Abomey-Calavi, 01 BP 2009 Cotonou, Rép. du Bénin

[2]Unité de Nutrition Humaine UMR 1019 INRA-UdA

[3]Laboratoire des Sciences Végétales et Fongiques Pharmaceutiques (SVFp)

[4]Institut de Chimie de Clermont-Ferrand (ICCF), Ecole Nationale Supérieure de Chimie de Clermont-Ferrand (ENSCCF), BP10448, F-63000 Clermont-Ferrand (France)

[5]UMR 6296, ICCF - CNRSF-63171 Aubière

[6] Laboratoire d'Analyse des Extraits Végétaux et des Arômes (LEXVA Analytique), 460 Rue du Montant, 63110 Beaumont France

[7]Laboratoire de Chimie des Huiles Essentielles, Université Blaise-Pascal, Clermont-Ferrand, Campus des Cézeaux, 63177 Aubière cedex, France

*Corresponding Author

(Received 22 March 2013; Revised 26 March-14 May 2013; Accepted 18 May 2013)

ABSTRACT

The present study reports the results of the analysis of chemical profiles and *in vitro* evaluation of the antiproliferative activities of *O. insignis*, *P. butyracea*, *S. aethiopicus* and of *X. aethiopica* essential oils on breast cancer cells (MCF-7). The four essential oils, obtained by hydrodistillation after different harvest periods, were studied by GC/FID and by GC/MS. The bioactivity of these volatile extracts was evaluated by testing their antiproliferative efficiency against cancer cells MCF-7 using a resazurin fluorescent test. The chromatographic analysis revealed major compounds depending of the essential oil analyzed. Thus, the *O. insignis* essential oil was mainly constituted of α-pinene (13.7%), β-pinene (13.7%) and of myrcene (58.9%). *P. butyracea* was potentially rich in β-caryophyllene (67.4%). Moreover, the major compounds contained in *S. aethiopicus* volatile extract are α-humulene (11.0%), β-caryophyllene (13.4%) and β-pinene (44.5%) while those marking *X. aethiopica* volatile extract are terpinen-4-ol (9.8%), myrtenal (13.2%) and 1,8-cineole (15.8%). The biological investigations showed that essential oils tested contain molecules that can delay or suppress cancer cells (MCF-7) activity. Following to these tests of evaluation of the volatile extracts antiproliferative potential targeted, the inhibitory concentration values (IC_{50}) determined are 127.99±28.80µg/ml (*X. aethiopica*), 133.48±2.6µg/ml (*P. butyracea*), 215.68±12.17µg/ml (*S. aethiopicus*) and IC_{50}>327.6µg/ml for *O. insignis*. The inhibitory activity observed could be attributed to the essential oils major compounds antiproliferative power which would have only acted or in synergy with the components appeared in low proportions.

Keywords: Extract; Volatile; Antiproliferative; Benin.

INTRODUCTION

The essential oils of aromatic plants and their components have a wide range of applications in ethno-medicine, preservation, food flavoring and fragrances and in the perfume industries (Unlu, et al., 2010). *O. insignis* was a small tree of the Sudano-Guinean regions of Africa, reaching 5-7m of height. It gave small flowers and gleaming black fruits (Akoègninou, et al., 2006). Its roots treated gonorrhea, purulent urethritis, syphilis. It's crushed leaves and boiled in some milk were used as an anthelmintic. Its bark scared for enteralgia and hepatic diseases. *P. butyracea* was a botanical species of the regions of Sudano-Guinean climate that extends from Sierra - Leone to Cameroon (Aubreville, 1936). Its parts (almonds, leaves, flowers, barks, roots) were involved in diet, aesthetic and in traditional medicine (Abbiw, 1990; Sinsin and Sinadouwirou, 2003). *S. aethiopicus* was itself a rhizomatous plant cultivated in tropical areas of Africa and carrying two various raised over ground stems. The aqueous decoction of its roots served traditionally to treat feminine infertility problems (Adjanohoun, et al., 1989). Finally, *X. aethiopica* was a high tree (20 m). It carried evergreen, oblong, lanceolate, obtuse or rounded, glaucous, glabrous and it was wide spread in the Western, Central and in the South Africa (Somova, et al., 2001). *X. aethiopica* fruit was a flavoring of the traditional culinary dishes (Addae-Mensah and Sofowora, 1979). It was a plant used in the treatment of the feminine infertility (Fish and Waterman, 1971; Couillerot, et al., 1996). These four plants (*O. insignis*, *P. butyracea*, *S. aethiopicus* and *X. aethiopica*) have been the subject of several investigations, as well in chemistry and in biology (Asekun and Adeniyi, 2004; Ayédoun, et al., 1996; Karioti, et al, 2004; Aïssi, et al., 2011, Tchobo, et al., 2007; Goulding and Robert, 1915; Makhuvha, et al., 1997; Mcgaw, et al., 1997; Lindsey, et al., 1999; Zschocke, et al., 2000; Holzapfel, et al., 2002; Stafford, et al., 2005). In Benin, the antiproliferative capacity of these plants was barely approached. By cons, in Nigeria, it was noted a remarkable antiproliferative character of the essential oil from *X. aethiopica* fruits (IC_{50} = 5mg/ml) on Hep-2cell line (Asekun and Adeniyi, 2004). Moreover, *P. butyracea* barks xanthones exhibited an interesting cytotoxic activity on MCF-7 (Zelefack, et al., 2009). The present work objected to study the chemical composition by GC/MS of the volatile extracts of *O. insignis*, *P. butyracea*, *S. aethiopicus*, *X. aethiopica* harvested in Benin and to assess their activities on breast cancer cells MCF-7 (Lacroix, 2004).

MATERIALS AND METHODS

The plants materials were harvested in the spontaneous state in Benin and these were identified by botanical specialists. The date and the place of harvesting of studied material were indicated in Table I. In the laboratory, the plants material were stored at 20-22°C and protected from sunlight during the extractions. The identification and authentication were made at Abomey-Calavi University National Herbarium (Benin). Voucher numbers (*O. insignis* Delile (*Anacardiaceae*):**AA6449/HNB**; *P. butyracea* Sabine (*Clusiaceae*):**AA6450/HNB**; *S. aethiopicus* (Schweinf.) B. L. Burtt (Zingiberaceae):**AA6451/HNB;** of *X. aethiopica* (Dunal) A. Rich. (*Annonaceae*):**AA6452/HNB)** were assigned to specimen.

Table-1: Harvest sites and the four plants parts studied.

Plant species	**Oi**	**Pd**	**Sa**	**Xa**
Harvested places	Cotiakou	Natitingou	Manigri	Banigbe
Harvested date	19-07-08	20-10-07	07-07-07	11-07-07
Harvested organ	leaves	stem-bark	leaves	fruit
Oi=*Ozoroa insignis*; **Pd**=*Pentadesma buyracea* ; **Sa**=*Siphonochilus aethiopicus*; **Xa**=*Xylopia aethiopica*				

***Volatile oil preparation*:** 250g of plants material (leaves, stem-bark, fruits or roots) were subjected to hydrodistillation with water (21) using a Clevenger-type apparatus for a period of three hours. Distillation was carried out twice for each plant, and the oils obtained for each were pooled, dried over anhydrous sodium sulfate and then stored at 4°C in amber glass vials until analysis.

Essential oil GC/FID and GC/MS Analysis. The essential oils were analyzed on a Hewlett-Packard gas chromatograph Model 5890, coupled with a Hewlett-Packard MS model 5871, equipped with a DB5 MS column (30m× 0.25mm, 0.25μm), programming from 50°C (5 min) to 300∘C at 5∘C/min, 5 min hold. Helium as carrier gas (1.0 ml/min); injection in split mode (1: 30); injector and detector temperature, 280 and 280°C respectively. The MS works in electron impact mode at 70eV; electronmultiplier: 2500eV; ion source temperature: 180°C; mass spectra data were acquired in the scan mode in *m/z* range 33–450. The essential oil was analysed on a Hewlett-Packard gas chromatograph Model 6890, equipped with a DB5 MS column (30m× 0.25 mm, 0.25μm), programming from 50°C (5 min) to 300°C at 5°C/min, 5 min hold. Hydrogen as carrier gas (1.0ml/min); injection in split mode (1: 60); injector and detector temperature, 280 and 300°C respectively. The essential oil was diluted in hexane: 1/30. The compounds found by GC in the different essential oils were identified by comparing their retention indices with those of reference compounds in the literature. Their identities were further confirmed using GC/MS and comparing their mass spectra with those of reference substances (Rösch, et al., 1999; Adams, 1995; Jennings and Shibamoto, 1980; Davies, 1990; Doimo, et al., 1999).

***Cancer cells*:** The cell line under investigation was the human breast adenocarcinoma MCF-7 obtained from the American Type Culture Collection. MCF-7 cell line was maintained in RPMI-1640 supplemented with 10% heat-inactivated Fetal Calf Serum (FCS), L-glutamine (2mM) and gentamicin (50µg/ml) at 37°C with 5% CO_2.

***Antiproliferative activity*:** MCF-7 cells (50000/ml) were seeded in 96-well plates in the supplemented medium. After 24 hours, cells were treated with volatile oils initially dissolved in dimethylsulfoxide (*O. insignis*: 0.04%, *P. butyracea*: 0.0175%, *S. aethiopicus*: 0.04%, *X. aethiopica*: 0.02%)) and sonicated (2 min 30 s; 2 cycles). The following concentration ranges were tested for each plant: *O. insignis* (0.015-0.04% or 122.8-327.6µg/ml); *P. butyracea* (0.0135-0.075% or 119.07-154.35µg/ml); *S. aethiopicus* (0.02–0.04% or 178.0-356.0µg/ml); *X. aethiopica* (0.005–0.02% or 46.2-184.6µg/ml). In parallel, a control with DMSO was realized for each tested essential oil. After 72 hours of treatment with essential oils, the proliferation of cancer cells was measured using a fluorescent test. For that, the culture medium was replaced with a solution of resazurin (25mg/ml) which is oxidized into resofurinin the presence of metabolically active cells (Jardé, et al., 2009). So the fluorescence intensity was proportional to the number of viable cancer cells. Fluorescence was then measured using an automatic plate reader (FluoroskanAscentFL®, Thermo Electron Corporation, France) using an excitation wavelength of 530 nm and an emission wavelength of 590nm. The cell proliferation assay was performed in triplicate for each independent assay (n = 3). Results were expressed as the percentage of inhibition of the cell proliferation relative to control cells (DMSO). The IC_{50} (50% inhibitory concentration) was calculated by plotting the percentage of cell survival against respective concentrations of volatile extracts used in the assay. Values for collected data were expressed as average±SD (SD=standard deviation) of at least three independent experiments. Statistical analysis was performed using the paired bilateral

Student's *t*-test with Stat View Software version 5.0 (SAS Institute Inc.). Differences at $P < 0.05$ (flagged as*) were considered statistically significant.

RESULTS

The essential oils yields values of the four plant species (*O. insignis, P. butyracea, S. aethiopicus* and *X. aethiopica*) were respectively 0.204%, 0.37%, 0.16% and 1.25%. The table-2 presents the different proportions of the chemical compounds which characterize the samples of essential oils studied. In this table, 25 to 41 compounds representing 91.6% to 99.7% of the essential oils were identified. The distribution of the large families of compounds varied according to the plant species. Thus, it was observed a preponderance of hydrogenated monoterpenes in *O. insignis* (87.2%) and in *S. aethiopicus* (67.0%), hydrogenated sesquiterpenes in *P. butyracea* (90.5%) and oxygenated monoterpenes in *X. aethiopica* (57.2%). The main constituents (> 10%) identified independently of essential oils various origin and organs distilled were β-caryophyllene (13.4 - 67.4%), myrcene (58.9%), β-pinene (13.7-44.5%), 1,8-cineole (15.8%), α-pinene (13.7%), myrtenal (13.2%), α-humulene (11.0-11.6%). The biological investigations carried out with the four essential oils have shown an inhibition of breast cancer cells proliferation in presence of volatile extracts of *P. butyracea, S. aethiopicus, X. aethiopica*, without influence of *O. insignis* essential oil. The table-3 below shows the influence of *P. butyracea* essential oil on MCF-7 proliferation. *P. butyracea* essential oil inhibited in a remarkable way the proliferation of cells at the concentration of 119.1µg/ml and 154.4µg/ml. At the highest tested concentration, more than 70% of these cells were inhibited. The IC_{50} obtained was 133.5±2.6µg/ml. The results presented on the table-4 indicate the proliferation of breast cancer cells MCF-7 in the presence of *S. aethiopicus* essential oil. We observed an inhibition of MCF-7 cells by *S. aethiopicus* essential oil with concentrations superior to 178.0 µg/ml. The inhibition was become important when the tested dose reached 267.0µg/ml. At 356.0µg/ml, the percentage of growth was almost inexistent (only 1.5%). The IC_{50} obtained was 215.68±12.17µg/ml. The table-5 reported the results concerning *X. aethiopica* essential oil effect on breast cancer cell proliferation. At the highest tested dose (184.6µg/ml), less than 25% of the cells have proliferated. The IC_{50} value obtained was 127.9±28.8µg/ml. The values recorded in the table – 6 shows that *Ozoroa insignis* essential oil did not inhibit significantly the cancer cells proliferation. At 327.6µg/ml, the proliferation percentage (82.53%) was very close to the value recorded when the cells were not treated with *O. insignis* essential oil.

DISCUSSION

This bio-reactivity observed was to be reported to the chemical composition of each of the essential oil of *P. butyracea, S. aethiopicus* and of *X. aethiopica*. Indeed, the GC/MS analysis showed that β-caryophyllene (67.4%) and β-pinene (44.5%) was the major compounds of *P. butyracea* and *S. aethiopicus* volatile extracts. These two hydrocarbons were for terpene character whereas 1,8-cineole (15.8%) and myrtenal (13.2%) were the dominating compounds identified in the essential oil extracted from *X. aethiopica* fruits. The analysis of the graphs above shows a difference of behavior of MCF-7 cells in the presence of four volatile extracts. These different behaviors assimilated to bio-reactions could be reported to the chemical compositions of essential oils studied whose major compounds acted only or by synergy effect on MCF-7 cells. However, it shows through the results obtained that there activities observed by determining the IC_{50} varied from an essential oil to another (*P. butyracea*: 133.5±2.2mg/ml; *S. aethiopicus*: 215.7±12.2mg/ml; *X. aethiopica*:

127.9±28.8mg/ml). The *P. butyracea* essential oil activity observed could be justified by the presence of β-caryophyllene (67.4%) previously recognized as possessing antiproliferative properties on MCF-7 cells (Jardé, et al., 2009; Sylvestre et, al., 2005; , Lampronti, et al., 2006; Sylvestre et, al., 2006; Salvador, et al., 2011). According to the results presented on the figures II and III, the activities generated on breast cancer MCF-7 cells could be attributed respectively to the preponderance of β-pinene (44.5%) in *S. aethiopicus* essential oil, of 1,8-cineole (15.8%) and myrtenal (13.2%) in *X. aethiopica* volatile extracts. Indeed, Lampronti et al. (2006) have reported anticancer properties of α-pinene. Also, it was brought back by Cole et al. (2007) that α-pinene would possess anticancer virtues. Concerning *X. aethiopica* essential oil the inhibition expected was observed beyond 100µg/ml but only 184.6µg/ml, the proliferation rate of cells exceeded 15%. Previously, it has reported such an activity of *X. aethiopica* on the line laryngeal carcinoma (Asékun et Adeniyi, 2004). These performances towards cells could be due to the major compounds at low concentrations and probably to other antagonist compounds of essential oils tested. Compared with *O. insignis* volatile extract, no interesting activity was observed (Table-6). According to all the observations above, Xa essential oil was more active than Pd, Sa and then Oi. Nevertheless, it is important to note that the reactivity of *X. aethiopica*, *P. butyracea* and *S. aethiopicus* essential oils remains extremely low compared with tamoxifen (IC_{50} = 3.77±0.03 µg/ml) having served as reference during these investigations. The works previously reported in the literature had to show the antiproliferative character of certain essential oils rich in terpenes (α and β-pinene, α-humulene), in 1,8-cineole and other oxygenated compounds on cancer cell lines (P388D1, U-373 MG, MDA-MB-231, LNCaP, MCF-7) (AitM'Barek, et al., 2007; Imelouane, et al., 2010; Palazzo, et al., 2009; Loizzo, et al., 2007).

CONCLUSION

This work was devoted to the determination of the chemical compositions by GC/FID and GC/MS then to the assessment of the antiproliferative activity of *O. insignis*, *P. butyracea*, *S. aethiopicus* and *X. aethiopica* volatile extracts harvested in Benin. These essential oils have been variously marked by several major compounds of the hydrogenated and oxygenated terpenes group. An inhibition of MCF-7 cells proliferation was observed at the level of *P. butyracea*, *S. aethiopicus* and *X. aethiopica* essential oils. This activity seems to be related to the chemical profile of these three essential oils whose active compounds were diverse nature. *O. insignis* volatile extract was not active.

REFERENCES

Abbiw, D.K., (1990): Useful plants of Ghana West African use of wild and cultivated plants. Intermadiate Tecnology Publications, London and the Royal Botanic Gardens Kew, Richmond, United Kingdom, pp. 337.

Adams, R.P., (2007): Identification of essential oils components by gas chromatography/ mass spectrometry, 4th Edition, Allured Publishing Corporation, USA.

Addae-Mensah, I., Sofowora, E.A., (1979): Conservation and sustainable use of medicinal plants in Ghana. *Planta Medica*, 35: 94-96.

Adjanohoun, E.J., Adjakidjè, V., Ahyi, M.R.A., Ake Assi, Akoègninou, A., d'Ameida, J., Apovo, F., Boukef, K., Chadaré, M., Cusset, G., Dramane, K., Eymé, J., Gassita, J.N., Gbaguidi, N., Goudoté, E., Guniko, S., Houngnon, P., Lo, I., Keita, A., Kiniffo, H.V., Koné-Bamba, D., Musampa Nseyya, A., Saadou, M., Sodogandji, Th., de Souza, S., Tchabi, A., Zinsou, D.C., Zohoun, Th., (1989): Medicine traditionnelle et

Pharmacopée, Contribution aux etudes ethnobotaniques et floristiques en République Populaire du Bénin, ACCT, Paris, 81.

Aïssi, M.V., Tchobo, F.P., Natta, A.K., Piombo, G., Villeneuve, P., Sohounhloue, D.C.K., Soumanou, M.M., (2011) : Effet des prétraitements, poste-récolte des amandes de *Pentadesma butyracea* (Sabine) sur la technologie d'extraction en milieu réel et la qualité du beurre. *Oléagineux, Corps gras, Lipide*, 18(6) : 384-392.

AitM'Barek, L., Ait Mouse, H., Jaâfari, A., Aboufatima, R., Benharref, A., Kamal, M., Bénard, J., El Abbadi, N., Bensalah, M., Gamouh, A., Chait, A., Dalal, A. and Zyad, A., (2007): Cytotoxic effect of essential oil of thyme (*Thymus broussonettii*) on the IGR-OV1 tumor cells resistant to chemotherapy. *Brazilian Journal of Medical and Biological Research*, 40: 1537-1544.

Akoègninou, A., Van Der Burg W.J., Van der Maesen, L.J.G., (2006) : Flore Analytique du Bénin. pp. 317.

Asekun, O.T., Adeniyi, B.A., (2004): Antimicrobial and cytotoxic activities of the fruit essential oil of *Xylopia aethiopica* from Nigeria. *Fitoterapia*, 75: 368-370.

Aubreville, (1936): La flore forestière de la Côte-d'Ivoire, Larose éd. Paris.

Ayédoun, A.M., Adeoti, B.S., Sossou, P.V., Leclercq, P.A., (1996): Influence of fruit conservation methods on the essential oil composition of *Xylopia aethiopica* (Dunal) A. Richard from Benin. *Flavour and Fragrance Journal*, 11: 245-250.

Cole, R.A., Bansal, A., Moriarity, D.M., Haber,W.A., Setzer, W.N., (2007): Chemical composition and cytotoxic activity of the leaf essential oil of *Eugenia zuchowskiae* from Monteverde, Costa Rica. *Natural Medicines*, 61(4): 414-417.

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

Doimo, L., Fletcher, R.J., D'Arcy, B.R., (1999): Comparison in the γ - lactone content of oils and extracts from white cypress pine (*Callitris glaucophylla* Thompson & Johnson). *Journal of Essential Oil Research*, 11(4): 415–422.

Fish, F., Waterman, P.G., (1971): "Chloroform-soluble alkaloids of the root bark of *Fagara viridis*." *Phytochemistry*, 10 (12): 3322.

Gouillerot, E., Caron, C., Audran, J.C., Jardillier, J.C., Chénieux, J.C., (1996): Furoquinoline alkaloid accumulation in *Fagara zanthoxyloïdes* cell cultures highly depends on the presence of exogenous benzylaminopurine. *Plant Growth Regulation*, 19: 203-206.

Goulding, E., Robert, O.D., (1915): Volatile Oil from Tubers of *Kaempferia* ethelae. *Journal of the Chemistry Society*, 107: 314–319.

Holzapfel, C.W., Marais, W., Wessels, P.L., Van Wyk, B.E., (2002): Furanoterpenoids from *Siphonochilus aethiopicus*, *Phytochemistry*, 59: 405-407.

Imelouane, B., Elbachiri, A.,Wathelet, J.P., Dubois, J., Amhamdi, H., (2010): Chemical composition, cytotoxic and antioxidant activity of the essential oil of *Lavandula dentata*, *World Journal of Chemistry*, 5(2): 103-110.

Jardé, T., Caldefie-Chézet, F., Goncalves-Mendes, N., Mishellany, F., Buechler, C., Penault-Llorca, F., Vasson, M.P., (2009): Involvement of adiponectin and leptin in breast cancer: clinical and in vitro studies. *Endocrine-Related Cancer*, 16(4): 1197-210.

Jennings, W., Shibamoto, T., (1980): Quantitative analysis of flavor volatiles by gas capillary chromatography, Academic Press, New York, USA.

Karioti, A., Hadjipavlou-Litina, D., Mensah, M.L.K., Fleischer, T.C., Skaltsa,H., (2004): Composition and antioxidant activity of the essential oils of *Xylopia aethiopica* (Dun.) A. Rich. (*Annonaceae*) leaves, stem - bark, root bark and fresh and dried fruits, growing in Ghana. *Journal of Agriculture and Food Chemistry*, 8094-8098.

Lacroix, M., (2004): Relevance of breast cancer cell lines as models for breast tumors: an update. *Breast Cancer Research and Treatment*, 83(3): 249-289.

Lampronti, I., Saab, A.M., Gambari, R., (2006): Antiproliferative activity of essential oils derived from plants belonging to the Magnoliophyta division. *International Journal of Oncology*, 29(4): 989-995.

Lindsey, K., Jäger, A.K., Raidoo, D.M., Van Staden, J., (1999): Screening of plants used by Southern African traditional healers in the treatment of dysmenorrhoea for prostaglandin synthesis inhibitors and uterine relaxing activity. *Journal of Ethnopharmacology*, 64: 9-14.

Loizzo, M.R., Tundis, R., Menichini, F., Saab, A.M., (2007): Giancarlo Antonio Statti and Francesco Menichini, Cytotoxic Activity of Essential Oils from Labiatae and Lauraceae Families Against *In Vitro* Human Tumor Models. *Anticancer Research*, 27: 3293-3300.

Makhuvha, N., Van Wyk, B.E., Van Der Bank, H., Van Der Bank, M., (1997): Genetic Polymorphism in Wild and Cultivated *Siphonochilus aethiopicus* (Zingiberaceae).*Biochemical Systematics and Ecology*, 25(4): 343-351.

McGaw, L.J., Jäger, A.K., Van Staden J., (1997): Prostaglandin Synthesis Inhibitory Activity in Zulu, Xhosa and Sotho Medicinal Plants. *Phytotherapy Research*, 11: 113-117.

Palazzo, M.C., Agius, B.R., Wright. B.S., Haber, W.A., Moriarity D.M., Setzer, W.N., (2009): Chemical Compositions and Cytotoxic Activities of Leaf Essential Oils of Four Lauraceae Tree Species from Monteverde, Costa Rica. *Records of Natural Products*, 3(1): 32-37.

Rösch, P., Popp, J., Kiefer, W., (1999): Raman and SERS investigations on lamiaceae. *Journal of Molecular Structure*, 121: 480–481.

Salvador, M.J., de Carvalho, J.E., Wisniewski-Jr, A., Kassuya, C.A.L., Santos, E.P., Riva, D., Alves Stefanello, M.E., (2011): Chemical composition and cytotoxic activity of the essential oil from the leaves of *Casearia lasiophylla*. *Brasilian Journal of Pharmacognosy*, 21(5): 864-868.

Sinsin, B., Sinadouwirou, A.T., (2003) : Valorisation socio-économique et pérennité du *Pentadesma butyracea* Sabine en galeries forestières au Bénin. *Cahier d'Agricultures*, 12: 1-5.

Somova, L.I., Shode, F.O., Moodley, K., Govender, Y., (2001): Cardiovascular and diuretic of kaurene derivatives of *Xylopia aethiopica* and *Alepidea amatymbica*. *Journal of Ethnopharmacology*, 77: 165-174.

Stafford, G.I., Jäger, A.K., Van Staden, J., (2005): Effect of storage on the chemical composition and biological activity of several popular South African medicinal plants, *Journal of Ethnopharmacology*, 97: 107-115.

Sylvestre, M., Legault, J., Dufour, D., Pichette, A., (2005): Chemical composition and anticancer activity of leaf essential oil of *Myrica gale* L. *Phytomedicine*, 12: 299-304.

Sylvestre, M., Pichette, A., Longtin, A., Nagau, F., Legault, J., (2006): Essential oil analysis and anticancer activity of leaf essential oil of *Croton flavens* L. from Guadeloupe. *Journal of Ethnopharmacology*, 103: 99-102.

Tchobo, F.P., Natta, A.K., Barea, B., Barouh, N., Piombo, G., Pina, M., Villeneuve, P., Soumanou, M.M., Sohounhloué, D.C.K., (2007): Characterization of *Pentadesma butyracea* Sabine butters of different Production Regions in Bénin. *Journal of the American Oil Chemists' Society*, 84(8): 755-760.

Unlu, M., Ergene, E., Vardar Unlu, G., Zeytinogluand, H.S., Vural, N., (2010): Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from *Cinnamomum zeylanicum* Blume (*Lauraceae*). *Food Chem. Toxicol.*, 48: 3274-3280.

Zelefack, F., Guilet, D., Fabre, N., Bayet, C., Chevalley, S., Ngouela, S., Ndjakou Lenta, B., Valentin, A., Tsamo, E., Dijoux-Franca, M.G., (2009): Cytotoxic and Antiplasmodial Xanthones from *Pentadesma butyracea*. *Journal of Natural Products*, 72:954–957.

Zschocke, S., Rabe, T., Taylor, J.L.S., Jäger, A.K., Van Staden, J., (2000): Plant part substitution – a way to conserve endangered medicinal plants. *Journal of Ethnopharmacology*, 71: 281 - 292.

Table-2: *O. insignis, P. butyracea, S. aethiopicus* and *X. aethiopica* essential oils compositions.

Components	KI	(%)				Components	KI	(%)			
		Oi	Pd	Sa	Xa			Oi	Pd	Sa	Xa
tricyclene	922	-	-	0.8	-	methyl eugenol	1408	-	0.4	-	-
α-thujene	930	-	-	6.2	1.0	β-caryophyllene	1424	1.1	67.4	13.4	-
α-pinene	934	13.7	-	-	2.6	*trans*-α-bergamotene	1435	-	-	0.1	-
α-fenchene	946	-	-	0.2	-	aromadendrene	1439	-	0.1	-	-
sabinene	970	-	-	8.5	2.2	(Z)-β-farnesene	1444	-	0.1	-	-
β-pinene	979	13.7	-	44.5	4.5	*trans*-muurola-3,5-diene	1454	-	0.3	-	-
myrcene	992	58.9	-	0.7	-	α-humulene	1457	0.2	11.6	11.0	-
p-mentha-1(7), 8-diene	999	-	-	0.1	-	*trans*-prenyl limonene	1459	-	-	0.1	-
m-mentha-1,8-diene	1013	-	-	0.1	-	aromadendr-9-ene	1467	-	0.1	-	-
α-terpinene	1018	0.1	-	0.2	1.2	8-himachalene	1483	-	0.1	-	-
p-cymene	1026	-	-	1.9	-	germacrene-D	1485	2.0	-	0.8	-
β-phellendrene	1028	-	-	3.2	-	α-zingiberene	1492	0.2	0.3	-	-
1,8-cineole	1030	3.0	-	-	15.8	bicyclogermacrene	1493	-	-	0.2	-
(E)-β-ocimene	1047	0.5	-	-	-	α-selinene	1494	-	0.2	-	-
8-terpinene	1059	0.1	-	0.4	2.0	α-muurolene	1501	-	0.4	-	-
cis-sabinene hydrate	1067	-	-	0.1	1.1	(Z)-α-bisabolene	1503	-	-	0.1	-
terpinolene	1086	0.2	-	0.2	-	α-bulnesene	1505	-	0.1	-	-
trans-sabinene hydrate	1099	-	0.1	0.1	-	β-bisabolene	1512	-	5.1	-	-
linalool	1100	0.4	-	-	0.8	8-cadinene	1517	-	0.1	-	-
nonanal	1105	0.1	-	0.1	-	δ-cadinene	1520	0.4	1.4	-	-
cis-p-menth-2-en-1-ol	1121	-	-	-	0.9	*trans*-calamenene	1526	-	0.7	-	-
α-campholenal	1126	-	-	-	1.7	cadina-1,4-diene	1530	-	0.3	-	-
trans-pinocarveol	1138	-	-	0.1	-	α-calacorene	1544	-	0.1	-	-
nopinone	1140	-	-	-	2.1	germacrene-B	1554	-	0.1	-	-
cis-sabinol	1143	-	-	-	6.7	(E)-nerolidol	1555	-	-	0.2	-
trans-verbenol	1145	-	-	-	1.7	(Z)-hex-3-enyl benzoate	1573	0.1	-	-	-
β-pineneoxide	1156	-	-	-	2.9	caryolan-8-ol	1581	-	1.7	-	-
sabinacetone	1159	-	-	-	2.1	davanone	1586	-	0.4	-	-
pinocarvone	1165	-	-	-	2.4	caryophyllene oxide	1587	0.1	-	2.6	-
p-mentha-1,5-dien-8-ol	1170	-	-	-	1.9	neryl isovalerate	1596	-	-	0.1	-
terpinen-4-ol	1183	0.2	-	0.8	9.8	epi-globulol	1606	-	0.4	-	-
myrtenol	1194	-	-	-	0.9	humulene epoxide II	1607	-	-	1.0	-
myrtenal	1196	-	-	-	13.2	1-epi-cubenol	1625	-	0.3	-	-
α-terpineol	1197	1.2	-	0.3	-	citronellyl pentanoate	1630	-	-	0.4	-
methyl chavicol	1198	-	0.1	-	-	caryophylla-4(14),8(15)-diene-5α	1635	-	-	0.2	-
n-decanal	1201	-	-	0.1	-	epi-α-cadinol	1646	0.3	-	-	-
verbenone	1205	-	-	-	3.4	cubenol	1652	-	-	0.1	-
trans-carveol	1217	-	-	-	0.9	α-cadinol	1659	0.3	-	-	-
cumin aldehyde	1242	-	-	-	2.0	14-hydroxy-9-epi-(E)-caryophyllene	1667	-	-	0.1	-
geraniol	1251	0.1	-	-		(Z)-nerolidyl acetate	1675	-	-	0.1	-
dihydrolinalyl acetate	1275	-	-	-	0.9	(6S, 7R)-bisabolone	1743	-	-	0.1	-
bornyl acetate	1281	-	-	0.1	-	benzyl benzoate	1856	-	0.2	-	-
8-terpin-7-al	1287	-	-	-	0.9	isophytol	1947	1.2	-	-	-
p-cymen-7-ol	1291	-	-	-	1.8	Monoterpene hydrocarbons		87.2	0.0	67.0	13.5
carvacrol	1297	-	0.2	-	-	Oxygenated monoterpenes		4.9	0.4	1.5	57.2
cis-pinocarveyl acetate	1305	-	-	0.2	-	Sesquiterpene hydrocarbons		4.3	90.5	26.0	0.0
myrtenyl acetate	1327	-	-	0.1	4.2	Oxygenated sesquiterpenes		0.7	2.8	4.1	0.0
α-cubebene	1349	-	0.3	-	-	**Total**		**98.5**	**94.6**	**99.7**	**91.6**
α-longipinene	1352	-	0.1	-	-						
linalyl isobutanoate	1373	-	0.1	-	-						
α-copaene	1379	0.3	1.5	0.1	-						
10-epi-italicene	1385	-	0.1	-	-						
β-cubebene	1390	0.1	-	-	-						
α-isocomene	1392	-	0.1	-	-						
cyperene	1402	-	0.1	-	-						

Oi = *Ozoroa insignis*;
Pd = *Pentadesma buyracea* ;
Sa = *Siphonochilus aethiopicus*;
Xa = *Xylopia aethiopica*,
KI = Kovats Index

Table-3: Average of breast cancer cells MCF-7 proliferation in the presence of *P. butyracea* (Pd) essential oil.

EO	Concentrations (µg/ml)			witness	IC_{50} (µg/ml)
	119.1	132.3	154.4		
	average of proliferation ± S D				average ± SD
Pd	68.7 ± 5.3	52.8 ± 3.6	28.5 ± 3.9	97.8 ± 1.4	133.5±2.6

- **SD** = Standard deviation, **EO** = Essential oil

Table-4: Average of breast cancer cells MCF-7 proliferation in the presence of *S. aethiopicus* (Sa) essential oil.

EO	Concentrations (µg/ml)				witness	IC_{50} (µg/ml)
	178.0	267.0	311.5	356.0		
	average of proliferation ± SD					average ± SD
Sa	86.0± 3.7	14.1± 2.3	2.9± 1.6	1.5± 0.8	93.4± 3.2	215.7± 12.2

- **SD** = Standard deviation, **EO** = Essential oil

Table-5: Average of breast cancer cells MCF-7 proliferation in the presence of *X. aethiopica* (Xa) essential oil.

EO	Concentrations (µg/ml)				witness	IC_{50} (µg/ml)
	46.2	69.2	92.3	184.6		
	average of proliferation ± SD					Average ± SD
Xa	94.5± 3.5	83.93 ± 3.2	64.75 ± 4.5	18.65 ± 0.9	97.44 ± 1.4	127.9 ± 28.8

- **SD** = Standard deviation, **EO** = Essential oil

Table-6: Average of breast cancer cells MCF-7 proliferation in the presence of *O. insignis* (Oi) essential oil.

EO	Concentrations (µg/ml)				witness	IC_{50} (µg/ml)
	122.8	163.8	245.7	327.6		
	average of proliferation ± SD					average ± SD
Oi	96.5± 1.3	96.6± 1.0	89.5 ± 9.7	82.5± 7.4	98.1 ± 0.9	$IC_{50} > 327.6$

- **SD** = Standard deviation, **EO** = Essential oil

