Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Biochemistry

Cytotoxic Activity of Leaves Essential oils of *Aeollanthus heliotropioides* and *Ocimum urticaefolium* (Lamiaceae) from Cameroon on the Breast Cancer (MCF-7) and Epithelial (ARPE-19) Cells

Issakou Bakarnga-Via[1, 2*], Nadlaou Bessymbaye[1], Lauve Rachel Yamthe Tchokouaha[2, 3*], Patrick Valere Tsouh Fokou[2, 4], Magali Gary-Bobo[5], Audrey Gallud[5], Marcel Garcia[5], Pierre Michel Jazet Dongmo[6], Fabrice Fekam Boyom[2], Chantal Menut[5]

[1]Université de N'Djaména, Faculté des Sciences de la Santé Humaine, Laboratoire de Pharmacologie et Toxicologie, B.P. 1027, N'Djaména, Tchad
[2]Antimicrobial and Biocontrol Agents Unit, Laboratory for Phytobiochemistry and Medicinal Plants Studies, Department of Biochemistry, Faculty of Science University of Yaounde I, P. O Box 812, Yaounde, Cameroon
[3]Institute of Medical Research and Medicinal Plants Studies (IMPM), Ministry of Scientific Research and Innovation, P.O. Box 13033, Yaounde, Cameroon
[4]Department of Biochemistry, Faculty of science University of Bamenda, Bambili, P. O Box. 39, Bamenda, Cameroon
[5]Equipe Glyco et nanovecteurs pour le ciblage thérapeutique, IBMM, Faculté de Pharmacie, 15 Avenue Charles Flahault, BP 14491, 34093 Montpellier, France
[6]Faculty of Science, University of Douala, P.O. Box 24157, Douala, Cameroon

DOI: 10.36347/sajb.2022.v10i11.006 | **Received:** 28.09.2022 | **Accepted:** 03.11.2022 | **Published:** 19.11.2022

***Corresponding authors:** Issakou Bakarnga-Via[1] and Lauve Rachel Yamthe Tchokouaha[2]
[1]Institute of Medical Research and Medicinal Plants Studies (IMPM), Ministry of Scientific Research and Innovation, P.O. Box 13033, Yaounde, Cameroon
[2]Université de N'Djaména, Faculté des Sciences de la Santé Humaine, Laboratoire de Pharmacologie et Toxicologie, B.P. 1027, N'Djaména, Tchad

Abstract **Original Research Article**

Background: The aim of this work was to study the cytotoxicity of leaves essential oils and their identified constituents of *Aeollanthus heliotropioides* and *Ocimum urticaefolium* (Lamiaceae) from Cameroon. ***Materials and Methods***: Leaves essential oils were obtained by hydrodistillation and were analysed by gas chromatography (GC) and GC/mass spectrometry (MS). The cytotoxic activity was evaluated in Human breast cancer (MCF-7) and normal epithelial (ARPE-19) cells line by MTT assay. ***Results***: The essential oils of *Aeollanthus heliotropioides* and *Ocimum urticaefolium* leaves were found to most constitute by sesquiterpene components with 52.8% and 67.3% respectively than monoterpenes. The major constituents of the essential oil of *Aeollanthus heliotropioides* are linalool (43.4%), (E)-(Z)-farnesene (27.7%), (δ- Cadinol (13.0%), germacrene D (3.7%), α-caryophyllene (3.1%). Eugenol (26%), (Z)-β-ocimene(5.3%), α- caryophyllene (5%), germacrene D (4.4%), δ-muurolène (17%) , spathulenol (14.2%), δ-Cadinol (11.3%), (E)-Z-farnesol (5.4%) and 6E)-2Z-farnesol (3.6%) are the main major components of *Ocimum urticaefolium* essential oil. The cytoxicity of *Aeollanthus heliotropioides* and *Ocimum urticaefolium* oils indicate that the oils have a good cytotoxicity against breast cancer cells (MCF-7) growth with IC_{50} values of 0. 620µL/mL and 0.422 µL/mL. The highest selectivity was obtained with the essential oil *Aeollanthus heliotropioides* (1.72) which was more cytotoxic against MCF-7 than against normal cell line (ARPE-19) with IC_{50} values of 0.620 µL/mL and 1.07 µL/mL, respectively. ***Conclusion***: This study suggests for the first time the ability of *Aeollanthus heliotropioides* and *Ocimum urticaefolium* essential oils to inhibit human tumor cell growth. They could be considered as a potent source of alternate lead compounds against breast cancer.

Keywords: Essential oils, *Aeollanthus heliotropioides*, *Ocimum urticaefolium*, cytotoxicity, MCF-7 and ARPE-19 cell lines.

INTRODUCTION

Cancer is the cause of more than six million deaths each year in the world (Izevbigive, 2003). Breast cancer is one of the main life-threatening diseases that a woman may have to face during her lifetime (Angelopoulos *et al*., 2004). The increasing incidence of breast neoplasia reported over the last a few decades has

led to development of new anticancer drugs, drug combinations, and chemotherapy strategies by methodical and scientific exploration of enormous pool of synthetic, biological, and natural products (Mukherjee *et al*., 2001). Breast cancer is the second cause of mortality in the majority of sub-saharian countries (Ahmedin *et al*., 2010). For a long time, plants are being used in the treatment of cancer (Hartwell, 1982). According to an estimate, 50% of breast cancer patients use herbal products (Richardson, 2001). The search for anticancer agents from plant sources started in the 1950s. More than 60% of currently used anticancer agents are derived in one way or another from natural sources (Cragg and Newman, 2003; Balunas and Kinghorn, 2005). Currently, more than 30 compounds of natural origin are in different phases of the clinical study for the treatment of different types of cancer (Harsha *et al*., 2020). Biological active components from plants are significant and important source of new drugs that are likely to lead to new and better treatments for breast cancer as chemotherapy destroys the normal cells along with cancer cells, sometimes cancer cells can develop resistance to treatment through mutations (Wiseman and Spencer, 1998). Recently, cancer chemoprevention with strategies using essential oils has been regarded as one of the most visible fields for cancer control (Modzelewska *et al*., 2005; Béliveau *et al.*, 2006). The pharmaceutical properties of aromatic plants such as *Aeollanthus heliotropioides* and *Ocimum urticaefolium* are partially attributed to essential oils that are natural, complex, multi-component systems composed mainly of terpenes (Edris, 2007).

Aeollanthus heliotropioides is an herb found in Africa and South america. In Africa, the leaves are stuffed into the ear like an earplug for relief from ear aches. Its soothing tea is also used in folk medicine for menstrual cramps. The flowers and leaves of *A. heliotropioides* have a high content of monoterpenes which may play a role in its anticonvulsant, sedative, anti-microbial and anti-inflammatory properties (Coelho *et al*., 1997; Re *et al*., 2000). *Ocimum urticaefolium* (Lamiaceae) is cultivated in its natural area, the Europe, Asia and America for its essential oils (chemotypes rich in thymol, eugenol "Clocimum", resp. geraniol) as a medicinal, spice, and perfume plant. The plant has been widely employed as a folk remedy (febrifuge, diaphoretic, antiseptic). The leaves are eaten in salads, used as a condiment for sauces, soups or meat, and infused into a drink (Pushpangadan *et al*., 1979).

Cytotoxicity has been reported for many essential oils but no previous study has been undertaken on the cytotoxic activity of essential oils from essential oils from *Aeollanthus heliotropioides* and *Ocimum urticaefolium*.

The studied was designed to investigate chemical composition of essential oils of *Aeollanthus heliotropioides* and *Ocimum urticaefolium* and to assess their anti-cancer effect of on MCF-7 and ARPE 19 cell lines.

MATERIAL AND METHODS

Plant material and extraction procedure

The leaves of *Aeollanthus heliotropioides* and *Ocimum urticaefolium* (Lamiaceae) were collected, respectively in Obala and Bamenda (Cameroon) in July 2012 and identified at the National Herbarium of Yaoundé for Cameroonian samples where voucher specimens (42756HNC, 49085HNC, respectively) have been deposited. Essential oils were extracted by hydrodistillation using a Clevenger type apparatus. The leaves were steam-distilled for about 5 h and dried on anhydrous sodium sulphate column (Na_2SO_4) and then preserved at approximately 4°C free from the light until used.

Culture Medium

DMEMF-12: Penicillin-streptomycin solution, ficoll-hypaque solution, trypsin-EDTA solution, RPMI-1640 medium, Dulbecco's modified Eagle's medium (DMEM/F-12), and 1% antibiotic-antimycotic solution were obtained from (Life Technologies GIBCO, Grand Island, NY, USA). Fetal bovine serum (FBS) was purchased from Sigma-Aldrich (St. Louis, MO).

Cells Culture

MCF-7 human breast cancer cell and epithelial cells (ARPE-19) line was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and was maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), glutamine, Phenol Red and 50 µg/mL gentamycin, in humidified atmosphere at 37°C and 5% CO_2 atmosphere.

Chemical analysis

The analysis of the chemical composition was made by gas chromatography (CPG), then by gas chromatography coupled with mass spectrometry.

Gas chromatography

The oil was analyzed on a Varian CP-3380 GC with flame ionization detector fitted with a fused silica capillary column (30 m x 0.25 mm coated with DB5, film thickness 0.25 µm); temperature program 60-220°C at 3°C/min, injector temperature 200°C, detector temperature 220°C, carrier gas N2, 1 mL/min. 0.5µL of essential oils diluted at 10% in dichloromethane was injected manually. The linear retention indices of the components were determined relatively to the retention times of a series of *n*-alkanes and the percentage compositions were obtained from electronic integration measurements without taking into account relative response factors.

Gas chromatography spectrometry

GC/MS analyses were performed using a Hewlett-Packard apparatus equipped with a TG fused silica column (30 m x 0.25 mm, film thickness 0.25 µm) and interfaced with a quadrupole detector (GC-quadrupole DSQ II). Column temperature was programmed from 60°C-220°C at 3°C/min; injector temperature was 200°C. Helium was used as carrier gas at a flow rate of 1.2 mL/min, the mass spectrometer was operated at 70eV.

Identification of components

The identification of the constituents was based on comparison of their relative retention indices with either those of authentic samples or with published data in the literature (Adams, 2007; Jennings and Shibamoto 1980) and by matching their mass spectra with those obtained with authentic samples and/or the NBS75K, Wiley 7th NIST 98 EPA/NIH, and FFNSC 2 libraries spectra.

Cytotoxicity evaluation of essential oils

Human breast cancer cells (MCF-7) and epithelial cells (ARPE-19) were used for the determination of cytotoxic activity using the protocol described by Lau *et al*. (2004). They were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) F12 supplemented with 10% of fetal bovine serum (FBS), glutamine, Phenol Red and 50 µg/mL gentamycin in humidified atmosphere at 37°C and 5% CO_2. Cells were seeded in 96-well plates at the density of 20000 cells/well in 200 µL of culture medium and allow growing for 24 h. Then various concentrations of essential oils dissolved at 10% in absolute ethanol were added to the cells culture at final concentrations ranging from 0.1 to 2µg/µL and were incubated at 37°C for 72 h. Each concentration was tested in triplicate.

MTT assay was used to determine cell viability. The MTT assay is a laboratory test which measures changes in color for measuring the activity of enzyme that reduce MTT to formazan, giving a purple color. After incubation, 20µL of 0.5 mg/mL MTT reagent was added to each well and incubated for additional 4 h. Then MTT precipitates were dissolved in (150µl of EtOH/DMSO (1:1) solution to each well to solubilize the formazan crystals. The plates were read for optical density at 540 nm, using a microplate reader (Multiskan). Inhibition percent of MCF-7 and ARPE-19 cells was calculated using optical density. The IC_{50} value in the MTT assay was defined as the concentration of test oil resulting in a 50% reduction of absorbance compared with untreated cells. The contents of each microplate well was observed under the microscope for the phenotypic characterization of cancer cells (MCF-7) brought into contact with the essential oil of *A. heliotropioides* and *O. urticaefolium* at different concentrations.

Selectivity indices were calculated for individual oil from the IC_{50} values against normal (ARPE-19) and breast cancer (MCF-7) cell lines as SI = IC_{50}ARPE-19/IC_{50}MCF-7. Safer essential oils were considered as those with SI > 1.5.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) followed Fisher test (F-test) and were considered significantly different when P <0.05 using STAGRAPHICS software 5.0 for Windows.

RESULTS AND DISCUSSION

1-Yields of extraction of essential oils

The hydrodistillation of the leaves of *Aeollanthus heliotropioides* gave a yield of 0.7%. This result is similar to those obtained with the others essential oils from Lamiaceae family (Ndoye, 2001, Mawussi, 2008). The yields were ranged between 0.09% to 4.1%. Nevertheless, *Aeollanthus heliotropioides* from Cameroon in another study gave a yield of 0.07% (Ngo Mback *et al*., 2019) and 0.1% (Nguimatsia *et al*., 2021). These variations could be explained by intrinsic and geographic differences (Braga *et al*., 2005).

The yield of essential oil of leaves from *Ocimum urticaefolium* was 0.27%. We have not found previous studies on this plant. *Ocimum urticaefolium* is the hybrid of *Ocimum gratissimum* L. × *Ocimum forskolei* Benth. The yields of essential oils of leaves of *Ocimum gratissimum* obtained by Ndoye, (2001) yields were ten folks higher (2.9%) than the yield of *Ocimum urticaefolium* obtained in this study.

2-Chemical composition of the essential oils

The results of the chemical analyses are presented in Table 1.

Table 1: Chemical composition of the essential oils of leaves of *Aeollanthus heliotropioides* and *Ocimum urticaefolium*

AI	component	***Aeollanthus heliotropioides (%)***	***Ocimum urticaefolium (%)***
Monterpene hydrocarbons		**0.4**	**5.5**
930	α-pinene	0.4	-
1032	(Z)-β-ocimene	0.1	5.3
1041	(E)-β-ocimene	0.2	0.2
Oxygenated monoterpenes		**46.1**	**26.0**
1102	linalool	43.4	0.1

AI	component	*Aeollanthus heliotropioides (%)*	*Ocimum urticaefolium (%)*
1189	α-terpineol	1.5	-
1249	geraniol	1.2	-
1361	eugenol	-	26.0
Sesquiterpene hydrocarbons		**37.3**	**30.8**
1377	α-copaene	-	0.9
1387	β-cubebene	-	0.2
1390	isocaryophyllene	0.2	0.4
1423	α- caryophyllene	3.1	5.0
1434	**γ-elemene**	0.1	-
1451	E)-β-farnesene	27.7	0.6
1456	(Z)- β-farnesene	1.5	0.4
1477	**γ-muurolene**	0.1	0.2
1484	germacrene D	3.7	4.4
1498	α-amorphene	0.2	0.7
1509	δ-muurolène	0.2	17.0
1514	**γ-cadinene**	0.2	0.2
1523	δ-cadinene	0.2	0.7
1530	α-cadinene	0.1	0.1
Oxygenated sesquiterpenes		**15.5**	**36.5**
1556	spathulenol	0.1	14.2
1581	Caryophyllene oxide	0.2	0.1
1588	cubenol	0.1	0.5
1643	**t-muurolol**	0.4	0.1
1656	α-cadinol	0.4	0.2
1666	t-cadinol	0.1	1.0
1687	δ- Cadinol	13.0	11.3
1695	(E)-(Z)-farnesol	0.6	5.4
1718	6(E)-2(Z)-farnesol	0.4	3.6
1739	2E-6E-farnesal	0.2	0.1
Liner compounds		**0.2**	**-**
1804	octadecanal	0.2	-
Total identified compounds		**99.3**	**98.8**
Extraction yields (%)		**0.7**	**0.27**

We noticed that sesquiterpenes are highly prevalent in the leaves oils of *Aeollanthus heliotropioides* and *Ocimum urticaefolium* with 52.8% and 67.3% respectively than monoterpenes (46.5% and 31.5%).

The major constituents of the essential oil of *Aeollanthus heliotropioides* are linalool (43.4%), (E)-(Z)-farnesene (27.7%), (δ- Cadinol (13.0%), germacrene D (3.7%), α-caryophyllene (3.1%). We have the presence of linear compound (octadecanal: 0.5%) in the essential oil of *Aeollanthus heliotropioides*.

Concerning the essential oil of leaves of *Ocimum urticaefolium* the major compounds are eugenol (26%), (Z)-β-ocimene (5.3%), α-caryophyllene (5%), germacrene D (4.4%), δ-muurolène (17%), spathulenol (14.2%), δ-Cadinol (11.3%), (E)-Z-farnesol (5.4%) and 6E)-2Z-farnesol (3.6%).

The main major compounds of *Aeollanthus heliotropioides* and *Ocimum urticaefolium* are respectively linalool (43.4%) and eugenol (26.0%). This result is similar with those obtained by Arthur *et al*., (2001) and Ntezurubanza *et al*., (1988) who respectively obtained linalool as a major compound of essential oils of leaves of *Aeollanthus heliotropioides* with a percentage of (41.86±2.73%) and eugenol as a major compound of essential oil of leaves of *Ocimum urticaefolium* from Rwanda, this component amounted to more than 50%. In the same line Ngo Mback *et al*., (2019) and Nguimetsia *et al*., (2021) also obtained from *A. heliotropioides*, linalool as major compound.

3- Cytotoxic activity

The percentage of MCF-7 and ARPE-19 cell s viability and the IC_{50} values recorded for both tested essential oils of the leaves of *Aeollanthus heliotropioides* and *Ocimum urticaefolium* are shown in Table 2.

Table 2: *In vitro* viability of MCF-7 and ARPE-19 cells exposed to *Aeollanthus heliotropioides* and *Ocimum urticaefolium* essential oils

Essential oils	**Concentrations (**µL/mL)	**Cell viability (%)**		**IC_{50} (**µL/mL)		
		MCF-7	***ARPE-19***	***MCF-7***	***ARPE-19***	***SI***
Aeollanthus heliotropioides	Control(0)	100 ± 7	100 ± 7	0.620	1.07	1.72
	0.1	75 ± 13	85 ± 9			
	0.2	47 ± 17	46 ± 9			
	0.4	3 ± 1	32 ± 4			
	2.0	0± 0	3 ± 5			
Ocimum urticaefolium	Control (0)	100 ± 12	100 ± 5	0.422	0.384	0.909
	0.1	92± 2.5	90± 3			
	0.2	61± 4	91 ± 7			
	0.4	8± 1	93 ± 8			
	2.0	0 ± 0	3 ± 0			

MCF-7: Human breast cancer cells; ARPE-19: Normal epithelial cells; IC_{50}: 50% Inhibitory Concentration; SI: selectivity indices

The cytotoxicity effects of essential oils from leaves of *Aeollanthus heliotropioides* and *Ocimum urticaefoliumon* on MCF-7 and ARPE-19 cell lines are shown in Figure 1. The plate content was examined microscopically for phenotypic characterization of inhibition effect.

At higher concentrations ranging from 0.1 to 2 µL/mL, all of the two tested essential oils induced significantly increased cell cytotoxicity. These effects could be explained by the presence sesquiterpenes in essential oils of *A. heliotropioides* and *O. urticaefolium*. We noticed that sesquiterpenes are highly prevalent in the leaves oils of *Aeollanthus heliotropioides* and *Ocimum urticaefolium* with 52.8% and 67.3% respectively. Sesquiterpenes have been known for their strong anticancer (Modzelewska *et al.,* 2005) against breast cancer, colon cancer, gastric cancer cells, and lung, ovarian and laryngeal cancer cell lines (Ren and Gould, 1998).

Among the two essential oils, essential oil from, *Ocimun urticaefolium* is the most cytotoxic against both cancer and normal cell lines (Figure 1, Table 2). This could be due to its high level of oxygenated sesquiterpenes (36.5%) such as spathulenol (14.2%). Spathulenol has been shown to have significant cytotoxic effect through an apoptosis-dependent mechanism in cancer cells SK-MEL-28 (Santos *et al*., 2020).

Interestingly, the highest selectivity was obtained with the essential oil of *Aeollanthus heliotropioides* (1.72) which was more cytotoxic against MCF-7 than against normal cell line (ARPE-19) with IC_{50} values of 0.620 µL/mL and 1.07 µL/mL, respectively (Table 2). Therefore, with a SI > 1.5, the essential oil of *Aeollanthus heliotropioides* is considered as safe essential oil as potential source of alternative against breast cancer. This preferential anticancer potential of *Aeollanthus heliotropioides* on MCF-7 cells could be due to the high level of monoterpenes mainly of geraniol in its essential oil. In fact, geraniol is known as potent biosafety anticancer compound (Gateva *et al*., 2019). The studies reported by Carnesecchia *et al.,* (2002) and Carnesecchia *et al.,* (2004) shown that the anticancer activity of geraniol could be explained by his inhibitor effect of tumoral growth of 5 fluorouracil. Monoterpenoids have been found to activate multiple antitumoral responses, like aoptosis, autophagic cell death, cyto stasis and necrosis (Dos Santos e Silva *et al*., 2022).

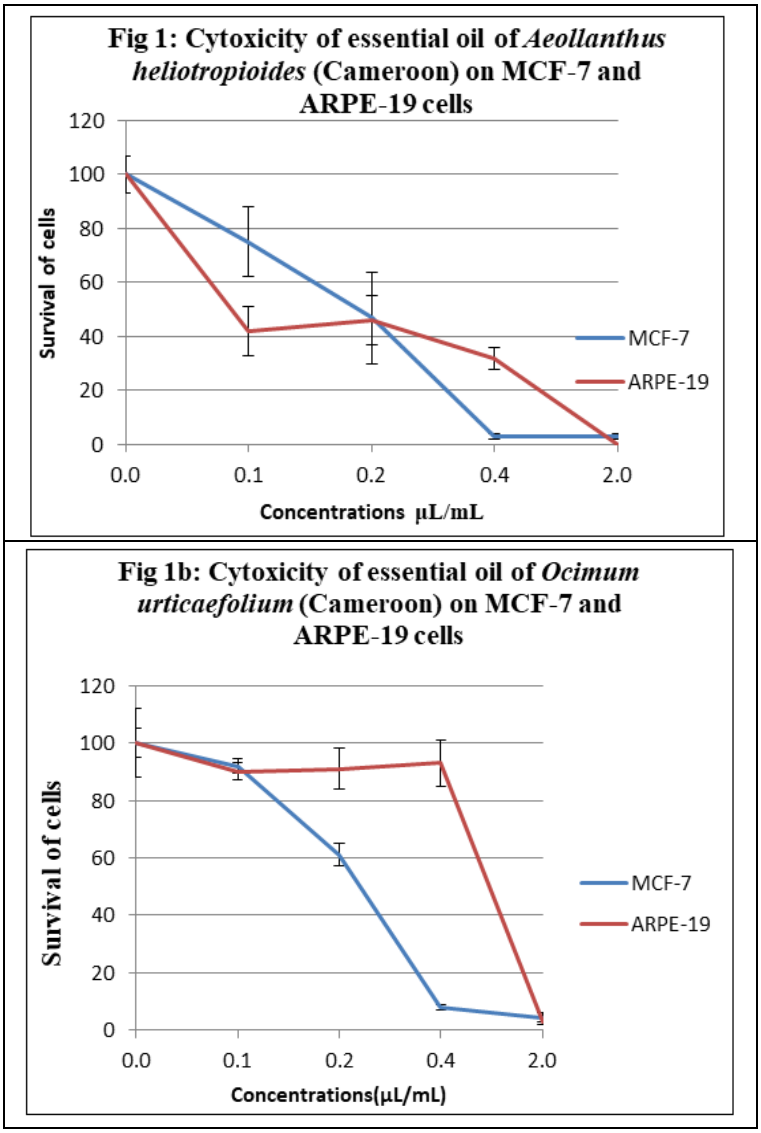

Figure 1: Dose-dependent cytotoxic effect of the essential oils of *Aeollanthus heliotropioides* (Fig1a) and *Ocimum urticaefolium* (Fig 1b)

Human breast cancer (MCF-7) and normal epithelial (ARPE-19) cell lines were incubated 72 h with increasing doses of essential oils from 0.1 to 2 µL/mL. After treatment, cytotoxicity was measured by MTT assay as described in Material and Methods. Values are means ± standard deviations of three independent experiments.

Phenotypic characterizations of MCF-7 cells before and after MTT treatment are shown in Figure 2. By microscopic observation, died cancer cells were characterized by budding of the membrane without loss of integrity or by bubbling of the plasma membrane; these results corroborated the observations of Cohen (1993) on this type of cells.

Fig 1a: Phenotypic charaterization of cells before treatment with MTT

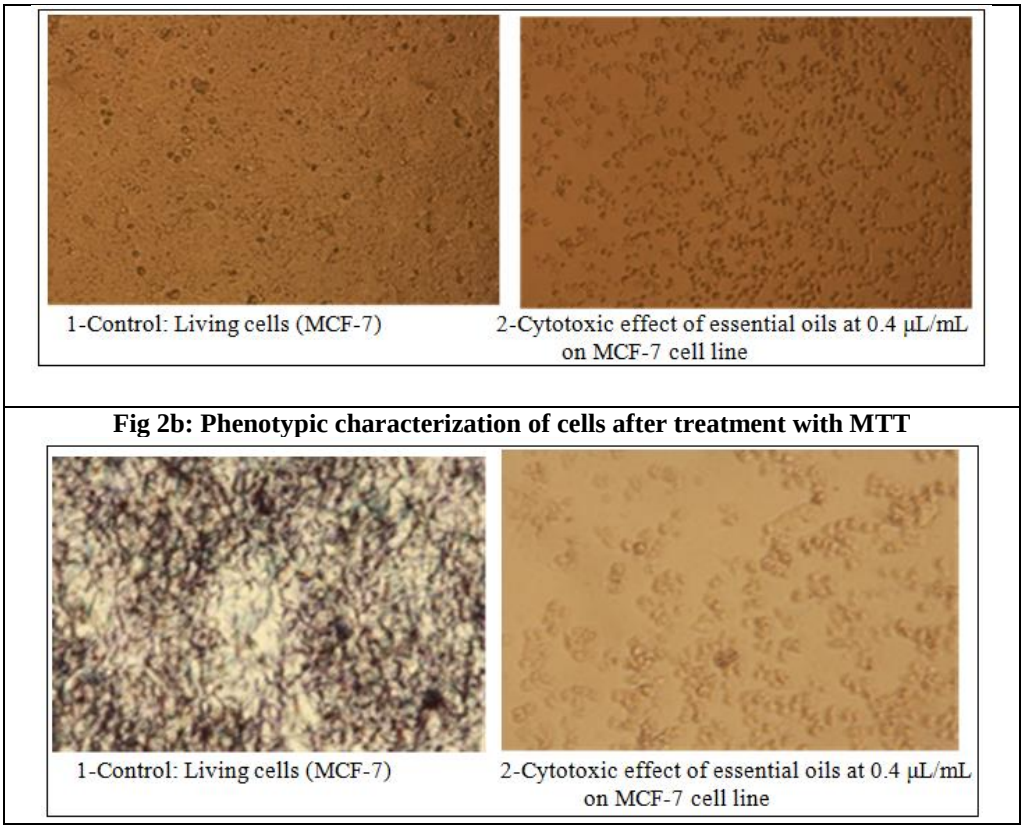

Fig 2b: Phenotypic characterization of cells after treatment with MTT

Figure 2: Phenotypic characterization of cells

CONCLUSION

This study revealed sesquiterpene components are the main group of components found in *Aeollanthus heliotropioides* and *Ocimum urticaefolium* leaves essential oils. *Aeollanthus heliotropioides* and *Ocimum urticaefolium* essential oils might have a good potential anti breast cancer effect and it might be useful in testing its further potential treatments of colorectal cancer and other cancers. Mechanism of action of active component of each plant essential oil could be done as further study.

Competing interests: The authors declare that they have no competing interests.

Authors' Contributions

FFB, PMJD and CM conceived and designed the study. IB-V, BN, LRYT, VPFT and MGB participated in plant selection and collection, spectral analyses and in the drafting and correction of the manuscript. IB-V, MG, AG, and CM performed GC, GC/SM analyses of the essential oils and carried out the cytotoxicity assay. They also contributed to data analysis and critically revised the manuscript. IB-V, BN, LRYT and VPFT extracted the essential oils. All the authors read and approved the final manuscript version.

ACKNOWLEDGEMENTS

This work received funding from the University of Adam Barka of Abeche (Chad) to Bakarnga-Via Issakou. We are mostly grateful to Mr Victor Nana from Cameroon National Herbarium for his assistance with the collection and identification of plant materials.

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