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Research Article

Antifungal, antiradical, anti-inflammatory and antineoplastic activities of essential oils of some medicinal plants of the Annonaceae family of Chad and Cameroon.

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Abstract

Yeasts infections, cancer and other diseases associated with free radical generation and inflammation are currently a critical public health issue that needs innovative control measures. In order to search for solutions, this study was designed to assess the antifungal, radical scavenging, anti-inflammatory, and antineoplastic activities of essential oils from four Annonaceae plants (*Monodora myristica*, *Xylopia aethiopica*, and *Xylopia parviflora*) collected in Chad and Cameroon. Essential oils were extracted by hydrodistillation. The antifungal activity of the oils was determined using the microdilution method; and their antiradical activity was determined using the DPPH free radical scavenging assay. The anti-inflammatory activity was assessed using the inhibitory effect of oils against the 5-lipoxigenase. Flow cytometry was used to assess the effect of essential oils on cell cycle. The essential oils of *X. aethiopica*, *X. parviflora*, and *M. myristica* inhibited the growth of *Candida albicans* ATCC24433, *Candida parapsilosis* ATCC22019, and *Cryptococcus neoformans* IP95026 with MIC values ranging from 5 to 10 mg/mL. Essential oils of *X. aethiopica*, *X. parviflora*, and *M. myristica* showed free radical scavenging potential with SC_{50} values between 8.9 and 11.74 g/L. The oils samples at 100 µg/mL from both origins significantly inhibited cancer cell (MCF-7) and normal epithelial cells of the eye (ARPE-19) growth, with a notable cell cycle arrest at phases G0/G1 and S at 72h respectively. According to the findings of this study, essential oils from Annonaceae plants contain bioactive secondary metabolites that have the potential to inhibit pathogenic yeasts, free radicals, inflammatory and cancer cells. Further research is needed to confirm these findings and formalize their eventual application to control the targeted affections.

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1. Introduction

Since synthetic drugs have several side effects on the human body, there is growing interest in the use of plants as a medicinal agent. Experiments revealed that many naturally occurring agents in plant extracts, including essential oils, have antioxidant, anti-inflammatory, antimicrobial, and anticancer potential in a variety of bioassay systems and animal models relevant to human disease [1]. As GLOBOCAN 2020 reports, there were 19.3 million new cancer cases diagnosed in 2020, and almost 10.0 million people died due to cancer. GLOBOCAN predicts that the number of cancer cases will increase to 28.4 million by 2040 [2, 3]. Female breast cancer is the most common type of cancer (2.26 million cases), with a significant disease burden and high mortality rates [4]. Several classes of anticancer drugs are currently available on the market, but issues such as toxicity, low efficacy, and solubility have reduced overall therapeutic indices [5]. As a result, new anti-cancer agents with distinct mechanisms of action, high efficacy, low toxicity, low cost, and short therapy duration profiles are being developed [5]. Targeting drugs, particularly to specific genes and proteins involved in cancer cell growth and survival, is a critical need for research worldwide [6].

The antioxidant, anti-inflammatory and anticancer properties of the majority of essential oils have been studied. Essential oils may have anticancer properties due to a link between the production of reactive oxygen species (ROS) and oxidative and inflammatory states that can lead to cancer. Besides, excessive ROS production is linked to chronic inflammation and can also cause DNA damage, increasing the mutation rate and the likelihood that cells will undergo oncogenic transformation. ROS are well known for their ability to modulate redox-mediated signaling pathways, which can lead to tumor development. Drugs used to treat cancer have the primary goal of inducing apoptosis or cell cycle arrest in cancer cells. Thus, essential oils capable of inducing apoptosis in cancer cells may be useful resources for cancer patients [7].

Furthermore, fungal infections have emerged as a major cause of morbidity and mortality in cancer patients [8]. The prevalence of fungal infections has increased significantly in recent years, particularly in immunocompromised individuals, with concomitant life-threatening increases. Furthermore, the epidemiology of systemic fungal infections has shifted, *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. parapsilosis* are the most common pathogens that cause bloodstream systemic infections [9]. Fungal infections remain a burden worldwide, thus underpinning the need for effective new therapeutic approaches.

The common thread connecting all of these components necessitates that new therapies have a broad spectrum of action, preferably synergistic. Natural substances derived from medicinal plants have proven to be the most reliable source of molecules with the desired profile in this regard. Specifically, essential oils have demonstrated multiple beneficial biological properties, potentially exploitable in the fight against fungal infections, oxidative stress, inflammation and cancer [7]. Aromatic and medicinal plants, especially those belonging to Annonaceae family, have been used for centuries in traditional medicine for the treatment of several pathologies with many therapeutic effects being associated with the presence of essential oils. In fact, these compounds have been described as bioactive agents, primarily due to their antifungal, antioxidant, anti-inflammatory [9] and anticancer properties. Indeed, Choumessi *et al*, [10] revealed that ethanolic extracts of *Xylopia aethiopica* from Cameroon exhibit anti-proliferative activity on cancer cell lines (HCT116 of colon cancer, U937 and KG of 1a leukemia). Similarly, Bakarnga-via *et al*, previously reported the chemical composition of the essential oils of the fruits of *Xylopia aethiopica, Xylopia parviflora* and *Monodora myristica* and their antiproliferative activity on breast cancer cells (MCF-7) [11]. However, how they exert their antiproliferative properties is not yet elucidated. Consequently, the goal of this study is to investigate the antifungal, radical-scavenging, anti-inflammatory, and the antineoplastic mechanism of action of essential oils from three Annonaceae plants (*Monodora myristica*, *Xylopia aethiopica*, and *Xylopia parviflora*) that were collected in Chad and Cameroon.

2. Materials and methods

2.1 Plant material and essential oil extraction procedure

The plant material used in this work consisted of seeds of *Monodora myristica*, fruits of *Xylopia aethiopica* and *Xylopia parviflora* of the *Annonaceae* family collected in Chad and Cameroon. Each plant was identified at the herbarium of the National Research Support Center (HCNAR) of Chad and the National Herbarium of Cameroon (HNC) according to the place of harvest. The dates and places of collection of each sample and the reference numbers are recorded in Table 1.

Table 1: Plant samples used in the study

Plant	Organ	Locality	Reference number
Monodora myristica (Cameroon)	Seed	Kribi	27690/SFR/CAM
Monodora myristica (Chad)	Seed	Goré	6814/HCNAR/CHAD
Xylopia aethiopica (Cameroon)	Fruit	Kribi	28725/SFR/CAM
Xylopia aethiopica (Chad)	Fruit	Goré	7563/HCNAR/CHAD
Xylopia parviflora (Cameroon)	Fruit	Kribi	42351/SFR/CAM
Xylopia parviflora (Chad)	Fruit	Goré	7557/HCNAR/CHAD

The essential oils of the seeds of *Monodora myristica*, the fruits of *Xylopia aethiopica* and *Xylopia parviflora* were extracted for 5 h by hydrodistillation using a Clevenger-type apparatus. The collected oil was dried on a column of anhydrous sodium sulphate (Na_2SO_4), weighed in a dark vial and stored at about 4°C away from light. Specific molecular content of the extract was previously reported [11] and their GC-MS profiles are presented in Fig. 1

2.2 Antifungal assays

The evaluation of the antifungal activity of essential oils was carried out at the Laboratory of Phytobiochemistry and Study of Medicinal Plants of the University of Yaoundé I (Cameroon). Antifungal tests of the extracts were done on three strains of fungi including: two pathogenic yeasts (*Candida albicans* ATCC24433, *Candida parapsilosis* ATCC22019 and *Cryptococcus neoformans* IP95026) as previously reported [12]. These fungi were maintained on Sabouraud Dextrose agar (SDA) medium. The tests were carried out in microplates of 96 wells using Sabouraud Dextrose broth (SDB) and phenol red as a colour indicator. In short, 150 µL of culture medium were introduced into the first twelve cups of line A and 100 µL into the rest of the plates; then, 50 µL of a 40 mg/mL solution of essential oil were taken and added to the 150 µL of the first wells. A series of 6 dilutions of geometric reason 2 was carried out from line A to line G; subsequently, 30 µL of a yeast suspension, adjusted to 2×10^4 CFU/mL were seeded into the cups of the plate, except those of columns 4, 8 and 12 which contained only the culture medium and served as blank; the wells of line H, containing the medium culture and inoculum were used as negative control. Nystatin, a positive control, was prepared under the same sterility conditions and then tested on yeasts at 2 mg/mL as the starting concentration. The tests were carried out in triplicate. The microplates were incubated at 37 °C for 48 hours. The MIC was determined at the end of the incubation period as the lowest concentration at which no color change was observed. The contents of the microplate wells that did not show discoloration were seeded on sterile SDA and incubated at 37 °C for 48 hours before being observed for possible growth. MFC was defined as the lowest concentration at which no fungal growth was observed after subculture.

2.3 Determination of anti-radical activity

The anti-radical activity of essential oils was evaluated at the Laboratory of Metabolic and Clinical Biochemistry (IBMM) of Montpelier I University (France), by the DPPH spectrophotometric method (2,2-diphenyl-1-picrylhydrazine) as described by Brand-Williams et *al.* [13]. A stock solution of DPPH solution (stock solution) was prepared at 0.39 g/L by dissolving 19.7 mg DPPH in 50 mL ethanol using a magnetic stirrer. From this solution, the working solution is prepared by taking 5 mL and dissolved in 45 mL of ethanol. Solution of BHT (Butyl-Hydroxy-Toluene) was prepared at 1 g/L by dissolving 50 mg of BHT in 50 mL of methanol. Each essential oils solution was prepared at 30 g/L in methanol, and then, a series of dilution was carried out so as to obtain 3 solutions of concentrations 5 g/L, 10 g/L and 15 g/L. Each spectrophotometric cell received 100 µL of pre-prepared essential oil solutions before receiving 1900 µL of DPPH solution (80 µM). Every ten minutes, optical densities are measured at 517 nm. The negative control is prepared under the same conditions, but

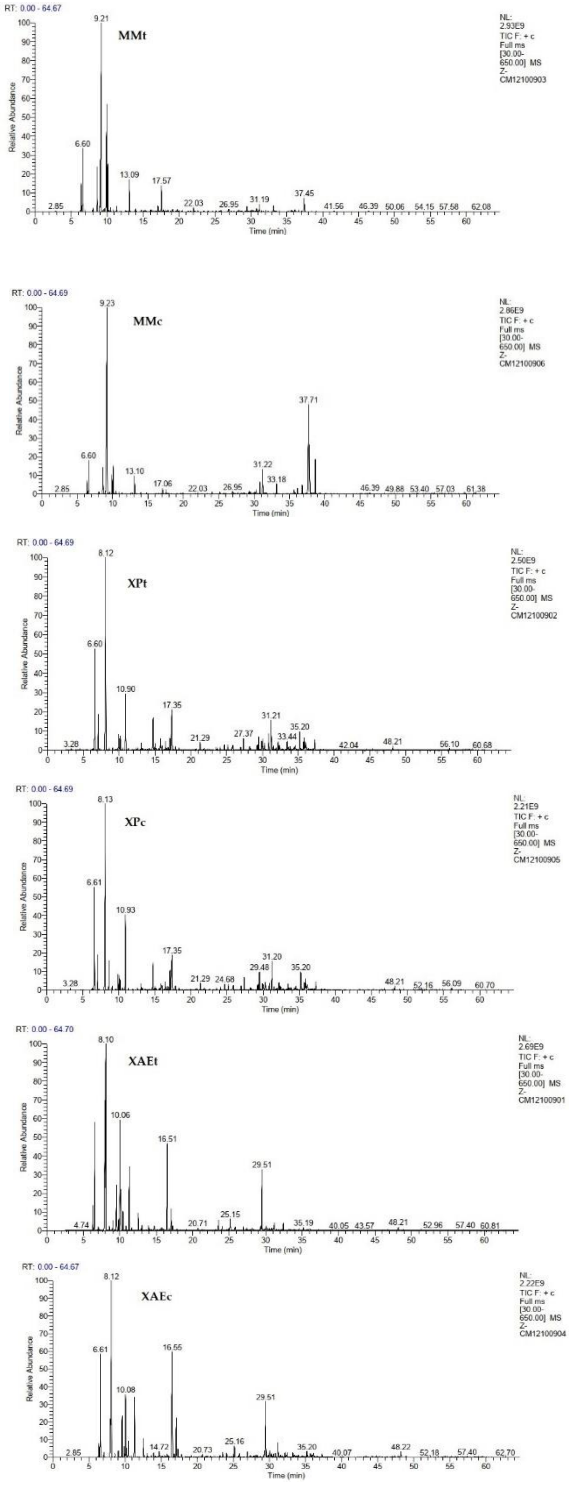

Figure 1. Total ion chromatogram of essential oils using GC–MS. The GC/MS system used is the HEWLETT-PACKARD 589 0 series II, equipped with a capillary column of fused silica 30 m x 0.25 mm and a grafted stationary phase of type HP5 (5%-phenyl-methylpolysiloxane). The mass detector is a quadrupole type (Model 5972) and the ionization energy used is 70 eV. MMt= *Monodora myristica* from Chad; MMc= *Monodora myristica* from Cameroon; XPt= *Xyplopia parviflora* of Chad; XPc= *Xyplopia parviflora* of Cameroon; XAEt= *Xyplopia aethiopica* from Chad; XAEc= *Xyplopia aethiopica* from Cameroon.

instead of the essential oil, 100 µL of methanol is added. Similarly, the positive control is prepared under the same conditions, with 100 µL of BHT (1 g/L) replacing the essential oil. The scavenging percentage is calculated using the following formula:

A_{ref} = Absorbance at t = 60 min of the control (DPPH solution without antiradical)

A_{mes} = Absorbance at t = 60 min of the DPPH solution containing the anti-radical

A 100 = Absorbance at the end of the reaction (close to 0) for total scavenging (0.015 and 0.08 depending on the staining of the solution) [14].

$$\%SC = \frac{A_{ref} - A_{(mes)}}{A_{ref} - A_{(100)}} X\ 100$$

Anti-radical concentrations to trap 50% of free radicals (SC_{50}) were calculated using the percentages of DPPH radical scavenge. The SC_{50} is calculated from a graph that shows the percentage of scavenging (% SC) as a function of concentration (g/L). This value is determined by the DPPH concentration used in the test. As a result, the inhibitors' 50% effective concentration (EC_{50}) and anti-radical power is determined. It is expressed in moles of inhibitor required to trap 1/2 mole of DPPH and is calculated as follows:

$$EC_{50} = \frac{SC_{50}}{[DPPH]}$$

Anti-radical Power (AP) is the inverse of effective concentration; it measures anti-radical effectiveness. The greater the value, the more effective the anti-radical is:

$$AP = \frac{1}{EC_{50}}$$

2.4 Determination of anti-inflammatory activity

The anti-inflammatory activity was determine as previously described by Braga *et al*. [15]. Phosphate buffer was made by dissolving 13.6 g of KH_2PO_4 in 900 mL of distilled water and adjusting the pH with 1M NaOH to 9. The volume was then adjusted to 1 liter using distilled water. The phosphate buffer was used

to prepare the substrate (linoleic acid) solution at 0.03 g/L and the enzyme (5-lipoxygenase) solution at 10 g/L. Essential oil solutions were prepared at three different concentrations: 10, 20, and 30 ppm, which correspond to 9.4 µg/mL, 18.8 µg/mL, and 28.2 µg/mL, respectively. Each spectrophotometric cell contains 2 mL of linoleic acid solution (substrate) and 10 L of the inhibitor solution at various concentrations. The negative control was a mixture of 2 mL of linoleic acid solution (substrate) and 10 L of ethanol. After leaving the spectrophotometric cells at 30 °C for 10 minutes, 50 µL of the enzyme solution is added to begin the reaction. Following rapid homogenization of the tube content, simultaneous absorbance measurements at 234 nm are acquired (multi-kinetic system). The rate constants are evaluated from the linear part of the curve, corresponding to the initial rate of the enzymatic reaction according to the formula:

$$K = \frac{A(t) - A(0)}{t}$$

t = 10 seconds in our experimental conditions
K = Rate constant
A (t) = absorbance at 10 seconds
A (0) = absorbance at 0 seconds.

The percentage of inhibition is evaluated from the following expression:

$$\%I = \frac{K_0 - K_E}{K_0} \times 100$$

%I = percentage of inhibition
K_0 = reaction rate constant in the absence of inhibitor
K_E = reaction rate constant in the presence of the essential oil [14].

The concentration corresponding to 50% inhibition (IC_{50}) is determined from the curve representing the percentage of inhibition as a function of concentration.

2.5 Mechanism of action of essential oils on cell lines: MCF-7 and ARPE-19

The cytotoxic activity of essential oils on breast cancer cell strain (MCF-7) and normal epithelial cells of the eye (ARPE-19) and the mechanism of action involved in cell death were evaluated at the IBMM Laboratory of Metabolic and Clinical Biochemistry. The study was performed using the colorimetric method with 3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) as described by Gary-Bobo *et al*. [16]. The cells (MFC-7, ARPE-19) were cultured in culture vials of 25 and 75 cm^2 in Dulbecco's Modified Eagle Medium (DMEM) F12 (Life Technologies GIBCO, Grand Island, NY, USA) supplemented with 10% inactivated bovine fetal serum (SBF) (Sigma-Aldrich St. Louis, MO), glutamine (2mM), phenol red and gentamycin (50 µg/mL). The culture was maintained in the incubator at 37 ^{0}C under humid atmosphere, at 5% CO_2. The environment was renewed every 72 hours. The antiproliferative effect of essential oils of *Monodora myristica*, *Xylopia aethiopica* and *Xylopia parviflora* was previously reported on breast cancer cells (MCF-7) and normal epithelial cells of the eye (ARPE-19) using MTT assay. The essential oils of *X. aethiopica*, *X. parviflora*, and *M. myristica* produced a significant ($p < 0.005$) antiproliferative effect on MCF-7 and ARPE-19 cells. The fruit essential oils of *X. parviflora* from Chad and Cameroon were the most active on cancer MCF-7 cells, with IC_{50} values of 0.155 and 0.166 µL/mL, respectively, while on normal ARPE-19 cells, they showed IC_{50} values of 0.910 and 0.920 µL/mL, indicating a selective cytotoxic effect (SI = 5.871 and 5.542, respectively) [11]. The mechanism of cell death (MCF-7 and ARPE-19) was analyzed by the flow cytometry method as described by Giret *et al.* [17]. One hundred and fifty thousand (150,000) normal epithelial cells (ARPE-19) and breast cancer cells (MCF-7) were seeded in 60 mm diameter Petri dishes and placed at the incubator under culture conditions for 24 hours. After incubation, the cells were treated with a homogeneous mixture of essential oil / culture medium of final concentration 100 µg/mL. After 24 hours, the cells were recovered and fixed with cold ethanol for 6 hours at 4 °C. The cells thus fixed were incubated with 1 mg/mL of RNASE A and 40 µg/mL of propidium iodide for 48 h in the dark at 4 °C. The cellular DNA was then analyzed by flow cytometry with a FACSCALIBUR flow cytometer coupled to the CellQuest software (Becton Dickinson). *In vitro* test results are expressed on average ± SD: Standard deviation.

2.6 Statistical Analysis

Results were expressed as mean ±standard deviation (SD) and statistical analyses were performed using the GraphPad Pris software. All in vitro data were analyzed using the one-way analysis of variance (ANOVA) with Tukey's post hoc test for multiple pairwise comparisons. Throughout this work, statistical differences were considered significant for probabilities below 5% ($p < 0.05$) unless otherwise stated.

3. Results and Discussion

3.1 Essential oils prevent the growth of yeasts in culture

Essential oils from *Monodora myristica* seeds, *Xylopia aethiopica* fruits, and *Xylopia parviflora* fruits were tested for antifungal activity against yeasts (*Candida albicans* ATCC24433, *Candida parapsilosis* ATCC22019, and *Cryptococcus neoformans* IP95026) using the microdilution method and phenol red as a growth indicator. Figure 1 depicts an assayed 96 well microplate after incubation. Indeed, Figure 2 summarizes the colorimetric shift from phenol red to

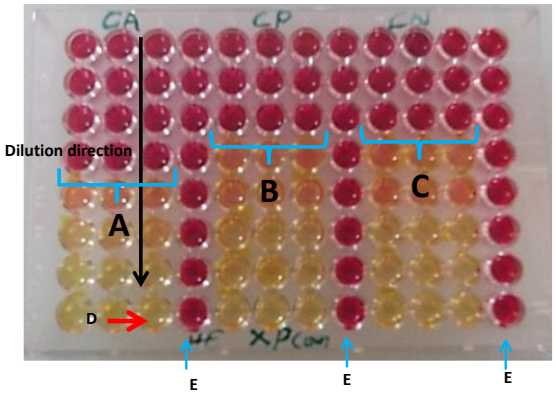

Figure 2: Evaluation of yeast growth using phenol red discoloration at various concentrations of Cameroonian essential oil *Xylopia parviflora*. The MICs of *Xylopia parviflora* essential oil (Cameroon) on *Candida albicans* (A); *Candida parapsilosis* (B) and *Cryptococcus neoformans* (C); D= negative control line; E= sterility control.

yellow in the wells containing yeasts in the presence of essential oils at different concentrations. This shift materializes cell viability. On the other hand, the wells showing no color change indicate the inhibition of growth or the death of yeasts. The MIC is defined as the smallest concentration of essential oil that prevents the growth of yeast (no color change) (Figure 2, Table 2).

The essential oils of the plants studied have antifungal properties and inhibit the growth of the three fungal strains (*Candida albicans* ATCC24433, *Candida parapsilosis* ATCC22019 and *Cryptococcus neoformans* IP95026) with MICs ranging from 5 to 10 mg/mL depending on the oils and yeasts tested (Table 2). At the same MIC (10 mg/mL), *Xylopia aethiopica* and *Monodora myristica* essential oils from Chad and Cameroon inhibit the growth of *Candida albicans* ATCC24433, *Candida parapsilosis* ATCC22019 and *Cryptococcus neoformans* IP95026 at the same MIC (10 mg/mL). While, *Xylopia parviflora* essential oils from Cameroon showed inhibitory activity on the growth of *Candida albicans* at 5 mg/mL. *Xylopia parviflora* oil from Chad, in particular, was found to be the most active oil by inhibiting the growth of yeasts with a MIC of 5 mg/mL. The antifungal activity of the essential oils studied, however, remains lower than that of nystatin, the reference molecule that inhibits the growth of the three yeasts at very low MICs ranging from 0.031 to 0.125 mg/mL.

The inhibition of the growth of *Candida albicans* ATCC24433 by the essential oils of *Xylopia parviflora* from Chad and Cameroon at 5 mg/mL and *Xylopia aethiopica* at 10 mg/mL corroborates the work of which showed that essences of *Xylopia parviflora*, *Xylopia aethiopica* and *Monodora myristica* harvested from Bayagam and Yaoundé inhibit the growth of *Candida albicans* at 4 mg/mL and >9 mg/mL respectively [18].

In addition, the essential oil of *Monodora myristica* harvested in Yaoundé, contains primarily α-phellandrene (29.2%), p-cymene (11.2%), α-pinene (9.7%), *cis*-sabinol (6.9%) and limonene (6.8%) and that of *Xylopia aethiopica*, which contains β-pinene (18.3 %), terpinen-4-ol (8.9%), sabinene (7.2%), α-phellandrene (7.1%) and (E)-β-ocimene (6.4%) showed antimicrobial activity on strains of bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis*) and fungi (*Aspergillus flavus*) [19]. Their high concentration of monoterpenes may explain their antifungal activity. Indeed, the monoterpene content of essential oils confers

Table 2: Antifungal activity of essential oils against yeasts

Essential oils and Nystatin	Parameters of antifungal activity of essential oils								
	C. albicans ATCC24433			*C. parapsilosis* ATCC22019			*C. neoformans* IP95026		
	MIC	CMF	CMF/CMI	MIC	CMF	CMF/CMI	MIC	CMF	CMF/CMI
X. aethiopica (Tchad)	10	>10	ND	10	10	1	10	10	1
X. aethiopica (Cam)	10	10	1	10	10	1	10	10	1
X. parviflora (Tchad)	5	5	1	5	5	1	5	5	1
X. parviflora (Cam)	5	5	1	10	10	1	10	10	1
M. myristica (Tchad)	10	10	1	10	10	1	10	10	1
M. myristica (Cam)	10	>10	ND	10	>10	ND	10	>10	ND
Nystatine	0.039	0.04	1	0.062	0.06	1	0.125	0.13	1

Table 3: Summary of SC_{50}, EC_{50} and PA essential oils and BHT

Treatment	SC_{50} (g/L)	EC_{50} (g/mol of DPPH)	AP
BHT	$7.02\ 10^{-3} \pm 0.00$	87.75	1.13×10^{-2}
Monodora myristica (Cameroun)	$10.22^{b} \pm 1.10$	12.77×10^{4}	7.83×10^{-6}
Monodora myristica (Tchad)	$11.74^{a} \pm 0.60$	14.67×10^{4}	6.80×10^{-6}
Xylopia aethiopica (Cameroun)	$9.46^{a} \pm 0.14$	11.82×10^{4}	8.46×10^{-6}
Xylopia aethiopica (Tchad)	$9.63^{a} \pm 0.45$	12.03×10^{4}	8.31×10^{-6}
Xylopia parviflora (Cameroun)	$9.70^{a} \pm 0.69$	12.12×10^{4}	8.25×10^{-6}
Xylopia parviflora (Tchad)	$8.90^{a} \pm 0.16$	11.12×10^{4}	8.99×10^{-6}

SC_{50}: scavenging concentration 50; EC_{50}: effective concentration at 50% scavenging; AP: anti-radical power. a and b link values that are not significantly different ($P < 0.05$) according to student's test.

inhibitory activity against *Candida albicans* [20]. Jazet *et al*. [21], Aouni *et al*. [22] and Hzounda *et al*. [23] also revealed that essential oils consisting of oxygenated monoterpenes exhibit antifungal properties. Similarly, Peana *et al*. [24] and Pitarokili *et al*. [25] demonstrated that essential oils high in 1,8-cineol and linalool inhibit the growth of *Candida albicans* and *Fusarium solani* at a MIC of 2 mg/mL. It should be noted that these compounds were also found in the Annonaceae species studied.

The subculture of yeasts on SDA medium of the contents of the wells having no visible fungal growth allow to determine the MFC defined as the smallest concentration at which no fungal regrowth is visible at the end of the subculture. According to Carbonnelle *et al*. [26], the MFC/MIC ratio classifies compounds with antifungal activity as fungicides when the quotient is less than or equal to 4, and fungistatic if it is found to be greater than 4. Thus, it appears from Table 2 that the MFC/MIC ratios are all less than 4, reflecting the fungicidal activity of the essential oils of the fruits of *Monodora myristica*, *Xylopia aethiopica* and *Xylopia parviflora* at the concentrations tested on *Candida albicans* ATCC24433, *Candida parapsilosis* ATCC22019 and *Cryptococcus neoformans* IP95026. The reference molecule, nystatin, also showed a fungicidal effect on the yeasts tested.

This study's findings suggest that essential oils, particularly those from the Annonaceae family, could be effective against *Candida* and *Cryptococcus neoformans* IP95026. This means that further research is needed to explore their potential as new treatments for mycoses. Given the chemical composition of these essential oils, no parameter stood out as the reason for this difference in activity. However, inherent properties of each of these essences such as the equilibrium of the relative concentrations of the constituents could cause synergistic interactions resulting in the increase in the cumulative effect of the essence on microorganisms. This observation also corroborates the idea that the complexity of essential oils coupled with other characteristics such as the origin and nature of plant material would give them specific powers.

3.2 Essential oils portrayed interesting anti-radical activities

The anti-radical activities were carried out in *vitro* using the DPPH free radical scavenging method with essential oils, in comparison with a reference antiradical, BHT. The scavenging percentages (%SC)

were calculated from the spectrophotometric data obtained, and the concentrations corresponding to 50 % scavenging was determined graphically (SC_{50}). From these SC_{50} values, effective concentrations (EC) and anti-radical powers were deduced. The results (Figure 3) show that BHT and essential oils exhibits anti-radical activity in a dose-dependent manner. BHT maximum activity (100% scavenging) was obtained at 0.05 g/L compared to the range tested. Figure 3 shows that the percentage of scavenging is proportional to the BHT concentration required to trap 50% of DPPH molecules (SC_{50}) is 7.02 $x10^{-3}$ g/L (Table 3). The mean of the free radical scavenging percentages of the two tests with different concentrations of essential oils of *Xylopia aethiopica, Xylopia parviflora* and *Monodora myristica* (Figure 3)

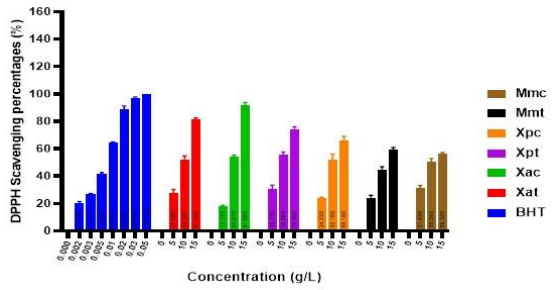

Figure 3: DPPH scavenging properties of essential oils. BHT**:** Butyl Hydroxy Toluene; MMt= *Monodora myristica* of Chad; Mmc= *Monodora myristica* of Cameroon; Xpt= *Xyplopia parviflora* of Chad; Xpc= *Xyplopia parviflora* of Cameroon; Xat= *Xyplopia aethiopica* of Chad; Xac= *Xyplopia aethiopica* of Cameroon

The scavenging percentage (%SC) of DPPH free radicals increases as the concentration of essential oils of *Xyplopia aethiopica* from Tchad and Cameroon increases, reaching a maximum of 81.3% and 91.66%, respectively, at a concentration of 15 g/L (Figure 3). The SC_{50} obtained are 9.63 g/L and 9.46 g/L for *Xyplopia aethiopica* essential oils from Tchad and Cameroon, respectively. However, these values remain higher than those obtained with BHT, indicating a lower anti-radical activity (7.02 10^{-3} g/L) than BHT. The SC_{50} essential oils of *Xylopia ethiopica* fruits from Chad and Cameroon (9.63 and 9.46 g/L, respectively) (Table 3) are statistically similar, but lower than Alitonou *et al.*'s SC_{50} of 15.0 g/L for the essence of the same plant's leaves harvested in Benin. This demonstrates that the essential oil of fruits scavenged more free radicals (DPPH) than the essential oil of leaves [27].

According to this figure 2, the percentages of free radical scavenging increase with the concentration of essential oils of *Xyplopia parviflora* fruits from Chad and Cameroon, with SC_{50} values of 8.90 g/L and 9.70 g/L, respectively (Table 3). This demonstrates that the essential oil of *Xyplopia parviflora* from Chad traps slightly more free radicals (DPPH) than the essential oil from Cameroon, but both less than the reference antiradical molecule BHT.

The anti-radical activity obtained from the essential oils of *Xyplopia parviflora* fruits from Chad and Cameroon supports the work of Woguem *et al.* [28], who demonstrated that the essential oil of *Xylopia parviflora* fruits harvested in Nde (West -Cameroon) has anti-radical properties with a SC_{50} of 590 µg/mL or 0.59 g/L, scavenging more free radicals of DPPH compared to essences of *Xylopia parviflora* harvested in Goré (Chad) and in Kribi (Cameroon). This variation in activity could be due to their chemical composition which is slightly rich in oxygenated monoterpenes (20.7%) as demonstrated by Jazet *et al*. [21].

Figure 3 shows that the percentages of free radical scavenging (DPPH) increase with the concentration of essential oils from *Monodora myristica* fruits from Chad and Cameroon. The essential oils of *Monodora myristica* from Chad and Cameroon had SC_{50} values of 11.74 g/L and 10.22 g/L (Table 3), indicating that the essence from Cameroon would scavenge more free radicals (DPPH) than that of Chad. The essential oils of *Monodora myristica* seeds from Chad and Cameroon have higher anti-radical activity than the essential oil of *Monodora myristica* leaves Benin, which SC_{50} is 15 g/L [27].

Table 3 shows that the essential oil *Xylopia aethiopica*, and *Xylopia parviflora* from Chad and Cameroon has a higher antiradical power ($8.25x10^{-6}$-$8.99x10^{-6}$) with a SC_{50} of 8.90-9.70 g/L than the essential oils of *Monodora myristica* from Chad and Cameroon. The SC_{50} values obtained differ, these differences are not statistically significant between essential oils of the genus *Xylopia* ($p>0.05$), whereas there is a significant difference

between essential oils of the genus *Monodora* ($p<0.05$). The essential oil of *Xylopia parviflora* from Chad scavenges relatively more free radicals of DPPH than that of *Xylopia parviflora* from Cameroon, with a non-significant difference. *Xylopia aethiopica* oils from Chad and Cameroon have nearly identical anti-radical activity. The essential oil of *Monodora myristica* from Chad has significantly higher antiradical activity ($p<0.05$) than that of *Monodora myristica* from Cameroon. Generally, essential oils rich in hydrocarbon monoterpenes found in the genera *Xylopia* and *Monodora* have low anti-radical activity as previously reported [27]. All of these values, however, are insignificant when compared to the anti-radical power of the reference molecule BHT ($1.13x10^{-2}$).

3.3 Essential oils poorly inhibit inhibition of 5-lipoxygenase

The anti-inflammatory potential of essential oil was determined by inhibiting 5-lipoxygenase activity. Figure 3 depicts the percentage inhibition of 5-lipoxygenase at various essential oils and Nordihydroguaiaretic acid (NDGA) concentrations. Overall, it appears from this study that the essential oils studied have only a very moderate effect compared to the NDGA used as a reference on the activity of 5-lipoxygenase at the concentrations tested (Figure 4). Therefore, it was not possible to determine their IC_{50} at these concentrations. Figure 4 shows that all essential oils tested have no inhibitory action of 5-lioxygenase at a concentration of 9.4 µg/mL. On the other hand, three essential oils out of the six studied cause an inhibition of 10% on average on the activity of soya lipoxygenase at the concentration of 18.8 µg/mL, as well as an inhibition between 12% and 19% to 28.2 µg/mL. The weak anti-inflammatory activity obtained with the essential oils of Annonaceae from Chad and Cameroon is corroborated by the work of Alitonou *et al*. with essential oils from the leaves of two Annonaceae (*Monodora myristica* and *Xylopia aethiopica*) harvested in Benin, which inhibited lipoxygenase to less than 30% at concentrations ranging from 9.4 µg/mL to 47 µg/mL [27]. It would therefore be wise to conclude that the species of both geographical origins (with regard to the samples studied) demonstrated biochemical homogeneity with respect to the action vis-à-vis 5-lipoxygenase. The percentage of inhibition of 5-lipoxygenase increases as NDGA concentration increases. These percentages were used to determine an IC_{50} of 0.53 µg/mL for NDGA.

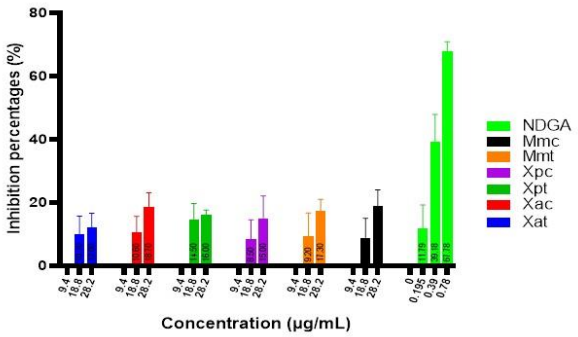

Figure 4: Inhibitory activity of essential oils on 5-lipoxygenase. The anti-inflammatory activity of oils of *Monodora myristica*, *Xylopia aethiopica*, *Xylopia parviflora* was evaluated on 5-lipoxygenase and the averages of percentages of inhibition are presented. NDGA: Nordihydroguaiaretic acid**;** Mmt= *Monodora myristica* from Chad; Mmc= *Monodora myristica* from Cameroon; Xpt= *Xyplopia parviflora* of Chad; Xpc= *Xyplopia parviflora* of Cameroon; Xat= *Xyplopia aethiopica* from Chad; Xac= *Xyplopia aethiopica* from Cameroon

3.4. Xylopia parviflora, Xylopia aethiopica, and Monodora myristica Causes cell cycle arrest in MCF-7 and ARPE-19

The essential oils of *Xylopia parviflora*, *Xylopia aethiopica* and *Monodora myristica* from Chad and Cameroon significantly inhibit cancer cells (MCF-7) after 72 hours of incubation compared to normal epithelial cells (ARP-19) whose selectivity index varies from 3.476 to 5.5 depending on the species and cell lines [11]. Based on their IC_{50}, essential oils of *Xylopia aethiopica*, *Monodora myristica* from Chad, *Xylopia parviflora* from Cameroon were selected to perform this study. The results of the flow cytometry study of the effect of essential oils on the cell cycle of cancer cells (MCF-7) and normal epithelial cells (ARPE-19) at a concentration of 100 µg/mL.

Figures 5 show that the essential oils of the fruits of *Xylopia aethiopica* (Chad), *Xylopia parviflora* (Cameroon), and seeds of *Monodora myristica* (Chad), incubated for 24 hours with breast cancer cells (MCF-7) induce cell cycle arrest at the G0/G1 phase and a

significant emergence of apoptosis during the sub-G1 phase. This conclusion follows from the appearance of a third peak in the cell cycle, with 1 to 4% of cells (MCF-7) dying by apoptosis. This peak corresponds to cells whose DNA content is decreased during apoptosis due to the loss of cleaved DNA fragments [29, 30]. In the case of normal epithelial cells (ARPE-19), essential oils of *Xylopia aethiopica*, *Xylopia parviflora* and *Monodora myristica* cause cell cycle arrest in phase S.

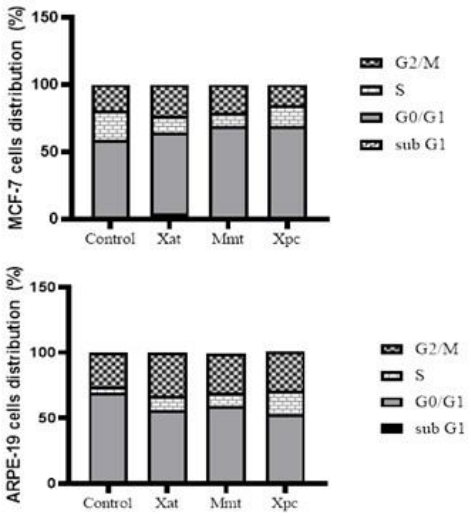

Figure 5: Essential oils induce cell cycle arrest at the G0/G1 phase in MCF-7 and at S phase in ARPE-19. Cell cycle analysis of cells (ARPE-19 & MCF-7) treated with ethanol (Control) for 24 hours or supplemented with essential oils at 100 µg/µL. Typical histogram of cells stained with PI showing the distribution of cells in various phases of the cell cycle. Xat= *Xylopia aethiopica* (Chad), Mmt= *Monodora myristica* (Chad), Xpc= *Xylopia parviflora* (Cameroon), G2/M: Mitosis phase (M), S: Synthesis phase corresponding to DNA replication, G0/G1: Cell activation phase, SubG1: Apoptosis phase

These findings point to a fundamental difference between normal and cancer cells in that normal cells (ARPE-19) can remain alive during the S (DNA replication) or G2/M (pre-mitotic) phases corresponding to RNA synthesis, whereas cancer cell cycle arrest (MCF-7) is observed in the G0/G1 phase and self-destruction via apoptosis. A similar study had previously shown that the combination of curcumin and citral treatment was found to induce apoptosis and cell cycle arrest at the G0/G1 phase in breast cancer cells [31]. Furthermore, the ability of normal cells (ARPE-19) to survive the S phase under the influence of essential oils shows that they can repair their DNA damaged during apoptosis by excision, as previously demonstrated by Sarasin in eukaryotic cells treated with growth inhibitors [32, 33].

The cell cycle arrest of cancer cells (MCF-7) and normal epithelial cells (ARPE-19) in phases G0/G1 and S, and their death by apoptosis caused by the cytotoxic effects of the essential oils tested, is supported by the work of Cha and Kim [34] who demonstrated that the essential oil of the leaves of *Cryptomeria japonica* containing compounds present in our essences such as α-pinene (6.07%), sabinene (8.86%), terpinen-4-ol (9.97%), and α-terpineol (6.13%) induce apoptosis of cancer cells (KB) of the oral epidermis. Indeed, these researchers discovered that this essential oil activated caspases (Caspases 3) and caused changes in the mitochondrial content of proteins from the Bcl 2 family and the Bax/Bcl2 ratio, resulting in the release of cytochrome c in the cytosol, forming a molecular model of apoptosis. Similarly, Wei *et al*. [35] demonstrated that essential oil extracted from pine needles (*Pinus sylvestrus*) induces apoptosis in hepatic cancer cells by inhibiting telomerase activity and expression of the Bcl2 gene (HepG2) [35]. It should be noted, however, that the essences of *Xylopia aethiopica* from Chad induce twice as much cell death by apoptosis in cancer cells as all other oils. Essential oils, on the other hand, have a stronger apoptotic effect on cancer cells (MCF-7) than on normal cells (ARPE-19).

4. Conclusion

The findings showed that essential oils from Annonaceae plants including *Xylopia parviflora*, *Xylopia aethiopica*, and *Monodora myristica* have bioactive secondary metabolites that can help protect against yeast infections, oxidative stress, inflammation, and cancer. Essential oils significantly reduced growth of cancer cells (MCF-7). The oils seem to preferentially inhibit the cell cycle of cancer cells in phase G0/G1, as opposed to normal epithelial cells (ARPE-19), which stop in phase S. While these findings are compelling, more research is needed to understand the mechanisms behind them, as well as to determine how they can be practically applied to control the targeted conditions.

Supplementary materials

They will be made available up request by the corresponding authors.

Author Contributions:

Conceptualization, FFB, PMJD, IB-V, JBHF, VPFT, LRYT, MGB, and YS; Methodology, IB-V, JBHF, VPFT, LRYT, MGB and YS; Formal Analysis, IB-V, JBHF, VPFT, LRYT, RK MGB; Investigation, IB-V, JBHF, VPFT, LRYT, MGB, and YS; Resources, FFB, CM; Writing – Original Draft Preparation, IB-V, JBHF, VPFT, LRYT, MGB, and YS; Writing – Review & Editing, FFB, PMJD, IB-V, VPFT, CM; Supervision, FFB, PMJD, CM; Funding Acquisition, IB-V.

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Conflicts of interest

The authors declare no conflict of interest.

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