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Bagora Bayala*, Ahmed Y Coulibaly, Florencia W. Djigma, Bolni Marius Nagalo, Silvère Baron, Gilles Figueredo, Jean-Marc A. Lobaccaro, Jacques Simpure

Chemical composition, antioxidant, anti-inflammatory and antiproliferative activities of the essential oil of *Cymbopogon nardus*, a plant used in traditional medicine

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Abstract: Objectives: Natural products commonly used in traditional medicine, such as essential oils (EOs), are attractive sources for the development of molecules with anti-proliferative activities for future treatment of human cancers, e.g., prostate and cervical cancer. In this study, the chemical composition of the EO from *Cymbopogon nardus* was characterized, as well as its antioxidative

properties and anti-inflammatory and antiproliferative activities on LNCaP cells derived from prostate cancer.

Methods: The chemical composition of the EO was determined by GC/FID and GC/MS analyses. The antioxidative properties were assessed using DPPH radical scavenging assay and ABTS+• radical cation decolorization assay, and the anti-inflammatory capacity was determined by the inhibition of the lipoxygenase activity. Antiproliferative activity was evaluated by MTT assay.

Results: Collectively, our data show that the major constituents of *C. nardus* EO are citronellal (33.06 %), geraniol (28.40 %), nerol (10.94 %), elemol (5.25 %) and delta-elemene (4.09 %). *C. nardus* EO shows modest antioxidant and anti-inflammatory activity compared to the standard gallic acid. *C. nardus* EO exhibits the best antiproliferative activity on the prostate cancer cell line LNCaP with an IC₅₀ of 58.0 ± 7.9 µg/mL, acting through the induction of the cell cycle arrest.

Conclusions: This study has determined that *C. nardus* EO efficiently triggers cytotoxicity and opens a new field of investigation regarding the putative use of this EO *in vivo*.

Keywords: *Cymbopogon nardus*; essential oil; gas chromatography; antioxidant; anti-inflammatory; antiproliferative.

***Corresponding author: Bagora Bayala**, Laboratoire de Biologie Moléculaire et de Génétique (LABIOGENE), Département de Biochimie-Microbiologie, Université Joseph KI-ZERBO, 03 BP 7021, Ouagadougou 03, Burkina Faso; Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA), 01 BP 216 Ouagadougou 01, Burkina Faso; Laboratoire Génétique, Reproduction & Développement, UMR CNRS 6293, INSERM U1103, Université Clermont Auvergne, 28, Place Henri Dunant, BP38, F63001, Clermont-Ferrand, France; Université Norbert ZONGO, BP 376 Koudougou, Burkina Faso, E-mail: bayalabagora@gmail.com

Ahmed Y Coulibaly, Université Norbert ZONGO, BP 376 Koudougou, Burkina Faso; Laboratoire de Biochimie et de Chimie Appliquées (LABIOCA), Département de Biochimie-Microbiologie, Université Joseph KI-ZERBO, 09 BP 848 Ouagadougou 09, Burkina Faso

Bolni Marius Nagalo, Division of Hematology and Oncology, Mayo Clinic, Arizona, 13400 E. Shea Blvd. Scottsdale, AZ 85259, USA

Silvère Baron, Jean-Marc A. Lobaccaro, Laboratoire Génétique, Reproduction & Développement, UMR CNRS 6293, INSERM U1103, Université Clermont Auvergne, 28, Place Henri Dunant, BP38, F63001, Clermont-Ferrand, France

Gilles Figueredo, LEXVA Analytique, Biopole Clermont-Limagne, Saint-Beauzire, France

Florencia W. Djigma, Jacques Simpure, Laboratoire de Biologie Moléculaire et de Génétique (LABIOGENE), Département de Biochimie-Microbiologie, Université Joseph KI-ZERBO, 03 BP 7021, Ouagadougou 03, Burkina Faso; Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA), 01 BP 216 Ouagadougou 01, Burkina Faso

Background

Cancer continues to be a major public health problem worldwide [1]. It is the second leading cause of morbidity, globally, with about 8.8 million deaths in 2015 [2]. Cervical cancer is the fourth most common cancer in women and the fourth leading cause of cancer death in women [3].

Prostate cancer is the second most common malignancy in men worldwide, and its incidence is increasing. The development of better strategies to prevent and to improve access to care and treatment in developing countries is mandatory. Interestingly, plants have been at the origin of important anti-cancer molecules such as paclitaxel, docetaxel, vinblastine, and vincristine [4–7]. Thus, isolated molecules from plants remain a critical area in the field of drug development.

Essential oils (EOs) are compounds extracted from aromatic plants. Their volatile constituents have been widely used for bactericidal purposes [8, 9], virucidal [10], fungicidal [11], antiparasitical [12], insecticidal [13], anticancer [14, 15], antioxidant [15], antidiabetic [16], cardiovascular [17], and cosmetic and food applications [18]. Aromatic grasses of the genus *Cymbopogon* (*Poaceae* family) represent a unique group of plants that produce a diverse composition of rich monoterpene EOs [19, 20]. These are of great value in flavor, fragrance, cosmetic, and aromatherapy industries [19]. Ethnopharmacology evidence shows that they possess a wide array of properties that justifies their use in many fields such as pest control, cosmetics or anti-inflammation agents [20]. These plants may also hold promise as potent anti-tumor and chemopreventive drugs [20].

C. nardus is particularly known for its antioxidant, anti-inflammatory, and antimicrobial properties [21–23]. The purpose of this work was to determine the chemical composition, chemotype and to analyse the anti-radical and anti-proliferative activity of the EO of *C. nardus*, used in Burkina Faso (West Africa), for the potential treatment of inflammatory and oxidative diseases.

Methods

Plant material and essential oil (EO) extraction

Leaves of *C. nardus* were collected in August 2017, at the National Institute of Applied Sciences and Technologies (IRSAT) in Ouagadougou, Burkina Faso. GPS location: 12°25'29.5"N 1°29'14.3"W 12.424853, -1.487297. Before harvesting for extraction, the plant was identified by Dr. Abdoulaye Seremé, Researcher in Plant Biology at IRSAT/CNRST, and then identified and authenticated by Professor Amadé Ouédraogo, Professor in Botany at University Joseph KI-ZERBO of Ouagadougou. A specimen was deposited in the herbarium of the Laboratory of Biology and Plant Ecology of University Joseph KI-ZERBO publicly accessible under ID: 17827 and sample number 6905.

Fractions of fresh plant material (1 Kg) were submitted to hydrodistillation using an alembic/Clevenger-type apparatus for 3 h, as described previously [14]. EOs were stored in airtight containers in a refrigerator at 4°C until GC-FID and GC/MS analyses and biological tests. EOs were diluted in hexane (1/30, v/v) for GC/FID analysis.

Chemical composition

Gas chromatography-flame ionization detector (GC/FID) analysis

The composition of the EO was determined as previously described [15]. Briefly, gas chromatography of hexane diluted EO was performed on an Agilent gas chromatograph model 6890 (Agilent, Palo Alto, Ca), equipped with a 30m x 0.25 mm, 0.25 µm film thickness column under a hydrogen flow, from 50°C (5 min) to 300°C with an increasing temperature of 5°C/min. Samples were injected in split mode, with injector and detector temperatures at 280 and 300°C, respectively [14].

Gas chromatography-mass spectrometry (GC/MS) analysis

Mass spectrometry analyses have been reported previously [15]. Briefly, an Agilent gas chromatograph model 7890 coupled to an Agilent MS model 5975 was used. Helium was used with an average flow of 1.0 mL/min. The oven temperature program was from 50°C (3.2 min) to 300°C at 8°C/min, 5 min post-run at 300°C. The sample was injected in split mode, injector and detector temperatures at 250°C and 280°C, respectively [14]. The MS working in electron impact mode at 70eV; electron multiplier, 1500 V; ion source temperature, 230°C; mass spectra data were acquired in the scan mode in m/z range 33–450 [14].

Identification of components

The main compounds present in the EO of *C. nardus* have been identified as previously described [15]. Using compound standards to identify EO components would have been the preferred state-of-the-art methodology, however, due to technical and resource constraints [14], we performed retention indices and comparisons using the NIST library [24] or literature [25]. Component relative percentages were calculated based on GC peak areas without using correction factors [14].

Cell cultures

LNCaP is an human prostate androgen-responsive adenocarcinoma cell line with a low metastatic potential derived from a lymph node metastasis [26]. HeLa cells are derived from a human cervical cancer [27] and P69 cells are immortalized non-cancerous epithelial cells from human prostate (a kind gift from Dr. Frederic Bost, Inserm C3M, Nice, France) [28, 29]. They were cultured and maintained at 37°C and 5% CO₂ in RPMI-1640 medium (Invitrogen) supplemented with 10% fetal calf serum (FCS, Biowest, Nuaille, France), 1% penicillin and 1% streptomycin (Invitrogen, Oslo, Norway).

Antioxidant activity

DPPH radical scavenging assay

DPPH (Sigma-Aldrich, L'Île d'Abeau, France) radical scavenging activity was measured as described by Velasquez [30]. Briefly, the EO of *C. nardus* at 8.8 mg/mL was first serially diluted in a 96-well plate. Then, 100 µL of each EO concentration was mixed with 100 µL of DPPH (30 mg/L in methanol). After 30 min of incubation in the dark, the absorbance was read at 517 nm using a UV/Visible spectrophotometer. Gallic acid was used as a control. The radical scavenging activity was expressed as a percentage inhibition according to the formula:

$$\text{Radical scavenging capacity (\%)} = \frac{\text{Absorbance Blank} - \text{Absorbance Sample}}{\text{Absorbance Blank}} \times 100$$

The concentrations were expressed in µg of extracts/µg of DPPH by the formula:

$$\text{Concentration} = \frac{\text{Mass of EO}}{\text{Mass of DPPH}}$$

or

$$\text{Concentration} = \frac{\text{Concentration of EO} \times \text{Volume of EO}}{\text{Concentration of DPPH} \times \text{Volume of DPPH}}$$

The concentration of extract capable of scavenging 50% of the DPPH radicals was then determined graphically.

ABTS+• radical cation decolorization assay

The spectrophotometric analysis of ABTS+• scavenging activity was determined according to the method of Re et

al. [31]. Briefly, the ABTS+• solution was prepared of by dissolving 10 mg of ABTS+• in 2.6 ml of distilled water. Then, 1.7212 mg of potassium persulfate was added, and the mixture was kept in the dark at room temperature for 12 h. The mixture was then diluted with ethanol in order to obtain an absorbance of 0.70 ± 0.02 to 734 nm. In 96-well plates, 50 µl of ethanolic extract solution at an initial concentration of 4.4 mg/mL was added to 200 µl of freshly prepared ABTS+• solution. The same process was carried out for gallic acid at an initial concentration of 1.25 mg/mL and used as a standard. The mixture made in the 96-well plates was then incubated in the dark at room temperature for 15 min, and the absorbance was read at 734 nm against a standard curve of 5,7,8-tetramethyl-2-carboxylic acid 6-hydroxy-2 (Trolox, Sigma-Aldrich) using a spectrophotometer. The activity of the EO from *C. nardus* on the radical cation ABTS+• was expressed in micromolar Trolox equivalent per gram of EO (µM TE/g) using the following formula: C = (cx D) / Ci. C, the concentration of the EO from *C. nardus* in µM TE/g; c, the concentration of the sample read; D, the dilution factor and Ci, the concentration of the stock solution.

Anti-inflammatory capacity

Lipoxygenase (EC 1.13.11.12) type I-B inhibiting activity was assayed spectrophotometrically as described by Lyckander and Malterud [32] with minor modifications. Briefly, the EO from *C. nardus* was initially diluted in the 96-well plate. Then, 100 µl of a solution of 15-lipoxygenase (200 U/ml) prepared in borate buffer (0.2 M, pH 9.0) was mixed with 25 µL of EO from *C. nardus* also prepared in borate buffer at different concentrations at 0.083 mg/mL than at 2.2 mg/mL and incubated for 3 min at 25°C. A negative control without EO and a blank without enzyme were made. The reaction was initiated by the addition of 125 µL of a solution of linoleic acid substrate (234 µM) and the variation of the velocity was monitored at 234 nm for 3 min. The percentage of inhibition was calculated using the following formula:

$$I\% = \frac{E - S}{E} \times 100$$

Where E is the activity of the enzyme without inhibitor (negative control), and S is the activity in the presence of the extract.

Antiproliferative activity

3[4,5-dimethylthiazol-2-yl]-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) assay was used to measure cell survival. Briefly, 50,000 cells/mL were seeded for 24 h in 96-well plates. After 24 h EO from *C. nardus* was added. After 72 h incubation, the number of living cells was measured as described [14, 15] using a microplate reader type Bio-Rad 11885 at 490 nm. Cisplatin was used as a reference compound and dissolved in dimethyl sulfoxide (DMSO). Data are the results of three independent experiments for each cell line performed in octuplet.

Flow cytometry analysis

LNCaP and HeLa cells were seeded at a concentration of 3×10^5 in 6-well plates and treated with either 220 $\mu\text{g/mL}$ or 110 $\mu\text{g/mL}$ of *C. nardus* EO for 72 h at 37°C. After the treatment, cells were harvested with trypsin, centrifuged and fixed with paraformaldehyde (4 %) for 15 min at room temperature, and then washed with PBS. 10^6 cells were prepared in suspension, centrifuged, and the supernatant removed. Then, 0.2 ml of FxCycle™ PI/RNase staining solution (Invitrogen, OR) was added to each tube and mixed well. Samples were incubated for 30 min at room temperature, protected from light, and analyzed by FACS using excitation at 488 nm; emissions were collected using a 585/42 bandpass filter.

Statistical analysis

In vitro experiments were performed in triplicate, each data point represents the average of at least three independent experiments. All data are presented as mean \pm standard deviation. The data were analyzed by analysis of variance followed by the Turkey multiple comparison test. The analysis was performed using XLSTAT 7.1 software. A *p* value of < 0.05 was used as a criterion for statistical significance.

Results

The results of the analysis of the EO *C. nardus* leaves (Table 1 and Figure 1) showed that this plant contained 43 compounds, including two not identified in the literature. Among them, the five most significant compounds were: citronellal (33.06%), geraniol (28.40%), nerol (10.94%),

elemol (5.25%) and delta-elemene (4.09%) (Figure 1 and 2).

The EO of *C. nardus* exhibited an antioxidant activity of $102.19 \pm 4.2 \mu\text{g extract}/\mu\text{g DPPH}$ compared to $0.11 \pm 0.04 \mu\text{g}$ for gallic acid ($p < 0.05$) (Table 2). The percentage of inhibition of DPPH radicals by the EO of *C. nardus* increased with concentration (Figure 3). In fact, at a concentration of 4.58 $\mu\text{g/mL}$ of the *C. nardus* EO, a percentage of inhibition of DPPH radicals was 6.72% and 62.14% at 146.66 $\mu\text{g/mL}$ (Figure 3). The antioxidant activity by the ABTS+• method, the EO has an activity of $0.009 \pm 0.0004 \mu\text{M TE/g}$ against $2.66 \pm 0.31 \mu\text{M TE/g}$ for the gallic acid (Table 2). The results of inhibition of lipoxygenase by the EO are shown in Table 2. At a concentration of 0.083 mg/mL, the EO showed an inhibition of $0.5 \pm 0.9\%$ compared to $59.64 \pm 2.12\%$ of the gallic acid used as standard ($p < 0.05$). Moreover, for a concentration of 2.2 mg/mL of the EO of *C. nardus*, an inhibition of $25 \pm 3\%$ was obtained (Table 2)

The results of the *C. nardus* EO tested on LNCaP, HeLa, and P69 cells are shown in Table 3. An IC_{50} was calculated from the dose-response curves (Figure 4) $58.0 \pm 7.9 \mu\text{g/mL}$, $142 \pm 6 \mu\text{g/mL}$ and $100.9 \pm 3.2 \mu\text{g/mL}$ ($p < 0.05$), respectively. Cisplatin presented IC_{50} values of 4.4 ± 0.5 , 7.8 ± 1.3 , 11.4 ± 1.2 ($p < 0.05$) on HeLa, LNCaP and P69 cells, respectively (Table 3). Figure 5 highlights the action of *C. nardus* EO on LNCaP prostate cancer cell morphology. Moreover, the cell cycle activity of the LNCaP cells was evaluated after *C. nardus* EO treatment (Figure 6).

Discussion

Chemical composition: EOs are a mix of complex molecules [33], are natural, complex, volatile, and odorous molecules synthesized by the secretory cells of aromatic plants [34]. The EO extracted from *C. nardus* leaves by hydrodistillation, determined that citronellal (33.06%), geraniol (28.40%), nerol (10.94%) and elemol (5.25%) is the chemotype of *C. nardus*. This chemotype was similar to those described by De Toledo *et al.* [21], and Aguiar *et al.* [35], and noticeably different from those of Wei and Wee [36] that identified 22 compounds, with citronellal being the primary compound (29.6%); Kandimalla *et al.* [37] reported a total of 95 compounds, citral, 2,6-octadienal-, 3,7-dimethyl-, geranyl acetate, citronellal, geraniol, and citronellol being the most abundant. This difference could be explained by several factors, including genetic factors, age of the plant, season of harvest or plant environment [38, 39]. Besides, our EO contains more monoterpene alcohols (40.77%), followed by monoterpene aldehydes

Table 1: Chemical composition of essential oils of *Cymbopogon nardus*.

Compounds	Retention time (min)	Percentage (%)	Compounds	Retention time (min)	Percentage (%)
α -Thujene	11.64	0.06	α -Copaene	26.07	0.05
α -Pinene	11.92	0.08	β -Elemene	26.21	0.22
Sabinene	13.38	0.31	β -Bourbonene	26.32	0.07
β -Pinene	13.57	0.09	δ -Elemene	26.43	4.09
3-Octanone	13.78	0.06	β -Caryophyllene	27.30	0.12
Myrcene	13.93	0.12	β -Copaene	27.54	0.04
δ -3-Carene	14.66	0.01	(Z)-Beta-Farnesene	27.92	0.04
α -Terpinene	14.96	0.02	α -Humulene	28.24	0.13
Para-Cymene	15.24	0.10	γ -Muurolene	28.66	0.19
Limonene	15.40	2.69	Germacrene-D	28.88	1.79
β -Phellandrene	15.47	0.06	Iso-Butanoate de Néryle	29.17	0.06
Eucalyptol	15.51	0.13	α -Muurolene	29.24	0.52
γ -Terpinene	16.39	0.09	γ -Cadinene	29.64	0.42
Terpinolene	17.31	0.07	δ -Cadinene	29.72	1.64
Linalol	17.78	0.50	Elemol	30.52	5.25
Isopulegol	19.50	0.55	Unknown MW 222	31.24	1.34
Citronellal	19.56	33.06	Unknown MW 220	33.06	0.31
δ -Terpineol	20.12	0.04	Total		98.05
α -Terpineol	20.90	0.04	Monoterpene hydrocarbons		3.69
Nerol	21.80	10.94	Monoterpene alcohols		40.77
Neral	22.11	0.26	Monoterpene aldehydes		33.32
Geraniol	22.57	28.40	Monoterpene ketones		0.06
Cis-Acetate de Pinocarveyle	22.97	0.37	Monoterpene ethers		3.94
Acetate de Citronellyle	25.16	1.47	Sesquiterpene hydrocarbon		9.38
Eugenol	25.39	0.30	Sesquiterpene alcohols		5.25
Acetate de Geranyle	25.94	1.96	Others*		1.65

*, value consists mainly of unidentified compounds with a molecular weight (MW) of 222 (1.34%) and 220 (0.31%)

(33.32%). The content of hydrocarbon sesquiterpene (9.38%), sesquiterpene alcohols (5.25%), monoterpene ethers (3.94%), monoterpene hydrocarbons (3.69%) and monoterpene ketones (0.06%) are respectively lower.

Antioxidant activity: Plants are generally a potential source of antioxidant molecules. Several studies have highlighted the strong antioxidant potential of EOs from medicinal plants used in Burkina Faso [14, 15]. The EO from *C. nardus* has demonstrated antioxidant activity against both DPPH and ABTS+• cation radicals. The activity

of gallic acid used as a standard is however greater. In addition, we identified antioxidant activity. Other studies showed that the EO from *C. nardus* has antioxidant activity [22], which could be explained by its high content of monoterpene alcohol (40.77%). Indeed, geraniol [40] is a significant monoterpene in the EO we studied. Compared to gallic acid used as standard, it should be noted that the activity of the EO from *C. nardus* is however, lower.

Anti-inflammatory capacity: Inhibition of lipoxygenase by the EO is concentration-dependent. At

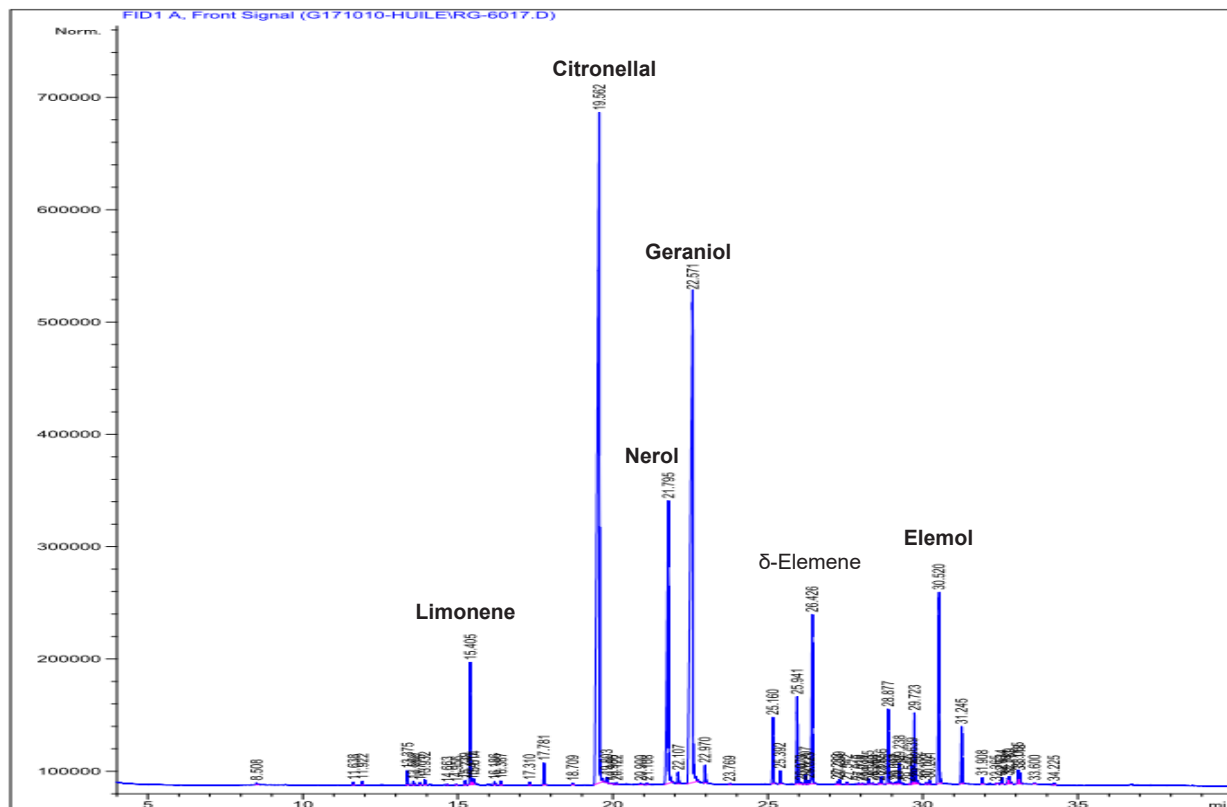


Figure 1: Chromatograms of the essential oil *Cymbopogon nardus* with its major compounds identified.

Monoterpene hydrocarbon	 Limonène (R)
Monoterpene aldehydes	 Citronellal
Monoterpene alcohols	 Nerol Geraniol
Monoterpene ethers	 Acetate Geranyle
Sesquiterpene alcohols	 Elemol

Figure 2: Chemical structures of the major compounds found in the analyzed *Cymbopogon nardus* essential oil.

low concentration (0.083 mg/mL), there was virtually no inhibition ($0.5 \pm 0.9\%$), while at a higher concentration (2.2 mg/mL) a $25 \pm 3\%$ inhibition was noted. This anti-inflammatory activity could be explained by its high citronellal content (33.06%). Previous studies have showed such activity by citronellal [41]. These results corroborate those of Kandimalla et al. [37]. In addition, several studies have shown anti-inflammatory activity of monoterpenes aldehydes; however the specificity of each compound within this group could contribute to increase or decrease activity [42].

Antiproliferative activity: Antiproliferative activity was studied to evaluate the putative antitumor properties of the EO from *C. nardus* on LNCaP and HeLa cells. Our results show for the first time that the EO inhibits the proliferation of both LNCaP and HeLa cancer cells. Antiproliferative activity was also discovered using non-cancerous P69 epithelial cells. The antiproliferative activity of the EO from *C. nardus* was dependent on concentration for both cancer cells and non-cancer cells (Figure 4). Determination of the IC_{50} showed that LNCaP cells are more sensitive than HeLa cells ($58.0 \pm 7.9 \mu\text{g/mL}$

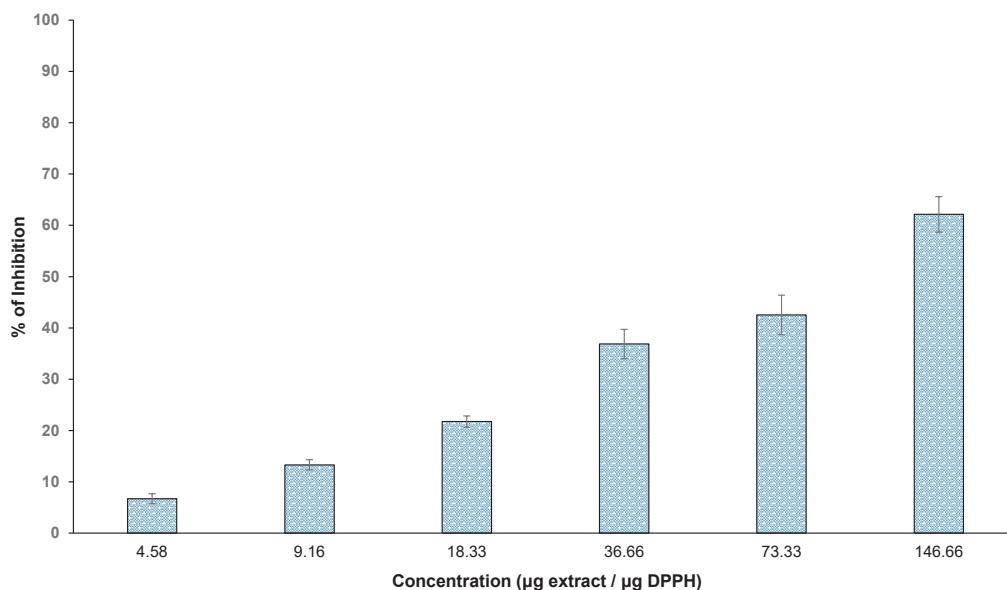


Figure 3: Percent inhibition of the DPPH radicals of *Cymbopogon nardus* essential oil as a function of concentration.

Table 2: Antioxidant and anti-inflammatory activity of *C. nardus* essential oil and gallique acid.

Essential oil (EO) and standard	Antioxidant activity		Anti-inflammatory activity
	DPPH (IC ₅₀ ; µg EO/µg DPPH)	ABTS (µMET/g)	% Inhibition of LOX
<i>C. nardus</i> EO	102.19 ± 4.2	0.009 ± 0.0004	0.5 ± 0.9 [§] 25 ± 3 [£]
Gallique acid	0.11 ± 0.04 ^{***}	2.66 ± 0,31 ^{***}	59.64 ± 2.12 ^{***§}

DPPH, (2,2-diphenyl-1-picrylhydrazyl); ABTS (2,20-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid]); values are expressed as mean values ± SD; n = 3 independent experiments in quadruplicate for the measurement of antioxidant activity; DPPH activities is expressed as IC₅₀ (µg EO/µg DPPH) and ABTS activities are given in µmol Throlox equivalent/g of *C. nardus* EO. Anti-inflammatory activity, values are expressed as mean values ± SD; n = 3 experiments in quadruplicate for the anti-inflammatory activity is expressed as percent inhibition of lipoxygenase (LOX); §, percent inhibition of lipoxygenase at 0.083 mg/mL; £, percent inhibition of lipoxygenase at 2.2 mg/mL; ***, (p < 0.05) values significantly different comparatively to gallique acid.

Table 3: IC₅₀ (µg/mL) of essential oil of *C. nardus* tested on LNCaP human prostate cancer cell lines, HeLa human cervical cancer cell lines and P69 non-cancerous human prostate epithelial cell lines.

Cell lines	IC ₅₀ (µg/mL)	
	EO of <i>C. nardus</i>	Cisplatin
HeLa	142 ± 6.0*	4.4 ± 0.5 ^{***£}
LNCaP	58.0 ± 7.9 ^{***}	7.8 ± 1.3 ^{***£}
P69	100.9 ± 3.2 ^{**}	11.4 ± 1.2 [£]

Values are expressed as mean values ± standard deviation; n = 3 independent experiments in sextuplicate; *, p < 0.05; **, p < 0.05; *** p < 0.05 values significantly different comparatively for each column; EO, essential oil

vs. 142 ± 6 µg/mL, respectively). The fact that the EO alters the cell proliferation is probably due to its high level of monoterpene alcohol (40.77%) of which geraniol (28.40%) and nerol (10.94%), as well to a significant amount of monoterpene aldehyde (33.32%) including citronellal (33.06%). The alcohol monoterpenes are known for their anticancer potential. Antiproliferative activities of citronellal [43] and geraniol [44, 45] have already been reported. This could also explain the cytotoxicity observed using the P69 cells [46]. The most significant effect was observed in the LNCaP cells, we further analyzed the role of the EO from *C. nardus* on the cell morphology (Figure 5) and cell cycle (Figure 6) by cytometry.

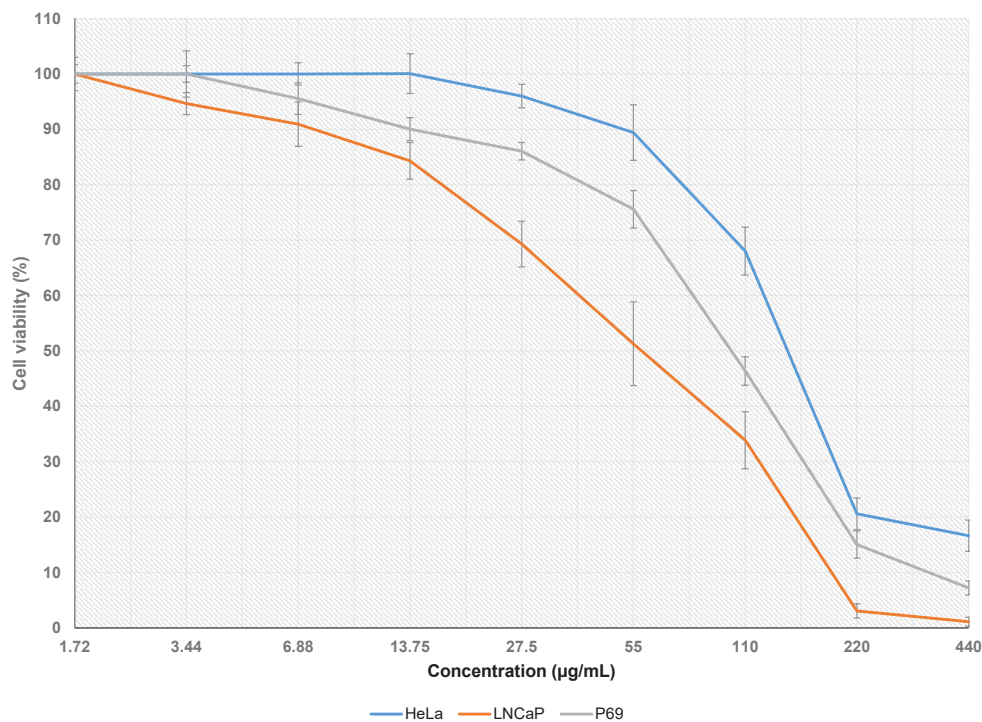


Figure 4: Dose-dependent anti-proliferative activity of *Cymbopogon nardus* essential oil. HeLa, LNCaP and P69 cells were treated for 72 h. Experiments were performed three times in sextuplicate.

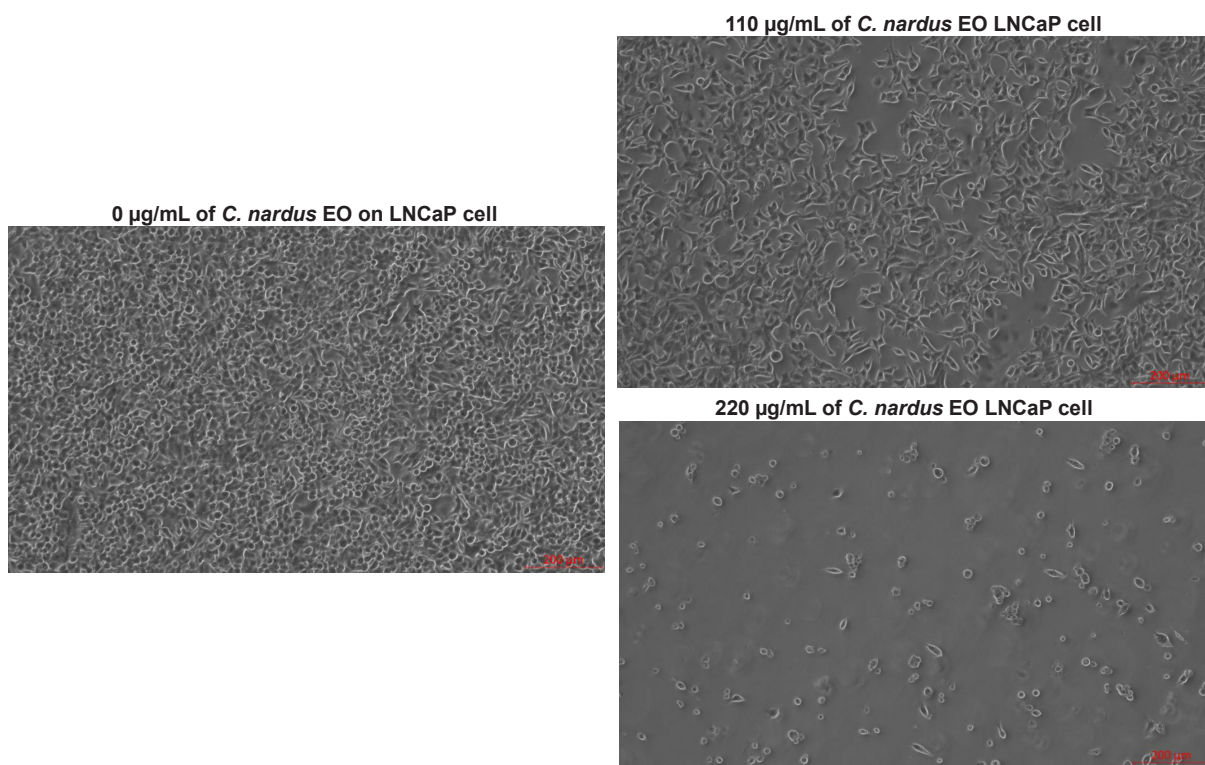


Figure 5: Action of *C. nardus* essential oil on the morphology of LNCaP cells.

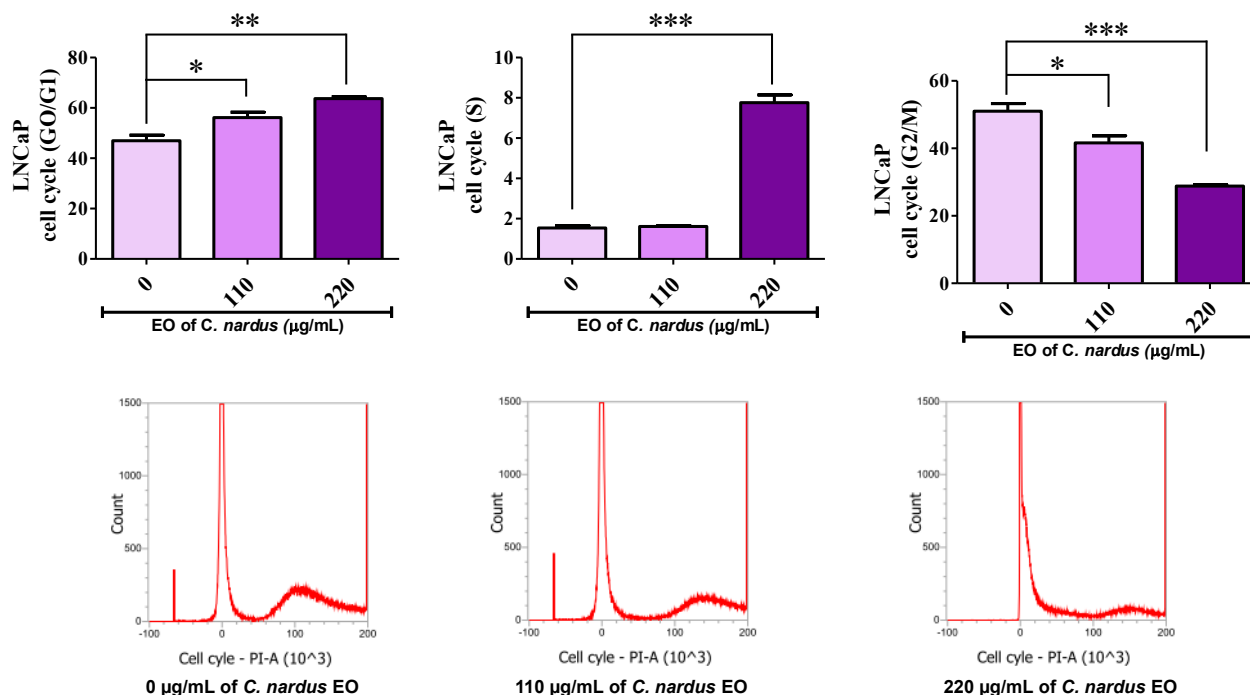


Figure 6: Effect of *C. nardus* essential oil on prostate cancer LNCaP cell cycle. Values are expressed as mean values \pm standard deviation; $n = 3$ independent experiments in triplicate; *, $p < 0.05$; **, $p < 0.05$; ***, $p < 0.05$ values significantly different compared to vehicle; EO, essential oil.

Cisplatin, a compound used in cancer chemotherapy, was used as a reference compound, and exhibited superior activity to that of *C. nardus* EO on both HeLa and LNCaP cancer cells and on immortalized P69 prostate cells. Cisplatin is a pure compound, unlike EOs, which consist of a mixture of various molecules. However, the P69 cells are immortalized and this could, in part, justify the toxicity of the EO and cisplatin on these cells.

The cell cycle is closely linked to tumorigenesis. Irregular cell cycle regulation and the resultant uncontrolled cell growth that occurs as a consequence are common characteristics for most tumors [47]. Morphology of LNCaP cells were drastically modified, and this was due to the arrest of the cell cycle at the G2/M phase in a dose-dependent manner.

Conclusion

C. nardus is commonly used in traditional medicine alone or in combination with other medicinal plants for the management of various diseases, mainly microbial and oxidative stress-related diseases in Burkina Faso, West Africa. Herein, we show that the EO of this plant has antioxidant properties through the inhibition of DPPH

radicals and radical cation ABTS+•, anti-inflammatory inhibition of lipoxygenase and for the first time demonstrated anti-proliferative activity on various cell lines derived from prostate (LNCaP and P69) or cervix (HeLa). These data highlight the need for more *in vitro* research to identify the compound(s) responsible for these effects as well as the use of animal models to establish the pharmacokinetics and toxicity profiles of this EO, and to further quantify its putative anti-tumor effect.

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Authors' contributions: BB, JS and JML designed the research; BB and GF performed chemical synthesis; and BB, AYC, and BMN performed the experiments and analyzed the data. BB, AYC, FD, SB, JS and JML wrote the manuscript. All authors read and approved the final manuscript.

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