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Chemical Composition of Essential Oil of *Cymbopogon schoenanthus* (L.) Spreng from Burkina Faso, and Effects against Prostate and Cervical Cancer Cell Lines

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Abstract: The aim of this research was to evaluate the essential oil of *Cymbopogon schoenanthus* (L.) Spreng. (*C. schoenanthus)* from Burkina Faso in cytotoxic activity against LNCaP cells, derived from prostate cancer, and HeLa cells, derived from cervical cancer. Antioxidant activities were evaluated *in vitro*. Essential oil (EO) was extracted by hydrodistillation and analyzed by GC / FID and GC / MS. Thirty-seven compounds were identified, the major compounds being piperitone (49.9%), alpha terpinene isomer (24.02%), elemol (5.79%) and limonene (4.31%). EO exhibited a poor antioxidant activity, as shown by the inhibition of DPPH radicals (IC_{50} =1730 ± 80 µg/mL) and $ABTS^{+}$ (IC_{50} = 2890 ± 26.9 µg/mL). Conversely, EO decreased the proliferation of LNCaP and HeLa cells with respective IC_{50}s of 135.53 ± 5.27 µg/mL and 146.17 ± 11 µg/mL. EO also prevented LNCaP cell migration and led to the arrest of their cell cycle in the G2/M phase. Altogether, this work points out for the first time that EO of *C. schoenanthus* from Burkina Faso could be an effective natural anticancer agent.

Keywords: *Cymbopogon schoenanthus*; essential oil; antioxidant; cytotoxic; antimigratory; cell cycle; cancer

1. Introduction

Medicinal plants have been widely handled in traditional medicine for several centuries for the treatment of many health-related ailments [1]. Hence, in low-income countries such as Burkina Faso, medicinal plants are used with significant results to treat many pathologies, *e.g.*, tumors, boils, or chronic wounds, especially in villages where modern health cares are totally inaccessible. *Cymbopogon schoenanthus* (L.) Spreng (*C. schoenanthus*) is a herbal plant of Southern Asia and Northen Africa, with fragrant foliage, suggesting leafs are enriched in essential oils (EO). This herbaceous plant is well known and used in Burkina Faso in traditional medicine, as well as in other African and Asian countries. Chemical characterization and *in vitro* analyses of EO of *C. schoenanthus* from various geographic area pointed out specific activities. EO extracted from the Sudanese plant has shown a high antiproliferative activity against human breast carcinoma and human colon adenocarcinoma cell lines [2]. EO extracted from the Saudi Arabian plant has strong protective effects against *Escherichia coli*, *Staphylococcus aureus*, *Methicillin-susceptible S. aureus* and *Klebsiella pneumoniae* [1]. The authors identified eight main components such as piperitone (14.6%), cyclohexanemethanol (11.6%),

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β-elemene (11.6%), α-eudesmol (11.5%), elemol (10.8%), β-eudesmol (8.5%), 2-naphthalenemethanol (7.1%) and γ-eudesmol (4.2%) [1]. EO of *C. schoenanthus* from Brazil presents an efficient anthelmintic activity, since the developmental of trichostrongylids obtained from naturally infected sheep is blocked *in vitro* [3]. Extracts from the Algerian plants shows strong antioxidant activities [4,5]. So far, only one study has been performed on EO of *C. schoenanthus* from Burkina Faso [6]. Sawadogo et al. focused their work on the putative antifungal activities. The authors studied the inhibition of both mycelial growth of *Aspergillus parasiticus* and *Aspergillus flavus*, and activity of aflatoxins B2 and G1.

Because EO of leafs of *C. schoenanthus* from Burkina Faso have been poorly studied so far, we have aimed to evaluate the chemical composition of this EO to investigate its biological activities on the survival characteristics of LNCaP and HeLa cells, respectively derived from prostate and cervical cancers. Effects on the cell cycle and migration were also investigated in LNCaP cells.

2. Results and discussion

2.1. Chemical composition of the essential oil

Gas chromatography coupled with a flame ionization detector and mass spectrometry (Figure 1) identified 37 compounds within the EO of C. schoenanthus, for a percentage of 98.46% (Table 1).

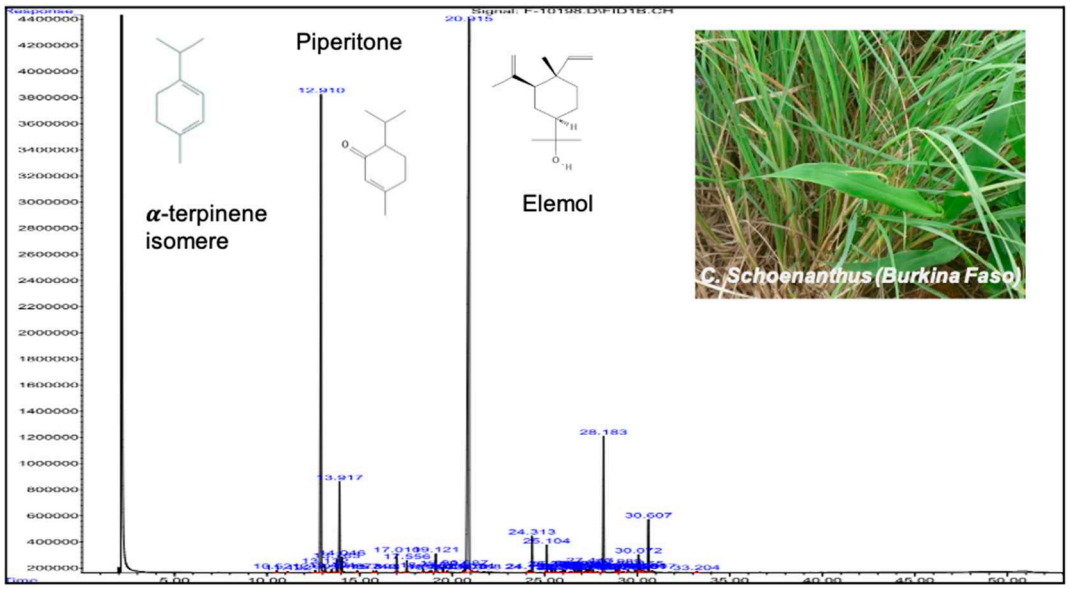

Figure 1. Chromatogram of identified compounds of C. schoenanthus EO. Structures of the three major identified compounds are drawn. Essential oil was extracted by hydrodistillation using an alembic / Clevenger-type apparatus for 3 hours and stored in the bottle at 4°C. Inset, photograph of Cymbopogon schoenanthus taken by Dr. Bagora BAYALA in Ouagadougou, Burkina Faso. GPS location: 12°25'29,5''N and 1°29'14,3''W.

Table 1. Chemical composition of essential oil of Cymbopogon schoenanthus.

Chemical compounds	Retention time (min)	Percentage (%)
α-pinene	10.521	0.11
1,8-Cineol dehydro	12.603	0.11
α-terpinene isomere	12.910	24.02
α-phellandrene	13.132	0.38
α-terpinene	13.494	0.13
P-cymene	13.763	0.58
Limonene	13.917	4.31
Eucalyptol	14.046	0.64
γ-terpinene	14.872	0.11

Terpinolene	15.740	0.09
α-Thujone	15.898	0.11
Menth-2-ene-1-ol-Cis para	17.010	0.81
Menth-2-ene-1-ol-Trans para	17.556	0.55
P-Cymene-8-ol	18.897	0.12
α-terpineol	19.121	1.06
Cis Piperitol	19.517	0.28
Piperitone	20.915	49.90
β-bourbonene	24.192	0.11
β-elemene	24.313	1.50
β-caryophyllene	25.104	1.26
β-gurjunene	25.371	0.21
Aromadendrene	25.561	0.20
α-humulene	25.986	0.14
Allo-aromadandrene	26.090	0.12
Germacrene-D	26.598	0.28
β-Selinene	26.804	0.32
α-selinene	26.955	0.34
Cuparene	27.262	0.22
γ-cadinene	27.344	0.12
δ-cadinene	27.443	0.35
Elemol	28.183	5.79
Spathulenol	28.844	0.15
Caryophyllene oxide	28.989	0.33
Viridiflorol	29.059	0.12
γ-Eudesmol	29.889	0.10
Epi- γ-eudesmol 10	30.072	0.83
α-Eudesmol	30.607	2.65
Total		**98.46**
Monoterpene hydrocarbons		29.73
Monoterpene ethers		0.75
Monoterpene ketones		50.01
Monoterpene alcohols		2.82
Sesquiterpene hydrocarbons		5.18
Sesquiterpene alcohols		9.64
Sesquiterpene oxides		0.33

The main compounds were piperitone (49.9%), isomeric alpha terpinene (24.02%), elemol (5.79%) and limonene (4.31%), representing almost 84% of the total composition. Monoterpene ketones, such as piperitone, represent up to 50% of the compounds. The levels of piperitone, a compound generally used for the production of synthetic menthol and thymol, is in accordance with the other studies with 59.1% [2] and 59.8% [6]. Interestingly, other authors have described qualitatively and quantitatively different compositions for EO extracted from C. schoenanthus. Hashim et al. [1] described that piperitone, cyclohexanemethanol, β-elemene, α-eudesmol and elemol were equally distributed in the EO. On the other side, Katiki et al. showed that the major compound quantified by gas chromatography was geraniol (62.5%) [3]. We have hypothesized that these differences were due to the collection area and/or season.

2.2. Antoxidant potential of the essential oil

The need of antioxidant compounds is important as oxidation is a process strongly involved in many pathologies. Inhibitory activity of DPPH and ABTS+· radicals proportionally reflects the antioxidant activity. Because, EO of C. schoenanthus was enriched in putative antioxidant molecules,

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inhibitions of the DPPH and $ABTS^{+\cdot}$ radicals were measured. Concentrations necessary to reach 50% of the inhibition (IC_{50}) were calculated as 1730 ±80 µg/mL and 2890 ± 26.9 µg/mL for DPPH and $ABTS^{+\cdot}$, respectively (Table 2).

Table 2. Antioxidant activity of C. schoenanthus EO.

Compound tested	DPPH test	ABTS test
EO of *C. schoenanthus*	1730.00 ± 80.00	2890.00 ± 26.90
Trolox	1.84 ± 0.07***	7.57± 1.8***

DPPH, (2,2-diphenyl-1-picrylhydrazyl); ABTS (2,2'-azino-bis-[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 and ABTS activities are expressed as IC50 (µg/mL); ***, $p < 0.05$, values significantly different for each test compared to the positive control Trolox ; EO, Essential Oil.

Compared to the Trolox used as a positive control, EO of C. schoenanthus is 900 and 300-fold less efficient on DPPH and ABTS+· radicals, respectively, which let us to conclude that this EO has a really poor antioxidant activity. Interestingly, EO of C. schoenanthus from Sudan has also a weak antioxidant activity [2], conversely to the extracts collected from Algeria [7] and South Tunisia [8]. The main differences between our EO and those described were the amount of cis- and trans-pmeth-2-en-1-ols (0.81 and 0.55% for our study, vs. 23 and 14%, respectively) in the Algerian EO [7], and the amount of limonene (above 11% vs. 4% for the Burkina Faso) and α-terpineol (above 7% vs. 1% for the Burkina Faso) for the Tunisian EO [8].

2.3. Cytotoxic activity of the essential oil

The imperfections of drugs currently used in therapy and the increasing problem of drug resistance have forced a search for new substances with therapeutic potential. Throughout history, numerous organisms have been rich sources of biologically active compounds [9]. To investigate whether C. schoenanthus could be such a source, effects of EO of C. schoenanthus were evaluated on LNCaP (derived from metastatic prostate cancer) and HeLa (derived from cervical cancer) cell viability.

As shown in Figure 2, EO from C. schoenanthus decreased the cell survival with reasonable IC_{50}s of 135.53 ± 5.27 µg/mL and 146.17 ± 11.83 µg/mL on LNCaP and HeLa cells, respectively (Table 3), compared to the positive control cisplatine (3.2 ± 0.5 and 5.2 ± 0.8 µg/mL on the cells, respectively, $p<0.001$).

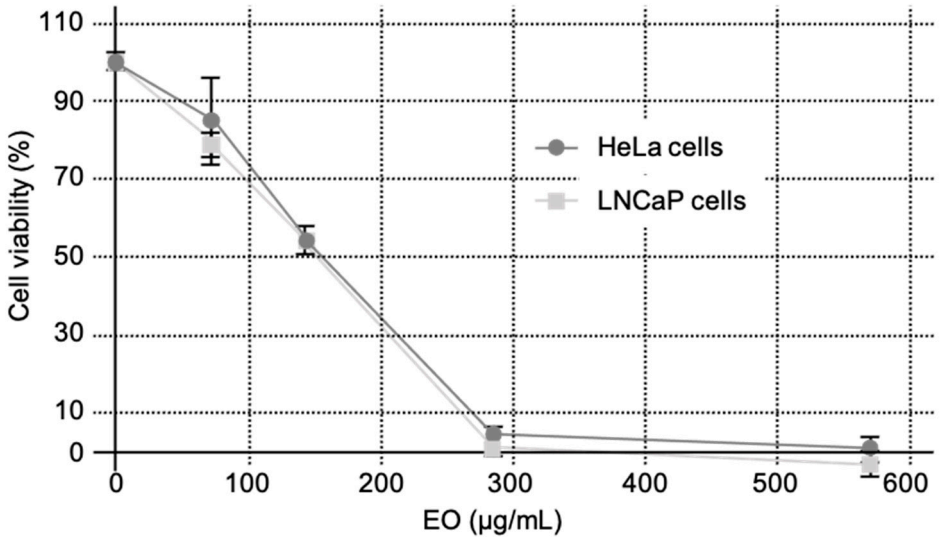

Figure 2. Effects of C. schoenanthus EO on LNCaP and HeLa cell survivals. LNCaP and HeLa cells were treated 24 hours after seeding with various concentration of EO for 72 hours. Values are expressed as mean values ± SD. n = 3 independent experiments in sextuplicate.

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Table 3. IC_{50} (µg/mL) of EO of Cymbopogon schoenanthus tested on LNCaP human prostate cancer cell lines and HeLa human cervical cancer cell lines.

Compound tested	LNCaP cells	HeLa cells
EO of *C. schoenanthus*	135.53 ± 5.27	146.17 ± 11.83
Cisplatin	3.20 ± 0.50***	5.20 ± 0.80***

Values are expressed as Mean ± Standard Deviation, n = 3 independent experiments in sextuplicate, EO; Essential Oil, ***(p<0.001); values significantly different compared to chemotherapeutic agent cisplatin (positive control) for each cell line.

Mohamed Abdoul-Latif et al. have shown that EO of C. schoenanthus from Djibouti have a more significant cytotoxic effects [10]. It should be noted that piperitone was totally absent in their EO, while 3-isopropenyl-5-methyl-1-cyclohexene and D-limonene were the main compound. Conversely, Hakkim et al. pointed that piperitone present at 39% in EO from Oman has a strong cytotoxic activity in triple negative breast cancer and cervical cancer cell lines [11]. Piperitone representing almost 50 of the EO could be responsible of the results we obtained on LNCaP and HeLa cells. We have also associated the cytotoxic activity to the high amount of terpinenes, known to have anticancer properties [12]. Monoterpene ketones (50.1%) and monoterpene hydrocarbons (29.73%) could also explain this activity. However, compared to cisplatin, used as control (Table 3), EO of C. schoenanthus is less efficient on LNCaP (135 vs. 3 µg/mL) and HeLa (146 vs. 5 µg/mL) cells. Altogether, It should thus be interesting to test the cytotoxic activity of piperitone, the major compound identified in our study, alone or in combination with the other compounds.

2.4. Effects of essential oil on migration and cycle of LNCaP cells

Killing cells and blocking their migration are important pharmacological targets in the clinical management of tumors [13,14]. As cell migration is a hallmark in tumor development, we performed scratch cell assays to measure the effects of the tested EO on the migration of the LNCaP cells (Figure 3). While, the wounded area was decreased by 32% (p<0.001) after 72h, EO used at the IC_{50} blocked the cell migration.

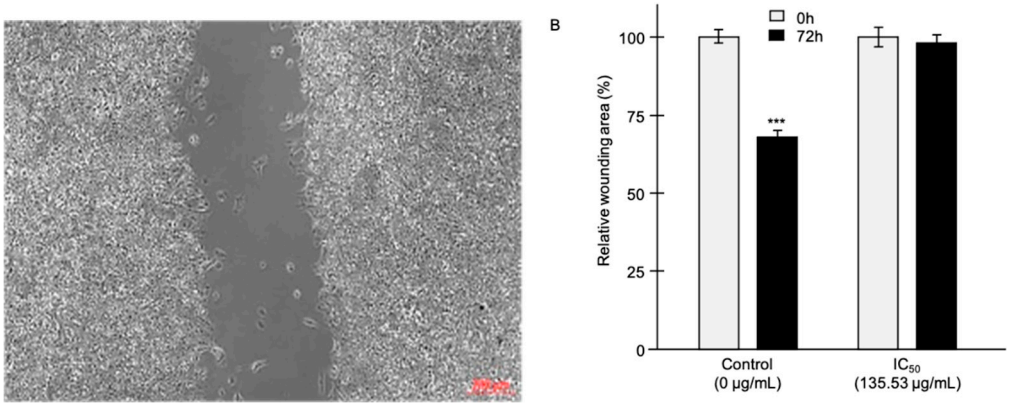

Figure 3. Activity of C. schoenanthus EO on cell migration. A. Significant picture of scratch cell assay. B. Relative wound healing area remaining after the incubation with EO at the IC_{50}. Values are expressed as mean values ± standard deviation; n = 3 independent experiments.***, p < 0.001 values significantly different compared to IC_{50} of EO after 72 Hours of induction 0h.

Cancer cells could also be characterized by a strong activity of division [15]. Whether EO from C. schoenanthus could modify the cell cycle of LNCaP cells was also investigated. Our experiments enlightened a significant activity of EO on the distribution of the various phases of the cell cycle (Figure 4). An increased accumulation of cells in the subG1 phase was observed together with a 2-fold increase in the G0/G1 (from 35% to 70%, for 0 and 285 µg/mL of EO, respectively) and S (from 2% to 13%, for 0 and 285 µg/mL of EO) phases. In parallel, a significant decrease in G2/M phase was shown in cells trated by 285 µg/mL of EO compared to the control cells (from 63% to 17%, p<0.01). It

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could thus be suggested that EO of C. Schoenanthus reduces the cell proliferation. Whether an early apoptosis is induced needs to be demonstrated.

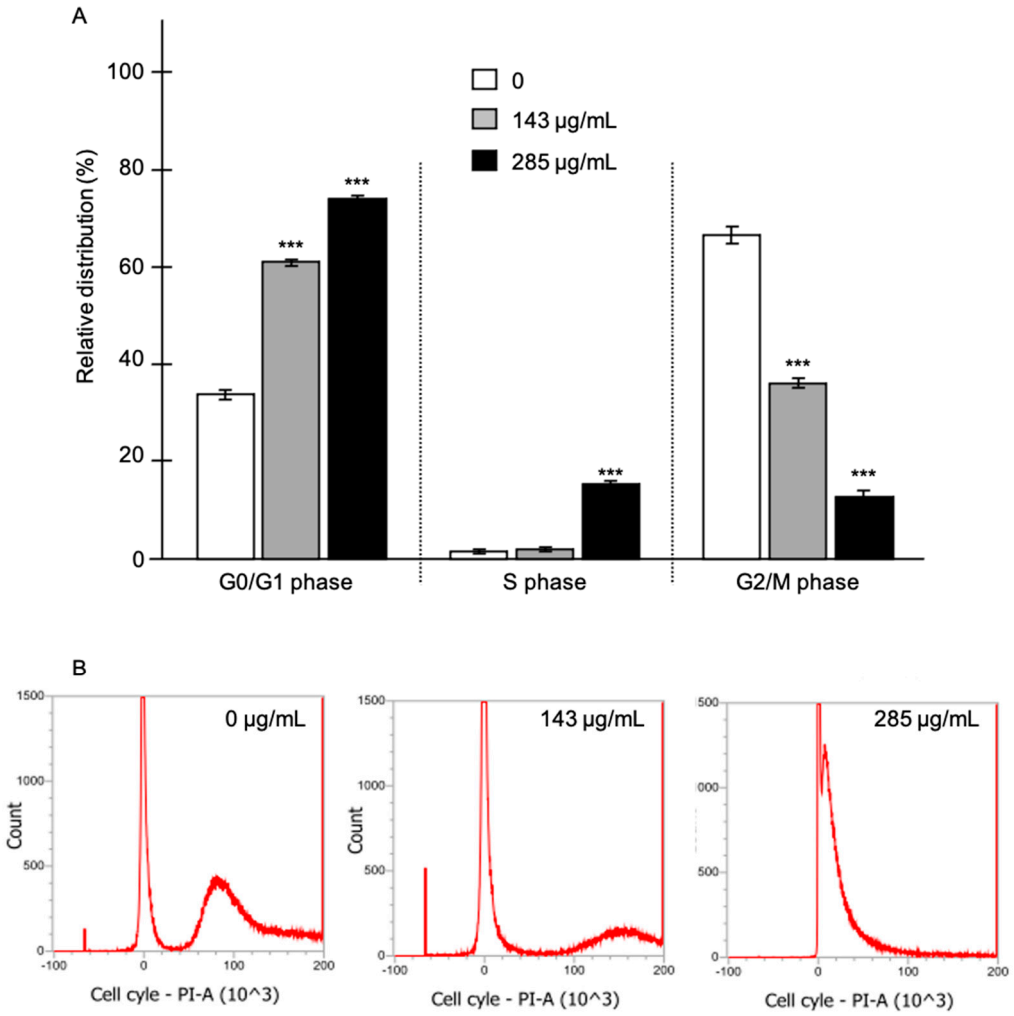

Figure 4. Effect of C. schoenanthus EO on LNCaP cell cycle. A) LNCaP cells were incubated without (DMSO 1/1000) or with (142 or 285 µg/mL) EO for 72h. Values are expressed as mean values ± standard deviation; n = 3 independent experiments in triplicate; ***, $p < 0.001$ values significantly different compared to vehicle-treated condition; EO, essential oil. Flow cytometry plots are shown in B).

3. Materials and Methods

3.1. Plant material and Essential Oil (EO) extraction

Leaves of *C. schoenanthus* were collected 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). Identification and authentication were performed by Dr. A. SEREME (IRSAT / CNRST, Ouagadougou, Burkina Faso). A specimen was deposited in the herbarium of the Laboratory of Biology and Plant Ecology of University Joseph KI-ZERBO, publicly accessible under ID: 15936 and sample number 03 of September 2019. Fresh leaves of *C. schoenanthus* (1 Kg) were submitted to hydrodistillation using an alembic / Clevenger-type apparatus for 3h as described previously [16]. EO was stored in airtight containers in a refrigerator at 4°C until GC-FID and GC/MS analyses and biological tests. EO was therefore diluted in hexane (1/30, v/v) for GC/FID analysis.

3.2. Chemical composition

Gas chromatography–flame ionization detector (GC/FID) analysis - Composition of EO was performed as previously mentioned [17]. Briefly, gas chromatography of hexane-diluted EO was

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performed on an Agilent gas chromatograph Model 6890 (Agilent, Palo Alto, Ca), equipped with a DB5 MS column (30 m x 0.25 mm, 0.25 µm film thickness). Hyrdogene was used as carrier gas (flow 1.0 mL/min). Oven temperature program was 50°C (5 min) to 300°C with an increasing temperature of 5°C/min. The sample (1 mL) was injected in split mode (1:60), injector and detector temperatures being respectively at 280 and 300°C [16].

Gas chromatography–mass spectrometry (GC/MS) analysis - Mass spectrometry analyses of EO were performed on an Agilent gas chromatograph Model 7890 coupled to an Agilent MS model 5975, equipped with a DB5 MS column (20 m x 0.20 mm, 0.20 mm film thickness), programming from 50°C (5 min) to 300°C at 8°C/min, 5 min hold, as described [17]. Briefly, helium was used as carrier gas (average flow of 1.0 mL/min). Oven temperature program was from 50°C (3.2 min) to 300°C at 8°C/min, 5 min post run at 300°C. Sample (1µL) was injected in split mode (1:150), injector and detector temperature being at 250°C and 280°C, respectively [16]. The MS worked 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 [16].

Identification of components - The main compounds in the EO of *C. schoenanthus* were identified by comparison of their retention indices with those of the literature, determined in relation to a homologous series of n-alkanes (C8–C32) under the same operating conditions [17]. In replace of standard compounds, we performed retention indices and comparisons with the NIST library [18] or literature [19]. Component relative percentages were calculated based on GC peak areas without using correction factors [16,20]. The major identified compounds are indicated on Figure 1.

3.3. Cell culture

LNCaP cells, derived from prostate cancer (ATCC # CRL-1740), or Hela cells, derived from cervical cancer (ATCC # CCL-2), were used. These cell lines were available in the GReD Institute (Université Clermont Auvergne, France) [21]. Cells were cultured and maintained at 37°C in a chamber moistened with 5% CO_2 in 75 cm^2 flasks in RPMI-1640 or DMEM medium (Invitrogen, Oslo, Norway) supplemented with 10% fetal calf serum (FCS, Biowest, Nuaillé, France), 1% penicillin and 1% streptomycin (Invitrogen).

3.4. Antioxidant activity

DPPH radical scavenging assay - As was already described [17], each 100µL of serial dilutions EO of *C. schoenanthus* starting at 3.8 mg/mL was mixed with 100µL of DPPH (30 mg/L in methanol) and incubated 30 min in darkness at room temperature. The absorbance was read at 517 nm against a blank (mixture without EO). Trolox (Sigma-Aldrich, L'Ile d'Abeau, France) was used as positive control. The radical scavenging activity was calculated according to the formula (absorbance blank-absorbance sample)/absorbance blank x 100. The concentration of extract able of scavenging 50% of the DPPH radicals was then determined graphically and expressed as µg of EO / µg of DPPH [21].

$ABTS^{+\cdot}$ radical cation decolorization assay - The spectrophotometric analysis of $ABTS^{+\cdot}$ scavenging activity was performed using 96-well plates with 50µL of ethanolic solution of EO of *C. schoenanthus* at an initial concentration of 3.8 mg/mL, added to 200µL of freshly prepared $ABTS^{+\cdot}$ solution, as previously described [17,21]. Briefly, $ABTS^{+\cdot}$ solution was prepared by dissolving 10 mg of ABTS in 2.6 mL of distilled water in which 1.7212 mg of potassium persulfate was added. ABTS was then incubated at room temperature for 12h and diluted with ethanol in order to obtain an absorbance of 0.70±0.02 to 734 nm. Trolox (Trolox, Sigma-Aldrich) was used as a positive control. The 96-well plates were then incubated in the dark at room temperature for 15 min and the absorbance read at 734 nm. The activity of EO of *C. schoenanthus* on the radical cation $ABTS^{+\cdot}$ was expressed in micromoles Trolox equivalent per gram of EO (µmol TE g^{-1}) [21].

3.5. Measurement of cell survival

3[4,5-dimethylthiazol-2-yl]-diphenyltetrazolium bromide (MTT) assay (Sigma-Aldrich) was used to measure the mitochondrial activity, which reflects the number of viable cells [16,17]. Briefly,

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12500 cells were seeded for 24 h in 96-well plates. EO of *C. schoenanthus* was added at various concentrations for 72 h. The number of living cells is directly proportional to the intensity of the violet color measured quantitatively by spectrophotometry using a microplate reader spectrophotometer Thermo Fisher Scientific SN 1510-02948 at 570 nm. Three independent experiments were performed in octuplicate for each cell line.

3.6. Measurement of cell survival

The effects of EO of *C. schoenanthus* on the migration of LNCaP and HeLa cells were evaluated by *in vitro* wound healing assay. Cells were seeded in 6-well plates at a rate of $35x10^4$ cells per well in a final volume of 2 ml of complete medium for 24 h. A scratch was performed on the cell monolayer with a sterile micropipette tip. Detached cells were then removed with phosphate buffered saline 1X (Life Technologies). Cells were next grown in medium containing EO of *C. schoenanthus* at the concentration corresponding to the IC_{50}. Multiple images were then taken for 72 hours by Zeiss AxioObserver 7 inverted microscope equipped with a Colibri 7 LED source, which made possible to determine the effect of the EO on LNCaP cells migration.

3.7. Flow cytometry analysis

LNCaP cells ($3x10^5$) were seeded in 6-well dishes and treated with EO after 24 hours at the dose of 0 µg/mL (control; DMSO, 0.001), 143 µg/mL and 285 µg/mL respectively for 72 hours at 37°C. After the treatment, cells were harvested with trypsine, 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, Oregon, USA) was added to each tube and mixed. The samples were incubated for 30 min at room temperature, protected from light, and then analyzed by FACS using excitation at 488 nm and the emissions were collected using a 585/42 bandpass filter.

3.8. Statistical analysis

The data were analyzed by analysis of variance followed by the Tukey multiple comparison test. The analyses were performed using XLSTAT 7.1 software. $P < 0.05$ was used as a criterion for statistical significance.

5. Conclusion

Our work demonstrates for the time the effects of EO of *C. Schoenanthus* from Burkina Faso on LNCaP and HeLa cells. The chemical composition points out the richness of EO in piperitone and terpenes. While the antioxidant capacity of the EO is quite low, cytotoxic activities on LNCaP and HeLa cells are significant. Besides, EO also prevents the migration of LNCaP cells and leads to the arrest of their cell cycle in the G2/M phase. Altogether, this work constitutes a scientific basis for the use of EO of *C. schoenanthus* from Burkina Faso for the traditional management of tumors.

Author Contributions: BB and JML designed the research; BB and LLC performed chemical synthesis and analysis; the experiments and analyzed the data. BB, LLC, FD, JS, JBN and JML wrote the manuscript. All authors read and approved the final manuscript.

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Data Availability Statement: Datasets generated during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Sample Availability: Samples of the compounds analyzed in this study are available from the authors.

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