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Chemical composition, antiproliferative, antioxidant and antibacterial activities of essential oils from aromatic plants growing in Sudan

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ABSTRACT

Objective: To explore the potential of essential oil, as therapeutic molecule source, from olibanum of *Boswellia papyrifera* (Burseraceae), leafy stems of *Cymbopogon schoenanthus* (Poaceae) and *Croton zambesicus* (Euphorbiaceae) and rhizome of *Cyperus rotundus* (Cyperaceae) found in Sudan. Respective essential oil was evaluated for anti-proliferative, antibacterial and antioxidant activity.

Methods: Essential oils were extracted by hydrodistillation and then analysed by gas chromatography coupled to mass spectrometry (GC–MS). Anti-proliferative activity was determined against human cell lines (MCF7 and MDA-MB231, HT29 and HCT116) by the thiazolyl blue tetrazolium bromide (MTT) procedure. Antioxidant activity was evaluated by diphenyl 2 pycril hydrazil (DPPH) assay. Antibacterial activity was determined against two Gram-positive and two Gram-negative bacteria by microdilution method.

Results: The essential oil from olibanum of *Boswellia papyrifera* contained mainly alcohol and ester derivatives (46.82%) while monoterpenes (69.84%) dominated in *Corton zambesicus* oil. Sesquiterpenes were the most highly represented classes of terpene derivatives in *Cyperus schoenanthus* (71.59%) and *Cyperus rotundus* (44.26%). Oil of *Cymbopogon schoenanthus* revealed the best anti-proliferative activity against HCT116 cell line with IC50 value at (19.1 ± 2.0) μg/mL. Oil of *Croton zambesicus* showed the best antioxidant activity [EC50 (4.20 ± 0.19) mg/mL]. All oils showed good antibacterial activity against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* with minimum inhibitory concentration (MIC) value ranged from 16 to 250 μg/mL.

Conclusions: The results suggest that the essential oils of these plants could be used as a source of natural anti-proliferative, antioxidant and antibacterial agents.

1. Introduction

In recent years, there has been great interest on the biological activity of essential oils as cosmetic, pharmaceutical and food processing industries seek natural alternatives [1,2]. Essential oils, which are complex mixtures of substances, are considered as valuable natural source of bioactive molecules that can be of therapeutic benefit in the treatment of various diseases [3,4]. In Sudan, essential oils have a rich history of use as a source of food, medicine and for cosmetic applications. For example, aerial part of *Cymbopogon schoenanthus* (*C. schoenanthus*) L. Spreng (Poaceae) is used to treat gout, prostate inflammation, kidney diseases and for stomach pains [5]. Olibanum of *Boswellia papyrifera* (*B. papyrifera*) (Del.) Hochst. (Burseraceae) is widely used as incense at home and for the treatment of cough and respiratory infections [6]. Aerial part of *Croton zambesicus* (*C. zambesicus*) Mull-Arg (Euphorbiaceae) is used to treat constipation, malaria and cough [7]. Rhizome of *Cyperus rotundus* (*C. rotundus*) L (Cyperaceae) is used to treat stomach disorders, bowels irritation, dyspepsia, diarrhoea,

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dysentery, ascites, cholera, ulcers, sores and fevers, as an anthelmintic, to cure wounds and for scorpion stings [8].

Data from literature showed that essential oils contain a large variety of substances with great potential as valuable source of bioactive molecules. They exhibited many biological effects such as antibacterial [9], antifungal [10], antiviral [11], anti-leishmanial [12], antioxidant [2] and anti-proliferative properties [13]. However, the literature survey revealed that limited works were undertaken on chemical composition and/or biological activities of aromatic plants growing in the Sudan. Thus, the aim of the present study was to determine the chemical constituents and evaluate the anti-proliferative, antioxidant and antibacterial activities of the essential oil extracted from olibanum of *B. papyrifera*, leafy stems of *C. schoenanthus* and *C. zambesicus* and rhizome of *C. rotundus* grown in Sudan.

2. Materials and methods

2.1. Plant material

Plant samples were collected from West Kordofan on January 2015. Botanical identification and authentication were performed and voucher specimens (No. 13/BP for *B. papyrifera*, No.13/CS for *C. schoenanthus*, No. 13/CZ for *C. zambesicus* and No. 13/CR for *C. rotundus*) have been deposited in Botany Department Herbarium, Faculty of Science, University of Khartoum, Sudan.

2.2. Preparation of essential oils

Essential oils from all the plant species (500 g) were extracted by hydrodistillation using a Clevenger-type apparatus for two to 4 h. The extracted oils were dried over anhydrous sodium sulphate and stored at 4 °C, in amber-coloured bottles, before use.

2.3. Gas chromatography/mass spectrometry (GC–MS) analysis

Analysis of the chemical composition of the essential oils were performed by gas chromatography coupled to mass spectrometry (Model GC-MS-QP2010 Plus, Shimadzu, Japan) equipped with a Rtx-5MS capillary column (5% diphenyl–95% dimethylsilicone, 30.00 m × 0.25 mm × 0.25 m). The oven temperature was programmed from 45 °C for 1 min and then increased at a rate of 3 °C min^{-1} to 300 °C, and held isothermally for 5 min. Helium was used as the carrier gas (with a flow rate of 1 mL min^{-1}). The detection was performed in the full scan mode, with a mass range of 50–650 *m/z*. Electron impact ionisation was employed with collision energy of 70 eV and the mass spectrometer ion source was maintained at 240 °C.

The volatile compounds were identified by matching mass spectra with spectra of reference compounds present in the National Institute of Standards and Technology (NIST 08) mass spectral library and by comparison of its retention index (RI) relative to C_{10}—C_{24} *n*-alkanes [14]. The relative amounts of individual components were expressed as percent peak areas relative to the total peak area.

2.4. Cell viability assay

2.4.1. Cell culture

Anti-proliferative activities of respective essential oils were evaluated with four cell lines established from human breast carcinoma samples (MCF7 and MDA-MB231) and from human colon adenocarcinoma samples (HT29 and HCT116). HCT116 and HT29 cells were cultivated in Dulbecco's minimum essential medium (DMEM, Eurobio, Courtaboeuf, France) supplemented with 10% (v/v) foetal calf serum (Eurobio), 1% Penicillin/streptomycin (Eurobio) and 2 mM L-glutamine (Eurobio). MCF7 and MDA-MB231 cells were grown in RPMI medium with the same additives. Cells were routinely seeded at 100 000 cells/mL and maintained weekly in a humidified atmosphere of 5% CO_2 at 37 °C.

2.4.2. MTT procedure

Cell viability assay was performed using the thiazolyl blue tetrazolium bromide (MTT) procedure as described by Mosman [15]. In brief, cancer cells were seeded in 96-well plate at 10 000 cells/well for HT29, MCF-7 and MDA-MB231 cells, at 5 000 cells/well for HCT116 cells (Greiner-Bio-One GmbH, Friekenhanusen, Germany). Twenty four hours after seeding, 100 μL of medium containing increasing concentrations of each essential oil (final concentration range from 0.5 to 400.0 μg/mL) were added to each well for 72 h at 37 °C. Essential oils were firstly diluted in DMSO at 50 mg (w/v)/mL or 200 mg (w/v)/mL. After incubation, the medium was discarded and 100 μL/well of MTT solution (0.5 mg/mL diluted in DMEM or RPMI medium) were added and incubated for 2 h. Water-insoluble formazan blue crystals were finally dissolved in DMSO. Each plate was read at 570 nm. IC_{50} was calculated using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Data are expressed as IC_{50} ± SD obtained from quadruplicate determinations of two independent experiments (n = 8).

2.5. Antioxidant activity assay

The antioxidant activity of essential oils was estimated by diphenyl 2 pycrilhydrazil (DPPH) assay adapted in 96-well plate [16]. Ascorbic acid was used as an antioxidant molecule reference (concentration range from 1 to 20 μg/mL). Each sample (starting from concentration at 200 mg/mL) was diluted in DMSO ($^1/_2$ to $^1/_{64}$) and tested. After 30 min incubation in the dark at room temperature, plates were read at 515 nm. Every analysis was done in triplicate. Antioxidant EC_{50} was calculated after the establishment of inhibition curve as a function of sample concentration. For each diluted sample, inhibition of DPPH oxidation was calculated using the formula:

$$\text{Inhibition(expressed in percentage)} = \left[1 - \left(\text{absorbance}_{\text{diluted sample}} / \text{absorbance}_{\text{control}}\right)\right] \times 100.$$

2.6. Antibacterial activity assay

2.6.1. Microorganisms

Standard strains of microorganism, obtained from Medicinal and Aromatic Institute of Research, National Research Center, Khartoum, were used in this study. The bacterial species used were the Gram-negative *Escherichia coli* (*E. coli*) (ATCC

25922) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC27853), and Gram-positive *Bacillus subtilis* (*B. subtilis*) (ATCC 6633) and *Staphylococcus aureus* (*S. aureus*) (ATCC 25923).

2.6.2. Minimum inhibitory concentration (MIC) assay

The two-fold serial microdilution method described by Eloff [17] was used to determine the MIC values for the essential oils against bacteria growth. All dilutions were prepared under aseptic conditions. A volume of 100 µL of the oil (1 mg/mL) dissolved in DMSO (5%) in duplicate was serially diluted two-fold with sterile distilled water and 100 µL of bacterial culture in MH broth, corresponding to 10^6 CFU/mL, was added to each well. Gentamicin and amoxicillin were used as positive controls and DMSO as negative control. Plates were incubated overnight at 37 °C. Afterwards, 40 µl of 0.2 mg/mL of *p*-iodonitrotetrazolium violet (INT) was added to each well to indicate microbial growth. The colourless salt of tetrazolium acts as an electron acceptor and is reduced to a red coloured formazan product by biologically active organisms. The solution in wells remains clear or shows a marked decrease in intensity of colour after incubation with INT at the concentration where bacterial growth is inhibited. Plates were further incubated at 37 °C for 2 h and the MIC was determined as the lowest concentration inhibiting microbial growth, indicated by a decrease in the intensity of the red colour of the formazan. The experiment was performed in triplicate.

3. Results

3.1. GC/MS profiles of essential oils

3.1.1. B. papyrifera

The yield of volatile oil of olibanum of *B. papyrifera* was 3% (w/w) on dry weight basis. The oil was pale yellow with agreable perfumery odour. A total of 16 compounds were identified comprised 99.30% of the essential oil (Tables 1 and 2). The essential oil was dominated by the presence of alcohol and ester derivatives (46.82%) followed by monoterpenes (16.30%), sesquiterpenes (27.70%) diterpenes (5.08%) and fatty acids which represent 5.51% of the total oil. Octyl acetate was the most prominent compound found in highest concentration (22.69%) followed by nerolidol-epoxyacetate (18.83%), 1-octanol

Table 2
Chemical composition of essential oil extracted from *B. papyrifera* olibanum.

Peak No	RI	Compound	Area (%)
1	948	α-Pinene	3.05
2	986	β-Myrcene	1.06
3	1 027	L-Limonene	7.11
4	1 038	β-Ocimene (Z)-	2.80
5	1 075	1-Octanol	16.16
6	1 104	Linalool	2.28
7	1 211	Octyl acetate	22.69
8	1 336	Bicycloelemene	1.16
9	1 395	β-Elemene	4.27
10	1 545	Elemol	0.79
11	1 607	(4E)-1,5,9-Trimethyl-1-vinyl-4,8-decadienyl formate	7.97
12	1 638	Nerolidol-epoxyacetate	18.83
13	1 969	n-Hexadecanoic acid	1.06
14	2 014	Heptadecene-(8)-carbonic acid-(1)	0.54
15	2 136	Elaidinsaeure	4.45
16	2 146	Verticiol	5.08

Extraction of essential oil and identification of volatile chemical compounds were performed as described in the materials and methods section.

Table 3
Chemical composition of essential oil extracted from *C. zambesicus* leafy stems.

No.	RI	Compound	Area (%)
1	920	α-Thujene	1.55
2	935	α-Pinene	3.79
3	973	Sabinene	1.13
4	979	β-Pinene	1.53
5	1 009	α-Phellandrene	2.59
6	1 028	Cymene	13.80
7	1 038	1,8-Cineole	27.07
8	1 099	L-Linalool	5.77
9	1 181	4-Terpineol	3.98
10	1 189	p-Cymen-8-ol	1.76
11	1 190	α-Terpineol	6.87
12	1 379	α-Copaene	3.06
13	1 388	β-Bourbonene	3.12
14	1 443	α-Guaiene	4.80
15	1 574	Spathulenol	4.64
16	1 969	Palmitic acid	1.43
17	2 139	Oleic Acid	3.31
18	2 177	Stearic acid	2.95

Extraction of essential oil and identification of volatile chemical compounds were performed as described in the materials and methods section.

Table 1
Composition of chemical compound families identified in essential oil.

Chemical compound	Relative percentage			
	B. papyrifera	*C. zambesicus*	*C. schoenanthus*	*C. rotundus*
	Olibanum	Leafy stems	Leafy stems	Rhizomes
Monoterpene hydrocarbons	14.02	24.39	1.60	2.99
Oxygenated monoterpenes	2.28	45.45	19.84	14.33
Sesquiterpenes hydrocarbons	5.43	10.98	11.02	5.49
Oxygenated sesquiterpenes	19.62	4.64	60.57	38.77
Diterpenes	5.08	–	0.14	2.81
Alcohol and ester derivatives	46.82	–	0.36	0.38
Fatty acids	5.51	7.69	–	7.25
Others	0.54	–	0.03	18.28
Total	99.30	93.15	93.56	90.30
Extraction yield	3.00	0.28	2.10	2.60

Extraction of essential oil and identification of volatile chemical compounds were carried out as described in the material and methods section. Results were expressed as percentage relative to total volatile chemical compounds extracted from respective plant.

(16.16%), (4E)-1,5,9-trimethyl-1-vinyl-4,8-decadienyl formate (7.97%) and L-limonene (7.11%) respectively.

3.1.2. C. zambesicus

The yield of the light yellowish essential oil of the leafy stems of *C. zambesicus* was 0.28% (w/w). In total, 18 components were identified representing 93.15% of the total oil composition and results are presented in Tables 1 and 3. The oil was dominated with monoterpenes (69.84%) where oxygenated monoterpenes represented by 45.45%. Predominance of hydrocarbons (10.98%) over oxygenated (4.64%) derivatives

Table 4

Chemical composition of essential oil extracted from *C. schoenanthus* leafy stems.

No.	RI	Compound	Area (%)
1	904	(+)-4-Carene	0.05
2	935	α-Pinene	0.01
3	951	Camphene	0.01
4	995	β-Myrcene	0.02
5	998	(+)-2-Carene	0.88
6	1 009	α-Phellandrene	0.10
7	1 028	p-Cymene	0.35
8	1 063	γ-Terpinene	0.23
9	1 080	L-Fenchone	0.04
10	1 169	2-Piperidineethanamine	0.03
11	1 183	Octyl acetate	0.36
12	1 184	endo-Borneol	0.03
13	1 188	α-Terpineol	0.56
14	1 210	trans-Piperitol	0.03
15	1 225	Dihydrocarveol	0.12
16	1 236	o-Mentha-1(7),8-dien-3-ol	0.24
17	1 244	Piperitone	18.48
18	1 260	Carvacrol	0.14
19	1 289	Thymol	0.20
20	1 373	Cyclosativene	0.37
21	1 379	α-Ylangene	0.04
22	1 385	Elemene	0.08
23	1 410	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	0.15
24	1 439	γ-Elemene	0.07
25	1 442	α-Guaiene	0.90
26	1 448	Humulene	0.09
27	1 456	Alloaromadendrene	0.57
28	1 458	Bicyclo(5,3,o)decane,2-methylene-5-(-1-methylvinyl)-8-methyl	3.56
29	1 460	Santalene	0.15
30	1 470	Aristolochene	0.06
31	1 477	γ-Muurolene	0.42
32	1 495	α-Silenene	0.54
33	1 502	Cuparene	1.25
34	1 504	α-Muurolene	0.08
35	1 507	Chamigrene	0.33
36	1 519	7-epi-α-selinene	2.04
37	1 548	Elemol	18.33
38	1 550	Selina-3,7(11)-diene	1.94
39	1 557	α-Calacorene	0.08
40	1 558	Dihydro-beta-agarofuran	0.17
41	1 599	Selina-6-en-4-ol	0.12
42	1 625	γ-Eudesmol	5.80
43	1 649	Agarospirol	0.24
44	1 652	α-Eudesmol	10.69
45	1 673	Bulnesol	7.08
46	1 682	tau-Cadinol	1.00
47	1 703	Eudesm-7(11)-en-4-ol	17.09
48	1 708	Farnesol	0.05
49	2 944	Andrographolide	0.14

Extraction of essential oil and identification of volatile chemical compounds were performed as described in the materials and methods section.

Table 5

Chemical composition of essential oil extracted from *C. rotundus* rhizomes.

No.	RI	Compound	Area (%)
1	945	α-Pinene	0.98
2	958	Camphene	0.04
3	973	β-Pinene	0.83
4	988	β-Myrcene	0.02
5	998	2-Carene	0.23
6	1 026	p-Cymene	0.76
7	1 028	Limonene	0.13
8	1 039	1,8-cineole	0.15
9	1 069	Octanol	0.03
10	1 092	Linalool	0.05
11	1 111	Camphenol,6-	0.03
12	1 118	β-Fenchol	0.12
13	1 146	Verbenol	0.17
14	1 157	Sabina ketone	0.03
15	1 164	Pinocarvone	0.26
16	1 168	Borneol	0.14
17	1 177	Terpinen-4-ol	0.35
18	1 182	1-Octyl acetate	0.35
19	1 190	α-Terpineol	1.00
20	1 191	Myrtenol	1.53
21	1 209	Verbenone	2.16
22	1 226	cis-Carveol	0.27
23	1 244	Carvone	0.14
24	1 245	Piperitone	7.37
25	1 292	Thymol	0.21
26	1 303	Carvacrol	0.35
27	1 353	Longipinene	0.11
28	1 354	α-Cubebene	1.11
29	1 376	α-Copaene	0.73
30	1 409	α-Gurjunene	0.07
31	1 410	Caryophyllene	0.11
32	1 441	α-Guaiene	0.20
33	1 454	Rotudene	0.20
34	1 456	Alloaromadendrene oxide-(1)	8.47
35	1 458	Alloaromadendrene	0.45
36	1 459	Humulene	0.13
37	1 460	Patechoulene	0.49
38	1 474	β-H,5 α-Eremophila	0.51
39	1 485	1,5-cycloundecadien, 8,8-dimethyl-1-9-methylene	0.68
40	1 489	β-Selinene	0.22
41	1 490	Guaia-1(10),11-diene	0.07
42	1 491	Tertacyclo(6,3,2,o(2,5), o(1,8)tridecan-9-ol	2.44
43	1 499	α-Muurolene	0.15
44	1 509	Cuparene	0.33
45	1 542	β-Elemol	7.14
46	1 544	Selina-3,7(11)-diene	3.67
47	1 563	Ledol	0.61
48	1 568	Palustrol	1.53
49	1 587	Caryophyllene oxide	0.13
50	1 592	Isoaromadendrene epoxide	3.26
51	1 626	γ-Eudesmol	0.83
52	1 648	Longiverbenone	4.30
53	1 649	Selina-6-en-4-ol	7.00
54	1 695	2,5,9-trimethylcycloundeca-4, 8-dienone	13.44
55	2 291	Androstan-17-one,3ethyl3-hydroxy-,(5-α)	2.31
56	2 308	Methyl (Z)-5,11,14,17-eicosatetraenoate	7.25
57	2 400	4,8,13-Duvatriene-1,3-diol	2.81
58	3 395	tricyclo(20,8,o,o(7,16) tricontane,1(22),7(16)diepoxy	1.85

Extraction of essential oil and identification of volatile chemical compounds were performed as described in the materials and methods section.

Table 6
Anti-proliferative activity of essential oils.

Essential oil	IC_{50} value (μg/mL)			
	HT29	HCT116	MCF7	MDA-MB231
B. papyrifera (olibanum)	34.80 ± 7.89	71.50 ± 9.24	50.35 ± 4.75	48.65 ± 2.10
C. zambesicus (leafy stem)	23.81 ± 4.53	25.40 ± 8.12	41.37 ± 6.55	53.77 ± 1.68
C. schoenanthus (leafy stem)	23.41 ± 8.22	19.16 ± 2.73	38.43 ± 4.48	41.30 ± 1.99
C. rotundus (rhizome)	28.81 ± 3.38	21.33 ± 4.19	41.28 ± 5.98	44.31 ± 2.03

Cell viability assay was performed as described in the biological methods section. Values were representative of quadruplicate determinations of two independent experiments (n = 8).

stands for the sesquiterpene fraction. The composition of the oil was marked by the presence of 1,8-cineole (27.07%) followed by cymene (13.80%), α-terpineol (6.87%) and L-linalool (5.77%).

3.1.3. C. schoenanthus

The dried leafy stems of *C. schoenanthus* yielded 2.1% (w/w) light yellowish oil. 49 components were identified representing 93.03% of the total oil composition (Tables 1 and 4). The proportion of sesquiterpenes (71.59%) was higher than that of monoterpenes (21.44%). The oil revealed the presence of monoterpenes (1.6%), oxygenated monoterpenes (19.84%), sesquiterpenes (11.02%), oxygenated and sesquiterpenes (60.57%) and other components as, ester and diterpene, were also detected at trace levels. The major components were the monoterpene piperitone (18.48%) followed by the sesquiterpenes elemol (18.33%), eudesm-11-en-1-ol (17.09%), α-eudesmol (10. 69%), bulnesol (7.08%) and γ-eudesmol (5.80%), respectively.

3.1.4. C. rotundus

Hydrodistillation of the dried rhizomes of *C. rotundus* yielded 2.6% (w/w) pale yellowish oil. A total of 58 components were identified representing 90.30% of the total oil composition (Tables 1 and 5). The oil was characterised by larger amounts of oxygenated sesquiterpenes (38.77%) and monoterperenes (14.33%). Hydrocarbons sesquiterpenes and monoterpenes represented 5.49% and 2.99% respectively. The major constituent of the oil was 2,5,9-trimethylcycloundeca-4,8-dienone (13.44%) followed alloaromadendrene oxide-(1) (8.47%), piperitone (7.37%), β-elemol (7.14%), selina-6-en-4-ol (7%) and longiverbenone (4.30%) respectively.

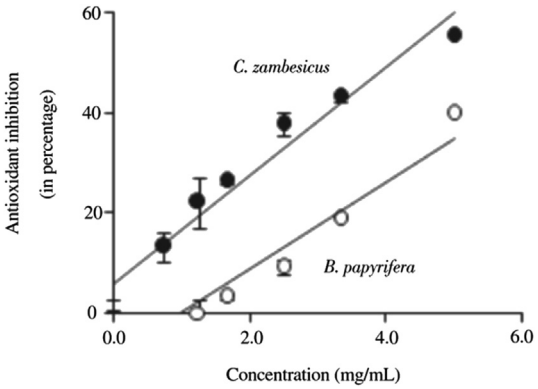

Figure 1. Antioxidant inhibition curve established *for C. zambesicus and B. papyrifera* essential oils. Antioxidant activity was determined as described in the biological methods section. Each point corresponds to mean ± SD of three determinations.

3.2. Biological activity

3.2.1. Cell viability assay

Essential oils of studied species were tested, *in vitro*, for their potential anti-proliferative activity against HT29, HCT116, MCF7 and MDA-MB231 cell lines (Table 6). *C. schoenanthus, C. zambesicus* and *C. rotundus* essential oils exhibited interesting anti-proliferative activity against HT29 and HCT116 cell lines with IC_{50} values in the range of 19.9–28.8 μg/mL. *B. papyrifera* essential oil was active against HT29 cell line but with cell viability activities higher than 30 μg/mL. IC_{50} values were estimated at 38.40–50.35 μg/mL when MCF7 cells were tested and at 41.30–53.77 μg/mL against MDA-MB231 cell line.

3.2.2. Antioxidant activity

The potential antioxidant activity of the oils was determined according to the basis of scavenging activity of the stable free radical DPPH. Only essential oils of *C. zambsicus* and *B. papyrifera* possessed DPPH free radical scavenging activity with EC_{50} estimated at (4.20 ± 0.19) and (5.90 ± 0.17) mg/mL while those *C. rotundus* and *C. schoenanthus* were inactive (Figure 1). The value EC_{50} of ascorbic acid was (5.37 ± 0.44) μg/mL.

3.2.3. Antibacterial activity

Results of MIC for the four essential oils against *E. coli, P. aeruginosa, B. subtilis* and *S. aureus* are presented in Table 7. *E. coli* was inhibited by *B. papyrifera, C. zambesicus* and *C. schoenanthus* at 16 μg/mL and by *C. rotundus* at 32 μg/mL. *P. aeruginosa* was less susceptible to the oils, it was inhibited by *C. rotundus* and *C. zambesicus* at 126 and 250 μg/mL respectively. *C. zambesicus* and *C. schoenanthus* displayed antibacterial activity against *B. subtilis* and *S. aureus* with MIC value of 16 μg/mL while *C. rotundus* and *B. papyrifera* inhibited the

Table 7
Antibacterial activity of essential oils of olibanum of *B. papyrifera*, leafy stems of *C. schoenanthus* and *C. zambesicus* and rhizome of *C. rotundus*.

Essential oil	MIC (μg/mL)			
	E.coli	*P. aeruginosa*	*B. subtilis*	*S.aureus*
B. papyrifera	16	>1000	250	32
C. zambesicus	16	250	16	16
C. schoenanthus	16	>1000	16	16
C. rotundus	32	126	32	16
Gentamicin	4	4	–	–
Amoxicillin	–	–	4	4

Results were obtained as described in the biological methods section. Gentamicin and amoxicillin were used as antibiotic references.

former at 32 and 250 μg/mL respectively and the latter at 16 and 32 μg/mL respectively.

4. Discussion

The chemical profile of olibanum of *B. papyrifera* in the present study was closer to that reported for *B. papyrifera* from Ethiopia [18]. Moreover, octyl acetate was the dominant compound but in lower concentration than those reported for samples collected in other countries [18–20]. Interestingly, the diterpene verticiol which represents 5.08% of the total oil has only been previously reported for *Boswellia elongate* collected from different locations in Yemen [21]. It could also be seen that some notable compounds such as incensole, incensyl acetate and geraniol, that were characteristics of *B. papyrifera*, were not identified in the present study.

Generally, hydrocarbons monoterpene presented the main fraction of most *Croton* species oils than their oxygenated counterparts and are marked with a high presence of β-caryophyllene and/or α/β-pinene as major constituents [22]. In this study the oil of leafy stems of *C. zambesicus* was dominated with oxygenated monoterpenes. The composition of the oil was marked by the presence of 1,8-cineole and cymene and the absence of borneol which was found to be in high amount in samples from Saudi Arabian and Cameroon [23,24]. Of note, 1,8-cineole was reported previously as the major compound from the essential oil of *C. sakamaliensis* stem from Madagascar [22].

Essential oil of leafy stems of *C. schoenanthus* was characterized by higher proportion of sesquiterpenes, however, samples collected from other countries like Burkina Faso [25], Togo [26], Tunisia [27], Algeria [28] and Brazil [29] showed that the proportion of monoterpenes was higher than that of sesquiterpenes. Moreover, the chemical composition of sample in this study was closer to those obtained from Burkina Faso and Togo species with piperitone (42%–61%) as the major oil compound [25,26,30–32].

The nature and proportion of compounds that constitute the essential oil of *C. rotundus* rhizomes in this study are not the same as those reported in previous studies; the rhizome oils of this plant from different countries showed compositional differences and four chemotypes (H-, K-, M- O-types), of the essential oils from different parts of Asia have been reported [33]. These four types besides oils of Brazilian [34], Germany [35], Hawaii [36,37], Nigerian [38] and Tunisian [39,40] species, were characterised by the presence of α-cyperone, cyperotundone, cyperene, cyperol, cyperotundone which were not detected in the present work or by others on rhizomes samples from the Sudan [41].

It was clear that oils obtained from these aromatic plants growing in Sudan showed quantitative and qualitative differences. As reported earlier by several researchers, the plant organ that the oil was extracted from, time of harvest, geographical and environmental factors were the key factors influencing the chemical composition, quality and quantity of the plant essential oil and could probably contribute to create a unique and spectacular chemical composition [22,42].

The ranking order of the investigated plants essential oils on the basis of their anti-proliferative activity against HT29 cell line (in terms of IC_{50} value) was *C. schoenanthus* > *C. zambesicus* > *C. rotundus* > *B. papyrifera*. Their ranking order on the basis of their antiproliferative activity against HCT116 and MCF7 cell lines was *C. schoenanthus* > *C. rotundus* > *C. zambesicus* > *B. papyrifera*. Thus, it was clear that *C. schoenanthus* revealed the best anti-proliferative activity. Terpenes and their derivatives were shown to possess anti-proliferative and chemopreventive activities in various models [43]. For example, caryophyllene oxide, α-cadinol, α-humulene, α-pinene, β-pinene, α-phellandrene, D-limonene, linalool and trans-caryophyllene were previously reported to exhibit moderate to strong anti-proliferative effects against these or different tumour cell lines [43,44]. Moreover, although the major components reflect the biological activities of essential oils, many authors suggested that the bioactivity of an essential oil is hardly due to a single active compound, but it is rather ascribed to a synergic activity of different kinds of chemicals that may not be necessarily the most abundant [1,45].

Essential oils of *C. zambesicus* and *B. papyrifera* possessed interesting DPPH free radical scavenging activity. Generally, Brazilian *Croton* species; *Croton zenthmeri, Croton nepetaefolius* and *Croton argyrophylloides* exhibited good antioxidant activities [46]. Essential oil of stem bark of *Croton lechleri* was reported to show antioxidant property with IC_{50} (DPPH) at (15.28 ± 0.94) mg/mL [47]. Antioxidant activity of olibanum of *Boswellia* species was previously evaluated for *Boswellia socotrana, Boswellia elongate, Boswellia dioscorides* where *Boswellia socotrana* exhibited high radical scavenging effect [21,48].

All oil samples inhibited both Gram positive and Gram negative bacteria. According to Salvat *et al.* [49], plant extracts with MIC's less than/or around 500 μg/mL indicate good antibacterial activity. Thus, it could be suggested that the oils samples in this study showed good antibacterial activity against all tested bacteria except *B. Papyrifera* and *C. Schoenanthus* which were less activity against *P. aeruginosa*. Furthermore, oxygenated monoterpenes were reported to be responsible for the antimicrobial activity of several essential oils [50].

Our findings show for the first time that the essential oils extracted from *C. schoenanthus, C. zambesicus* and *C. rotundusm* with their complex mixtures of volatile substances, presented anti-proliferative property against several human cancer cell lines as demonstrated by cell viability assay. Moreover, they showed good antibacterial activity. Furthermore, essential oils of *C. zambesicus* and *B. papyrifera* possessed DPPH free radical scavenging activity. Thus, these results demonstrated the potential of these oils to exert beneficial anti-proliferative and antibacterial effect and could be a natural source for antioxidant agents. Further studies are needed to isolate active compounds of these essential oils and to investigate in depth their modes of action.

Conflict of interest statement

We declare that we have no conflict of interest.

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