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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