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Journal of Acute Medicine 3 (2013) 138–141

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Original Research

Essential oil from the stem bark of *Cordia sebestena* scavenges free radicals

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Received 22 May 2013; accepted 18 July 2013
Available online 7 September 2013

Abstract

Background: Essential oils have been reported to possess various medicinal properties in folkloric medical practices. Their application in modern medicine has also increased recently.
Materials and methods: The chemical composition of the essential oil from the stem bark of *Cordia sebestena* obtained by hydrodistillation was determined using gas chromatography–mass spectrometry and analyzed for its free radical scavenging potential using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.
Results: A total of nineteen compounds were identified with the major compounds being 9-octadecene (E) (20.26%), 5-octadecene (E) (18.68%), 9-eicosene (13.99%), cyclopropane, nonyl (12.42%), 3-eicosene (E) (7.29%), phenol, 2,4-bis(1,1-dimethylethyl) (4.71%), 1-nonadecene (3.17%), 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (2.70%), and 2,6-diisopropylnaphthalene (2.17%). The DPPH radical scavenging potential of the oil was higher than the standard, butylated hydroxyanisole, with IC_{50} of 2.00 ± 0.31 μg/mL and 47.00 ± 1.27 μg/mL, respectively. At 50 μg/mL, the antioxidant potential of the butylated hydroxyanisole was 75% whereas the oil had 82% free radical scavenging activity. Several hydrocarbons contained in the essential oil may have contributed to the aromatic and antioxidant properties of the plant. The hydrocarbons could be useful for chemotaxonomic characterization of *Cordia sebestena*.
Conclusion: The essential oil may be further explored for its potential as an antioxidant contributor in food and phytotherapeutic medicine.

Keywords: Antioxidant; Butylated hydroxyanisole; *Cordia sebestena*; Essential oil; Gas chromatography–mass spectrometry

1. Introduction

Volatile oils have characteristic fragrances and tastes and are completely volatilized at room temperature. Volatile oils are mixtures of known and partially unknown compounds such as hydrocarbons, and contain terpene alcohols, aldehydes, ketones, phenols, and esters.[1] The essential oils containing these volatiles have found applications in food and pharmaceutical industries.[2] Essential oils have been reported to possess various medicinal properties in folkloric medical practices. The antimicrobial activity of essential oils and their applications in the food system have been investigated.[3–6] Essential oils from plant sources have recently gained more attention in the production of perfumes, aromatic soaps, fragrant lotions, and cosmetics as well as pharmaceuticals.

Cordia sebestena, also called Geiger tree, is a species of a flowering plant belonging to the family Boraginaceae and native to the American tropics where it grows up to 30 m high, bearing green or white scenty fruits of about 7.5 cm.[7,8] The plant is grown as an ornamental tree in Nigeria (Fig. 1) where its medicinal importance is unclear. The chemical compositions of the petroleum ether and ethyl acetate extracts of the flowers have been reported.[9,10] The dyeing potential of the flower have also been evaluated.[11] Bioassay guided fractionation of the ethyl acetate extract of the fruit of the plant have led to the isolation of sebestinoids A–D, which exhibit a moderate inhibition of aspartic protease.[12] However, the chemical composition and free radical scavenging activity of essential oils of *C. sebestena* stem bark have not been studied

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2211-5587/$ - see front matter
http://dx.doi.org/10.1016/j.jacme.2013.07.002

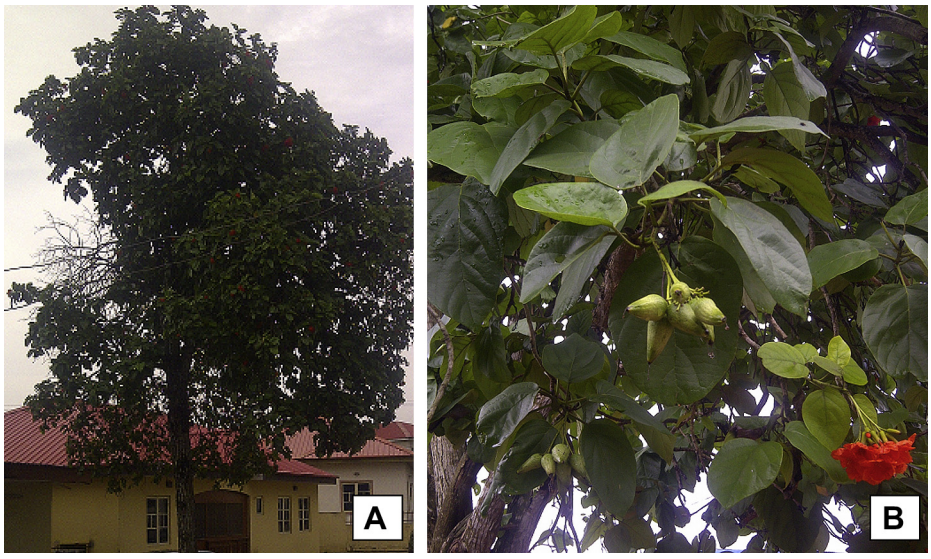

Fig. 1. (A) *Cordia sebestena* as an ornamental tree; (B) *C. sebestena* in fruiting season in Nigeria.

to date. The present study focused on the qualitative and quantitative determination of the chemical profile of the essential oil from *C. sebestena* stem bark obtained via hydrodistillation, and evaluation of the free radical scavenging activities of the oil.

2. Materials and methods

2.1. Plant material

Stem bark of *C. sebestena* was obtained from the tree in November 2012 from the premises of the Redeemer's University, Mowe, Ogun State, Nigeria. Taxonomic identification of the plant was performed by a botanist at the Herbarium of the Botany Department of the University of Lagos, Lagos, Nigeria, where the voucher specimen (LUH 5551) was deposited.

2.2. Hydrodistillation

The wet plant sample (2000 g) was subjected to hydrodistillation for 5 hours, using a Clevenger-type apparatus, according to the European Pharmacopoeia.[13] The volatile distillate was collected over anhydrous sodium sulfate, stored in an air-tight glass vial with screw lid and refrigerated at 4°C until the time of analysis. The yield of the oil was 0.2 g.

2.3. Free radical scavenging potential of the essential oil

The free radical scavenging activity of the essential oil was examined *in vitro* using the standard 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. This spectrophotometric assay was carried out according to the method previously described.[14,15] The DPPH free radical was prepared at a 0.1mM concentration in methanol and protected from light after preparation. Stock solutions of the oil (1 mg/mL) was prepared and diluted to final concentrations of 500 μL/mL, 250 μL/mL, 200 μL/mL, 100 μL/mL, and 50 μL/mL in methanol. A 1 mL aliquot of 0.1mM DPPH methanol solution was added to solutions of the sample as well as the standard (butylated hydroxyanisole, BHA) and incubated for 30 minutes in the dark. The absorbance was determined at 518 nm. A blank experiment was also carried out to determine the absorbance of DPPH prior to interacting with the sample. The antioxidant activity, AA was calculated using the equation given below:

$$\%AA = 100 \times \left[\left(Abs_{control} - Abs_{sample}\right)\right] / \left(Abs_{control}\right) \quad (1)$$

The IC_{50} was determined on GraphPad Prism 3 software (San Diego, USA) through a nonregression analysis. The IC_{50} was taken as the concentration that scavenged 50% of the radicals.

2.4. Gas chromatography–mass spectrometry analysis

The gas chromatography (GC)–mass spectrometry (MS) analyses were realized using Agilent Technology 7890A (USA) gas chromatograph equipped with a fused silica capillary column HP-5MS (30 m × 0.32 m, 0.5 μm film thickness) on ultrapure helium gas and coupled to a mass selective detector (mass spectrometer). The injector and interface were operated at 250°C and 380°C, respectively. The oven temperature was raised from 60°C to 300°C at a heating rate of 5°C/min and then held isothermally at that temperature. The sample was injected in a splitless mode. The MS was operated at an ionization voltage of 70 eV over an acquisition mass range. The constituents of the essential oil were identified based on their linear retention indices and comparing their MS spectral with data obtained from the National Institute of Standard and Technology, USA (2008). The relative proportions of the constituents were percentages from the GC peak areas without any corrections.

3. Results and discussion

Qualitative composition and relative abundances of the compounds in the essential oil from the stem bark of *C. sebestena* are presented in Table 1. A total of 19 compounds were identified with the major compounds being 9-octadecene (E) (20.26%), 5-octadecene (E) (18.68%), 9-eicosene (13.99%), cyclopropane, nonyl (12.42%), 3-eicosene (E) (7.29%), phenol, 2,4-bis(1,1-dimethylethyl) (4.71%), 1-nonadecene (3.17%), 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (2.70%), and 2,6-diisopropylnaphthalene (2.17%). Others are below 2%. Aliphatic hydrocarbons accounted for 72.73%, cyclic hydrocarbons for 13.89%, aromatic hydrocarbon for 4.71%, and oxygenated aliphatic compounds for 3.78% (Fig. 2). Cyclic oxygenated compound was (2.7%) whereas the least was aromatic oxygenated compound (2.17%).

3.1. Result of the free radical scavenging activity

The free radical scavenging activity (antioxidant potential) of the essential oil was examined using the DPPH assay. This is a spectrophotometric assay that uses a stable DPPH radical as a reagent.[16] The hydrogen atoms or electron-donating ability of the corresponding essential oil and butylated hydroxyanisole (as a positive control) was determined from the bleaching of purple-colored methanol solution of the DPPH.[17] The IC_{50} of the oil and BHA are shown in Table 2 and its free radical scavenging potential is shown in Fig. 3. The spread over the range of concentration described in the experimental section is depicted in Fig. 4. As shown in Fig. 3, the essential oil from *C. sebestena* constantly had higher antioxidant potential even at very low concentrations compared to the standard, BHA. The result indicated that the essential oil is more potent as an antioxidant than BHA. It is assumed that the essential oil has compounds that work in synergy to produce the pronounced antioxidant effects.

Hydrocarbons are the major class of compounds identified in the essential oil of the plant. Apart from terpenoids, aliphatic hydrocarbons such as undecane, tridecane, and pentadecane are commonly situated on the surface of plants.[18] For example, 7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione has been detected in mango skin.[19] Phenol, 2,4-bis(1,1-

Table 1
Qualitative and quantitative profile of the essential oil obtained from the stem bark of *Cordia sebestena*.

S/N	Compounds	RT	%Yield
1	Cyclododecane	11.819	1.47
2	Cyclopropane, nonyl	17.232	12.42
3	Oxalic acid, allyl undecyl ester	17.398	0.71
4	5-Tetradecene (E)	17.529	1.22
5	3-Tetradecene	17.769	1.02
6	Phenol, 2,4-bis(1,1-dimethylethyl)	20.556	4.71
7	9-Octadecene (E)	22.095	20.26
8	7-Hexadecene (Z)	22.336	1.73
9	3-Hexadecene	22.564	1.27
10	2,6-Diisopropylnaphthalene	24.893	2.17
11	5-Octadecene (E)	26.438	19.91
12	3-Octadecene (E)	26.650	1.89
13	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	28.973	2.70
14	9-Eicosene	30.369	13.99
15	1-Eicosene	30.793	0.98
16	n-Heptadecanol-1	32.092	1.46
17	n-Nonadecanol-1	32.137	1.61
18	3-Eicosene (E)	33.980	7.29
19	1-Nonadecene	37.310	3.17

RT = retention time; S/N = serial number.

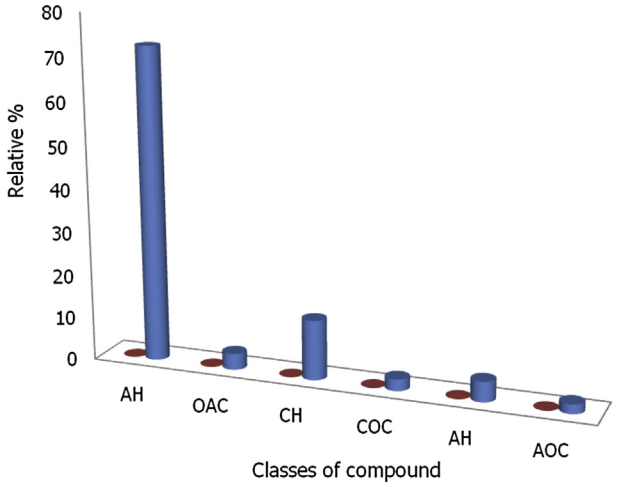

Fig. 2. Relative percentages of different classes of compounds obtained from the essential oil. AH = aliphatic hydrocarbons; AH = aromatic hydrocarbon; AOC = aromatic oxygenated compound; CH = cyclic hydrocarbons; COC = cyclic oxygenated compound; OAC = oxygenated aliphatic compounds.

Table 2
1,1-diphenyl-2-picrylhydrazyl antioxidant assay.

Samples	IC_{50} (μg/mL)
Cordia sebestena oil	2.00 ± 0.31
BHA	47.00 ± 1.27

Data are presented as mean ± SD.
BHA = butylated hydroxyanisole; IC_{50} = 50% inhibition concentration. Results represent mean ± standard error of the mean of duplicate determinations.

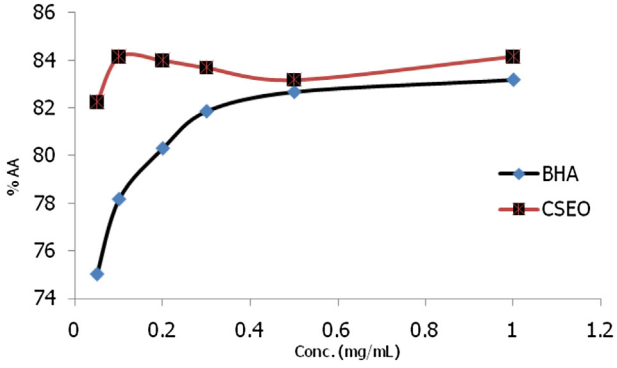

Fig. 3. Antioxidant capacity of BHA and essential oil. %AA= percentage antioxidant activity; BHA = butylated hydroxyanisole; CSEO = *Cordia sebestina* essential oil.

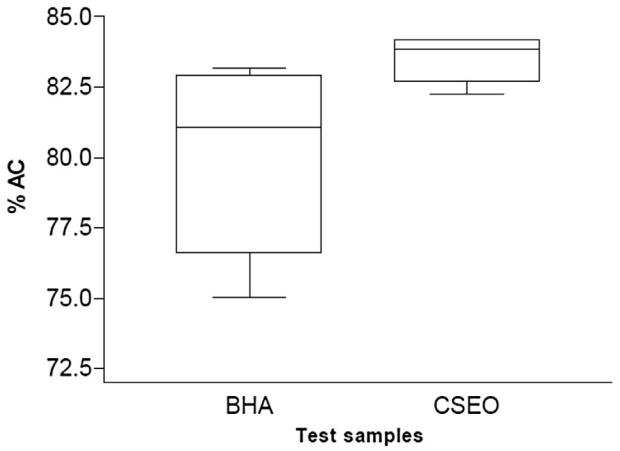

Fig. 4. Box diagram indicating the spread of the percentage antioxidant capacity of BHA and essential oil with standard error of the mean. % AC = percentage antioxidant capacity; BHA = butylated hydroxyanisole; CSEO = *Cordia sebestina* essential oil.

dimethylethyl) has also been identified in the mycelium of *Tuber borchii* Vitt. and of the volatile constituent of chorizo de Pamplona.[20,21] Similarly, hydrocarbons such as undecane, tridecane, docosane, and docosene have been detected in the volatile constituent of *Moringa oleifera* leaves.[4]

In conclusion, *C. sebestena* stem bark essential oil obtained through hydrodistillation has been analyzed using GC-MS and a total of 19 compounds are identified. The major classes of compounds identified are aliphatic hydrocarbons (72.73%) and cyclic hydrocarbons (13.89%). The essential oil showed promising antioxidant potential in the *in vitro* analysis. The established antioxidant properties of *C. sebestena* stem bark oil thus broaden the scope for its utilization in medicine as well as in the food industry. The essential oil showed promising antioxidant potential suggesting that *C. sebestena* could be exploited further for utilization in medicine and the food industry.

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