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Antioxidant and chemical properties of essential oil extracted from blend of selected spices

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ABSTRACT

Objective: To investigate the chemical properties of essential oil extracted from blends of selected Nigerian spices as well as its antioxidant protective potentials against free radical *in vitro*.

Methods: Essential oil was extracted from selected spices blend consisting of *Monodora myristica, Myristica fragrans, Tetrapleura tetraptera*, and *Aframomum sceptrum* using a Clevenger type apparatus. Oil obtained was subjected to phytochemical and gas chromatography-mass spectrometer analysis as well as analyzed for antioxidant activity which covers for 1,1-diphenyl-2 picrylhydrazyl, nitric oxide scavenging activities and reducing property.

Results: Gas chromatography-mass spectrometer analysis revealed over 50 compoundfs with α-phellandrene being the most predominant compound (27.32%), which was followed by (-)-β-bourbonene (15.78%) and 5-(1-methylethyl)-α-phellandrene (11.80%). Phytochemical analysis showed high flavonoid content and a lower phenolic content. The oil showed a dose like dependent effect on the1-diphenyl-2 picrylhydrazyl and nitric oxide scavenging activities, these activities increased with increasing concentration. The same was also observed for the reducing power properties of the oil.

Conclusions: The antioxidant activities exhibited by the essential oil *in vitro* signify its protective potential against free radicals. The chemical constituents, α-phellandrene in particular and the studied phytochemicals may be responsible for these effects. However, *in vivo* study is needed to further authenticate this potency.

1. Introduction

Increasing interest in plant-based antioxidants has led to widespread screening of plants for the development and utilization of antioxidants of natural origin[1,2]. These antioxidants protect the body against the adverse effects of free radical or reactive oxygen species (ROS) generation involve in various related physiological processes and diseases such as aging, cancer and atherosclerosis[3].

Spices have been recognized for their nutritional and medicinal properties. They play major role in the food and flavor industry owing to their aroma, colour and taste. The chemopreventive and bioprotectant properties of spices against cancer cells have been reported. They have also been reported to possess strong antimicrobial and antioxidant activity and may slow down other serious brain diseases like multiple sclerosis and Alzheimer's disease[4].

Attraction for effective alternatives to synthetic drugs has led to the growth and development of several natural therapeutic products from plant sources. Of such products are the essential oils. Characterized by a strong odour, essential oils are natural, complex compounds composed of lipophilic and highly volatile secondary plant metabolites such as aldehydes, amines, esters, ethers, ketones, terpenes, and thiols[5-7]. They have been reported to constitute effective alternatives in nutritional, pharmaceutical, and agricultural fields due to reported antimicrobial, antiviral, nematicidal, antifungal, insecticidal, and antioxidant properties[8,9].

This paper aims to report the chemical properties of essential oil extracted from blends of selected Nigerian spices as well as

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investigate its antioxidant protective potentials against free radical *in vitro*.

2. Materials and methods

2.1. Plant material

Selected spices consisting of *Monodora myristica, Myristica fragrans, Tetrapleura tetraptera*, and *Aframomum sceptrum* were purchased from a local market in Benin City, Nigeria. They were dehulled and air-dried at room temperature. After which, they were grounded to fine powder, using a laboratory mill. The powders were mixed in the ratio of 1:1:1:1 and stored in air-tight containers for laboratory analysis.

2.2. Extraction of essential oil

The blended sample was subjected to hydrodistillation for 3 h using a Clevenger type apparatus. Oil obtained was dried over sodium sulphate over night, filtered, and then stored at -2 ℃ until further analysis.

2.3. Gas chromatography-mass spectrometry (GC-MS) instrumentation

2.3.1. GC

Agilent 6890N gas chromatograph, flame ionization detector at 220, N_2 at 1.0 mL/min, ZB-5 HT capillary column (30 m × 0.53 mm, inner diameter 0.32 mm), split ratio 1:30 injector temperature of column 260 ℃, temperature of column maintained at 70 ℃ for 3 min and then raised to 235 ℃ (5 ℃/min) followed by 5 min at 260 ℃.

2.3.2. GC-MS

Hewlett Packard 6890 gas chromatograph combined with a Jeol JMS-HX 110 mass spectrometer with source at 270 ℃ at 70 eV. Injector was set at 270 ℃ with splitting ratio 1:30. A mass spectral survey was performed using the National Institute of Standards and Technology mass spectral program 2008. The concentrations of the identified compounds were calculated using area normalization over flame ionization detector response method.

2.4. Determination of total phenol and flavonoid contents

Total phenolic and flavonoid compound contents were determined by the Folin-Ciocalteau and colorimetric aluminium chloride methods respectively[10].

2.5. Determination of reducing property

The reducing property of the essential oil was determined by assessing the ability of the sample extracts to reduce $FeCl_3$ solution using gallic acid as reference[11].

2.6. Free radical scavenging assay

The free radical scavenging ability of the essential oil against 1,1-diphenyl-2 picrylhydrazyl (DPPH) free radical was evaluated using gallic acid as reference[12].

2.7. Nitric oxide (NO) radical scavenging assay

This was carried out according to the method described by Govindarajan *et al.*[13].

2.8. Statistical analysis

To address the biological variability, each set of experiments was repeated at least three times. Differences between the groups were analyzed by One-way ANOVA with the aid of SPSS software version 17. The P-values < 0.05 were considered statistically significant for differences in mean using the least of significance difference, and data were reported as mean ± SD.

3. Results

Over 50 compounds were identified after GC-MS analysis of the oil as showed in Table 1 and Figure 1. Alpha-phellandrene was observed to be the most predominant compound (27.32%), which was followed by (-)-β-bourbonene (15.78%) and 5-(1-methylethyl)-α-phellandrene (11.80%). Traces of fatty acids consisting of 9-octadecenoic acid, hexadecenoic acid, oleic acid and 9,17-octadecadienal were also observed.

Table 1
Identified compounds of essential oil extracted from blends of selected spices.

Peak No.	Retention time (min)	Area (%)	Compounds
1	3.99	3.27	1R-α-pinene
2	5.12	0.54	Beta-pinene
4	5.59	27.32	Alpha-phellandrene
6	6.06	11.80	5-(1-methylethyl)-α-Phellandrene
7	8.16	0.52	1,6-octadien-3-ol,3,7-dimethyl-
8	11.06	0.44	L-α-terpineol
14	15.11	0.97	3-Methyl-4-isopropylphenol
16	15.96	0.77	Beta-ocimene
19	18.14	1.42	1,3,6,10-Dodecatetraene,3,7,11-trimethyl-,(Z,E)-
20	18.66	0.82	4,4-Dimethyl-non-5-enal
32	23.17	15.78	(-)-β-bourbonene
36	25.26	0.52	Alpha-Cadinol
38	27.61	2.36	Benzenamine,2,4-dimethoxy-
42	33.46	4.36	1,1'-biphenyl,2-methyl-
45	36.50	0.25	9-octadecenoic acid (Z)-,2,3-dihydroxypropyl ester
46	36.68	0.32	Hexadecenoic acid, Z-11-
47	37.67	0.21	Oleic acid
50	38.84	0.49	9,17-Octadecadienal, (Z)-

Phytochemical analysis revealed a high flavonoid content and

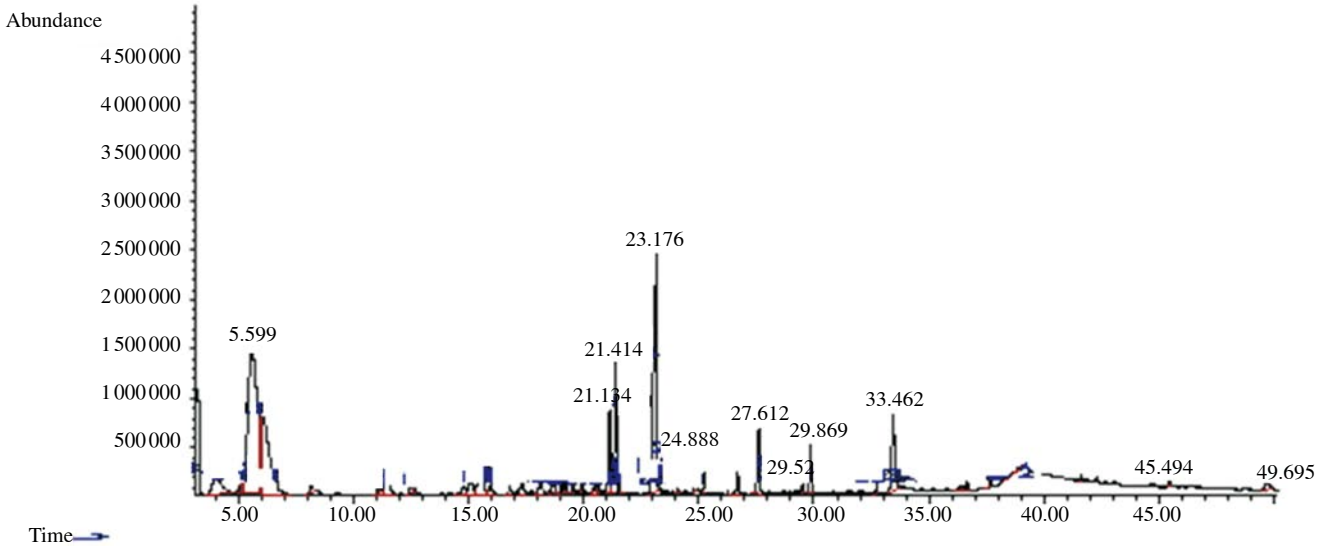

Figure 1. GC-MS spectra of identified compounds of essential oil extracted oil from blends of selected spices.

lower phenolic content (Figure 2).

A dose like dependent effect was observed in the free radical scavenging properties of the oil as portrayed by the DPPH and NO scavenging activities in Figures 3 and 4 respectively. The scavenging activities increased with increasing concentration but were rather low compared to ascorbic acid. The same was also observed in the reducing properties of the oil as depicted in Figure 5.

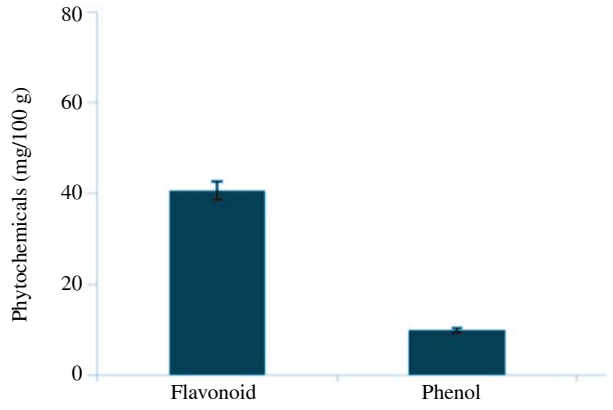

Figure 2. Phytochemicals of essential oil extracted from blends of selected spices.

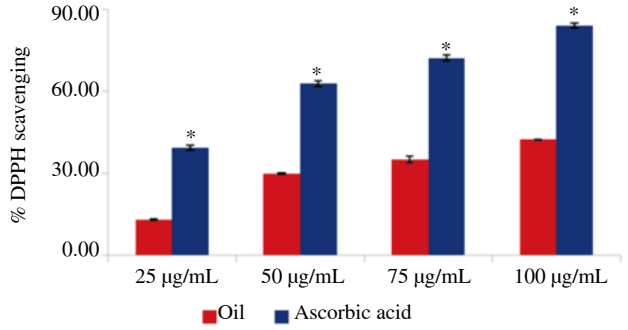

Figure 3. DPPH scavenging activity of essential oil extracted from blends of selected spices.

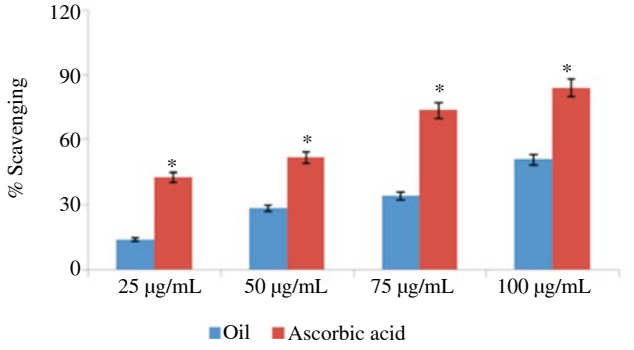

Figure 4. NO scavenging activity of essential oil extracted from blends of selected spices.

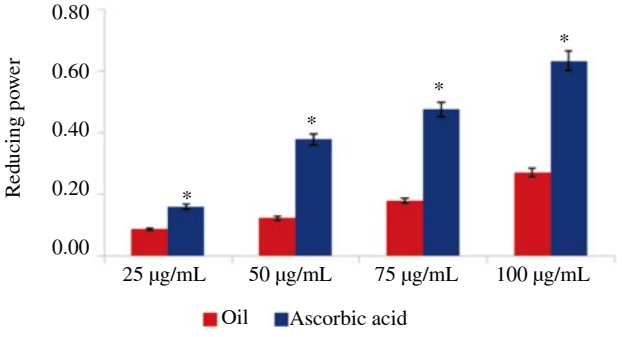

Figure 5. Reducing power activity of essential oil extracted from blends of selected spices.

4. Discussion

Essential oils are natural products composed mainly of terpenes and terpene-derivatives in addition to some other non-terpene components[8]. These components have been reported to have medicinal properties and also responsible for the medicinal properties of essential oils. In this study, the antioxidant properties of essential oil from blends of selected spices were investigated as well as its chemical properties.

Alpha-phellandrene (5-isopropyl-2-methyl-1,3-cyclohexadiene)

has been described as an active compound and constituent of most essential oils[14]. In this study, α-phellandrene and its derivative (5-(1-methylethyl)-α-phellandrene) were observed to be the most prominent of the essential oil constituents. Alpha-phellandrene has been reported to increase p-p53, p-H2A.X, 14-3-3-σ , and MDC1 protein expression in murine leukemia cells[15]. Lin *et al*. further reported the promotion of immune responses in normal mice by α-phellandrene via enhancement of macrophage phagocytosis and natural killer cell activities[15]. The observed high concentration of α-phellandrene in the extracted essential oil, is therefore of tremendous health benefits. It may be responsible for the observed antioxidant activities.

The protective properties of antioxidants against ROS have been reported in several studies. The role of phenols and flavonoids as powerful antioxidants is well documented[16]. Flavones and catechins were reported to be the most powerful flavonoids with the highest antioxidant potency[17]. The antioxidant activity of phenolics has been attributed to their redox activities allowing them act as reducing agents, metal chelators and free radical quenchers[18]. The appreciable amount observed in the essential oil is of tremendous health benefits.

The observed scavenging activities of the essential oil against DPPH and NO respectively may be attributed to the observed phytochemicals and chemical constituents. This activity connotes an antioxidant protective potential of the essential oil against oxidative stress. Oxidative stress has been implicated in several degenerative diseases and ailments[19]. It sets in when there is an imbalance between oxidants (ROS) and antioxidants in favour of the oxidants. Several studies have reported the antioxidant potency of essential oils and this has attributed to the chemical constituents[4]. The observed reducing property of the oil also reflects its antioxidant activity.

The antioxidant activities exhibited by the essential oil *in vitro* signify a protective potential against free radicals. The chemical constituents, α-phellandrene in particular and the studied phytochemicals may be responsible for these effects. An *in vivo* study is however, needed to further authenticate the potency.

Conflict of interest statement

We declare that we have no conflict of interest.

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