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Chemical profiling and DPPH radical scavenging activity of fruit essential oil of *Eucalyptus camaldulensis* Dehnh. native to north-central, Nigeria

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Abstract: This study investigated the chemical constituents and antioxidant activity of the volatile oil from the fruit of *Eucalyptus camaldulensis.* Pulverized fruits (500 g) of the plant afforded 0.58% (w/w) of essential oil after hydro-distillation. The chemical profile of the oil was characterized by GC-MS, which revealed twenty-one (21) compounds accounting for 99.4% of the total composition. The principal components of the oil included: β-ocimene, α-pinene, β-pinene, 2-carene, limonene, and β-caryophyllene. The essential oil scavenged DPPH radical with an IC_{50} value of 9.86±0.02 µL/mL, showing activity comparable to that of ascorbic acid, which was used as a reference compound. Therefore, the oil may serve as a natural antioxidant for oxidative stress management and its complications, pending clinical validation.

Keywords: Fruit volatile oil; DPPH radical; β-ocimene; α- pinene; *Eucalyptus camaldulensis*

Introduction

Eucalyptus is a native of Australia and one of the genera of the Myrtaceae family that consists of over 800 species and subspecies.[1] Being a family of aromatic plants, it is a major source of essential oils with antifungal, anti-inflammatory, and antibacterial effects.[2] The most often considered species include *Eucalyptus globulus*, *Eucalyptus camaldulensis, Eucalyptus terticornis* and *Eucalyptus citriodora*.[3,4,5] In this present work, we focused on the volatile oil obtained from the fruits of *E. camaldulensis* growing in north-central Nigeria

E. camaldulensis is an important ethno-medicinal plant. Extracts from its aerial parts have been used traditionally for the treatment of sore throat and other bacterial infections of the respiratory tracts, diarrhea, dysentery, healing of wounds and fungal infections. [6,7] The leaves, fruits and aerial parts of the plant possess volatile oils, and the phytochemical composition of the oils has been reported. The ethereal oils from the leaves and fruits of the plant from Cyprus contained *p*-cymene, eucalyptol, α-pinene, and α-terpineol as major compounds. However, *p*-cymene was the chemotype of the leaf oil , while that of the fruit oil was eucalyptol.[1] Similarly, *p*-cymene was also the chemotype of the volatile leaf oil of *E. camaldulensis* from Palestine, while aromadendrene was present in appreciable quantities.[8]

Ebadollahi and Setzer[9] characterized the ethereal leaf oil of *E. camaldulensis* from USA. The oil contained *p*-cymene as the principal component. Cryptone and spathulenol were also identified in detectable amounts in the oil. Meanwhile, the trunk bark ethereal oil of the Tunisian-grown *E. calmadulensis* analyzed using GC-FID and GC-MS was chiefly constituted by δ-8,9-dehydro-4-hydroxythymoldimethyl ether, modephen-8-β-ol, and thymohydroquinone methyl ether.[10] The leaf volatile oils isolated from saline and non-saline vegetation in Pakistan were analyzed as 1,8-cineole chemotypes. Other major phytochemicals in the oils were ledol, α-pinene, *t*-pinocarveole and γ-terpinene.[11]

The phytochemicals of the seed, aerial part and leaf essential oils of *E. camaldulensis* of south-western and northern Nigerian origin have been documented.[12,13,14] Meanwhile, as far as we know, there is no reported work on the phytochemical profiling and antioxidant activity of fruit essential oil of *E. camaldulensis* from north-central Nigeria. We therefore investigated the phytoconstituents and antioxidant activity of

the fruit volatile oil of Nigerian (north-central) grown *E. camaldulensis*.

Materials and methods

Plant sample

Fresh fruits of *E. camaldulensis* were harvested in Ilorin-South, Kwara State, Nigeria. The sample was taken to the Herbarium of the Plant Biology Department, University of Ilorin, for identification. Thereafter, voucher specimens (UILH/006/0097) were deposited.

Isolation of essential oil

Five hundred grams of fruits of *E. camaldulensis* were blended and hydrodistilled for 4 hours using Clevenger apparatus following British Pharmacopia specifications.[15] The extracted oil was refrigerated at 4^{o}C until further analyses.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Characterization of the ethereal oil was carried out using GC equipment (Hewlett-Packard ITP5890A). The equipment was interfaced with a 70-250 S double-focusing VG analytical mass spectrometer (433 HP-5). Helium was the carrier gas, and the flow rate was 1.2 mL/min. The conditions of operation of the MS were: transfer line temperature of 300 ^{o}C; ion source of 250 ^{o}C and an ionization voltage of 70 eV. The GC was fitted to a 25 m × 0.25 mm fixed silica capillary column coated with phenyl methyl siloxane at a split ratio of 1:50 and a 0.25 µm film thickness. An online desktop computer equipped with a disk memory was used to process the acquired MS data. The GC peak areas were used to obtain the percentage composition of the phytochemicals in the oil. The chemical components were identified by: (i) the use of straight chain alkanes (C_7–C_{30}, Supelco Bellefonte, PA, US) co-injection with the standards under identical experimental conditions to calculate the retention indices; (ii) comparison of the retention indices with the available data in NIST 08 and Wiley 275 libraries and; (iii) comparing the fragmentation pattern in the mass spectra of each constituent with the data obtained from NIST 08 and Wiley 275 libraries.[16,17,18]

Antioxidant study

Spectroscopic technique was used to examine the essential oil's antioxidant activity as reported by Ilhami.[19] This was based on the tendency of the oil to scavenge the free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH).[19] The procedure involves mixing 1.5 mL of 90 µM DPPH in a 95% methanolic solution with 1.5 mL of the volatile oil at a concentration range of 1.5 - 48 µL/mL. The mixture was incubated for an hour. The control (Ascorbic acid) was separately tested for antioxidant activity using the same procedure. The absorbances of blank and the resulting solutions were recorded. A spectrophotometer (at 520 nm) was used to monitor the disappearance of DPPH radicals. Scavenging (%) of DPPH free radical by the essential oil was estimated accordingly:

$$\textbf{Scavenging}\,(\%) = \frac{\boldsymbol{B - A}}{\boldsymbol{A}}\ \boldsymbol{X}\ \ \mathbf{100}$$

Where B is the absorbance of the control solution (containing all reagents except the oil), while A is the absorbance of the mixture containing the oil. The 50% radical scavenging concentration of the volatile oil was estimated from the graph of percentage inhibition against the oils' concentration.[13]

Statistical analysis

The antioxidant test was conducted in triplicate and the mean values were obtained. Standard deviation (SD) was calculated, and the mean ± SD (n = 3) was used to express the various biochemical parameters, which were then compared with a one-way analysis of variance (ANOVA) test and Dunnett multiple comparison test with equal sample size test. Statistically, values with $p<0.05$ were considered statistically significant. The IC_{50} values were calculated by non-linear regression analysis from the mean values. SPSS for Windows version 10 was used to assess the statistics.

Results and discussion

The yield of essential oil isolated from the fruits of *E. camaldulensis* was 0.58% (w/w). The yield was lower than that which was afforded by the fruits of the Turkish-grown *E. camaldulensis*.[1] Environmental factors such as soil conditions affect plants' physiology and subsequently influence the number of secretory cells in plant organs. The lower oil yield was due to the lower number of secretory cells as a result of unfavourable environmental factors in the north-central Nigeria compared to that of Turkey. The retention indices, identities and percentage composition of phytochemicals of essential oil from the fruit of *E. camaldulensis* are shown in Table 1.

Twenty-one (21) compounds represents 99.4% of the oil extracted. Hydrocarbon monoisoprenoids constituted 77.1%; oxygenated monoisoprenoids constituted 5.2% ; hydrocarbon and oxygenated sesquiisoprenoids were found in the percentages of 15.0% and 2.1%, respectively.

The principal compounds in the oil were: β-ocimene (29.0%), β-pinene (24.7%), 2-carene (10.5%), limonene (10.5%), and β-caryophyllene (6.7%). Linalool (2.2%), copaene (2.5%), α-terpineol (2.2%), ϒ-elemene (2.0%), δ-cardinene (1.4%), alloaromadendrene (1.0%), globulol (1.0%), and terpinen-4-ol (0.8%) were detected in moderate quantities. Isoprenoids that were identified in minor quantities were: cubenol (0.4%), α-cubebene (0.3%), *p*-cymene (0.4%) and 2-bornene (0.4%). The GC chromatogram is presented in Supplementary Data 1. The oil was of β-ocimene and β-pinene chemotype since the compounds were present in the highest percentages among other isoprenoids in the oil. In the earlier report, a mixed chemotype of eucalyptol and *p*-cymene was documented for the essential oil from the fruit of the plant that was indigenous to Mersin, Turkey.[1] The disparity in the oils' chemotypes could be linked to the variance in environmental factors of each of the locations of the plant.

The synthases of mono- and sesquiterpenoids that were present in the highest percentages in a volatile oil are well known as the enzymes that catalyze the chemical transformation of their respective precursors to isoprenoids through carbocationic intermediates.[20,21] The categories and quantities of the terpenoid synthesized relied on the activity of the enzymes, which is determined by the climatic conditions of the location of the plant.[22] β-Ocimene and caryophyllene synthases were responsible for the formation of the detected terpenoids in the fruit volatile oil of *E. camaldulensis* since both isoprenoids were present in the highest quantities in the oil. The biosynthesis of the isoprenoids is presented as Schemes 1 and 2 in the supplementary data 2. Scheme 1 showed the β-ocimene synthase catalyzed ionization of neryl pyrophosphate **(1)** to neryl cations **(2)** in the fruit of the plant. *Cis*-linalyl **(3)** cation was formed by isomerization of neryl carbocation **(2)**. Deprotonation of the intermediate cation **(3)** at C_3 formed β-ocimene **(4)** in the fruit. Linalool **(5)** was formed after the cation **(3)** was hydrated. Electrophilic addition of the cation **(3)** to the double bonds at C_6-C_7 gave α-terpinyl cation **(6)**. The ion **(6)** was hydrated to form α-terpineol **(7)**. The synthase of β-ocimene aided the loss of a proton by the cation **(6)** at C_8 to give D-limonene **(8)** in the fruits of the plant. Folding of the α-terpinyl cation which, subsequently attack the C_2-C_3 double bond by electrophilic addition at C_2 and C_3, produced pinyl **(9)** and bornyl cations (**10**), respectively. Deprotonation of the cations **(9 and 10)** at C_{10} and C_1 formed β-pinene **(11)** and 2-bornene **(12)** in the fruits. Terpinyl-4-yl cation **(13)** was formed after the α-terpinenyl cation had undergone 6,7-hydride shift. Terpinene **(14)** and γ-terpinene **(15)** were formed when the terpinly-4-yl cation was deprotonated at C_1 and C_5. Protonation of α-terpinene and then subsequent loss of a proton at C_{10} gave β-terpinene **(16)** in the fruits of the plant. Hydration of the cation **(13)** produced terpinen-4-ol **(17)**. Abstraction of a proton at C_1 of the α-terpinyl cation and subsequent attack of the carbanion formed on the carbocation of the same intermediate ion formed 2-carene **(18)**.

In Scheme 2, the synthase of β-caryophyllene catalyzed the formation of E, Z-farnesyl **(20)** and E,E-farnesyl **(21)** cations from E,Z-farnesyl pyrophosphate **(18)** and E,E-farnesyl pyrophosphates **(19)**. Electrophilic addition of the carbocation **(21)** to the double bond of C_{10}-C_{11} via C_{11} formed humullyl carbonium ion **(22)**. The ion **(21)** lost a proton at C_9 to produce α-humullene **(23)**. Markovnikov's addition of the cation **(21)** towards the double bond of C_2-C_3 at C_2 gave caryophyllyl cation **(24),** and the ion was deprotonated at C_{15} to form β-caryophyllene **(25)** in the oil. The E,Z-farnesyl cation attacked the double bond of C_{10}-C_{11} by electrophilic addition at C_{10} to give E,Z-germacredienyl cation **(26)**. The carbocation **(26)** undergoes a series of hydride shifts and subsequently attacks the double bond of C_6-C_7 at C_6 to form cadinyl cation **(27)**. Loss of a proton by the cation formed δ-cadinene **(28)** in the oil. The cadinyl cation undergoes 1,2-hydride shift and then hydration to form cubenol **(29)**. E,E-germacredienyl cation **(30)** was formed after the E,E-farnesyl cation attacked the double bond of C_{10}-C_{11} at C_{10}. The cation **(30)** lost a proton at C_{12} to produce germacrene A **(31)**. Another intermediate cation **(32)** was formed when the germacrene A was protonated at the double bond of C_6-C_7 at C_7 of the compound **(31)**. The cation **(32)** undergoes electrophilic addition on the double bond of C_2-C_3 at C_2 to produce eudesmanyl cation **(33)**. The ion **(33)** lost a proton at C_4 to produce α-selinene **(34)** in the oil. Subsequent hydration of the double bond of C_{11}-C_{12} in the α-selinene gave γ-eudesmol **(35)**. The synthase of β-caryophyllene catalyzed the 1,2-hydride shift of the cation (**32**) to form another cationic intermediate carbocation **(36)**. Guaiyl cation (**37**) was formed when the intermediate ion **(36)** undergoes electrophilic addition on the double bond of C_2-C_3 pi bond at C_2 of the ion **(36)**. The cation **(37)** was deprotonated at C_2 to give α-guaiene **(38)** in the oil. Protonation of the double bond of C_{11}-C_{12} of α-guaiene resulted in the formation of a cationic intermediate (**39**). The cation (**39**) was deprotonated at C_1, followed by the electrophilic addition of the cation on the deprotonated carbon to form isoledene **(40)** in the oil. The

isoledene undergoes protonation and then a 6,7-hydride shift to form allo-aromdendryl cation **(41)**. The cation **(41)** later undergoes deprotonation at C_{15} to form aromadendrene **(42)** in the oil. The hydrocarbon **(42)** subsequently undergoes hydration to produce globulol **(43)**.

Table 1: Chemical composition (%) of essential oil from the fruit of *E. camaldulensis*

S/N	Compound	RI	% Composition
1	2-Bornene	932	0.4
2	**β-Pinene**	943	**24.7**
3	2-Carene	948	10.5
4	**β-Ocimene**	976	**29.0**
5	β-Terpinene	993	0.1
6	ϒ-Terpinene	998	1.5
7	Limonene	1018	10.5
8	p-Cymene	1042	0.4
9	Linalool	1082	2.2
10	Terpinen-4-ol	1137	0.8
11	α-Terpineol	1143	2.2
12	α-Copaene	1221	2.5
13	α-Cubebene	1344	0.3
14	Aromandendrene	1386	1
15	ϒ-Elemene	1431	2
16	δ-Cadinene	1469	1.4
17	β-Caryophyllene	1494	6.7
18	Globulol	1530	1
19	Humulene	1579	1.1
20	Cubenol	1580	0.4
21	ϒ-Eudesmol	1626	0.7
	Hydrocarbon monoisoprenoids		77.1
	Oxygenated monoisoprenoids		5.2
	Hydrocarbon sesquiisoprenoids		15
	Oxygenated sesquiisoprenoids		2.1
	Total Identified Phytochemicals (%)		**99.4**

Figure 1 shows the scavenging activity of DPPH radical by the volatile oil obtained from the fruits of *E. camaldulensis* and ascorbic acid, which was the reference sample. Steadily, theactivity of the essential oil increased from 1.5±0.01 – 48±0.03 µL / mL as presented in the figure. The 50% inhibitory concentration (IC_{50}) of the oil and that of ascorbic acid were 9.86±0.02 and 4.97±0.01 µL/mL, respectively. The IC_{50} values revealed that the radical scavenging ability of ascorbic acid was twice that of the essential oil. Essential oils that are rich in phenolic compounds and/or oxygenated monoterpenoids have been established to show antioxidant activities.[23] Thus, the activity of the ethereal oil from this work could be associated with α-terpineol, linalool and terpinen-4-ol that were identified in the oil. Therefore, the volatile oil could be used as a natural substitute to commercial synthetic antioxidants for the treatment and management of oxidative stress and its related health issues after pharmacological validation.

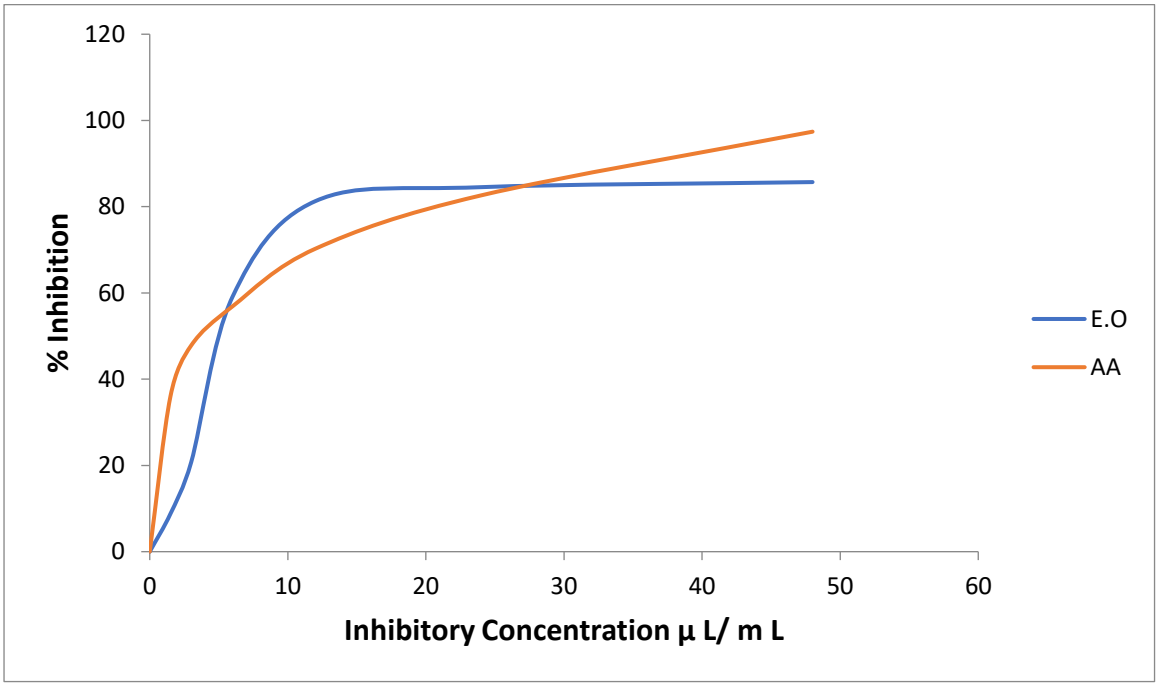

Figure 1: DPPH radical scavenging activity of essential oil (E.O) and ascorbic acid (AA) from the fruits of *E. camaldulensis*

Conclusion

This study is the first to report the constituents and antioxidant activity of essential oil from the fruits of a Nigerian (north-central) grown *E. camaldulensis* Dehnh. The oil contained β-ocimene, α-pinene, limonene, 2-carene, β-caryophyllene, and β-pinene as the major constituents. It also exhibited antioxidant activity and scavenged free DPPH radicals, and its antioxidant activity was linked to the presence of oxygenated compounds in the oil. Hence, the volatile oil may serve as a potential natural antioxidant pending further pharmacological validation.

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Conflict of interest

Authors declare no conflict of interest

Supplementary data

The chromatogram and reaction schemes are provided as supplementary data

Authors contributions

Conceptualization and Methodology: IRO, LAU, GTO; Methodology, Writing-review and Editing: IRO, ULA, GTO, OMA, IMM, TST.

The Authors have read and approved the manuscript.

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