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Corrigendum: Antioxidant assessment of characterised essential oils from Calophyllum inophyllum Linn using 2,2-diphenyl-1- picrylhydrazyl and hydrogen peroxide methods

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In the version of the article initially published, Ojah, E.O., Moronkola, D.O. & Osamudiamen, P.M., 2020, 'Antioxidant assessment of characterised essential oils from *Calophyllum inophyllum* Linn using 2,2-diphenyl-1- picrylhydrazyl and hydrogen peroxide methods', *Journal of Medicinal Plants for Economic Development* 4(1), a83. https://doi.org/10.4102/jomped.v4i1.83, a reference was omitted on pages 2, 4, 6, 7 and 9. The text is now updated as follows:

The last paragraph in the Introduction section on page 2 should read:

Calophyllum inophyllum Linn is the most abundant species in genus Calophyllum and is widespread in tropical areas, with a wide variety of uses ranging from traditional, medicinal and industrial applications (Dweck & Meadowst 2002). The extracted oil from the fruit of C. inophyllum Linn is used as a remedy for sciatica, shingles, neuritis, rheumatism, ulcers and skin diseases, whilst the seed oil is reported to have medicinal and healing properties. The plant's dried leaves and its decoction are widely used in curing rheumatism, skin infections, cuts and sores (Uma et al. 2012). Its leaf and stem bark extracts have shown anti-hyperglycaemic and anti-hyperlipidaemic activities, whilst the leaf extract was identified to inhibit OS (Varsha et al. 2016). Its fruits are effectively utilised in the treatment of dermatitis (Yu et al. 2016). The broad spectrum of biological activities exhibited by C. inophyllum may be associated with the chemical composition of its different parts (Figures 1-3). Ojah et al., reported the chemical constituents and toxicity levels of ten essential oils from this plant. GC-MS analysis of volatile constitituents from the plant revealed that the plant is furnished with non-toxic volatile constituents with promising biological activities (Ojah et al. 2019). This article was therefore designed to evaluate the antioxidant properties of gas chromatography-mass spectrophotometry (GC-MS) characterised EOs from 10 parts of C. inophyllum Linn using the generally reliable 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide models.

The first paragraph under the heading 'Essential oil composition of *C. inophyllum* Linn' in the Results and discussion section on page 4 should read:

The GC-MS characterisations of the leaf, leaf stalk, flower oil, pod, peel, stem wood, stem bark, root wood and root bark EOs extracted from *C. inophyllum* Linn showed a total of 102 compounds, which are mostly monoterpenes, sesquiterpenes and their oxygenated derivatives as shown in Table 2 (Ojah et al. 2019).

The footnote for Table 2a and Table 2b on page 6 should read:

Source: Ojah, E.O., Moronkola D.O., Riccardo P., Nzekoue F.K., Loredana C., Cristiano G., Marcel J. & Jioji N.T., 2019, 'Chemical Composition of ten Essential oils from *Calophyllum inophyllum* and their Toxicity against *Artemia salina*', European Journal of Pharmaceutical and Medical Research 6(12),185–194.

The third last paragraph under the heading 'Essential oil composition of *C. inophyllum* Linn' under the Results and discussion section on page 7 should read:

Stem bark oil contains nine compounds that make up 69.38% of it. This oil is rich in hexadecanal (46.80), E-anethole (6.12) and limonene (3.24). The oil is dominated by non-terpenes (60.90%) and monoterpenes (8.48%), whilst sesquiterpenes were absent. Root wood oil contains 51 compounds that make up 58.73% of it. This plant part is rich in non-terpenes (45.80%) and sesquiterpenes (12.83%), whilst monoterpenes were absent. This oil is rich in hexanedioic acid (9.86), E-nerolidol (5.83) and α -bisabolol (4.36). The oil also contains methyl eugenol, a phenylpropanoid. Root bark oil has 24 compounds that make up 74.66% of it, which are mainly with monoterpenes (44.01), diterpenes (15.05) and non-terpenes (14.46). This oil is rich in cembrene-3Z (15.05), limonene (13.93) and hexadecanal (10.61) (Ojah et al. 2019).

Note: DOI of original article: https://doi.org/10.4102/jomped.v4i1.83.



The last paragraph under the heading 'Essential oil composition of *C. inophyllum* Linn' under the Results and discussion section on page 7 should read:

The high content of γ -terpinene in leaf stalk (13.06%), seed coat (6.77%) and root bark (7.75%) oils of C. inophyllum Linn is responsible for the anti-inflammatory and antioxidant effects, thus supporting the plant's anti-osteoarthritic activity. The presence of Terpinene in Hyptis species inhibited gastric lesions, reduced volume and acidity of the gastric juice and increased gastric wall mucus (Marcelo, Rafael & Lucio 2015). Limonene, which is found in an appreciable amount in stem heartwood (23.79%), stem bark (3.24%) and root bark (13.93%) EOs of C. inophyllum Linn, is known to have sedative and stimulative effects in Lippia alba (Vale et al. 2002; Viana, Vale & Matos 2000). Consumption of diets containing fruits and vegetables rich in monoterpenes, such as limonene, is known to reduce the risk of developing cancer of the colon, mammary gland, liver, pancreas and lung. Limonene, which is known to possess high anticancer properties (Chistani et al. 2007; Marostica et al. 2009),

is abundant in *C. inophyllum* Linn: leaf stalk (25.40%), seed (25.40%) and root bark (13.93%) oils. The presence of phenylpropanoids, norisoprenoids and other non-ubiquitous compounds, such as β -alaskene, β -acoradiene and E-anethole, is a unique feature of oils from *C. inophyllum* Linn as shown in Table 2 (Ojah et al. 2019).

In the references list on page 8, the following reference should be added:

Ojah, E.O., Moronkola D.O., Riccardo P., Nzekoue F.K., Loredana C., Cristiano G., Marcel J. & Jioji N.T., 2019, 'Chemical Composition of ten Essential oils from *Calophyllum inophyllum* and their Toxicity against *Artemia salina'*, *European Journal of Pharmaceutical and Medical Research* 6(12),185–194.

This correction does not alter the study's findings of significance or overall interpretation of the study's results. The authors apologise for any inconvenience caused.





Antioxidant assessment of characterised essential oils from *Calophyllum inophyllum*Linn using 2,2-diphenyl-1-picrylhydrazyl and hydrogen peroxide methods



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Background: Oxidative stress is a multifactorial global health disorder that disrupts all levels of cell function. Therefore, therapeutic intervention using reliable, affordable and non-toxic natural sources is crucial.

Aim: The aim of this article was to determine the chemical constituents and antioxidant activity of 10 essential oils (EOs) from *Calophyllum inophyllum* Linn using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide (H₂O₂) methods.

Setting: Plant sample was collected at the Botanical Garden, University of Ibadan, Nigeria. Extractions and antioxidant assay were performed at the Organic Chemistry Research Laboratory, University of Ibadan, Nigeria. Gas chromatography-mass spectrophotometry (GC-MS) analysis was carried out at the School of Pharmacy, University of Camerino, Italy.

Methods: The chemical constituents were determined using GC-MS. The oils were extracted using an all-glass Clevenger-type apparatus and the antioxidant activity was determined using DPPH and hydrogen peroxide assays.

Results: A total of 102 compounds were identified in EOs from *C. inophyllum* Linn, which are mostly monoterpenes, sesquiterpenes and their oxygenated derivatives. The oils exhibited concentration-dependent activity with reference to standard synthetic antioxidants. Root wood had the highest antioxidant activity with the half maximal inhibitory concentration (IC $_{50}$) of 3.19 mg/mL compared to ascorbic acid (2.84 mg/mL) and butylated hydroxyl anisole (BHA) (2.97 mg/mL). In the $\mathrm{H_2O_2}$ antioxidant assay, root wood had the highest antioxidant activity with IC $_{50}$ of 2.78 mg/mL compared to ascorbic acid (2.20 mg/mL) and BHA (2.92 mg/mL).

Conclusion: The *in vitro* chemical compositional analysis of EOs from *C. inophyllum* Linn confirms the presence of compounds responsible for the antioxidant properties of the plant.

Keywords: *Calophyllum inophyllum*; essential oils; antioxidant; oxidative stress; hydrodistillation.

Introduction

Recently, researches on the antioxidant evaluation of volatile constituents from medicinal plants have increased geometrically as a result of gross increase in health disorders triggered by oxidative imbalance. This imbalance is caused by reactive oxygen species (ROS), which is because of the inability of antioxidants in the body to scavenge the effects of free radicals generated in the human system. Excess amount of ROS is deleterious because they can initiate biomolecular oxidative chain reactions (Bhaskara et al. 2015; Rattan 2006). When reactive radicals are generated in the body, the process disrupts all levels of cell function, resulting in oxidative stress (OS). Oxidative stress is associated with increased production of oxidising species or a significant decrease in the effectiveness of antioxidant defences. It can result in numerous diseases and disorders, such as ageing, cancers, rheumatoid arthritis and cardiovascular diseases (Saiket et al. 2010). Antioxidants are molecules that can safely react with free radicals and terminate the chain reaction before vital molecules are damaged (Ajiboye, Moronkola & Adesomoju 2017). These free radicals and ROS may oxidise nucleic acids, proteins, lipids or deoxyribonucleic acid (DNA) and can trigger several degenerative

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diseases, such as atherosclerosis, stroke, diabetes and cancer, in humans (Ushio-Fukai & Nakamura 2008). Antioxidants are believed to be prophylactic for the mentioned deleterious diseases. Human cells possess an inherent ROS scavenging mechanism, but this becomes inefficient and insufficient with age and under undue environmental stresses. Hence, dietary supplementation with synthetic antioxidants is necessary (Barros et al. 2011).

Plants have been utilised from time immemorial in the alternative and complementary treatment of several disease conditions, especially in developing economies like Nigeria where affordability and access to modern treatment is a major setback. Chemical constituents in medicinal plants possess several pharmacological potentials, which have been the focus of researches targeted at prospection of reliable, affordable and potent drugs (Mohammadhosseini et al. 2016; Nunes & Miguel 2017). These constituents could be found in plant extracts or essential oils (EOs) with great activity useful for several therapeutic applications (Camilo et al. 2017; Ganesan & Xu 2017; Pavunraj, Ramasubbu & Baskar 2017).

Calophyllum inophyllum Linn is the most abundant species in genus Calophyllum and is widespread in tropical areas, with a wide variety of uses ranging from traditional, medicinal and industrial applications (Dweck & Meadowst 2002). The extracted oil from the fruit of C. inophyllum Linn is used as a remedy for sciatica, shingles, neuritis, rheumatism, ulcers and skin diseases, whilst the seed oil is reported to have medicinal and healing properties. The plant's dried leaves and its decoction are widely used in curing rheumatism, skin infections, cuts and sores (Uma et al. 2012). Its leaf and stem bark extracts have shown anti-hyperglycaemic and antihyperlipidaemic activities, whilst the leaf extract was identified to inhibit OS (Varsha et al. 2016). Its fruits are effectively utilised in the treatment of dermatitis (Yu et al. 2016). The broad spectrum of biological activities exhibited by C. inophyllum may be associated with the chemical composition of its different parts (Figures 1-3). This article was therefore designed to evaluate the antioxidant properties of gas chromatography-mass spectrophotometry (GC-MS) characterised EOs from 10 parts of C. inophyllum Linn using the generally reliable 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide models.

Materials and methods

Materials

Plant material

Fresh samples of *C. inophyllum* Linn were collected from the Department of Botany, University of Ibadan, Ibadan, Oyo State, Nigeria. The samples were authenticated in the herbarium of the Department of Botany, University of Ibadan, Nigeria, where voucher samples were deposited with specimen voucher number UIH – 22659. The plant was sorted into 10 parts: leaf, stalk, flower, seed, pod, peel, stem wood, stem bark, root wood and root bark.



FIGURE 1: Fresh aerial part of Calophyllum inophyllum Linn.



FIGURE 2: Root wood of Calophyllum inophyllum Linn.



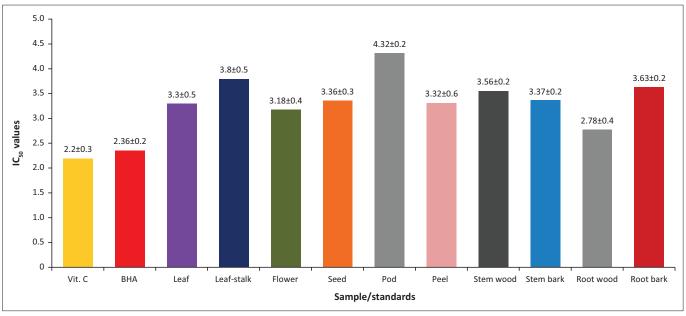
 $\textbf{FIGURE 3:} \ \textbf{Root bark of} \ \textit{Calophyllum inophyllum Linn}.$

Solvents and chemicals

Methanol (American Chemical Society [ACS] grade), hexane (ACS grade), butylated hydroxyl anisole (BHA), ascorbic acid, DPPH (95%), hydrogen peroxide (H₂O₂) distilled water, anhydrous sodium sulphate, sodium carbonate, deionized water (DI) and Whattman filter paper no. 3, 6-mm diameter were purchased from Sigma-Aldrich, Oakville, Ontario, Canada.

Extraction of essential oil

Each separated part (leaf, stalk, flower, seed, pod, peel, stem wood, stem bark, root wood and root bark) of *C. inophyllum* Linn was air-dried, pulverised and hydrodistilled for 3 h in an all-glass Clevenger-type apparatus designed according to British Pharmacopeia (BP) specifications (Figure 4). Essential oils were procured in 0.219% volume per weight (v/w) to 0.560% volume per weight (v/w) yields (Table 1).



BHA, butylated hydroxyl anisole.

Note: All the results are mean ± standard deviation (SD) where n = 3. The half maximal inhibitory concentration (IC_{so}) was obtained in mg/mL using non-linear regression analysis in Microsoft Excel. FIGURE 4: The half maximal inhibitory concentration values (mg/mL) for hydrogen peroxide inhibition of Calophyllum inophyllum.

 $\textbf{TABLE 1:} \ \ \textbf{Percentage (\%) yield of 10 essential oils from \textit{Calophyllum inophyllum}$

LIIVIN.					
S/N	Plant parts	% Yield	Colour	Odour	
1.	Leaf	0.333	Pale yellow	Leafy	
2.	Leaf stalk	0.313	Colourless	Herbal	
3.	Flower	0.288	Colourless	Floral	
4.	Seed	0.305	Cloudy white	Pleasant	
5.	Pod	0.506	Pale red	Nut-like	
6.	Peel	0.560	Pale yellow	Fruity	
7.	Stem wood	0.341	Pale yellow	Woody	
8.	Stem bark	0.307	Colourless	Nut-like	
9.	Root wood	0.219	Pale red	Woody	
10.	Root bark	0.279	Pale red	Nut-like	

S/N, serial number

The oils had a distinct characteristic smell. The EOs were refrigerated until the assay was carried out.

Identification of essential oils by gas chromatographymass spectrometry analyses

Gas chromatography-mass spectrometry analyses were carried out by using an Agilent 7890B-5977B GC-MS (Santa Clara, California, United States) system operating in the EI mode at 70 eV, using an HP-5MS capillary column (5% phenylmethyl polysiloxane, 30 m, 0.25 mm internal diameter (i.d.) and $0.1 \,\mu m$ film thickness) (Jenning and Walter Scientific, Folsom, California, United States), which was programmed with the following conditions: 60 °C for 4 minutes, then up to 4 °C/minutes to 160 °C, then 11 °C/min up to 280 °C, held for 15 min, and finally 15 °C/min up to 300 °C. The carrier gas was helium at a flow rate of 1.2 mL/min, the injector temperature was 280 °C whilst the transfer line temperature was 300 °C, the injection volume was 1 μ L, the split ratio was 1:100, the run time was 57 min and the acquisition mass range was 29 atomic mass unit (amu) - 400 amu. Identification of the EO components was based on their retention indices (experimentally determined using homologous series of

C8-C30 alkanes) and by comparison of their mass spectral fragmentation patterns in computer matching against library linear retention index and mass spectra taken from Adams and NIST 17 [25] FFNSC2 and MAGGI libraries (Adams 2007; FFNSC2 2012; NIST 17 2017). Relative peak area percentages were obtained by peak area normalisation without using correction factors and were the mean of the three determinations with an relative standard deviation (RSD%) in all cases below 10%.

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl assay: The free radical scavenging activity of EOs from C. inophyllum Linn was determined using the stable DPPH radical (Ebrahimzadeh & Bahramian 2009; Njenga & Mugo 2020). The dark purple colour of DPPH is lost when it is reduced to non-radicals by antioxidants and decreases in its absorbance when monitored at a characteristic wavelength of 517 nm. A 0.1 mM concentration of DPPH was prepared by dissolving 3.94 mg in 100 mL of methanol. An amount of 2 mg of the EO was dissolved in 2 mL of methanol to prepare a 1.0 mg/mL concentration of the EO, which was the stock solution. This stock solution was vortexed and serially diluted with methanol to obtain sample solutions of various concentrations, ranging from 1.0 mg/mL to 0.3125 mg/mL. The six serially dilute concentrations (1.0 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.0625 mg/mL and 0.03125 mg/mL) of EOs and standards were prepared in triplicates. Ascorbic acid (vitamin C) and BHA were used as standard positive controls. About 0.5 mL of each of these concentrations of the triplicates was added to 3 mL of pure methanol solution of DPPH (0.1 M). The absorbance of each sample concentration against methanol solution of DPPH blank was measured at 517 nm using an ultraviolet (UV) spectrophotometer.

All readings were taken after 30 min of reaction time at room temperature. The decrease in absorbance of DPPH on the addition of test samples to the blank was used to calculate the percentage inhibition (I %) using the following equation:

DPPH Inhibition (%) =
$$\frac{\text{Abs}(Blank) - \text{Abs}(Essential oil)}{\text{Abs}(Blank)} * 100$$

[Eqn 1]

where Abs (*Blank*) is the absorbance measurement of the blank and Abs (*Eo*) is the absorbance reading of EOs at 517 nm.

Hydrogen peroxide scavenging activity: The ability of EOs from C. inophyllum Linn to scavenge hydrogen peroxide was determined using the hydrogen peroxide scavenging assay at different concentrations (1.0 mg/mL - 0.03125 mg/mL) (Kamalanathan et al. 2015; Njenga & Mugo 2020; Serhat et al. 2012). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer at pH 7.4. The concentration of hydrogen peroxide was determined by absorption at 230 nm using a UVD. Essential oils and standards in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide was determined after 30 min against a blank solution containing phosphate buffer without hydrogen peroxide. The absorbance value of the reaction mixture was recorded at 230 nm using ascorbic acid (vitamin C) and BHA as standard. The percentage of hydrogen peroxide scavenged by the EOs and standards was calculated as follows:

Hydrogen Peroxide inhibition (%)

$$= \frac{\text{Abs}(control) - \text{Abs}(EO)}{\text{Abs}(control)} * 100$$
[Eqn 2]

where Abs (*Blank*) is the absorbance measurement of the blank and Abs (*Eo*) is the absorbance of EOs at 230 nm.

Statistical analysis

The experiments were conducted three times, all determinations were performed in triplicates (n=3) and the results were expressed as mean \pm standard deviation (SD). Statistical analysis was performed by non-linear regression analysis on Microsoft Excel. The half maximal inhibitory concentration (IC $_{50}$) values of 10 parts of *C. inophyllum* Linn were determined using non-linear regression analysis on Microsoft Excel in comparison with standards.

Ethical consideration

This article followed all ethical standards for a research without direct contact with human or animal subjects.

Results and discussion

Percentage yield of essential oils from C. inophyllum Linn

Essential oils obtained from *C. inophyllum* Linn gave characteristic odours (herbal, floral and woody). The oils were procured in 0.219% to 0.506% yields (Table 1), with the highest yield from fruit pulp, which gave 0.560%, and the lowest yield (0.219%) from the root, which may be because of its high fibre content.

Essential oil composition of C. inophyllum Linn

The GC-MS characterisations of the leaf, leaf stalk, flower oil, pod, peel, stem wood, stem bark, root wood and root bark EOs extracted from *C. inophyllum* Linn showed a total of 102 compounds, which are mostly monoterpenes, sesquiterpenes and their oxygenated derivatives as shown in Table 2.

A total of 71 compounds were characterised in leaf oil, which corresponded to 54.94% of the identified peaks. This oil consists mainly of sesquiterpenes (22.18%) and nonterpenes (27.89%). The predominant compounds in leaf oil are cis-cadina-1(6), 4-diene (6.50%), hexadecanal (6.16%) and cis-calamenene (5.41). The oils contain non-ubiquitous norisoprenoids, such as α-cyclocitral, β-cyclocitral and β-ionone. Leaf stalk essential oil gave 22 compounds, which constitute about 79.55% of it and are dominated by monoterpenes (75.62%). The leaf stalk oil is a rich source of monoterpenes, such as limonene (23.79), γ -terpinene (13.06) and p-cymene (9.28). The presence of non-ubiquitous phenylpropanoids, such as methyl chavicol and methyl eugenol, may be responsible for the diverse bioactivities expressed by the leaf stalk. Flower oil had 25 identified compounds, which make up 51.24% of it. Over 50% of the oils are sesquiterpenes (32.87%). The presence of characteristic chemical constituents, such as cis-cadina-1(6), 4-diene (15.42), β -alaskene (9.63) and γ -bisabolene (7.20), may be responsible for the impact notes of the plant. A sum of 25 compounds was identified in the cloudy white seed oil, which makes up about 89.39% of it. The compound is predominant in compounds such as limonene (25.40), γ -terpinene (14.00) and p-cymene (10.03). About 83.81% of identified oils are classified as monoterpenes. Phenylpropanoids such as methyl chavicol were also present. Pod oil is made up of 69 compounds which constitute 73.80% of it. The pod is a rich source of non-terpenes (48.82) and monoterpenes (24.98). Limonene (16.85), γ -terpinene (9.82) and p-cymene (6.70) are predominant in this part of the plant. 15 compounds were characterised in peel oil (46.10%), which is a good source of cis-cadina-1(6), 4-diene (15.6%), β-alaskene (8.4%) and β-acoradiene. This oil is exclusively rich in sesquiterpenes (45.80%). Fifty-five compounds were characterised in stem wood oil, which make up 59.40% of it. Predominant compounds in the stem wood oil include hexadecanal (6.87%), E-nerolidol (5.86%) and 1,8-cineole (5.63). This oil is rich in monoterpenes (22.96) and non-terpenes (28.11).

 TABLE 2a: Chemical constitution of 10 essential oils from Calophyllum inophyllum Linn.

S/N.	RI	Compound	Class	Leaf	Leaf stalk	Flower	Seed	Pod	Peel	Stem wood	Stem bark	Root wood	Root bark
1.	784	3-Hexanone	Alkanone	0.13	0.05	-	-	4.76	-	0.14	1.72	0.17	0.07
2.	789	2-Hexanone	Alkanone	0.50	0.07	-	-	3.21	-	1.00	1.85	0.82	0.12
3.	793	3-Hexanol	Alkanol	0.09	-	-	-	3.16	-	0.12	-	0.11	-
l.	800	Hexanal	Alkanal	0.92	0.42	0.06	0.67	3.82	-	0.84	-	1.24	0.16
j.	844	(E)-2-Hexenal	Alkanal	1.05	-	-	-	-	-	0.06	-	0.10	-
i.	846	3-Hexen-1-ol	Alkanol	0.23	-	-	-	-	-	-	-	-	-
7.	857	(E)-2-Hexen-1-ol,	Alkanol	0.99	-	-	-	-	-	-	-	-	-
3.	859	n-Hexanol	Alkanol	3.33	-	-	-	-	-	0.14	-	0.09	-
Э.	923	Acetonyl acetone	Alkanone	0.34	-	-	-	-	-	2.71	-	0.71	-
10.	926	α-Thujene	Monoterpene	-	1.96	-	2.34	-	-	0.35	-	-	1.09
11.	932	α-Pinene	Monoterpene	0.21	7.88	0.07	9.39	-	-	1.28	-	0.02	4.44
12.	945	Acetoxyhexane	Ether	-	-	-	-	4.80	-	-	-	-	-
13.	947	Camphene	Monoterpene	-	0.62	-	0.70	-	-	-	-	-	0.39
14.	958	Benzaldehyde	Aldehyde	0.09	-	-	-	-	-	-	-	-	0.5
15.	969	Sabinene	Monoterpene	-	0.72	-	0.93	-	-	-	-	-	-
L6.	975	eta-Pinene	Monoterpene	0.11	4.41	-	5.13	-	-	0.73	-	-	2.54
L7.	978	1-Octen-3-ol	Alkanol	0.04	-	-	-	-	-	0.54	-	0.25	-
L8.	987	6-methyl-5-Hepten-2-one,	Alkanone	0.10	-	-	-	-	-	0.11	-	0.17	-
L9.	991	Myrcene	Monoterpene	0.30	2.81	-	3.21	-	-	1.19	-	-	1.67
20.	1002	trans-2-(2-Pentenyl)furan	Aromatic	0.05	-	-	-	-	-	-	-	-	-
21.	1004	α-Phellandrene	Monoterpene	-	0.27	-	0.32	-	-	0.26	-	-	-
22.	1010	δ-3-Carene	Monoterpene	0.08	0.36	-	0.41	-	-	0.13	-	0.21	-
23.	1016	α-Terpinene	Monoterpene	0.09	2.40	-	2.54	-	-	0.39	-	-	1.41
24.	1024	p-Cymene	Monoterpene	0.34	9.28	0.06	10.03	8.50	-	1.42	2.29	-	5.39
25.	1028	Limonene	Monoterpene	0.75	23.79	0.18	25.4	9.71	-	3.47	3.24	-	13.93
26.	1030	1,8-Cineole	Oxygenated MT	0.30	5.33	-	5.69	-	-	5.63	-	0.39	3.54
27.	1034	2,2,6-trimethyl Cyclohexanone,	Alkanone	0.05	-	-	-	-	-	-	-	-	-
28.	1039	β-Ocimene	Monoterpene	-	0.56	-	0.65	-	-	0.11	-	-	0.36
29.	1043	Benzeneacetaldehyde	Aldehyde	0.08	-	-	-	-	-	-	-	-	-
30.	1049	, (E)- β-Ocimene	Monoterpene	-	0.84	-	0.90	-	-	0.15	-	-	0.51
31.	1058	γ-Terpinene	Monoterpene	0.56	13.06	0.10	14.00	6.77	-	2.14	2.95	0.27	7.75
32.	1065	Acetophenone	Alkanone	0.61	-	-	-	-	-	0.43	-	0.21	-
33.	1071	1-Octanol	Alkanol	-	-	-	-	_	-	-	-	0.31	_
34.	1087	Terpinolene	Monoterpene	-	0.70	-	0.84	-	-	-	-	-	0.53
35.	1100	Linalool	Oxygenated MT	0.12	0.63	-	0.62	_	-	2.62	-	1.96	0.46
36.	1105	Nonanal	Alkanal	0.31	-	_	-	_	_	-	-	1.79	-
37.	1116	α-Cyclocitral	Norisoprenoid	0.04	-	_	_	_	-	-	_	1.75	_
38.	1134	,	Alkanone	-	_	_	_	_	_	0.03	_	0.02	_
39.		Camphor	Oxygenated MT		_	_	0.29	_	_	0.07		0.02	
10.	1160	(E)-2-Nonenal	Alkanal	0.08	-	_	0.23		-	0.33		0.69	_
‡0. ‡1.	1176	Terpinen-4-ol	Oxygenated MT	-	-	-	-	-	-	1.34	-	0.09	_
+1. 12.	1170	α-Terpineol	Oxygenated MT	-	-	-	-	-	-	1.48	-	1.30	-
+2. 13.	1193	Methyl salicylate	Aromatic	0.08	-	-	-	-	-	1.40	-	-	-
+3. 14.	1195	Myrtenol	Alkanol	-	-	-	-	-	-	-	-	0.64	-
		·						-					
15. 16	1197	Methyl chavicol	Phenylpropanoid	- 0.10	0.59	-	0.73	-	-	0.09	-	-	- 0.06
16.	1198	Safranal	Alkanal	0.10	-		-	-	-		-	- 0.72	0.06
17.	1206	Decanal 9 Cyclesityal	Alkanal	0.09		-		-		0.46		0.73	-
18. 10	1220	β-Cyclocitral	Norisoprenoid	0.26	-	-	-	-	-	- 0.09	-	-	-
19. -0	1238	Ascaridole	Oxygenated MT	1.05	-	-	-	-	-	0.08	-	-	-
50.	1258	Edulan II	Ether	1.05	-	-	-	-	-	1.07	-	0.99	-
51.		2-Decenal	Alkanal	0.11	-	-	-	-	-	0.09	-	0.08	-
52.	1271	α-Citral	Alkanal	0.04	-	-	-	-	-	0.12	- 6.42	0.14	-
53.	1285	E Anethole	Ether	0.13	2.80	-	3.66	25.45	-	0.87	6.12	-	2.04
54.	1293	(E,Z)-2,4-Decadienal	Alkanal	-	-	-	-	-	-	-	-	0.55	-
5.	1301	Carvacrol	Oxygenated MT	-	-	-	0.42	-	-	0.12	-	-	-
56.	1314	Edulan I	Ether	1.69	-	-	-	-	-	-	-	-	-
57.	1316	(E,E)-2,4-Decadienal	Alkanal	-	-	-	-	-	-	-	-	1.34	-
58.	1350	α-Cubebene	Sesquiterpene	-	-	0.07	-	-	-	-	-	-	-
59.		1,2-dihydro-1,1,6-trimethyl naphthalene		0.14	-	-	-	-	-	-	-	-	-
50.	1363	2-Undecenal	Alkanal	0.07	-	-	-	-	-	-	-	-	-

Table 2a continues on the next page \rightarrow



 TABLE 2a(Continues...): Chemical constitution of 10 essential oils from Calophyllum inophyllum Linn.

S/N.	RI	Compound	Class	Leaf	Leaf stalk	Flower	Seed	Pod	Peel	Stem wood	Stem bark	Root wood	Root bark
61.	1367	Cyclosativene	Sesquiterpene	0.04	-	-	-	-	-	-	-	-	-
62.	1376	Copaene	Sesquiterpene	0.17	-	0.31	-	-	0.40	0.10	-	0.15	-
63.	1382	(3Z)-3-Hexenyl hexanoate	Ester	0.05	-	-	-	-	-	-	-	-	-
64.	1384	β -Bourbonene	Sesquiterpene	0.06	-	0.27	-	-	-	-	-	-	-
65.	1387	n-Hexyl hexanoate	Ester	0.77	-	-	-	-	-	-	-	-	-
66.	1391	7-epi-Sesquithujene	Sesquiterpene	0.47	-	0.58	-	-	0.40	-	-	-	-
67.	1399	Cyperene	Sesquiterpene	-	-	-	-	-	-	-	-	-	1.14
68.	1400	Tetradecane	Alkane	0.10	-	-	-	-	-	-	-	4.19	-
69.	1405	Methyl eugenol	phenylpropanoid	0.03	-	-	-	-	-	0.05	-	0.10	-
70.	1413	β-Cedrene	Sesquiterpene	0.21	-	0.69	-	-	0.80	-	-	-	-
71.	1424	β-Copaene	Sesquiterpene	-	-	-	-	-	0.20	-	-	-	-
72.	1428	α-lonone	Ester	0.44	-	-	-	-	-	-	-	-	-
73.	1454	6,10-dimethyl 5,9-Undecadien-2-one	Alkanone	0.89	-	0.09	-	-	-	0.39	-	0.97	-
74.	1458	β-Farnesene	Sesquiterpene	0.15	-	-	-	-	0.3	-	-	-	-
75.	1463	Cis-Cadina 1,6 4 diene	Sesquiterpene	6.50	-	15.42	0.37	-	15.60	0.45	-	0.84	_
76.	1467	β-Acoradiene	Sesquiterpene	2.54	-	5.72	0.15	-	5.60	0.25	-	-	-
77.	1477	γ-Muurolene	Sesquiterpene	0.34	-	0.22	-	-	0.70	0.15	-	1.11	_
78.	1481	Germacrene	Sesquiterpene	0.20	-	3.74	-	_	1.30	-	-	-	-
79.	1486	(E)-β-Ionone	norisoprenoid	1.94	-	_	-	-	-	0.07	-	1.31	_
80.	1496	β-Alaskene	Sesquiterpene	2.73	-	9.63	-	-	8.40	0.44	-	1.56	-
81.	1509	β-Bisabolene	Sesquiterpene	0.16	-	0.28	_	_	-	-	_	-	_
82.	1516	γ -Bisabolene	Sesquiterpene	_	-	7.20	_	_	4.70	_	_	_	-
83.	1517	(Z)-γ-Bisabolene	Sesquiterpene	2.00	-	-	_	_	-	-	_	1.21	-
84.	1524	δ-Cadinene	Sesquiterpene	1.20	-	0.86	_	_	2.00	0.27	_	0.82	_
85.	1534	Cis-Calamenene	Sesquiterpene	5.41	-	1.88	_	_	5.70	0.65	_	5.83	_
86.	1565	E-Nerolidol	Sesquiterpene	-	_	-	_	_	3.70	5.86	_	-	
87.	1580	(3E,7E)-4,8,12-Trimethyltrideca-1,3,7, 11-tetraene	Alkane	0.20	-	-	-	-	-	-	-	0.92	-
88.	1660	Neointermedeol	Alcohol	-	-	-	-	-	-	-	-	1.48	-
89.	1682	(Z)-3-Heptadecene,	Alkane	0.77	-	-	-	-	-	-	-	-	_
90.	1689	α-Bisabolol	Alcohol	0.28	-	0.50	-	-	-	-	-	4.36	-
91.	1818	Hexadecanal	Alkanal	6.16	-	2.54	-	3.62	-	6.87	46.80	-	10.61
92.	1848	Hexahydrofarnesyl acetone	Polyacetylene	0.77	-	-	-	-	-	0.18	-	0.72	-
93.	1973	Cembrene A 3Z	Diterpene	-	-	_	-	-	-	-	-	-	15.05
94.	1881	1-Hexadecanol	Alkanol	1.53	-	0.11	-	-	-	5.40	4.41	3.04	-
95.	1922	Farnesyl acetone	Alkanone	0.63	-	-	_	_	-	-	-	1.12	-
96.	1974	n-Hexadecanoic acid	Alkanoic acid	-	-	_	_	_	_	_	_	9.86	-
97.	1997	9-Octadecenal	Alkanal	1.35	-	0.19	-	-	-	2.95	-	0.67	_
98.	2085	n-Octadecanol	Alkanol	-	_	-	_	_	_		_	-	0.90
99.	2085	2-Octadecen-1-ol	Alkanol	1.20		0.47	-	_	_	2.74	_	0.86	0.50
100.	2496	Pentacosane	Alkane	1.20	-	-	_	-	_	2.74	_	1.08	_
100.	2599	Hexacosane	Alkane	-	-	-	-	-	-	0.24	-	0.67	-
101.	2900		Alkane	-	-	-	-	-	-	0.24	-	0.87	-
	onotern	Nonacosane	AIKAIIE	-		-	-	-	-	0.23		0.32	

MT, monoterpenes.

TABLE 2b: Chemical constitution of 10 essential oils from Calophyllum inophyllum Linn.

			rom carepnym	,						
Compound	Leaf	Leaf stalk	Flower	Seed	Pod	Peel	Stem wood	Stem bark	Root wood	Root bark
Total	54.94	79.55	51.24	89.39	73.80	46.10	59.40	69.38	58.73	74.66
No. of compounds	71	22	25	25	69	15	55	09	51	24
Monoterpenes	2.86	75.62	0.41	83.81	24.98	-	22.96	8.48	-	44.01
Sesquiterpenes	22.18	-	32.87	0.52	-	45.80	8.24	-	12.83	1.14
Diterpenes	-	-	-	-	-	-	-	-	-	15.05
Norisoprenoids	1.98	-	-	-	-	-	-	-	-	-
Phenylpropanoids	0.03	0.59	-	0.73	-	-	0.09	-	0.10	-
Non-terpenes	27.89	3.34	17.96	4.33	48.82	0.30	28.11	60.90	45.80	14.46

Stem bark oil contains nine compounds that make up 69.38% of it. This oil is rich in hexadecanal (46.80), E-anethole (6.12) and limonene (3.24). The oil is dominated by nonterpenes (60.90%) and monoterpenes (8.48%), whilst sesquiterpenes were absent. Root wood oil contains 51 compounds that make up 58.73% of it. This plant part is rich in non-terpenes (45.80%) and sesquiterpenes (12.83%), whilst monoterpenes were absent. This oil is rich in hexanedioic acid (9.86), E-nerolidol (5.83) and α -bisabolol (4.36). The oil also contains methyl eugenol, a phenylpropanoid. Root bark oil has 24 compounds that make up 74.66% of it, which are mainly with monoterpenes (44.01), diterpenes (15.05) and non-terpenes (14.46). This oil is rich in cembrene-3Z (15.05), limonene (13.93) and hexadecanal (10.61).

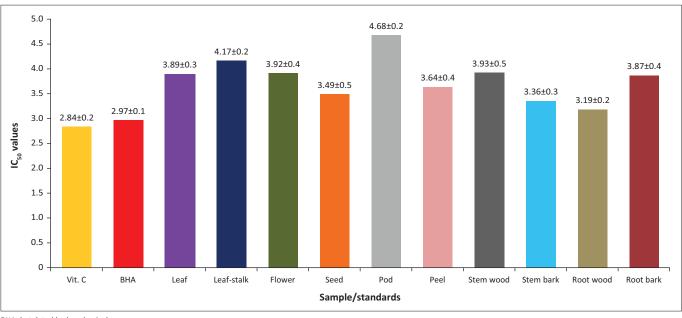
Generally, the essential oils are dominated by cymene, terpinene and limonene. Cymene, which is present in a relatively large percentage in eight of the oils from *C. inophyllum* Linn, has been reported as a good antioxidant, anti-inflammatory, anti-nociceptive, anxiolytic, anticancer and antimicrobial agent (DeOliveira et al. 2015), which corroborates the ethno-medicinal applications of the plant. In a recent *in vivo* investigation on an experimental animal model, p-cymene was found to increase the activity of antioxidant enzymes, thereby reducing the OS; the high antimicrobial potential of *Carum copticum* EO was also attributed to the abundance of cymene and terpinene (Hassan et al. 2016).

The high content of γ -terpinene in leaf stalk (13.06%), seed coat (6.77%) and root bark (7.75%) oils of *C. inophyllum* Linn is responsible for the anti-inflammatory and antioxidant effects, thus supporting the plant's anti-osteoarthritic activity. The presence of Terpinene in *Hyptis* species inhibited gastric lesions, reduced volume and acidity of the gastric juice and increased gastric wall mucus (Marcelo, Rafael & Lucio 2015). Limonene, which is found in an appreciable amount in stem heartwood (23.79%), stem bark (3.24%) and root bark (13.93%) EOs of *C. inophyllum* Linn, is known to have sedative and stimulative effects in *Lippia alba* (Vale et al. 2002; Viana, Vale & Matos 2000). Consumption of diets containing fruits and vegetables rich in monoterpenes, such as limonene, is known to reduce the

risk of developing cancer of the colon, mammary gland, liver, pancreas and lung. Limonene, which is known to possess high anticancer properties (Chistani et al. 2007; Marostica et al. 2009), is abundant in *C. inophyllum* Linn: leaf stalk (25.40%), seed (25.40%) and root bark (13.93%) oils. The presence of phenylpropanoids, norisoprenoids and other non-ubiquitous compounds, such as β -alaskene, β -acoradiene and E-anethole, is a unique feature of oils from *C. inophyllum* Linn as shown in Table 2.

2,2-diphenyl-1-picrylhydrazyl antioxidant activity of *Calophyllum inophyllum* Linn

The percentage inhibition obtained for standard antioxidants (ascorbic acid and BHA) was relatively high for the concentration range used (1.0 mg/mL - 0.03125 mg/mL). A maximum percentage inhibition of 92.68% and 91.67% was obtained at 1.0 mg/mL for ascorbic acid and BHA, respectively. The 10 oils (leaf, leaf stalk, flower, seed, pod, peel, stem wood, stem bark, root wood and root bark) exhibited concentration-dependent inhibition with reference to standard synthetic antioxidants used as a positive control. Percentage inhibitions of standards were in close range with pod EO, with inhibition efficiency of 78.32% at 1.0 mg/mL as indicated in Table 2. A graph of percentage DPPH inhibition versus concentration (mg/mL) of EOs was plotted from which the IC₅₀ values were obtained for each oil using linear regression analysis in reference to standards (Figure 5). An inverse relationship exists between the percentage inhibition efficiency and the IC_{50} values. The higher the IC_{50} value, the lower the activity of the EOs and vice versa. The following IC₅₀ values were obtained in the determination of DPPH inhibition: (leaf, 3.89 mg/mL; leaf stalk, 4.17 mg/mL; flower, 3.92 mg/mL; seed, 3.49 mg/mL; pod, 4.68 mg/mL; peel, 3.64 mg/mL; stem wood, 3.93 mg/mL; stem bark, 3.36 mg/mL; root wood, 3.19 mg/mL; and root bark, 3.87 mg/mL)



BHA, butylated hydroxyl anisole.

Note: All the results are mean \pm standard deviation (SD) where n=3. The half maximal inhibitory concentration (IC_{so}) was obtained in mg/mL using non-linear regression analysis in Microsoft Excel. **FIGURE 5:** The half maximal inhibitory concentration values (mg/mL) for 2,2-diphenyl-1-picrylhydrazyl inhibition of *Calophyllum inophyllum Linn*. compared to standard antioxidants (vitamin C [2.84 mg/mL] and BHA [2.97]). The standard antioxidants with lowest IC $_{50}$ values exhibited the highest antioxidant inhibition activity followed closely by the root wood (3.19 mg/mL). The least activity was expressed by pod oil (4.68 mg/mL). The high antioxidant activity of the root wood EO in comparison with standards must be because of the presence of some important bioactive phyto-constituents.

Hydrogen peroxide scavenging activity of Calophyllum inophyllum Linn

Optimum percentage inhibitions of 90.61% and 89.24% were obtained for ascorbic acid and BHA at 1.0 mg/mL and decreased slightly to 45.61% and 45.24% at 0.03125 mg/mL, respectively. This trend indicates that the percentage inhibition of the standard used in this study is concentration dependent. The 10 oils (leaf, leaf stalk, flower, seed, pod, peel, stem wood, stem bark, root wood and root bark) exhibited concentration-dependent inhibition similar to standard synthetic antioxidants used. A graph of percentage inhibition versus concentration (mg/mL) of EOs was plotted from which the IC₅₀ values were obtained for each oil using linear regression analysis in reference to the central standard. An inverse relationship exists between the percentage inhibition efficiency and the IC₅₀ values (Figure 4). The higher the IC₅₀ value, the lower the activity of the EOs and vice versa. The following IC₅₀ values were obtained in the determination of α-amylase inhibition: (leaf, 3.3 mg/mL; leaf-stalk, 3.8 mg/mL; flower, 3.18 mg/mL; seed, 3.18 mg/mL; pod, 4.32 mg/mL; peel, 3.32 mg/mL; stem wood, 3.56 mg/mL; stem bark, 3.37 mg/mL; root wood, 2.78 mg/mL; and root bark, 3.63 mg/mL) compared to standards (vitamin C [2.20 mg/mL] and BHA [2.92.36]).

Results obtained from the antioxidant assay are very consistent with the reported works in literature (Ajiboye et al. 2017; Bhaskara et al. 2015; Njenga & Mugo 2020).

Conclusion

The antioxidant activity of characterised compounds of C. inophyllum Linn was presented for the first time and extends the knowledge in the broad range of biological activities and therapeutic prospects associated with this medicinal plant. Results from both DPPH and hydrogen peroxide assays established that C. inophyllum Linn EOs possess antioxidant and radical scavenging potential. However, the antioxidant properties were found to be slightly lower compared to standard antioxidants used (ascorbic acid and BHA). Despite having lower activity compared to standards, the antioxidant activity of C. inophyllum Linn was found to be significant for potential applications in pharmaceutical industries and could act as a potential alternative to more toxic synthetic antioxidants. Because of the toxic nature of EOs, further studies on the toxicity and other biological properties of the extract are needed prior to possible applications.

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Competing interests

The authors have declared that no competing interests exist.

Authors' contributions

D.O.M. designed the study, coordinated the collection and supervised all laboratory experiments. E.O.O. carried out the plant collection, essential oil extractions, the antioxidant activity of the plant and wrote the first draft of the manuscript. P.M.O. made substantial contribution by revising the manuscript critically. All authors read and approved the final manuscript.

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Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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