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Original Research Article

Antioxidant activity of phytol dominated stem bark and leaf essential oils of *Celtis zenkeri* Engl.

Ejike Onwudiegwu Okpala[1], Patricia Akpomedaye Onocha[2]✉*, And Muhammad Shaiq Ali[3]

[1]Federal University Lokoja, Kogi State, Nigeria
[2]Natural Products/Medicinal Chemistry Unit, Department of Chemistry, University of Ibadan, Nigeria
[3]H.E.J. Research Institute of Chemistry, International Centre for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi, Pakistan

ABSTRACT

The essential oils (EOs) of the air-dried stem bark and leaves of *Celtis zenkeri* Engl. were extracted by hydrodistillation and characterized using gas chromatography-mass spectrometry. Twenty-three and nine compounds were respectively identified in the leaf and the stem bark EOs. Phytol (46.1%) was the most abundant compound in the leaf EO, while the major compounds in the stem bark EO were cycloeicosane (34.3%) and phytol (27.0%). The antioxidant activities of the obtained EOs were tested using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The percentage inhibition of the leaf EO (89.0%) was comparable to that of ascorbic acid (90.0%) and butylated hydroxy anisole (90.0%) at 250 µg/mL and higher than α-tocopherol (59.0%) at the same concentration. The EO of the stem bark of *Celtis zenkeri* Engl displayed good antioxidant activities when compared to the standard antioxidant drugs. The presence of these major constituents and the antioxidant activities of the EOs justify the ethnomedicinal uses of the *Celtis zenkeri* Engl. organs.

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1. Introduction

Essential oils (EOs) are liquid mixtures of volatile compounds characterized by their distinctive strong odor and are formed as by-products of plant metabolism, namely secondary metabolites (Gupta et al., 2010; Martin et al., 2010). These compounds comprise hydrocarbons, *e.g.*, monoterpenes and sesquiterpenes along with oxygenated derivatives such as phenols, alcohols, aldehydes, ketones, and esters (Pichersky et al., 2006). EOs are liquids at room temperature and can readily change from liquid to gaseous state without decomposition at room or slightly higher temperatures. Steam distillation, hydrodistillation, hydrodiffusion, solvent extraction, enfleurage, solvent-free microwave extraction, microwave-assisted hydrodistillation, headspace assisted analysis, and headspace solid-phase microextraction are the some of the traditional and advanced methods for extracting EOs as well as volatile fractions from a variety of plant materials (Mohammadhosseini, 2017; Mohammadhosseini et al., 2017). The development of resistance by organisms to synthetic drugs and the high cost of treatment consequent upon this resistance has necessitated a search for new, safe, efficient, and effective ways in the management of infectious diseases (El-Mahmood and Doughari, 2008).

Celtis zenkeri Engl. belongs to the Cannabaceae family. In Nigeria, various organs of the plant are used in traditional medicine. In this relation, an herbal preparation from the macerated wood of *Celtis zenkeri* Engl. is applied to cuts on the skin. Stem bark decoctions are drunk to treat cough and powdered stem bark with palm oil is rubbed on the body as an analgesic while a decoction mixture of the stem bark and leaf is used to treat arthritis and fever (Ugbogu and Oyebola, 2013). Previous phytochemical and biological

✉Corresponding author: Patricia Akpomedaye Onocha
Tel: +2347036015339; Fax: +2347036015339
E-mail address: triciaonocha@yahoo.com, **doi: 10.30495/tpr.2022.1952985.1246**

analysis of ethyl acetate fraction of the stem bark of *C. zenkeri* Engl. resulted in the isolation of new *iso*-benzofuranone propanamide named zenkeramide, *trans*-N-coumaroyltyramine, β-sitosterol and β-sitosterol-3-*O*-β-D-glucopyranoside which exhibited good urease inhibitory activity (Okpala et al., 2021). The compositions of leaves and stem of *Celtis sinensis* EO have been reported, with a total of 44 compounds identified in the leaves while 52 compounds were found in the stem oil. The most abundant compounds in both organs of *C. sinensis* were diisobutyl ester and cyclohexanone (Qiang and Wen-hu, 2020).
In continuation of our search for new potent antioxidants from plant sources (Okpala et al., 2019; Onocha et al., 2015; Onocha et al., 2016), we report the chemical composition and antioxidant activities of EOs obtained from the leaves and stem bark of *C. zenkeri* Engl. which to the best of our knowledge has not been reported in the literature.

2. Experimental

2.1. Plant material

The leaves and stem bark of *Celtis zenkeri* Engl. were collected from Ikire (7º22› 20''N; 4º 11'14''E), at an altitude of 305 m, Osun State, Nigeria, in August 2016. The plant was identified and authenticated by Dr. S.A. Odewo at the herbarium of the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria, where a voucher specimen was deposited as FHI-110554.

2.2. Extraction of the essential oil

The air-dried and pulverized samples of *C. zenkeri* Engl. were weighed and subjected to hydrodistillation for 3 hours using an all-glass Clevenger distillation unit designed according to the British Pharmacopoeia specification (British Pharmacoepia, 1980). The EOs extracted in 2.0 mL of *n*-hexane were collected in a pre-weighed sample vial and refrigerated at 4 ºC before analysis.

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

C. zenkeri Engl. EOs were analyzed using GC-MS Agilent Technologies, Model-7890A gas chromatograph, coupled with a 5975C mass spectrometer Agilent Technology. The gas chromatography capillary column type was an HP-5MS, with a column length of 30 m, an internal diameter of 0.320 mm, and a film thickness of 0.25 µm. The carrier gas used was helium at a constant flow rate of 1.4123 mL/min and an average velocity of 43.311 cm/sec, while the pressure was put at 1.5 PSI. Temperature programming started with an initial column temperature set at 80 ºC for 2 min and was increased to 240 ºC at the rate of 10 ºC/min. The volume of the EO injected was 1 µL and the split ratio was 50:1 and the split flow at 70.615 mL/min. The total chromatogram was auto-integrated and the constituents were identified by comparison of the GC-MS data with published mass spectral database (NIST 11. L) and literature data.

2.4. Antioxidant assay

The antioxidant activity of *C. zenkeri* Engl. EOs was determined using the DPPH+ (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging ability method (Shimadu et al., 1992). The concentrations (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL) of the EOs were mixed with 100 µM methanolic DPPH+ solution (2.0 mL), prepared by dissolving 3.94 mg of DPPH+ in 100 mL of methanol to give a purple solution. The mixture was shaken vigorously and left to incubate for 30 minutes in the dark at room temperature and the absorbance was then measured at 517 nm and recorded as $A_{(sample)}$ using a GS UV-12, UV-VIS spectrophotometer. In its radical form, DPPH+ absorbs, but its absorption reduces upon reduction by antioxidant species. A blank experiment was carried out applying the same procedure without EO ($DPPH^+$ + methanol) and the absorbance was recorded as $A_{(control)}$. Ascorbic acid, butylated hydroxy anisole (BHA) and α-tocopherol were used as standard antioxidants for comparison. The free radical scavenging activities of each EO were then calculated as percentage inhibition according to the following equation.

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad \text{(Eq. 1)}$$

3. Results and Discussion

3.1 Yields and properties of the *C. zenkeri* Engl. EOs

The weight of leaf and stem bark of *C. zenkeri* Engl. used in hydrodistillation extraction, the weight of the EOs obtained and corresponding yields (w/w%) are presented in Table 1. The GC-MS chromatograms of the EOs are also given in Fig. 1 and Fig. 2.
Chemical constituents of the EOs from the leaf and the stem bark of *Celtis zenkeri* Engl. are shown in Table 2. The activity for the scavenging ability on DPPH+ radical of the EOs of the leaf and stem bark of *C. zenkeri* Engl. assays are summarized in Table 3 and Table 4.
On the other hand, the DPPH+ scavenging activities of the stem bark and leaf EOs of *C. zenkeri* Engl. were investigated and compared with known standard antioxidants, *e.g.*, ascorbic acid, butylated hydroxyl anisole (BHA) and α-tocopherol.

3.2. Chemical composition of *C. zenkeri* Engl. EOs

Twenty-three compounds were identified in the leaf EO, representing a total percentage composition of 99.9% while nine compounds were responsible for 98.9% composition in the stem bark EO. The most abundant compound in the leaf EO was phytol (46.1%). Minor

Table 1
Yields and properties of the *C. zenkeri* Engl. EOs.

S/N	Plants name & parts	Weight of plant material (g)	Weight of oil (g)	Approximate (w/w) % yield of oil	Color of oil
1	*C. zenkeri* Engl. leaves	200	1.16	0.58	Colorless
2	*C. zenkeri* Engl. stem bark	200	1.27	0.64	Coloress

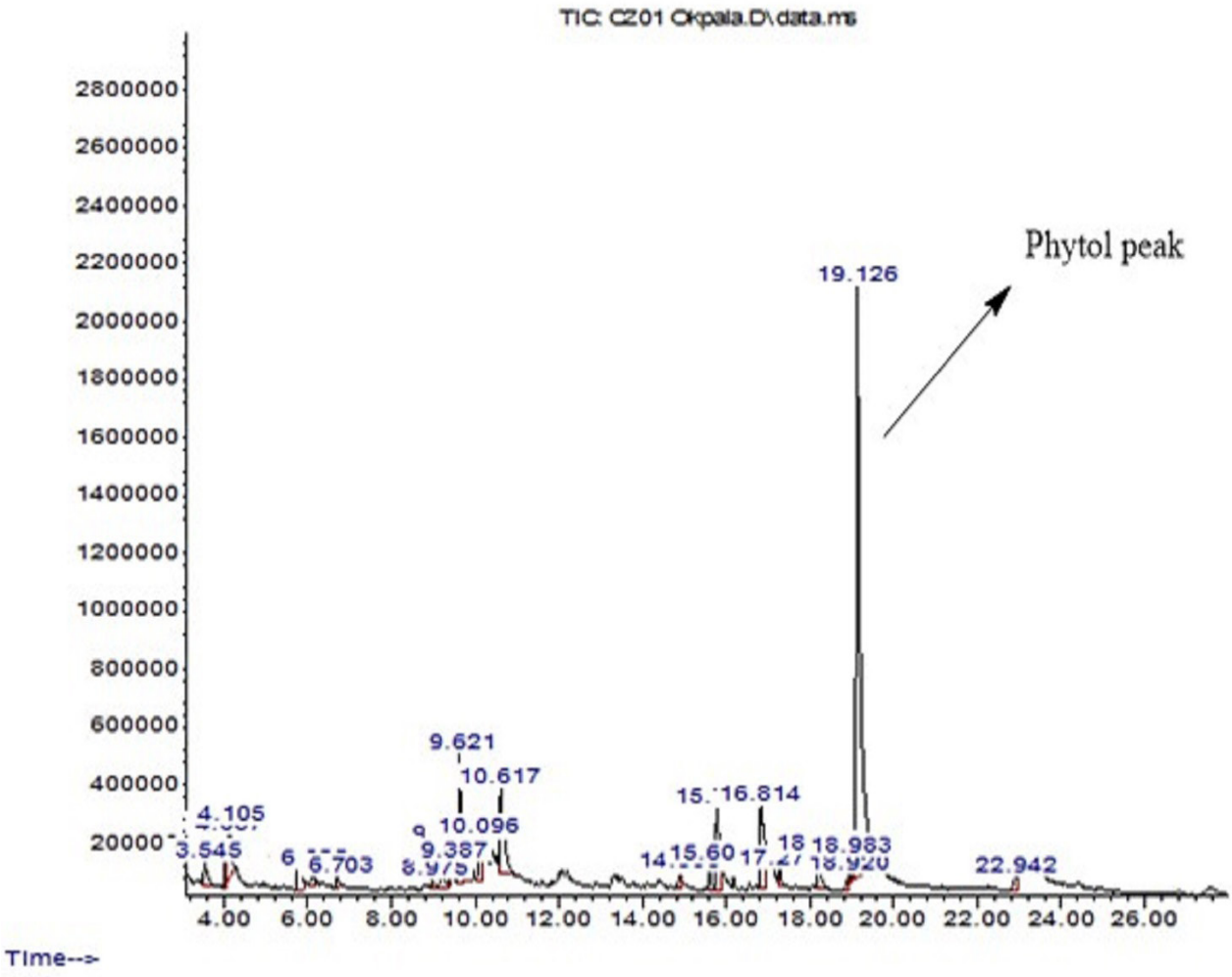

Fig. 1. The GC-MS chromatogram of the leaf of *C. zenkeri* Engl. essential oil.

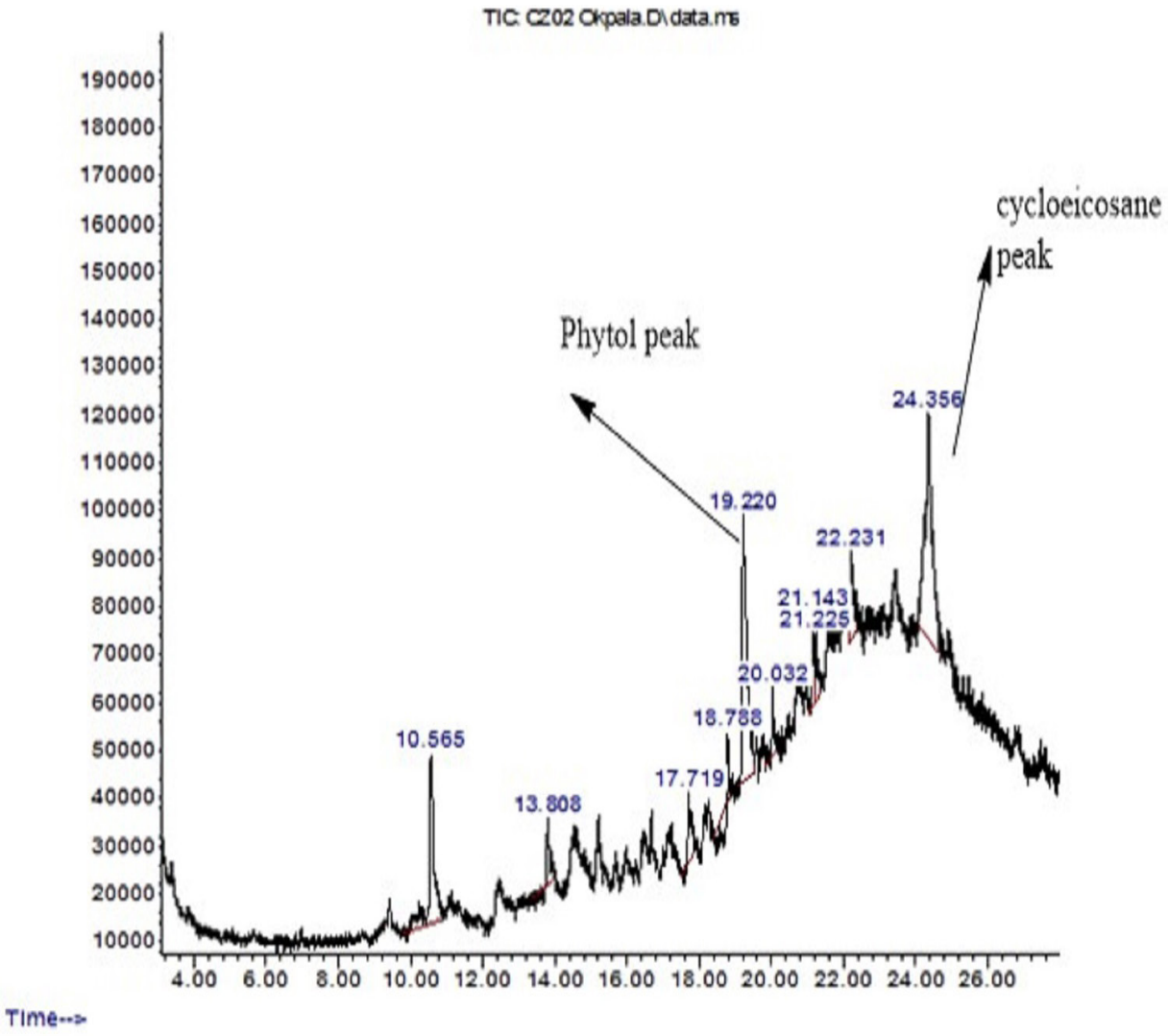

Fig. 2. The GC-MS chromatogram of the stem bark of *C. zenkeri* Engl. essential oil.

Table 2
Chemical composition of stem bark and leaves of *C. zenkeri* Engl. essential oils.

S/N	RT(min)	KI[a]	KI[b]	Compounds	Composition (%)	
					Leaf	Stem bark
1	3.54	878	700	2,4-Dimethylpenta-1,3-diene	1.4	-
2	4.03	1315	1062	*trans*-4-thujanol	1.7	-
3	4.1	1480	1518	Geranyl isobutyrate	3.4	-
4	5.75	1066	1205	Safranal	2	-
5	6.08	1035	1314	Epidolichodial	0.9	-
6	6.7	979	1261	β-Cyclo-homocitral	0.5	-
7	8.97	1283	1564	Phorone	0.6	-
8	9.19	1216	1486	Ethyl-4-t-butylbenzoate	2.9	-
9	9.38	1355	1421	α-Caryophyllene	0.7	17.3
10	9.62	1378	1433	α-Ionone	6.9	-
11	9.77	1300	1439	Dihydro-β-Ionone	2.3	-
12	10.09	1300	1434	*trans*-Geranylacetone	3.3	-
13	10.61	1322	1434	β-Ionone	7.6	-
14	13.8	1545	-	Tritetracontane	-	3.1
15	14.88	1899	1613	α-Eudesmol	0.5	-
16	15.6	1746	1836	Neophytadiene	1	-
17	15.76	1973	1847	Hexahydrofarnesylacetone	5.7	-
18	16.81	1592	1915	Farnesylacetone	7.2	-
19	17.27	1699	-	[2,6,6-trimethyl-4-(3-methylbut-2-enyl)-1-cyclohexenylmethanol	0.7	-
20	17.71	1788	1875	Tridecyl-2-methoxyacetate	-	4.5
21	18.19	1165	1531	Nerolidol	2	-
22	18.78	1785	-	*cis*-8-methyl-exo-tricyclo[5.2.1.0(2.6)] decane	-	Tr.
23	18.92	1868	1228	3,5-Dimethyl-1,6-heptadiene	0.6	-
24	18.98	1236	2103	Methyl oleate	1	-
25	19.12	1896	2122	Phytol	46.1	27
26	20.03	1831	2497	Carbonic acid, eicosyl vinyl ester	-	3.2
27	21.22	1627	2760	2-Hexyl-1-decanol	-	3.9
28	22.23	1729	1885	1-Nonadecene	-	5.5
29	22.94	2399	2397	Oleamide	0.9	-
30	24.36	2081	-	Cycloeicosane	-	34.3
Alkanes and Alkenes					2	43
Monoterpene hydrocarbons					16.9	-
Oxygenated Monoterpenes					5.1	-
Sesquiterpenes hydrocarbons					0.7	17.3
Oxygenated Sesquiterpenes					2.5	-
Diterpenes Hydrocarbon					1	-
Oxygenated diterpenes					46.1	27
Fatty acid and fatty acid esters					1	7.7
Ketone					16.7	-
Others					7.9	3.9
Total					**99.9**	**98.9**

RT = Retention time in minutes; [a] Linear retention index experimentally determined using homologous series of C_6-C_{30} alkanes; [b] Linear Kovats retention index (Literature); Tr. = Less than 0.1%.

Table 3
Absorbance values from scavenging effect of essential oils from *C. zenkeri* Engl. and standards at 517 nm of DPPH method of antioxidant assay.

Conc. (µg/mL)	Leaf	Stem bark	Asc. acid	BHA	α-TP
1000	0.057 ± 0.002	0.027 ± 0.001	0.016 ± 0.000	0.018 ± 0.001	0.093 ± 0.006
500	0.043 ± 0.001	0.030 ± 0.001	0.018 ± 0.002	0.019 ± 0.001	0.040 ± 0.002
250	0.023 ± 0.000	0.030 ± 0.000	0.020 ± 0.005	0.020 ± 0.000	0.082 ± 0.003
125	0.025 ± 0.009	0.030 ± 0.001	0.025 ± 0.001	0.024 ± 0.001	0.038 ± 0.000
62.5	0.031 ± 0.002	0.032 ± 0.000	0.024 ± 0.001	0.031 ± 0.013	0.030 ± 0.000

Asc.Acid = Ascorbic acid; BHA= Butylated hydroxyl anisole; α-TP = α-Tocopherol; ± Standard deviation for measurement based on three replicate analysis.

Table 4
Percentage inhibition of essential oils from *C. zenkeri* Engl. and standards.

Conc.(µg/mL)	Leaf	Stem bark	Asc. Acid	BHA	α-TP
1000	71	87	92	91	53
500	78	85	91	91	80
250	89	85	90	90	59
125	87	85	88	88	81
62.5	84	84	88	85	85

constituents included β-ionone (7.6%), farnesylacetone (7.2%), α-ionone (6.9%), and hexahydrofarnesylacetone (5.7%). The major compounds in the stem bark EO were cycloeicosane (34.3%), phytol (27.0%) and α-caryophyllene (17.3%). The leaf and stem bark EOs of *C. zenkeri* Engl. were of different classes of compounds. The leaf EO was dominated by oxygenated diterpene (46.1%), monoterpenes (16.9%), alkanone (16.7%) and oxygenated sesquiterpenes (2.5%) while the stem bark EO was dominated by alkanes and alkenes (43.0%), oxygenated diterpene (27.0%), sesquiterpenes (17.3%), fatty acid and fatty acid esters (7.7%). Comparing the constituent of the studied EOs, phytol is the major component of both EOs with higher content of 46.1% and 27.0% in the leaves and stem bark respectively. The α-caryophyllene content of stem bark EO is far greater than that of leaves. There were 30 non-common components identified in leaves and stem bark EOs of *C. zenkeri* Engl., this may be attributed to the physiology of the two organs of the plant. Also, the comparison of the composition pattern of the leaves and stem bark EOs showed notable qualitative and quantitative differences. Monoterpenes, oxygenated monoterpenes, oxygenated sesquiterpenes and ketone were found only in the leaves' EO.

The ethnomedicinal uses of the *C. zenkeri* Engl. plant's organs in analgesic, antimicrobial, antitussive activities and treatment of wounds, arthritis, fever and diseases associated with oxidative stress may be attributed to the presence of phytol, the major chemical constituent in the stem bark and leaves EOs. Phytol has been shown to have anti-nociceptive and antioxidant activities (De-Menezes et al., 2013); anti-inflammatory and anti-allergic effects (Ryu et al., 2011) as well as anti-schistosomal activity (De-Moraes et al., 2014). The medicinal properties of the plant are also enhanced by the presence of α-caryophyllene in the EOs which has also been reported to possess both topical and systemic anti-inflammatory properties (Fernandes et al., 2007) and is an effective analgesic when taken topically, orally, or by aerosol (Rogerio et al., 2009). β-Ionone an analogue of β-carotenoids and an intermediate in vitamin E preparation was reported to have antibacterial and fungicidal properties (Aloum et al., 2020; Shi, et al., 2020).

3.3. Antioxidant activity of *C. zenkeri* Engl. EOs

The measured absorbance increased with decreasing concentrations of *C. zenkeri* Engl. EO of the stem bark, while the absorbance decreased with decreasing concentrations in the case of the leaf EO. From the results, the % inhibition of the leaf EO has the highest antioxidant activity (89.0%), being comparable to that of ascorbic acid (90.0%) and butylated hydroxyl anisole (90.0%) at 250 µg/mL and higher than α-tocopherol (59.0%) at the same concentration as shown in Table 4. The antioxidant activity of stem bark EO was also higher than α-tocopherol at all concentrations (1000-125 µg/mL). The antioxidant activity of the EOs from the leaves and stem bark was comparable to that of known antioxidants (Ascorbic acid and Butylated Hydroxy Anisole), which are used as reference. *Celtis australis* fruit ethanolic extract has been shown to have considerable antibacterial activity against *Pseudomonas aeuroginosa* and *Escherichia coli* (Badoni et al., 2010). Water, ethanol, and methanolic extracts of *Celtis tournefortii* fruits have also been reported to have significant radical scavenging activity (Keser et al., 2017).

4. Concluding remarks

C. zenkeri Engl. plant's organs are used in traditional medicine for analgesic, antimicrobial, antitussive activities and in the treatment of wounds, arthritis, fever and diseases associated with oxidative stress. Phytol, the most abundant constituent of the oils is known for its anti-nociceptive, antioxidant, anti-inflammatory, anti-allergic and anti-schistosomiasis activities while α-caryophyllene, another constituent of the EOs has also been reported to possess anti-inflammatory, analgesic, antineoplastic, wound healing and angiogenesis properties. Also, β-ionone was reported to have antibacterial and fungicidal properties. The good radical scavenging activities exhibited by the EOs of the leaves and stem bark of *C. zenkeri* Engl. due to inhibition of DPPH+ radical may be attributed to the chemical constituents of the EOs. The chemical constituents and antioxidant activities of the EOs from *C. zenkeri* Engl. are reported for the first time. The antioxidative activity of EOs extracted from the plant's leaves and stem bark indicated that the oils have good free radical scavenging ability and may therefore be used as natural antioxidant sources.

Conflict of interest

The authors declare that there is no conflict of interest.

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References

Aloum, L., Alefishat, E., Adem, A., Petroianu, G., 2020. Ionone is more than a violet's fragrance: A review. Molecules 25, 5822.

Badoni, R., Semwa, D.K., Rawat, U., 2010. Fatty acid composition and antimicrobial activity of *Celtis australis* fruits. J. Sci. Res. 2(2), 397-402.

British Pharmacopeia. 1980. London: H. M., Stationery Office, PA, p. 109.

De-Menezes, C.C., Santos, P., Salvadori, M.S., Mota, V.G., Costa, L.M., De-Almeida, A.A.C., De-Oliveira, G.A.L., Costa, J.P., De Sousa, D.P., De Freitas, R.M., De-Almeida, R.N., 2013. Antinociceptive and antioxidant activities of phytol *in-vivo* and *in-vitro* models. J. Neurosci. 2314-4262.

De-Moraes, J., De-Oliveira, R.N., Costa, J.P., Junior, A.L.G., De-Sousa, D.P., Freitas, R.M., Allegretti, S.M., Pinto, P.L.S., 2014. Phytol, a diterpene alcohol from chlorophyll, as a drug against neglected tropical disease *schistosomiasis mansoni*. PLoS. Negl. Trop. Dis. 8(1), e2617.

El-Mahmood, A.M., Doughari, J.H., 2008. Phytochemical screening and antibacterial evaluation of leaf and root extracts of *Cassia alata* Linn. African J. Pharm. Pharmacol. 2(7), 124-129.

Fernandes, E.S., Passos, G., Medeiros, R., Da Cunha, F., Ferreira, J., Campos, M., Pianowski, L., Calixto, J., 2007. Anti-inflammatory effects of compounds *alpha*-humulene and *(-)-trans*-caryophyllene isolated from the essential oil of *Cordia verbenaceae*. Eur. J. Pharmacol. 569(3), 228.

Gupta, V., Mittal, P., Bansal, P., Khokra, S.L., Kaushik, D., 2010. Pharmacological potential of *Matricaria reculita*. Int. J. Pharma. Sci. Drug Res. 2, 67-71.

Keser, S., Keser, F., Kaygil, O., Tekin, S., Turkoglu, I., Demir, E., Turkoglu, S., Karatepe, M., Sandal, S., Kirbag, S., 2017. Phytochemical compounds and biological activities of *Celtis tournefortii* fruits. Anal. Chem. let. 7(3), 344-355.

Martin, A., Verona, S., Navarrete, A., Cocero, M. J., 2010. Encapsulation and co-precipitation processes with supercritical fluids: Applications with essential oils. The Open Chem. Eng. J. 4, 31-41.

Mohammadhosseini, M., 2017. Essential oils extracted using microwave-assisted hydrodistillation from aerial parts of eleven *Artemisia* species: Chemical compositions and diversities in different geographical regions of Iran. Rec. Nat. Prod. 11(2), 114-129.

Mohammadhosseini, M., Akbarzadeh, A., Flamini, G., 2017. Profiling of compositions of essential oils and volatiles of *Salvia limbata* using traditional and advanced techniques and evaluation for biological activities of their extracts. Chem. Biodivers. 14(5), e1600361.

Okpala, E.O., Oloyede, G.K., Onocha, P.A., 2019. Chemical composition, antimicrobial and antioxidant activities of volatile oil of *Euphorbia graminea* JACQ from Nigeria. Int. J. Adv. Sci. Eng. Technol. 7(4), 50-54.

Okpala, E.O., Onocha, P.A., Ali, M.S., Zikr-Ur-Rehmen S., Lateef, M., 2021. Zenkeramide: a new iso-benzofuranone propanamide and urease inhibitory constituents of *Celtis zenkeri* Engl stem bark (Ulmaceae). Nat. Prod. Res. 1-6.

Onocha, P.A., Oloyede, G.K., Johnny, E.U., 2016. Free radical scavenging activity and toxicity of node weed (*Synedrella nodiflora*) extracts. Chem. J. 6(1), 93-97.

Onocha, P.A., Oloyede, G.K., Odeja, O.O., 2015. Hydroxyl radical scavenging, toxicity and antimicrobial investigation of stem bark extracts of *Sterculiar tragacantha* Lindl (Malvaceae). Int. J. Env. Eng. 2(2), 20-26.

Pichersky, E., Noel, J.P., Dudareva, N., 2006. Biosynthesis of plant volatiles: nature's diversity and ingenuity. Sci. 311(5762), 808-811.

Qiang, W., Wen-hu, G., 2020. Chemical components of volatile oil from leaves and stems of *Celtis sinensis* Pers. J. Essen. Oil Bearing Plant 25(4), 772-778.

Rogerio, A.P., Andrade, E.L., Leite, D.F.P., Figueiredo C.P., Calixto, J.B., 2009. Preventive and therapeutic anti-inflammatory properties of the sesquiterpene *alpha*-humulene in experimental airways allergic inflammation. British J. Pharmacol. 158, 1074-1087.

Ryu, K.R., Choi, J.Y., Chung, S., Kim, D.H., 2011. Anti-scratching behavioral effect of the essential oil and phytol isolated from *Artemisia princeps* Pamp in mice. Planta. Med. 77, 22-26.

Shi, J., Cao, C., Xu, J., Zhou, C., 2020. Research advances on biosynthesis, regulation, and biological activities of apocarotenoid aroma in horticultural plants. J. Chem. 2020, 11.

Shimadu, K., Fujikawa, K., Yahara, K., and Nakamura, T., 1992. Antioxidative properties of xanthan of soybean oil in on the autoxidation cyclodextrin emulsion. J. Agric. Food. Chem. 40, 945-948.

Ugbogu, O.A., Oyebola, T.O., 2013. Checklist of medicinal plants in Omo forest reserve in the rain forest zone. J. For. Res. Manag. 10, 107-119.