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Research Article

Chemical composition, antioxidant and antimicrobial activity of the essential oil from the rhizome of *Curcuma longa* L.

Omolara Omowumi Fatunmibi[1, 4], Isaac Saint Njoku[1,2], Olayinka Taiwo Asekun[1] and James Ode Ogah[3]

1. Department of Chemistry, Faculty of Science, University of Lagos, Akoka-Yaba, Lagos, Nigeria.
2. Department of Chemistry, Faculty of Basic Medical and Applied Sciences, Trinity University Yaba, Lagos, Nigeria.
3. Department of Microbiology, Faculty of Science, University of Lagos, Akoka-Yaba, Lagos, Nigeria.
4. Department of Production, Analytical and Laboratory Management, Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.

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Corresponding Author
Isaac Saint Njoku
E-mail: saintnjokuphd@gmail.com
Tel: +2347037980140

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Abstract

The benefits of essential oils are well known for ages due to the presence of the major and minor components in the oils and their synergistic effects. The essential oil of air-dried rhizome of *Curcuma longa* L. obtained through hydro distillation using Clevenger apparatus was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) while the antimicrobial assay was carried out using the agar diffusion method. *In vitro* antioxidant assay was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, ferric reducing antioxidant power and nitric oxide radical scavenging on the extracted essential oil. The major components of the essential oil of *Curcuma longa* L. were p-Menthane-1,8-diol (17.26%), 3-Ethylthiophene (9.47%), trans-Ascaridol glycol (7.98%), 3-Methyl-2-(3-methylpentyl)-3-buten-1-ol (7.93%), Carvone hydrate (6.78%), Bis(2-ethylhexyl) phthalate (6.35%) 2-Ethylcyclohexanone (5.30%), Cyclohexanone, 4-hydroxy-4-methyl- (4.62%), α-Phellandrene-8-ol (4.32%) and Sobrerol (5.94%). Results of the antioxidant activities of the essential oil of *Curcuma longa* L. showed promising antioxidant potentials when compared to the positive control (ascorbic acid), strong nitric oxide scavenging activity was observed in the essential oil (IC_{50} of 23.19 µg/mL) than that of the standard drug used (IC_{50} of 34.24 µg/mL). The antimicrobial activities of the essential oil against the most frequently encountered microorganisms which include *Staphylococcus aureus* (ATCC 29213), *Streptococcus mutans, Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231) showed significant broad spectrum antibacterial and antifungal activities with zones of inhibition (mm) against *Staphylococcus aureus* (21.00±1.4), *Streptococcus mutans* (29.00±4.9), *Escherichia coli* (38.00±2.8) and *Candida albicans* (23.00±0.6).

1. Introduction

Curcuma longa L. is a rhizomatous, herbaceous, perennial herb, a member of the ginger family (*Zingiberaceae*). The rhizome of C. *longa* L. (underground stem) is thick and ringed with the bases of old leaves. Turmeric was probably cultivated at first as a dye and then became valued as a condiment as well as for cosmetic purposes [1-3]. The components of *Curcuma longa* L. (Turmeric) include diarylheptanoids, a class including numerous curcuminoids, such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin [2-5]. It was reported that major components of turmeric oil were α-zingiberene (27.70–36.75 %), aromatic-turmerone (19.54–32.24 %), β-sesquiphellandrene (13.14–18.23 %), α-turmerone (3.72–6.50 %), β-turmerone (2.86–5.60 %), and β-bisabolene (2.50–3.46 %) [6].

Studies revealed that oxygenated compounds were the most represented class of volatiles (63.6%), including *ar*-turmerone (45.5%) and α-turmerone (13.4%) as major compounds. Sesquiterpene hydrocarbons were the second class (18.0%) with α-zingiberene (5.3%) as predominant. Another major constituent was the monoterpene hydrocarbon α-phellandrene (6.3%) [7]. It was also reported that the major essential oils from turmeric rhizomes are Eucalyptol (76.46%), α-Terpinene (4.41%), γ-Terpinene (3.32%), p-Cymene (1.31%) and α-Terpineol (0.62%) [8]. The major ones were ar-turmerone (22.7%), turmerone (26%) and curlone (16.8%) [9].

Studies indicated that turmeric essential oil is an extremely strong antioxidant and antimicrobial (antifungal) agent with potential application in the food and pharmaceutical industries as a safer alternative to the synthetic antioxidants and antimicrobial agents [9]. It is a potent scavenger of a variety of reactive oxygen species (ROS) including superoxide anion, hydroxyl radical, singlet oxygen, peroxynitrite and nitric oxide [10]. Turmeric essential oil was found to have *in vitro* antioxidant activity and $IC_{(50)}$ for scavenging superoxides, hydroxyl radicals, and lipid peroxidation were 135 μg/ml, 200 μg/ml, and 400 μg/ml, respectively. The ferric-reducing activity for 50 μg of turmeric essential oil was found to be 5 mM. These results demonstrated that turmeric oil has potential health benefits as it can scavenge free radicals and produce significant anti-inflammatory and antinociceptive activities [11].

Studies show that the rhizomes contain compounds that may have therapeutic effects, which appear to support some of its uses in traditional medicine. Turmeric has been shown to have anti-bacterial, anti-fungal, antioxidant and anti-inflammatory effects, to which can be added possible anti-ulcer, wound-healing, liver-protective and anti-cancer properties [3]. The rhizome prevents skin diseases, and is a useful application on swellings and boils. A decoction is used as a cooling lotion in conjunctivitis, boiled rhizome in milk and sweetened with sugar is a popular remedy for cold, and is also used in jaundice and other liver ailments [4]. It was also reported that washing in turmeric improves skin tone and reduces hair growth, extracts have been added to creams and ointments as a colouring agent, anti-inflammatory and antioxidant, traditionally women would rub turmeric into their cheeks to produce a golden glow due to curcumin the main yellow pigment in turmeric [3, 12].

Curcuminoids are major components responsible for various biological actions. Curcumin acts as a pro-oxidant in the presence of transition metal ions (Cu and Fe) and is a potent bioprotectant with a potentially wide range of therapeutic applications [10]. It is an ingredient in modern pharmaceutical products effective in senile pruritis, anti-fungal, anti-inflammatory, anti-bacterial and to fight decaying metabolism to prevent cancer [13, 14].

This research analyzed the chemical composition, of the essential oil extracted from the rhizome of *Curcuma longa* L. grown in *North West* Nigeria and compare with research reports on oil extracted from rhizomes grown in North Central and South West of Nigeria. The antimicrobial and antioxidant activities of the essential oil was also evaluated to give detailed informations on the benefits of incorporating the essential oil in cosmetics products as natural preservative because of its antioxidant and antimicrobial activity.

2. Materials and methods

2.1 Plant material and essential oil extraction technique.

The healthy rhizome of *Curcuma longa L.* (Turmeric) (Fig. 1) was collected from Gwagwada Town of Chikun LGA of Kaduna State, *North West*, Nigeria in June, 2021.

Figure 1. Rhizome of *Curcuma longa L.* (Turmeric)

The botanical identification and authentication were done by Dr. Nodza George in the Herbarium of the Department of Botany, University of Lagos, Nigeria

with authentication number LUH: 8800. The fresh rhizome of *Curcuma longa L.* were cut into small pieces, air-dried for a week and pulverized using electric blender (BLK-828) prior to extraction. The essential oils were obtained by hydro distillation (Fig 2) of 300 g of the pulverized rhizome using the Clevenger apparatus for 4 hours [15]. The oil was dried over anhydrous sodium sulphate and stored in vials in a refrigerator prior to analysis.

Figure 2. Hydro-distillation using Clevenger setup.

2.2 GC-MS analysis of volatile oil

The analysis of the essential oil of the rhizome of *Curcuma longa L.* was carried out using an Agilent 7820A gas chromatograph coupled to 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies). The stationary phase of separation of the compounds was HP-5 capillary column coated with 5% Phenyl Methyl Siloxane (30m length x 0.32mm diameter x 0.25µm film thickness) (Agilent Technologies). The carrier gas was Helium and it was used at constant flow of 1.4871mL/min and an initial nominal pressure of 1.4902 psi and average velocity of 44.22cm/sec. 1µL of the samples were injected in splitless mode at an injection temperature of 300 °C. Purge flow to spilt vent was 15 mL/min at 0.75 min with a total flow of 16.654 mL/min; gas saver mode was switched off. The oven was initially programmed at 40 °C for (1 min) then ramped at 12 °C/min to 300 °C (10 min). The run time was 32.667 min. with a 5 min. solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C and transfer line temperature of 280 °C. Acquisition of ion was via Scan mode (scanning from m/z 45 to 550 amu at 2.0s/scan rate) [16]. Relative percentage amounts of the essential oil components were evaluated from the total peak area by apparatus software. Identification of components in the volatile oil was based on the comparison of their mass spectra and retention time with literature data and by computer matching with NIST 2017 and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

2.3 Antioxidant Assay

2.3.1 DPPH free radical scavenging assay

The free radical scavenging capacity of the essential oil was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method [16]. A solution of 0.1mM DPPH in ethanol was prepared, 1mL of the solution was added to 1 mL of the essential oils at different concentrations (25, 50, 75, 100 µg/mL). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Ascorbic acid (4 mg/mL in ethanol) was used as positive control while ethanol was used as negative control. Then the absorbance was measured at 517 nm by using Ultraviolet Visible Spectrophotometer TG 50 Plus UV-Vis microplate reader (Molecular Devices, GA, USA). Data were presented as mean and standard deviation for triplicate analysis. The percent DPPH scavenging effect was calculated using the following equation

$$\text{DPPH Scavenging effect (\%)} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance of the prepared of essential oils. The IC_{50} value represented the concentration of the compounds that caused 50% inhibition of DPPH radical formation.

2.3.2 Ferric reducing antioxidant power assay (FRAP)

In the FRAP assay, the method of Pham-Huy *et al.* [16] was adopted and the absorbance at 700 nm was measured using Ultraviolet Visible Spectrophotometer against a blank. Gallic acid was used as the control. The percentage FRAP Scavenging effect was calculated using the equation.

$$\text{FRAP Scavenging effect (\%)} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance of the prepared essential oils [16].

2.3.3 Nitric oxide radical scavenging assay.

The method described by Okoh *et al*. [17] was adopted. Nitric oxide radicals were generated from a sodium nitroprusside solution; Sodium nitroprusside (1 mL of

10 mM) was mixed with 1 mL of oils to give concentrations of 0.025–0.50 mg/mL in phosphate buffer. The mixture was incubated at 25 °C for 150 min. To 1 mL of the incubated solution, 1 mL of Griess' reagent was added. The absorbance was measured at 546 nm using a UV/VIS TG 50 Plus UV-Vis microplate reader (Molecular Devices, GA, USA). Ascorbic acid was used as the positive control. The % inhibition of nitric oxide radical by the oil was calculated using the equation.

Nitric oxide scavenged (%) = $((A_{control} - A_{test})/A_{control}) \times 100$

where $A_{control}$ = absorbance of control sample and A_{test} = absorbance in the presence of the samples of extracts or standards.

2.3.4 Lipid peroxidation

In this assay, 10 µL of Essential oils at different concentrations of 25,50,75 and 100 µg/mL or standard solution, (1,1,3,3-tetramethoxypropane, TEP) and 40 µL of 20 mM phosphate buffer (pH 7.0) were added to a test tube on an ice bath. In each tube, 50 µL of 3% sodium dodecyl sulfate (SDS), 200 µL of 0.1 N HCl, 30 µL of 10% phosphotungstic acid, and 100 µL of 0.7% of 2-thiobarbituric acid (TBA) were added. The tubes were firmly closed and boiled at 100°C for 30 min in water bath. The reaction mixture was mixed with 400 µL of n-butanol and then centrifuged at 3000 rpm for 10 min. Supernatants were collected and passed through a UV/VIS spectrophotometer at a wavelengths of 515 nm/555 nm [18]. The percentage of inhibition of lipid peroxide was calculated using the equation described in the DPPH assay.

Lipid Perioxidation (%) = $((A_{control} - A_{test})/A_{control}) \times 100$

where $A_{control}$ = absorbance of control sample and A_{test} = absorbance in the presence of the samples of extracts or standards.

2.4 Antimicrobial Assay

The antimicrobial activity of the essential oil of *Curcuma longa* L. was assayed using Agar well diffusion technique [19-20]. The inocula were prepared from the typed bacterial *S. aureus* (ATCC 29213), *S. mutans, E. coli* (ATCC 25922) and yeast cultures *Candida albicans* (ATCC 10231) which were maintained in glycerol-peptone water at 4°C in the pure culture laboratory of Microbiology Department, University of Lagos, Akoka-Yaba, Lagos, Nigeria and were sub-cultured into sterile peptone water in McCartney bottles. The densities of the bacterial suspensions were determined by diluting the broth cultures 1:100 (mixing 0.1mL of the inoculum and 9.9 mL of sterile normal saline). These were compared with 0.5 McFarland standards. These suspensions were estimated to 1.0 x106 - 107 CFU/mL. Standard broad spectrum antibiotic discs -Ciprofloxacin and Pefloxacin (10µg –Maxicare Med. Lab, Nig) were used as positive control while Hexane served as negative control.

3. Results and discussion

3.1 Chemical composition

The yield of rhizome essential oil obtained by hydro distillation was 1.4 % volume per dry weight of the sample. The analysis by Gas Chromatography-Mass Spectrometry (GC-MS) identified 37 volatile compounds. The essential oil of *Curcuma longa* L analyzed was composed mainly of monoterpenes, sesquinterpenes and diterpenes accounting for 75.95% of the essential oil. The major components are p-menthane-1,8-diol (17.26%), 3-ethylthiophene (9.47%), trans-ascaridol glycol (7.98%), 3-methyl-2-(3-methylpentyl)-3-buten-1-ol (7.93%), carvone hydrate (6.78%), bis(2-ethylhexyl) phthalate (6.35%) 2-ethylcyclohexanone (5.30%), cyclohexanone, 4-hydroxy-4-methyl- (4.62%), α-phellandrene-8-ol (4.32%) and sobrerol (5.94%) as shown in Table 1 and Fig 3. The composition of the oil was found to be different from the rhizome oil of North Central and South-West grown *Curcuma longa* L. in Nigeria [21-22]. Usman *et al.* reported that the major constituents of rhizome essential oil of *Curcuma longa* L. from north-central Nigeria were, β-bisabolene (13.9%), trans-ocimene (9.8%), myrcene (7.6%),1,8-cineole (6.9%), α-thujene (6.7%) and thymol (6.4%) in contrast to ar-tumerone the most abundant constituent of the oil obtained from the rhizome of the south-west grown *Curcuma longa* L. [21. In addition, studies showed that five different varieties of *C. longa* were cultivated in North Alabama, the rhizome essential oils obtained by hydro distillation, and analyzed by gas chromatographic techniques. The major components in the essential oils were α-phellandrene (3.7–11.8%), 1,8-cineole (2.6–11.7%), α- zingiberene (0.8–12.5%), β-sesquiphellandrene (0.7–8.0%), ar-turmerone (6.8–32.5%), α-turmerone (13.6–31.5%), and β-turmerone (4.8–18.4%) [22] which is similar in composition to the rhizome grown in the North Central Nigeria [21].

Table 1. Chemical composition of the essential oil of rhizome of *Curcuma longa* L.

Compounds	Composition (%)	RI_{cal}	RI
6-Methyl-2-heptyne	0.43	772	770
Cyclopropanecarboxylicacid	0.48	780	777
Camphocean	0.58	793	795
7-Oxabicyclo[4.1.0] heptane, 2-methylene	0.56	795	797
Octane	0.64	819	816
3-Ethylthiophene	9.47	898	894
Cis-8-methyl-exo-tricyclo[5.2.1.0(2.6)]decane	0.61	944	942
2-Fluoro-4-methylanisole	1.12	956	958
β-PicolineN-oxide	1.03	996	999
2,6-Dimethylcyclohexanol	0.9	1028	1030
2-Ethylcyclohexanone	5.3	1047	1051
Benzenamine,N,N,2-trimethyl-	0.54	1053	1055
trans-2,7-Dimethyl-4,6-octadien-2-ol	0.51	1103	1100
Cyclohexanone,4-hydroxy-4-methyl-	4.62	1104	1102
α-Phellandrene-8-ol	4.32	1128	1125
Menthane,1,2,4-trihydroxy	2.37	1126	1128
Fenchol	0.42	1134	1138
endo-Borneol	0.42	1140	1138
Terpineol	0.89	1141	1143
β-Terpineol	0.98	1160	1158
cis-Dihydrocarvone	0.47	1182	1179
Myrtenol	2.27	1194	1191
3-Methyl-2-(3-methylpentyl)-3-buten-1-ol	7.93	1198	1196
Carvone hydrate	6.78	1204	1202
2-Hydroxy-1,8-cineole	0.48	1249	1247
trans-Ascaridolglycol	7.98	1261	1259
Fenchyl acetate	0.6	1275	1277
p-Menthane-1,8-diol	17.26	1280	1282
Menthoglycol	0.51	1324	1320
Sobrerol	5.94	1345	1349
Perillartine	1.91	1376	1374
Eugenol	0.93	1394	1392
5,5-Dimethyl-4-(3-oxobutyl) dihydro-2(3H)-furanone	2.71	1457	1455
(-)-Myrtenylisovalerate	0.63	1550	1548
Oxalicacid,6-ethyloct-3-ylisobutylester	0.46	1617	1619
2-cis,cis-9,12-Octadecadienyloxy-ethanol	0.6	2340	2344
Bis(2-ethylhexyl)phthalate	6.35	2703	2704
Total oil content	**100%**		

RI_{cal}: Retention index determined relative to *n*-alkanes (C7-C30) on the HP-5ms column. RI: literature retention indices

Zhang et al. [23] collected samples from 20 different locations in China and found that the composition and bioactivity of the essential oils varied. Among the most abundant components, they found ar-turmerone, β-turmerone, α-zingiberene, ar-curcumene, and β-sesquiphellandrene. These authors found antioxidant and antimicrobial activities, which varied according to the origin of the samples used. Several factors can interfere with the chemical variation of essential oils in plants, such as temperature, humidity, luminosity, altitude, pluviometry, ultraviolet radiation, soil and nutrient conditions, seasonality, circadian cycle, method of collection, drying and part of the plant [24].

3.2 Antimicrobial activity

The antimicrobial potential of essential oil of rhizome of *Curcuma longa* L. using Agar well diffusion technique exhibited significant in vitro antimicrobial activity against *Escherichia coli* (38.00±1.0 mm) and *Streptococcus mutans* (29.00±0.0 mm) as shown in Table 2.

Table 2. Antimicrobial activity of *Curcuma longa L.* essential oil using the Agar well diffusion method.

Microorganisms	Zones of inhibition		
	CL	CPX	PEF
Staphylococcus aureus	21.00±1.4	29.00±0.7	25.00±0.0
Escherichia coli	38.00±2.8	27.00±0.0	26.00±0.7
Streptococcus mutans	29.00±4.9	27.00±0.7	27.00±0.0
Candida albicans	23.00±0.6	29.00±0.0	26.00±0.0

Abbreviations: *CL- Curcuma longa L.*; CPX, Ciprofloxacin; PEF, Pefloxacin. Results are means of triplicate values.

The results compare well with that of the standard drugs (25.00-29.00 mm). The promising antimicrobial activity of the essential oil could be attributed to the presence of curcuminoids. It was reported that the essential oil of *Curcuma longa* L. has antimicrobial activity against Gram- positive bacteria (*Bacillus subtilis and Staphylococcus aureus)* and *Pennicilium citrinum*. No antimicrobial activity was found against *Escherichia coli, Salmonella Enteritidis* and *Aspergillus niger* [8]. Turmeric essential oil is extracted from the roots of turmeric plant and just like turmeric powder, it has anti-allergic, anti-bacterial, anti-microbial, anti-fungal and anti-parasitic qualities. It is also loaded with antioxidants [24].

3.3 Antioxidant activity

The antioxidant activity of the essential oil of *Curcuma longa* L. was evaluated by measuring its radical scavenging activity using DPPH, Ferric Reducing antioxidant power (FRAP), Nitric Oxide and Lipid peroxidation methods. The IC_{50} radical scavenging ability of DPPH (68.14 µg/mL), FRAP (10879.57 µg/mL), Nitric oxide Scavenging activity (23.19 µg/mL), lipid peroxidation scavenging activity (107.08 µg/mL) was obtained as shown in Table 3.

Comparatively, strong nitric oxide scavenging activity was observed in the essential oil (IC_{50} of 23.19 µg/mL) compared to that of the standard drug (IC_{50} of 34.24 µg/mL), while weak antioxidant activity was observed in Lipid Peroxidation scavenging activity of

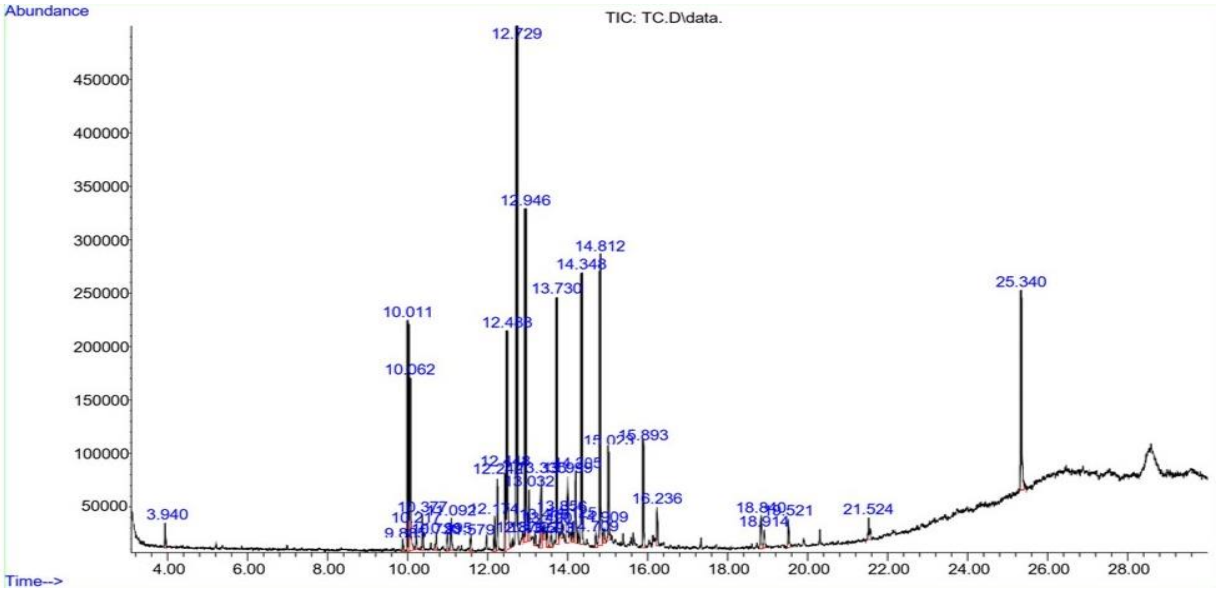

Figure 3. Total ion chromatogram of air-dried rhizome essential oil of *Curcuma longa L.*

Table 3. Antioxidant Assays of the essential oil of *Curcuma longa L.*

Antioxidant assays	IC_{50} µg/mL (*Curcuma longa L.)*	IC_{50} µg/mL (Ascorbic acid and gallic acid)
DPPH (%Inhibition)	68.14	29.61
FRAP (%Inhibition)	10879.57	8466.44
NO (%Inhibition)	23.19	34.24
LP (%Inhibition)	107.08	34.24

DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, Ferric reducing antioxidant power; NO: Nitric oxide; LP, Lipid peroxidation.

observed in Lipid Peroxidation scavenging activity of the oil (IC_{50} of 107.08 µg/mL) compare to the standard drug used (IC_{50} of 34.24 µg/mL). The standard drug (Ascorbic acid) used demonstrated higher antioxidant activity for DPPH and Ferric reducing antioxidant power (FRAP) than the essential oil. The strong nitric oxide scavenging activity of the essential oil indicates its ability to counteract the effect of nitric oxide formation and in turn may be of considerable interest in preventing the ill effects of nitric oxide on the skin.

4. Conclusions

The essential oil *Curcuma longa* L. was composed majorly of monoterpenes, sesquiterpenes and diterpenes. The composition of the rhizome oil grown in North West Nigeria was found to be different from the rhizome oil of North Central and South West grown *Curcuma longa* L. in Nigeria. The essential oil showed promising antibacterial and antioxidant capacity. The results propose the incorporation of the essential oil of rhizome of *Curcuma longa* L in cosmetic products to eliminate skin infections and as preservatives.

Authors' contributions

Conceptualization, O.T.A.; Methodology, O.T.A. and J.O.O.; Formal analysis, O.O.F, I.S.N and J.O.O; investigation, O.O.F.; Resources, O.O.F; Data Curation, I.S.N; Writing - Original Draft Presentation, O.O.F; Writing- Review & Editing, O.T.A., O.O.F and I.S.N.; Supervision, O.T.A.; Project Administration, O.T.A.

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Availability of data and materials

All relevant data are within the paper and its supporting information files. Additional data will be made available on request according to the journal policy.

Conflicts of interest

The authors agreed to this work to be published in this journal.

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