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Extraction and Characterization of the Essential Oil from the Leaves of *Ocimum basilicum* and Evaluation of its Antioxidant Properties

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Authors' contributions

This work was carried out in collaboration among all authors. Authors LL and AC designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors LL, AC and AA managed the analyses of the study. Author AC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The essential oil was extracted from the leaves of *Ocimum basilicum* using a modified steam distillation method. The chemical composition and free radical inhibition property of the plant essential oil were investigated. Results from gas chromatography-mass spectrometry revealed the presence of 39 different compounds in the essential oil of *Ocimum basilicum*. The major components, Terpinen-4-ol (21.31%), Eucalyptol (18.97%), α-Terpineol (14.41%), γ-Terpinene (12.61%), Caryophyllene (11.90%), α-Pinene (6.11%), and β-Myrcene (5.54%), were found in high percentages. The antioxidant activity of the essential oil was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide free radical scavenging assays, with Ascorbic acid as a standard drug. The results showed that the essential oil extracted from *Ocimum basilicum* leaves exhibited positive effects at different concentrations when tested using both methods. At a concentration of 2.5 µL/mL, the *Ocimum basilicum* essential oil significantly scavenged 60.89% of

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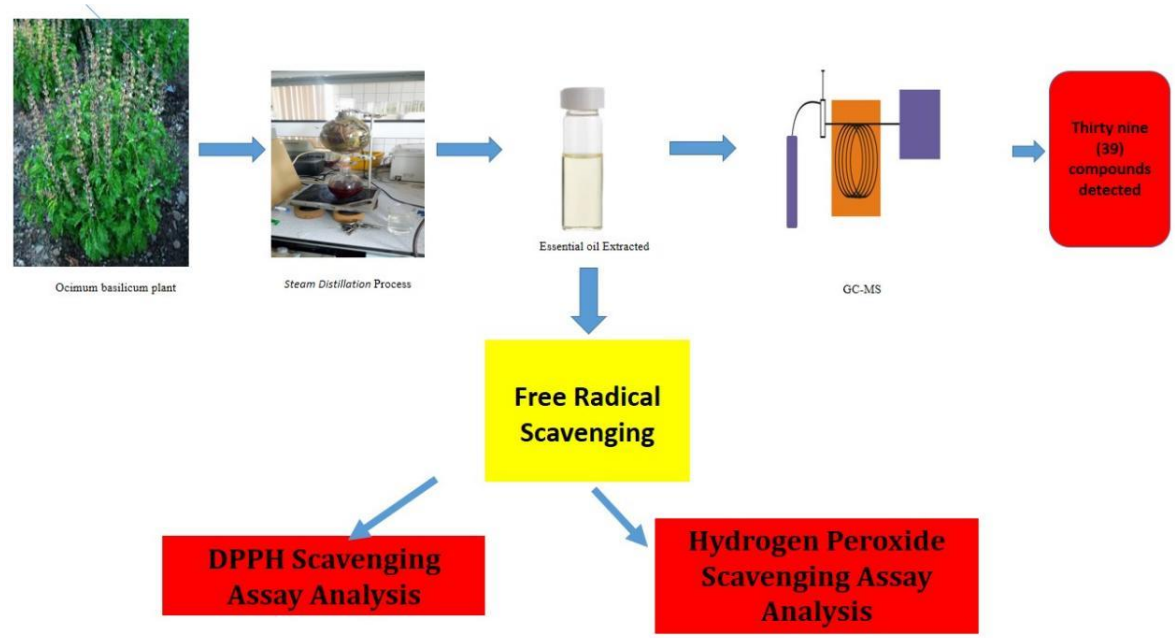
hydrogen peroxide radicals, while the standard Ascorbic acid scavenged 79.13%. Similarly, at a concentration of 10 µL/mL, the plant's volatile oil inhibits 87.12% of DPPH radicals, while the standard Ascorbic acid inhibits 90.40%. This study highlights the potential of the essential oil derived from *Ocimum basilicum* leaves as a valuable source of compounds with free radical scavenging properties, consistent with its traditional medicinal applications.

Graphical Abstract showing the entire experimental process:



Keywords: Antioxidant; radical; concentration; Ocimum basilicum; scavenging activity.

1. INTRODUCTION

"The term "essential oil" refers to volatile substances derived from plants, consisting of a complex mixture of terpenic hydrocarbons and oxygenated derivatives such as aldehydes, ketones, esters, and alcohols" [1]. "Over 1,200 compounds, including terpenes and their respective aldehydes, ketones, alcohols, phenylpropanoids, hydrocarbons, esters, oxides, and sulfur compounds, have been identified in essential oils. Generally, essential oil constituents include terpenes (monoterpenes and sesquiterpenes), aromatic compounds (aldehydes, alcohols, phenols, methoxy derivatives, etc.), and terpenoids (isoprenoids)" [1-3].

"Basilicum, derived from the Latin translation of the Greek word "basilikon" meaning king, holds the name "Herbe Royale" in French. The Urdu/Punjabi name Niazbo also reflects its pleasant fragrance" [4]. "Aromatic plants have long been used for various purposes such as food, food additives, cosmetics, and medicine. In recent decades, there has been an increase in the general population's preference for herbal medications over synthetic drugs due to their inherent safety and cost-effectiveness" [5]. "*Basilicum* is also cultivated for ornamental purposes" [6]. "In the early 1600s, the English used *basilicum* in food and as a means to ward off unwelcome pests like flies and evil spirits" [7]. "The *Ocimum* genus comprises more than 150 species and has been traditionally employed in treatments for diuretic function, constipation, intestinal pain, as a galactogogue, for headaches, coughs, diarrhea, warts, worms, kidney issues, and as an anti-inflammatory and antispasmodic agent" [8,9]. "Basil has demonstrated antioxidant, antimicrobial, antitumor, antiviral, anticancer, and antibacterial activities attributed to its phenolic acids and aromatic compounds. The medicinal value of any plant is reliant on its bioactive phytochemical components such as phenolics, alkaloids,

terpenes, flavonoids, tannins, and saponins, as these components produce specific physiological effects in the human body" [10-12]. "Traditionally, basil has been used as a medicinal plant for various ailments such as headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunctions" [13]. "Moreover, it has been utilized in folk medicine for managing fever, nausea, migraines, poor digestion, abdominal cramps, gastroenteritis, insomnia, exhaustion, and dysentery" [14].

Ocimum basilicum is a rich source of β-carotene, which helps prevent cellular damage caused by free radicals. Additionally, basil contains essential nutrients like iron, calcium, potassium, and vitamin C [15].

This research aims to extract and investigate the therapeutic properties of essential oils derived from the leaves of *Ocimum basilicum*. The objectives include determining the presence of essential oils in the plant, extracting the essential oils using the steam distillation method, analyzing the essential oil composition through Gas Chromatography/Mass Spectrometry, and evaluating the antioxidant activity of the extracted essential oils. These findings will lay the foundation for the plant's potential applications in traditional medicine or pharmacy.

2. EXPERIMENTAL DETAILS

2.1 Sample Collection

Ocimum basilicum, a member of the *Lamiaceae* family, was collected from Sukur Settlement, Madagali Local Government Area of Adamawa State, Nigeria on August 14th, 2019, in the early morning. Random leaf samples were collected and labeled accordingly, then stored in an ice cooler until transported to the laboratory for extraction and further analysis [16].

2.2 Steam Distillation Extraction Essential Oil

1 kg leaves of *Ocimum basilicum* samples collected was used for extraction per time to prevent loss of essential oils due to the drying process. The extraction was carried out using a modified steam distillation apparatus, where the receiver end passed through another vessel containing ice. The essential oils of the plant was collected over water and later kept at 4 °C until further required. The isolation process took approximately 2½ hours [17]. This process was repeated for each plant batch until a total mass of 2.6835kg was used for extraction. The steam distillation apparatus is Clevenger-like as described in the British Pharmacopoeia (BP) [18].

2.3 Spectrometric Analysis of Essential Oil

The essential oil extracted from the leaves of *Ocimum basilicum* was analyzed for its chemical constituents using gas chromatography coupled with mass spectrometry (GC-MS). An Agilent 190915-433:469.56509 Gas Chromatography–Mass Spectrometry System, equipped with a split-splitless injector operating at a pressure of 11.649 psi, was utilized. Helium was used as the carrier gas at a flow rate of 1 mL/min. The capillary column used was HP-5MS (30 m × 250 µm × 0.25 µm), with a stationary phase of 5 % phenyl methyl silox. The initial temperature was set at 60 °C for 0.5 minutes, followed by an increase of 10 °C/min up to 300 °C for 3 minutes, and then a constant temperature at 310 °C for 22.5 minutes. "A sample volume of 0.2 µL was injected into the column programmed at 310 °C, and the components were identified by comparing their retention indices and mass spectra with those obtained from the NIST library" [19].

2.4 Free Radical Inhibition Analysis

2.4.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

"The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed following a standard method with minor modifications. The hydrogen atom or electron donating abilities of the compounds were measured based on the decolorization of the purple-colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). In this spectrophotometric assay, the stable free radical DPPH was used as the reagent. Various concentrations (2.5 µL/mL) of the essential oil in ethanol were added to 4 mL of a 0.004 % methanol solution of DPPH. After incubation at room temperature for 30 minutes, the absorbance was measured at 517 nm and compared to the standard antioxidant, Ascorbic acid (vitamin C)" [20]. The DPPH radical scavenging effect was calculated as the percentage of inhibition (I %) using the following formula:

I % = absorbance of A Blank - absorbance of A sample / absorbance of A Blank X 100

where A blank is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound. The inhibition values were determined for various extract concentrations, and the tests were performed in triplicate and the mean values reported.

2.4.2 Hydrogen peroxide scavenging activity

The ability of the essential oil to scavenge hydrogen peroxide (H_2O_2) was determined using a modified method. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer with a ρH of 7.4. Volatile oil (2.5-25 µg/mL) in methanol were added to the H_2O_2 solution (0.6 mL, 40 mM). The absorbance of the reaction mixture was measured at 230 nm. The blank solution contained the phosphate buffer without H_2O_2 [21]. The percentage of H_2O_2 scavenging was calculated as:

> H_2O_2 scavenging effect (%) = absorbance of A control- absorbance of A sample / absorbance of A control x 100

where A control is the absorbance of the control, and A sample is the absorbance in the presence of the sample or standards.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield of the Essential Oils from *Ocimum basilicum*

2.6835 kg of *Ocimum basilicum* fresh leaves were subjected to steam distillation for the extraction of essential oil components from the plants. Results obtained showed that the essential oil of *Ocimum basilicum* has a percentage yield of 0.11%. The percentage yield was obtained by using the relation:

> % Yield= Weight of oil (g) / Weight of plant (g) x 100

3.2 Essential Oil Compositions of *Ocimum basilicum*

The essential oil of the leaves of *Ocimum basilicum* was extracted using a steam distillation method and appeared as colorless liquids. Subsequently, a detailed GC–MS analysis was conducted to determine the volatile constituents. In total, 39 volatile constituents were identified in the essential oil of *Ocimum basilicum* leaves. The identified compounds, their relative area percentages, and retention indices are summarized in Table 2. The spectrum from the GC-MS machine is shown in Fig. 1.

While the essential oil of *Ocimum basilicum* contained high percentages of the same group of terpenes, significant variability in chemical compositions was observed among different varieties. A total of 32 constituents, accounting for 99.99% of the oil, were identified. The dominant components in the essential oil were Terpinen-4-ol (21.31%), Eucalyptol (18.97%), α-Terpineol (14.41%), γ-Terpinene (12.61%), Caryophyllene (11.90%), α-Pinene (7.52%), β-Myrcene (5.54%), trans-β-Ocimene (1.79%), (+)-4-Carene (1.38%), and Dihydrocarvyl acetate (1.06%). This composition of essential oil is similar to the findings of Pripdeevech et al [22], where the essential oil of *Ocimum basilicum* leaves contained α-Pinene, Camphene, β-Pinene, γ-Terpinene, β-(E)-Ocimene, Terpinen-4-ol, α-Terpinene, γ-Terpinen-4-ol, δ-Terpinen-4-ol, iso-Bornyl acetate, and (E)-Caryophyllene.

“In a related study, the essential oil of *Ocimum basilicum* was found to contain linalool (69%), eugenol (10%), t-α-bergamotene (3%), and thymol (2%) as the major constituents” [23]. Similarly, Klimankova et al*.* [24] reported “Linalool, methyl chavicol, and eugenol as the major compounds in their investigation”. “Another study conducted in the Islamic Republic of Iran identified twenty-five constituents in the essential oil of *Ocimum basilicum* leaves, including methyl chavicol (85.19%), 1,8-cineol (3.96%), trans-alpha bergamotene (1.185%), linalool (1.03%), eugenol (0.7%), and γ-terpinene (0.53 %)” [25]. The results of hydrodistillation of essential oil from *Ocimum basilicum* revealed the presence of α-Pinene, Camphene, β-Pinene, α-Terpinene, trans-Caryophyllene, γ-Terpinene, and 4-Terpineol [26], which are similar constituents found in the present study.

Furthermore, researchers [27] reported that “the GC–MS analysis of the essential oil of *Ocimum basilicum* revealed fifty-nine compounds, with the major compounds being estragole (52.3%), linalool (15.7%), trans-α-bergamotene (7.29%), and 1,8-cineol (5.56%). These major compounds accounted for 80.9 % of the overall composition of the essential oil”. According to Tshilanda et al*.* [28], “the overall composition of the essential oil of *Ocimum basilicum* consisted of monoterpenes (0.67%), oxygenated monoterpenes (26.75%), non-oxygenated sesquiterpenes (14.41%), oxygenated sesquiterpenes (10.21%), oxygenated hydrocarbons (0.04%), aromatic hydrocarbons (0.02%), and oxygenated aromatic hydrocarbons (46.00%)”. In a study conducted in the Western Ghats of North West Karnataka,

India, [29] identified β-Pinene, Terpin-4-ol, and α-Terpineol as related constituents in the essential oil of *Ocimum basilicum*.

The presence of α-pinene, camphene, β-pinene, β-myrcene, trans-β-ocimene, δ-3-carene, γ-terpinene, α-terpinene, terpinen-4-ol, β-caryophyllene, and Ugbogu et al.caryophyllene oxide was reported in the essential oil of *O. gratissimum*, another species of the genus *Ocimum*, by Ugbogu et al. [30]. In the essential oil of *Ocimum basilicum*, methyl chavicol, geranial, neral, beta-caryophellene, 6-methyl-5-heptene-2-one, alpha-humulene, and germacrene-D were among the dominant components by percentage area [31]. Poonkodi [32] identified the main constituents in the essential oil of *Ocimum basilicum* as Linalool, 1,8-cineol, eugenol, methyl cinnamate, camphor, methyl eugenol, methyl chavicol, β-elemene, β-ocimene, camphene, carvacrol, α-bergamotene, α-cadinol, and geranial. Similarly, Stan et al. reported that the essential oils of *Ocimum basilicum* varieties, Yellow basil and Red-violet basil, were characterized by high concentrations of linalool, accounting for 32.66 % and 52.18 % of the respective compositions, compared to the other compounds [33].

Table 1. Percentage yield of the essential oils of *Ocimum basilicum*

Plant	Plant Part Used	Weight (kg) of Plant	Weight of Oil (g)	Appearance	% Yield (WO/WP) x 100
Ocimum basilicum	Fresh Leaves	2.6835	2.83	colourless	0.11

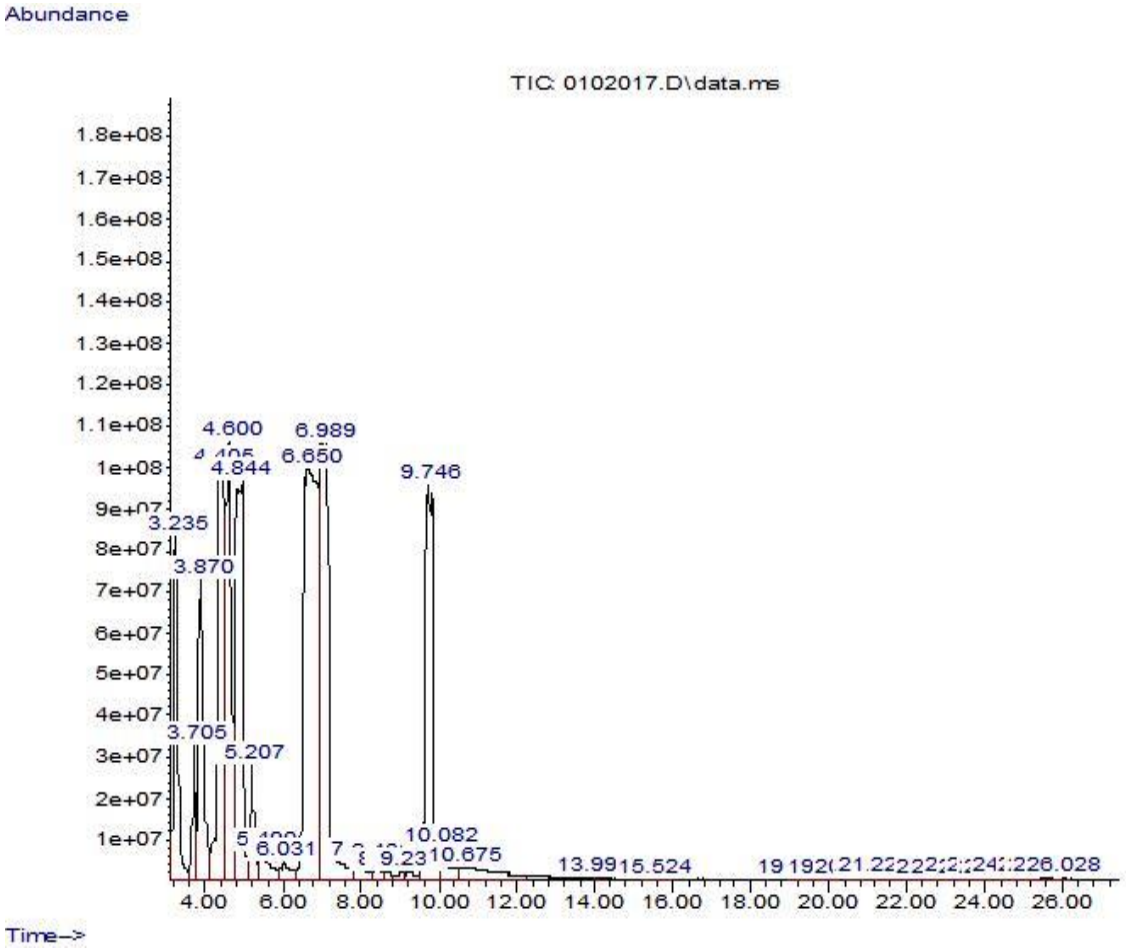


Fig. 1. GC-MS Spectrum of essential oil extracted from *Ocimum basilicum*

Table 2. GC-MS analysis of the essential oil from *Ocimum basilicum* leaves

S/N	Constituents	Molecular Formula	Retention Time (Min)	Area (%)
1.	*α*-Pinene	$C_{10}H_{16}$	3.231	6.11
2.	Bicyclo [3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	$C_{10}H_{16}$	3.708	1.41
3.	*β*-Myrcene	$C_{10}H_{16}$	3.867	5.54
4.	Eucalyptol	$C_{10}H_{18}O$	4.405	10.93
	Eucalyptol	$C_{10}H_{18}O$	4.601	8.04
5.	*γ*-Terpinene	$C_{10}H_{16}$	4.844	12.61
6.	(+)-4-Carene	$C_{10}H_{16}$	5.207	1.38
7.	Dihydrocarvyl acetate	$C_{12}H_{20}O_2$	5.487	1.06
8.	1,4-Cyclohexanedimethanol	$C_6H_8O_2$	6.032	0.63
9.	Terpinen-4-ol	$C_{10}H_{18}O$	6.653	21.31
10.	*α*-Terpineol	$C_6H_{10}O$	6.986	14.41
11.	Bornyl acetate	$C_{12}H_{20}O_2$	7.909	0.58
12.	Myrtenyl acetate	$C_{12}H_{18}O_2$	8.431	0.36
13.	2-Carene	$C_{10}H_{16}$	8.636	0.17
14.	Cyclohexene, 3-methyl-6-(1-methylethylidene)-	$C_{10}H_{16}$	9.029	0.20
15.	Camphene	$C_{10}H_{16}$	9.241	0.27
16.	Caryophyllene	$C_{15}H_{24}$	9.748	11.90
17.	Santolina triene	$C_{10}H_{16}$	10.081	0.91
18.	trans-*β*-Ocimene	$C_{10}H_{14}$	10.679	1.79
19.	Alloaromadendrene	$C_{15}H_{24}$	13.994	0.03
20.	1,5,9,13-Tetradecatetraene	$C_{14}H_{22}$	15.523	0.02
21.	Tetracosane	$C_{24}H_{50}$	19.149	0.01
22.	Tricosane, 2-methyl-	$C_{24}H_{56}$	19.883	0.01
23.	Nonadecane, 1-chloro-	$C_{19}H_{40}$	20.617	0.02
24.	Phthalic acid, dodecyl oct-3-yl ester	$C_{30}H_{58}O_4$	21.116	0.05
25.	Bis(2-ethylhexyl) phthalate	$C_{30}H_{50}O_4$	21.253	0.08
26.	Tricosane, 2-methyl-	$C_{24}H_{50}$	22.040	0.04
27.	Undecane, 1-bromo-	$C_{11}H_{23}Br$	22.706	0.02
28.	Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester	$C_{15}H_{24}O_2$	23.099	0.00
29.	1-Chloroeicosane	$C_{20}H_{14}Cl$	23.410	0.03
30.	Octadecane, 1-chloro-	$C_{18}H_{37}Cl$	23.758	0.00
31.	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	24.008	0.02
32.	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	24.333	0.00
33.	13-Methyl-Z-14-nonacosene	$C_{30}H_{60}$	24.416	0.01
34.	Eicosane	$C_{20}H_{42}$	24.659	0.02
35.	1-Octadecene	$C_{18}H_{36}$	25.317	0.02
36.	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	25.506	0.00
37.	Methoxyacetic acid, heptadecyl ester	$C_{20}H_{40}O_3$	25.612	0.00
38.	Erucic acid	$C_{22}H_{42}O_2$	25.711	0.00
39.	Estra-1,3,5(10)-trien-17β-ol	$C_{18}H_{24}O$	26.029	0.00

The hydrodistillation of the essential oil of two species of the genus *Ocimum, Ocimum basilicum L.* and *O. minimum L*., revealed a total of 49 and 41 components, respectively. The main components in the essential oil of *Ocimum basilicum* were methyl eugenol (78.02%), α-cubebene (6.17%), nerol (0.83%), and (-muurolene (0.74%). On the other hand, O. minimum contained geranyl acetate (69.48%), terpinen-4-ol (2.35 %), and octan-3-yl acetate (0.72%) as the main components [34]. Another study by Chenni et al. compared the essential oil composition of *Ocimum basilicum* using two extraction methods: Hydro-Distillation (HD) and Solvent-Free Microwave Extraction (SFME). The main components identified were linalool (43.5% SFME, 48.4% HD), methyl chavicol (13.3% SFME, 14.3% HD), 1,8-cineole (6.8% SFME, 7.3% HD), methyl eugenol (6.1% SFME, 3.7% HD), and α-Bergamotene (2.7% SFME, 2.5%

HD) [35]. Furthermore, the analysis of *Ocimum basilicum* revealed that the major components in the essential oils were linalool (48.69%), 1,8-cineole (14.00%), trans-α-bergamotene (8.23%), and eugenol (6.64%) [14]. Studies on two species of basil, *Ocimum basilicum L.* and *Ocimum americanum L.*, showed that Linalool (41.2%) and estragole (30.1%) were the major compounds in the essential oil of *Ocimum basilicum*, while carvotanacetol (38.4%) and estragole (27.5 %) were the main compounds identified in the essential oil of *O. americanum* [3]. Moreover, Avetisyan et al. reported that *Ocimum basilicum var. purpureum* predominantly contained methyl chavicol (57.3%) and linalool (18 %) as major components, *Ocimum basilicum var. thyrsiflora* contained methyl chavicol (20%) and linalool (68%), and *Ocimum citriodorum* contained Nerol (23%), Geranial (15%), Methyl chavicol (9.45 %), β-Bisabolenene (8.31%), and β-Caryophyllene (7.80%) as the main compounds [36].

The variation in volatile oil composition observed in this present study compared to other researches from other location might be as a result of the difference in environmental and ecological conditions, as well as genetic factors , as affirmed by Pripdeevech et al. [22].

3.3 Percentage Inhibition of DPPH Free Radical by Essential Oils of *Ocimum basilicum* leaves at 517nm

The present study aimed to investigate the free radical scavenging activity of essential oils extracted from *Ocimum basilicum* leaves by using DPPH assay. The results obtained are presented in Table 3. The scavenging ability of the essential oil and that of Ascorbic acid at various concentrations (2.5, 5, 7.5, and 10 µL/mL) were measured, and the percentage inhibition dependent on activity profile. The plant's essential oil showed a strong free radical scavenging activity at 10 µL/mL (87.12%), which was comparable to the standard. The results suggests that the plant is an effective antioxidant compared to Ascorbic acid, having a scavenging activity of 90.40%. A similar investigation conducted by Shafique et al. [37] also revealed that *Ocimum basilicum* essential oil has significantly higher antioxidant activity than the standard Butylated hydroxytoluene (BHT), with the percentage inhibition of DPPH ranging from 90.04% at the lowest concentration to 96.16% at the highest concentration. In addition, Lavany et al. [38] reported that *in vitro*, *Ocimum basilicum* extracts possess good free radical scavenging activity using DPPH and catalase method.

The antioxidant property of *Ocimum basilicum* is useful in retarding oxidative deterioration of food materials, especially those with high lipid contents, and also protects living cells from oxidative damage that occurs due to the formation of free radicals and reactive oxygen species during metabolic activity [37]. The damage caused by free radicals contributes to the etiology of various chronic health problems, such as cardiovascular and inflammatory disease, cataract, and cancer [39]. According to reports by Jafri et al. [40] Hassan et al. [41], the percentage free radical scavenging activity of *Ocimum basilicum* against DPPH were 49.80 ± 1.76, 45.63 ± 1.51 and 13.38 ± 1.94%, for ferric reducing antioxidant power (FRAP), the results obtained are 46.57 ± 0.78, 44.54 ± 0.45 and 14.22 ± 1.20 while for 2,2-azinobisethylbenzothiozoline-6-sulphonic acid (ABTS) assays the results are 47.75 ± 0.80, 47.49 ± 0.66, 14.96 ± 1.52 % respectively for ethanolic, methanolic and aqueous extracts. Furthermore, [42] confirmed that "the free radical inhibition of the extracts used showed significant activity against DPPH at the concentration of 4 mg/mL, with *Ocimum basilicum* dichloromethane extract exhibiting the highest scavenging activity (64.12 ± 0.23%), while *Ocimum basilicum* leaves hexane extract displayed the lowest scavenging activity (23.85 ± 0.09%)". "In addition, the 2,2-azinobisethylbenzothiozoline-6-sulphonic acid (ABTS) Cation de-Colorization assay of *Ocimum basilicum* extracts showed that all the extracts possessed good antioxidant activity at a concentration of 100 g/mL. The DPPH scavenging activity of the extracts were used to monitor the ability of the ethanol and hexane extracts of *Ocimum basilicum* seeds to mop up free radicals that may be found in animals and humans" [43].

A related research study revealed that two varieties of *Ocimum basilicum* (Fino Verde Variety and Genovese Variety) demonstrated strong antioxidant activity as Verde Variety showed DDPH scavenging activity of IC_{50} 110 ±10 and 153.33 ±30.5 µg/mL at the vegetative and flowering stages, respectively, while the Genovese Variety showed DPPH activity of IC_{50} 6.13 ± 4.8 and 133.33 ± 23.09 µg/mL for the vegetative and flowering stages, respectively [44]. This results showed that the flowering stage of both varieties of *Ocimum basilicum* investigated has higher antioxidant activity than

its vegetative stage. According to Al-Maskria et al, "the DPPH radical scavenging activity of *Ocimum basilicum* (Omani basil) oil (at an initial concentration of 150 µg/mL) in various seasons were in the following order: summer (85.4%) > spring (75.2%) > winter (72.8%)" [26].

Similarly, the leaf extracts of *Ocimum gratissium* of the family *Lamiaceae* have high antioxidant potentials due to the presence of antioxidant compounds in its plant parts such as thymol, eugenol, methyl chavical, gratissimol, alkaloids, tannins, flavonoids, etc [45]. In their research, [46], reported on the antioxidant activities of T*hymus vulgaris, Mentha pulegium,* and *Mentha* belonging to the family *Lamiaceae* against DPPH, Reactive Oxygen Species (ROS), and ABTS free radicals. Furthermore, [27] evaluated the antioxidant activity of basil essential oil using DPPH and FRAP assays. The essential oil exhibited remarkable antioxidant activity in DPPH (IC_{50} 5.92 µg/mL) and FRAP (23.4 µmol Fe/g), and the high antioxidant activity of *Ocimum basilicum* essential oil may potentially relate to the bioactive compounds compared to the inhibition activity of the standard, Quercetin, with values of IC_{50} 34 µg/mL, 4.96 µmol Fe/g, and 72.3 % for DPPH and FRAP, respectively. Similarly, Tshilanda et al [28] reported that the essential oil of the plant exhibited antioxidant potential, IC_{50} value of 1.180 ± 0.015) mg/mL against DPPH assay. Another report, Aldosary et al. [47], revealed that the essential oil of the genus *Thymus* of the family *Lamiaceae* showed 20.80% inhibition compared to Ascorbic acid at 48.20% inhibition at the concentration of 10 µg/mL, while at 100 µg/mL, it showed inhibition of 81.70% against 96.30% for Ascorbic acid using the DPPH scavenging assay.

More so, Anand et al [48] reported that 100 µg/mL concentrations of the aqueous and methanolic extracts of *basilicum* have exhibited radical-scavenging of 77.85% and 80.43% against DPPH free radicals, respectively. Eid et al*.* [49] conducted a study to test the free radical scavenging activity of the extracted *Ocimum basilicum* essential oil by the DPPH radical method using trolox as a reference standard. They found that *Ocimum basilicum* essential oil has good antioxidant activity (IC_{50} value of 23.44 ± 0.9 µg/mL) compared to the standard antioxidant compound trolox (IC_{50} of 2.7 ± 0.5 µg/mL). *Ocimum basilicum* contains several active antioxidant compounds, and its antioxidant property is due to the polyphenoid rosmarinic acid, a derivative of cinnamic acid [6]. In their results, Onyenibe et al*,* reported the DPPH scavenging activity of the leaf extracts of *Ocimum gratissium* of the family *Lamiaceae* were 85.45 µg/mL (acetone extract), 80.50 µg/mL (methanol extract), 79.90 µg/mL (ethanol extract) and 78.50 µg/mL (aqueous extract). The FRAP activity of the leaf extracts were highest in the methanol extract (508.19 µg/mL), followed by the acetone extract (346.51 µg/mL), the aqueous extract (159.83 µg/mL) and the least was the ethanol extract (51.20 µg/mL) [45].

Poonkodi reported IC_{50} antioxidant activities of essential oil of *Ocimum basilicum* from four different studies with the following values 0.96 g/L, 83.54 mg/mL, 25 µg/mL, 4.8-6.7 µg/mL, and percentage inhibition of two other research investigations as 55.67± 3.38 and 92.5-94.6%. In a similar study, the antioxidant activity of *Ocimum basilicum* essential oil against DPPH assay showed IC_{50} values of 15.47± 0.5 0 and 0.85 ± 0.01 g/L, respectively, for the Yellow basil (Aromat de Buzau) and Red-violet basil (Serafim) varieties of the plant investigated [33]. Furthermore, *Ocimum basilicum* essential oil extracted by SFME exhibited a dose-dependent increase with a radical scavenging activity of 86.13 ± 2.8% at 20 mg/mL, which is higher than the DPPH % inhibition of the *Ocimum basilicum* essential oil extracted by HD with values of 76.13 ± 2.6%, at the same concentration [35]. At the same time, according to Ahmed et al, the essential oil of *Ocimum basilicum* exhibited IC_{50} 145.35 µg/mL against DPPH assay, while the standard Ascorbic acid shows a high scavenging potency for DPPH radical with IC_{50} 35.1 µg/mL [14].

Table 3. Antioxidant activity of *Ocimum basilicum* essential oil against Ascorbic Acid using DPPH Assay

(Blank solution 2.312)

S/N	Concentration (µL/mL)	Essential oil (%)	Ascorbic acid *(%)*
1	2.5	50.87	52.56
2	5	48.75	65.26
3	7.5	73.36	79.06
4	10	87.12	90.40

Table 4. Percentage Inhibition of H_2O_2 by the *Ocimum basilicum* essential oils and Ascorbic Acid at 517nm

(Blank solution 0.992)

S/N	Concentration (µL/mL)	Essential oil (%)	Ascorbic acid *(%)*
1	2.5	60.89	79.13
2	5	73.69	86.59
3	7.5	83.67	88.71
4	10	88.41	90.12

3.4 Hydrogen Peroxide Radical Scavenging Activity of Essential Oil of *Ocimum basilicum* Leaves Against Ascorbic Acid

The essential oil of *Ocimum basilicum* leaves was evaluated for its ability to scavenge hydrogen peroxide, with results presented in Table 4. The highest concentration tested was 10 µL/mL, and the essential oil showed 88.41% scavenging activity, while the standard antioxidant, Ascorbic acid, demonstrated 90.12 % activity at the same concentration. The essential oil and standard antioxidant inhibited activity in a concentration-dependent manner. Previous research assessed the scavenging potential of ethanol and hexane extracts of *Ocimum basilicum* using the hydrogen peroxide scavenging method [43]. The IC_{50} values of the reference standard, gallic acid, as well as the ethanol and hexane extracts were 204.4 ± 0.65, 623.5 ± 0.27, and >1000.0 µg/mL, respectively. In contrast, other research reported high free radical inhibition, while one investigation reported only 14.6% inhibition activity of *Ocimum basilicum* against H_2O_2 assays [27,41]. The latter study used various methods to examine the in vitro antioxidant potential of *Ocimum basilicum* essential oil, including DPPH, H_2O_2, 2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical (ABTS), nitric oxide (NO), hydroxyl radical (HO), and nitrite (NO_2) scavenging properties. The results indicated that *Ocimum basilicum* essential oil has effective free radical scavenging activities. Furthermore, the study by Ahmed et al. demonstrated that the volatile oil of *Ocimum basilicum* has potential scavenging efficacy using the β-carotene-linoleic acid bleaching assay, further highlighting the plant's anti-oxidative potency [14]. The *tyrosinase* inhibitory activity of *Ocimum basilicum var. thyrsiflora, Ocimum basilicum var. purpureum,* and *O. x citriodorum* essential oils and arbutin acid was also determined. The values calculated were 20.1 ± 1.4%, 11.5 ± 0.3%, 17.4 ± 0.9%, and 81.5 ± 2.6%, respectively [36].

Another study evaluated the scavenging of superoxide anion radicals in low concentrations (50 µg/mL) of *Ocimum basilicum* extracts and found that the methanolic extract exhibited 81.25 % scavenging activity, which exceeded that shown by the aqueous extract (71.52%) [48]. Furthermore, Tulsi (*Ocimum basilicum*) extract from leaves and stem were reported to contain phenolic compounds and exhibited great antioxidant actions, strengthening the claims of its potential antioxidant properties [50].

4. CONCLUSION

Based on the results of the antioxidant activities from the leaves of the essential oil of *Ocimum basilicum*, it has been observed that the essential oils also exhibited equivalent high radical scavenging activities when compared with standard Ascorbic acid. This suggests that the essential oil of *Ocimum basilicum* can be a viable source of natural compounds with potential applications in traditional medicine, owing to its free radical scavenging properties.

The compositional analysis of the isolated essential oils, carried out using GC-MS techniques, identified thirty-nine (39) components. However, the pharmacological effects of this plant may depend on the identified phyto-compounds. Thus, there is a need for further studies to isolate, identify, and purify the specific phyto-compounds involved in preventing and treating ailments. These studies may ultimately pave the way towards drug development.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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