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Phytochemical, GC-MS Analysis and Acute Toxicity Evaluation of Algerian *Ocimum basilicum L* in Rats

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Abstract

Sweet Basil (*Ocimum basilicum* L.) is regarded as a significant plant. They include a wide variety of bioactive substances, particularly phenolic substances which contribute to the plant's alleged health advantages. The objective of this study was to identify and characterize the phytochemical profile of sweet basil using GC-MS analysis. By using standard protocols, Bioactive molecules were extracted and qualitative tests of phytochemicals were also released as well as quantitative analyses of total phenols, total flavonoids, and total hydrolysable tannins. Using GC-MS, volatile compounds can be identified. About 147 volatile compounds in this plant were identified by the GC-MS analysis. The chemical constituents higher than 0.5 % found in aqueous extract were propanoic acid, 2-hydroxy-, ethyl ester (3.503%), butanoic acid, 4-hydroxy- (1.657%), cyclopentasiloxane, decamethyl- (1.346%), cyclotetrasiloxane, octamethyl- (0.983%), heptadecane, 2,6,10,15-tetramethyl- (0.785%), bis(tert-butyldimethylsilyl) 2,3-bis((tert-butyldimethylsilyl)oxy) fumarate (0.703%), cyclononasiloxane, octadecamethyl- (0.672%), phthalic acid, 8-bromoctyl isobutyl ester (0.655%), 3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane (0.535%), and cyclononasiloxane, octadecamethyl- (0.506%). Moreover, the TPC, TFC, and THTC of sweet basil was systematically assessed. The results demonstrate the value of sweet basil (*Ocimum basilicum L*.) which may be used in the food and health industries as promising sources of phenolic and volatile chemicals.

Key word: *GC-MS; Ocimum basilicum L.; Phytochemical screening; Phenolic compounds.*

Introduction

Medicinal plants have long played important roles in the treatment of diseases all over the world (1). Their pharmaceutical properties are based on the presence and abundance of secondary metabolites, such as terpenoids, phenolics, alkaloids, and flavonoids (2). Since 2600 BC, people have utilized plant metabolites and during the next 4,000 years, secondary metabolites were mostly used for food, medicine, and poison (3). Many of these herbal products exhibit medicinal properties as anticancer, anti-inflammatory, antioxidant, antiviral, and antimicrobial actions (4). Furthermore, medicinal plants are used in various food, beverage, and pharmaceutical industries (5). Plant-based bioactives have drawn a significant interest due to

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their advantageous effects on health, especially polyphenolic chemicals (6). Common basil is among the most essential aromatic plants (7) belonging to the family Lamiaceae and is also a yearly herb that is planted in numerous diverse parts of the world (8). Since ancient times, *Ocimum basilicum* L. has been growing and used (9). This plant named usually as sweet basil (10) and was famous as a medicinal plant and culinary herb due to its phytochemical contents (11). Different basil extracts have been found to contain a various of chemical functionalities, including phenolic acids and their esters, flavonoids, anthocyanins, tannins, phytosterols, phenylpropanoid derivatives, monoterpenes, and triterpenes (12). Because of their antioxidant, anti-inflammatory characteristics, plant-based natural compounds are being investigated more and more for pharmacological uses, either as preventative or therapeutic agents (13). Common basil is one of the most significant species from the genus of *Ocimum* (14) that including more than 60 species comprising *basilicum* (15). The aim of this study was to screen the phytochemicals of an aromatic medicinal plant (*Ocimum basilicum* L.) leaves qualitatively and based on the presence of metabolites study further restricted to explore the plant extract to quantify the volatile compounds by GC-MS studies.

Materials and Methods

Plant and extract preparation

The *Ocimum basilicum* L. plant utilized in the present research was collected in August 2022 from the El-Oued (Guemar) region. The leaves were cleaned and then dried away from direct sunlight and at room temperature. By using a mechanical grinder, the dry leaves were ground into a fine powder. Until the experiment begins, *Ocimum basilicum* L. powder is kept at room temperature in airtight containers. Prof. Youcef Hellis identified the plant material (Arid Region Scientific and Technical Research Center, Station of Touggourt).

Figure 1. Sweet basil leaves.

Aqueous extract was prepared by boiling 10 g of dried *Ocimum basilicum* L. leaves powder in 100 ml of distilled water for two hours at 50 °C. The extract was first filtered through Whatman filter paper, then cooled and macerated to room temperature for 24 hours. After that, it was evaporated using a rotary evaporator and dried in an oven (16). A yield percentage of plant extract product was calculated according to the following equation used by Okoduwa et *al*. (17):

Plant aqueous extract was subjected to standard methodology for the qulitative phytochemical analysis.

$$Percentage\ yield\ \%\ (\frac{w}{w}) = \ (\frac{Weight\ of\ extract\ (g)}{Weight\ of\ plant\ sample\ (g)})\ x\ 100 \qquad (1)$$

Quantitative phytochemical contents

The Folin-Ciocalteu method was used to estimate the total phenolic content (18). Briefly, 1000 µl of Folin-Ciocalteu (10%) reagent was mixed with 200 µl of basil aqueous extract. 800 µl of saturated sodium carbonate (7.5%) was added after 4 minutes. The absorbance at 765 nm was measured following a 2 hours incubation period at room temperature. For the standard calibration curve, gallic acid was used.

The method of (19) was used to determine the flavonoid content of plant extract.

Briefly, 500 µl of $AlCl_3$ (2%) reagent was added to 500 µl of the basil extract. The absorbance was measured at 420 nm after 1 hour at room temperature. The calibration curve established with quercetin.

Using the Folin-Ciocalteu colourimetric method, the total hydrolysable tannin content was calculated. A calibration curve was created using tannic acid as the standard. Briefly, 1 ml of plant extract was added to a 10 ml test tube together with 0.7 ml of Na_2CO_3 (7%) solution, 0.5 ml of Folin-Ciocalteu (10%) reagent, and 8.4 ml of distilled water. After 30 minutes of incubation, the absorbance was measured at 700 nm against a blank (20).

The results were expressed as milligrams of gallic acid, quercetin and tannic acid equivalents per gram of dry extract (mg of GAE, QE and TAE / g). All the experiments were carried out in triplicate.

GC-MS analysis

Plant extract of leaves were prepared in universal solvent methanol and for which 1µl plant extract was employed to quantify the volatile compounds by GC-MS analysis. For extraction of volatiles headspace solid-phase micro-extraction (SPME) with DVB/CAR/PDMS fibre was used. Firstly, the fibre was conditioned in the GC injection port at 270°C for 4 h. Then the fibre was putted to the vial with the sample using adapter for 15 min at room temperature. After that the fibre was putted to the injection port of a gas chromatograph for desorption. Desorption time was 10 min at 260°C in the splitless mode. For analysis was used a 7890A GC system (Agilent Technologies, Santa Clara, United States) coupled to a 5975C VL Triple-Axis mass detector (Agilent Technologies, Santa Clara, United States). Separation was run on a DB-5MS capillary column (25 m × 0.2 mm; 0.33 µm film thickness; J&W, Folsom, California) with helium as a carrier gas at a flow rate of 0.6 mL/min. The temperature of injector and transfer line were 260°C and 280°C, respectively. The oven program of temperature was: the initial temperature at 40°C was held for 3 min, then increased at 4°C/min to 160°C and further increased at 10°C/min to 280°C, with the final temperature held for 3 min. The masses were scanned from 33 to 333 Da. The ionization energy value was set to 70 eV.

Result interpretation of GC-MS data was estimated with the aid of the database of National Institute Standard and Technology (NIST). The comparative estimation aided to characterize unknown volatile compounds when compared with stocked NIST library to explore the available data of basil extract.

In-vivo acute toxicity

In vivo acute toxicity was performed using healthy albino rats of Wistar strain. The animals were divided into three groups of two rats each and administered orally with a single dose of aqueous extract of *Ocimum basilicum* L (control, 2 and 5 g/kg of the rat's body weight). The rats were observed for 24h to monitor their behaviour as well as mortality. The results were expressed as mean ± standard deviation (SD), calculated from duplicate determinations and the linear relationship was visually determined.

Results and Discussion

The aqueous extraction of basil (*Ocimum basilicum* L.) dried leaves allowed us to obtain yield of approximately 19.063 ± 0.321 % (Table 1).

Table 1. Percentage yield of crude extract.

Plant species	Percentage yield (%)
Ocimum basilicum L.	19.063 ± 0.321

Phenols, flavonoids, catechic tannins, saponins, reducing sugars, alkaloids, and terpenes were present in this *Ocimum basilicum* L. aqueous extract (Table 2).

Table 2. Phytochemical compounds of *Ocimum basilicum* L. aqueous extract.

Phytochemical compounds	Test	Basil extract
Phenols	Ferric chloride test	+
Flavonoids	Magnesium test	+
Catechic tannins	Ferric chloride test	+
Saponins	Froth test	+
Reducing sugars	Fehling test	+
Alkaloids	Dragendorff's test	+
Terpenes	Salkowki's test	+

Total phenols, flavonoids and hydrolysable tannins contents of *Ocimum basilicum* L. obtained from water solvent revealed important concentrations; 63.60 ± 1.53 mg of GAE, 13.537 ± 0.281 mg of QE, and 27 ± 0.830 mg of TAE/g extract respectively (Figure 2,3,4,5).

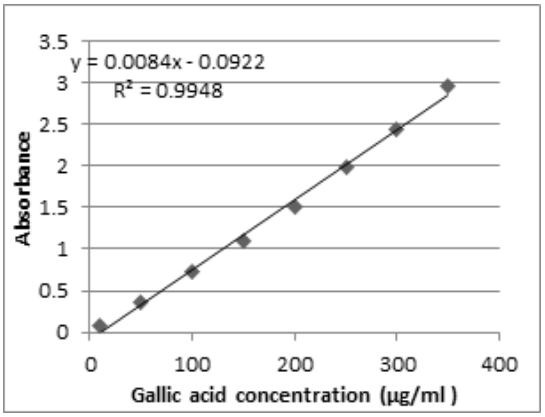

Figure 2. Gallic acid calibration curve for the quantitative determination of phenols.

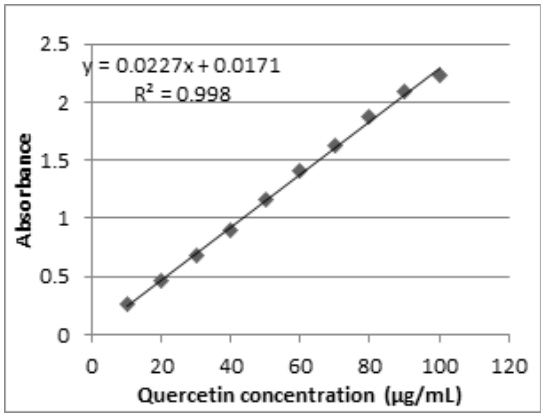

Figure 3. Quercetin calibration curve for the quantitative determination of flavonoids.

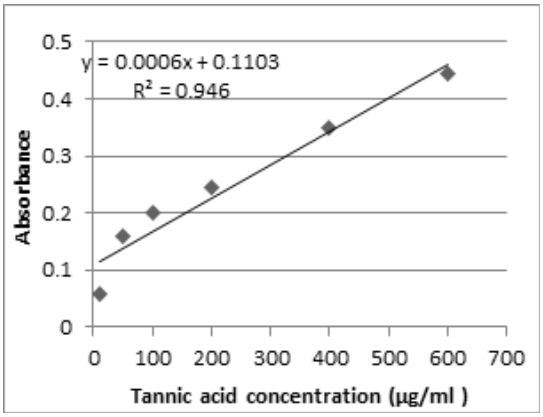

Figure 4. Tannic acid calibration curve for the quantitative determination of hydrolysable tannins.

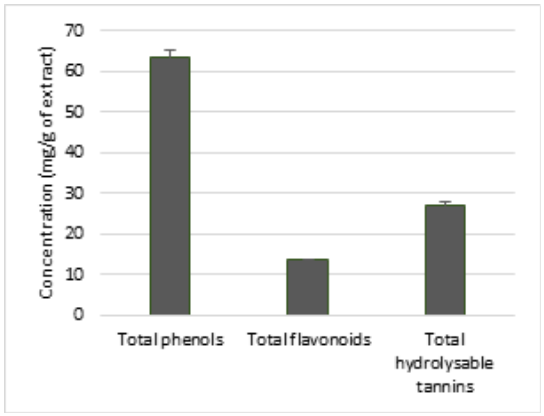

Figure 5. Total phenols, total flavonoids and total hydrolysable tannins concentrations in basil extract.

Gas chromatography-mass spectroscopy analysis

Characterization of volatile compounds from *Ocimum basilicum* L. extract was conducted through GC-MS chromatogram (Figure 6). Using GC-MS technique, about 147 components were identified (Table 3). GC-MS result showed that the chemical constituents higher than 0.5 % found in aqueous extract were propanoic acid, 2-hydroxy-, ethyl ester (3.503%), butanoic acid, 4-hydroxy- (1.657%), cyclopentasiloxane, decamethyl- (1.346%), cyclotetrasiloxane, octamethyl- (0.983%), heptadecane, 2,6,10,15-tetramethyl- (0.785%), bis(tert-butyldimethylsilyl) 2,3-bis((tert-butyldimethylsilyl)oxy)fumarate (0.703%), cyclononasiloxane, octadecamethyl- (0.672%), phthalic acid, 8-bromoctyl isobutyl ester (0.655%), 3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane (0.535%), and cyclononasiloxane, octadecamethyl- (0.506%).

Table 3: Quantification of volatile compounds by GC-MS of extract leaves of *Ocimum basilicum* L. plant and their various characters.

Peak	Name	Formula	RT (s)	Area (%)
1	Propanoic acid, 2-hydroxy-, ethyl ester	$C_5H_{10}O_3$	289.405	3.503
2	Butanoic acid, 4-hydroxy-	$C_4H_8O_3$	427.257	1.657
3	Lycorenan-7-one,9,10-dimethoxy-1-methyl-	$C_{18}H_{21}NO_4$	430.676	0.006
4	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	525.997	0.983
5	2-Propanol, 1-(2-methoxypropoxy)-	$C_7H_{16}O_3$	544.928	0.038
6	Benzene, 1-methyl-3-(1-methylethyl)-	$C_{10}H_{14}$	558.773	0.056
7	1-Hexanol, 2-ethyl-	$C_8H_{18}O$	560.792	0.144
8	Cyclohexene, 4-ethenyl-1,4-dimethyl-	$C_{10}H_{16}$	563.79	0.071
9	Benzyl alcohol	C_7H_8O	567.088	0.119
10	Benzeneacetaldehyde	C_8H_8O	578.822	0.237
11	Linalyl acetate	$C_{12}H_{20}O_2$	637.621	0.45
12	Nonanal	$C_9H_{18}O$	642.028	0.096
13	1,5,7-Octatrien-3-ol, 3,7-dimethyl-	$C_{10}H_{16}O$	642.329	0.074
14	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	691.471	1.346
15	Octanoic acid	$C_8H_{16}O_2$	705.073	0.214
16	1,5,5-Trimethyl-6-methylene-cyclohexene	$C_{10}H_{16}$	734.686	0.075
17	Benzene, 1-methoxy-4-(1-propenyl)-, (Z)-	$C_{10}H_{12}O$	741.029	0.099
18	(3S,4R,5R,6R)-4,5-Bis(hydroxymethyl)-3,6-dimethylcyclohexene	$C_{10}H_{18}O_2$	744.729	0.048
19	Benzothiazole	C_7H_5NS	770.123	0.118
20	1,4-Benzenedicarboxaldehyde	$C_8H_6O_2$	776.346	0.015
21	Carvone	$C_{10}H_{14}O$	786.8	0.019
22	(+)-3-Carene	$C_{10}H_{16}$	792.754	0.055
23	Nonanoic acid	$C_9H_{18}O_2$	798.399	0.136
24	1H-Indene-4-carboxaldehyde, 2,3-dihydro-	$C_{10}H_{10}O$	813.245	0.045

25	Tetrasiloxane, 3,5-diethoxy-1,1,1,7,7,7-hexamethyl-3,5-bis(trimethylsiloxy)-	$C_{16}H_{46}O_7Si_6$	820.45	0.015
26	1-[p-Chlorophenyl]-3-[4-[[2-(diisopropylamino)ethyl]amino]-6-methyl-2-pyrimidinyl]-guanidine	$C_{20}H_{30}ClN_7$	839.416	0.021
27	Pentadecane	$C_{15}H_{32}$	858.385	0.026
28	Pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	$C_{12}H_{24}O_3$	888.138	0.163
29	Hexadecane	$C_{16}H_{34}$	922.883	0.221
30	Dodecanal	$C_{12}H_{24}O$	931.63	0.096
31	Caryophyllene	$C_{15}H_{24}$	953.638	0.285
32	Phthalic acid, 4-fluoro-2-nitrophenyl methyl ester	$C_{15}H_{10}FNO_6$	974.316	0.02
33	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	$C_{14}H_{20}O_2$	988.069	0.124
34	Ethanone, 1-(6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)-	$C_{10}H_{14}O$	999.114	0.027
35	Decyl octyl ether	$C_{18}H_{38}O$	1013.58	0.036
36	Oxirane, dodecyl-	$C_{14}H_{28}O$	1017.11	0.034
37	Butylated Hydroxytoluene	$C_{15}H_{24}O$	1023.99	0.111
38	1H-Indene, 1-methyl-3-propyl-	$C_{13}H_{16}$	1026.14	0.006
39	Lilial	$C_{14}H_{20}O$	1036.3	0.038
40	Benzene, (1-butylhexyl)-	$C_{16}H_{26}$	1041.35	0.072
41	Undecane, 5-methyl-	$C_{12}H_{26}$	1044.53	0.015
42	3-[(4-Fluoroanilino)carbonyl]-1,2,2-trimethylcyclopentanecarboxylic acid	$C_{16}H_{20}FNO_3$	1045.61	0.007
43	Tetradecane, 3-methyl-	$C_{15}H_{32}$	1057.89	0.023
44	Oxirane, tetramethyl-	$C_6H_{12}O$	1085.53	0.003
45	Hexadecane	$C_{16}H_{34}$	1086.64	0.167
46	Diethyl Phthalate	$C_{12}H_{14}O_4$	1087.54	0.093
47	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	$C_{16}H_{30}O_4$	1089.69	0.166
48	Hexestrol, O-trifluoroacetyl-	$C_{20}H_{21}F_3O_3$	1096.71	0.006
49	Benzene, (1-pentylhexyl)-	$C_{17}H_{28}$	1115.07	0.025
50	Benzene, (1-butylheptyl)-	$C_{17}H_{28}$	1117.68	0.05

51	Phenol, 2,6-bis(1,1-dimethyleth-yl)-4-(1-methylpropyl)-	$C_{18}H_{30}O$	1121.14	0.029
52	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	$C_{13}H_{22}O_3$	1132.63	0.137
53	Octane, 1,1'-oxybis-	$C_{16}H_{34}O$	1135.59	0.042
54	3-Isopropoxy-1,1,1,7,7,7-hexa-methyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	$C_{18}H_{52}O_7Si_7$	1138.39	0.535
55	Nonane, 3,7-dimethyl-	$C_{11}H_{24}$	1140.85	0.015
56	Decane, 1-chloro-	$C_{10}H_{21}Cl$	1146.63	0.011
57	Salicylic acid, isopropyl ether, isopropyl ester	$C_{13}H_{18}O_3$	1152.88	0.026
58	Decyl acrylate	$C_{13}H_{24}O_2$	1157.24	0.019
59	1-(2-Aminopropoxy)-2-methoxyethane	$C_6H_{15}NO_2$	1163.35	0.001
60	Pentadecane, 2,6,10,14-tetramethyl-	$C_{19}H_{40}$	1166.7	0.06
61	Hydrazinecarboxamide	CH_5N_3O	1167.88	0.005
62	2-(4,5-Dihydroxy-2-methylphenyl)-4-hy-droxy-6-methoxybenzoic acid, 4TMS	$C_{27}H_{46}O_6Si_4$	1172.97	0.142
63	Phenethyl isocyanate	C_9H_9NO	1191.03	0.021
64	Tetradecanoic acid	$C_{14}H_{28}O_2$	1203.52	0.036
65	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	1240.94	0.045
66	Benzene, (1-methylnonadecyl)-	$C_{26}H_{46}$	1243.15	0.016
67	Salicylic acid, 1-methylpropyl ester	$C_{11}H_{14}O_3$	1245.87	0.021
68	Pentadecanal-	$C_{15}H_{30}O$	1246.84	0.063
69	Bis(tert-butyldimethylsilyl) 2,3-bis((tert-bu-tyldimethylsilyl)oxy)fumarate	$C_{28}H_{60}O_6Si_4$	1253.32	0.703
70	Nickel tetracarbonyl	C_4NiO_4	1264.2	0
71	Isoamyl laurate	$C_{17}H_{34}O_2$	1265.09	0.188
72	Dodecanoic acid, 1,1-dimethylpropyl ester	$C_{17}H_{34}O_2$	1267.65	0.034
73	4-Fluorobenzylamine, N,N-dibutyl-	$C_{15}H_{24}FN$	1275.68	0.009
74	Phthalic acid, 8-bromoctyl isobutyl ester	$C_{20}H_{29}BrO_4$	1285.64	0.655
75	Semioxamazide	$C_2H_5N_3O_2$	1288.89	0.002
76	Sulfurous acid, 2-ethylhexyl nonyl ester	$C_{17}H_{36}O_3S$	1301.97	0.028
77	Hydrazinecarboxamide	CH_5N_3O	1309.44	0.007
78	2-(Diethylamino)ethyl 4-amino-2-hydroxy-benzoate	$C_{13}H_{20}N_2O_3$	1312.77	0.404
79	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	1319.53	0.049

80	Nickel tetracarbonyl	C_4NiO_4	1324.45	0
81	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	1340.11	0.057
82	1,4-Dibutyl benzene-1,4-dicarboxylate	$C_{16}H_{22}O_4$	1348.04	0.128
83	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	1355.76	0.672
84	Decane, 6-ethyl-2-methyl-	$C_{13}H_{28}$	1367.14	0.019
85	Hydrazinecarboxamide	CH_5N_3O	1391.66	0.001
86	Piceatannol, 4TMS	$C_{26}H_{44}O_4Si_4$	1395.26	0.324
87	Hydrazinecarboxamide	CH_5N_3O	1406.69	0
88	Hydrazinecarboxamide	CH_5N_3O	1430.48	0.005
89	(1-Methoxy-pentyl)-cyclopropane	$C_9H_{18}O$	1436.74	0.009
90	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	1446.12	0.035
91	Cyclononasiloxane, octadecamethyl-	$C_18H_{54}O_9Si_9$	1449.57	0.506
92	5-Hexyl-5-methyloxolan-2-one	$C_{11}H_{20}O_2$	1457.25	0.004
93	Hydrazinecarboxamide	CH_5N_3O	1482.2	0.001
94	Benzoic acid, tetradecyl ester	$C_{21}H_{34}O_2$	1494.32	0.028
95	Semicarbazide	CH_5N_3O	1500.05	0.002
96	Nickel tetracarbonyl	C_4NiO_4	1509.8	0.005
97	Hydrazinecarboxamide	CH_5N_3O	1511.62	0.001
98	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	1534.56	0.364
99	Hydrazinecarboxamide	CH_5N_3O	1540.15	0.001
100	Hydrazinecarboxamide	CH_5N_3O	1553.07	0.001
101	Benzoic acid, tridecyl ester	$C_{20}H_{32}O_2$	1553.75	0.036
102	Semicarbazide	CH_5N_3O	1573.77	0.001
103	Semicarbazide	CH_5N_3O	1577.54	0.002
104	2-Hexanamine	$C_6H_{15}N$	1580.48	0.005
105	Benzoic acid, hexadecyl ester	$C_{23}H_{38}O_2$	1610.8	0.036
106	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	1612.91	0.29
107	Semicarbazide	CH_5N_3O	1621.61	0.002
108	L-Alanine, α-N-methyl-N-benzyl-, methyl ester	$C_{12}H_{17}NO_2$	1637.98	0.02
109	Semicarbazide	CH_5N_3O	1645.43	0.007
110	Carbonic acid, bis(2-ethylhexyl) ester	$C_{17}H_{34}O_3$	1656.43	0.017
111	Semicarbazide	CH_5N_3O	1664.98	0.001
112	Benzoic acid, pentadecyl ester	$C_{22}H_{36}O_2$	1665.58	0.031
113	Benzyldiethyl-(2,6-xylylcarbamoylmeth-yl)-ammonium benzoate	$C_{28}H_{34}N_2O_3$	1680.52	0.086

114	Phthalic acid, 2-ethylhexyl pentadecyl ester	$C_{31}H_{52}O_4$	1682.77	0.031
115	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	1686.3	0.274
116	Sulfurous acid, 2-ethylhexyl hexyl ester	$C_{14}H_{30}O_3S$	1712.83	0.04
117	Vinyl 2-ethylhexanoate	$C_{10}H_{18}O_2$	1717.17	0.175
118	dl-Alanyl-l-alanine	$C_6H_{12}N_2O_3$	1730.68	0.003
119	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	1732.27	0.785
120	Hydrazinecarboxamide	CH_5N_3O	1733.46	0.004
121	Hydrazinecarboxamide	CH_5N_3O	1754.55	0.001
122	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	1757.18	0.304
123	Carbonic acid, bis(2-ethylhexyl) ester	$C_{17}H_{34}O_3$	1758.38	0.011
124	Semicarbazide	CH_5N_3O	1760.82	0.001
125	Hydrazinecarboxamide	CH_5N_3O	1768.11	0.001
126	1,3-Benzenedicarboxylic acid, bis(2-ethyl-hexyl) ester	$C_{24}H_{38}O_4$	1785.34	0.041
127	Semicarbazide	CH_5N_3O	1805.8	0.001
128	Benzenemethanol, α-(1-aminoethyl)-	$C_9H_{13}NO$	1833.93	0.013
129	Squalene	$C_{30}H_{50}$	1835.62	0.108
130	Semicarbazide	CH_5N_3O	1836.47	0.001
131	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	1839.37	0.28
132	2-Hydroxybenzene-1,3-dicarboxylic acid, trimethylsilyl ether, bis(trimethylsilyl) ester	$C_{17}H_{30}O_5Si_3$	1850.8	0.311
133	Semicarbazide	CH_5N_3O	1863.63	0.001
134	Hydrazinecarboxamide	CH_5N_3O	1880.66	0.002
135	Hydrazinecarboxamide	CH_5N_3O	1882.03	0.001
136	Hydrazinecarboxamide	CH_5N_3O	1891.75	0.001
137	Semicarbazide	CH_5N_3O	1893.9	0.005
138	Benzenemethanol, α-(1-aminoethyl)-	$C_9H_{13}NO$	1904.71	0.003
139	Hydrazinecarboxamide	CH_5N_3O	1909.88	0.001
140	Benzenemethanol, α-(1-aminoethyl)-	$C_9H_{13}NO$	1911.59	0.001
141	Semicarbazide	CH_5N_3O	1917.4	0.001
142	Semicarbazide	CH_5N_3O	1927.25	0.001
143	Semicarbazide	CH_5N_3O	1932.07	0.001
144	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	1941.29	0.485
145	Semicarbazide	CH_5N_3O	1945.74	0.001
146	Semicarbazide	CH_5N_3O	1946.04	0.001
147	Semicarbazide	CH_5N_3O	1958.37	0.001

In-vivo acute toxicity

Wistar albino rats were used in this experiment to test acute toxicity over the course of 24 hours. Our plant is administered at doses of 2 g and 5 g per kilogram of rats. No mortality and no alteration in the other physiological parameters of the rats, which indicates that the aqueous extract of *Ocimum basilicum* L. had no toxic and no adverse effects on the rats during the treatment period (Table 4).

Table (4) : Effect of basil extract on physiological parameters of Wistar albino rats.

Parameter	Dose	Toxicity time				
		0 h	3 h	7 h	14 h	24 h
Death rats	Control	None	None	None	None	None
	2 g/ kg	None	None	None	None	None
	5 g/ kg	None	None	None	None	None
Eyes	Control	Normal	Normal	Normal	Normal	Normal
	2 g/ kg	Normal	Normal	Normal	Normal	Normal
	5 g/ kg	Normal	Normal	Normal	Normal	Normal
Sleep	Control	Normal	Normal	Normal	Normal	Normal
	2 g/ kg	Normal	Normal	Normal	Normal	Normal
	5 g/ kg	Normal	Normal	Normal	Normal	Normal
Diarrhea	Control	Normal	Normal	Normal	Normal	Normal
	2 g/ kg	Normal	Normal	Normal	Normal	Normal
	5 g/ kg	Normal	Normal	Normal	Normal	Normal

Discussion

The bioactive substances in *Ocimum basilicum* L. leaves aqueous extract were qualified and quantified using the qualitative and quantitative phytochemical analysis and GC-MS methodology.

Our study revealed the presence of phenols, flavonoids, catechic tannins, saponins, reducing sugars, alkaloids, and terpenes in basil aqueous extract. The results of the current study on phytochemical screening are consistent with those of Nadeem et *al*. who found that basil leaves water extract consist of phenols, alkaloids, tannins, flavonoids, steroids, terpenoids, and glycosides (21). Moreover; tannins, flavonoids, terpenoids, saponins, and reducing sugars were reported as the present phytochemical compounds aqueous extract from sweet basil leaves (22). According to Pushpalatha et *al*., preliminary secondary metabolites screening of aqueous extract of *Ocimum basilicum* L. showed the existence of phytochemicals such as carbohydrates, tannins, saponins, flavonoids, anthocyanin, cardiac glycosides, terpenoids, triterpenoids, phenols, and steroids (23). According to reports, the active substances in medicinal plants that give them their pharmacological potentials are their phytochemicals (24). Phenolic and flavonoids compounds found in plant secondary metabolites have pharmacological effects such as anti-allergic, antibacterial, antiviral, anti-inflammatory, antioxidant, anti-diabetic, anticancer and neurodegenerative effect (25).

Concerning the quantification of phytochemical molecules, the present results are supported by the by the results of Nadeem et *al*. who found 70.7 mg GAE/g of total phenolic content, 6.49 mg QE/g of total flavonoids and 13.3 mg GAE/g of total tannins content in O*. basilicum* aqueous extract (21).

In this work, the identification and characterisation of 147 volatile compounds from basil water extract were conducted through GC-MS. The identification of basil extracts was carried out by Kaya and Keskin using GC-MS Which showed the presence of various bioactive compounds (26). GC-MS result showed that the compounds higher than 4 % found in aqueous extract by Ababutain were Propane, 3-chloro-1,1,14,5-Dichloro-1,3-dioxolan-2-one (49.87 %), phenol (6.22 %), propane, 3-chloro-1,1,1-trifluoro (4.26 %), and gamma-sitosterol (4.19 %) (27). D´ıaz-Maroto et *al.* in their study could identifying various volatile components in basil extracts using (GC/MS), linalool being the major component (28). Numerous investigations of the volatile composition of *Ocimum basilicum* L. have been conducted (29). Volatile organic components in plant, the low-molecular-weight molecules, have elevated chemical reactivities due to their functional groups, such as the structure with hydroxy, α, β-unsaturated carbonyl, phenyl, alkoxyl and sulfhydryl, and ester groups, bringing high polarity or electrophile capacity to these molecules (30). The basil volatile components, as well as their antifungal, antibacterial, and antioxidant properties, have been extensively studied (31). A previous study has shown that the chemical profiles of leaf volatile compounds from a plant genus are highly diverse (32). The results of the acute toxicity study revealed that the oral administration of basil aqueous extract to rats at doses of 2 and 5 g/kg did not result in any toxicity symptoms or animal death. Acute toxicity is a single-dose test that identifies symptoms and the extent to which toxicity affects animals (33). The principal aim of evaluating the safety of any medicinal plant is to identify the nature and significance of adverse effect and to establish the exposure level at which this effect is observed (34). Based on their long-term use by humans one might expect plants used in traditional medicine to have low toxicity (35). The pharmacological potential of a medicinal plant depends on its secondary metabolites (36).

Conclusion

The volatile components from *Ocimum basilicum* L. were successfully identified using the GC-MS analysis; it has a distinctive compounds content, ester, alcohols, ketones, fatty acids, aldehydes and hydrocarbons. From this plant, about 147 chemicals were provably discovered. Basil was tentatively found to include phenols, flavonoids, catechic tannins, saponins, reducing sugars, alkaloids and terpenes. This research will help the extensive use of basil in the food, nutrition, and pharmaceutical industries and will provide useful information for the future utilization of volatile chemicals.

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