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Research Article

DETECTION OF PHYTOCHEMICAL CONSTITUENT IN FLOWERS OF *VIOLA ODORATA* BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

Objective: *Viola odorata* has a characteristic as antifungal, antibacterial, anticancer, antioxidant, antiasthmatic, anti-inflammatory, anti-HIV, and antipyretic agents. The aim of this study was detected about bioactive compounds in the methanolic extract of *V. odorata.*

Methods: The methanolic extract was analyzed through gas chromatography-mass spectrometry (GC-MS) for the identification of different compounds.

Results: The current study investigated about phytochemicals in flowers of *V. odorata*. GC-MS analysis of the methanol extract of flowers showed 84 compounds. The highest concentration was for components which include ethanol, 2-(9,12-octadecadienyl oxy) -, (Z,Z)-; pentadecanoic acid; 1-pentacosanol; 1-pentacosanol; 2-furan carboxaldehyde, 5-(hydroxymethyl)-; 1,2 benzenedicarboxylic acid, diisooctyl ester; and docosane, 11-butyl- and gamma-sitosterol. The peak area and retention time for each components, respectively, were (15.709, 25.51%), (14.015, 19.51%), (29.914, 4.69%), (27.292, 3.95%), (5.707, 4.05%), (20.357, 3.91%), (18.289, 2.48%), and (30.431, 2.37%). While the others components ranged the peak area from 2.03% to 0.05%.

Conclusions: These results indicate that the flowers of *V. odorata* contain the numerous components which have medical importance and this study was one of the first studies to detect phytochemicals in *V. odorata.*

Keywords: *Viola odorata*, Gas chromatography-mass spectrometry analysis, Phytochemicals, Pentadecanoic acid, Gamma-sitosterol.

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INTRODUCTION

The medicinal herbs obtained much importance in recent years due to extensive applications of its bioactive molecules. The different strategies have been advanced for the selection of specific herbs for the study. The herbs selected were screened for the active phytoconstituents. The specific component present in the herbs was active subjected to isolation with different analytical techniques. The analogs of isolated molecules are characterized, and structural modification has been done to enhance the desired activity and minimize the unfavorable side effects [1]. The Violaceae is a family which contains about 900 species belonging to 22 genera; this family is a medium-sized of perennial or rarely annual herbs or shrubs, including the violets or pansies. It is cosmopolitan, but more typical of the temperate regions and tends to be restricted to higher mountainous areas. The Viola L. is the largest genus of the family and has about 400 species in the world [2].

Viola odorata perennial herb, rhizome short, thick leaves are heart-shaped, slightly downy, especially beneath, on stalks rising alternately from a creeping rhizome or underground stem. The flowers are generally deep purple, giving their name to the color which was called for it, pale rose-colored or white variations are also frequent, and all these tints may sometimes be discovered in different plants growing on the same land. The flowers are full of honey and are constructed for bee visitors, but bloom before it is really bee time, so that it is rare that a violet flower is found setting seed [3] *V. odorata* has a characteristic as antifungal, antibacterial, anticancer, antioxidant, antiasthmatic, anti-inflammatory, anti-HIV, and antipyretic agents [4]. The flowers infusion used for jaundice, eczema, febrifuge, antiallergic, blood cleansing, and migraine [5,6]. So Shafi and Tabassum [7] revealed the importance of herbal remedies, such as of fruits of Eriobotrya japonica has beneficial effects on serum glucose levels. While Gulilat *et al*. [8] showed the importance of gas chromatography-mass spectrometry (GC-MS) in the phytochemical analysis which revealed the presence of various secondary metabolites, namely, flavonoids, alkaloids, terpenoids, saponins, and carotenoid in rue extract, it has a property antimicrobial agents in drugs development of infectious disease. This study aimed to detect about bioactive compounds in the methanolic extract of *V. odorata*.

METHODS

Plant material

V. odorata L. plant which authenticated by the National Herbarium of Iraq Botany Directorate at Abu-Ghraib was collected in April and July. The flowers dried at room temperature 25°C in the shade for (10) days then it was crushed into powder by electric Grinder and weighted.

Preparation of extracts

100 g of crushed powder (flowers) of *V. odorata* L. Plant was macerated for 36 h with shaking at room temperature 25°C in 1 L methanol, and the resulting extract was filtered. The residue was re-extracted twice for complete exhaustion. The obtained filtrates were combined and concentrated using a rotary evaporator to get the dry extract. The dried extract was dissolved in methanol and stored at 4°C in a refrigerator [9].

GC-MS analysis

The methanolic extract was analyzed through GC-MS for the identification of different compounds.

Instruments and chromatographic conditions GC Program

Column: Elite-5MS (5% diphenyl/95% dimethyl polysiloxane), 30 × 0.25 mm × 0.25 m df. Equipment: GC Shimadzu ap 2010 plus. Carrier gas: 3 ml/min, Split: 10:1. Detector: Mass detector Quader mass gold-Perkin Elmer.

Software: Turbomass 5.2. Sample injected: 2 µl.

Oven temperature program

- 80°C - 2 min hold. Up to 300°C 120 min at the rate of 10°C/min.
- Injector temperature 280°C. Total GC is running time 30 min.

MS program

- Library used NIST 10 Version-year 2010. Inlet line temperature 280°C.
- Source temperature 200°C. Electron energy: 70 eV. Mass scan (m/z): 40–600.
- Solvent delay: 0–2 min. Total MS running time: 30 min.
- The extract was dissolved in methanol and filtered with Elite- 5 MS column and analyzed in GC-MS for different constituents. The phytoconstituents obtained as a result was interpreted on mass spectrum GCMC using NIST (2010).

RESULT

GC-MS of the methanol extract of *V. odorata* flowers showed 84 peaks. The constituents were shown in Fig. 1 and Table 1 with their retention time (RT), molecular formula, molecular weight, concentration (peak area %), and chemical structures. GC-MS analysis showed 84 compounds the highest concentration was for components which include ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-; pentadecanoic acid; 1-pentacosanol; 1-pentacosanol; 2-furancarboxaldehyde, 5-(hydroxymethyl)-; 1,2 benzenedicarboxylic acid, diisooctyl ester; docosane, and 11-butyl- and gamma-sitosterol. The RT and peak area for each components, respectively, were (15.709, 25.51%), (14.015, 19.51%), (29.914, 4.69%), (27.292, 3.95%), (5.707, 4.05%), (20.357, 3.91%), (18.289, 2.48%), and (30.431, 2.37%). While the remain of the components ranged the peak area from 2.03% to 0.05%.

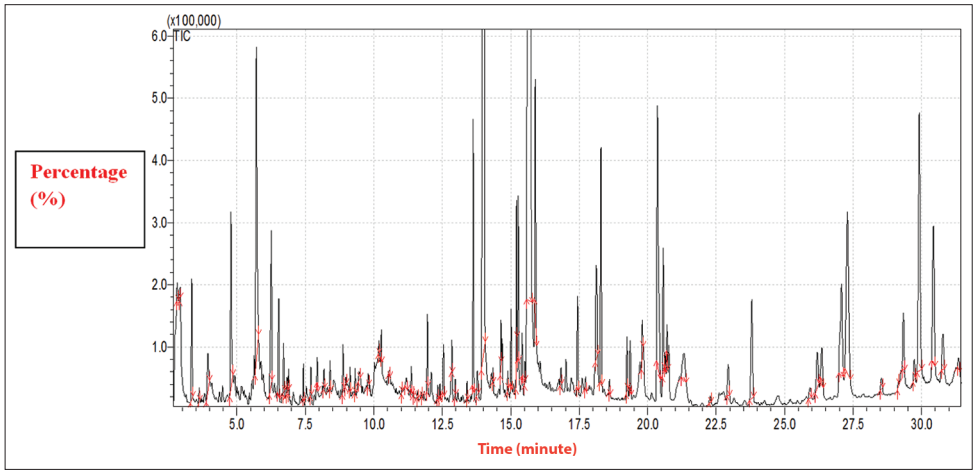
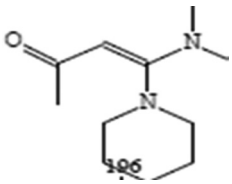
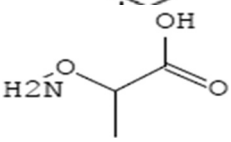
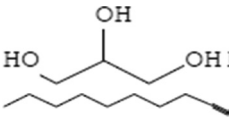
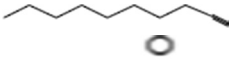
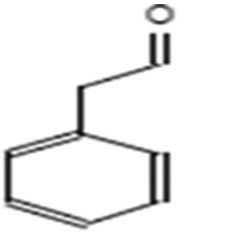


Fig. 1: Gas chromatography-mass spectrometry chromatogram of methanol extract of *Viola odorata*

Table 1: The compounds identified from methanol extract of *V. odorata* flowers by gas chromatography-mass spectrometry

NO	RT	Compound	Molecular formula	MW	Peak area	Chemical structure
1	2.593	3-Buten-2-one, 4-(dimethylamino)-4-(1-piperidinyl)	$C_{11}H_{20}N_2O$	196	0.05	
2	2.820	Propanoic acid, 2-(aminooxy)-	$C_3H_7NO_3$	105	0.13	
3	2.930	Glycerin	$C_3H_8O_3$	92	0.06	
4	3.347	1-Decyne	$C_{10}H_{18}$	138	1.23	
5	3.624	Benzene acetaldehyde	C_8H_8O	120	0.11	

(Contd...)

Table 1: (*Continued)*

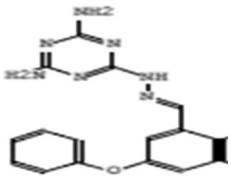
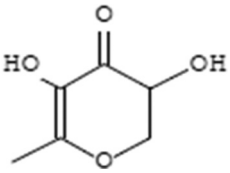
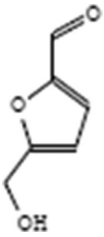
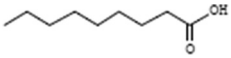
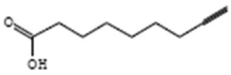
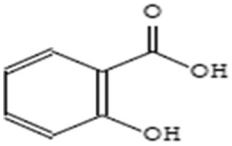
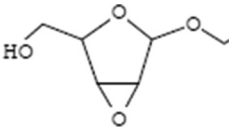
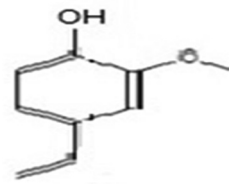
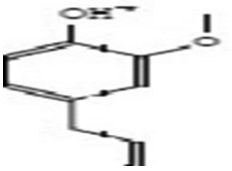
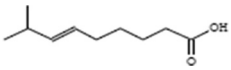
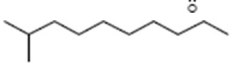
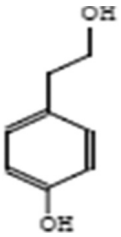
NO	RT	Compound	Molecular formula	MW	Peak area	Chemical structure
6	3.938	Benzaldehyde, 3-phenoxy-, (4,6-dimethyl-1,3,5-triazin-2-yl) hydrazone	$C_{16}H_{15}N_7O$	321	0.51	
7	4.784	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	1.83	
8	5.707	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	4.05	
9	6.258	Nonanoic acid	$C_9H_{18}O_2$	158	1.75	
10	6.522	8-Nonynoic acid	$C_9H_{14}O_2$	154	0.90	
11	6.703	Salicylic acid	$C_7H_6O_3$	138	0.14	
12	6.829	Methyl 2,3-anhydro-.beta.-d-ribofuranoside	$C_6H_{10}O_4$	146	0.11	
13	6.890	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.13	
14	7.425	Eugenol	$C_{10}H_{12}O_2$	164	0.22	
15	7.703	8-Methyl-6-nonenoic acid	$C_{10}H_{18}O_2$	170	0.19	
16	7.933	Decane, 2-methyl-	$C_{11}H_{24}$	156	0.19	
17	8.181	Benzeneethanol, 4-hydroxy-	$C_8H_{10}O_2$	138	0.15	

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Table 1: (*Continued*)

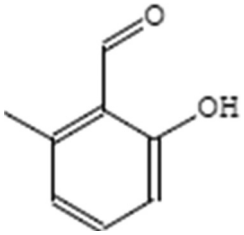
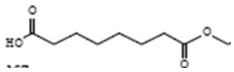
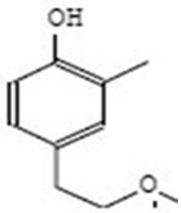
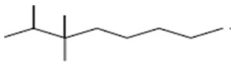
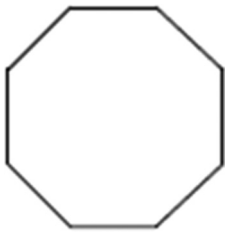
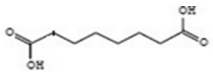
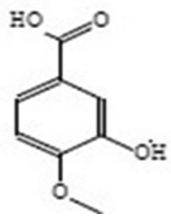
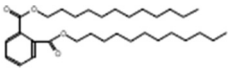
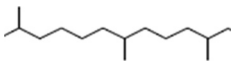
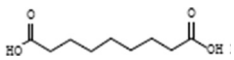
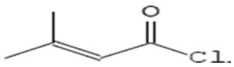
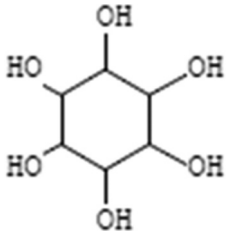
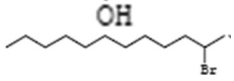
NO	RT	Compound	Molecular formula	MW	Peak area	Chemical structure
18	8.399	Benzaldehyde, 2-hydroxy-6-methyl-	$C_8H_8O_2$	136	0.22	
19	8.873	Suberic acid monomethyl ester	$C_9H_{16}O_4$	188	0.33	
20	8.979	4-(2-Methoxyethyl)-2-methylphenol	$C_{10}H_{14}O_2$	166	0.07	
21	9.129	Octane, 2,3,3-trimethyl-	$C_{11}H_{24}$	156	0.14	
22	9.317	Cyclooctane	C_8H_{16}	122	0.16	
23	9.467	Octanedioic acid	$C_8H_{14}O_4$	174	0.11	
24	9.789	3-Hydroxy-4-methoxybenzoic acid	$C_8H_8O_4$	168	0.11	
25	10.184	Didodecyl phthalate	$C_{32}H_{54}O_4$	502	0.06	
26	10.273	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	212	0.17	
27	10.561	Azelaic acid	$C_9H_{16}O_4$	188	0.05	
28	11.019	3,3-Dimethylacryloyl chloride	C5H7ClO	118	0.03	
29	11.188	Epi-inositol	$C_6H_{12}O_6$	180	0.12	
30	11.365	2-Bromo dodecane	$C_{12}H_{25}Br$	248	0.15	

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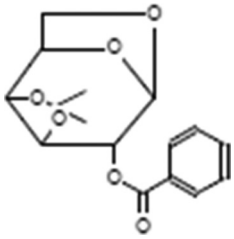
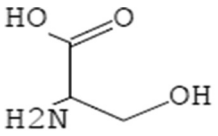
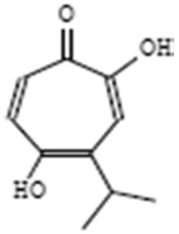
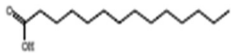
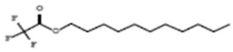
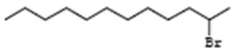
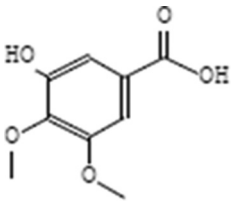
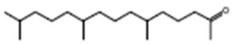
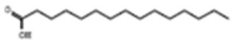
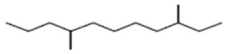
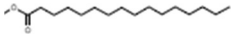
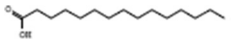
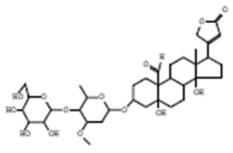
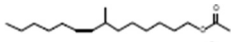
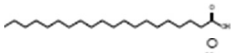
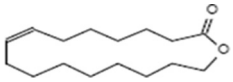
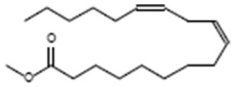
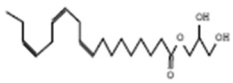
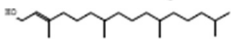
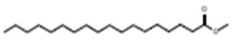
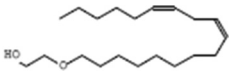
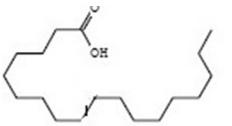
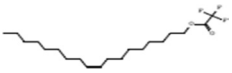
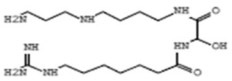
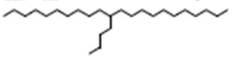
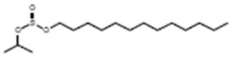
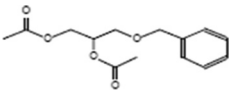
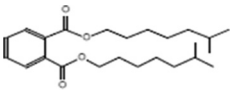
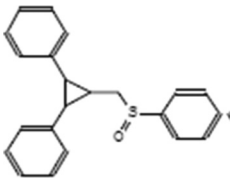
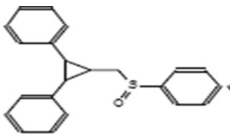
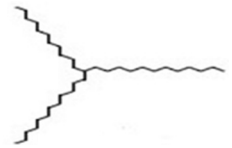
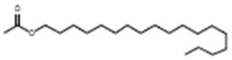
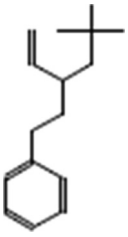
Table 1: (*Continued)*

NO	RT	Compound	Molecular formula	MW	Peak area	Chemical structure
31	11.445	2-Benzoyl-3,4-acetone-d-galactosan	$C_{16}H_{18}O_6$	306	0.02	
32	11.595	dl-Serine	$C_3H_7NO_3$	105	0.05	
33	11.758	2,5-Dihydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one	$C_{10}H_{12}O_3$	180	0.03	
34	11.958	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.48	
35	12.334	Undecyl trifluoroacetate	$C_{13}H_{23}F_3O_2$	268	0.09	
36	12.407	2-Bromo dodecane	$C_{12}H_{25}Br$	248	0.09	
37	12.540	3-Hydroxy-4,5-dimethoxybenzoic acid	$C_9H_{10}O_5$	198	0.49	
38	12.581	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	0.16	
39	12.973	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.10	
40	13.403	Undecane, 3,8-dimethyl-	C13H28	184	0.13	
41	13.626	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.00	
42	13.776	2-Tridecenal, (E)-	$C_{13}H_{24}O$	196	0.26	
43	14.015	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	19.51	
44	14.352	Card-20 (22)-enolide, 3-[(2,6-dideoxy-4-O-. beta.-D-glucopyranosyl-3-O-methyl-. beta.-D-ribo-hexopyranosyl) oxy]-5,14-dihydroxy-19-oxo	$C_{36}H_{54}O_{14}$	710	0.07	
45	14.637	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	0.31	
46	14.889	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.14	
47	15.011	Oxacycloheptadec-8-en-2-one	$C_{16}H_{28}O_2$	252	0.62	
48	15.213	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$	294	1.12	
49	15.274	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z)-	$C_{21}H_{36}O_4$	352	1.11	
50	15.414	Phytol	$C_{20}H_{40}O$	296	0.32	

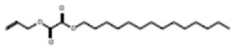
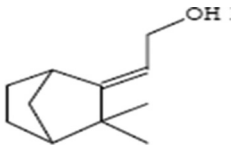
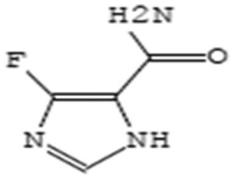
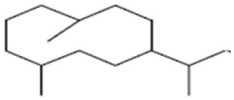
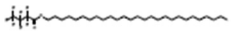
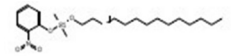
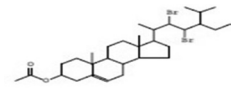
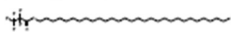
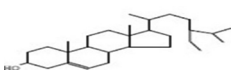
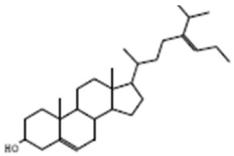
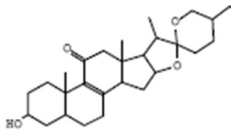
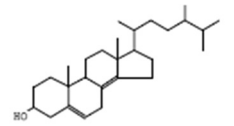
(Contd...)

Table 1: (*Continued)*

NO	RT	Compound	Molecular formula	MW	Peak area	Chemical structure
51	15.513	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	0.14	
52	15.709	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z, Z)-	$C_{20}H_{38}O_2$	310	25.51	
53	15.896	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.05	
54	16.829	Oleyl alcohol, trifluoroacetate	$C_{20}H_{35}F_3O_2$	364	0.15	
55	17.436	Eicosane	$C_{20}H_{42}$	282	0.88	
56	17.734	Cyclopentaneundecanoic acid, methyl ester	$C_{17}H_{32}O_2$	268	0.12	
57	18.119	Deoxyspergualin	$C_{17}H_{37}N_7O_3$	387	1.28	
58	18.289	Docosane, 11-butyl-	$C_{26}H_{54}$	366	2.48	
59	18.604	Sulfurous acid, 2-propyl tridecyl ester	$C16H_{34}O_3S$	306	0.12	
60	19.244	1,2-Propanediol, 3-benzyloxy-1,2-diacetyl-	$C_{14}H_{18}O_5$	266	0.48	
61	19.360	Tetratetracontane	$C_{44}H_{90}$	618	0.50	
62	19.804	Undecane, 2-methyl-	$C_{12}H_{26}$	170	0.36	
63	20.357	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	3.91	
64	20.564	(2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans-	$C_{22}H_{20}OS$	332	1.26	
65	20.635	(2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans-	$C_{22}H_{20}OS$	332	0.09	
66	20.704	Pentacosane, 13-undecyl-	$C_{36}H_{74}$	506	0.23	
67	21.325	Acetic acid n-octadecyl ester	$C_{20}H_{40}O_2$	312	0.74	
68	22.281	Benzene, (3-ethenyl-5,5-dimethylhexyl)-	$C_{16}H_{24}$	216	0.09	

(Contd...)

Table 1: (*Continued)*

NO	RT	Compound	Molecular formula	MW	Peak area	Chemical structure
69	22.939	Oxalic acid, allyl tetradecyl ester	$C_{19}H_{34}O_4$	326	0.49	
70	23.799	1-Octacosanol	$C_{28}H_{58}O$	410	1.69	
71	25.931	Ethanol, 2-(3,3-dimethylbicyclo[2.2.1]hept-2-ylidene)-	$C_{11}H_{18}O$	166	0.19	
72	26.193	Imidazole, 4-fluoro-5-aminocarbonyl-	$C_4H_4FN_3O$	129	0.63	
73	26.353	1,7-Dimethyl-4-(1-methylethyl) cyclodecane	$C_{15}H_{30}$	210	0.50	
74	27.078	Heptacosyl heptafluorobutyrate	$C_{31}H_{55}F_7O_2$	592	2.03	
75	27.292	1-Pentacosanol	$C_{25}H_{52}O$	368	3.95	
76	28.530	1-Heptatriacotanol	$C_{37}H_{76}O$	536	0.15	
77	29.154	Silane, dimethyl (2-nitrophenoxy) tetradecyloxy-	$C_{22}H_{39}NO_4Si$	409	0.05	
78	29.339	3-Eicosene, (E)-	$C_{20}H_{40}$	280	0.69	
79	29.730	22,23-Dibromostigmasterol acetate	$C_{31}H_{50}Br_2O_2$	612	0.26	
80	29.914	Triacontyl pentafluoropropionate	$C_{33}H_61F_5O_2$	584	4.69	
81	30.431	Gamma.-Sitosterol	$C_{29}H_{50}O$	414	2.37	
82	30.871	Cholest-5-en-3-ol, 24-propylidene-, (3.beta.)-	$C_{30}H_{50}O$	426	0.63	
83	31.348	3-Hydroxyspirost-8-en-11-one	$C_{27}H_{40}O_4$	428	0.15	
84	31.883	Ergost-5,8 (14)-dien-3-ol	$C_{28}H_{46}O$	398	0.28	

V. odorata: Viola odorata, MW: Molecular weight, RT: Retention time

DISCUSSION

GC analysis was not detected for the flower of *V. odorata* previously, while Hammami *et al*. [10] showed of the active components in volatile oils in Viola odorata by using GC , it revealed of the presence 63 identified volatile constituents, the main components were including: 1-phenyl butanone (22.43%), linalool (7.33% %),benzyl alcohol (5.65%), α-cadinol (4.91), globulol (4.32%) and viridiflorol (3.51%). Pulegone(3.33%), epi-α-cadinol (3.05%), terpinen-4-ol (2.31%), germacrene A (1.99%) and paramethyl anisole (1.09%) were found to be the main compounds [11] showed in GC analysis of *Hybanthus enneaspermus* which belongs to the family Violaceae, it contains the major phytoconstituents were (5E,13E)-5,13-Doco sadienoic acid (20.90%) and Cedran-diol, 8S, 14- (13.02). The results shown that the flower *V. odorata* contains active compounds that have medical importance such as eugenol has the characteristic as anti-tumor necrosis factor, antioxidant, antiprostaglandin, antipyretic, antiradicular, anti-salmonella, antiseptic, antiseptic, fungicide, antiestrogenic, antigenotoxic and antiviral [12], and anti-tumor [13]. While gamma-sitosterol used as anti-diabetic, anti-angiogenic, anticancer, antimicrobial, anti-inflammatory, anti-diarrheal, and antiviral [14]. Phytol has the property as antimicrobial, anti-inflammatory, antioxidant, diuretic, antimicrobial, anticancer, anti-inflammatory, anti-diuretic, immunostimulatory and anti-diabetic, and antimycobacterial activity [15]. While tetradecanoic acid used as antioxidant, lubricant, hypercholesterolemic, cancer-preventive, and cosmetic. Whereas hexadecanoic acid, methyl ester has the

ability as antioxidant, flavor, antifibrinolytic, hypocholesterolemic, anti-androgenic, lubricant, hemolytic, 5-alpha reductase inhibitor, nematicide, and anti-alopecic [16]. As for the compound octacosanol used as anticancer, cholesterol-lowering effect, anticoagulant, increase stamina and improve strength and reaction time for athletes [17]. Octadecanoic acid, methyl ester has the property as antioxidant, antibacterial, antifungal, anti-inflammatory, antiarthritic, antihistimic, anti-coronary, hypocholesterolemic, anticancer, hepatoprotective action, soap, lumbricant, and cosmetics [18].

CONCLUSION

This study showed through the GC analysis, the flowers of *V. odorata* contains many active compounds which have the medical importance and bioactivity. These compounds can be isolated and tested for the cellular toxicity to determine the safety of their usage in the treatment of diseases.

CONFLICTS OF INTEREST

The authors have not declared any conflict of interest. But have a contribution to some research in Ministry of science and technology \ Directorate of Water and Environment\ Iraq.

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