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, ditriosteryl 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.

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 from a creeping rhizome or underground stem. The flowers are full of honey and are constructed for 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 they are restricted to higher mountainous areas. The Viola L. is the largest genus of the family and has about 400 species in the world [2].

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-Ghrab 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 pks. 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-5MS 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, disooyctyl 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%.

**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) | C₆H₁₀N₂O       | 196 | 0.05      | ![Chemical structure](image1) |
| 2  | 2.820 | Propanoic acid, 2-(aminoxy)-                  | C₅H₇NO₂        | 105 | 0.13      | ![Chemical structure](image2) |
| 3  | 2.930 | Glycerin                                      | C₃H₈O₂         | 92  | 0.06      | ![Chemical structure](image3) |
| 4  | 3.347 | 1-Decyne                                      | C₁₀H₁₇        | 138 | 1.23      | ![Chemical structure](image4) |
| 5  | 3.624 | Benzene acetaldehyde                           | C₉H₁₄O        | 120 | 0.11      | ![Chemical structure](image5) |

Fig. 1: Gas chromatography-mass spectrometry chromatogram of methanol extract of *Viola odorata*
| NO | RT   | Compound                                      | Molecular formula | MW | Peak area |
|----|------|-----------------------------------------------|-------------------|----|-----------|
| 6  | 3.938| Benzaldehyde, 3-phenoxy-, (4,6-dimethyl-1,3,5-triazin-2-yl) hydrazone | C_{16}H_{15}N_{7}O_{3} | 321 | 0.51      |
| 7  | 4.784| 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | C_{6}H_{8}O_{4} | 144 | 1.83      |
| 8  | 5.707| 2-Furancarboxaldehyde, 5-(hydroxymethyl)-      | C_{6}H_{6}O_{3} | 126 | 4.05      |
| 9  | 6.258| Nonanoic acid                                 | C_{9}H_{18}O_{2} | 158 | 1.75      |
| 10 | 6.522| 8-Nonynoic acid                               | C_{9}H_{14}O_{2} | 154 | 0.90      |
| 11 | 6.703| Salicylic acid                                | C_{7}H_{6}O_{3} | 138 | 0.14      |
| 12 | 6.829| Methyl 2,3-anhydro-β-D-ribofuranoside        | C_{6}H_{10}O_{4} | 146 | 0.11      |
| 13 | 6.890| 2-Methoxy-4-vinylphenol                      | C_{9}H_{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_{16}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_{10}H_{22}O_{2} | 138 | 0.15      |

(Contd...)
| NO | RT  | Compound                                           | Molecular formula | MW | Peak area |
|----|-----|----------------------------------------------------|-------------------|----|-----------|
| 18 | 8.399 | Benzaldehyde, 2-hydroxy-6-methyl-                  | C₆H₇O₂            | 136| 0.22      |
| 19 | 8.873 | Suberic acid monomethyl ester                     | C₁₀H₁₄O₂          | 188| 0.33      |
| 20 | 8.979 | 4-(2-Methoxyethyl)-2-methylphenol                  | C₂₄H₂₄O₂          | 166| 0.07      |
| 21 | 9.129 | Octane, 2,3,3-trimethyl-                           | C₁₁H₂₄            | 156| 0.14      |
| 22 | 9.317 | Cyclooctane                                        | C₈H₁₆             | 122| 0.16      |
| 23 | 9.467 | Octanedioic acid                                   | C₁₀H₁₈O₄          | 174| 0.11      |
| 24 | 9.789 | 3-Hydroxy-4-methoxybenzoic acid                   | C₈H₈O₄            | 168| 0.11      |
| 25 | 10.184| Didodecyl phthalate                                | C₂₂H₂₄O₄          | 502| 0.06      |
| 26 | 10.273| Dodecane, 2,6,11-trimethyl-                        | C₁₂H₂₂            | 212| 0.17      |
| 27 | 10.561| Azelaic acid                                       | C₁₄H₂₀O₄          | 188| 0.05      |
| 28 | 11.019| 3,3-Dimethylacryloyl chloride                     | C₅H₇ClO            | 118| 0.03      |
| 29 | 11.188| Epi-inositol                                       | C₆H₁₁O₆           | 180| 0.12      |
| 30 | 11.365| 2-Bromo dodecane                                   | C₁₂H₂₄Br          | 248| 0.15      |

(Contd...)
| 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_{4}H_{7}NO_{3}   | 105 | 0.05      |                                  |
| 33 | 11.758 | 2,5-Dihydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one     | C_{21}H_{18}O_{5} | 180 | 0.03      |                                  |
| 34 | 11.958 | Tetradecanoic acid                                        | C_{14}H_{24}O_{2}  | 228 | 0.48      |                                  |
| 35 | 12.334 | Undecyl trifluoroacetate                                  | C_{12}H_{22}F_{2}O | 268 | 0.09      |                                  |
| 36 | 12.407 | 2-Bromo dodecane                                          | C_{12}H_{24}Br     | 248 | 0.09      |                                  |
| 37 | 12.540 | 3-Hydroxy-4,5-dimethoxybenzoic acid                       | C_{11}H_{16}O_{5}  | 198 | 0.49      |                                  |
| 38 | 12.581 | 2-Pentadecanone, 6,10,14-trimethyl-                        | C_{16}H_{28}O      | 268 | 0.16      |                                  |
| 39 | 12.973 | Pentadecanoic acid                                        | C_{15}H_{26}O_{3}  | 242 | 0.10      |                                  |
| 40 | 13.403 | Undecane, 3,8-dimethyl-                                   | C_{13}H_{28}      | 184 | 0.13      |                                  |
| 41 | 13.626 | Hexadecanoic acid, methyl ester                           | C_{16}H_{30}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_{28}O_{2}  | 242 | 19.51     |                                  |
| 44 | 14.352 | Card-20 (22)-enolide, 3-[(2,6-dideoxy-4-O-...             | C_{36}H_{54}O_{14} | 710 | 0.07      |                                  |
|    |      | beta-D-glucopyranosyl-3-O-methyl-                          |                   |     |           |                                  |
|    |      | beta-D-ribo-hexopyranosyl oxy]-5,14-dihydroxy-19-oxo     |                   |     |           |                                  |
| 45 | 14.637 | 7-Methyl-Z-tetradec-1-ol acetate                          | C_{16}H_{30}O_{2}  | 268 | 0.31      |                                  |
| 46 | 14.889 | Eicosanoic acid                                           | C_{20}H_{42}O_{3}  | 312 | 0.14      |                                  |
| 47 | 15.011 | Oxacycloheptadec-8-en-2-one                               | C_{16}H_{30}O_{2}  | 252 | 0.62      |                                  |
| 48 | 15.213 | 9,12-Octadecadienoic acid (Z, Z)-, methyl ester           | C_{18}H_{32}O_{2}  | 294 | 1.12      |                                  |
| 49 | 15.274 | 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z)- | C_{21}H_{34}O_{4} | 352 | 1.11      |                                  |
| 50 | 15.414 | Phytol                                                    | C_{20}H_{40}O      | 296 | 0.32      |                                  |

(Contd...)
| NO | RT  | Compound                                      | Molecular formula | MW  | Peak area | Chemical structure |
|----|-----|-----------------------------------------------|-------------------|-----|-----------|--------------------|
| 51 | 15.513 | Octadecanoic acid, methyl ester              | C₁₉H₃₈O₂           | 298 | 0.14      |                    |
| 52 | 15.709 | Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)- | C₂₀H₃₈O₂           | 310 | 25.51     |                    |
| 53 | 15.896 | Octadecanoic acid                            | C₁₈H₃₆O₂           | 284 | 2.05      |                    |
| 54 | 16.829 | Oleyl alcohol, trifluoroacetate               | C₂₀H₃₅F₃O₂         | 364 | 0.15      |                    |
| 55 | 17.436 | Eicosane                                      | C₂₀H₃₈             | 282 | 0.88      |                    |
| 56 | 17.734 | Cyclopentaneundecanoic acid, methyl ester    | C₁₉H₃₈O₂           | 268 | 0.12      |                    |
| 57 | 18.119 | Deoxyspergualin                               | C₁₈H₃₇N₇O₃        | 387 | 1.28      |                    |
| 58 | 18.289 | Docosane, 11-butyl-                           | C₂₂H₄₄             | 366 | 2.48      |                    |
| 59 | 18.604 | Sulfurous acid, 2-propyl tridecyl ester      | C₁₆H₃₄O₅S          | 306 | 0.12      |                    |
| 60 | 19.244 | 1,2-Propanediol, 3-benzyloxy-1,2-diacetyl-   | C₁₀H₁₆O₅           | 266 | 0.48      |                    |
| 61 | 19.360 | Tetratetracontane                             | C₄₄H₹₀             | 618 | 0.50      |                    |
| 62 | 19.804 | Undecane, 2-methyl-                           | C₁₃H₂₆             | 170 | 0.36      |                    |
| 63 | 20.357 | 1,2-Benzenedicarboxylic acid, diisooctyl ester| C₂₈H₅₄O₄          | 390 | 3.91      |                    |
| 64 | 20.564 | (2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans- | C₂₅H₂₆OS          | 332 | 1.26      |                    |
| 65 | 20.635 | (2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans- | C₂₇H₂₈OS          | 332 | 0.09      |                    |
| 66 | 20.704 | Pentacosane, 13-undecyl-                      | C₃₆H₇₄             | 506 | 0.23      |                    |
| 67 | 21.325 | Acetic acid n-octadeyl ester                 | C₁₀H₂₀O₂           | 312 | 0.74      |                    |
| 68 | 22.281 | Benzene, (3-ethyl-5,5-dimethylhexyl)-        | C₂₄H₂₄             | 216 | 0.09      |                    |

(Contd...)
| 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_{4}  | 410 | 1.69      |                   |
| 71 | 25.931 | Ethanol, 2-(3,3-dimethylbicyclo[2.2.1]hept-2-ylidine)-| C_{11}H_{18}O_{16} | 166 | 0.19      |                   |
| 72 | 26.193 | Imidazole, 4-fluoro-5-aminocarbonyl            | C_{11}H_{18}FN_{4}Si | 409 | 0.05      |                   |
| 73 | 26.353 | 1,7-Dimethyl-4-(1-methylethyl) cyclodecane     | C_{21}H_{30}        | 210 | 0.50      |                   |
| 74 | 27.078 | Heptacosyl heptafluorobutyrate                 | C_{21}H_{36}F_{7}O_{2} | 592 | 2.03      |                   |
| 75 | 27.292 | 1-Pentacosanol                                 | C_{25}H_{52}O_{4}  | 368 | 3.95      |                   |
| 76 | 28.530 | 1-Heptatriacocanol                             | C_{25}H_{52}O_{4}  | 536 | 0.15      |                   |
| 77 | 29.154 | Silane, dimethyl (2-nitrophenoxy) tetracycloxy-| C_{22}H_{39}NO_{4}Si | 409 | 0.05      |                   |
| 78 | 29.339 | 3-Eicosene, (E)-                               | C_{20}H_{40}O_{4}  | 280 | 0.69      |                   |
| 79 | 29.730 | 22,23-Dibromostigmasterol acetate              | C_{25}H_{52}Br_{6}O_{4} | 612 | 0.26      |                   |
| 80 | 29.914 | Triacetyl pentafluoropropionate                | C_{23}H_{39}FO_{4} | 584 | 4.69      |                   |
| 81 | 30.431 | Gamma-Sitosterol                               | C_{29}H_{50}O_{4}  | 414 | 2.37      |                   |
| 82 | 30.871 | Cholest-5-en-3-ol, 24-propylidene- (3.beta.)- | C_{29}H_{50}O_{4}  | 426 | 0.63      |                   |
| 83 | 31.348 | 3-Hydroxyxistrost-8-en-11-one                 | C_{28}H_{50}O_{4}  | 428 | 0.15      |                   |
| 84 | 31.883 | Ergost-5,8(14)-dien-3-ol                       | C_{28}H_{50}O_{4}  | 398 | 0.28      |                   |

**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 of 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-Docosadienoic 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, 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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