

DETECTION OF PHYTOCHEMICAL COMPOUNDS OF DUTCH IRIS (*IRIS HOLLANDICA*) BY USING GAS CHROMATOGRAPHY AND MASS SPECTROMETRY (GC-MS) TECHNIQUE

Ashwak Falih Kaizal and Hussein J. Hussein*

Department of Biology, College of science for women, University of Babylon, Iraq.

Abstract

A current investigation was conducted to screen the phytochemical compounds of the Dutch Iris (*Iris hollandica*) by using gas chromatography-mass spectrometry (GC-MS) Technique. The detection of phytochemical mixtures was relied on the four important factors, as following: the retention time, the molecular weight, the peak area, and the molecular structure. The aim of the study was analysis and detected of phytochemical compounds that present in *Iris hollandica* by means of a Technique of gas chromatography and mass spectrometry. Examination of *Iris hollandica* disclosed the presence of twenty of phytochemical compounds as following: (D-Glucose, $6-O-\alpha-D$ -galactopyranosyl-, Dodecanoic acid, 3-hydroxy-, Tertbutyloxyformamide, N-methyl-N-[4-(1-pyrrolidinyl,)], β -D-Glucopyranose, $4-O-\beta$ -D-galactopyranosyl- (1-pyrrolidinyl), 2, 4-Dimethylhexanedioic acid, 1-Nitro-2-acetamido-1, 2-dideoxy-d-mannitol, D-Alanine, N-propargyloxycarbonyl - tridecyl ester, D-Alanine, α -D-Glucopyranoside, $O-\alpha$ -glucopyranosyl-(1.fw), Methyl 6-oxoheptanoate, 3-Trifluoroacetoxydodecane, 1-Gala-1-ido-octonic lactone, N-methyl-N-4-[1-(pyrrolidinyl)-2-butynyl], α -D-Glucopyranoside, $O-\alpha$ -D-Glucopyranosyl-(1.fw), 9-Hexadecenoic acid, 9, 12, 15-Octadecatrienoic acid, 2, 3-bis (acetyloxy), 1-Hexadecanol, 2-methyl-, 2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-o, d-Allo-dec-2-enonic acid, 5, 8-anhydro-2, 3, 4, 9-tetra, E-8-Methyl-7-dodecen-1-ol acetate, and 1-Hexadecanol, 2-methyl-). Finally, *Iris hollandica* has wide range of phytochemical compounds might be using in different field of scientific such as antibacterial, antifungal, antioxidant, anticancer, and anti-inflammatory.

Key Words : Phytochemical compounds, Dutch Iris, Iris hollandica, Gas Chromatography-Mass spectrometry.

Introduction

The exceptional range of biochemicals products with a wide range of biological compounds of plants enable them to play a significant role in our life compared with animals (Buckingham, 1999). The technique of the Gas chromatography (GC) is a broadly applied strategy in various parts of knowledge and modernization. In the course of the most recent fifty years, Gas chromatography has expected a main role in choosing what number of mixes and to what degree they exist in a mix. Nonetheless, the capacity to build up the nature and synthetic structure of these isolated and evaluated mixes is equivocal and diminished, and needs a spectroscopic discovery method. The maximum utilized, is the (mass spectrometric detector), that permits to getting the "unique mark" of the molecules of compound, such as spectrum of mass. Mass spectra give data on the elemental composition and

*Author for correspondence : E-mail : huss2010feb@yahoo.com

molecular weight, and the high resolution mass spectrometer give information about the functional groups, and, in some circumstances give information about the isomerism geometrical arrangements of the molecule belonging to the compounds (Stashenko and Martínez, 2014). In the family Iridaceae, phytochemical studies on 18 Iris species all over the world have been informed to give forty six isoflavonoid aglycones (Rastogi and Mehrotra, 1991). In addition to three isoflavones like Iristectorin, Iriginen, and Tectoregenin, and also quinine Irisoquin 3, six novel compounds belonging for alkylating p-benzoquinones (Irisoquin A-F) were isolated and distinguished by the rhizomes Iris kumaonensis (Williams et al., 1997). Iris kumaonensis was used to isolate Two Isoflavones such as Iriskumonin and Irisdin (Wu, 1980). The Volatile oils were extracted from Iris plant that might have medicinal properties (Hadfield et al., 2000). Genus Iris encloses in excess (260 species) which are generally spread through the North part of the earth. Dutch Iris is

principally utilized as the attractive plants in the public gardens and houses, because of their vivid blooms, or then again in the aroma business, because of their violetlike scent, Dutch iris plants were additionally utilized in numerous regions of the world as therapeutic plants for recuperating of wide spectra of sicknesses. Scientists study now provides new information on phytochemical compositions of root, leaves, and flower for Iris plants, and their conceivable medication. In Iris species, flavonoids and isoflavonoids are the most prevalent secondary metabolites. While, the flavones, quinones, and xanthones the second most prevalent groups of secondary metabolites in Iris (Kaššak, 2012). Iris is a bulb plant or a rhizome, the name has been derived from Greek word means "Rain bow" (Bryan, 2002). Larson, (1980) mention that, Iris hollandica has been arisen from a hybridization process between Iris xiphium (var. praecox) and Iris tingitana or Iris Lusitanica. Undoubtedly, the plant kingdom is the natural factories and the vast reservoir of secondary metabolites produced by the plant for various purposes such as resistance to predator attacks and parasitic diseases. This immense abundance inspired people to think carefully about the use of these compounds in various aspects of life. The aim of the current study was analysis and identification of phytochemical compounds that present in Iris hollandica by using gas the technique chromatographic and mass spectrometric System.

Materials and Methods

Iris hollandica Collection

The target part (leaves) of *Iris hollandica* was collected in Al Hillah region one of city of Iraq. Plant leaves were collected during February, 2018.

Extraction of Iris hollandica leaves

About 20gm of plant material was soaked 200ml of absolute methanol and leave it overnight at room temperature to complete extraction of plant material, after that infiltrate by using Whatman No. 1, extraction of plant it was again filtered through sodium sulphate (Altameme *et al.*, 2015).

Gas chromatography and mass spectrum detection

A process of Gas chromatography and mass spectrum analysis was achieved by using (SHIMADZU apparatus QP 2010 Plus) in computer regulator at 70 eV according to (Mohammed and Imad, 2013; Hameed *et al.*, 2015).

Results

Due to the increasing role played by the active

compounds that derived from medicinal plants in various types of science, researchers from different countries have to work diligently in order to diagnosing these compounds. The process of analytical with Gas chromatography and mass spectroscopy for detection to the phytochemical compounds was approved in methanolic extract of Iris hollandica leaves. According to the GC-MS spectrum, the results revealed that, twenty of phytochemical mixes were detected in methanolic extract of Iris hollandica leaves. The results of current study uncovered that, the first one of these compound is D-Glucose, 6-O- α -D-galactopyranosyl-, (Fig.1), and the second phytochemical compound was detected to be Dodecanoic acid, 3-hydroxy- (Fig. 2). In addition to detection of the following phytochemical compounds such as Tertbutyloxyformamide, N-methyl-N-[4-(1pyrrolidinyl)], (Fig. 3), β-D-Glucopyranose, 4-O-β-Dgalactopyranosyl-, (Fig. 4), 2, 4-Dimethylhexanedioic acid, (Fig. 5), 1-Nitro-2-acetamido-1, 2-dideoxy-d-mannitol (Fig. 6), D-Alanine, N-propargyloxycarbonyl - tridecyl ester (Fig. 7), D-Alanine, α -D-Glucopyranoside, O- α glucopyranosyl-(1.fw), (Fig. 8), Methyl 6-oxoheptanoate, (Fig. 9), 3-Trifluoroacetoxydodecane, (Fig. 10), 1-Gala-1ido-octonic lactone, (Fig. 11), Formamide, N-methyl-N-4-[1-(pyrrolidinyl)-2-butynyl], (Fig. 12), α -D-Glucopyranoside, O- α -D-Glucopyranosyl-(1.fw), (Fig. 13), 9-Hexadecenoic acid, (Fig. 14), 9, 12, 15-Octadecatrienoic acid, 2, 3-bis (acetyloxy), (Fig. 15), 1-Hexadecanol, 2-methyl-, (Fig. 16), 2H-Indeno[1,2b]furan-2-one, 3, 3a, 4, 5, 6, 7, 8, 8b-o (Fig.17), d-Allodec-2-enonic acid, 5, 8-anhydro-2, 3, 4, 9-tetra, (Fig. 18), E-8-Methyl-7-dodecen-1-ol acetate, (Fig. 19), and finally, 1-Hexadecanol, 2-methyl-, (Fig. 20).

Discussion

Mass spectrometric techniques and the association of separation is the key factor that opens rich multidirectional prospects in the detection and analysis of complex compounds with high efficiency represented with high sensitivity, selectivity and specificity (Stashenko and Martínez, 2014). Hameed et al., (2015) were Identified twenty one phytochemical compounds by analyzing methanolic extract of Mentha viridis with GC-MS technique. Hussein et al., (2016a) were identified fifty six of phytochemical compounds from Foeniculum vulgare seeds methanolic extract by using gas chromatography and mass spectrometry technique and Fourier transform infrared spectrophotometer (FTIR). Hussein et al., (2016b) were identified twenty three of phytochemical compounds from Methanolic Leaves Extract of Cordia Myxa by using GC-MS technique. Hussein et al., (2017) were identified thirty one of

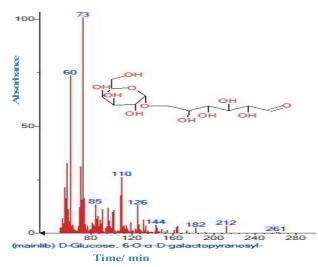


Fig. 1: Mass spectrum, for D-Glucose, 6-O-α-Dgalactopyranosyl-RT (min): 3.213, Molecular Weight: 342.11621.

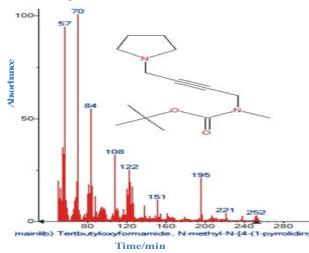


Fig. 3: Mass spectrum, for Tertbutyloxyformamide, N-methyl-N-[4-(1-pyrrolidinyl)] RT (min): 3.831, Molecular Weight: 252.183778.

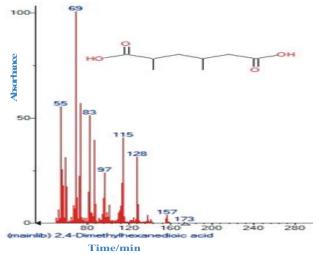


Fig. 5: Mass spectrum, for 2, 4-Dimethylhexanedioic acid RT (min): 3.962, Molecular Weight: 174.089209.

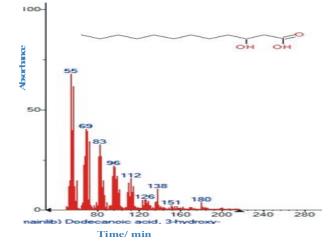


Fig. 2: Mass spectrum, for Dodecanoic acid, 3-hydroxy-RT (min): 3.402, Molecular Weight: 216.1725445.

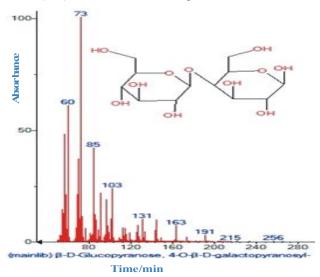


Fig. 4: Mass spectrum, for β-D-Glucopyranose, 4-O-β-D-galactopyranosyl- (1-pyrrolidinyl), RT (min): 3.928, Molecular Weight: 342.11621.

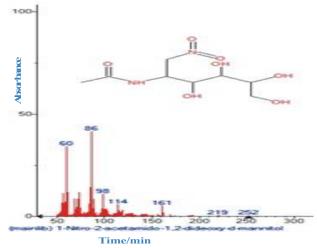


Fig. 6: Mass spectrum, for 1-Nitro-2-acetamido-1, 2-dideoxyd-mannitol RT (min): 4.271, Molecular Weight: 252.095751.

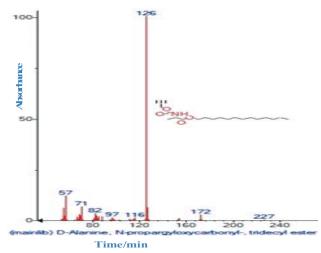


Fig. 7: Mass spectrum, for D-Alanine, N-propargyloxycarbonyl - tridecyl ester RT (min): 4.512, Molecular Weight: 353.256609.

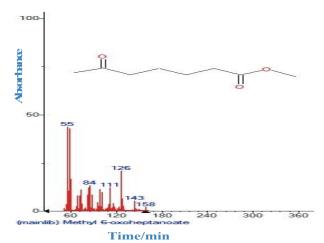
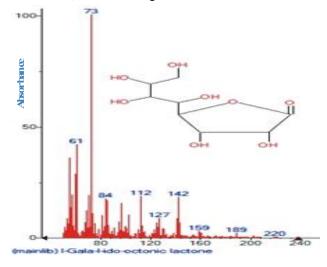


Fig. 9: Mass spectrum, for Methyl 6-oxoheptanoate RT (min): 5.290, Molecular Weight: 158.094295.



Time/min

Fig. 11: Mass spectrum, for l-Gala-l-ido-octonic lactone RT (min): 5.936, Molecular Weight: 238.068868.

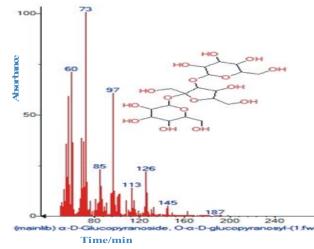
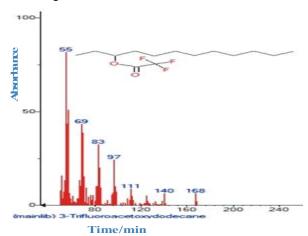
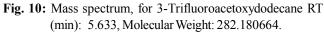


Fig. 8: Mass spectrum, for D-Alanine, α-D-Glucopyranoside, O-α-glucopyranosyl-(1.fw) RT (min): 4.918, Molecular Weight: 504.169035.





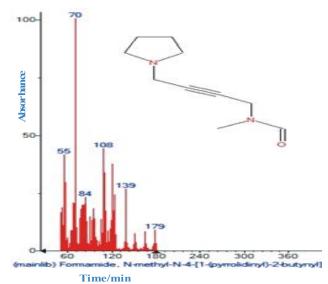


Fig. 12: Mass spectrum, for Formamide, N-methyl-N-4-[1-(pyrrolidinyl)-2-butynyl] RT (min): 6.365, Molecular Weight: 180.126264.

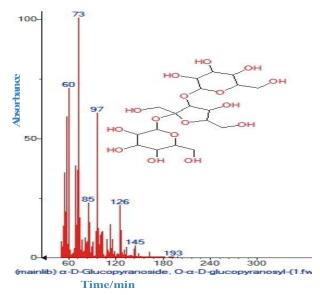


Fig. 13: Mass spectrum, for α-D-Glucopyranoside, O-α-D-Glucopyranosyl-(1.fw) RT (min): 6.537, Molecular Weight: 504.169035.

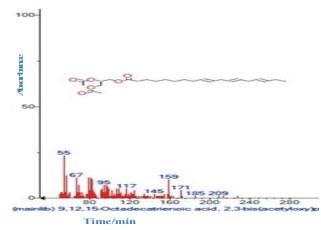


Fig. 15: Mass spectrum, for 9, 12, 15-Octadecatrienoic acid, 2, 3-bis (acetyloxy) RT (min): 7.979, Molecular Weight: Weight: 436.28249.

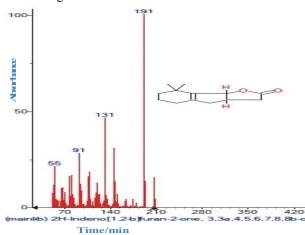


Fig. 17: Mass spectrum, for 2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-o RT (min): 8.580, Molecular Weight: Weight: 206.13068.

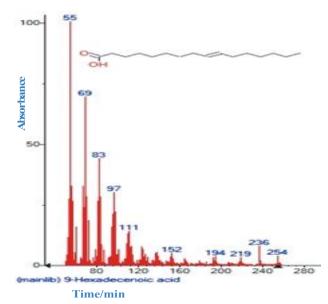


Fig. 14: Mass spectrum, for 9-Hexadecenoic acid RT (min): 6.737, Molecular Weight: 254.22458.

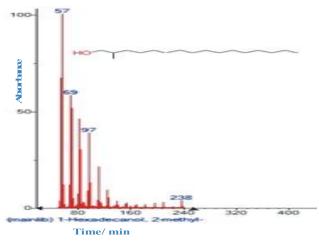


Fig. 16: Mass spectrum, for 1-Hexadecanol, 2-methyl- RT (min): 8.374, Molecular Weight: Weight: 256.276615.

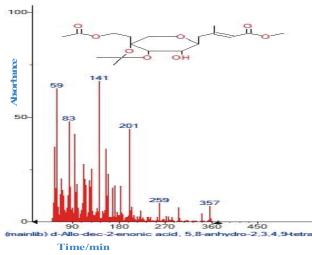


Fig. 18: Mass spectrum, for d-Allo-dec-2-enonic acid, 5, 8anhydro-2, 3, 4, 9-tetra RT (min): 9.873, Molecular Weight: Weight: 372.178417.

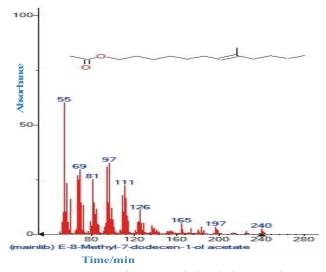


Fig. 19: Mass spectrum for E-8-Methyl-7-dodecen-1-ol acetate RT (min): 10.159, Molecular Weight: Weight: 240.20893.

phytochemical compounds from Methanolic Leaves Extract of Lepidium sativum by using GC-MS technique. Asghar et al., (2011) were identified eleven compounds from the petroleum ether extract of Iris germanica by using GC-MS analysis technique and the bioassay of these compounds were showed antioxidant and antimicrobial efficacy. On the other hand, Iris aitchisonii (Bakar) Boiss., Had antifungal and antibacterial efficacy against Aspergillus niger and Pseudomonas aeruginosa (Ajaib et al., 2013). Methanolic rhizome extract of five species belonging to Iris genus such as Iris kashmiriana, Iris hookeriana, Iris croceae, Iris ensata, and Iris germanica were showed antibacterial activity against wide spectrum of microorganisms (Wani et al., 2012). Iris pseudacorus was showed antibacterial activity against many pathogenic microorganisms (Ramtin et al., 2014). Finally, Iris hollandica plant needs further study by separating these compounds individually and using them in various fields of science to know their future effects and uses.

Conclusion

Iris hollandica has wide range of phytochemical compounds might be using in different field of scientific such as antibacterial, antifungal, antioxidant, anticancer, and anti-inflammatory.

References

- Ajaib, M.U.H.A.M.M.A.D., Z. Khan, M.A. Abbasi and T. Riaz (2013). Antimicrobial screening of Iris aitchisonii (Bakar) Boiss. *Biologia*, **59(1)**: 51-55.
- Altameme, H.J., I.H. Hameed and M.A. Kareem (2015). Analysis of alkaloid phytochemical compounds in the ethanolic extract of Datura stramonium and evaluation of

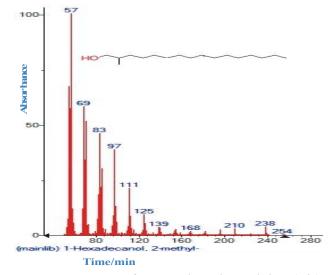


Fig. 20: Mass spectrum, for 1-Hexadecanol, 2-methyl-RT (min): 11.115, Molecular Weight: Weight: 256.276615.

antimicrobial activity. *African Journal of Biotechnology*, **14(19):** 1668-1674.

- Asghar, S.F. and M.I. Choudahry (2011). Gas chromatographymass spectrometry (GC-MS) analysis of petroleum ether extract (oil) and bio-assays of crude extract of Iris germanica. *International Journal of Genetics and Molecular Biology*, **3(7):** 95-100.
- Bryan, J.E. (2002). Bulbs, Revised edition, Timber press, Portland, Oregan U.S.A.
- Buckingham, J. (1999). Dictionary of Natural Compounds. Chapman and Hall, U.K:14-20.
- Hadfield, J.A., A.T. McGowan and J. Butler (2000). A highyielding synthesis of naturally occurring antitumour agent iris-quinone. *Molecules*, 5: 82-88.
- Hameed, I.H., H.J. Hussein, M.A. Kareem and N.S. Hamad (2015). Identification of five newly described bioactive chemical compounds in methanolic extract of Mentha viridis by using gas chromatography-mass spectrometry (GC-MS). Journal of Pharmacognosy and Phytotherapy, 7(7):107-125.
- Hussein, H.J., M.Y. Hadi and I.H. Hameed (2016). Study of chemical composition of Foeniculum vulgare using Fourier transform infrared spectrophotometer and gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*, 8(3): 60-89.
- Hussein, H.J., I.H. Hameed and M.Y. Hadi (2017). Using Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Compounds of Methanolic Leaves extract of Lepidium sativum. *Research Journal of Pharmacy and Technology*, **10(11):** 3981-3989.
- Hussein, J.H., J.M. Ubaid and I.H. Hameed (2017). Gas chromatography-mass spectrum analysis of volatile components of methanolic leaves extract of Cordia myxa. *International Journal of Current Pharmaceutical Review and Research*, **7(6):** 16-22.

- Kaššαk, P. (2012). Secondary metabolites of the choosen genus Iris species. Acta Universitatis agriculturae et silviculturae Mendelianae brunensis, **60(8):** 269-280.
- Larson, R. A. (1980). Introduction to Floriculture, First edition, Academic. Press, New York.
- Mohammad, A. and H. Imad (2013). Autosomal STR: From locus information to next generation sequencing technology. *Research Journal of Biotechnology*, **8(10)**: 92-105.
- Ramtin, M., A. Massiha, M.R.M. Khoshkholgh-Pahlaviani, K. Issazadeh, M. Assmar and S. Zarrabi (2014). In vitro antimicrobial activity of Iris pseudacorus and Urtica dioica. Zahedan Journal of Research in Medical Sciences, 16(3): 35-39.

- Rastogi, R.P. and B.N. Mehrotra (1991). In: Rastogi, R.P. (Ed.), Compendium of Indian Medicinal Plants, Vol. 2. CDRI Lucknow & PID, CSIR, New Delhi, 394-395.
- Stashenko, E. and J.R. Martínez (2014). Gas Chromatography-Mass Spectrometry, in: Guo, X. (Ed.), Advances in Gas Chromatography. Intech, Croatia. 1.
- Wani, S.H., A. Amin, M.A. Rather, J. Parray, A. Parvaiz and R.A. Qadri (2012). Antibacterial and phytochemical screening of different extracts of five Iris species growing in Kashmir. *Journal of Pharmacy Research*, 5(6): 3376-3378.
- Williams, C., J. Harborne and M. Colasante (1997). *Biochem. Syst. Ecol.*, **25:**309.
- Wu, Z.X., (1980). Pharmacological study of anit-cancer drug irisquinone. *Huanue Xuebao*, **38**: 156-159.