

Research Article

Phytochemical characterization using GC–MS analysis of bioactive constituents of ethanolic extract of bulbs of *Tulipa gesneriana* L.

Widad A. Abd AL-BEHADILI*¹, Salah H. FARAJ²

¹Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Misan, Maysan, Iraq.

²Department of Biology, College of Science, University of Misan, Maysan, Iraq.

*Email: widad84ss@uomisan.edu.iq

Abstract: The current study aims to analyze the biochemical composition of tulip (*Tulipa gesneriana*) bulbs. GC/MS analysis revealed the presence of various biologically active compounds, including hexadecanoic acid, oleic acid, campesterol, clionasterol, and stigmasterol. Furthermore, gamma-sitostenone and melezitose were identified in the bulb's extracts. Fatty acid analysis indicated the presence of compounds such as linoleic acid, linoelaidic acid, oleic acid, octanoic acid, eicosanoic acid, and palmitic acid. This thorough investigation offers a comprehensive analysis of tulip bulbs and emphasizes the existence of physiologically active substances such as stigmasterol, furfural, 2-furan-methanol, and 8,11-octadecadienoic acid, methyl ester. The results of this study shed new light on the possible biological and pharmacological effects of these bulbs.

Keywords: Bulbs, Therapeutics properties, Phytochemicals, GC-MS.

Citation: AL-Behadili, W.A.A. & Faraj, S.H. 2023. Phytochemical characterization using GC–MS analysis of bioactive constituents of ethanolic extract of bulbs of *Tulipa gesneriana* L.. Iranian Journal of Ichthyology (Special Issue 1): 327-334.

Introduction

Medicinal plants have valuable components that can be used in drug synthesis and development. Furthermore, these plants are essential to the development of human cultures worldwide, and the World Health Organization (WHO) has called for a return to natural remedies and a reduction in the use of chemically produced ones due to their detrimental side effects (Al-Mayah et al. 2020). Numerous studies in this field have shown how well plants can treat various illnesses; some even outperform synthetic medications in this regard, resulting in fewer side effects (Sanaye et al. 2015; Alrashedi et al. 2021). Current progressions in plant biotechnology have proved the potential of genetic transformation methods to develop the creation of medicinal compounds (The effect of *Agrobacterium tumefaciens* strains on callus induced from the shoot tips of ginger (Al-Mayah et al. 2020) in the

production of some medicinal active compounds estimated by RP-HPLC. Similar approaches exist to be explored for many other plants to optimize the biosynthesis of their phytochemical constituents.

One of the most widespread decorative plants in the world is the tulip (*Tulipa gesneriana* L.) (Li et al. 2022), a member of the Liliaceae family (Wang et al. 2022). Rich in nutritional and therapeutic value for humans and animals, this family is one of the most important and varied plant groups. Estimates place the number of species between 40-55 (Hall 1940; Stork 1984; Kutlunina et al. 2013). The Pamir Alai and Tien Shan Mountain ranges in \ Central Asia are the native habitats of *T. gesneriana* L. Their habitat stretches from the north-west of China to southern Europe, North Africa, Anatolia, and Iran (Pourkhaloee et al. 2018). Because of its extended blossoming period, attractive aroma, and variety of colors, it is thought to have great commercial value.

In terms of horticulture, tulips have been shown to have significant medicinal, ecological, aesthetic, and commercial horticulture significance. Additionally, it also holds great cultural value in many countries around the world, including Turkey, Holland, and Iran, where it is considered the national flower (Veldkamp & Zonneveld 2012; Marasek-Ciolakowska et al. 2012; Mu et al. 2020; Li et al. 2022). The genus currently has 102 recognized taxa and about 6700 cultivars, most of which are hybrids of tulips (Ágoston et al. 2020).

The genus's taxonomic classification is hampered by the broad features displayed by the progeny of Tulip hybridization (Zonneveld 2009). Nonetheless, they can endure and adjust to changes in their surroundings due to their wide genetic diversity. Consequently, the conservation of plant genetic resources depends on this variety (Montalvo et al. 1997). Additionally, native plants with high variety levels have valued gene pools for hybrid breeding in contemporary plant breeding (Valerie 1999). Tissue culture procedures have been extensively applied for the effectual propagation and preservation of medicinal plants, confirming a sustainable source of bioactive compounds. Studies on *Zingiber officinale* have demonstrated successful in vitro regeneration through shoot tip culture, enabling large-scale production of medicinal plant material (Al-Taha et al. 2020). Such techniques could be practical to *T. gesneriana* to improve its obtainability for phytochemical extraction and pharmaceutical claims. The present research objectives are to analyze the phytochemical composition of *T. gesneriana* bulbs using GC-MS technology to classify bioactive compounds with latent medicinal applications. By describing these compounds, the study pursues to deliver a scientific basis for future research on the pharmacological benefits of tulip bulbs.

Materials and Methods

Healthy, disease-free plants were collected from nurseries in the Basra Governorate, meticulously cleaned, dried for one week at 30°C, and

subsequently milled into a powder. The inquiry was conducted in the research laboratories of the University of Misan-College of Pharmacy. Fifty milliliters of 96% pure ethyl alcohol were combined with five grams of pulverized dry plant material (bulbs) to produce an extract of the *Tulipa gesneriana* plant component. Following a 12-hour vibration of the samples, the solution was filtered through filter paper into a beaker and subsequently cooked to 35°C for 24 hours in an electric oven to get a powdered form. In accordance with Santana et al. (2013), the powder was dissolved in 3 milliliters of pure ethyl alcohol. The active ingredients were quantified using a gas chromatography-mass spectrometry (GC-MS) system. To get a potent medicinal extract, the extraction process must be fine-tuned to generate more bioactive metabolites (Hateet et al. 2021).

Phytochemical components of Tulip bulb plant extracts analyzed by GC-MS: An ethanolic tulip extract was tested for phytochemical profiling using gas chromatography-mass spectrometry (GC-MS). With the help of a Millipore filter syringe (0.45 µm), one milligram of each extract was mixed with five milliliters of pure ethanol. Then, half a milliliter of the solution was combined with 4.5 milliliters of 100% ethanol. The GC-MS analysis was carried out following the method described by Santana et al. (2013). The procedure for injecting 1 µl of each extract using a MassHunter GC-MS system is described (Table 1). The NIST GC-MS library was used for comparison after the compounds in the extract were identified. A score greater than 90 indicated that the compounds were most comparable to the library data, and those were recorded.

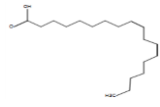
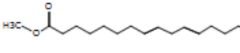
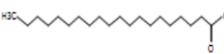
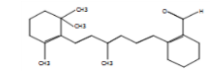
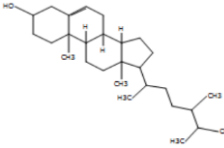
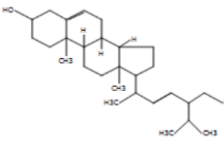
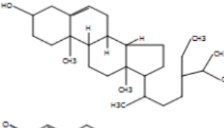
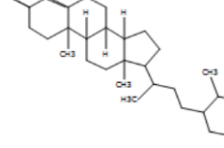
Results and Discussion

Among the bioactive compounds detected in Tulip bulb extract was linoelaidic acid (C₁₈H₃₂O₂) with the highest peak area of 24.66% and retention time of 24.182 minutes followed by n-hexadecanoic or palmitic acid (C₁₆H₃₂O₂) with a peak area of 16.75% and retention time 22.556 minutes followed by Clionasterol (C₂₉H₅₀O) 4.58%, gamma. –Sitostenone

Table 1. Chemicals identified in a GC-MS study of a Tulip bulb ethanolic extract.

No.	R.T.	Compound name	Molecular formula	structure	M. W. (g/mol)	Area%
1	5.518	2-Pentanol, 3-chloro-4-methyl-	C ₆ H ₁₃ ClO		136.620	0.54
2	5.588	2-Heptanol, 3-methyl-	C ₈ H ₁₈ O		130.23	0.55
3	6.29	Furfural	C ₅ H ₄ O ₂		96.08	2.38
4	6.853	2-Furanmethano	C ₅ H ₆ O ₂		98.10	0.43
5	8.944	Pyrazole-4-carboxaldehyde, 1-methyl-	C ₅ H ₆ N ₂ O		110.11	0.81
6	10.118	Alpha-L- Rhamnose	C ₆ H ₁₂ O ₅		164.16	0.48
7	11.807	Melezitose	C ₁₈ H ₃₂ O ₁₆		504.4	0.46
8	12.516	d-Glycero-d-ido-heptose	C ₇ H ₁₄ O ₇		210.18	1.55
9	13.648	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃		126.11	0.53
10	14.843	Octanoic acid, 7-oxo-	C ₉ H ₁₀ O ₂		150.17	2.32
11	17.199	(R)-1-Methyl-4-(6-methylhept-5-en-2-yl) cyclohexa-1,4-diene	C ₁₅ H ₂₄		204.35	0.97
12	22.556	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂		256.42	16.75
13	23.731	Oleic Acid	C ₁₈ H ₃₄ O ₂		282.5	0.49

Table 1. Continued.

No.	R.T.	Compound name	Molecular formula	structure	M. W. (g/mol)	Area%
14	24.182	Linoelaidic acid	C ₁₈ H ₃₂ O ₂		280.4	24.66
15	24.683	8,11-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂		294.5	1.73
16	25.968	Eicosanoic acid	C ₂₀ H ₄₀ O ₂		315.5	1.75
17	27.462	ethyl cholate	C ₂₆ H ₄₄ O ₅		436.6	0.73
18	33.382	Campesterol	C ₂₈ H ₄₈ O		400.7	1.91
19	33.827	Stigmasterol	C ₂₉ H ₄₈ O		412.7	1.41
20	34.779	Clionasterol	C ₂₉ H ₅₀ O		414.7	4.58
21	37.1	Gamma. -Sitostenone	C ₂₉ H ₄₈ O		412.7	2.72

(C₂₉H₄₈O) 2.72%, Furfural (C₅H₄O₂) 2.38%, Octanoic acid, 7-oxo- (C₉H₁₀O₂) 2.32%, Campesterol (C₂₈H₄₈O) 1.91%, Eicosanoic acid (C₂₀H₄₀O₂) 1.75% and 8,11-Octadecadienoic acid, methyl ester (C₁₉H₃₄O₂) 1.73% in addition to other important compounds such as Stigmasterol 1.41%, d-Glycero-d-ido-heptose 1.55%, (R)-1-Methyl-4-(6-methylhept-5-en-2-yl) cyclohexa-1,4-diene 0.97%, Pyrazole-4-carboxaldehyde, 1-methyl- 0.81%, ethyl cholate 0.73%, Oleic Acid 0.49%, 5-Hydroxymethylfurfural 0.53%, Melezitose 0.46%, 2-Pentanol, 3-chloro-4-methyl-, 2-Heptanol, 3-methyl- 0.54%, 2-Furanmethano 0.43% and Alpha-L-Rhamnose 0.48% along with different compounds identified by GC-MS (Table 2 and Figures 1-11). Traditional medicine has an extensive history of

treating human health problems utilizing herbal treatments derived from *Tulipa* plants, reports Aali et al. (2018). Throughout the experiment many bioactive substances were found: linoelaidic acid, palmitic acid, gamma-sitostenone, clionasterol, and stigmasterol. These molecules have great application in medicine because of their strong antibacterial and antifungal qualities. In research, plant-based antibacterial compounds have demonstrated encouraging effects. Medical plants have been proposed by Banu et al. (2010) and AL-Behadili et al. (2019) to help with current health problems.

According to Aali et al. (2018), experiments against several bacterial strains at various solvent concentrations revealed that tulip bulb extracts possessed an antibacterial effect. The isolates fell

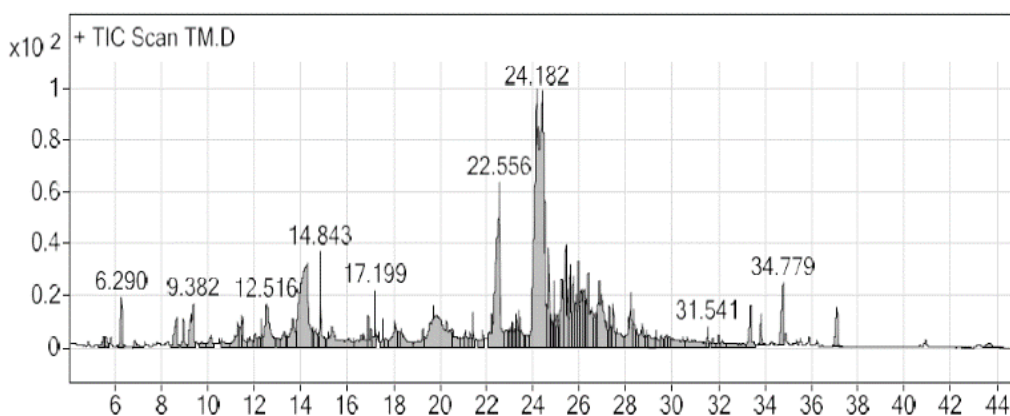


Fig.1. GC-MS chromatogram of the bulbs leaves of tulip.

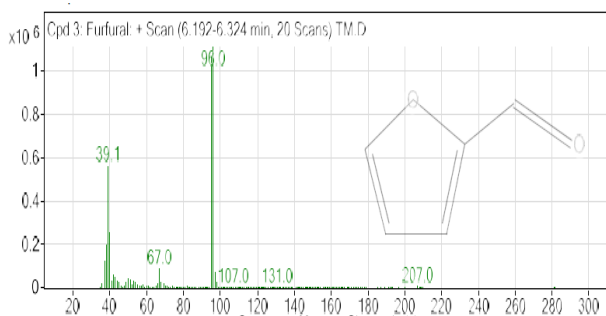


Fig. 2. Mass spectrum of Furfural.

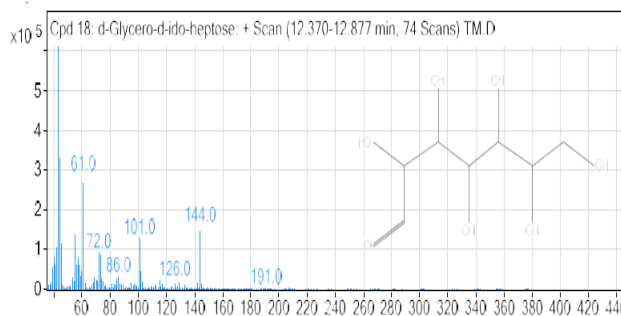


Fig.3. Mass spectrum of d-Glycero-d-ido-heptose.

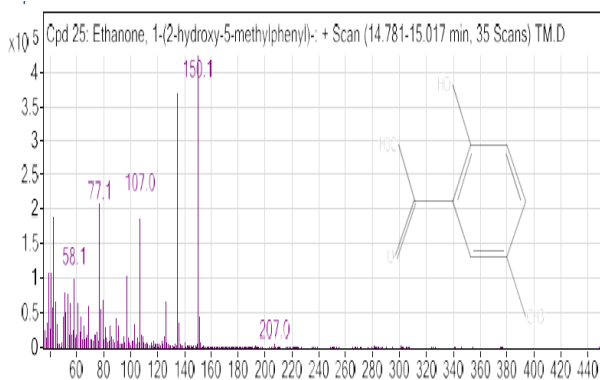


Fig.4. Mass spectrum of Ethanone, 1-(2-hydroxy-5-methylphenyl).

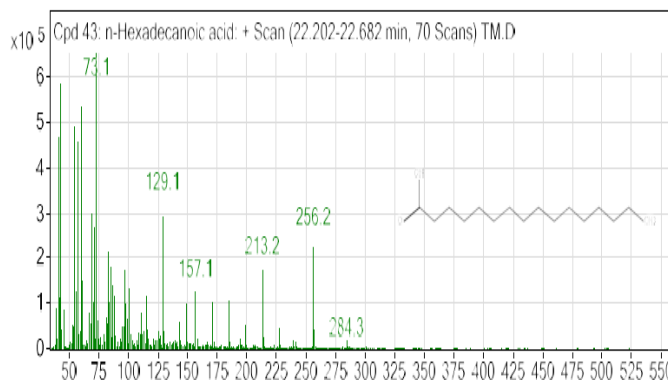


Fig.5. Mass spectrum of n-Hexadecanoic acid.

into either Gram-positive or Gram-negative classification. The bulb extracts' high flavonoid and phenolic content suggested they would be efficient DPPH and ABTS radical scavengers. Tulip bulbs may have antibacterial and antifungal properties, given their long history of medicinal usage and great antioxidant content. Based on the findings of this study, investigating tulip bulb antibiotics and antioxidants could be a fascinating direction for subsequent studies.

The antioxidant properties and bioactive elements of this plant might assist in lessening the severity of several health problems (Lawi et al. 2021). Al-Abboodi (2023) claims that lowering parasitological contamination in vegetables depends on bioactive compounds, compromising public health and food safety. They also allow one to investigate general breast cancer treatment as well as the isolation of particular compounds. The great concentration of bioactive compounds in this plant might aid in

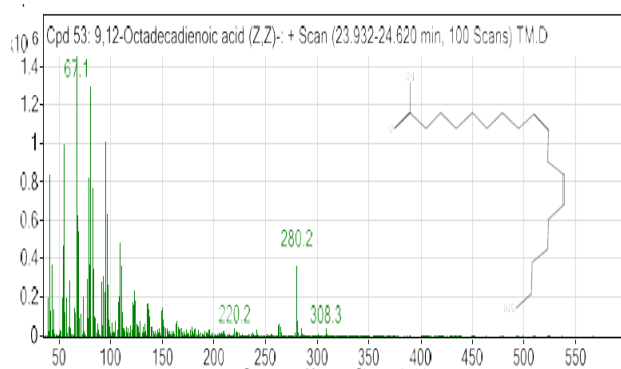


Fig.6. Mass spectrum of Linoelaidic acid.

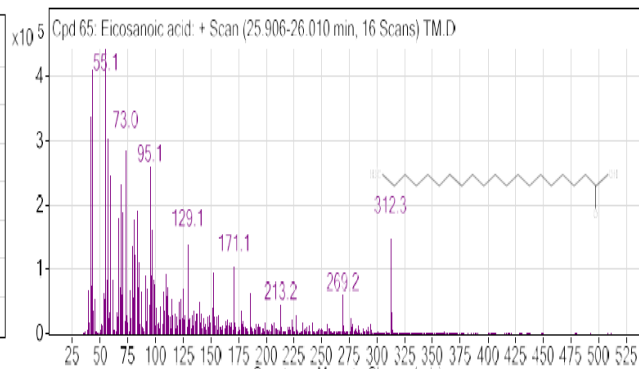


Fig.7. Mass spectrum of Eicosanoic acid.

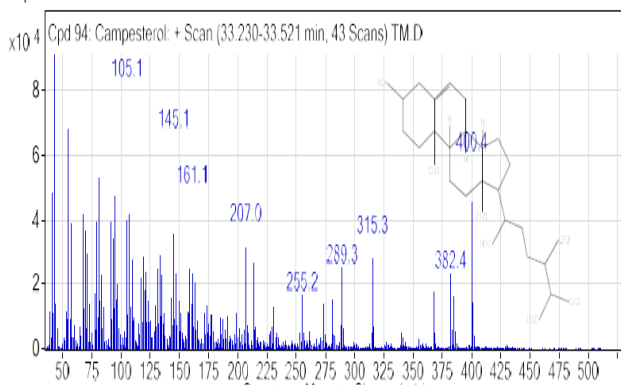


Fig.8. Mass spectrum of Campesterol.

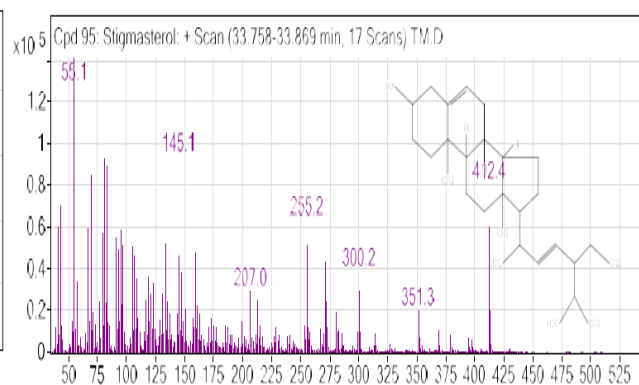


Fig.9. Mass spectrum of Stigmasterol.

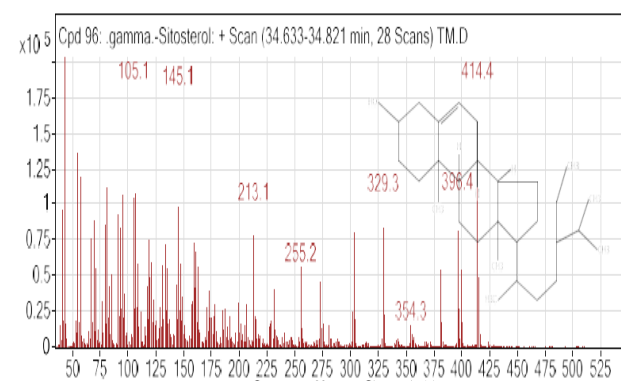


Fig.10. Mass spectrum of Clionasterol.

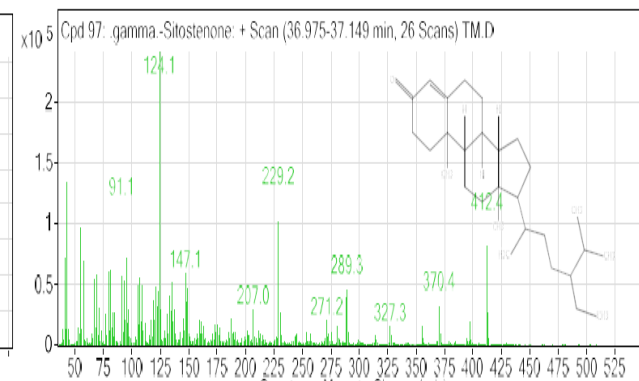


Fig.11. Mass spectrum of gamma-sitostenone.

lowering air pollution. Observing the outward symptoms of stress that plants show when exposed to air pollution helps one to evaluate the condition of the ecosystem (Fadhil et al. 2023).

Conclusions

The ethanolic extract of *Tulipa gesneriana* bulbs was assessed in this work using gas chromatography-

mass spectrometry (GC-MS), therefore exposing many bioactive components. Among other bioactive compounds in tulip bulbs, phytochemicals, sterols, and fatty acids have pharmacological and therapeutic action. Further studies on the possible health advantages of tulips confirm their historical use as a natural remedy and give validity as well. More research on the therapeutic possibilities of these

chemicals is justified, considering their outstanding antioxidant, antifungal, and antibacterial characteristics. Finding and separating active elements is the main objective of this project. Moreover, the results highlight the significance of phytopharmaceuticals in contemporary medicine by opening fresh paths for synthesizing natural therapeutic compounds based on tulip bulbs.

References

- Aali, M.H.; Ismaiel, N.J. & Majid, F.A.A. 2018. Antioxidant, and antimicrobial activities of phenolic and flavonoid rich medicinal plants (*Fritillaria zagrica* and *Tulipa kurdica*) bulbs collected in Kurdistan Region of Iraq. Zanco Journal of Pure and Applied Sciences (ZJPAS) 30(5): 1-16.
- Ágoston, J.; Almási, A.; Salánki, K. & Palkovics, L. 2020. Genetic diversity of potyviruses associated with tulip breaking syndrome.[J]. Plants (Basel Switzerland) 9(12).
- Al-Abboodi, A.K. 2023. Parasitological contamination of raw vegetables collected from selected local markets in Maysan Province, South of Iraq. Nigerian Journal of Parasitology 44(2).
- AL-Behadili, W.A.A.; Faaz, R.A. & Tarmooz, A.A. 2019. Antifungal activity of *Carum carvi* L. extraction against *Candida albican* and *Aspergillus niger*. Plant Archives 19(2): 1799-1805
- Al-Mayah, A.A.; Al-Taha, H.A. & Al-Behadili, W.A.A. 2020. The effect of *Agrobacterium tumefaciens* Strains on callus induced from the shoot tips of ginger (*Zingiber officinale* var. *Roscoe*) in the production of some medicinal active compounds estimated by RP-HPLC. Periódico Tchê Química 17(36): 706-719.
- Al-Mayah, A.A.; Al-Taha, H.A., & Al-Behadili, W.A. 2020. The effect of *Agrobacterium tumefaciens* strains on callus induced from the shoot tips of ginger (*Zingiber officinale* var. *Roscoe*) in the production of some medicinal active compounds estimated by RP-HPLC. Periódico Tchê Química 17(36): 706-719.
- Alrashedi, H.S.; Al-Ataie, S.S.K.; Banoon, S.R. & Fayed, M.I. 2021. Potential role of medicinal plants for the treatment of respiratory viruses: A review. Egyptian Journal of Chemistry 64(12): 7495-7508.
- Al-Taha, H.A.; Al-Mayah, A.A. & Abd Al-Behadili, W.A. 2020. Efficient in vitro regeneration of *Zingiber officinale* Rosc. var. White through shoot tips culture. Plant Archives 20(1): 434-437.
- Banu, B.; Harun, O., Hanifi, K. & Nilufer, S. 2010. Antimicrobial Activity of *Tulipa sintenisii* International J omml of Molecular Medicine and Advance Sciences 6(2): 31-33.
- Esteban, J.L.; Martínez-Castro, I.; Morales, R.; Fabrellas B. & Sanz, J. 1996. Rapid. identification of volatile compounds in aromatic plants by automatic thermal desorption-GC-MS. Chromatographia 43(1-2): 63-72.
- Fadhil, A.A.; Mohammed, S.J. & Aswan, Al-Abboodi. (2023). Morphological responses of plants to air pollutants: A comparative study on leaf changes in five species. Iranian Journal of Ichthyology, 10: 286-293.
- Hall, D. 1940. The genus *Tulipa*. London: Royal Horticultural Society.
- Hateet, R.R.; Hassan, Z.A.; Al-Mussawi, A.A. & Banoon, S.R. 2021. Optimization of cultural conditions affecting improved bioactive metabolite production by endophytic fungus *Trichoderma harzianum*. Revista Bionatura 6(4): 2187-2192.
- Kutlunina, N.A.; Polezhaeva, M.A. & Permyakova, M.V. (2013). Morphologic and AFLP analysis of relationships between tulip species *Tulipa biebersteiniana* (Liliaceae). Russian Journal of Genetics 49: 401-410.
- Lawi, Z.K.K.; Merza, F.A.; Banoon, S.R.; Jabber Al-Saady, M.A.A. & Al-Abboodi, A. 2021. Mechanisms of Antioxidant Actions and their Role in many Human Diseases: A Review. Journal of Chemical Health Risks, 11.
- Li, Y.; Chen, L.; Zhan, X.; Liu, L.; Feng, F.; Guo, Z.; Wang, D. & Chen H. 2022. Biological effects of gamma-ray radiation on tulip (*Tulipa gesneriana* L.). PeerJ 10: e12792.
- Marasek-Ciolakowska, A.; Ramanna, M.S.; Arens, P. & Van-Tuyl, J.M. 2012. Breeding and cytogenetics in the genus *Tulipa*. Floricult Ornament Biotechnol 6: 90-97.
- Montalvo, A.M.; Williams, S.L.; Rice, K.J.; Buchmann, S.L.; Cory, C.; Handel, S.N.; Nabhan, G.P.; Primack, R. & Robichaux, R.H. 1997. Restoration biology: a population biology perspective. Restoration Ecology 5: 277-290.
- Mu, H.; Fan, L.; Zhu, S. & Sun, T. 2020. Effects of arbuscular mycorrhizal fungi on root growth and architecture of Tulip *Gesneriana*. Land Science 2: 60-

66.

- Pourkhaloee, A.; Khosh, K.M.; Arens, P.; Salehi, H.; Razi, H.; Niazi, A.; Afsharifar, A. & Van, T.J. 2018. Molecular analysis of genetic diversity population structure and phylogeny of wild and cultivated tulips (*Tulipa L.*) by genic microsatellites [J]. Horticulture Environment and Biotechnology 59(6).
- Sanaye, M.M.; Joglekar, C.S. & Pagare, N.P. 2015. Mimosin- A brief overview. Journal of Pharmacognosy and Phytochemistry 4(2): 182-187.
- Santana, P.M.; Miranda, M.; Payrol, J.A.; Silva, M.; Hernández, V. & Peralta, E. 2013. Gas chromatography-mass spectrometry study from the leaves fractions obtained of *Vernonanthura patens* (Kunth) H. Rob. International Journal of Organic Chemistry 3: 105-109.
- Stork, A.L. (1984). *Tulipes sauvages et cultivées*. Serie documentaire 13 des Conservatoire et Jardin botaniques, Geneve.
- Valerie, M.W. 1999. Plant nematode resistance genes [J]. Current Opinion in Plant Biology 2(4).
- Veldkamp, J.F. & Zonneveld, B.J.M. 2012 The infrageneric nomenclature of *Tulipa* (Liliaceae) [J]. Plant Systematics and Evolution 298(1).
- Wang, Y.; Chen, L.; Yang, Q.; Hu, Z.; Guo, P.; Xie, Q. & Chen, G. 2022. New insight into the pigment composition and molecular mechanism of flower coloration in tulip (*Tulipa gesneriana L.*) cultivars with various petal colors. Plant Science 317: 111193.
- Zonneveld, B. 2009. The systematic value of nuclear genome size for all species of *Tulipa L.* (Liliaceae). Plant Systematics and Evolution 281: 217-245.