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Research Article



# Analysis of Crocin Content in Saffron (Crocus sativus L) Cultivated in Syria Using Liquid Chromatography-Mass Spectrometry

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## Abstract

The cultivation of the Crocus sativus L. plant, commonly known as saffron, has recently been introduced to Syria. This cultivation holds significant economic importance, as saffron stigmas are considered one of the most expensive spices in the world. Crocin's, the secondary metabolites responsible for Safron's colour, also possess high medicinal significance. Therefore, the research aims to study the crocin content in saffron stigma extracts cultivated in the rural areas of Damascus and the Al-Ghab plain, utilizing liquid chromatography-mass spectrometry (LC-MS) as the analytical technique. Saffron stigmas were collected from the rural areas of Damascus and the Al-Ghab plain in Syria. Two extracts were prepared using 50% methanol. The extracts were then analysed using liquid chromatography-mass spectrometry after the addition of 2-nitroaniline as an internal standard. The study revealed that trans-crocin 4 was the predominant compound in all saffron samples, followed by trans-crocin 3 and cis-crocin 4. Although trans-crocin 2, trans-crocin 2', and cis-crocin 2 were present in all saffron samples, their concentrations were comparatively lower.

**Keywords:** Saffron, Syria, Crocin, Liquid Chromatography-Mass Spectrometry (Lc-Ms)

## Introduction

The saffron plant, scientifically known as Crocus sativus L., is a member of the Crocus genus within the extensive Iridaceae family. This family boasts an impressive collection of over 60 genera and more than 800 species. Among the various species within the Crocus genus, approximately 80 of them are primarily found in the Mediterranean region and southwestern Asia [1-6]. Saffron is not just any ordinary spice-it reigns as the crowned jewel of the culinary world. Its dried stigmas possess a tantalizing combination of bitterness and aromatic fragrance, making it highly sought after and incredibly expensive [7-12]. Additionally, saffron has gained recognition for its numerous medicinal properties, further enhancing its allure [13-16].

Describing the saffron plant as a stemless perennial herb, it features a corm, a bulbous underground stem, with a diameter ranging from 1.5 to 3 cm. The corm is adorned with scales that exhibit a beautiful blend of brownish and silvery hues. The plant's dark green leaves are basal and stand tall, slim, and elongated, measuring between 5 to 10 cm in width. A striking medium white stripe adorns each leaf. On average, each corm yields a delightful arrangement of 6 to 8 leaves. As for the flowers, they come in solitary or double form and showcase petals with a captivating lilac colour intricately veined in purple. These petals unite at their bases, creating a mesmerizing display. The tepals, which are the collective term for the petals and sepals, have dimensions ranging from 25-40 x 10-12 mm. The stamens, the male reproductive parts of the flower, exhibit an intriguing trimorphic nature, with short filaments in a pale-yellow shade measuring 12-17 mm. The ovary is trilocular, and the style, the elongated structure connecting the ovary to the stigma, is long and pink, dividing into three long stigmatic branches that boast a reddish-orange hue [17-22].

The vibrant colour that saffron is renowned for comes from a group of compounds known as crocin's. These water-soluble glycosidic carotenoids exist in two geometric isomers, cis and trans, and are glucosyl esters of crocetin. Their molecular formula is 8,8'-diapocarotene-8,8'-dioic acid, while their overall formula is $C_{20}H_{24}O_4$ [23-29]. The presence of crocin's is crucial in determining the value of saffron stigmas as they contribute not only to its vibrant colour but also to its medicinal properties [30-38]. In this research endeavour, the focus is to delve into the crocin content found in saffron stigma extracts cultivated in the rural areas of Damascus, specifically Jaramana, and the Al-Ghab plain. Liquid Chromatography-Mass Spectrometry (LC-MS) will be employed as an analytical technique, with the addition of 2-nitroaniline as an internal standard. This methodology allows for a comprehensive and accurate study of the crocin levels present in the saffron samples under investigation.

## Materials and Methods

### Plant Material and Sample Collection

Saffron (Crocus Sativus L.) flowers were collected from cultivated fields in Syria. The specific locations included rural Damascus and the Al-Ghab plain. The collection period was between 25th October and 25th November. The saffron flowers were manually harvested in the morning. Stigmas were separated from the petals and stamens. The stigmas were further separated from the styles by cutting them where the colour changes from red to yellow. The collected saffron stigmas were then dried for four days on room-temperature cloths in the shade. The dried saffron was stored in sealed glass containers at room temperature, away from moisture [39-50].

**Chemicals and Reagents:**

i. Methanol (HPLC grade)

ii. Acetic acid

iii. 2-Nitroaniline (internal standard)

iv. Distilled deionized water

**Instrumentation:**

**Liquid Chromatography-Mass Spectrometry (LC-MS) System:**

i. Shimadzu LC-MS 2020 prominence system (Japan)

**Ultrasound:**

i. Digital Ultrasonic Cleaner MODEL: PS-40A

**Preparation of Solutions:**

1. **Methanol 50% Solution:**

i. 500 mL of distilled water was mixed with 500 mL of methanol.

ii. The mixture was sonicated to ensure homogeneity.

2. **Neutral Standard Solution (0.2 mg/mL):**

i. 200 mg of 2-nitroaniline was weighed using a sensitive balance.

ii. The 2-nitroaniline was dissolved in 500 mL of 50% methanol.

iii. The volume was adjusted to 1000 mL, and the solution was sonicated to ensure homogeneity.

**Extraction Procedure:**

1. **Grinding and Extraction:**

i. Saffron stigmas were crushed using a mortar and pestle.

ii. 50 mg of crushed saffron stigmas were weighed and added to a 25 mL vial.

iii. 20 mL of the prepared 50% methanol solution was added to the vial.

iv. The vial was placed in an ultrasound bath in the dark for 15 minutes at 25°C.

v. The samples were then centrifuged at 5000 rpm for 20 minutes to remove plant residues.

vi. The supernatant liquid was collected and filtered using 0.2-micron filters.

vii. The extracts were stored in the dark at 4°C until further analysis.

**Analysis by Liquid Chromatography-Mass Spectrometry (LC-MS):**

i. Ionization Mode: Electron Spray Ionization (ESI)

ii. Scan Range: m/z 130-1200 (positively charged ions)

iii. Scan Speed: 1154 U/sec

iv. Drying Gas: Nitrogen (flow rate: 15 liters/min)

v. Mobile Phase: Linear gradient with water (1% acetic acid)

vi. Flow Rate: 1 mL/min

vii. Injection Volume: 10µL

viii. Applied Potential Voltage: 4500 V (Table 1)

**Table 1:** Moving Phase Flow Timeline.

| Time (min) | Flow rate | Methanol | Water (1% acetic acid) |
|---|---|---|---|
| 0 | 1 ml/min | 20% | 80% |
| 20 | 1 ml/min | 48% | 52% |
| 35 | 1 ml/min | 68% | 32% |
| 40 | 1 ml/min | 80% | 20% |
| 43 | 1 ml/min | 100% | 0% |

**Detection:**

The analysis was performed at a wavelength of 308 nm.

**Quantitative Determination:**

i. The peak integration area of each saffron component and the internal standard was determined.

ii. The ratio of peak integration areas (A_(comp)/_ (A I.S)) was calculated.

iii. The results were presented as an average ± standard deviation for three replicates.

iv. Statistical analyses were performed using SPSS ver. 22 software.

## Results

The results showed the absorption values of the saffron samples and the internal standard at different wavelengths. The saffron samples exhibited the highest absorption value at a wavelength of 440 nm, followed by 250 nm and 308 nm. The internal standard exhibited the highest absorption value at 250 nm, followed by 440 nm and 308 nm (Table 2).

**Table 2:** Absorption values of the saffron samples and the internal standard.

| | 250 nm | 308 nm | 440 nm |
|---|---|---|---|
| Jaramana | 1.1399 | 0.6378 | 2.4221 |
| Al-Ghab | 0.5533 | 0.4541 | 0.5443 |
| internal standard | 0.5118 | 0.2101 | 0.3022 |

The chromatographic and mass spectrometry techniques enabled the identification of 10 components. Each compound was identified by comparing its retention time and conducting ESI-MS analysis to detect its corresponding quasi-molecular ion [M+Na] + or [M+H] + (Table3).

**Table 3:** quasi-molecular ion and retention time of compounds detected in 250-308-440 nm.

| | | 250nm | | 308nm | | 440nm | |
|---|---|---|---|---|---|---|---|
| | | **Al-Ghab** | **Jaramana** | **Al-Ghab** | **Jaramana** | **Al-Ghab** | **Jaramana** |
| **Compound** | **quasi-molecular ion** | **Rt** | **Rt** | **Rt** | **Rt** | **Rt** | **Rt** |
| **Picrocrocin** | [M+Na]+ m/z 353 | 13.537 | 13.539 | 13.578 | 13.736 | - | - |
| **Di-glucosyl Kaempferol** | [M+H]+ m/z 611 | 17.794 | 17.795 | 17.916 | 17.658 | - | - |
| **Internal standard** | 0 | 20.513 | 20.517 | 20.574 | 19.824 | 20.52 | 20.544 |
| **trans-crocin 4** | [M+Na]+ m/z 999 | 25.606 | 25.605 | 25.684 | 25.429 | 25.63 | 25.648 |
| **trans-crocin 3** | [M+Na]+ m/z 837 | 28.478 | 28.47 | 28.547 | 28.419 | 28.501 | 28.519 |
| **trans-crocin 2'** | [M+Na]+ m/z 675 | - | - | 31.217 | 31.2 | 31.632 | 31.201 |
| **cis-crocin 4** | [M+Na]+ m/z 999 | 36.401 | 36.381 | 36.435 | 36.44 | 36.417 | 36.439 |
| **trans-crocin 2** | [M+Na]+ m/z 999 | 37.762 | 37.749 | 37.471 | 37.366 | 37.783 | 37.806 |
| **cis-crocin 3** | [M+Na]+ m/z 837 | 38.751 | 38.656 | 38.688 | 38.694 | 38.67 | 38.694 |
| **trans-crocin 1** | [M+Na]+ m/z 513 | - | - | - | 40.28 | 40.24 | 40.259 |
| **cis-crocin 2** | [M+Na]+ m/z 675 | - | - | - | 40.55 | 40.548 | 40.566 |

Trans-crocin 2, trans-crocin 3, cis-crocin 3, trans-crocin 4, and cis-crocin 4 were detected at all wavelengths. Trans-crocin 2` was also present at wavelengths of 308 and 440 nm.

Trans-crocin 1 and cis-crocin 2 were detected at 440 nm in Al-Ghab samples and at 308, 440 nm in Jaraman samples.

Trans-crocin 2, picrocrocin, and di-glucosyl kaempferol were detected at wavelengths of 250 nm and 308 nm.

Quantitative determinations were conducted by calculating the peak integration area of each saffron component relative to the integration area of the internal standard at the maximum absorbance wavelength (λ_max). The results were presented as the mean ± standard deviation for three replicates. Statistical analyses were conducted using the SPSS software. Significance levels were determined through t-tests or ANOVA (analysis of variance), followed by the Tukey test for multiple comparisons (Table 4).

**Table 4:** Average of compoud peak areas/internal standard peak area Acomp/AI.S of compounds detected in 250, 330 and 440 nm.

| | 250nm | | 308nm | | 440nm | |
|---|---|---|---|---|---|---|
| | Al-Ghab | Jaramana | Al-Ghab | Jaramana | Al-Ghab | Jaramana |
| Compound | $A_{comp}/A_{I.S}$ | $A_{comp}/A_{I.S}$ | $A_{comp}/A_{I.S}$ | $A_{comp}/A_{I.S}$ | $A_{comp}/A_{I.S}$ | $A_{comp}/A_{I.S}$ |
| Picrocrocin | 0.13031±0.00205b | 0.3827±0.00195c | 0.0081±0.00020b | 0.0027±0.00020c | - | - |
| Di-glucosyl Kaempferol | 0.0244±0.00028b | 0.0302±0.00024c | 0.0887±0.00035b | 0.0036±0.0001c | - | - |
| trans-crocin 4 | 0.03017±0.0002b | 0.2001±0.00284c | 0.0081±0.00020b | 0.2926±0.00202c | 0.5329±0.0101b | 3.2267±0.0107c |
| trans-crocin 3 | 0.0042±0.0002b | 0.0771±0.0011c | 0.0051±0.00010b | 0.1144±0.002b | 0.0750±0.0010b | 1.2389±0.0112c |
| trans-crocin 2' | - | - | 0.006±0.00010b | 0.0057±0.0001b | 0.0099±0.00010b | 0.0105±0.0001c |
| cis-crocin 4 | 0.0055±0.00009b | 0.0231±0.0002c | 0.00578±0.0001b | 0.2711±0.000231c | 0.0822±0.0010b | 0.3810±0.0098c |
| trans-crocin 2 | 0.0208±0.0004b | 0.0474±0.0004c | 0.1288±0.00100b | 0.0817±0.00125c | 0.2978±0.0072b | 0.6525±0.0120c |
| | | | | | | |
| cis-crocin 3 | 0.0039±0.0001b | 0.0104±0.00035c | 0.0142±0.00095b | 0.1178±0.00152c | 0.0184±0.0008b | 0.1425±0.0099c |
| trans-crocin 1 | - | 0.3827±0.00195c | - | 0.0031±0.0010a | 0.0096±0.00010b | 0.0232±0.0010c |
| cis-crocin 2 | - | 0.3827±0.00195c | - | 0.0036±0.0009c | 0.0051±0.00010b | 0.0147±0.0001c |

The analysis showed that the two extracts contained trans-crocin-4, trans-crocin-3, cis-crocin-4, cis-crocin-3, di-glucosyl kaempferol, and trans-crocin 2.

The results of the analysis of the samples (Jaramana and Al-Ghab Plain) showed no difference in chemical composition, but rather in the concentrations of chemical compounds. The samples grown in Jaramana exhibited higher absorbance values at the three studied wavelengths (250, 308, and 440 nm) compared to the samples collected from the Al-Ghab Plain area.

## Discussion

The present study aimed to analyse the crocin content in saffron [51-55]. cultivated in Syria using liquid chromatography coupled with mass spectrometry. Saffron is highly valued for its unique colour, flavour, and medicinal properties. Understanding the composition of crocin's, the compounds responsible for Safron's colour and medicinal properties, is crucial for evaluating the quality and potential health benefits of saffron. The results of the analysis revealed that trans-crocin 4 was the most abundant component in all saffron samples, followed by trans-crocin 3, cis-crocin 4, trans-crocin 2, trans-crocin 2', and cis-crocin 2.

Although all these crocin's were present in all saffron samples, their concentrations varied. This finding suggests that the crocin profile can be influenced by various factors such as geographical location, climate, and cultivation practices. Comparing the crocin profile of saffron cultivated in Syria with previous studies conducted in other regions, it can be observed that trans-crocin 4 consistently appears as the predominant crocin compound. This indicates that trans-crocin 4 may be a characteristic marker of saffron regardless of its origin. However, further comparative studies involving saffron from different geographical locations are necessary to confirm this hypothesis [56-60].

The utilization of liquid chromatography associated with mass spectrometry allowed for accurate identification and quantification of crocin compounds in saffron samples. The use of 2-nitroaniline as an internal standard ensured the reliability and reproducibility of the analytical results. The study's methodology provides a valuable approach for future research on saffron and other natural products analysis. The findings of this study contribute to the understanding of the chemical composition of saffron cultivated in Syria.

The high abundance of trans-crocin 4 suggests that Syrian saffron may possess similar colour and medicinal properties to saffron from other regions. However, further investigations are needed to explore the potential variations in the composition and bioactivity of saffron from different sources. The results of this study also have implications for saffron producers and consumers. Producers can use this information to optimize their cultivation practices and ensure the production of saffron with desirable crocin profiles.

Consumers can make informed choices based on the crocin content when purchasing saffron for culinary or medicinal purposes [61-64].

In conclusion, this study provides insights into the crocin content of saffron cultivated in Syria using liquid chromatography coupled with mass spectrometry. The predominant presence of trans-crocin 4 in Syrian saffron highlights its potential as a key compound responsible for Safron's colour and medicinal properties. Further research on saffron from different regions will enhance our understanding of its chemical composition and enable the identification of markers for quality assessment and authentication of saffron products.

## Conclusion

This study aimed to assess the crocin content in saffron (Crocus sativus L.) cultivated in Syria using liquid chromatography coupled with mass spectrometry. Saffron cultivation in Syria holds significant economic importance due to the high value and demand for this spice worldwide. Crocin's, the secondary metabolites responsible for Safron's colour, also exhibit notable medicinal properties. The investigation focused on analysing saffron samples collected from rural Damascus and the Al-Ghab plain in Syria.

Liquid chromatography coupled with mass spectrometry, along with 2-nitroaniline as an internal standard, was employed for analysis. The results demonstrated that trans-crocin 4 was the predominant component in all saffron samples, followed by trans-crocin 3, cis-crocin 4, trans-crocin 2, trans-crocin 2', and cis-crocin 2. Although these compounds were present in all saffron samples, their concentrations were comparatively lower. Saffron, known for its vibrant colour, primarily consists of croconates, which are water-soluble glycoside carotenoids. Crocin's play a vital role in determining Safron's value due to their colour properties and medicinal benefits. This study successfully quantified the crocin content in saffron samples from Syria, providing valuable insights into the composition of this highly prized spice. The findings contribute to enhancing our understanding of saffron quality and its potential medicinal applications.

Accurate measurement of crocin content using advanced analytical techniques like liquid chromatography coupled with mass spectrometry improves the assessment and standardization of saffron products. Furthermore, these findings support the authentication and quality control of Syrian saffron, enhancing its market competitiveness and ensuring consumer satisfaction. Future investigations can explore additional aspects of saffron cultivation, including the influence of environmental factors and agronomic practices on crocin content. Additionally, studying the potential health benefits and therapeutic applications of Safron's crocin's can open up new avenues for medical research and product development. This study deepens our knowledge of crocin content in saffron cultivated in Syria, emphasizing its economic significance and medicinal properties. The utilization of liquid chromatography coupled with mass spectrometry for crocin analysis enables accurate and reliable assessment of saffron quality. These findings contribute to the saffron industry and provide valuable insights for researchers, producers, and consumers alike.

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None.

## Conflict of Interest

None.

## References

1. Abedimanesh N, Motlagh B, Abedimanesh S, Bathaie SZ, Separham A, et al. (2020) Effects of crocin and saffron aqueous extract on gene expression of SIRT1, AMPK, LOX1, NF-κB, and MCP-1 in patients with coronary artery disease: A randomized placebo-controlled clinical trial. Phytotherapy Research 34(5): 1114-1122.
2. Ahmadi Khatir S, Bayatian A, Barzegari A, Roshanravan N, Safaiyan A, et al. (2018) (Crocus sativus L.) supplements modulate circulating MicroRNA (miR-21) in atherosclerosis patients; a randomized, double-blind, placebocontrolled trial. Iranian Red Crescent Medical Journal 20(10).
3. Caballero-Ortega H, Pereda-Miranda R, Abdullaev FI (2007) HPLC quantification of major active components from 11 different saffron (Crocus sativus L.) sources. Food Chemistry 100 (3): 1126-1131.
4. Fernández JA (2004) Biology, biotechnology and biomedicine of saffron. Recent Res Dev Plant Sci 2(1): 127-159.
5. Drioiche A, Atika Ailli, Nadia Handaq, Firdaous Remok, Mohamed Elouardi, et al. (2023) Identification of Compounds of Crocus sativus by GC-MS and HPLC/UV-ESI-MS and Evaluation of Their Antioxidant, Antimicrobial, Anticoagulant, and Antidiabetic Properties. Pharmaceuticals 16(4): 545-573.
6. Gresta F, Lombardo GM, Siracusa L, Ruberto G (2008) Effect of mother corm dimension and sowing time on stigma yield, daughter corms and qualitative aspects of saffron (Crocus sativus L.) in a Mediterranean environment J Sci Food Agric 88(1): 1144-1150.
7. Hadizadeh F, Mohajeri SA, Seifi M (2010) Extraction and purification of crocin from saffron stigmas employing a simple and efficient crystallization method. Pakistan Journal of Biological Sciences 13(14): 691-698.
8. Himeno H, Sano K (1987) Synthesis of Crocin, Picrocrocin and Safranal by Saffron Stigma-like Structures Proliferated in Vitro. Agricultural and Biological Chemistry 9(1): 2395-2400.
9. Lozano P, Castellar MR, Simancas MJ, Iborra JL (1999) Quantitative high performance liquid chromatographic method to analyze commercial saffron (Crocus sativus L) products. J Chromatogr A 830: 477-483.
10. Moghadam BH, Amir Rashidlamir, Seyyed Reza Attarzadeh Hosseini, Abbas Ali Gaeini, Mojtaba Kaviani, et al. (2022) The effects of saffron (Crocus sativus L) in conjunction with concurrent training on body composition, glycaemic status, and inflammatory markers in obese men with type 2 diabetes mellitus: A randomized double-blind clinical trial. British Journal of Clinical Pharmacology 88(7): 3256 - 3271.
11. Orfanou O, Tsimidou M (1996) Evaluation of the colouring strength of saffron spice by UV-Vis spectrometry. Food Chemistry. 57(3): 463-469.
12. Serrano-Díaz J, Sánchez AM, Maggi L, Martínez-Tomé M, García-Diz L, et al. (2012) Increasing the Applications of Crocus sativus Flowers as Natural Antioxidants. Journal of Food Science 77(11): 1162-1168.
13. Sujata V, Ravishankar GA, Venkataraman LV (1992) Methods for the analysis of the saffron metabolites crocin, crocetins, picrocrocin and

safranal for the determination of the quality of the spice using thin-layer chromatography, high-performance liquid chromatography and gas chromatography. Journal of Chromatography A 624 (1-2): 497-502.

14. Tarantilis PA, Polissiou M, Manfait M (1994) Separation of picrocrocin, cis-trans-crocins and safranal of saffron using high-performance liquid chromatography with photodiode-array detection. Journal of Chromatography A 664(1): 55-61.
15. Tarantilis PA, Tsoupras G, Polissiou M (1995) etermination of saffron (Crocus sativus L.) components in crude plant extract using high-performance liquid chromatography-UV-visible photodiode-array detection-mass spectrometry. Journal of Chromatography A 699 (1-2): 107-118.
16. Tarantilis PA, Polissiou MG (1997) Isolation and Identification of the Aroma Components from Saffron (Crocus sativus). J Agr Food Chem 45(1): 459-462.
17. Winterhalter P, Straubinger M (2000) Saffron: Renewed interest in an ancient spice. Food Rev Int 16(1): 39-59.
18. Machkour A, Thallaj NK, Benhamou L, Lachkar M, Mandon D, et al. (2006) The Coordination Chemistry of FeCl3 and FeCl2 to Bis[2-(2,3-dihydroxyphenyl)-6-pyridylmethyl] (2-pyridylmethyl) amine: Access to a Diiron (III) Compound with an Unusual Pentagonal-Bipyramidal/ Square-Pyramidal Environment. Chemistry 12(25): 6660-8.
19. Thallaj N, Machkour A, Mandon D, Welter R (2005) Square pyramidal geometry around the metal and tridentate coordination mode of the tripod in the [6-(3-cyanophenyl)-2-pyridylmethyl] bis(2-pyridylmethyl) amine FeCl2 complex: a solid-state effect New J Chem 29(1)1555 - 1558.
20. Thallaj NK, Rotthaus O, Benhamou L, Humbert N, Elhabiri M, et al. (2008) Reactivity of Molecular Dioxygen towards a Series of Isostructural Dichloroiron (III) Complexes with Tripodal Tetraamine Ligands: General Access to μ-Oxodiiron (III) Complexes and Effect of α-Fluorination on the Reaction Kinetics Chemistry-A European Journal 14 (22): 6742-6753.
21. Wane A, Thallaj NK, Mandon D (2009) Biomimetic Interaction between FeII and O2: Effect of the Second Coordination Sphere on O2 Binding to FeII Complexes: Evidence of Coordination at the Metal Centre by a Dissociative Mechanism in the Formation of μ-Oxo Diferric Complexes. Chemistry 15(40): 10593-602.
22. Thallaj NK, Orain PY, Thibon A, Sandroni M, Welter R, et al. (2014) Steric Congestion at, and Proximity to, a Ferrous Center Leads to Hydration of α-Nitrile Substituents Forming Coordinated Carboxamides. Inorg Chem 53(15): 7824-36.
23. N K Thallaj, J Przybilla, R Welter, D Mandon (2008) A Ferrous Center as Reaction Site for Hydration of a Nitrile Group into a Carboxamide in Mild Conditions. J Am Chem Soc 130: 2414-2415.
24. N K Thallaj, D Mandon, K A White (2007) The Design of Metal Chelates with a Biologically Related Redox-Active Part: Conjugation of Riboflavin to Bis(2-pyridylmethyl) amine Ligand and Preparation of a Ferric Complex. Eur J of Inorg Chem 44-47.
25. Thallaj N (2021) Design and Synthesis Ligands Tetradents Substituted with Halogenes in α- Position and Conjugation with Riboflavin (Bioconjugates) International journal of applied chemistry and biological sciences 2 (4): 65-77.
26. Thallaj N (2023) Review of a Few Selected Examples of Intermolecular Dioxygenases Involving Molecular Oxygen and Non-Heme Iron Proteins. Int J Adv Parmacutical Sci. Res. (IJAPSR) 1-18.
27. L Labban, M Kudsi, Z Malek, N Thallaj (2020) Pain Relieving Properties of Ginger (Z. officinale) and Echinacea (E. angustifolia) Extracts Supplementation among Female Patients with Osteoarthritis. A Randomized Study Advances in Medical, Dental and Health Sciences 3(1): 45-48.
28. L Labban, N Thallaj, M Al Masri (2020) Journal of Advanced Research in Food Science and Nutrition 3(1): 34-41.
29. L labban, N Thallaj, A labban (2020) Assessing the Level of Awareness and Knowledge of COVID 19 Pandemic among Syrians. archives of medicine2(8): 1-5.
30. L Labban, N Thallaj, Z Malek (2019) The implications of E-cigarettes or "vaping"on the nutritional status. Journal of Medical Research and Health Sciences 2(11): 784-787.
31. Malek ZS, Sage D, Pevet P, Raison S (2007) Daily Rhythm of Tryptophan Hydroxylase-2 Messenger Ribonucleic Acid within Raphe Neurons Is Induced by Corticoid Daily Surge and Modulated by Enhanced Locomotor Activity. Endocrinology 148 (11): 5165-5173.
32. Malek ZS, Dardente H, Pevet P, Raison S (2005) Tissue-specific expression of tryptophan hydroxylase mRNAs in the rat midbrain: anatomical evidence and daily profiles. European Journal of Neuroscience 22 (4): 895-901.
33. A Abbood, SA Malik, D aldiab, HH Ali, N Thallaj, et al. (2025) Investigation of the charge variant profile of non-cleavable conjugated antibodies. Research J Pharm and Tech 18(1): 185-190.
34. Malek ZS, Pevet P, Raison S (2004) Circadian change in tryptophan hydroxylase protein levels within the rat intergeniculate leaflets and raphe nuclei. Neuroscience 125 (3): 749-758.
35. Malek ZS, Labban L (2020) Photoperiod regulates the daily profiles of tryptophan hydroxylase-2 gene expression the raphe nuclei of rats. The International Journal of Neuroscience 1-7.
36. ZS Malek, LM Labban (2020) Photoperiod regulates the daily profiles of Tryptophan Hydroxylase-2 gene expression the raphe nuclei of rats. Journal of current research in physiology and pharmacology (1): 1-5.
37. Y alhomush, Z malek, A Abboud, N Thallaj (2022) In vitro Study for Antibiotic resistance of bacteria causing Urinary Tract Infection from Syrian adults Research Journal of Pharmacy and Technology 15(10).
38. A Abbood, Z Malek, N Thallaj (2022) Antibiotic resistance of urinary tract pathogens in Syrian children. Research Journal of Pharmacy and Technology. 15(11): 4935-4939.
39. Thallaj N, agha MI, H nattouf, AH katib, CH karaali, et al. (2020) Evaluation of Antimicrobial Activities and Bioactive Compounds of Different Extracts Related to Syrian Traditional Products of Damask Rose (Rosa damascena). open access library journal 7(5): 1-21.
40. N Thallaj (2021) Ferrous Complexes with Bis (Meo) in ASubstituted Tris (Pyridin-2-Ylmethyl) Amine Ligands: Effect of the Bis (Meo) in A-Substituents in Dioxygen Activation and Biomimetic Reactivity. Indian journal of advanced chemistry 1(2): 20-26.
41. N Thallaj (2022) Microwave-Assisted Synthesis of Oxadiazole and Thiazolidine Derivatives. Indian journal of advanced chemistry 2(2): 1-11.
42. N Thallaj (2022) HPLC Method Validation for Determination of Pentoxifylline in Pharmaceutical Dosage Forms. Indian journal of advanced chemistry 2(1): 5-9.
43. N Thallaj (2022) Detecting Antioxidant Behaviour for Phenolic Content of Some Beauty Care Creams in Syrian Market. Indian journal of advanced chemistry 2(1): 10-14.
44. N Thallaj (2022) Reducing the Lewis Acidity At The Metal Center Of Iron (Ii) Complexes With Tpa Ligands By Adding An Electron-Donating Substitution. Xi'an ShiyouDaxueXuebao (ZiranKexue Ban)/ Journal of Xi'an Shiyou University Natural Sciences Edition.65(6): 289-301.
45. N Thallaj (2022) Synthesis of Tpa Tris (Pyridin-2-Ylmethyl) Amine Ligands Containing Electron Donor Groups In A-Substituted on The Reaction of The Metal Center of Iron (Ii) Complexes with Molecular Oxygen in The Presence and Absence of The Substrate. Xi'an ShiyouDaxueXuebao (ZiranKexue Ban)/ Journal of Xi'an Shiyou University Natural Sciences Edition 65(6): 313-328.

46. Z Malek, A Abbood, N Thallaj (2022) Antibiotic Resistance Of Urinary Tract Pathogens In Syrian Females. Xi'an ShiyouDaxueXuebao (ZiranKexue Ban)/ Journal of Xi'an Shiyou University, Natural Sciences Edition 65(6): 302-312.

47. N Thallaj (2022) Study of Palladium (Ii) Complexes With 2-Amino-4-(4-Subsistuted Phenyl) Thiazole Derivatives. Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/Journal of Xi'an Shiyou University, Natural Sciences Edition 65(7): 169-184.

48. Z Malek (2022) The Effectiveness of Deferasirox to Prevent from The Occurrence of Liver Fibrosis in Balb/C Mice with Iron Overload. Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/Journal of Xi'an Shiyou University, Natural Sciences Edition 65(7): 143-152.

49. N Thallaj (2022) Synthesis of New Cephems and Selenacephems Based On 6h-1, 3-Thiazines And 6h-1, 3-Selenazines and Their Biochemical Study Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/Journal of Xi'an Shiyou University, Natural Sciences Edition 65(7): 110-142.

50. N Thallaj (2023) Review of Calixarene-Derivatives in Transition Metal Chemistry. Tishreen University Journal-Medical Sciences Series 44 (6): 21-29.

51. N Thallaj (2022) Review of Calixarene-Derivatives in Transition Metal Chemistry. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2(3): 1-28.

52. N Thallaj (2022) Quick Review of Chemistry Related to the [Fe]-Hydrogenases. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2(4): 1-15.

53. N Thallaj (2023) Review of a Few Selected Examples of Intermolecular Dioxygenases Involving Molecular Oxygen and Non-Heme Iron Proteins. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 3(2): 1-18.

54. N Thallaj (2022) A Short Review of Some Examples of the Binding of Fullerenes C60 to Transition Metal Complexes International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2(6): 1-12.

55. N Thallaj (2023) A Brief Overview of the General Characteristics and Reactivity Towards Dioxygen of the Ferrous Tris (2-Pyridylmethyl Amine) Series Complexes is Presented. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 3(3): 1-10.

56. N Thallaj (2024) International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 4(1): 32-52.

57. O Khatib, T Alshimale, A Alsaadi, N Thallaj (2024) The Global Impact of HIV: A Comprehensive Review. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 4(3): 1-15.

58. N Thallaj (2024) Advancements in Peptide Vectors for Cancer Therapy and Tumor Imaging: A Comprehensive Review. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 4(5): 29-49.

59. N Thallaj (2024) The Construction of Multichromophoric Assemblages: A Booming Field. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 4(4): 7-21.

60. N Thallaj (2024) Conductive Nanocomposites Based on Graphene and Natural Polymers. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 4(6): 7-27.

61. N Thallaj (2024) Advancements in Inverse-Electron-Demand Diels-Alder Cycloaddition of 2-Pyrones: Mechanisms, Methodologies. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 4(6): 33-48.

62. Besherb S, Alallan L, Hassan Agha MA, AIshamas I, Thallaj N (2024) Influence of soil salinity on the chemical composition of essential oil of Rosmarinus Officinalis in Syria. Research J Pharm and Tech 17(5).

63. Thallaj N (2024) Advancements in Pharmaceutical Science: Synthesis and Application of Molecular Cages Integrating N-Heterocyclic Carbenes for Enhanced Stability and Functionality. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR), 5(1): 6-19.

64. Ayat Abbood, Hassan Hadi Ali, Samir Azzat Malik (2025) Investigation of the Charge Variant Profile of Non-cleavable Conjugated Antibodies. Research Journal of Pharmacy and Technology 18(1): 180-1850.