



Research Article:

Characterization of Constituent Therapeutic Components of *Alcea kurdica* Flowers and Leaves Using HPLC Technique

Dilbreen H. Abdulqader ¹ , Sami R. AL-Zubaydi ¹, Enas Jawad Kadhim ²,¹ College of Pharmacy, University of Duhok, Duhok, Iraq.² Department of Pharmacognosy, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Article Information

Article history:

Received on: 28 March 2023

Revised on: 10 April 2023

Accepted on: 11 April 2023

Published on: 01 June 2023

Keywords:

Alcea kurdica,
Flavonoids,
HPLC,
Sterols.

Abstract

Background: *Malvaceae* is a large herbal family with more than a hundred genera and thousands of species. Some of these species are well-known for their therapeutic activity, including *Alcea*. **Aim:** The purpose of this study is to isolate and identify the biologically and therapeutically active constituents of the flowers and leaves of *Alcea kurdica* alongside the characterization of the extract composition. **Methods:** Extraction was done with 70% ethanol using Soxhlet extraction method. The fractionated extracts were condensed, dried, and labeled as (LEA) for organic leaf extract, (LW) for aqueous leaf extract, (FEA) for organic flower extract, and (FW) for aqueous flower extract using ethyl acetate with an equal volume of water and shaken three times. **Results:** The isolated flavonoids and sterols were identified by comparison with the chromatograms of the standard compounds, which were prepared under standard circumstances. Flavonoids and sterols were found in the leaves and flower extract of *Alcea kurdica* after HPLC analysis.

2023 [Iraqi Journal of Pharmacy](#). Published by [University of Mosul](#), Iraq. This is an open access article licensed under CC BY: (<https://creativecommons.org/licenses/by/4.0>)

1. Introduction

Malvaceae, a cosmopolitan family of 244 genera and probably more than 4225 species. The family is identified by the presence of mucilage cavities, cells, or ducts that are either lysigenous or schizogenous (1). The high concentration of polyphenols, which serve as chemotaxonomic markers in the *Malvaceae* family of plants, sets them apart from other leafy plants. Moreover, they showed a significant concentration of cyclopropane acids, which was not seen in plants from other families. The *Alcea* species have received relatively little attention in study to

date. Representative image for the species *Alcea kurdica* shown in [Figure 1](#) (2).



Figure 1. Representative image for the plant *Alcea kurdica*.

***Corresponding author:** Dilbreen H. Abdulqader, College of Pharmacy, University of Duhok, Duhok, Iraq.

E-mail: phcdilbreen@gmail.com

How to cite:

Abdulqader, D., H., AL-Zubaydi, S., R., & Kadhim, E., J. (2023). Characterization of constituent therapeutic components of *Alcea kurdica* flowers and leaves using HPLC technique. *Iraqi J. Pharm.* 20(1), 41-47.

DOI: <https://doi.org/10.33899/iphr.2023.139258.1031>

The Highlands of Turkey, Armenia, and Kurdistan have been regarded as the foci of speciation for the genus *Alcea*, one of the largest genera of the *Malvaceae* family with almost 70 species worldwide (3). Researchers have become interested in the *Alcea* species because of their possible antibacterial properties in addition to their antioxidant, anti-inflammatory, and cytotoxic effects, notably because of flavonoids and other phenolic components (4). *Alcea* is a botanical source for various pharmaceutically active components. Even though different parts of the plant, including the flowers and herbage, contain a variety of active substances, e.g., starch, mucilage, pectin, sucrose, and phenolics (5).

The vast class of polyphenolic chemicals known as flavonoids is generally present in all foods that are derived from plants, including flavones, flavanones, flavanols, isoflavones, anthocyanidins, and flavanols (6). Common fruits like grapefruit, plant-based beverages, vegetables including parsley, onions, oranges, tea, chamomile, wheat sprouts, and various seasonings are all rich sources of apigenin (a flavone) (7). Resveratrol (a polyphenol) is not widely distributed across plant species and has only been identified in a small number of fruits and vegetables used for human consumption, raising the hypothesis that it is virtually exclusively found in red wine among dietary components (8). Silybin, a flavolignan that occurs naturally, is isolated from *Silibum marianum*'s fruits (9). As a flavanol, rutin is widely distributed in plants like apple, buckwheat, tea, and passion flower. It is an essential part of food's nutrition. Buckwheat contains rutin, also known as rutoside, quercetin-3-rutinoside, and sophorin, a citrus flavonoid glycoside. Chemically, it is a glycoside made up of the disaccharide rutinose and the flavonolic aglycone quercetin (10).

The three phytosterols (β -sitosterol, campesterol, and stigmasterol), which make up 65%, 30%, and 3% of the food contents, respectively, are the most prevalent sterols in human herbal nutrition (11). The unsaturated plant sterol stigmasterol, sometimes referred to as stigmasterin or wulzen anti-stiffness factor, is found in many therapeutic plants. In Calabarbohne in 1906, *Adolf Wind Form and A. Hauth* isolated it for the first time (12). One of the main sterols in plant cell plasma membranes, stigmasterol, is involved in cell proliferation and the activation of the plasma membrane H^+ -ATPase (13). A naturally occurring micronutrient called β -sitosterol is present in the cell membranes of all plants that produce oils, including fruits, vegetables, cereals, seeds, and trees. It has been established that β -sitosterol is a safe, non-toxic nutritional supplement with tremendous potential health benefits in a wide range of uses (14). The primary usage and benefit of β -sitosterol are to decrease cholesterol. Yet, research suggests that the mentioned phytochemical may also boost health in other ways, including by alleviating the symptoms of benign prostatic enlargement, lowering cancer risk, and preventing oxidative damage through its antioxidant action (15).

High-performance liquid chromatography, also known as high pressure liquid chromatography (HPLC), is one of the most widely used, cutting-edge, potent, and adaptable chromatographic separation techniques. It has been regularly used to separate, identify, and quantify components from complex mixtures, such as herbal extracts or products, and to create chemical profiles or fingerprints of unprocessed mixtures (16). These days, it is crucial to evaluate and monitor the quality of medicines and natural products because the effectiveness of the goods is directly

impacted by the quality of the contents. Techniques for HPLC fingerprinting have been widely used to evaluate the quality of functional goods and traditional Chinese medicines (17).

The flavonoids quercetin, luteolin, rutin, apigenin, and kaempferol were frequently recognized and quantified in the chromatogram of *Ocimum sanctum* and *Ocimum basilicum* produced by HPLC using UV-Vis as a detector. Phenolic acids (gallic, caffeic, ferulic, sinapic, and syringic) and flavonoids (quercetin, luteolin, and rutin) were commonly identified and quantified in the chromatogram of *Ocimum sanctum* and *Ocimum basilicum* carried out by HPLC using UV-Vis as detector (18). Although it is often done in reverse phase, analytical liquid chromatography has not been widely used to ascertain the sterol composition of vegetable oils. The total amount of phytosterols can also be accurately quantified using normal-phase HPLC techniques, but these techniques have poor chromatographic resolution and do not provide detailed information on the sterol composition (19). The present study aimed to separate the therapeutically active sterols and flavonoids from *Alcea kurdica* in our locality.

2. Material and Methods

2.1. Plants source and collection

Fragments of *Alcea kurdica* were gathered from Duhok's natural fields. Specimens are stored in the pharmacy college's herbarium at Duhok University. The entire plant was cleaned, washed, cut into pieces, and then shade dried for two weeks at room temperature in a ventilated area. Separately, the dried leaves and flowers were ground using a mortar and pestle, and the resulting powders were put into a container that was well-sealed in preparation for the extraction process (18,19).

2.2. Preparation of extracts

The Soxhlet extraction method was used to extract the phytochemicals. A 50g of dried plant powder (flowers and leaves were extracted separately) was used for this extraction, which was carried out using 500 ml of 70% ethanol. The extraction procedures took nine hours to complete. Rotary evaporators were used to condense the volume of the extracted material, which was subsequently transferred to an oven to complete drying. The fractionated extracts were condensed, dried, and labeled as (LEA) for organic leaves extract, (LW) for aqueous leaves extract, (FEA) for organic flower extract, and (FW) for aqueous flower extract after the dried extract was dissolved in 250 ml of ethyl acetate and shaken with an equal volume of water three times. The dried extracts were moved to HPLC for additional analysis, following extraction the organic and water layers were condensed and dried again after separation and subsequent identification (19,20).

2.3. Sample preparation for flavonoids isolation

A mixture of 3g of samples and 60 ml of methanol/water (40/60) was blended for 24 hours. The mixture was filtered, and the filtrate was then heated to 40°C and concentrated to a volume of 5 ml under vacuum. Using 5 ml of 2N NaOH, this solution was hydrolyzed for 30 minutes. With the help of 2N HCl, the mixture's acidity was raised, and ethyl acetate was used to extract the phenolic acids by a liquid-liquid process (20mlx3). The ethyl acetate was then extracted at decreased pressure after the extracts were

mixed. A small volume of 10 μ l of the residue's solution in 7 ml of methanol was used for the HPLC analysis (20,21).

2.4. HPLC conditions for flavonoids

A C18-ODS column (250mm, 4.6 mm, 5 μ m) was used in the high-performance liquid chromatography (HPLC) examination, which was carried out using a SYKAMN HPLC system (Germany). The system was injected with samples (100 μ l). 95% acetonitrile plus 0.01% trifluoroacetic acid (solvent A) and 5% acetonitrile plus 0.01% trifluoroacetic acid (solvent B) were used in the mobile phase at a flow rate of 1 ml/min. The gradient program looked like this: 10% A from 0 to 5 minutes, 25% A from 7 to 15 minutes, and finally a return to the initial conditions. A UV-visible detector operating at 278 nm was used to find phenolic chemicals (20) (Figure 2).

2.5. Sample preparation for sterol isolation

A 2.5 g of dried plant material was pulverized, extracted using Soxhlet apparatus using 70% ethanol over two days at 37°C, subjected to ultrasound treatment for 30 min, and the solvent was evaporated to dryness in a rotary evaporator to produce 2.36% (w/w) crude extract. An accurately weighted 10 mg of solvent-free dried extract was dissolved in 5ml of methanol to prepare a concentration of 2mg/ml. Prior to

injection, the aliquot was filtered via a 0.45mm membrane to obtain a filtrate (22).

2.6. HPLC condition for sterols:

A C18-ODS column (250mm, 4.6 mm, 5 μ m) was used in the high-performance liquid chromatography (HPLC) examination, which was carried out using a SYKAMN HPLC system (Germany). The system was injected with samples (100 μ l). Acetonitrile, DW, and acetic acid were the components of the mobile phase, which had a flow rate of 1 ml/min. A UV-visible detector operating at 220 nm was used to detect sterols (21) (Figure 2).

3. Results and Discussion

The organic and aqueous extracts from the flowers and leaves with characteristic HPLC profiles of flavonoids are shown in Figure 3. The flavonoids were identified by comparison with the chromatograms of the standard flavonoid compounds, which were prepared under standard circumstances. The chromatograms of the standard flavonoids rutin (Rt = 3.90 min), silybin (Rt = 5.12 min), resveratrol (Rt = 7.32 min), and apigenin (Rt = 10.00 min) are shown, respectively, in Figure 4. On the other hand, the distinctive HPLC traces of the sterols from the organic and aqueous fractions of the *Alcea kurdica* flowers and leaves extracts are depicted in Figure 5.

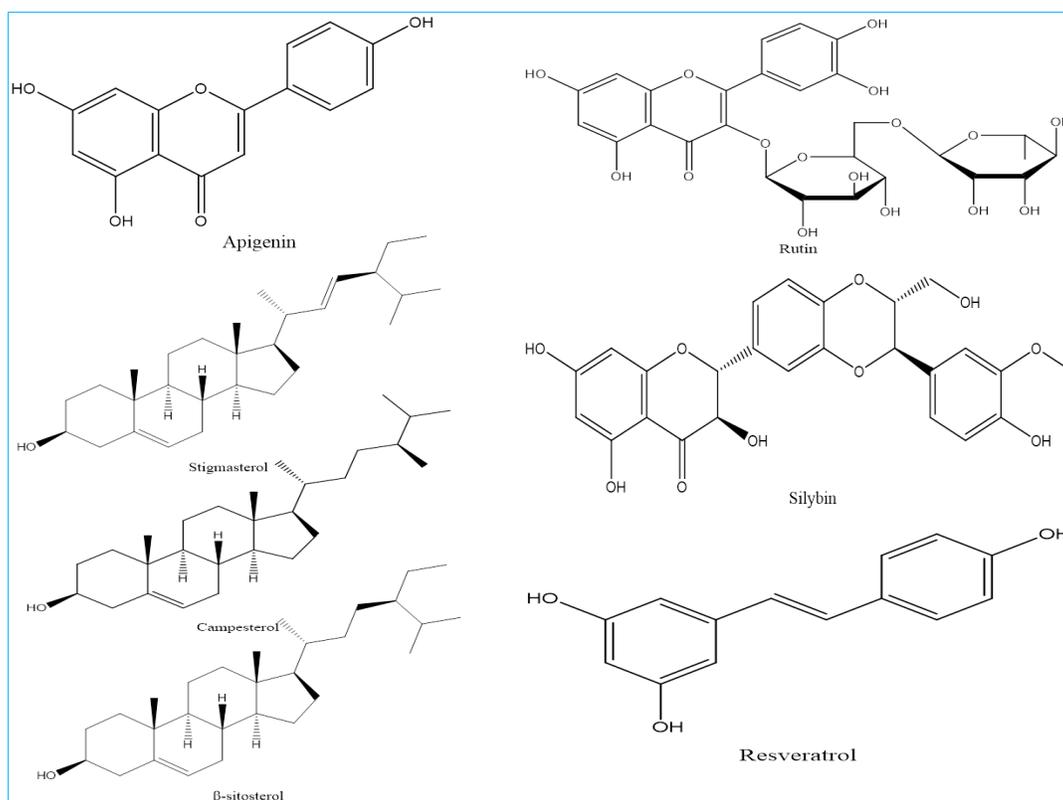


Figure 2. The chemical structure of the flavonoids and sterols present in *Alcea kurdica*.

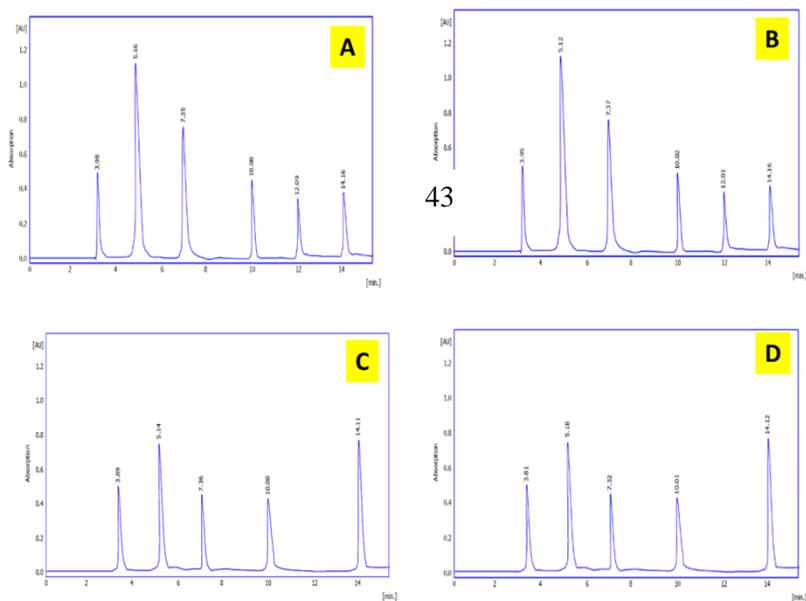


Figure 3. HPLC chromatograms of the *Alcea kurdica* flavonoids A) organic, B) aqueous fractions of the flower extracts, C) organic and D) aqueous fractions of the leaves extracts

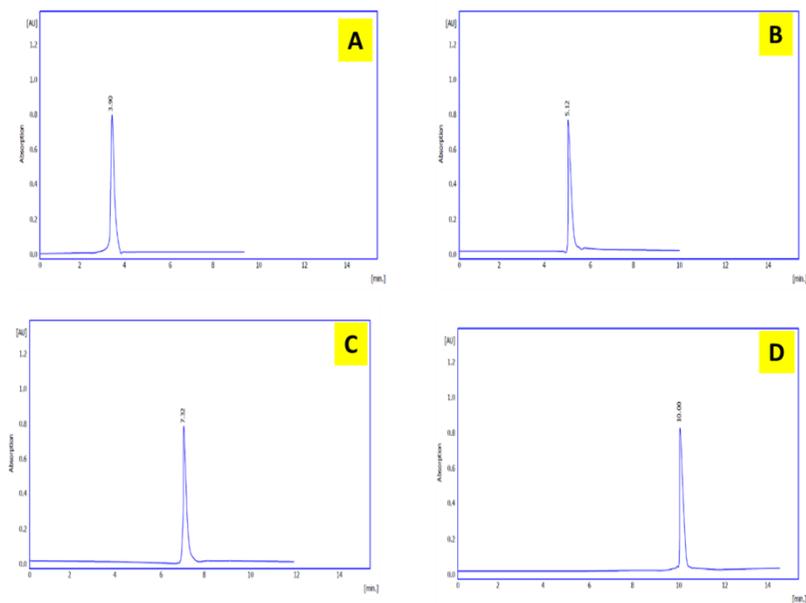


Figure 4. HPLC chromatograms of standard flavonoids A) rutin, B) silybin, C) resveratrol, and D) apigenin

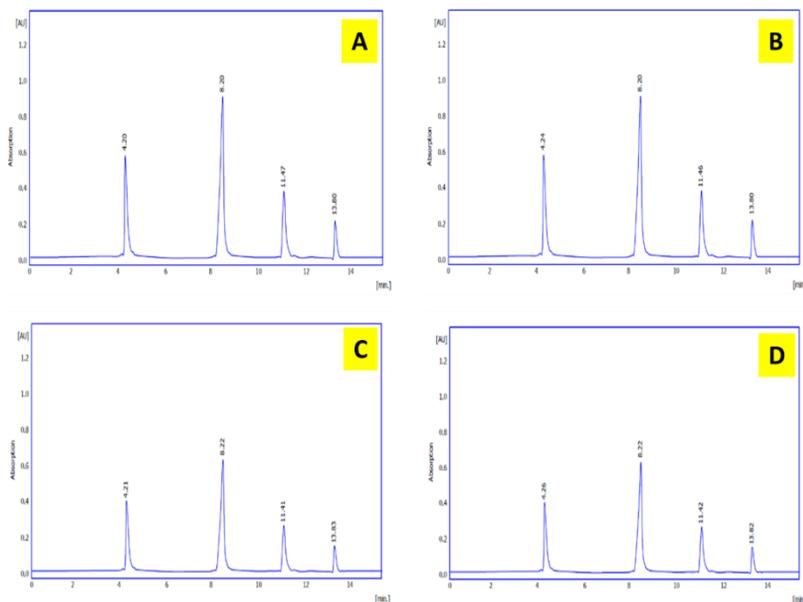


Figure 5. HPLC chromatograms of the *Alcea kurdica* sterols A) organic, B) aqueous fractions of the flower extracts, C) organic and D) aqueous fractions of the leaves extracts

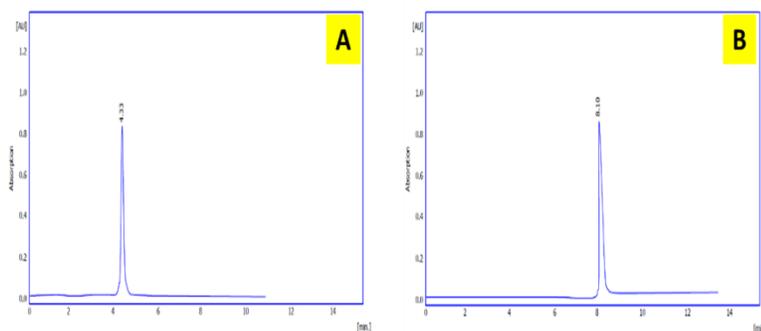


Figure 6. HPLC chromatograms of standards of A) beta-sitosterol and B) stigmasterol

Despite the fact that the total phenolic content of the *Alcea kurdica* species was quantified (22) and antioxidant activity was assessed (23), however, no clear data were found about the nature of flavonoids in *Alcea kurdica* species. In the present study, apigenin, resveratrol, rutin, and silybin were identified in both flowers and leaves. The findings were consistent with other *Alcea* species; apigenin has been found in *Alcea fasciculiflora*. *Alcea calvertii* contains resveratrol (24). *Alcea rosea* contained rutin, a flavonoid glycoside (25).

Figure 6 shows the chromatograms of the standard sterols β -sitosterol (Rt =4.33 min) and stigmasterol (Rt =8.10 min), respectively. The flavonoids and sterols in the aqueous and organic fractions of the *Alcea kurdica* leaves and flower extracts were quickly analyzed using this approach.

Moreover, apigenin is found in members of the *Malvaceae* family such as *A. augustum* L, *A. theophrasti*, *B. ceiba* L, *H. sabdariffa* L, *S. sabdariffa* L, and *K. vitifolia* L. Also, apigenin is found in additional plants from the *Malvaceae* family such as, *A. indicum* (Link) sweet, *K. vitifolia* L, *L.* & *T. populnea* (L.) Sol. ex *Correa*'s

hermaphrodita plant (26). Several *Malvaceae* family members, such as *H. sabdariffa*, contain resveratrol (27). Some species of the *Malvaceae* family, including *Abelmoschus cailei*, *Hibiscus asper*, *H. rosa-sinensis*, *H. sabdariffa*, *H. schizopetalus*, *Malvaviscus arboreus*, *Sida acuta*, and *Sida rhombifolia*, showed positive results when tested for the presence of sterols (28).

The present study has identified β -sitosterol and stigmasterol in both flowers and leaves, despite the fact that there is no information available regarding the nature of sterols in *Alcea kurdica* species. The findings were comparable to those of other *Alcea* species, such as *Althaea rosea*, which had β -sitosterol and stigmasterol in their flowers, leaves, and seeds (29). *Althaea armeniaca* and *Althaea nudiflora* are other *Alcea* species that contain sterols (30).

4. Conclusion

Chromatographic analysis of *Alcea kurdica* plant leaves and flowers revealed the presence of various sterols and

flavonoids. Apigenin, resveratrol, rutin, and silybin flavonoids were identified in both flowers and leaves. The findings were consistent with other *Alcea* species; apigenin has been found in *Alcea fasciculiflora*. β -sitosterol and stigmasterol are found in both flowers and leaves, despite the fact that there is no information available regarding the nature of sterols in *Alcea kurdica* species. The existence of flavonoids and sterols was verified following the hydrolysis of the ethanol fraction, which resulted in the liberation of various flavonoids that were not detectable in the ethyl acetate fraction.

5. Acknowledgments

The authors are grateful to the College of Pharmacy/University of Duhok and the College of Pharmacy/University of Baghdad for their assistance and support.

6. Conflict of interest

There is no conflict of interest.

7. References

- Bayer C, Kubitzki K. Malvaceae. Springer Berlin Heidelberg, 2003.
- Ertas A, Boga M, Gazioglu I, Yesil Y, Hasimi N, Ozaslan C, Yilmaz H, Kaplan M. Fatty acid, essential oil and phenolic compositions of *Alcea pallida* and *Alcea apterocarpa* with antioxidant, anticholinesterase and antimicrobial activities. *Chiang Mai Journal of Science*. 2016;43(1):1143-53.
- Khodayari H, Arabameri M. *Alcea acaulis* and *A. fasciculiflora*, two new records for the flora of Iran. *Rostaniha*. 2019;20(2):173-81.
- Esmaelian B, Kamrani YY, Amoozegar MA, Rahmani S, Rahimi M, Amanlou M. Anti-cariogenic properties of malvidin-3, 5-diglucoside isolated from *Alcea longipedicellata* against oral bacteria. *International Journal of Pharmacology*. 2007;3:468-74.
- Shah SA, Akhtar N, Akram M, Shah PA, Saeed T, Ahmed K, Asif HM. Pharmacological activity of *Althaea officinalis* L. *Journal of Medicinal Plants Research*. 2011;5(24):5662-6.
- Ali F, Rahul, Naz F, Jyoti S, Siddique YH. Health functionality of apigenin: A review. *International Journal of Food Properties*. 2017;20(6):1197-238.
- Shukla S, Gupta S. Apigenin: A Promising Molecule for Cancer Prevention. *Pharmaceutical Research*. 2010;27(6):962-78.
- Jebir RM, Mustafa YF. Natural Coumarin-Lead Compounds: A Review of Their Medicinal Potentials. *Iraqi Journal of Pharmacy*. 2022;18(2):139-61.
- Borsari M, Gabbi C, Ghelfi F, Grandi R, Saladini M, Severi S, Borella F. Silybin, a new iron-chelating agent. *Journal of inorganic biochemistry*. 2001;85(2-3):123-9.
- Ganeshpurkar A, Saluja AK. The pharmacological potential of rutin. *Saudi pharmaceutical journal*. 2017;25(2):149-64.
- Mustafa YF, Oglah MK, Bashir MK. Conjugation of sinapic acid analogues with 5-Fluorouracil: Synthesis, preliminary cytotoxicity, and release study. *Systematic Reviews in Pharmacy*. 2020;11(3):482-9.
- Kaur N, Chaudhary J, Jain A, Kishore L. Stigmasterol: a comprehensive review. *International Journal of Pharmaceutical Sciences and Research*. 2011;2(9):2259.
- Aboobucker SI, Suza WP. Why do plants convert sitosterol to stigmasterol?. *Frontiers in plant science*. 2019;10:354.
- Sen A, Dhavan P, Shukla KK, Singh S, Tejovathi G. Analysis of IR, NMR and antimicrobial activity of β -sitosterol isolated from *Momordica charantia*. *Science secure journal of biotechnology*. 2012;1(1):9-13.
- Bulama JS, Dangoggo SM, Mathias SN. Isolation and characterization of beta-sitosterol from ethyl acetate extract of root bark of *Terminalia glaucescens*. *International Journal of Scientific and Research Publications*. 2015;5(3):1-3.
- Nahar L, Onder A, Sarker SD. A review on the recent advances in HPLC, UHPLC and UPLC analyses of naturally occurring cannabinoids (2010-2019). *Phytochemical Analysis*. 2020;31(4):413-57.
- Tao H, Cui B, Zhang H, Bekhit AE, Lu F. Identification and characterization of flavonoids compounds in cassava leaves (*Manihot esculenta* Crantz) by HPLC/FTICR-MS. *International journal of food properties*. 2019;22(1):1134-45.
- Ullah S, Naseem RA, Hussain A, Sheikh IA, Farooq M. HPLC profile of phenolic acids and flavonoids of *Ocimum sanctum* and *O. basilicum*. *International Journal of Plant Based Pharmaceuticals*. 2022;2(2):205-9.
- Perez-Castano E, Ruiz-Sambles C, Medina-Rodriguez S, Quiros-Rodriguez V, Jimenez-Carvelo AM, Valverde-Som L, Gonzalez-Casado A, Cuadros-Rodriguez L. Comparison of different analytical classification scenarios: application for the geographical origin of edible palm oil by sterolic (NP) HPLC fingerprinting. *Analytical Methods*. 2015;7(10):4192-201.
- Ngamsuk S, Huang TC, Hsu JL. Determination of phenolic compounds, procyanidins, and antioxidant activity in processed *Coffea arabica* L. leaves. *Foods*. 2019;8(9):389.
- Maji AK, Pandit S, Banerji P, Banerjee D. A validated RP-HPLC method for simultaneous determination of betulin, lupeol and stigmasterol in *Asteracantha longifolia* nees. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2014;6(5):691-5.
- Qader SW, Awad HM. Evaluation of antioxidant, antimicrobial and cytotoxicity of *Alcea kurdica* Alef. *Jordan Journal of Biological Sciences*. 2014;7(3):205-9.

23. Bouayed J, Piri K, Rammal H, Dicko A, Desor F, Younos C, Soulimani R. Comparative evaluation of the antioxidant potential of some Iranian medicinal plants. *Food Chemistry*. 2007;104(1):364-8.
24. Ozturk RB, Zengin G, Sinan KI, Montesano D, Zheleva-Dimitrova D, Gevrenova R, Uba AI, Cakılcıoğlu U, Kaplan A, Jugreet S, Dall'Acqua S. Which Extraction Solvents and Methods Are More Effective in Terms of Chemical Composition and Biological Activity of *Alcea fasciculiflora* from Turkey?. *Molecules*. 2022;27(15):5011.
25. Keser S, Keser F, Tekin S, Kaygılı O, Turkoglu I, Demir E, Karatepe M, Kirbag S, Yilmaz O, Sandal S, Turkoglu S. In vitro Antiradical, Antimicrobial and Antiproliferative Activities and Phytochemical Compositions of Endemic *Alcea calvertii* (Boiss) Boiss. Flowers. *Duzce Universitesi Bilim ve Teknoloji Dergisi*. 2020;8(1):693-701.
26. Vadivel V. Distribution of flavonoids among Malvaceae family members-A review. *International Journal of Green Pharmacy*. 2016;10(1):S33.
27. Ojulari OV, Lee SG, Nam JO. Beneficial effects of natural bioactive compounds from *Hibiscus sabdariffa* L. on obesity. *Molecules*. 2019;24(1):210.
28. Focho DA, Nkeng EA, Lucha CF, Ndam WT, Afegenui A. Ethnobotanical survey of plants used to treat diseases of the reproductive system and preliminary phytochemical screening of some species of malvaceae in Ndop Central Sub-division, Cameroon. *Journal of Medicinal Plants Research*. 2009;3(4):301-14.
29. Fahamiya N, Shiffa M, Aslam M. A comprehensive review on *Althaea rosea* Linn. *Journal of Pharmaceutical Research*. 2016;6(11).
30. Egamberdieva D, Mamedov N, Ovidi E, Tiezzi A, Craker L. Phytochemical and pharmacological properties of medicinal plants from Uzbekistan: A review. *Journal of Medicinally Active Plants*. 2017;5(2):59-75.

توصيف المكونات العلاجية المكونة لأزهار وأوراق نبات الختمية باستخدام تقنية الاستشراب

الخلاصة:

المقدمة: الخبازيات هي عائلة عشبية كبيرة تضم أكثر من مائة جنس وآلاف الأنواع. بعض هذه الأنواع معروفة بنشاطها العلاجي، بما في ذلك نبات الختمية. **الهدف:** الغرض من هذه الدراسة هو عزل وتحديد المكونات النشطة علاجياً لأزهار وأوراق نبات الختمية جنباً إلى جنب مع توصيف تركيبة المستخلص. **طريقة العمل:** تم الاستخلاص باستخدام 70% من الإيثانول باستخدام طريقة استخلاص بالسوكسليت. تم تكثيف المستخلصات المجزأة وتجفيفها وتصنيفها على أنها (LEA) لمستخلص الأوراق العضوية، و (LW) لمستخلص الأوراق المائية، و (FEA) لمستخلص الزهور العضوية، و (FW) لمستخلص الزهور المائية باستخدام أسيتات الإيثيل مع حجم متساو من الماء وتهتز ثلاث مرات. **النتائج:** تم تحديد مركبات الفلافونويد والستيرول المعزولة بالمقارنة مع كروماتوجرام المركبات القياسية، والتي تم تحضيرها في ظل الظروف القياسية. تم العثور على مركبات الفلافونويد والستيرول في مستخلص أوراق ومستخلص زهرة نبات الختمية بعد تحليل الاستشراب.

الكلمات المفتاحية: نبات الختمية، الفلافونويدات، الاستشراب، الستيرولات