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Phytochemical investigation of some active constituents from N-Butanol fraction of *Tropaeolum majus* L. cultivated in Iraq

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Abstract*---Tropaeolum majus*, Tropaeolaceae family, is anornamental plant and has a long history of use as a medicinal plant; it is cultivated currently in most countries of the world, including Iraq. The aim of this work was to isolate and identify three compounds from the leaves of *Tropaeolum majus* (N-butanol fraction) cultivated in Iraq. The constituents were isolated by preparative high-Performance Liquid Chromatography and their structures were determined using precise NMR spectroscopic techniques.

Keywords*---Tropaeolum majus,* Preparative HPLC, NMR.

Introduction

Traditional remedies remain the first line of pharmacotherapy for many millions of people around the world. The archetypal examples of traditional medicines being transformed into modern drugs are antimalarial quinine and antipyretic analgesic aspirin (1). Traditional herbal medicines are defined by the World Health Organization as natural, plant-derived products that have undergone little or no industrial processing and have been utilised to cure sickness in local or regional healing traditions. Because of its natural origins and absence of side effects, traditional herbal medicine and its preparations have been widely utilised for thousands of years in both developing and developed countries (2). The use of herbal products has increased dramatically over the last three decades all over the world. Herbal medications are utilized as part of basic health care by over 80% of the world's population, mainly in developing countries, as part of their primary health care needs (3, 4). It is often assumed that plants are responsible for 25% of all medications used today. According to this estimate, plant-derived

pharmaceuticals account for a significant share of natural products-pharmaceutics related (5).

Drug development of bioactive molecules from plants was previously time-consuming, with the process of isolating and determining the chemical structures of bioactive chemicals from an extract taking months or years. High-performance liquid chromatography (HPLC) coupled to mass spectrometry, liquid chromatography-mass spectrometry (LC-MS), higher magnetic field-strength nuclear magnetic resonance (NMR) devices, and robots have considerably reduced time (2).

Tropaeolum majus, also known as garden nasturtium, is a Tropaeolaceae family member that originated in the South American Andes and has been grown as a crop plant since ancient times (6). It is now grown as an ornamental plant in most countries around the world, including Iraq. It is widely cultivated as both a decorative and therapeutic plant (7). Popularly, it is well-known for its diuretic, antiseptic, purgative, hair tonic, anti-inflammatory, antiscorbutic, antihypertensive, and antidepressant properties. It is also used to clean the skin and eyes, as well as to treat skin disorders, pulmonary disorders, amyotrophic lateral sclerosis, psoriasis, eczema, and scrofula (8, 9).

Previous phytochemical studies on this plant have revealed the existence of some chemical components in specific parts of the plant, but no complete characterization has been performed. Polyphenols, flavonoids, alkaloids, saponins, tannins, anthocyanins, carotenoids, and terpenoids are some of the chemical constituents (10-12).

Materials and Methods

Plant material collection

The leaves of *Tropaeolum majus* of the Family (Tropaeolaceae) were collected from Babil in January and February 2021. The plant was identified and authenticated by Prof. Dr Sukaena Abass /Department of Biology /College of Sciences/ University of Baghdad. Leaves were washed thoroughly, dried under shade, and ground in a mechanical grinder to a fine powder.

Method of work

The leaves' air-dried powder is weighed, then defatted with n-hexane to remove chlorophyll and waxy material, then extracted in soxhlet with 80% ethanol for 18 hours, then mixed and dried using a rotary evaporator, the dry extract is weighted and the yield of extraction is calculated. The dry extract is dissolved in water and partitioned 2-3 times with various solvents of increasing polarity such as petroleum ether, chloroform, ethyl acetate and N-butanol then each fraction is dried and weighted. The chromatographic method preparative HPLC will be used to isolate several chemicals from the N-butanol fraction. The separated chemicals will be identified using the NMR spectroscopic technique**.**

Isolation and Identification of the Major Polyphenolic Compound

The isolation of the compounds in the *Tropaeolum majus* leaves (N-butanol) fraction was carried out by preparative HPLC technique using a Shimadzu, LC-10-AD HPLC system with an SPD-20A-PDA detector equipped with a preparative column (RP-C18, 5µm, 250 mmX 10 mm, Kromasil). For system control and data acquisition, Sweden software (Shimadzu-lc-labsolutions) was used. The sample solvent was methanol with a concentration of 1 mg/mL; filtration using a 0.45 µm syringe filter was then carried out. The mobile phase system consisted of two phases: acetonitrile: acidified water (1% Triflouroacetic acid) mixtures at different ratios. The flow rate was 3 mL/min and the injection volume was 100 µL (13, 14). The structures of the isolated compounds were elucidated by ^{1}H and ^{13}C NMR spectroscopical analyses. The NMR spectra were taken by dissolving the sample in 0.2 ml of deuterated dimethyl sulphoxide (DMSO) and transferred in a 3 mm NMR tube and then run on NMR Spectrometer. The NMR measurement was carried out at the Center for Drug Discovery, Research and Development, Faculty of Pharmacy, Ain Shams University using (Bruker®, AVANCE III HD, 400 MHz) spectrometer apparatus (^{1}H NMR was determined at 400.1 MHz and ^{13}C-NMR was determined at 100 MHz), (15).

Result and Discussion

Isolation of compounds from N-butanol fraction

HPLC is a technology often employed for natural material separation. Preparative HPLC chromatography is an outstanding purification technology that has been created to assure the isolation and identification of important components in the chemical, pharmaceutical, biotechnological, and biochemical sectors. Preparative HPLC is a costly process when compared to other classic techniques (distillation, crystallisation, or extraction). Optimization, which is required to get a high purity result, was the most significant aspect of preparative liquid chromatography. Following a synthetic or natural extract procedure, preparative HPLC is used to separate and refine high-purity target chemicals from mixed solutions. The goal of HPLC, a prepared, high-performance liquid chromatography (high-purity fraction), is to seperate pure substances in the shortest amount of time feasible and then deposit the sample in the sample collector, which is required for further analysis, evaluation, and operations. The isolation of the compounds (M1, M2, and M3) from *Tropaeolum majus*was achieved byPreparative HPLC. Subsequent injections and collections, the chemicals were separated in sufficient amount and purity to provide high-quality spectra. All data were analysed statistically as described previously (16, 17).

Identification of the isolated compounds

The identification of the isolated compounds structures were achieved by ^{1}H NMR and ^{13}C NMR spectroscopical analyses.

1- Compound (M1)

The ^{1}H NMR and ^{13}C NMR of the isolated compound (M1) are shown in table 1.

Table 1: The Observed ^{13}C NMR and ^{1}H NMR Spectroscopic Data of M1

Position	^{13}C NMR	^{1}H NMR
2	156.87	
3	133.74	
4	177.82	
5	161.66	
6	99.14	5.35 (1H, d)
7	164.51	
8	94.05	6.39 (1H, d)
9	157.07	
10	104.42	
1'	121.63	
2'	115.68	6.42 (1H, d)
3'	145.19	
4'	148.85	
5'	116.72	6.85 (1H, d)
6'	122.05	7.59 (1H, m)
1"	101.63	5.09 (1H, d)
2"	74.52	3.73-3.04 (6H, M)
3"	76.89	
4"	70.45	
5"	76.35	
6"	67.45	
1'"	101.19	4.37 (1H, d)
2'"	70.83	3.73-3.04 (4H, M)
3'"	71.1	
4'"	72.30	
5'"	68.70	
6'"	18.19	1.01 (1H, d)

Based on the spectral data and the related literatures (18, 19), we could conclude that compound M1 was rutin as shown in figure 1.

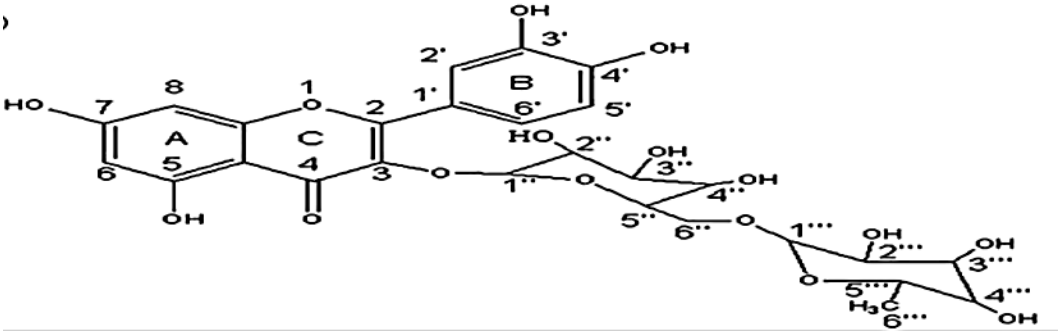

Figure 1: Chemical structure of rutin

2- Compound (M2)

The ^{1}H NMR and ^{13}C NMR of the isolated compound (M2) are shown in table 2.

Table 2: The Observed ^{13}C NMR and ^{1}H NMR Spectroscopic Data of M2

Position	^{13}C NMR	^{1}H NMR
C2	79.14	5.6 (1H, dd)
C3	42.54	3.2 (1H, M), 2.73(1H, dd)
C4	197.69	
C5	163.39	12.1 (1H, s)
C6	96.96	6.15 (1H, d)
C7	165.77	
C8	95.90	6.19 (1H, d)
C9	165.67	
C10	103.71	
C1'	129.08	
C2', C6'	128.91	7.4 (2H, d)
C3', C5'	115.65	6.8 (2H, d)
C4'	158.28	9.6 (1H, s)
C1''	100.06	4.9 (1H, d)
C2''	73.48	5.35 (1H, d)
C3''	76.77	3.38 (2H, m)
C4''	69.95	5.05 (1H, d)
C5''	77.54	3.38 (2H, m)
C6''	61.02	3.43 (1H, m), 3.67 (1H, m)

Based on the spectral data and the related literatures (20), we could conclude that compound M2 was naringenin7-O-β-D-glucoside as shown in figure 2.

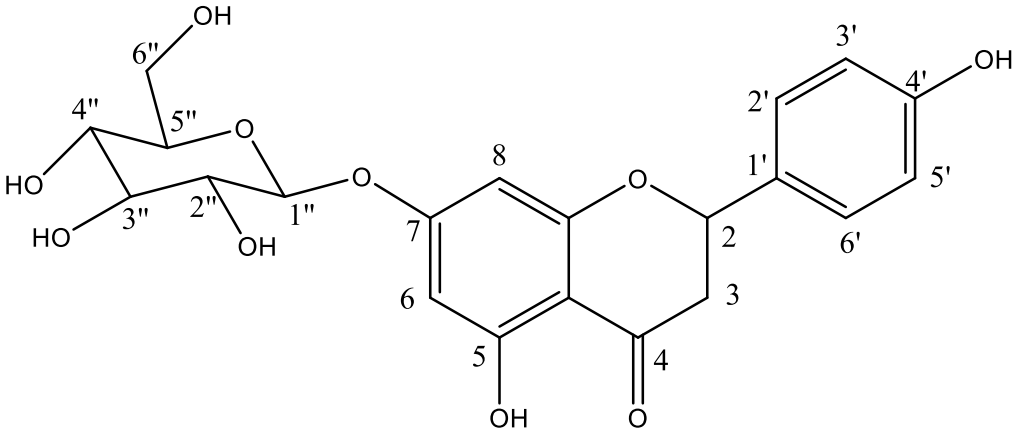

Figure 2: Chemical structure ofnaringenin7-O-β-D-glucoside

3- Compound (M3)

The ^{1}H NMR and ^{13}C NMR of the isolated compound (M3) are shown in table 3.

Table 3: The Observed ^{13}C NMR and ^{1}H NMR Spectroscopic Data of M3

Position	^{13}C NMR	^{1}H NMR
2	77.72	6.45 (1H, dd)
3	56.41	3.65 (2H, d)
4	182.42	
5	163.40	
6	99.94	6.91 (1H, s)
7	164.77	
8	95.46	6.94 (1H, s)
9	161.57	
10	105.79	
1'	122.70	
2'	100.49	7.63 (1H, s)
3'	148.72	
4'	157.38	
5'	110.71	7.45 (1H, d)
6'	116.42	7.6 (1H, d)
-O-CH3	61.09	3.86 (1H, s)
1″	100.49	5.05 (1H, d)
2″	73.60	3.32-3.8 (6H, m)
3″	73.60	
4″	70.07	
5″	76.93	
6″	61.09	

Based on the spectral data and the related literatures (21, 22), we could conclude that compound M3 was hesperetin-7-O-β-D-glucoside as shown in 3.

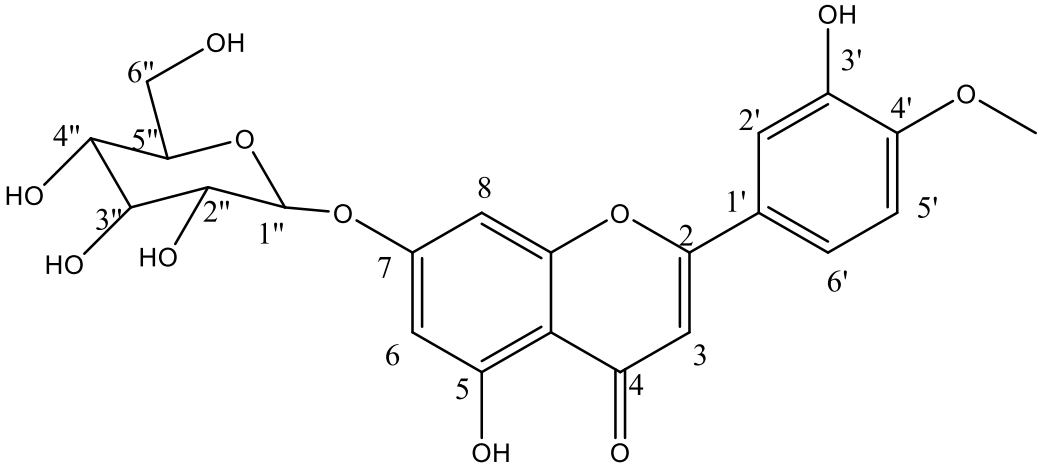

Figure 3: Chemical structure of hesperetin-7-O-β-D-glucoside

The three compounds were separated from *Tropaeolum majus*, which are rutin, naringenin-7-O-β-D-glucoside, and hesperetin-7-O-β-D-glucoside. They were all flavonoid glycosides, and the last two compoundswere isolated for the first timefrom this plant.

Conclusion

The current study's findings proved the existence of bio-active phytochemicals in the leaves extract. Using preparative HPLC and NMR spectroscopic techniques, three compounds including rutin, naringenin7-O-β-D-glucoside, and hesperetin-7-O-β-D-glucoside were successfully separated and identified from an extract of *Tropaeolum majus*. The data also suggested that the naringenin7-O-β-D-glucoside and hesperetin-7-O-β-D-glucoside were isolated and identifiedfor the first time in this plant. Thus, it may be concluded that the leaves of *Tropaeolum majus* have great potential for producing healthy and highly nutritive products.

References

1. Corson TW, Crews CM. Molecular Understanding and Modern Application of Traditional Medicines: Triumphs and Trials. Cell. 2007;130(5):769–74.
2. Qazi Majaz A, Molvi Khurshid I. Herbal medicine: A comprehensive review. Int J Pharm Res. 2016;8(2):1–5.
3. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Neurol. 2014;4 JAN(January):1–10.
4. Thakur L, Ghodasra U, Patel N, Dabhi M. Novel approaches for stability improvement in natural medicines. Pharmacogn Rev. 2011;5(9):48–54.
5. Schmidt BM, Ribnicky DM, Lipsky PE, Raskin I. Revisiting the ancient concept of botanical therapeutics. Nat Chem Biol. 2007;3(7):360–6.
6. Munir M, Alhajhoj MR, Khakwani AA, Baloch JUD. Flowering time response of nasturtium (Tropaeolum majus L.) cultivar 'Empress of India' to photoperiod, light integral and temperature using photo-thermal model. Songklanakarin J Sci Technol. 2015;37(3):247–54.
7. Huxley AJ, Griffiths M. Dictionary of gardening. Stockton Press; 1992.
8. Brondani JC, Cuelho CHF, Marangoni LD, de Lima R, Guex CG, Bonilha I de F, et al. Traditional usages, botany, phytochemistry, biological activity and toxicology of Tropaeolum majus L. - A review. Bol Latinoam y del Caribe Plantas Med y Aromat. 2016;15(4):264–73.
9. Cock IE, Van Vuuren SF. The traditional use of southern African medicinal plants in the treatment of viral respiratory diseases: A review of the ethnobotany and scientific evaluations. J Ethnopharmacol [Internet]. 2020;262(March):113194.
10. Jakubczyk K, Janda K, Watychowicz K, Łukasiak J, Wolska J. Garden nasturtium (Tropaeolum majus L.) - a source of mineral elements and bioactive compounds. Rocz Panstw Zakl Hig. 2018;69(2):119–26.
11. Jurca T, Baldea I, Filip GA, Olteanu D, Clichici S, Pallag A, et al. A Phytocomplex Consisting of Tropaeolum majus L. And Salvia officinalis L. Extracts Alleviates the Inflammatory Response of Dermal Fibroblasts to Bacterial Lipopolysaccharides. Oxid Med Cell Longev. 2020;2020.
12. Bazylko A, Granica S, Filipek A, Piwowarski J, Stefańska J, Osińska E, et al. Comparison of antioxidant, anti-inflammatory, antimicrobial activity and chemical composition of aqueous and hydroethanolic extracts of the herb of Tropaeolum majus L. Ind Crops Prod. 2013;50:88–94.
13. Asha S, Thirunavukkarasu P, Sadiq AM. Preparative HPLC method for the isolation of compounds from Euphorbia hirta Linn. ethanol extract. Res J

Pharm Technol. 2018;11(6):2541–5.
14. Mansour R Ben, Wided MK, Cluzet S, Krisa S, Richard T, Ksouri R. LC-MS identification and preparative HPLC isolation of Frankenia pulverulenta phenolics with antioxidant and neuroprotective capacities in PC12 cell line. Pharm Biol [Internet]. 2017;55(1):880–7. Available from: http://dx.doi.org/10.1080/13880209.2016.1278452
15. Elissawy AM, Ebada SS, Ashour ML, El-Neketi M, Ebrahim W, Singab ANB. New secondary metabolites from the mangrove-derived fungus Aspergillus sp. AV-2. Phytochem Lett [Internet]. 2019;29(November 2018):1–5.
16. Al-gharban, H. A., and Dhahir, S.H. (2015). Serological diagnosis of persistent infection with Anaplasma marginale bacteria in cattle. *The Iraqi Journal of Veterinary Medicine*, *39*(1), 33-39
17. Gharban, H. A. J., and Yousif, A. A. (2021). First Isolation and Molecular Phylogenetic Analysis of *Coxiella burnetii* in Lactating cows, Iraq. *Bulgarian Journal of veterinary medicine*, *24*(4), 508-519.
18. Sathyadevi M, Subramanian S. Extraction, isolation and characterization of bioactive flavonoids from the fruits of physalis peruviana linn extract. Asian J Pharm Clin Res. 2015;8(1):152–7.
19. Kuruüzüm-Uz A, Güvenalp Z, Kazaz C, Demirezer LO. Phenolic compounds from the roots of Anchusa azurea var. azurea. Turkish J Pharm Sci. 2013;10(2):177–84.
20. Hernández-García E, García A, Avalos-Alanís FG, Rivas-Galindo VM, Delgadillo-Puga C, Camacho-Corona M del R. Nuclear magnetic resonance spectroscopy data of isolated compounds from Acacia farnesiana (L) Willd fruits and two esterified derivatives. Data Br [Internet]. 2019;22(December):255–68.
21. Lahmer N, Belboukhari N, Cheriti A, Sekkoum K. Hesperidin and hesperitin preparation and purification from Citrus sinensis peels. Der Pharma Chem. 2015;7(2):1–4.
22. Jadeja YS, Kapadiya KM, Jebaliya HJ, Shah AK, Khunt RC. Dihedral angle study in Hesperidin using NMR Spectroscopy. Magn Reson Chem. 2017;55(6):589–94.