Journal of Medicinal and Chemical Sciences

Journal homepage: http://www.jmchemsci.com/

Original Article

Isolation and Characterization of Furanocoumarins from Golden Delicious Apple Seeds

Ban Ali Ahmed[1], Yasser Fakri Mustafa[2,*], Bassam Yahya Ibrahim[3]

[1]*Department of Pharmacognosy, College of Pharmacy, University of Mosul, Ninavah-41002, Iraq*
[2]*Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Ninavah-41002, Iraq*
[3]*Department of Plant Protection, College of Agriculture and Forestry, University of Mosul, Ninavah-41002, Iraq*

ARTICLE INFO

Article history

Received: 2022-01-01
Received in revised: 2022-01-24
Accepted: 2022-01-26
Manuscript ID: JMCS-2201-1380
Checked for Plagiarism: **Yes**
Language Editor:
Ermia Aghaie
Editor who approved publication:
Dr. Behrooz Maleki

DOI:10.26655/JMCHEMSCI.2022.4.9

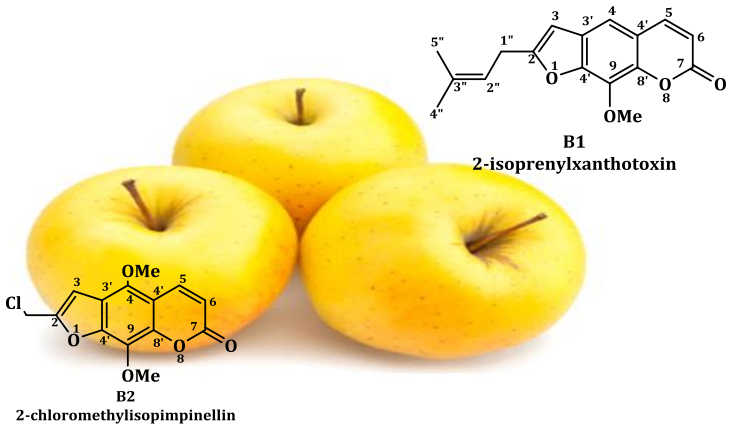
KEYWORDS

Characterization
Extraction
Furanocoumarin
Golden Delicious apple
Seeds

ABSTRACT

The isolation, characterization, and exploration of the pharmacological potentials of natural-obtained products are the starting steps in discovering new pharmaceutical scaffolds. Coumarin-based products attract much of the research concern as secondary metabolites due to their structural variety and various phytobiological activities. In this study, the seeds of Golden Delicious apple were extracted by four solvents of various polarities, including water, ethanol, chloroform, and ethyl ether. This process was accomplished by applying three extracting techniques: kinetic, ultrasound, and microwave-supported maceration. Each technique was performed in three fashions based on the sequence of solvents' utilization. The resultant 36 extracts were scanned for the presence of certain phytoconstituents, and based on the gathered results, one of the extracts was selected to isolate its content of coumarin-based products. The isolated products were purified via column chromatography and the results acquired from the TLC technique revealed the isolation of two coumarins. The chemical structures of these two products were identified by their scanning on FTIR, ^{1}H-NMR, ^{13}C-NMR spectroscopies. From the acquired outcomes and the gathered evidence from the literature, it was concluded that the isolated coumarin-based products belong to the furanocoumarin category and are chemically identified as 2-isoprenylxanthotoxin and chloromethylisopimpinellin.

GRAPHICAL ABSTRACT



* Corresponding author: Yasser Fakri Mustafa
✉ E-mail: Email: Dr.yassermustafa@uomosul.edu.iq

Introduction

Naturally, coumarin-based products have been identified and isolated from many resources, such as plants, microorganisms, and naval beings [1]. In the green vegan world, coumarin-based products are originated via the shikimic acid pathway and viewed as multifunctional secondary metabolites [2]. These products showed a high structural diversity that results in various biological potentials such as anticancer, anticoagulant, antioxidant, antidiabetic, antibacterial, antiviral, antihistamine, antifungal, and anti-inflammatory [3–6].

The isolation and characterization of the natural products constitute a rooted target of the current global trend to find bioactive agents that can treat the common human-health threats [7]. This target can be satisfied by utilizing various extracting and characterizing techniques [8]. Methods such as kinetic-, microwave- and ultrasound-supported macerations are the most applicable extraction techniques for isolating the natural products [9]. While spectroscopic instruments like those that detect the infrared (FTIR), nuclear magnetic resonance (NMR), and mass spectra are widely serviceable for characterizing these products [10].

Although there are many apple cultivars, the Golden Delicious one can be easily distinguished from the others. As shown in Figure 1, this apple brand can be recognized by its small-to-medium size, pale green-to-golden yellow color, and oblong-to-conical shape [11]. Many reports concerning the health benefits of consuming this apple brand have been found in the literature [12]. Despite that, the data concerning the phytochemical composition of the apple flesh [13], peel [14], and pomace [15] are limited. Besides, the phytochemical analysis of the seeds of this apple brand is not being investigated until now.

Figure 1: The physical manifestation of Golden Delicious apple

This study aims to isolate the coumarin-based products found in Golden Delicious apple (GDA) seeds and characterize their chemical structures. This aim was accomplished by extracting the seeds with four solvents of various polarity indexes in three distinct methods, isolating the extracted coumarin-based products, and finally characterizing their chemical backbones based on the analysis of the spectra acquired from the employed spectroscopic techniques.

Materials and Methods

Solvents and Equipment

The instruments utilized in the extraction were included the Swbr 17 Shell Lab shaker water-bath, sonicator (Power sonic 410, 40 kHz, 350 W), and microwave-oven (Moulinex, MW531070) for kinetic-, ultrasound-, and microwave-supported maceration techniques, respectively. The solvents used to extract the powdered seeds, follow up the spots on chromatograms and elute the column chromatography were acquired from authenticated international suppliers. Column chromatography used for isolating and purifying the natural coumarin-based products was CHX column (25 × 2.9 cm^2). The melting points, wavelengths of maximum absorption (λ_{max}), and FTIR spectra of the isolated products were measured by CIA 9301 electrothermal melting point apparatus, UVD-2950 instrument, and Bruker-α-ATR-FTIR spectrophotometer, respectively. Besides, Bruker Analytische Messtechnik GmbH (400 MHz) was used to scan the isolated products' proton- and carbon-NMR spectra.

Preparation of the Powdered Seeds

The purchased apple batch (152.36 kg) consisted of 19 boxes. Each one has an approximated weight of eight kilos. One apple sample was selected for authenticating by fruit professionals from the College of Agriculture and Forestry/the University of Mosul from each box. Each GDA was washed by running water, knife-cut into four slices, and posteriorly, the seeds were acquired manually. The seeds were shade-desiccated at room environment for two weeks, hashed by domestic blender, and sifted to afford a uniformed powder. The hashed material (234.67

g) was positioned in a well-enclosed glass container and preserved in a refrigerator [16].

Extraction of the Powdered Seeds

The acquired powder was extracted using different polarity index (PI) values solvents. These solvents included water (H_2O, PI=9.0), ethanol (EtOH, PI=5.2), chloroform ($CHCl_3$, PI=4.1), and ethyl ether (Et_2O, PI=2.8). The employed extracting methods included kinetic-, ultrasound-, and microwave-supported techniques. Each was accomplished by using the solvents mentioned above in three manners. In the first one, the extracting solvent was used separately for the extraction. For the second manner, the seeds powder was extracted with H_2O, the aqueous mixture was filtered, and the residue was re-extracted by EtOH. The alcoholic mixture was filtered, and the solid was re-extracted with $CHCl_3$. The organic mixture was filtered, and the material was re-extracted with Et_2O and then filtered. The same steps were carried out for the third manner, but the order of using the solvents was inversed [17,18].

Kinetic-supported maceration (KSM)

Seeds powder (2 g) was macerated by the assistance of shaking in the extracting solvent (20 mL) at 30 °C for 72 h. The soaked mixture was filtered, and the acquired filtrate was preserved in a refrigerator until subjected to the phytochemical investigation [19].

Ultrasound-Supported Maceration (USM)

A mixture of seeds powder (2 g) in the extracting solvent (20 mL) was macerated via a sonicator water-bath adjusted to 30 °C for 30 min. The macerated mixture was filtered, and the filtrate was preserved in a refrigerator [20].

Microwave-supported maceration (MSM)

A mixture of seeds powder (2 g) in the extracting solvent (20 mL) was macerated using a microwave oven operated at 100 W for 5 min. The irradiated mixture was then filtered, and the filtrate was preserved in a refrigerator [21].

Phytochemical Investigation

The presence of certain phytochemicals, including flavonoids, alkaloids, tannins, coumarins, and fixed oils in the 36 obtained extracts, was investigated by applying the scanning tests proposed by Harborne [22].

Inspection for flavonoids

Flavonoids were inspected in the extract by applying two checks: Pew and lead acetate tests. In the first assay, seven drops of conc. HCl was added to the 0.1 g powdered zinc metal mixture in 5 mL extract. The red, cherry or purple color development revealed a positive result. In the second assay, 1 mL of 10% lead acetate solution was mixed with 1 mL extract. The formation of yellow precipitate revealed a positive result.

Inspection for alkaloids

The presence of alkaloids was examined by using Mayer's test. In which Mayer's reagent (7 drops) was added to the hot mixture of HCl (1 mL, 10%) and extract (1 mL). The turbidity of the solution or the green coloration revealed a positive outcome.

Inspection for tannins

The presence of tannins was detected by applying Braymer's test. In which three drops of aqueous ferric chloride solution (5%) were added to the mixture of H_2O (1 ml) and extract (2 mL). The formation of green precipitate revealed a positive outcome.

Inspection for Coumarins

Coumarins were inspected in the extract by applying two checks: NaOH and fluorescence tests. The first one was accomplished by shaking an aqueous NaOH solution (3ml, 10%) with the extract (2 mL). The yellow coloration revealed a positive result. In the second test, a filter paper wetted with 1N NaOH was used to cover the top of a test tube containing 1 ml extract. This tube was immersed in a boiling water bath for 10 min, and the dried filter paper was examined under UV light at 366 nm. The visualization of yellow fluorescence revealed a positive outcome.

Inspection for fixed oils

Two tests were employed to detect the presence of fixed oils in the extract, named spot and saponification tests. A small amount of the extract was inserted between two filter papers and rubbed in the first one. The generation of an oil spot revealed a positive outcome. In the second

test, a mixture of ethanolic KOH (2 mL, 0.5N), a drop of phenolphthalein solution, and 2 mL extract was refluxed for 30 min. The partial decline in the basicity of the mixture or the generation of soap revealed a positive outcome.

Isolation of the Coumarin-Based Products

The $CHCl_3$ extract of the powdered seeds (160 g) obtained from the USM method executed by the first manner of extraction was evaporated via a rotary evaporator. The crude (6.21 g) was suspended in 62 mL of 1N NaOH, and the resulted mixture was heated for 45 min at 50 °C and filtered. A cold solution of 1N HCl was added dropwise to the acquired deep yellowish filtrate in an ice bath until the solution color vanished. The mixture was refrigerated for 48 h to complete the formation of crystals, which were acquired by filtration affording a mass-weighted 2.14 g [23,24].

An ascending TLC method was used to identify the number of coumarin-based products found in this mass by dissolving a small amount of the crude in 1 ml $CHCl_3$. Drops of the prepared solution were spotted on the TLC plate and eluted by an eluent composed of $CHCl_3$: ethyl acetate (4:1). The separated spots were visualized by UV-light (366 nm). The outcomes of triple trials revealed the existence of two spots [23,24].

The separation and purification of the coumarin-based products were accomplished by column chromatography. In this technique, silica gel (120 µm) particles were the stationary phase. The mobile phase consisted of ether mixtures: ethyl acetate in the ratios started from 9:1 and ended at 1:9. Two coumarin-based **B1** and **B2** were separated and detected in various eluent systems [23,24].

Results and Discussion

Phytochemical Investigation

The extraction process was performed by applying KSM, USM, and MSM methods. Each method worked in three manners symbolized as the first, second, and third ones. The resultant 36 extracts were scanned via phytochemical tests to detect the existence of specific phytoconstituents. Our target is to find an extract that exhibited positive results for coumarins and negative results for flavonoids, tannins, alkaloids, and fixed oils. This is because the presence of these phytoconstituents may interfere with the chemical basis utilized for isolating the coumarins from the crude mixture [16–18].

Tables 1-9 exhibited the outcomes acquired from the phytochemical investigation performed on the obtained extracts. The analysis of these outcomes revealed that coumarin-based products could be indicated in more than one solvent-phenotype. This can be attributed to two factors; firstly, the nature of the functional groups substituted on the coumarin nucleus. Secondly, the amount of extracting solvent is considered high compared to natural coumarin-based products [25].

Table 1: Names of the phytochemical scanning tests and their outcomes for extracts obtained from KSM operated on the first manner

Phytoconstituents	Test name	H_2O extract	EtOH extract	$CHCl_3$ extract	Et_2O extract
Flavonoids	Pew's test	+	+	+	-
	Lead acetate test	+	+	-	-
Alkaloids	Mayer's test	+	-	-	-
Tannins	Ferric chloride test	-	+	+	-
Coumarins	NaOH test	-	+	+	-
	UV-light test	-	+	+	-
Fixed oils	Spot test	-	-	+	+
	Saponification test	-	-	+	+

Table 2: Names of the phytochemical scanning tests and their outcomes for extracts obtained from USM operated on the first manner

Phytoconstituents	Test name	H_2O extract	EtOH extract	$CHCl_3$ extract	Et_2O extract
Flavonoids	Pew's test	+	+	-	-
	Lead acetate test	+	+	-	-
Alkaloids	Mayer's test	+	-	-	-
Tannins	Ferric chloride test	-	+	-	-
Coumarins	NaOH test	-	+	+	-
	UV-light test	-	+	+	-
Fixed oils	Spot test	-	-	-	+
	Saponification test	-	-	-	+

Table 3: Names of the phytochemical scanning tests and their outcomes for extracts obtained from MSM operated on the first manner

Phytoconstituents	Test name	H_2O extract	EtOH extract	$CHCl_3$ extract	Et_2O extract
Flavonoids	Pew's test	+	+	-	-
	Lead acetate test	+	+	-	-
Alkaloids	Mayer's test	+	-	-	-
Tannins	Ferric chloride test	+	+	-	-
Coumarins	NaOH test	-	+	+	-
	UV-light test	-	+	+	-
Fixed oils	Spot test	-	-	+	+
	Saponification test	-	-	+	+

Table 4: Names of the phytochemical scanning tests and their outcomes for extracts obtained from KSM operated on the second manner

Phytoconstituents	Test name	H_2O extract	EtOH extract	$CHCl_3$ extract	Et_2O extract
Flavonoids	Pew's test	+	+	-	-
	Lead acetate test	+	+	-	-
Alkaloids	Mayer's test	+	-	-	-
Tannins	Ferric chloride test	-	+	+	-
Coumarins	NaOH test	-	+	+	-
	UV-light test	-	+	+	-
Fixed oils	Spot test	-	-	+	+
	Saponification test	-	-	+	+

Table 5: Names of the phytochemical scanning tests and their outcomes for extracts obtained from USM operated on the second manner

Phytoconstituents	Test name	H_2O extract	EtOH extract	$CHCl_3$ extract	Et_2O extract
Flavonoids	Pew's test	+	+	-	-
	Lead acetate test	+	+	-	-
Alkaloids	Mayer's test	+	-	+	-
Tannins	Ferric chloride test	-	+	-	-
Coumarins	NaOH test	-	+	+	-
	UV-light test	-	+	+	-
Fixed oils	Spot test	-	-	+	+
	Saponification test	-	-	+	+

Table 6. Names of the phytochemical scanning tests and their outcomes for extracts obtained from MSM operated on the second manner

Phytoconstituents	Test name	H_2O extract	EtOH extract	$CHCl_3$ extract	Et_2O extract
Flavonoids	Pew's test	+	+	-	-
	Lead acetate test	+	+	-	-
Alkaloids	Mayer's test	+	-	-	-
Tannins	Ferric chloride test	-	+	+	-
Coumarins	NaOH test	-	+	+	-
	UV-light test	-	+	+	-
Fixed oils	Spot test	-	-	+	+
	Saponification test	-	-	+	+

Table 7: Names of the phytochemical scanning tests and their outcomes for extracts obtained from KSM operated on the third manner

Phytoconstituents	Test name	Et_2O extract	$CHCl_3$ extract	EtOH extract	H_2O extract
Flavonoids	Pew's test	-	-	+	-
	Lead acetate test	-	-	+	-
Alkaloids	Mayer's test	-	-	+	-
Tannins	Ferric chloride test	-	+	+	-
Coumarins	NaOH test	-	+	+	-
	UV-light test	-	+	+	-
Fixed oils	Spot test	+	-	-	-
	Saponification test	+	-	-	-

Table 8: Names of the phytochemical scanning tests and their outcomes for extracts obtained from USM operated on the third manner

Phytoconstituents	Test name	Et_2O extract	$CHCl_3$ extract	EtOH extract	H_2O extract
Flavonoids	Pew's test	-	-	+	+
	Lead acetate test	-	-	+	+
Alkaloids	Mayer's test	-	-	+	+
Tannins	Ferric chloride test	-	+	+	-
Coumarins	NaOH test	-	+	+	-
	UV-light test	-	+	+	-
Fixed oils	Spot test	+	-	-	-
	Saponification test	+	-	-	-

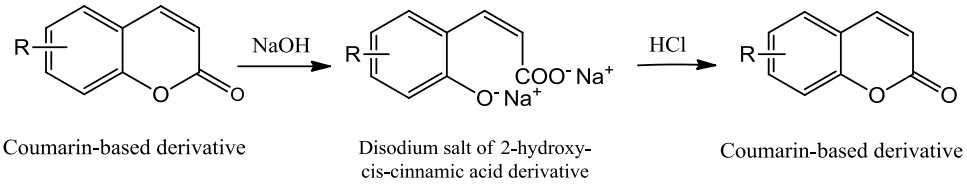
Table 9: Names of the phytochemical scanning tests and their outcomes for extracts obtained from MSM operated on the third manner

Phytoconstituents	Test name	Et_2O extract	$CHCl_3$ extract	EtOH extract	H_2O extract
Flavonoids	Pew's test	-	-	+	+
	Lead acetate test	-	-	+	+
Alkaloids	Mayer's test	-	-	+	-
Tannins	Ferric chloride test	-	+	+	-
Coumarins	NaOH test	-	+	+	-
	UV-light test	-	+	+	-
Fixed oils	Spot test	+	+	-	-
	Saponification test	+	+	-	-

Isolation of Coumarin-Based Products

The characteristic structural feature of the coumarin-based derivatives is the presence of cyclic ester, which is susceptible to nucleophilic attack. Strong nucleophiles like NaOH can hydrolyze this ester, yielding disodium salt of 2-hydroxy-*Z*-cinnamic acid derivative. The dropwise addition of concentrated inorganic acid like HCl recycles this salt generating the original coumarin-based derivative. As shown in Scheme

1, this chemical basis was utilized to separate the coumarin-based products from the crude mixture [26].



Scheme 1: The chemical theme applied for the isolation of coumarin-based products

Characterization of Coumarin-Based Products

The two spots revealed by the TLC technique indicated the presence of two coumarin-based products, which were symbolized as **B1** and **B2**. These products were separated and purified by column chromatography. The physical appearance, eluent system, % yield, melting point (mp, °C), λ_{max} (EtOH), and R_f value for each of the isolated products are listed in Table 10.

Table 10: The physicochemical properties of the **B1** and **B2** products

Product symbol	Physical appearance	Eluent system Et_2O: ethyl acetate	% yield out of 160 g	mp (°C)	λ_{max} (EtOH) nm	R_f value
B1	Off-white powder	9:1	0.58% (0.92 g)	191-194	322	0.74
B2	White powder	7:3	0.76% (1.22 g)	176-179	275	0.61

The IUPAC names of the isolated coumarins, and their spectroscopic data were displayed below:

B1: *9-methoxy-2-(3"-methylbut-2"-en-1-yl)-7H-furo[3,2-g]chromen-7-one*; IR ν_{max} (cm^{-1}) 3087, 3055 (=C-H), 2915 (-C-H), 1732 (C=O, pyrone), 1671 (C=C, exocyclic), 1628 (C=C, furan), 1588 (C=C, pyrone), 1550 (C=C, aromatic), 1243, 1048 (C-O-C, aryl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm): δ=7.76 (1H, d, *J*= 8 Hz, H-5), 7.11 (1H, s, H-4), 6.46 (1H, d, *J*= 8 Hz, H-6), 6.24 (1H, s, H-3), 5.17 (1H, t, H-2''), 3.71 (3H, s, OCH_3-9), 3.08 (2H, d, H-1''), 1.70 (6H, s, CH_3-4'', 5''); ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 163.0 (C, C-7), 157.2 (C, C-2), 146.8 (CH, C-5), 138.7 (C, C-9'), 137.1 (C, C-9), 134.4 (C, C-3''), 132.0 (C, C-8'), 126.3 (C, C-3'), 124.1 (C, C-4'), 115.4 (CH, C-2''), 114.5 (CH, C-6), 113.8 (CH, C-4), 105.2 (CH, C-3), 52.3 (CH_3, OCH_3), 26.1 (CH_3, C-4''), 20.0 (CH_3, C-5'').

B2: *2-(chloromethyl)-4,9-dimethoxy-7H-furo[3,2-g]chromen-7-one*; IR ν_{max} (cm^{-1}) 3063 (=C-H), 2893 (-C-H), 1735 (C=O, pyrone), 1630 (C=C, furan), 1587 (C=C, pyrone), 1554 (C=C, aromatic), 1248, 1052 (C-O-C, aryl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm): δ=7.81 (1H, d, *J*= 8 Hz, H-5), 6.45 (1H, d, *J*= 8 Hz, H-6), 6.24 (1H, s, H-3), 4.56 (2H, s, H-1''), 3.80 (6H, s, OCH_3-4, 9), ; ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 163.1 (C, C-7), 157.2 (C, C-2), 146.7 (CH, C-5), 143.8 (C, C-4), 140.0 (C, C-9'), 134.4 (C, C-8'), 130.6 (C, C-9), 118.4 (CH, C-6), 115.2 (C, C-3'), 111,1 (C, C-4'), 105.3 (CH, C-3), 52.7 (CH_3, OCH_3-4), 52.1 (CH_3, OCH_3-9), 47.3 (CH_2, C-1'').

Based on the aforelisted spectral data and evidence gathered from the literature [27–29], it was concluded that the isolated products, as shown in Figure 2, are belonged to the furanocoumarin category. The product **B1** is a derivative of xanthotoxin and chemically named 2-isoprenylxanthotoxin. Besides, the **B2** product is a derivative of isopimpinellin and chemically named 2-chloromethylisopimpinellin.

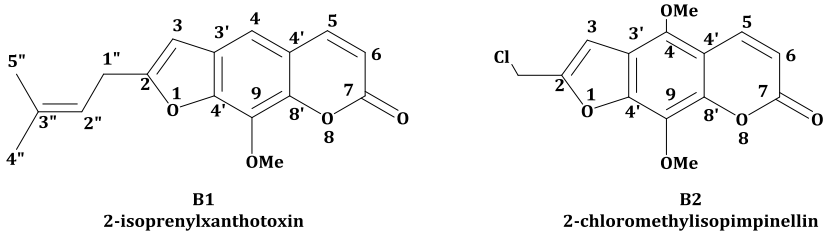


Figure 2: Chemical structures of the products **B1** and **B2**

Conclusions

This study reported the extraction of Golden Delicious apple' seeds by four solvents of different polarity indexes. This process was performed via three extracting methods; each was carried out in three different modes. The phytochemical analysis of the gathered extracts revealed the presence of coumarin-based products in most of these extracts. Their purification, followed by the isolation of the natural coumarins via column chromatography, revealed the presence of two products. From the characterization data and overview of the literature, it was concluded that the isolated products belong to the furanocoumarin group, and they are chemically termed 2-isoprenylxanthotoxin and chloromethylisopimpinellin, respectively.

Acknowledgments

The authors are very grateful to the University of Mosul/College of Pharmacy for their provided facilities, which helped to improve the quality of this work.

Financial Support: The work is self-funded.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

ORCID

Ban Ali Ahmed:
https://www.orcid.org/0000-0002-8971-4215
Yasser Fakri Mustafa:
https://www.orcid.org/0000-0002-0926-7428
Bassam Yahya Ibrahim:
https://www.orcid.org/0000-0003-0285-6067

References

[1]. Ranjan Sahoo C., Sahoo J., Mahapatra M., Lenka D., Kumar Sahu P., Dehury B., Padhy R.N., Paidesetty S.K., *Arab. J. Chem.,* 2021, **14**:102922 [Crossref], [Google Scholar], [Publisher]
[2]. Oglah M.K., Mustafa Y.F., Bashir M.K., Jasim M.H., *Syst. Rev. Pharm.,* 2020, **11**:472. [Google Scholar], [Publisher]
[3]. Mustafa Y.F., Abdulaziz N.T., *Syst. Rev. Pharm.,* 2020, **11**:438 [Google Scholar], [Publisher]
[4]. Mustafa Y.F., Abdulaziza N.T., Jasima M.H., *Egypt. J. Chem.,* 2021, **64**:1807 [Crossref], [Google Scholar], [Publisher]
[5]. Bashir M.K., Mustafa Y.F., Oglah M.K., *Syst. Rev. Pharm.,* 2020, **11**:175 [Crossref], [Google Scholar], [Publisher]
[6]. Bashir M.K., Mustafa Y.F., Oglah M.K., *Period. Tche Quim.*, 2020, **17**:871 [Google Scholar], [Publisher]
[7]. Altemimi A., Lakhssassi N., Baharlouei A., Watson D.G., Lightfoot D.A., *Plants,* 2017, **6**:42 [Crossref], [Google Scholar], [Publisher]
[8]. Mustafa Y.F., *J. Med. Chem. Sci.*, 2021, **4**:612 [Crossref], [Google Scholar], [Publisher]
[9]. Zhang Q.W., Lin L.G., Ye W.C., *Chin. Med.,* 2018, **13**:1 [Crossref], [Google Scholar], [Publisher]
[10]. Pasciu V., Baralla E., Varoni M.V., Demontis M.P., *Drug Chem. Toxicol.,* 2019, 1. [Crossref], [Google Scholar], [Publisher]
[11]. Almeida D.P.F., Gião M.S., Pintado M., Gomes M.H., *Int. J. Food Prop.,* 2017, **20**:2206 [Crossref], [Google Scholar], [Publisher]
[12]. Patocka J., Bhardwaj K., Klimova B., Nepovimova E., Wu Q., Landi M., Kuca K., Valis M., Wu W., *Plants*, 2020, **9**:1408 [Crossref], [Google Scholar], [Publisher]
[13]. Liang X., Zhu T., Yang S., Li X., Song B., Wang Y., Lin Q., Cao J., *Molecules,* 2020, **25**:4153 [Crossref], [Google Scholar], [Publisher]
[14]. Song B., Wang H., Xia W., Zheng B., Li T., Liu R.H., *Food Funct.,* 2020, **11**:6170 [Crossref], [Google Scholar], [Publisher]
[15]. Tao B., Ye F., Li H., Hu Q., Xue S., Zhao G., *J. Agric. Food Chem.,* 2014, **62**:7166 [Crossref], [Google Scholar], [Publisher]

[16]. Mustafa Y.F., Bashir M.K., Oglah M.K., *Syst. Rev. Pharm.*, 2020, **11**:598 [Google Scholar], [Publisher]
[17]. Oglah M.K., Bashir M.K., Mustafa Y.F., Mohammed E.T., Riyadh R., *Syst. Rev. Pharm.,* 2020, **11**:717 [Google Scholar], [Publisher]
[18]. Mustafa Y.F., Mohammed N.A., *Biochem. Cell. Arch.*, 2021, **21**:1991 [Google Scholar], [Publisher]
[19]. Majoumouo M.S., Sibuyi N.R.S., Tincho M.B., Mbekou M., Boyom F.F., Meyer M., *Int. J. Nanomedicine,* 2019, **14**:9031. [Crossref], [Google Scholar], [Publisher]
[20]. Montero-Calderon A., Cortes C., Zulueta A., Frigola A., Esteve M.J., *Sci. Rep.,* 2019, **9**:1 [Crossref], [Google Scholar], [Publisher]
[21]. Mustafa Y.F., *Appl. Nanosci.*, 2021 [Crossref], [Google Scholar], [Publisher]
[22]. Harborne, J.B. (1998) Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 5th Edition, Chapman and Hall Ltd, London, 21-72. [Publisher]
[23]. Mustafa Y.F., Khalil R.R., Mohammed E.T., *Egypt. J. Chem.*, 2021, **64**:3711 [Crossref], [Google Scholar], [Publisher]
[24]. Mustafa Y.F., Khalil R.R., Mohammed E.T., *Syst. Rev. Pharm.,* 2020, **11**, 382 [Google Scholar], [Publisher]
[25]. Kielesiński Ł., Morawski O.W., Sobolewski A.L., Gryko D.T., *Phys. Chem. Chem. Phys.,* 2019, **21**:8314 [Crossref], [Google Scholar], [Publisher]
[26]. Toan V.N., Thanh N.D., *Synth. Commun.,* 2020, **50**:3603 [Crossref], [Google Scholar], [Publisher]
[27]. Ozek G., Yur S., Goger F., Ozek T., Andjelkovic B., Godjevac D., Sofrenic I., Aneva I., Todorova M., Trendafilova A., *Chem. Biodivers.,* 2019, **16**:e1800672 [Crossref], [Google Scholar], [Publisher]
[28]. Fang H., Ji H., *Anti-Cancer Agents Med. Chem.*, 2020, **19**:2091 [Crossref], [Google Scholar], [Publisher]
[29]. Kubrak T., Czop M., Kołodziej P., Ziaja-Sołtys M., Bogucki J., Makuch-Kocka A., Aebisher D., Kocki J., Bogucka-Kocka A., *Molecules*, 2019, **24**:1824 [Crossref], [Google Scholar], [Publisher]

HOW TO CITE THIS ARTICLE
Ban Ali Ahmed, Yasser Fakri Mustafa, Bassam Yahya Ibrahim. Isolation and Characterization of Furanocoumarins From Golden Delicious Apple Seeds*, J. Med. Chem. Sci.*, 2022, 5(4) 537-545
DOI: 10.26655/JMCHEMSCI.2022.4.9
URL: http://www.jmchemsci.com/article_143907.html