RESEARCH ARTICLE

Phytochemical Study of Iraqi *Cydonia oblonga* Miller Fruit

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Received: 31st July, 2022; Revised: 22nd April, 2022; Accepted: 08th September, 2022; Available Online: 25th September, 2022

ABSTRACT

Quince (*Cydonia oblonga*) fruit has a lot of phytochemicals that draw the attention of many researchers nowadays for its important medicinal values. This study concerns the identification and isolation of two flavonoids (Astragalin) flavonol glycoside and (Isorhamnetin) from fruit ethyl acetate extract of Iraqi cultivated *Cydonia oblonga*. The identification was carried out by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) analysis techniques. The yield of isolated compound was (1.6 and 1.3 %) for astragalin and isorhamnetin, respectively. This was achieved by using preparative thin layer chromatography glass plate (20×20) cm. This is followed by structure elucidation of isolated compounds through fourier transform infrared (FT-IR), ultraviolet (UV), nuclear magnetic resonance (^{1}HNMR) and carbon-13 nuclear magnetic resonance (^{13}CNMR). analysis procedures.

Keywords: *Cydonia oblonga*, Phytochemical, Flavonoids

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.3.54

How to cite this article: Obaid, N.S., Al-Ani, W.M. Phytochemical Study of Iraqi *Cydonia oblonga* Miller Fruit. International Journal of Drug Delivery Technology. 2022;12(3):1256-1260.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Nature has provided an enormous source of herbal medicinal products for the last thousand years with impressive modern medicinal products isolated from natural sources. Many isolations have been based on the usage of agents in traditional medicine. The traditional medicinal systems are still part of healthcare, with almost 80% of the world's population mostly relying on traditional primary medical products. (*Cydonia oblonga* Miller).[1] It is a member of Pireas tribe, which belongs to the Maloideae subfamily of the Rosaceae family, and genus *Cydonia*. The quince fruit has limited use in the fresh produce market due to its bitter, astringent pulp and its inherent hardness. However, when the fruit is mature, it will have a very pleasant, long-lasting, and intense flavor. Manufacturers request numerous varieties of jams, jellies, and cakes to make with it.[2] *C. oblonga* fruit contains important phenolic compounds, including 3-O-caffeoylquinic, 4-O-caffeoylquinic, 5-O-caffeoylquinic and 3,5-dicaffeylquinic acids. Additionally, it contains C-flavonoids such as lucenin-2, vicennine-2, stellarine-2, isoschaftoside, shaftoside, chrysoreiole 6-C-pentosyl-8-C-glucoside, 6-Clucosyl-8-C-pentoside and apigenin.[3,4]

In addition, the fruit also produces flavon-3-ol flavonoids such as quercetin-3-O-galactoside (Hyproside), quercetin-3-O-rutinosides (rutin) and kampferol-3-O-glucoside (Astragaline) and kampferol-3-O-rutinoside (Nicotiflorin).[5]

This fruits conation many important phytochemical compounds such as organic acids, amino acids, vitamins, reducing sugars, and minerals.

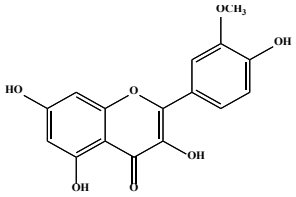
This work identified and isolated isorhamnetin and astragalin from ethyl acetate fruit (pulp and peel) of Iraqi cultivated *C. oblonga*.

MATERIALS AND METHODS

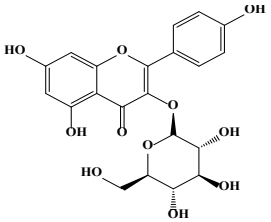
C. oblonga fresh unripe fruit was collected in September 2020 from Iraqi farms located in Karbala, Al wind town. After separating seeds from fruits, the remaining parts (Peel and Pulp) were grated to very fine particles by grater, and left to dry at room temperature for four days and then weighted.

Plant Extraction

A total of 100 grams of dried powder were defatted by maceration with (2.5 L) of n-hexane (99.8%) for 5 days. After that the marc was macerated with (2.5 L, 80%) of ethanols for



3-O-methoxy-3, 4, 5, 7-tetrahydroxy flavonol (isorhamentin)



kaempferol-3-O- -D-glucoside

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five days. Then ethanolic extract was evaporated to 50 mL by rotary evaporator

The following step was partitioning aqueous ethanol extract with 50 mL ethyl acetate three times. The combined Ethyl acetate fraction was concentrated under vacuum and used to identify the phytochemicals.

Detection of Phytochemicals by Thin Layer Chromatography

Detection of phytochemicals by TLC in comparison with standards. A spot from the concentrated fruit extract was applied on analytical TLC plate. Finally, the developed spots were examined under ultraviolet light at 254 and 366 nm, then the Rf value was calculated for the separated compounds and compared with the Rf value of the standards. The solvent systems summarized in Table 1.

Detection of Phytochemical by High-Performance Liquid Chromatography

The HPLC was performed to recognize and estimate phenolic compounds in ethyl acetate extract. The HPLC analysis was carried out by prominence HPLC system (Shimadzu) with a degasser (DGU-20A) and the separation was performed in reverse phase (RP) C18 column (250 x 4.6 mm i.d). The mixture was passed through 0.45 µm disposable filters and then 100 µL of each sample was injected into the HPLC system. The analysis was done for Fruit (pulp and peel) extract: The chromatographic condition for detection of astragalin was made using isocratic mixtures, eighty percent methanol as solvent A and 20% (water with 0.1% acetic acid) as solvents (B). A 10-minute of 0.8 mL/min flow rate, detection was done by UV at 360. The total run time was 12 minutes. The retention time of the compound was compared with the retention time of it is external standards.[6]

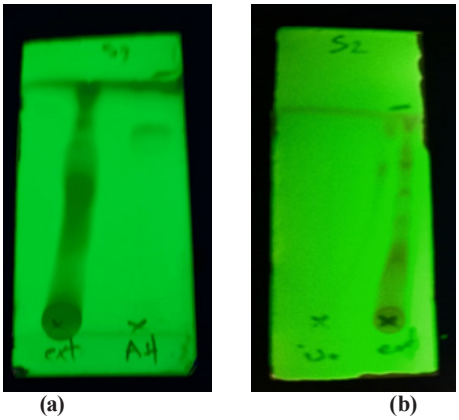
While isorhamnetin identification was carried out by using other chromatographic conditions through elution with isocratic mixture of 75% methanol as solvent A and water as solvent B. Flow rate was set at 0.9 mL/min for 5 minutes, detected by UV at 370 nm. The compound was detected according to retention time of it is standard.[7]

Isolation of Phenolic Compounds from Ethyl Acetate Extract

3 grams of fruit extract were dissolved in 30 mL of 99.8% methanol, spread on pre-activated preparative TLC (20×20) cm plate at base line and left to dry then developed in (20×20) cm covered glass jar with S2 solvent system. The bands of astragalin were detected under UV light at 254 nm wavelength.

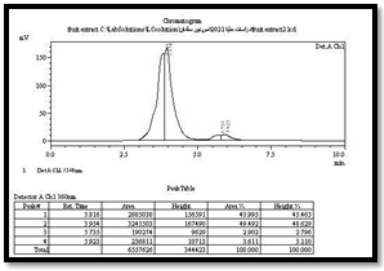
Table 1: Mobile phases used in TLC

Name of mobile phase	*Composition*
S1	Acetone:chloroform:water (80:10:10)
S2	Ethylacetate:formic acid:water (80:10:10)
S3	Chloroform:methanol (80:20)
S4	Toluene:ethylacetate:formic acid (58:33:9)
S5	Chloroform:ethylacetate (60:40)

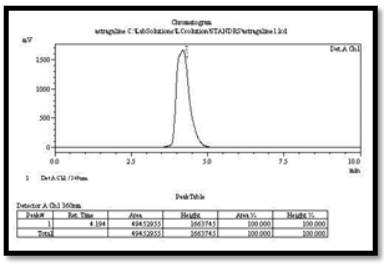


(a) (b)

Figure 1: TLC plates of detected phytochemicals in ethyl acetate fruit extract of Iraqi *C. oblonga* [(a) astragalin in S2, (b) isorhamnetin in S3] mobile phases.



(a)



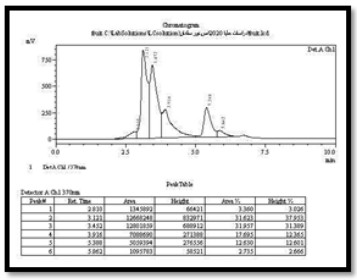
(b)

Figure 2: HPLC Chromatogram of (a) ethyl acetate fruit extract of Iraqi *C. oblonga*, (b) astragalin standared.

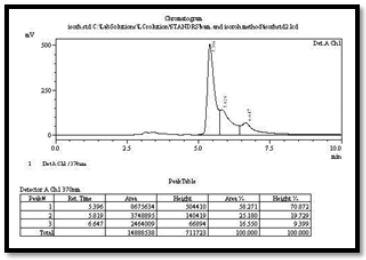
Furthermore, the bands were scratched and washed with 50 mL absolute methanol and the filtrate left to dry till became powder. The purity of isolated compound was checked by applying it on another preparative TLC plate and using different solvent systems. Furthermore, the isolation of isorhamnetin was achieved by developing a preparative TLC plate in different solvent systems (S4) and the same steps of astragalin isolation were done to isolate isorhamnetin.

Structure Elucidation of Isolated Compounds

The structure of isolated compounds was elucidated through using Spectral analysis by FT-IR method and Ultraviolet spectroscopy (UV) spectra (MeOH) was calibrated for maximum absorption of the isolated compounds between 200 and 400 nm. In addition to Proton nuclear magnetic resonance (H1 NMR) and Carbon nuclear magnetic resonance (C13NMR) analysis was done in Iran at the University of Tehran.



(a)



(b)

Figure 3: HPLC Chromatogram of: (a) ethyl acetate fruit extract of Iraqi *C. oblonga*, (b) isorhamnetin standared.

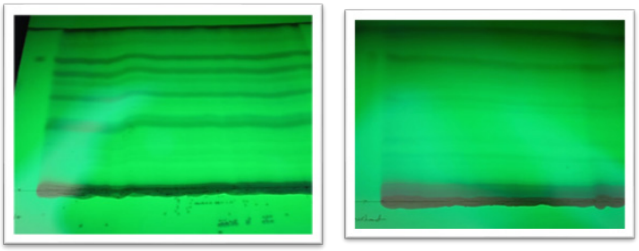


Figure 4: Isolation and purification of : (a) astragalin, (b) isorhamnetin by PLC.

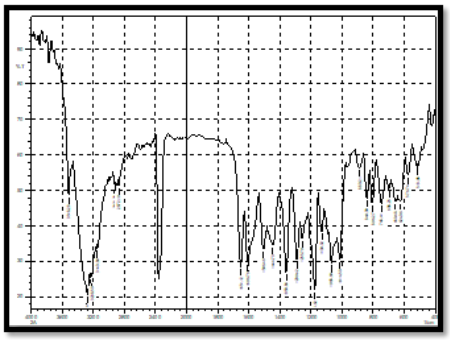


Figure 5: IR Spectrum of isolated astragalin

RESULTS AND DISCUSSION

The identification of phenolic compounds in ethyl acetate fruit extract of Iraqi *C. oblonga* by TLC analysis method revealed the presence of astragalin and isorhamnetin as major flavonoids. As astragalin was detected by using S1, S2 and S3 mobile phases with Rf values (0.72, 0.71, and 0.77), respectively. While Isorhamnetin detection was done using S3, S6 and S4 solvent systems, Rf values were recorded as (0.73, 0.61, and 0.60), respectively (Figure 1).

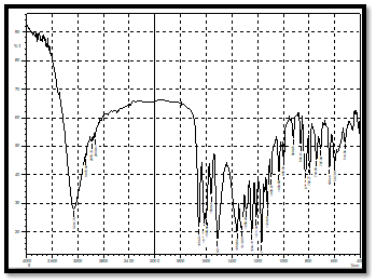


Figure 6: IR Spectrum of isolated isorhamentin

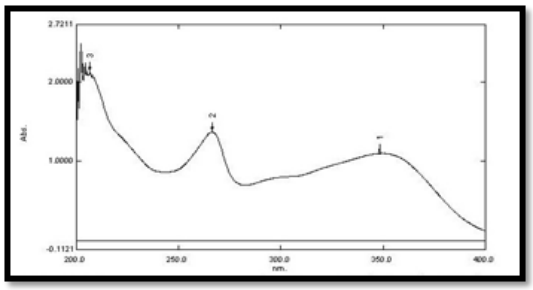


Figure 7: UV Spectrum of isolated astragalin

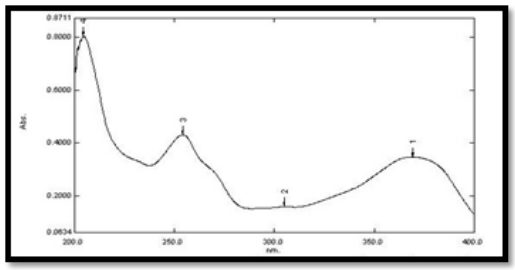


Figure 8: UV Spectrum of isolated isorhamntin

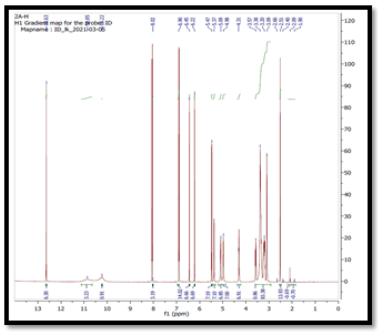


Figure 9: H^1 NMR Spectrum of isolated astragalin

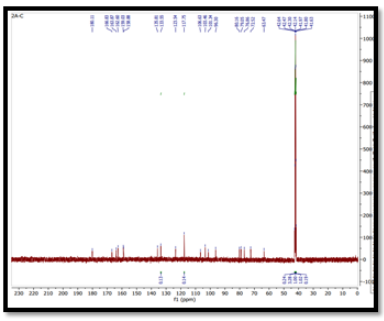


Figure 10: C^{13} NMR Spectrum of isolated astragalin

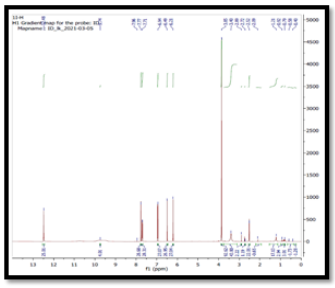


Figure 11: H^1 NMR Spectrum of isolated isorhamntin

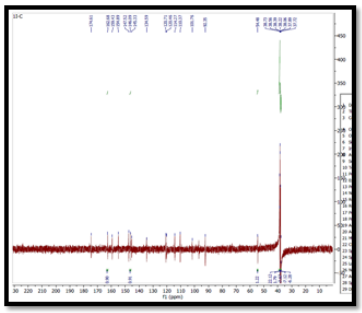


Figure 12: C^{13} NMR Spectrum of isolated isorhamentin

Table 2: C^{13} NMR Spectrum data of isolated astragalin

Group	*Chemical shift (ppm)*	*interpretation*
C=O	180.11	Carbonyl group of flavonol ring
C=C	158,159 and 163, 166	Carbons signals of aromatic C=C group
C	162	Carbon of flavonol ring
C	135	Carbon of flavonol ring adjacent to oxygen atom
C-H	117–133	Carbon signals of ring B
C-H	96–106	Carbon signals of ring A
C-H	72–80	Carbon signals of sugar moiety
C-H2	63.47	CH2 group of sugar part

Also, HPLC analysis showed the presence of astragalin and isorhamnetin with a retention time (3.954 and 5.388 minutes), respectively, and these results matching with the retention time of external standards under the same chromatographic conditions (Figures 2 and 3).

The identified compounds were isolated on a preparative TLC (PLC) plate and developed in the S2 mobile phase for astragalin and S4 mobile phase for isorhamnetin. The yield was (48 mg, 1.6%) and (39 mg, 1.3%) for astragalin and isorhamnetin, respectively which correspond to (100 g) of *C. oblonga* dried fruit (Figure 4).

FT-IR analysis of isolated compounds gives bands of astragalin (phenolic -OH-3304, -CH-2924 and 2872, -C=O-1651, aromatic –C=C- 1606 and 1508, -C-O-C-1290 and -C-OH-1136) cm^{-1}[8]. Bands for isorhamnetin (phenolic –OH-326, aromatic –CH- 3076, -CH- 2968 and 2939, -C=O-1656, aromatic –C=C-1510-1599 and -C-OH-1211) cm^{-1} (Figures 5 and 6).[9]

Table 3: C^{13} NMR Spectrum data of isolated isorhamentin

Group	*Chemical shift (ppm)*	*interpretation*
CH3	54.48	Signal of methoxy group (-O-CH3)
C-H	92.35	Signal of (C-H) group of benzene ring A
C-H	101.76	Signal of (C-H) group of benzene ring A
C-H	110–120.46	Signals of (C-H) group of benzene ring B
C	120.71	Signal of Carbon at ring B
C	146.059 and 147.52	Signals of Carbon at ring B
C	145.33 and 134.59	Signals of flavonol ring
C	154–162.68	Signals of carbons at ring A
C	174.61	Signal of(C=O) group

In addition UV Spectrum analysis show the γ_{max} of isolated astragalin at (348.2 and 266.2) nm wavelength as presented in it is absorption spectrum (Figure 7). Furthermore, (γ_{max}) for isolated isorhamnetin at (369.4 and 254) nm wavelength (Figure 8). These two peaks are related to cinnamoyl and benzoyl parts, respectively.

^{1}HNMR Spectrum of isolated compound gives multiple proton signals that indicate the chemical structures of isolated compounds. The peaks of astragalin proton analysis at δ (8.02 doublet, 2H and 6.90 doublet, 2H) ppm belong to protons of aromatic ring B, While peaks at δ (6.45 singlet,1H and 6.22 singlet, ^{1}H) ppm related to protons of aromatic ring A. As well as the protons of sugar moiety appeared at (3.57, 3.38,3.2 and 3.09 multiplate) ppm; these overlapped signals that starting from δ (2.5-4) are the characteristic feature of glcosides.[8,10] As well as the C13 NMR Spectrum of isolated astragalin demonstrate the presence of characteristic carbon signals of this glycoside. These signals are presented in Table 2. The ^{1}HNMR and C^{13} NMR Spectrum (Figures 9 and 10).

Isorhamnetin proton signals δ 12.48 singlet (^{1}H, flavonol-OH), δ 6.21 singlet and δ 6.49 singlet (^{1}H, aromatic-CH), δ 6.94 singlet (^{1}H, aromatic-OH), δ 7.71 singlet (^{1}H, aromatic-CH), δ 7.77 doublet and 7.96 doublet (^{1}H,aromatic-CH), δ 3.85 singlet (^{3}H, methoxy group) (Figure 11). While the C^{13} NMR Spectrum of isolated isorhamentin showed the characteristic signals of (CH3) group at (54.48) ppm as well as other carbon signals as presented in Figure 12 and Table 3.

CONCLUSION

From the above study, it can be concluded that Iraqi cultivated *C. oblonga* fruit rich with many active secondary metabolites, namely flavonol flavonoids like (astragalin and isorhamnetin) that could be isolated from the ethyl acetate extract.

Furthermore, those major flavonoids are structurally elucidated by using different Spectroscopic procedures (UV, FT-IR, ^{1}HNMR and C^{13}NMR).

REFERENCES

1. Owolabi OJ, Omogbai EK, Obasuyi O. Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigelia africana (Bignoniaceae) stem bark. African Journal of Biotechnology. 2007;6(14).
2. Okigbo R, Eme U, Ogbogu S. Biodiversity and conservation of medicinal and aromatic plants in Africa. Biotechnology and Molecular Biology Reviews. 2008;3(6):127-34.
3. Silva BM, Andrade PB, Ferreres F, Domingues AL, Seabra RM, Ferreira MA. Phenolic profile of quince fruit (Cydonia oblonga Miller)(pulp and peel). Journal of Agricultural and Food Chemistry. 2002;50(16):4615-8.
4. Magalhães AS, Silva BM, Pereira JA, Andrade PB, Valentão P, Carvalho M. Protective effect of quince (Cydonia oblonga Miller) fruit against oxidative hemolysis of human erythrocytes. Food and Chemical Toxicology. 2009;47(6):1372-7.
5. Silva BM, Andrade PB, Ferreres F, Seabra RM, Beatriz M, Oliveira P, et al. Composition of quince (*Cydonia oblonga* Miller) seeds: phenolics, organic acids and free amino acids. Natural product research. 2005;19(3):275-81.
6. Fawzi F, Mahdi MF, Abaas IS. Isolation of Astragalin from Cressa cretica cultivated in Iraq. Journal of Pharmaceutical Sciences and Research. 2019;11(1):185-90.
7. Merken HM, Beecher GR. Measurement of food flavonoids by high-performance liquid chromatography: a review. Journal of agricultural and food chemistry. 2000;48(3):577-99.
8. Saito S, Silva G, Santos RX, Gosmann G, Pungartnik C, Brendel M. Astragalin from Cassia alata induces DNA adducts in Vitro and repairable DNA damage in the yeast Saccharomyces cerevisiae. International Journal of Molecular Sciences. 2012;13(3):2846-62.
9. Azeez RAK, Abaas IS, Kadhim EJ. Isolation and Characterization of Isorhamnetin and Kaempferol from Elaeagnus Angustifolia (F: Elaeagnaceae) Cultivated in Iraq. Al-Mustansiriyah Journal of Pharmaceutical Sciences (AJPS). 2019;19(1):12-21.
10. Ghavam-Haghi F, Sadeghi Dinani M. Isolation and identification of Astragalin and 2-methoxy tyrosol from the bulbs of Allium paradoxum. Journal of Herbmed Pharmacology. 2017;6(3).