Original Article

Photochemical study of flavonoid "isovitexin" present in the whole plant Anastatica hieroch untica L. cultivated in Iraq

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Abstract

This study detects the presence of an important flavonoids "isovitexin" present in the whole plant Anastitica hierochuntica L. cultivated in Iraq. The absence of any study concerning the isovitexin content of this medicinal plant in Iraq and the medicinal importance isovitexin depending on its uses for treatment some disease, acquired this study its value. This study concerned with extraction, identification, isolation, and purification of isovitexin from the whole plant Anastatica hierochuntica L. Extraction of this compound was carried out using two methods. Identification of this compound was done using thin layer chromatography (TLC) where different solvent systems had been tried. This identification was further augmented by using high performance liquid chromatography (HPLC) and then this flavonoid was isolated and purified. The identification of isolated isovitexin was carried out using Melting point (M.P), Thin layer chromatography (TLC), Infrared spectroscopy (IR) and High performance liqude chromatography (HPLC). This study confirms the presence of isovitexin in the whole plant Anastatica hierochuntica L. cultivated in Iraq. Also the result of this study showed that the extraction method NO.2 was the best, because the amount of both extract and isovitexin were higher than from extraction method NO.1.

Keywords: Anastatica hierochuntica, flvonoids, isovitexin

Introduction

Anastatica hierochuntica is a small, winter annual herb of Brassicaceae family, a found in the Sahara - Arabian deserts, which rolls in wards under dry conditions. In Saudi Arabia, Jordan and Egypt it is known as Rose of Jericho and it has many Arabic names like keff mariyam or fatma. The whole plant contains many active constituents like the flavonoids (isovitexin, luteolin-7 glucoside, kaemmpferol - 7-glucoside and isovitexin), sterols and different sugars, therefore this plant has many pharmacological including used for cold, reduces the pain of pain and facilitates childbirth, act as a pain - killer, an emmenagogue and for epilepsy. It is also used as a tea to treat asthma and respiratory diseases, dysentery, fevers and headaches (1,2).

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Material And Methods

Plant material

The plant materials of Anastatica hierochantica L. were collected from the botany garden in college of agriculture, university of Baghdad, during the months (June – July) (2020). The plant materials were cleaned and dried at room temperature, then these plant materials were coarsely powdered by mechanical grinder and weighted.

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Extraction

Extraction method No.1 (3)

A 100 g of dried powdered plant material was placed in a thimble of a soxhlet extractor and the plant material was extracted with 500 ml of 75% ethanol for 10 hours at (40 C). The ethanolic extract was then filtered and the filtrate was evaporated to dryness under reduced pressure at a temperature not exceeding (40 C) and then the ethanolic extract residue was weight and subjected to identification (Figure 1).

Extraction method No.2 (3)

A100 g of dried powdered plant material was defatted with 500 ml of n-hexan in a soxhlet extractor for six hours. The residual plant material was dried at room temperature and then was extracted in a soxhlet extractor with 500 ml of 80% methanol for 10 hours at (40 C). The methanolic extract was then filtered and the filtrate was evaporated to dryness under reduced pressure at a temperature not exceeding (40 C) and then the methanolic extract was weighted and subjected to identification (Figure 2)

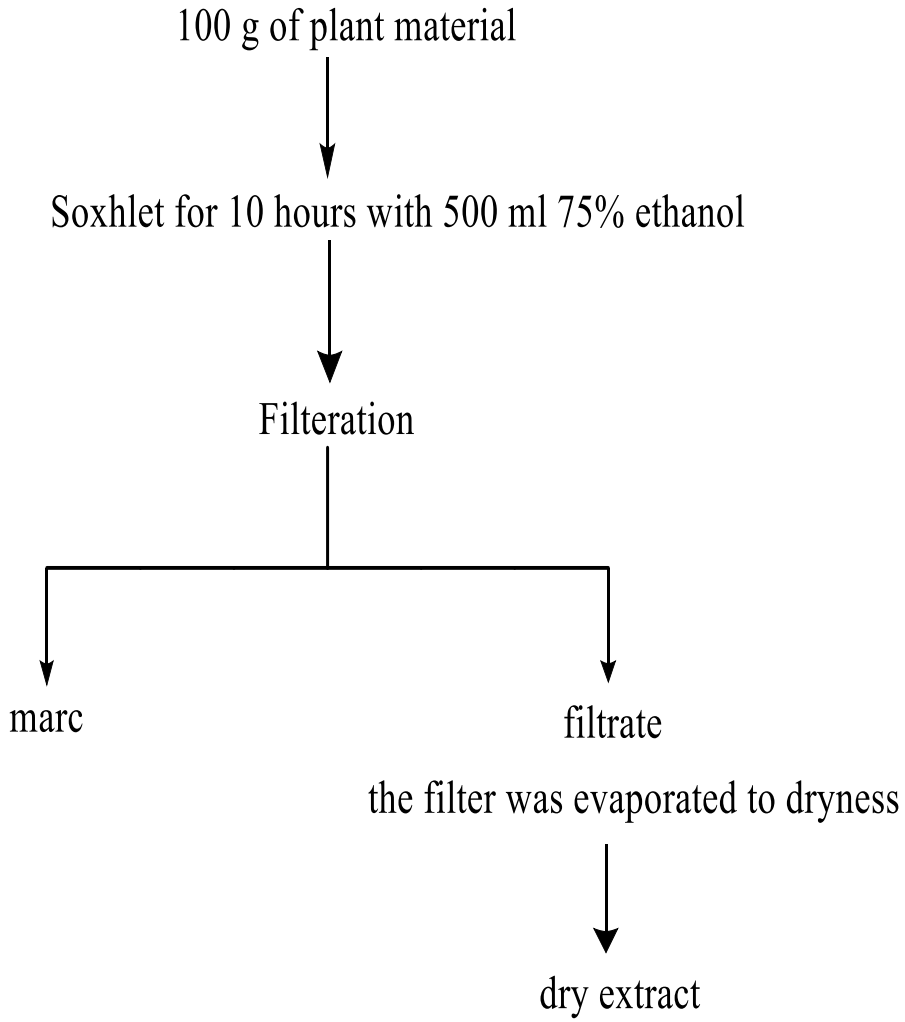


Fig.1: General scheme for method (No. 1) for extraction of flavonoids from Anastatica hierochuntica

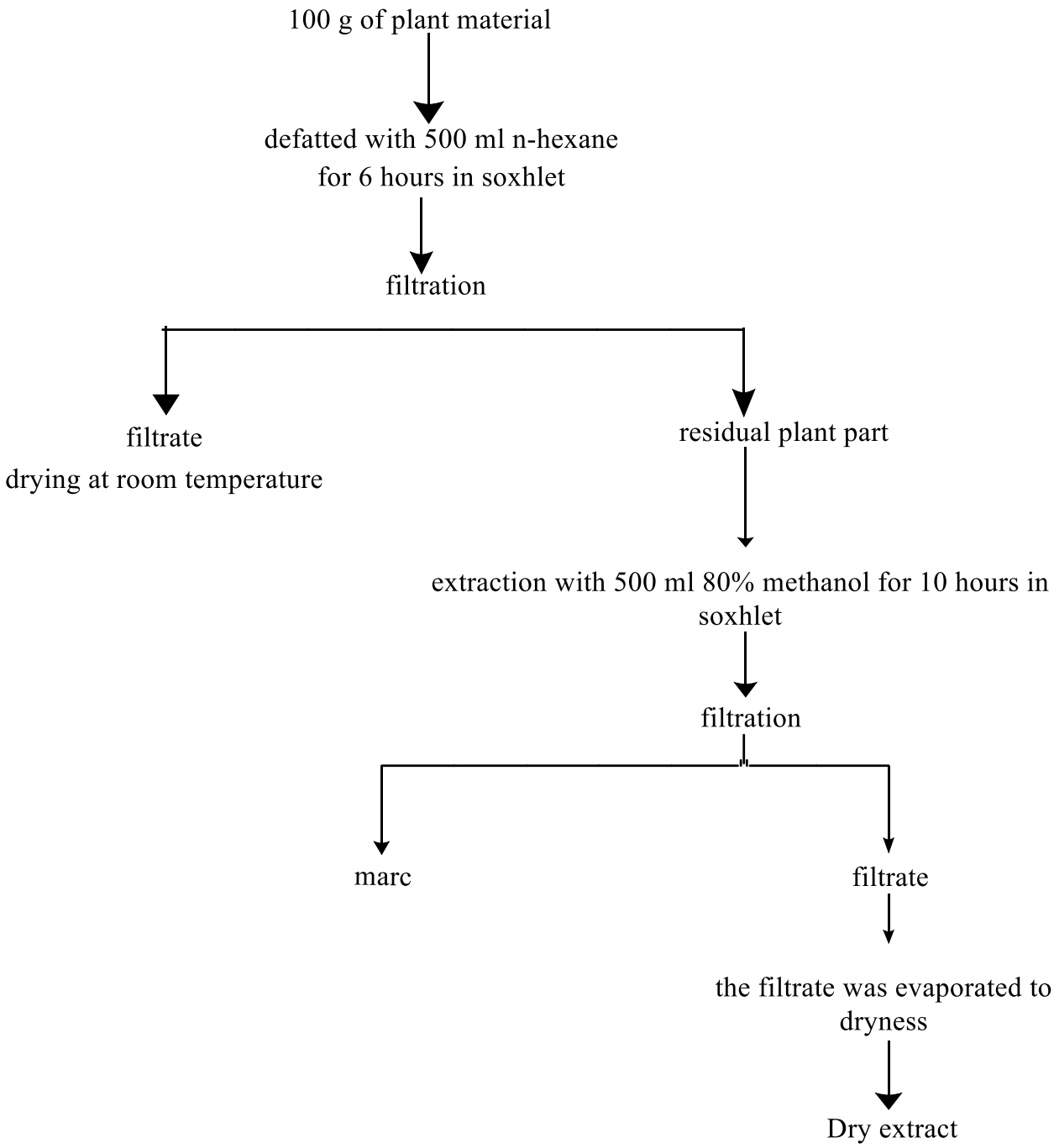


Fig.2: General scheme for method (No. 2) for extraction of flavonoids Anastatica hierochuntica.

Thin layer chromatography (TLC)

In this qualitative identification a ready made aluminum plates of silica gel 60 F 254, (20 x 20) cm of 0.25 mm thickness were used, these plates were activated at 110 C for one hour before use.

High performance liquid chromatography (HPLC)

Qualitative and quantitative estimation of isovitexin compound in the crud extract obtained by extraction methods was carried out by using high performance liquid chromatography (HPLC).

The identifications were made by comparison of retention time of Isovitexin component in the crud extracts with that of authentic standard at identical chromatographic conditions. HPLC analysis was done by using the following condition (4) :

1-Mobile phase: Methanol-Water (70:30).

2- Column: C18 5 mm x 150 mm. (Name of the colom

3- Flow rate: 0.5 ml/min.

4- Detection: UV. Detector at 2, 270 nm.

5- Injection volume: 20 µL..

D. Isolation and purification of the flavonoid "Isovitexin":

E. Identification and Characterization of the Isolated flavonoid "Isovitexin":

1.TLC Analytical TLC was performed by using ready made plate. The purified Isovitexin s applied on silica gel plate as one spot by using capillary tube along with its standard, using the vent system (S1, S2 and S3) (5,6). The detection was done by using UV light at 254 nm and 366 nm.

Where,

SI-Chloroform: Methanol: Formic acid (70:30:2).

S2 Ethyl acetate: Methanol: Formic acid: Water (50:2:3:6).

S3Butanol: Acetic acid: Water (65:15:20).

2. Melting point: The melting point (M. P.) of the purified flvonoid "Isovitexin" was done and compared with that of the available standard Isovitexin

3.FTIR: Infrared spectra was carried out by using KBr disc for both purified "Isovitexin" and its standard.

4. HPLC analysis: HPLC analysis was made by comparison of retention times obtained at identical chromatographic conditions of analysed purified flavonoid "Isovitexin" and its standred. (HPLC conditions as mentioned in pag 7).

Result And Discussion

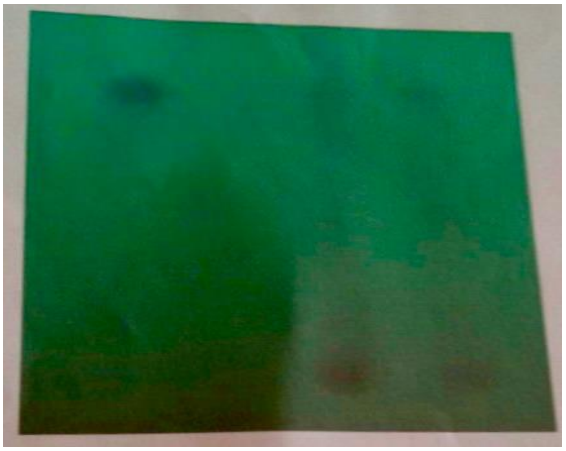
Extraction methods Two methods of extraction of the flavonoid Isovitexin were tried to select the best one. Result showed that the method No.2 was better, because the yield of crude extract was higher than obtained method No. 1. In addition quantitative estimation by using HPLC analysis showed that the amount of isovitexin obtained by No.2 was much more compared with that obtained by method No.1 as showed in table (1). So we select method No. 2 as an extraction procedure in our work.

Table 1: Quantitative of crud extracts and Isovitexin obtained from the whole plant Anasutica hierochuntica by extraction methods.

Extraction methods	*crud extract (mg)*	*isovitexin %*
NO.I	2.49	12.5
NO.2	25.48	33

Identification of the flavonoid "Isovitexin" by TLC

TLC of the crud extracts obtained from the whole plant Anasatica hierochuntica by the extraction method NO. I and No. 2, confirms the presence of Isovitexin in these extracts in comparison with isovitexin standard. As presented in table (2) and figures (3, 4 and 5)



S 1 2

Fig.3: TLC of plant Amammatica hierochantica obtained by extraction methods 1 and 2 using silica gel 60 F254 as adsorbent and (S1) as a mobile phase. (S: isovitexin.1: method NO.1,2: method NO.2)

S 1 2

Fig.4: TLC of plant Anastatica hierochuntica obtained by extraction methods (1 and 2) using silica gel 60 F254 as an adsorbent and (S2) as a mobile phase. (S: isovitexin, 1: method NO.1, 2: method NO.2).

S 1 2

Fig.5: TLC of plant Anastatica hierochuntica obtained by extraction methods 1 and 2 using silica gel 60 F254 as an adsorbent and (S3) as a mobile phase. S: isovitexin, 1: method NO.1.2: method NO.2).

Table 2: Re values of Isovitexin obtained from the whole plant Anastatica hierochumica by extraction methods and its standard in different developing solvent systems in TLC

Solvent system	*S1*	*S2*	*S3*
RF value of standard Isovitexin	0.583	0.444	0.74
RF value of isolated Isovitexin	0.58	0.445	0.74

S1: Chloroform: Methanol: Formic acid (70:30:2)

S2: Ethyl acetate: Methanol: Formic acid (50:2:3:6).

S3: Butanol: Acetic acid: Water (65:15:20)

1.Analytical TLC

Isolated isovitexin appeared as a single spot having the RF value as that of reference standard.

2.Measuring melting point

The isolated isovitexin was identified from its sharp melting point of (219-221 "C) compared to standard isovitexin melting point (220-221 °C).

3.FTIR

The IR spectra of isolated isovitexin was given identical results with that of isovitexin standard, as shown in figures (6 and 7).

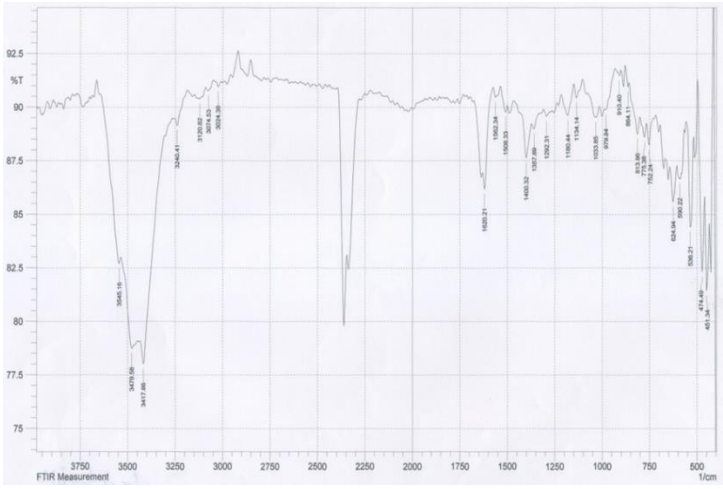


Fig.6 :FT-IR spectrum of the standared Isovitexin

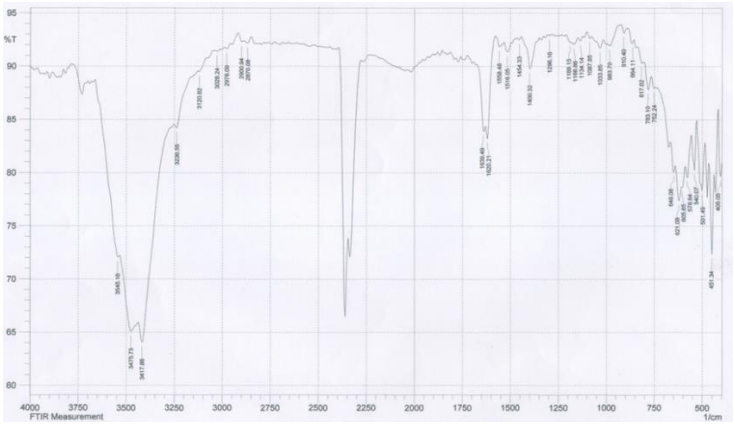


Fig.7: FT-IR spectrum of the isolated Isovitexin.

Table (3-6): The most significant group frequencies from FT-IR spectrum of isolated Isovitexin (Ma1)

Origin	*Group frequency wave number (Cm -1)*	*Assignment*
O-H	3417, 3479	O-H stretching of phenol
C = C-H	3028	C - H stretching of aromatic ring
C = O	1620	C=O stretching of keton conjugated system
C = C	1639	C=C stretching of conjugated system
C=C-H	1400 , 1508, 1560	C=C stretching of aromatic ring
O-H	1357	O-H bending of phenol
C - O – C	1033	C-O-C stretching of ether
C – H	813 , 864	C-H of aromatic group out of plane

4.HPLC analysis

The retention time for the isolated isovitexin was identical to the main peak of the standard reference as showed in figures (8 and 9).

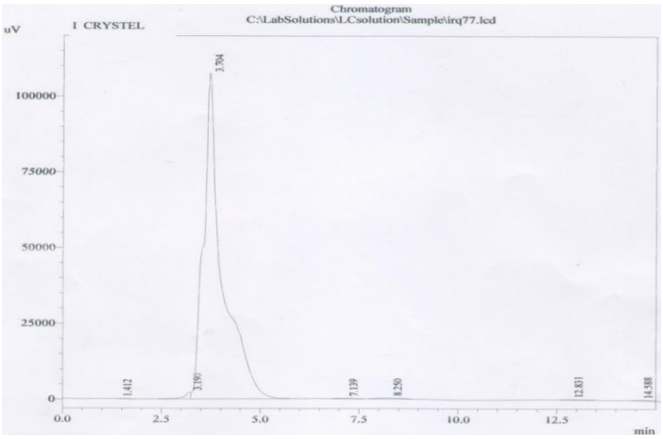


Fig.8: HPLC analysis of the isolated Isovitexin .

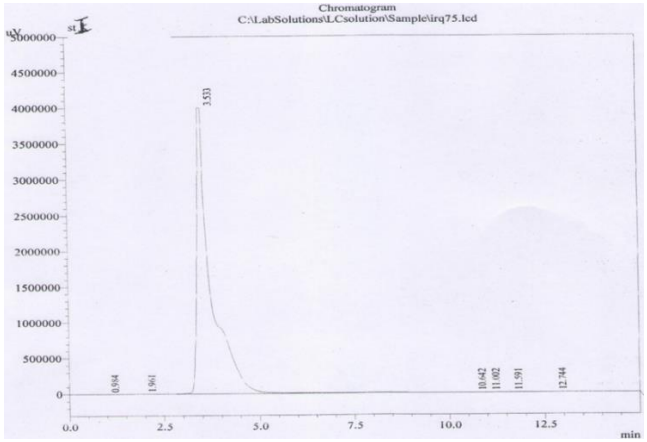


Fig.9: HPLC analysis of Isovitexin standard.

Conclusions

Pytochemical investigation of the whole plant Anastatic hierachuntica cultivated in Iraq revealed the presence of important medicinal natural product isovitexn" belonging to flavonoids Isovitexin was extracted by using two extraction methods, and identified by using TLC and HPLC method. using preparative TLC plates The identification of isolated isovitexin was carried out using melting point. Thin layer chromatography infrared spectroscopy and HPLC.also the study showing the amount of isovitexin obtained by No.2 was much more compared with that obtained by method No.1.

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