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Phytochemical Study of Flavonoid "Rutin"Present in the leaves of Cordia myxa L. Cultivated in Iraq.

Nuha M. Aboud[1], and Zainab J. Awad[2].

[1]College of Pharmacy, University of Basrah, Basrah, Iraq.
[2]College of Pharmacy, University of Baghdad, Baghdad,Iraq.

ABSTRACT

This study detects the presence of an important flavonoid "Rutin" in the leaves of *Cordia myxa* L. grown in Iraq. The pharmaceutical importance of Rutin arise from its consideration as anti-oxidative activity, and the absence of any study concerning Rutin content of this medicinal plant in Iraq, gave this study its importance. This study concerned with the extraction, identification, isolation and purification of Rutin from the leaves of Cordia myxa L. The extraction of this compound was carried out using one method. Identification of this compound was done by Thin Layer Chromatography (TLC) in which three different solvent system has been tried. This identification was further augmented by using High Performance Liquid Chromatography (HPLC) and then this compound was isolated and purified. Identification of the isolated Rutin was carried out using melting point (M.P.), Thin Layer Chromatography (TLC), and Infrared spectroscopy (IR). This study confirms the presence of Rutin in the leaves of Cordia myxa L. grown in Iraq.

Keywords: Cordia myxa, Flavanoid , Rutin.

Corresponding author

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INTRODUCTION

Cordia myxa L. (family: Boragenaceae),figure (1), is a tree or shrub, ca. 7–12 m high, which grows on deep moist soils, such as river banks. The tree keeps its leaves for most of the year. These are broad, alternate, ovateelliptic shaped. The inflorescence carries numerous white flowers. Fruits are round to ovoid shaped drupes, about 15–20 mm in diameter, arranged in clusters . Fruit is drupe, round shaped, green at maturity and yellowish brown at full ripening . The origin of C.myxa L.is less clear but it is suspected from the area stretching from the eastern Mediterranean region to eastern india.Further it is introduce in tropical Asia , Africa, Australia.it is cultivated in the desert region of iraq (quite common in basra district ,rare further north), habbaniya cantonment , kut, al imara [1,2].

Chemical screening of leaves showed the presence of flavonoid (rutin), has been shown to be effective as antioxidant that have been exploited in human medicine and nutrition. Conventionally, it is used as an antimicrobial, antifungal, and anti-allergic agent. However, current research has shown its multi-spectrum pharmacological benefits for the treatment of various chronic diseases, such as cancer, diabetes, hypertension, and hypercholesterolemia [3].

Figure 1: *Cordia myxa* L.

EXPERIMENTAL

Material and Instruments

The plant materials (leaves) of *Cordia myxa* L. were collected from private garden, during the months of September and October (2015), they were cleaned and dried in shed, then these plant materials were coarsely powdered by mechanical grinder and weighted. The dried powdered plant materials were extracted using one method.the following chemicals were used in this study:Ethanol(99.8%),N-hexan,Ethylacetate,N-butanol,Glacial acetic acid,Formic acid,distilled water and Rutin standard purchased from Biopurifyphytochemicals company.

Identification and characterization occurred by thin layer chromatography(TLC) using a readymade aluminum plates of silica gel GF-254,melting point by using Electro–thermal Melting point, Fourier transform infrared (FT-IR), FT-IR spectra were scanned on Shimadzu FT-IR-84005 Infrared Spectrometer and High Performance Liquid Chromatography(HPLC).

Extraction method [4]

A quantity of 50 g of dried powdered leaves of *Cordia myxa* L. was extracted by using soxhlet apparatus for 10 hours with aqueous ethanol (500 ml) 75% (V/V) and the resulting extract was filtered and concentrated using rotary evaporator until we get dry extract, For the isolation of flavonoids, the extract was partitioned successively between an equal volumes of water and n-Hexane, this step called (defatting step) then the residual water phase was extracted with an equal volume of ethyl acetate to draw all the flavonoids containing compounds in to the organic phase and the ethyl acetate phase was concentrated by means of rotary evaporator, then the dry extract was weighted and subjected for identification of flavonoids. Figure (2) represents the general scheme for extraction of flavonoids from the leaves of *Cordia myxa* L.

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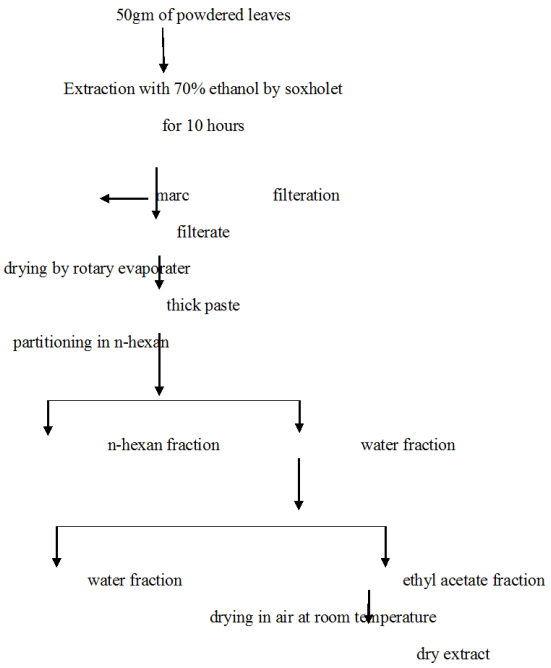

Figure 2: General scheme for extraction of Flavonoids from Cordia myxa

Identification of the Flavonoid(rutin)in the crude extract

Thin layer chromatography (TLC)

Using Thin Layer Chromatography (TLC) technique for qualitative identification of Rutin. In this identification, using a readymade aluminum plates of silica gel GF-254. and a comparison was made with three different developing solvent systems that were [5,6].

Solvent 1 (S1) : Butanol :glacial acetic acid: water(4:1:5)(upper layer)
Solvent 2 (S2) :Ethylacetate : ethanol : acetic acid : water)(16:1.5:1:1)
Solvent3(S3): Ethylacetate: Formic acid: acetic acid: water(100:11:11:27)

Freshly prepared ethanolic solutions of the standard reference, Rutin, and ethanolic solutions of the dried leaves of *Cordia myxa L*. extract were applied to TLC plates manually by using capillary tubes , and then developed by the ascending technique. The solvent migration limits was 10-12 cm from the base line. The above three developing systems were tried, and placed in a glass tank (22.5cm x 22cm x 7cm), and covered with a glass lid and allowed to stand for 45 minutes before use for saturation. After development, the plates were allowed to dry at room temperature and were detected using UV-light,(254 nm).

High Performance Liquid Chromatography(HPLC)

Qualitative identification of Rutin in extracts obtained from extraction methods above was authenticated by HPLC, this identification was made by comparison of the retention time obtained at identical chromatographic conditions .The analyzed sample and the authentic standard conditions were:

1- Mobile phase: Isocratic: Methanol / Water (70/ 30)
2- Column: C18 (250 x 4.6 mm)
3-column temp.: 25ºC
3- Flow rate: 1.5 ml /min.
4- Detection: UV. Detector at λ 280 nm.
5- Injection volume: 5 µL
6- Injection concentration: 1 mg /ml

Isolation and purification

After locating of Rutin of the extract, preparative Thin Layer Chromatography was done to isolate and purified Rutin. The preparative TLC was performed by using a readymade glass plates 20x20 cm, which were coated with silica gel; layer thickness 1 mm; made by MERCK, Germany, these plates were activated at 100□C for one hour before use.

The dried *Cordia myxa* L. leaves extract was dissolved in ethanol, then applied as a concentrated solution in a raw of spots using capillary tubes, the mobile phase for rutin was S1**=** butanol : glacial acetic acid:water (4:1:5)(upper layer) , the standard sample was applied in one side of the plate, the separated compound appeared as a band identified using UV-light detection method. The band was then assigned and the assigned silica gel was scrapped out and collected in a beaker and mixed with hot ethanol and then filtered. The silica gel on the filter paper was washed again with hot ethanol. The ethanol solutions were evaporated to dryness under reduced pressure to give the corresponding precipitates. These precipitates then recrystallized using aqueous ethanol and maintained for TLC , HPLC , FT-IR and measuring melting point.

Identification and characterization of the isolated Rutin

For further identification of the isolated Flavonoid, Rutin, from the dried leaves of *Cordia myxa* L., the following methods were used:-

- TLC **:** Analytical TLC was performed by using a readymade plates of 20x20 cm, which are coated with silica gel layer of 0.25 mm thickness.
- The isolated Flavonoid, Rutin, obtained by preparative TLC was applied on silica gel plates as one spot by using a capillary tube along with the standard, the mobile phase was S1.
- Melting point **:** Melting point was estimated by using Electro-Thermal melting point apparatus for the isolated compound and compared with that of the available standard of Rutin.
- IR **:** Infrared spectra were recorded using KBr disk.
- HPLC analysis (conditions as in 2.3.2).

RESULTS AND DISCUSSION

Extraction method

Ethanol was selected as a right choice for extraction of flavonoid because it is environmentally benign, relatively safe to human health and interacts with the flavonoid probably through non covalent interactions and promotes a rapid diffusion into the solution [7]. After fractionation with n-hexan (defatting step),the aqueous layer fractionated with ethyl acetate which contained highest amount of flavonoid [8]**.**

Table 1: Percentages of crude extracts and Rutin obtained from the leaves of Cordia myxa L.

% Yield of crude extract	12.22%
% Yield of Rutin	0.6233%

Identification of Rutin in crude extract by TLC

TLC of the extract obtained from the leaves of *Cordia myxa* L., confirms the presence of Rutin in comparison with the standard by using different solvents .. As presented in table (2) and figure (4).

Table 2: Rf values of flavonoid (Rutin) as compared with the standard reference in different developing systems in TLC.

Solvent system	S1	S2	S3
Rf of the standard Rutin	0.55	0.35	0.34
Rf of Rutin of extract	0.57	0.37	0.40

S1 : butanol : glacial acetic acid:water (4:1:5)(upper layer)
S2 : Ethylacetate : ethanol : acetic acid : water)(16:1.5:1:1)
S3 : Ethylacetate: Formic acid: acetic acid: water(100:11:11:27)

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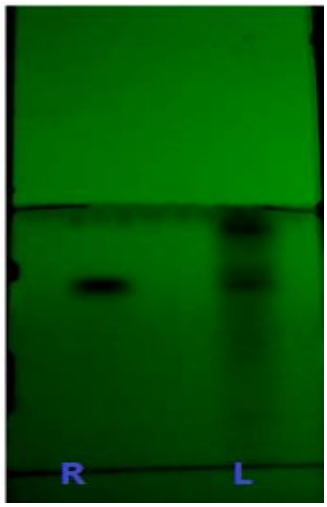

Figure 3: TLC chromatogram of qualitative analysis of the rutin compound using silica gel GF254 as adsorbent and S1 in as a developing solvent system. Detection by UV at 254 nm. (L=leaves extract, R= Rutin standard).

Charactrization of isolated Rutin

TLC

Isolated compound (Rutin) appeared as a single spot having the Rf value as that of reference standard as shown in figure (4).

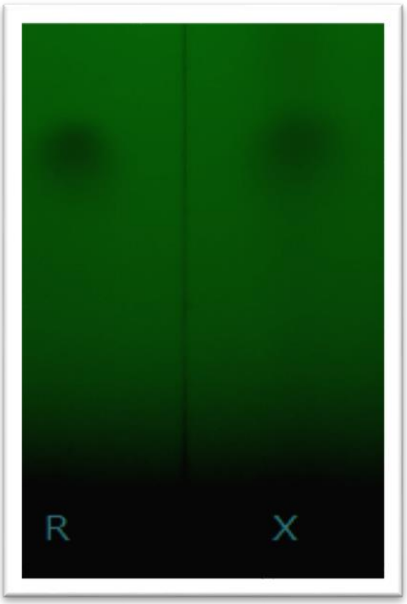

Figure (4): TLC chromatogram of qualitative analysis of the isolated compound (rutin) using silica gel GF254 as adsorbent and S1 in as a developing solvent system. Detection by UV at 254 nm,(R : Rutin reference standard, X: isolated Rutin.)

Melting Point

The isolated compound was identified as Rutin from its sharp melting point .Since the compound showed a melting point of (192 – 194) □C compared to Rutin standard (195)□C.

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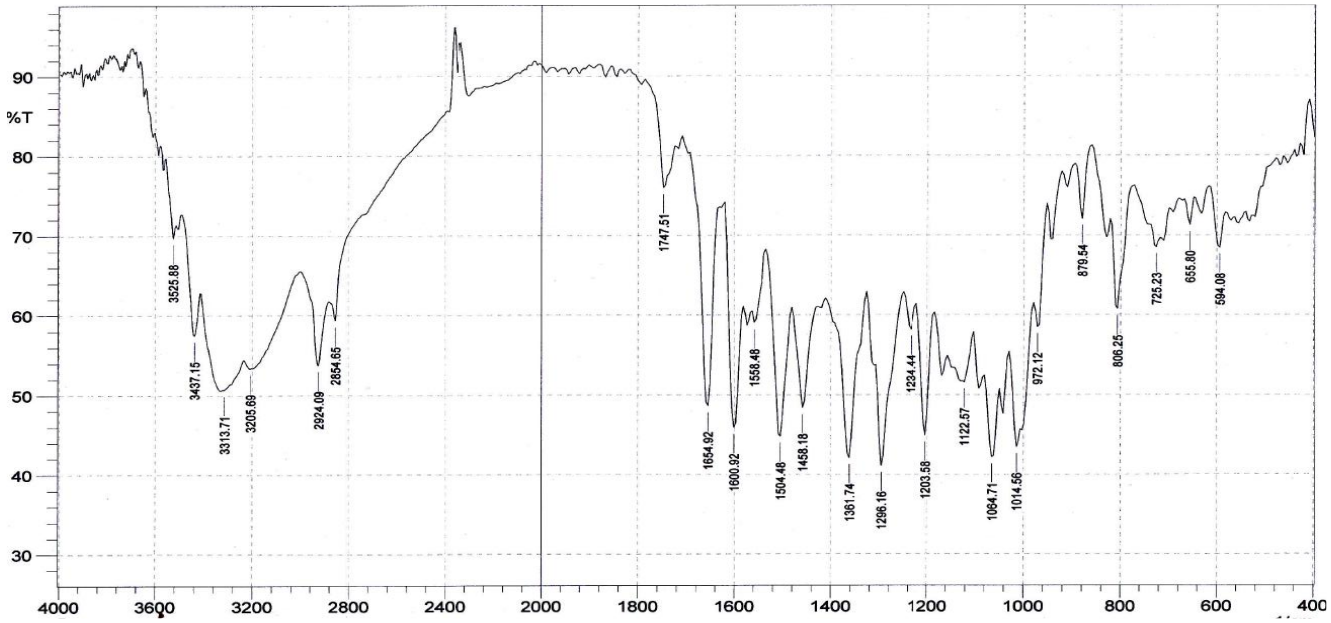
FT-IR

For further characterization of Rutin isolated from the dried leaves of *Cordia myxa* L., FT-IR spectroscopy analysis was performed using Rutin standard as a reference. The IR spectra of the isolated Rutin compared with the authentic Rutin standard gave us an identical results indicating that the isolated compound from *Cordia myxa* L. was Rutin as shown in figure (5).

Figure 5: IR spectra of the isolated Rutin



HPLC analysis

The HPLC analysis of both of the authentic Rutin and the isolated compound showed an identical retention time, which is considered as a conclusive evidence that the compound was Rutin .As shown in Figures (6 and 7).

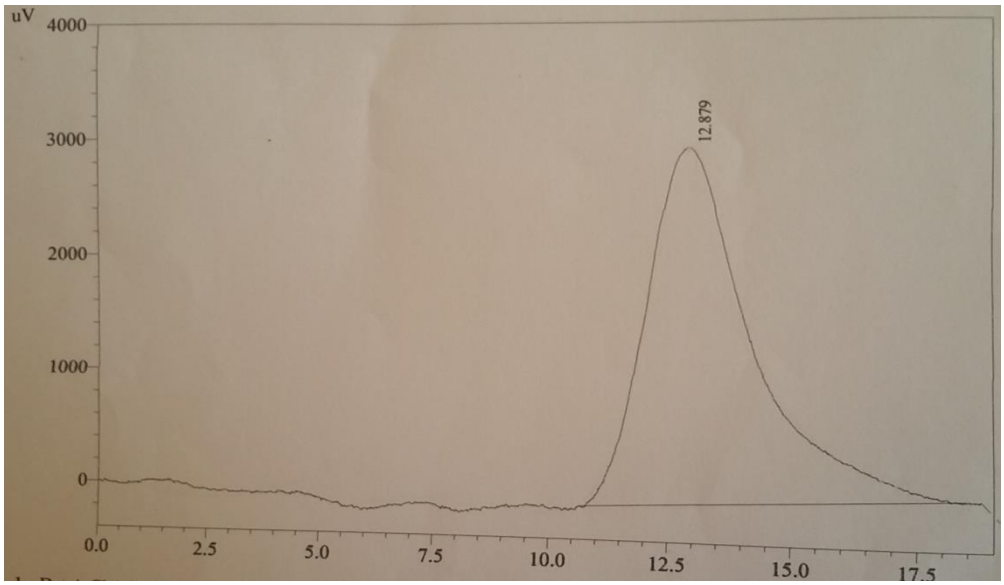


Figure 6: HPLC analysis of Rutin standard

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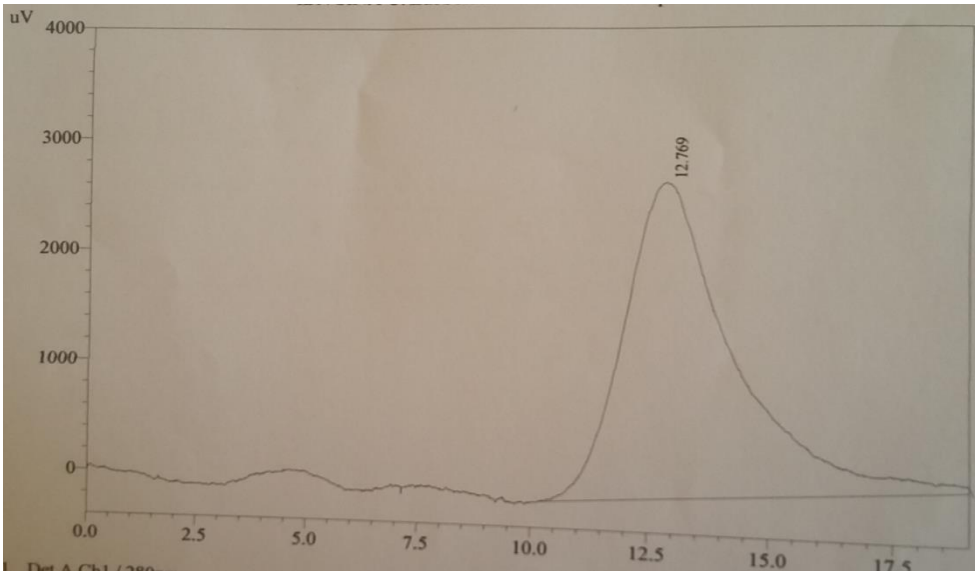

Figure 7: HPLC analysis of isolated Rutin

CONCLUSION

The phytochemical investigation of *Cordia myxa* leaves, grown in Iraq revealed the presence of important medicinal natural product "Rutin", which is a flavonoid. The extracted Rutin was identified using TLC and HPLC methods then it was purified and isolated from the dry crude extract by using a preparative TLC technique. The identification of the isolated compound, Rutin, was made using TLC , melting point and IR spectroscopy and HPLC.

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