

PHYTOCHEMICAL INVESTIGATION OF LEAVES AND SEEDS OF *CORCHORUS OLITORIUS* L. CULTIVATED IN IRAQ

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## ABSTRACT

**Objective:** The aim of this study was to investigate chemical constituents of leaves and seeds of *Corchorus olitorius* since no phytochemical investigation had been done previously in Iraq.

**Methods:** Leaves and seeds of *C. olitorius* were defatted by maceration in hexane for 24 h. The defatted plant materials were extracted using Soxhlet apparatus, the aqueous methanol 85% as a solvent extraction for 24 h, and fractionated by petroleum ether, chloroform, ethyl acetate, and n-butanol. The ethyl acetate, n-butanol, and n-butanol after hydrolysis fractions for each part (leaves and seeds) were analyzed by high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) for its phenolic acid and flavonoid contents. The petroleum ether fraction from the leaves was analyzed using Gas chromatography/mass spectrometer (GC/MS). Flavonoids and phenolic acid derivative were isolated from the ethyl acetate of leaf fraction and n-butanol after hydrolysis fraction of the seeds and identified by mass spectrometry, infrared, HPLC, and HPTLC.

**Results:** The different chromatographic and spectroscopic results revealed the presence of luteolin, quercetin, astragalins, isoquercetin, catechins, and 3,5-dicaffeoylquinic acid (3,5-DCQA) in both leaves and seeds of *C. olitorius* and also 17-octadecynoic acid, 9-octadecanoic acid (oleic acid), hexadecenoic acid (palmitic acid), 9,12-octadecanoic acid (linoleic acid), octadecadien-1-ol ( $\beta$ -sitosterol), and  $\alpha$ -tocopherol (Vitamin E) in the petroleum ether fraction of *C. olitorius* leaves.

**Conclusion:** The results of the current study proved the presence of 3,5-DCQA, astragalins, and isoquercetin in the ethyl acetate fraction of *C. olitorius* leaves and catechin in the n-butanol after hydrolysis fraction of *C. olitorius* seeds.

**Keywords:** *Corchorus olitorius*, Flavonoids, Phenolic acid,  $\beta$ -sitosterol, High-performance liquid chromatography, High-performance thin-layer chromatography.

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## INTRODUCTION

The genus *Corchorus* (jute), which belongs to the family of Tiliaceae, involves about 50–60 species allocated in the tropics, subtropics, and warm temperate areas of the world. On the other hand, there is an assumption that the origin of *Corchorus olitorius* and most other species are from Africa. Jute cultivation is thought to have started 200 years past in the tropics. *C. olitorius* is an important green leafy vegetable in many areas including Egypt, Southern Asia, Japan, India, China, Lebanon, Palestine, Syria, Jordan, Tunisia, and Nigeria. It is a leading leaf vegetable in Nigeria, Cameroon, Sudan, Kenya, Uganda, and Zimbabwe. It is also cultivated as a leafy veggie in the Caribbean, Brazil, India, Bangladesh, China, and the Middle East. The plant is widely grown in the tropics for the viscosity of its leaves. *C. olitorius* is tall, annual herbs, reaching a height of 2–4 m. The plant could be unbranched or with only a few side divisions. The leaves alternately distributed, simple, lanceolate, finely serrated, or lobed margin. The flowers are small (2–3 cm in diameter) and yellow, and it has five petals. The fruit of the plant contains many seeds inside in the form of a capsule [1]. It is a cultivated plant here in Iraq.

Linnaeus was the first scientist who describes the *Corchorus* genus in 1753. The ancient Greeks used to call a potherb as "Korkhoros," from which the common name *Corchorus* is obtained [2].

*Corchorus* species (Tiliaceae family) is one of the main genera containing cardiac glycosides, especially in the seeds [3].

It has been used pharmaceutically and in cosmetic fields [4]. Different parts of *C. olitorius* have been utilized to relieve pain, aches, chronic

cystitis, dysentery, enteritis, and pectoral pain [5]; the leaves have been utilized in case of gonorrhoea, chronic cystitis, fever, and tumors [6]; and the seeds were utilized as demulcent, diuretic, and purgative and also used in chronic cystitis, in cases of cardiac diseases such as heart failure due to its content of cardenolides cardiac glycosides [7,8]. Studies done by Adegoke and Adebayo-Tayo [9] and Barku *et al.* [10] demonstrated that the methanolic extract of the *C. olitorius* leaves shows a considerable antibacterial activity and high susceptibility against *Escherichia coli* with a high zone of inhibition, while Ilhan *et al.* [7] proved that the petroleum ether extract of *C. olitorius* leaves exerts a good activity against *E. coli*. In general, phytochemical verification that performed on the leaves of the plant revealed the presence of sterols, triterpenes, carotenoids, coumarins, saponins, tannins, flavonoid, carbohydrates, all, and cardiac glycosides in the seeds of the plant [11,12]. From these chemical groups, flavonoids and phenolic acids are the most important.

The flavonoids have 15 carbon skeleton (benzopyran) C6-C3-C5 backbone structure and it is composed of two benzene rings, namely, ring A and ring B connected by heterocyclic ring called ring C. It is subdivided to many classes such as flavones, flavonols, flavanones, flavanonols, isoflavones, and other, depending on the degree of oxidation, type, and manner of substitution of ring C; the difference between individual flavonoids depends on type and manner of substitution on rings A and B. It appears in nature as aglycones or glycosides in which the carbohydrates such as D-glucose and galactose attached to carbon number 3 or 7 and as methylated compounds. Flavonoids have several biological activities such as antioxidant, hepatoprotective, antibacterial, anti-inflammatory, anticancer, and antiviral activity [13–15]. Flavonoid is potential as antibacterial agents against *Staphylococcus epidermidis* and

therefore justifies their usage in traditional medicine for the treatment of body odor [16], and phenolics and polyphenolics compounds have considerable value with respect to antioxidant activities [17].

**Phenolic acids:** These are non-flavonoid polyphenolic compounds, it is subdivided into two major classes, benzoic acid derivatives such as gallic acid and cinnamic acid derivatives such as caffeic acid depending on C1-C6 and C3-C6 skeleton [18].

Caffeic acid occurs regularly as the quinic acid ester, chlorogenic acid, or its derivatives such as 1,5 or 3,5-dicaffeoylquinic acid [19].

Phenolic acids have several biological activities such as antioxidant, cardioprotective, antibacterial, and anticancer [20].

Several types of flavonoids and phenolic compound had been detected in the plant such as astragalol (kaempferol 3-O- $\beta$ -D glucopyranoside) [21,22], tolifolin (kaempferol 3-O- $\beta$ -D galactopyranoside), jugalanin (kaempferol 3-O- $\alpha$ -L-arabinopyranoside) [22], isoquercetin (quercetin 3-O- $\beta$ -D-glucopyranoside) [21-26], quercetin-3-galactoside [26], quercetin-3-(6 malonyl glucoside) [25], catechin [27], corchoroside-A and corchoroside-B [28], 5-caffeoylquinic acid (chlorogenic acid) [21,25,26], and 3,5-dicaffeoylquinic acid (3,5-DCQA) (isochlorogenic acid) [24-26]. The plant also contains fatty acids such as linoleic acid [25,29], sterols such as  $\beta$ -sitosterol [22,23,30] and vitamins such as Vitamin C and E ( $\alpha$ -tocopherol) [25,31].

## METHODS

### Collection of plant materials

*C. olitorius* leaves were harvested from a farm in al-Utadiyah district in Baghdad City, during July, and the seeds were collected in September 2017. 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, and the seeds were cleaned from unwanted materials, dried in an oven at 40°C for 2 days, and ground in a mechanical grinder to a fine powder.

### Equipment and chemical

The instruments used were rotary evaporator (BÜCHI Rotavapor R-205, Swiss), sonicator (Branson Sonifier, USA), high-performance liquid chromatography (HPLC) (SYKAM Germany), and high-performance thin-layer chromatography (HPTLC) (Eike Reich/CAMAG Laboratory, Switzerland). All chemicals and solvents used were of analytical grade and obtained from Riedel-de Haen, Germany, except trifluoroacetic acid, and methanol which is HPLC grade was purchased from Sigma-Aldrich, Germany. The standard luteolin, quercetin, astragalol, isoquercetin, catechin, and 3,5-DCQA were purchased from Chengdu Biopurify Phytochemicals, China (purity >97). TLC aluminum plates pre-coated with silica gel 60 F 254 (100 mm×100 mm, 0.2 mm thick) used were obtained from E. Merck Ltd., India.

### Extraction

1. 250 g of shade-dried pulverized leaves of *C. olitorius* were defatted by maceration with hexane for 24 h and then allowed to dry at room temperature. The defatted plant materials were extracted by Soxhlet using aqueous methanol 85% as a solvent for 24 h. The extract was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator to get a dry extract named part A. The residue was suspended in 400 ml water and partitioned successively with petroleum ether (B.P. 30–60), chloroform, ethyl acetate, and n-butanol (3×300 ml) for each fraction. The first three fractions were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness.
2. The same procedure was applied to the seeds; the dry extract obtained named part B.

### Hydrolysis of n-butanol fractions

3 g of n-butanol fractions of leaves and seeds were hydrolyzed by 200 mL of 5% HCl for 2 h under reflux, cooled, and partitioned with

200 mL × 3 ethyl acetate. The organic layers were combined, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure using a rotary evaporator; this was used to separate the flavonoid aglycone part from the sugar part, which in turn would simplify the isolation and detection procedures.

### Preparations of standards and samples for analysis

Standard solutions for HPLC of luteolin, quercetin, astragalol, isoquercetin, catechin, and 3,5-DCQA were prepared by dissolving 0.04 mg in 1 ml of methanol HPLC grade. Dried samples were prepared for HPLC analysis by dissolving them in methanol and subjecting them to ultrasonication at 60% duty cycles for 25 min at 25°C followed by centrifugation at 7500 rpm for 15 min. The clear supernatant of each sample was evaporated under vacuum. The residues were resuspended individually, in 1 ml of methanol HPLC grade, homogenizing using vortex mixer, and passing them through 2.5  $\mu$ m disposable filter, and stored at 4°C for further analysis. 20  $\mu$ l of the sample was injected into the HPLC system for analysis. Standards used for HPTLC analysis (astragalol, isoquercetin, catechin, and 3,5-DCQA) were prepared by dissolving 1 mg of each standard in 1 ml methanol, while the samples were prepared by dissolving few milligrams from each sample in 1 ml methanol.

### Preliminary phytochemical investigation

#### Test for flavonoids

Few milligrams of aqueous methanol plant extracts were suspended in ethanol and few drops of 5% ethanolic KOH were added, and then, few drops of 5% HCl were added. The changes in colors were recorded.

#### Test for phenols

Few milligrams of aqueous methanol plant extracts were treated with few drops of 1% FeCl<sub>3</sub>. Formation of dark greenish-blue color indicates the presence of phenols.

### HPLC analysis

HPLC technique (SYKAM, Germany) was applied for the detection of different constituents found in the ethyl acetate, n-butanol, and n-butanol after hydrolysis fractions as flavonoids and phenolic acids for both leaves and seeds of *C. olitorius* using a mobile phase composed of solvent A: Methanol: Solvent B: 0.05% trifluoroacetic acid in water for HPLC, gradient program from A=70% (0–5 min), A=40% (5–8 min), and A=90% (8–15 min). Column used: C18-ODS (25 cm x 4.6 mm), Detector: UV-280 nm, Injection volume: 20  $\mu$ L, Flow rate: 1.0 ml/min.

### HPTLC analysis

HPTLC, ethyl acetate for the leaves, and n-butanol after hydrolysis for the seeds fractions were analyzed also for its flavonoids and phenolic acid contents utilizing HPTLC (Eike Reich/CAMAG Laboratory, Switzerland), using silica gel GF254 plates developed in a mobile phase composed of ethyl acetate:formic acid:acetic acid:water (100:11:11:27 V/V) examined at 254 and 366 nm wavelength. the percentage of the compound in the plant was calculated depending on the following equation:

### Gas chromatography (GC)/mass analysis

The petroleum ether fraction of the *C. olitorius* leaves was analyzed using GC/mass (Agilent Single Quadrupole GC/mass, USA) Column used: Agilent 190915-433UI, HP-5ms Ultra Inert, 60–325°C (350°C): 30 m×250  $\mu$ m×0.25  $\mu$ m, Initial pressure: 7.037 psi, Flow rate: 0.9 ml/min. Average velocity: 34.772 cm/s. Holdup time: 1.4379 min.

- Conditions used for GC/mass spectrum (MS) analysis were as follows:

Injection temperature: 275.0°C, Injection mode: Splitless, Column temperature: 60.0°C, pressure: 7.037 psi, column flow: 0.9 mL/min, average velocity: 34.772 cm/s, holdup time: 1.4379 min, total elution time: 4.20 min, ion source: EI, source temperature (°C): 230, quad

temperature (°C): 150, scan time segments: 4.20, start mass: 30, end mass: 700, threshold: 150, and scan speed: 1,562] N=2. The oven temperature programs for column and injector used for petroleum ether fraction are shown in Table 1.

**Isolation of flavonoid from n-butanol fraction after hydrolysis fraction from the seeds by preparative HPLC**

1 g from the n-butanol fraction after hydrolysis fraction from the seeds was dissolved in 5 ml gradient methanol for the isolation of catechin using the same conditions for HPLC analysis mentioned above, and the target peak was collected by fractions collector after monitoring it according to the time, i.e., (time from the beginning of each peak appearance until disappearance of peak), which was between 11.4 and 12.6 min.

**Isolation of flavonoids and phenolic acid derivative by preparative TLC**

Flavonoids and phenolic derivative were isolated by preparative TLC, utilizing ethyl acetate fraction of *C. olitorius* leaves.

**Preparation of stationary phase**

Readymade silica gel GF254 plates with a layer thickness of 0.5 mm dimension 20 cm×20 cm. The plates were reactivated by heating in the oven at 120°C for 15–20 min, left to cool, and used for application after allocation of the baseline and the solvent front.

**Preparation of mobile phase (solvent system)**

The constituents of the mobile phase for coumarin (ethyl acetate:formic acid:acetic acid:water [100:11:11:27 V/V]) were mixed in a conical flask and introduced in the jar. The jar was lined with a filter paper, closed tightly, and left for saturation.

**Application of sample**

About 0.5 g of the sample was dissolved in absolute methanol and applied on the baseline of TLC plates using a capillary tube.

**Detection of separated compounds**

Detection was done by examination under UV light with wavelengths, namely, 254 nm. The purity of each band was checked by analytical TLC until a single spot on TLC plate is obtained for identification with a reference standard.

**Identification of isolated flavonoid and phenolic derivatives from ethyl acetate fraction of leaves and n-butanol after hydrolysis fraction of seeds**

The isolated flavonoid and phenolic derivatives were identified by different spectroscopic and chromatographic techniques listed below:

**Table 1: The oven temperature programs for column and injector**

Rate	Temperature (°C)	Hold time (min)
0.0	60.0	3.00
5°C/min	150.0	2.00
5°C/min	280.0	5.00

**Table 2: DI temperature program**

Rate (°C/min)	Final temperature (°C)	Hold time (min)
80	150	0.0
40	300	1.0

**Table 3: Preliminary examination of the aqueous methanol extract for phenols and flavonoids**

Phytochemicals	Leaf extract	Seeds extract
Flavonoids	+	+
Phenols	+	+

- Using GC/mass chromatography (Shimadzu GCMS-QP2010Ultra) by direct sample injection method under the following conditions: (GCMS-QP2010 Ultra), ion source temperature: 150.00°C, interface temperature: 40.00°C, start time: 0.00 min, end time: 6.62 min, ACQ mode: scan, and sample inlet unit: DI (direct sample injection). The oven temperature programs for column and injector used are shown in Table 2.
- Infrared (IR): IR spectra for the isolated compounds were recorded in the range of scanning 4000–400/cm.
- HPTLC: As listed before.

**RESULTS**

Preliminary examination of the ethyl acetate fraction results is shown in Table 3.

The HPLC results of analyzed fractions of leaves and seeds are demonstrated in Tables 4 and 5. The identification of compounds by HPLC is usually performed by comparing the obtained retention times with the ones of related standards. Their chromatograms are shown in Figs. 1 and 2 compared with that of standard 3,5-DCQA, astragaline, isoquercetin, catechin, quercetin, and luteolin showed in Figs. 3-8, respectively.

The HPLC for the isolated flavonoid (catechin) from the n-butanol fraction after hydrolysis fraction from the seeds is shown in Fig. 9.

HPTLC results are shown in Figs. 10 and 11, and preparative TLC used for isolation of flavonoids and phenolic compounds and observed at 254 nm wavelength is shown in Fig. 12.

HPTLC for isolated flavonoids and phenolic compounds is shown in Fig. 13.

The GC/MS for the petroleum ether fraction is shown in Fig. 14.

**GC/MS fragmentation chromatogram of the isolated compounds**

The mass peaks of the isolated compounds were described as follows:

- Molecular ion peak at m/z 516 [M]<sup>+</sup> of the isolated 3,5-DCQA and its fragmentation is shown in Fig. 15.
- Molecular ion peak at m/z 448 [M]<sup>+</sup> of the isolated astragaline and its fragmentation is shown in Fig. 16.

**Table 4: Retention times of the detected compounds in minutes in all leaf fractions corresponding to the related standards**

Ethyl acetate	n-butanol before hydrolysis	n-butanol after hydrolysis	Standard compound	Retention time in a min for standards
-	4.11	4.09	3,5-DCQA	4.1
-	5.27	5.26	Luteolin	5.18
6.52	6.52	6.52	Quercetin	6.56
7.32	7.32	7.32	Isoquercetin	7.28
8.13	8.13	8.16	Astragaline	8.35
11.98	11.98	11.98	Catechin	11.9

3,5-DCQA: 3,5-dicaffeoylquinic acid

**Table 5: Retention times of the detected compounds in minutes in all seeds fractions corresponding to the related standards**

Ethyl acetate	n-butanol before hydrolysis	n-butanol after hydrolysis	Standard compound	Retention time in a min for standards
-	4.12	4.11	3,5-DCQA	4.1
-	5.27	5.27	Luteolin	5.18
6.52	6.25	6.52	Quercetin	6.56
7.32	7.32	7.32	Isoquercetin	7.28
8.13	8.13	8.13	Astragaline	8.35
-	11.98	11.98	Catechin	11.9

3,5-DCQA: 3,5-dicaffeoylquinic acid

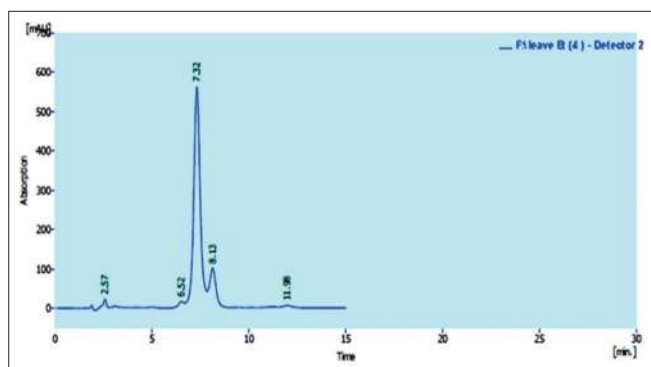


Fig. 1: High-performance liquid chromatogram of ethyl acetate fraction of the leaves

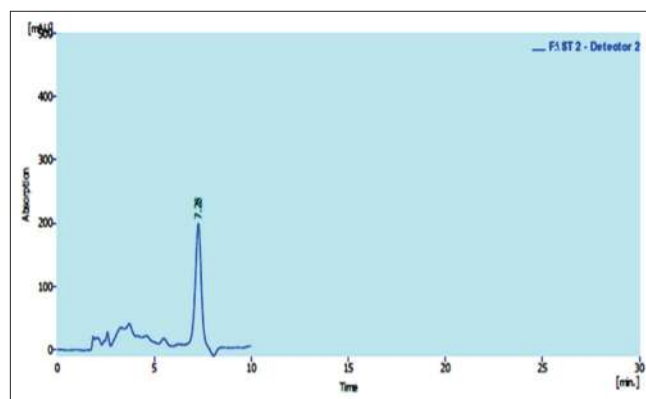


Fig. 4: High-performance liquid chromatogram of astragalins standard

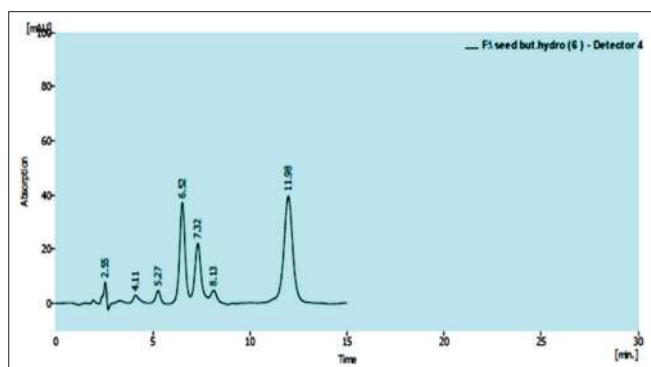


Fig. 2: High-performance liquid chromatogram of n-butanol after hydrolysis fraction of the seeds

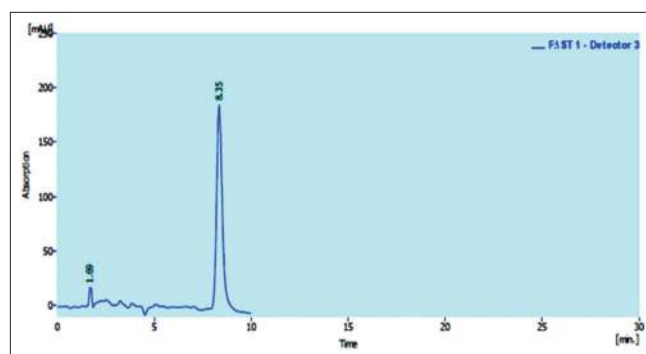


Fig. 5: High-performance liquid chromatogram of isoquercetin standard

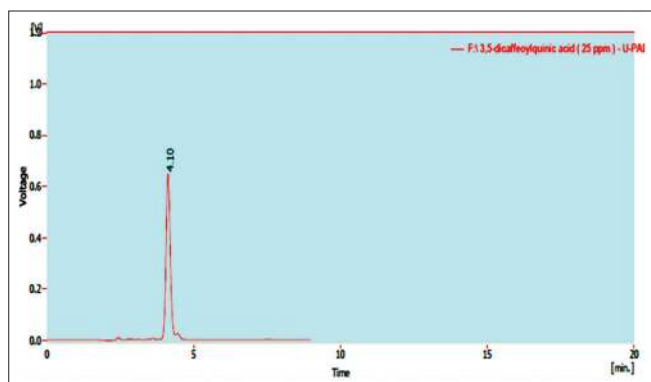


Fig. 3: High-performance liquid chromatogram of 3,5-dicaffeoylquinic acid standard

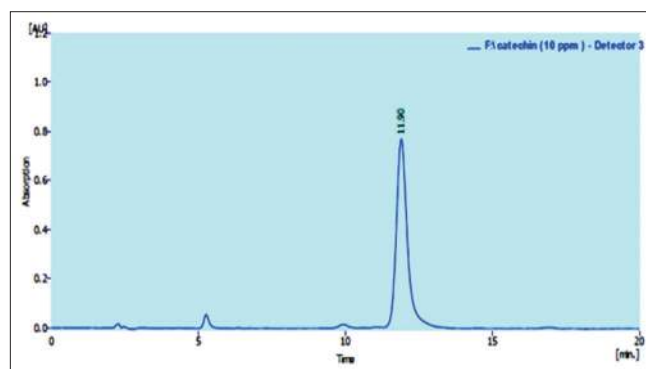


Fig. 6: High-performance liquid chromatogram of catechin standard

3. Molecular ion peak at  $m/z$  464 [M]<sup>+</sup> of the isolated isoquercetin and its fragmentation is shown in Fig. 17.
4. Molecular ion peak at  $m/z$  290 [M]<sup>+</sup> of the isolated of catechin and its fragmentation is shown in Fig. 18.

**IR spectroscopy**

The IR spectral analysis of the isolated compounds illustrates the characteristic band frequencies for each compound as follows:

1. The IR spectral analysis (Fig. 19) of the isolated 3,5-DCQA showed the peaks at 3500–3140 (broadband), 3064, 2966, 2910, 2852, 1712, 1683, 1365, 1350, 1269, 1624, 1559, 1442, 922, 854, 781, 729, and 684/cm.
2. The IR spectral analysis (Fig. 20) of the isolated astragalins showed the peaks at 3626–3444, 3101, 2994, 2904, 1666, 1396, 1359, 1286, 1249, 1606, 1437, 902, 854, 833, 731, 705, and 688/cm.
3. The IR spectral analysis (Fig. 21) of the isolated isoquercetin showed the peaks at 3562–3504, 3147, 3076, 2947, 2848, 1662, 1357, 1294, 1230, 1599, 1452, 1440, 906, 862, 810, 792, 763, and 694/cm.

4. The IR spectral analysis (Fig. 22) of the isolated catechin showed the peaks at 3649-3564, 3057, 2997, 2852, 1637, 1398, 1280, 1217, 1606, 1413, 871, 837, 769, and 698/cm.

The IR spectra of the isolated 3,5-DCQA, astragalins, isoquercetin, and catechin are shown in Figs. 19-22.

The GC/MS spectrum for the petroleum ether fraction shows the presence of several compounds; that have been listed in Table 6.

**DISCUSSION**

Plants are generally a good source of many important pharmaceutical compounds. Specifically, the plants rich in secondary metabolites. Plant-derived drugs played an important role in the benefits of human health and wellness.



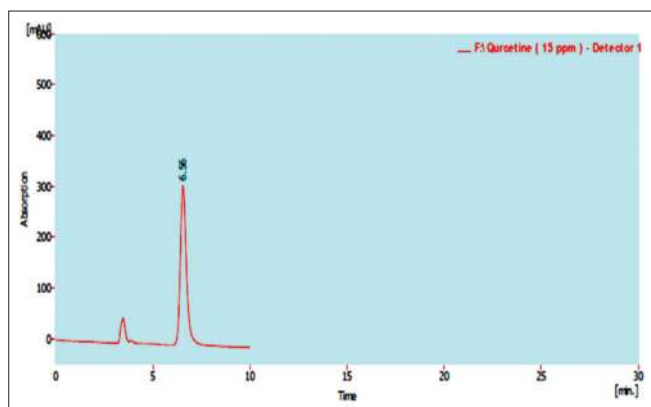


Fig. 7: High-performance liquid chromatogram of quercetin standard

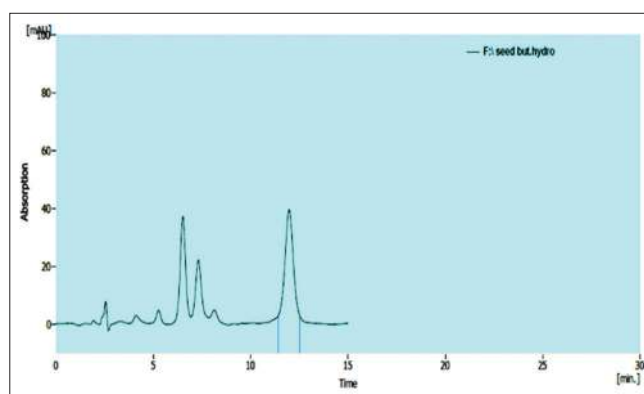


Fig. 9: Preparative high-performance liquid chromatogram of n-butanol fraction after hydrolysis fraction from the seeds

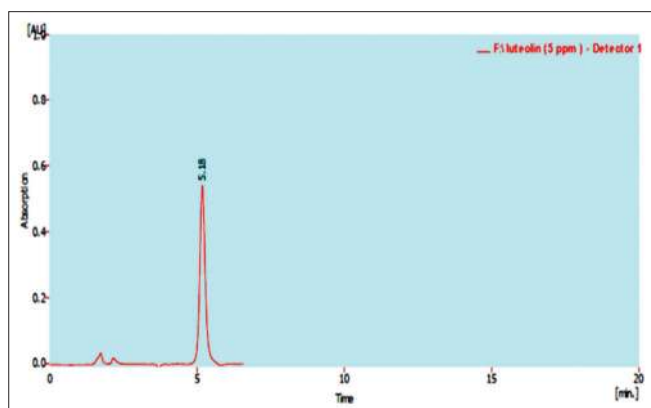


Fig. 8: High-performance liquid chromatogram of luteolin standard

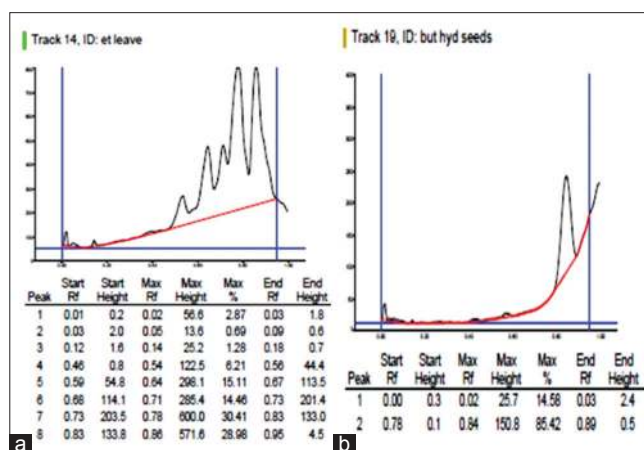


Fig. 10: High-performance thin-layer chromatography of a - ethyl acetate fraction of leaves. b - HPTLC of n-butanol after hydrolysis fraction for seeds

The preliminary phytochemical analysis confirmed the presence of phenols and flavonoids.

The HPLC results show the presence of flavonoids (in both forms as aglycones and as glycosides) and phenolic acid in the leaves and seeds of the plant, such as luteolin, quercetin, isoquercetin, astragal, catechin, and 3,5-DCQA.

The compound 3,5-DCQA does not appear in the HPLC chromatogram of ethyl acetate fractions of the leaves; but appeared as a peak having a small area under the curve in the n-butanol after hydrolysis fraction of seeds. This is due to the low concentration of the compound, and the difference in absorption scales used in analyzing the ethyl acetate fraction (absorption scale 0-1000) and the rest of fractions (absorption scale 0-100).

HPLC result revealed that astragal, isoquercetin, and quercetin are the major compounds in the leaves and seeds of *C. olitorius*.

Luteolin appeared in the n-butanol and n-butanol after hydrolysis but not the ethyl acetate fraction for both the leaves and seeds of the plant.

HPTLC results revealed the presence of astragal, isoquercetin, and 3,5-DCQA in the ethyl acetate fraction of the leaves and the presence of catechin in the hydrolyzed n-butanol fraction of the seeds.

The explanations for the obtained IR spectral analysis are shown in Table 7.

Regarding MS fragmentation, chromatogram of the isolated compounds is explained as follows:

1. Molecular ion peak at  $m/z$  516 [M]<sup>+</sup> that corresponds to a molecular formula of 3,5-DCQA (C<sub>25</sub>H<sub>24</sub>O<sub>12</sub>). Ion peaks were also observed at  $m/z$  353 due to loss of one caffeic acid moiety from the original compound, 163 for caffeic acid, and 191 for quinic acid which is the result of losing the second caffeic acid moiety, and these are in good agreement with reported values of the 3,5-DCQA structure[32]. According to all the data obtained from HPLC, HPTLC, IR, and mass fragmentation, Fig. 23 demonstrates the predicted chemical structure for the compound 3,5-DCQA.
2. Molecular ion peak at  $m/z$  448 [M]<sup>+</sup> that corresponds to a molecular formula of Astragal (C<sub>21</sub>H<sub>20</sub>O<sub>11</sub>). Ion peaks were also observed at  $m/z$  285 which represents the kaempferol aglycon moiety, 163 represents the sugar part (glucose), and 117 and 152 which are in a good agreement with reported values of astragal structure [33]. According to all the data obtained from HPLC, HPTLC, IR, and mass fragmentation, Fig. 24 demonstrates the predicted chemical structure for the compound astragal.
3. Molecular ion peak at  $m/z$  464 [M]<sup>+</sup> that corresponds to a molecular formula of isoquercetin (C<sub>21</sub>H<sub>20</sub>O<sub>12</sub>). Ion peaks were also observed at  $m/z$  301 which represents the quercetin aglycon moiety, 163 represents the sugar part (glucose), and 133 and 152 which are in a good agreement with reported values of isoquercetin structure[33]. According to all the data obtained from HPLC, HPTLC, IR, and mass fragmentation, Fig. 25 demonstrates the predicted chemical structure for the compound isoquercetin.
4. Molecular ion peak at  $m/z$  290 [M]<sup>+</sup> that corresponds to a molecular formula of catechin (C<sub>15</sub>H<sub>14</sub>O<sub>6</sub>). Ion peaks were also observed at  $m/z$  152, 139, and 123 which are in a good agreement with the reported

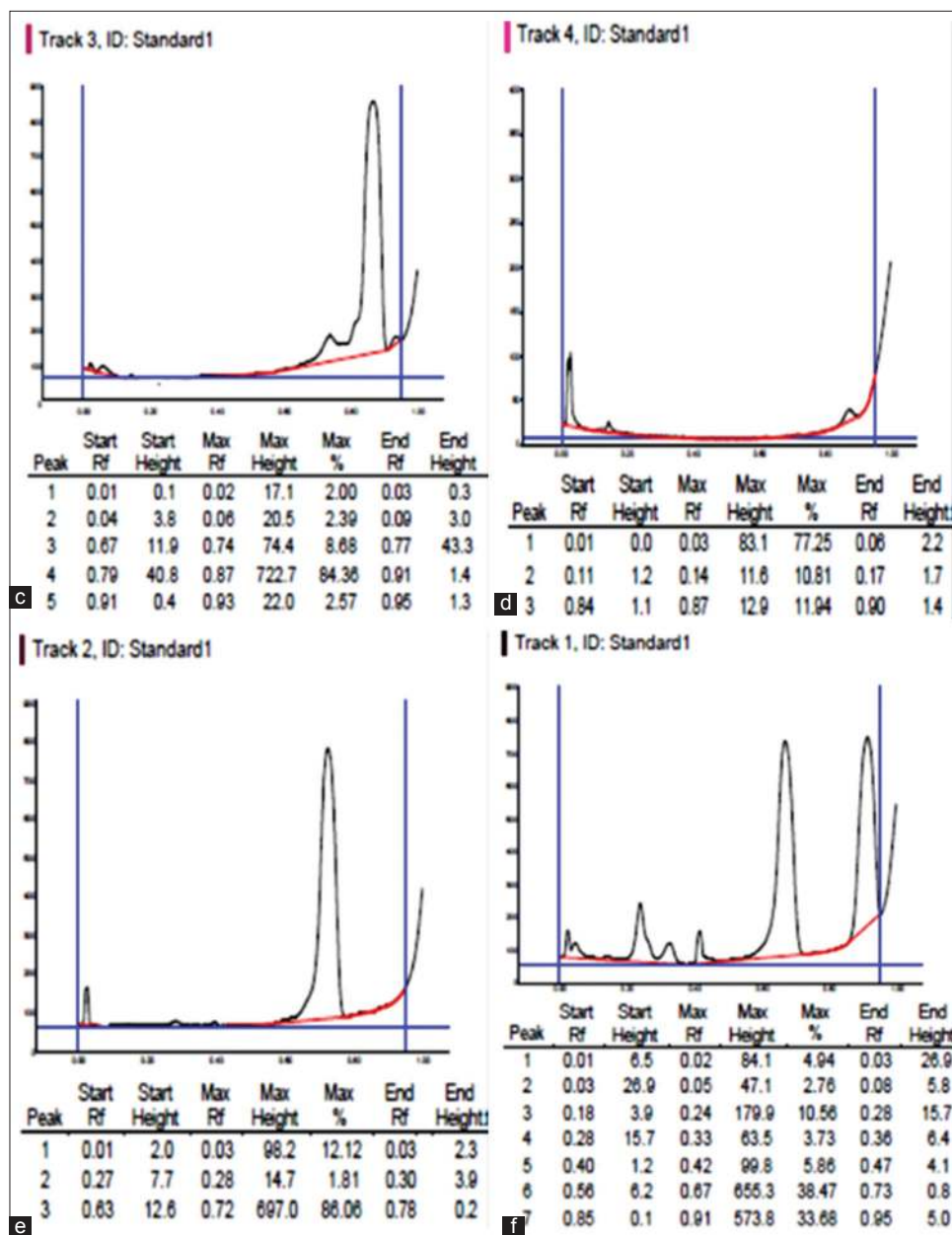


Fig. 11: High-performance thin-layer chromatography for c - 3,5-dicafeoylquinic acid standard, d - catechin standard, e - astragaline standard, and f - isoquercetin standard

Table 6: The compounds detected by GC-mass in the petroleum ether fraction of the leaves

Ref. No.	Compound name	Molecular weight g/Mol	Molecular formula
1	17-Octadecynoic acid	294	C <sub>19</sub> H <sub>39</sub> O <sub>2</sub>
2	9-Octadecanoic acid (oleic acid)	282	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>
3	Hexadecenoic acid (palmitic acid)	256	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
4	9,12-Octadecanoic-acid (linoleic acid)	280	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>
5	Octadecadien-1-ol	266	C <sub>18</sub> H <sub>34</sub> O
6	β-sitosterol	414	C <sub>29</sub> H <sub>50</sub> O
7	α-tocopherol (Vitamin E)	430.7	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>

3,5-DCQA: 3,5-dicafeoylquinic acid, GC: Gas chromatography

values of catechin structure [33]. According to all the data obtained from HPLC, HPTLC, IR, and mass fragmentation, Fig. 26 demonstrates the predicted chemical structure of the compound catechin.

**Also named 3-O-(glucoside)-5,6,3',4'-tetrahydroxy-flavonol**

The petroleum ether fraction trapped the compounds with low polarity as mentioned in the Table 6.

This is the first phytochemical investigation done in Iraq concerning *C. olitorius* and comparison between flavonoids and phenolic acids contents of leaves and seeds of this plant. Further steps are needed to be done to isolate the different chemical constituents detected.

**CONCLUSION**

1. The results of the current study showed the presence of flavonoids such as luteolin, astragaline, isoquercetin, quercetin, and catechin and the presence of phenolic acid 3,5-DCQA in the leaves and seeds of *C. olitorius* L. cultivated in Iraq.
2. The results of the current study show the isolation of flavonoids (astragaline and isoquercetin) and phenolic acid 3,5-DCQA from the ethyl acetate fraction of the leaves by TLC and show the isolation of flavonoid catechin by HPLC from the hydrolyzed n-butanol fraction of the seeds.

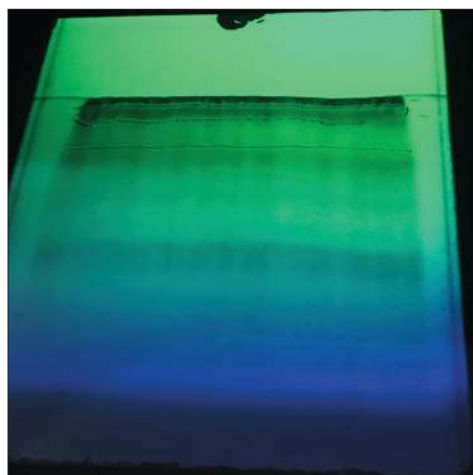


Fig. 12: Preparative thin-layer chromatography chromatogram of flavonoids and phenolic acid isolation, observed at 254 nm wavelength

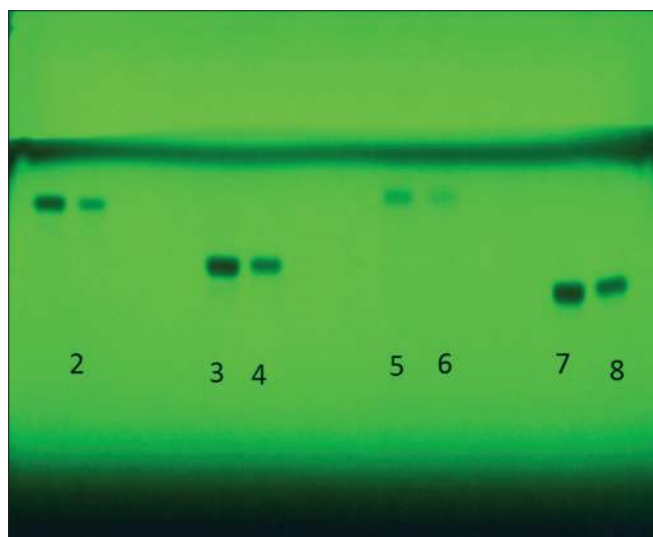


Fig. 13: HPTLC plate for isolated compounds (2, 4, 6, and 8) and related standards (1: DCQA, 3: Astragalin, 5: Isoquercetin, and 7: Catechin), respectively, and observed at 254 nm wavelength

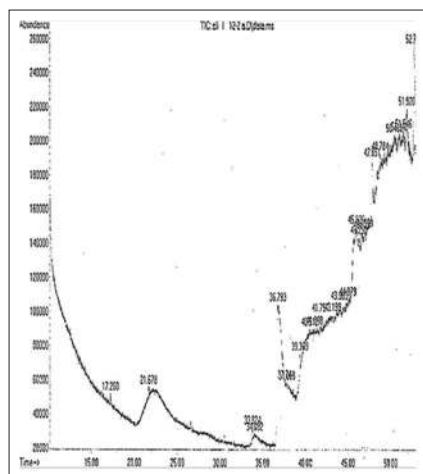


Fig. 14: Mass spectrum for petroleum ether fraction of the leaves

3. The study showed the presence of several compounds such as 17-octadecynoic acid, 9-octadecanoic acid (oleic acid), hexadecenoic

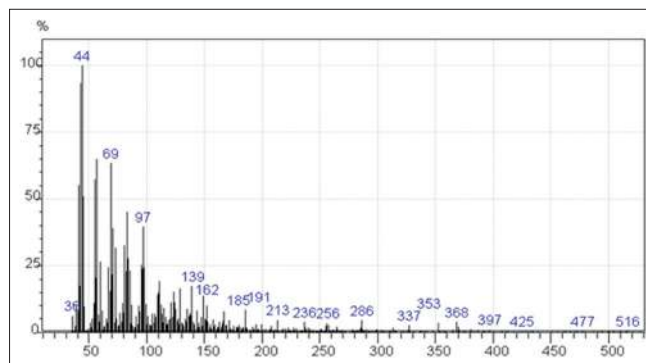


Fig. 15: Mass fragmentation spectra of the isolated dicaffeoylquinic acid

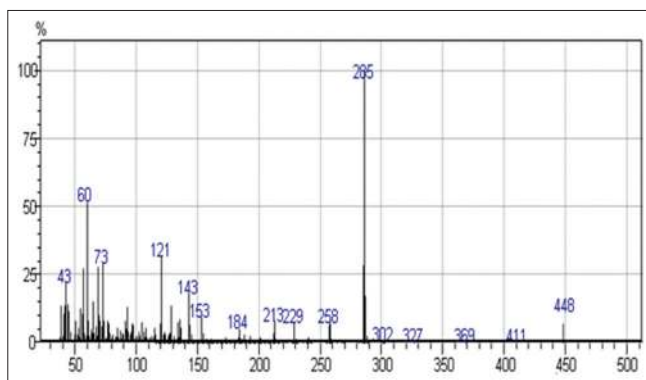


Fig. 16: Mass fragmentation spectra of the isolated astragalgin

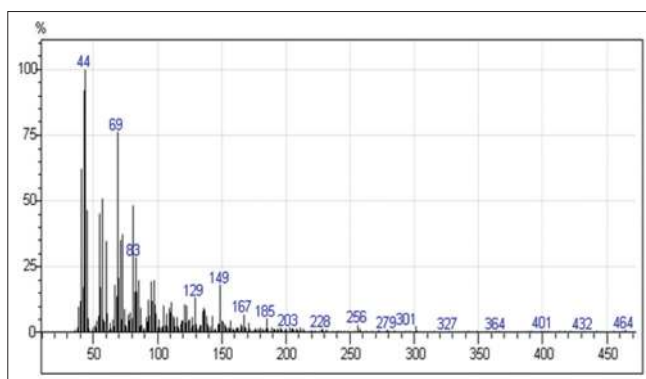


Fig. 17: Mass fragmentation spectra of the isolated isoquercetin

acid (palmitic acid), 9,12-octadecanoic acid (linoleic acid), octadecadien-1-ol, B-sitosterol, and  $\alpha$ -tocopherol (VIT E) in the petroleum ether fraction of the leaves.

4. The study shows that no major differences were found between the flavonoids and phenolic acids contents of the leaves and seeds of *C. olitorius*.

**ACKNOWLEDGMENT**

We are deeply grateful to the College of Pharmacy, University of Baghdad, for giving us the opportunity and facilities to achieve this work.

**AUTHORS' CONTRIBUTIONS**

Enas J Khadim has provided the design, intellectual content, innovation, and protocol for conducting the experiment in the laboratory along with

Table 7: The explanation of characteristic FT-IR absorption bands (in cm<sup>-1</sup>) of the isolated compounds

Functional group	Group frequency wave number (in cm <sup>-1</sup> )				Assignment
	Measured for isolated compound 3,5-DCQA	Measured for isolated compound astragalin	Measured for isolated compound Isoquercetin	Measured for isolated compound catechin	
O-H	Broadband 3500–3140	3626–3444	3562–3504	3649–3564, the additional band 3500–3200	O-H stretching of phenol. Carboxylic acid in most cases appears as a dimer
C=C-H	3064	3101, 370	3147, 3076	3057	C-H stretching of aromatic alkene
C-H	2966, 2910, 2852	2994, 2904	2947, 2848	2997, 2852	Asymmetric and symmetric stretching of CH <sub>2</sub> and CH <sub>3</sub>
C=O	1712 for ester, and 1683 for carboxylic acid	1666	1662	1637	C=O stretching (conjugation and H-bonding)
O-H	1365, 1350	1396, 1359	1357	1398	O-H bending of phenol
C-O-C	1269, 1257	1286, 1249	1294, 1230, 1200	1280, 1217	C-O-C stretching of ether
C=C	1624, 1559	1606	1599	1606	C=C stretching of aromatic alkene
C-H	1442	1437	1452, 1440	1413	C-H bending of CH <sub>2</sub> and CH <sub>3</sub>
C-H and C=C	922, 854, 812, 781, 729, 684	902, 854, 833, 802, 731, 705, 688	906, 862, 810, 792, 763, 694	871, 837, 815, 769, 698	C-H and C=C bending of aromatic (out- and in-plane)
C-H	1087, 1033	1130, 1099, 1076	1166, 1134, 1118	1217, 1145	C-H bending of aromatic (in the plane)

3,5-DCQA: 3,5-dicafeoylquinic acid

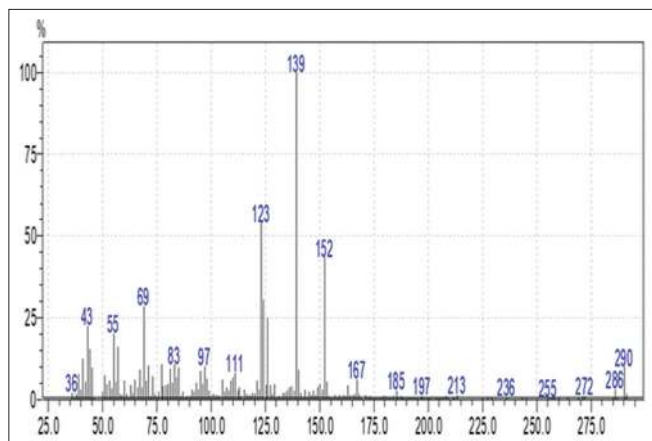


Fig. 18: Mass fragmentation spectra of the isolated catechin

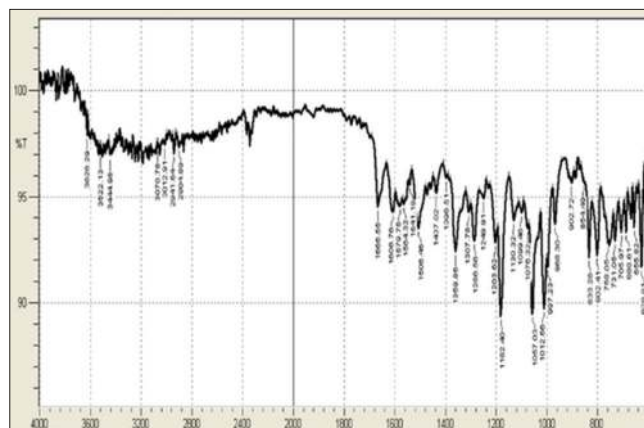


Fig. 20: Infrared spectrum of isolated astragalin

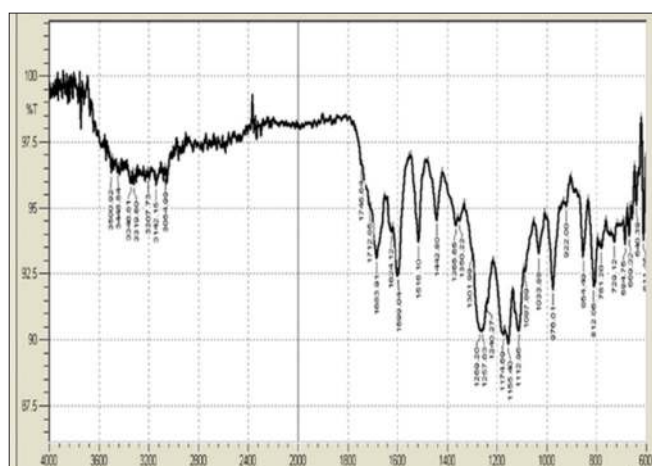


Fig. 19: Infrared spectrum of isolated 3,5-dicafeoylquinic acid

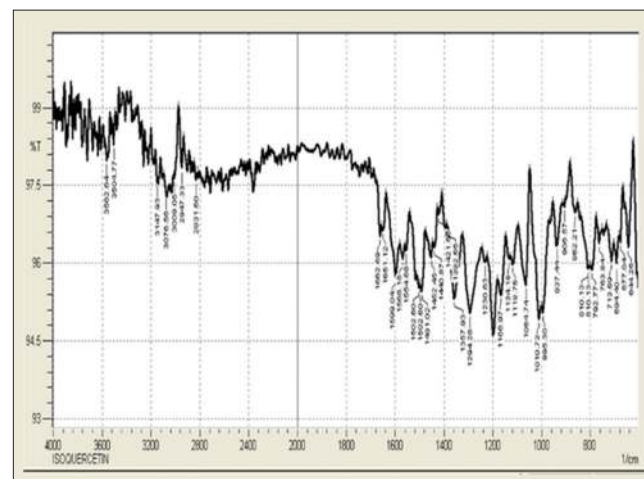


Fig. 21: Infrared spectrum of isolated compound isoquercetin



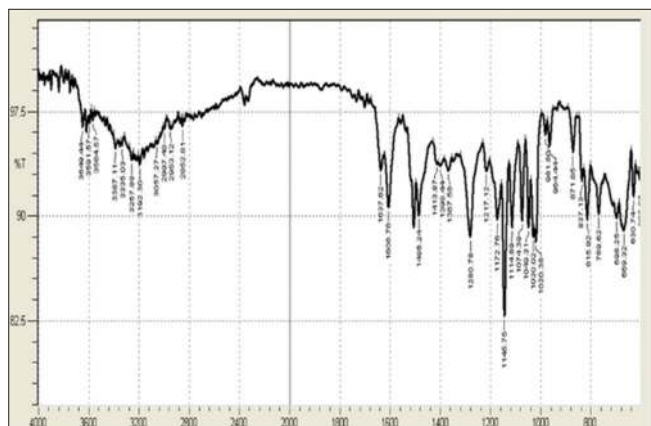


Fig. 22: Infrared spectrum of isolated catechin

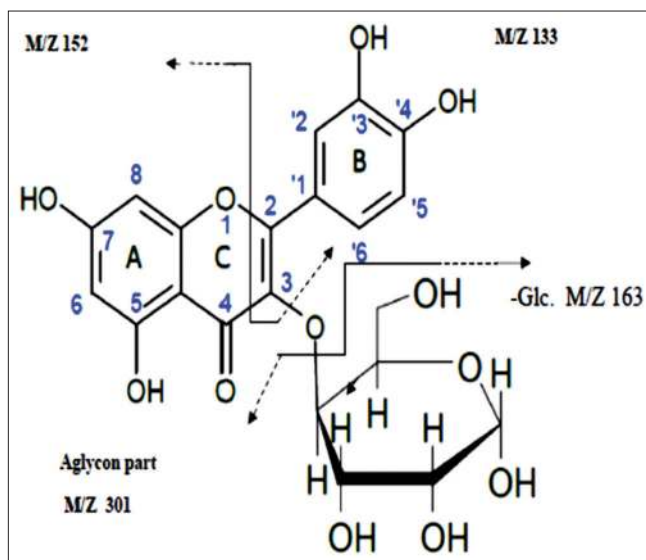


Fig. 25: Chemical structure and mass fragmentation of isolated compound (isoquercetin)

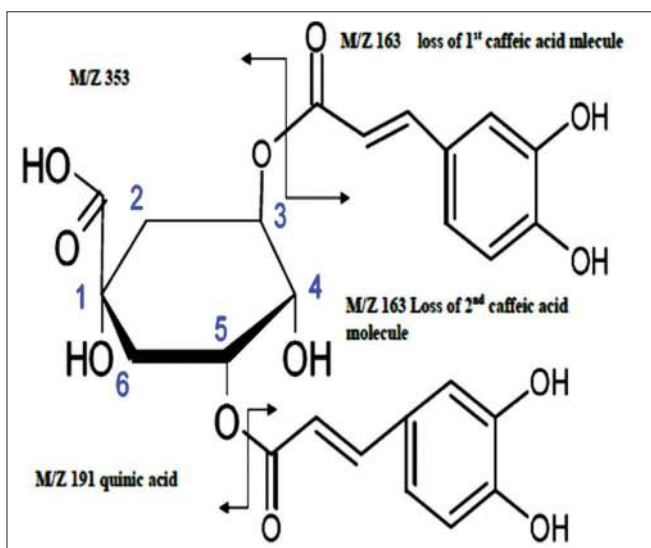


Fig. 23: Chemical structure and mass fragmentation of isolated compound (3,5-dicaffeoylquinic acid)

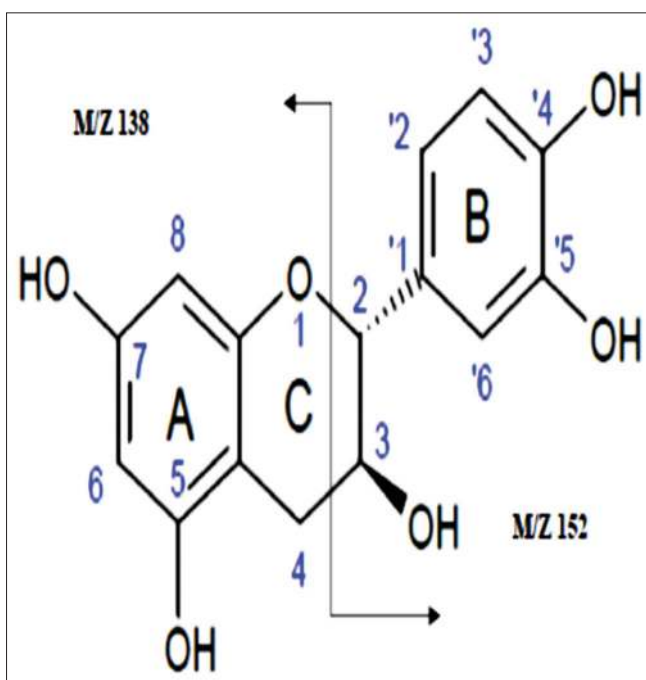


Fig. 26: Chemical structure and mass fragmentation of isolated compound (catechin). Also named 5,6,4',5'- tetrahydroxy flavan-3-ol

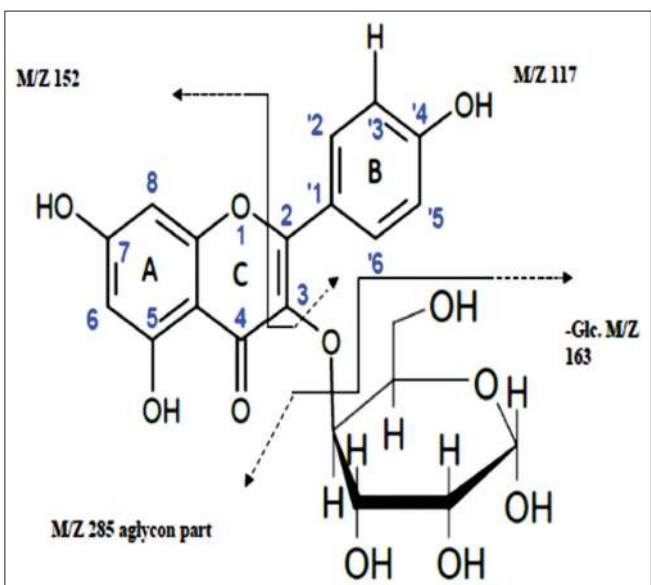


Fig. 24: Chemical structure and mass fragmentation of isolated compound (astragalin). Also named 3-O-(glucoside)-5,6,4'- trihydroxy-flavonol or kaempferol-3-O-glucoside

mentorship. Hayder T Hasan has majorly performed the experiment in the laboratory and analysis of obtained data.

#### CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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