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Original Article

# QUALITATIVE PHYTOCHEMICAL COMPARISON BETWEEN FLAVONOIDS AND PHENOLIC ACIDS CONTENTS OF LEAVES AND FRUITS OF *MELIA AZEDARACH* (FAMILY: MELIACEAE) CULTIVATED IN IRAQ BY HPLC AND HPTLC

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**ABSTRACT**

**Objective:** The aim of our study was to compare between flavonoids and phenolic acids contents of leaves and fruits of *Melia azedarach* since no phytochemical investigation had done previously in Iraq.

**Methods:** The leaves and fruits of *Melia azedarach* were extracted by soxhlet using 80% ethanol then the dried extract was suspended in water and fractionated using petroleum ether, chloroform, ethyl acetate, and n-butanol. The n-butanol fraction was hydrolyzed by acid and partitioned with ethyl acetate. The different fractions containing flavonoids and phenolic acids were analyzed by HPLC and HPTLC.

**Results:** The HPLC results revealed the presence catechin-7-O-glycoside in fruit only, while kaempferol-7-O-glycoside is found in the leaves only. Catechin and its glycosides are more abundant in the fruits than in the leaves. The HPTLC results revealed that kaempferol and quercetin are present in all fractions of leaves and fruits as aglycones and as glycosides. Free chlorogenic was found in both leaves and fruits**.**

**Conclusion:** No major differences were found between the flavonoids and phenolic acids contents of the leaves and fruits of *Melia azedarach.*

**Keywords:** *Melia azedarach L,* Flavoniods, HPLC, HPTLC


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## INTRODUCTION

*Melia azedarach* also known as Chinaberry, umbrella tree, white cedar and Persian lilac is an important medicinal plant which belongs to Meliaceae family [1]. It is a deciduous small to medium size, shade tree with a rounded crown can reach a height of 5-15 meter at maturity having a width of 5-7 meters [2]. The plant has an average lifespan about 20 y [3]. It is excessively distributed in tropical and subtropical countries, native to South-East Asia and Australia [4]. It contains many phytochemical compounds like alkaloids, tannins, saponins, phenols, glycosides, steroids, terpenoids and flavonoids [5, 6].

Leaves and fruits of *M. azedarach* have anti-fee dent activity and used in pest control due to biologically active triterpenoids such as azadirachtin with an anti-alimentary effect [7, 8]. The plant show antipyretic activity [9], antiviral activity due to meliacarpin [10], antimicrobial andantioxidant [11], antifungal [12, 13], anti-fertility activity [14], anti-nephrolithiasis [15], antiulcer [16], antihyperglycemic [17], anthelmintic activity [18], antiprotozoal [19], anti-complementary [20], wound healing property [21] and cytotoxic [22].

One of the important constituents are flavonoids which are polyphenolic compounds possess a common phenyl benzopyrone structure based on a $C_{15}$ ($C_6$-$C_3$-$C_6$) nucleus [23], biosynthesized by phenylpropanoid pathway in response to microbial infection and cannot be biosynthesized by humans and animals, thus the source of flavonoids in animals are of plant origin rather than being biosynthesized in situ. Flavonoids in food are generally responsible for color, taste, prevention of fat oxidation, and protection of vitamins and enzymes [24]. The effectiveness or actions of flavonoids are structure dependent [25]. They have been shown to exert antimicrobial, antiviral, anti-atherosclerosis, cardioprotective, anti-ulcerogenic, cytotoxic, antineoplastic, mutagenic, anti-diabetic, anti-inflammatory, antioxidant, anti-aging, anti-hepatotoxic, anti-hypertensive, hypolipidemic, antiplatelet [26, 27], neuroprotective and anticonvulsant activities [28].

Several flavonoids had been detected and isolated from different extracts of *Melia azedarach.* Rutin, quercetin were identified in leaves extract by high-performance thin layer chromatography (HPTLC) method [29], gallic acid, caffeic acid and naringenin were detected in leaves extract by high pressure liquid chromatography (HPLC) [30] while rutin, kaempferol-3-O-robinobioside, kaempferol-3-O-rutinoside, and isoquercitrin were isolated from methanolic extract of *M. azedarach* leave by column chromatography [31]. Chlorogenic-conjugates, p-coumaric-conjugates, gentisic-conjugate, kaempferol-conjugates, quercetin-conjugates, chlorogenic acid, kaempferol-3-O-β-rutinoside,quercetin-3-O-β-D-glucoside, and rutin were detected in 50% aquouse ethanol and water extracts from *M. azedarach* leaf by HPLC method [32] acylated quercetin tri glycoside, quercetin-3-*O*-[rhamnosyl 1→6(4″-lactoyl glucoside)]-4′-*O*-glucoside, kaempferol-3-*O*-rutinoside, quercetin-3-*O*-rutinoside, and the aglycones quercetin and kaempferol were isolated from leaves [33].

The main objective of this study was to investigate the flavonoids and phenolic acids contents of *Melia azedarach* cultivated in Iraq since there were no previous studies concerning the Iraqi species and also to compare the flavonoids and phenolic acids contents of leaves and fruits.

## MATERIALS AND METHODS

### Collection of plant materials

*Melia azedarach* leaves and fruits were obtained from Al-mesayab in Babel. The plant was identified and authenticated by Professor Dr. Ali Al-Musawy/Department of Biology/College of Sciences/University of Baghdad. A voucher sample was kept at the Department of Pharmacognosy/College of Pharmacy/University of Baghdad.

### Equipment and chemicals

The instruments used were rotary evaporator (BŬCHI Rotavapor R-205, Swiss), sonicator (Baranson sonifier, USA), HPLC (Shimadzu

10AV-LC, Japan) and HPTLC (Eike Reich/CAMAG–Laborator, Switzerland).

All chemicals and solvents used were of analytical grade and obtained from Riedel-de Haen, Germany except trifluoroacetic acid and methanol which are HPLC grade purchased from Sigma-Aldrach, Germany. The standard rutin, kaempferol, quercetin, caffeic acid and chlorogenic acid were purchased from Chengdu Biopurify phytochemicals, China (purity>97). Apigenin, leuteolin, catechine, kamepferol-3-O-glycoside, quercetin-7-O-glycoside, catechin-7-O-glycoside, apegenin-7-O-glycoside, kamepferol-7-O-glycoside and catechin-5-O-glycoside standards data were obtained from the database of the HPLC instrument. Thin layer chromatography (TLC) aluminum plates pre-coated with silica gel 60 F 254 (100x 100 mm, 0.2 mm thick) used were obtained from E. Merck Ltd, India.

**Extraction**

Leaves and fruits of *Melia azedarach* were thoroughly washed, dried on the shade for 15 d. The dried plant was powdered in a mechanical grinder. 250 g of both leaves and fruits powders of *Melia azedarach,* were individually packed in the thimble of soxhlet apparatus and extracted with 1500 ml of aqueous ethanol (ethanol-water 80:20, v/v) for 12 h. Each extract was filtered and concentrated under vacuum using a rotary evaporator to get a dry residue. 10 g of each residue was suspended in water and subsequently fractionated by partitioning with petroleum ether, chloroform, ethyl acetate and n-butanol successively using 100 ml x 3 from each solvent. The first three fractions were dried over anhydrous sodium sulfate, filtered and evaporated to dryness under vacuum. 1 g of n-butanol fraction from each of leaves and fruits extracts were hydrolyzed separately by refluxing with 50 ml of 5% hydrochloric acid for 6 h, cooled and partitioned with 50 ml x 3 ethyl acetate. The organic layers were combined together, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness.

**Preparations of standards and samples for analysis**

Standard solutions for HPLC of rutin, quercetin, kamepferol, 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 µm disposable filter, and stored at 4˚C for further analysis. 20 µl of the sample was injected into HPLC system for analysis. Standards used for HPTLC analysis (rutin, quercetin, kamepferol, chlorogenic acid and caffeic acid) 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**

Preliminary investigations for the chemical constituents were done using 5% ethanolic potassium hydroxide (KOH) for detection of flavonoids, Mayers and Dragendorffs reagents for detection of alkaloids, and 1% ferric chloride ($FeCl_3$) for detection of phenolic acids.

**HPLC analysis**

Ethyl acetate, n-butanol fractions before and after hydrolysis of both parts were analyzed for their flavonoids contents utilizing HPLC separation technique (Shimadzu 10AV-LC), using a mobile phase composed of 0.05% trifluoroacetic acid in deionized water (solvent A) and solvent B was 0.05% trifluoroacetic acid in methanol pH 2.5, gradient program from 0% B to 100% B for 15 min with flow rate 1.2 ml/min, wavelength 280 nm, and a column nucludar C-18-DB, 3 µm particle size (50x20 mmI. D).

**HPTLC analysis**

Ethyl acetate and n-butanol fractions before and after hydrolysis of both parts were analyzed also for their flavonoids and phenolic acids contents utilizing HPTLC (Eike Reich/CAMAG–Laboratory, Switzerland), using silica gel GF254 plate and a mobile phase composed of organic layer of a mixture of ethyl acetate: acetic acid: formic acid: H2O (84:4:4:10) and examined at 280 nm wavelength.

**RESULTS**

Preliminary investigations revealed the presence of flavonoids and phenolic compounds in ethyl acetate, n-butanol fractions before and after hydrolysis of both parts of plants and absence of alkaloids. The HPLC result of analyzed fractions shows the presence of the following flavonoids which are listed in table 1.

**Table 1: Flavonoids content of analyzed ethyl acetate and n-butanol fractions**

| Sample | Ethyl acetate fraction | N-butanol fraction before hydrolysis | N-butanol fraction after hydrolysis |
|---|---|---|---|
| Leaves | quercetin | quercetin | quercetin |
| | rutin | rutin | apigenin |
| | catechin | apigenin | kaempferol |
| | kaempferol | leuteolin | |
| | kaempferol-3-O-glycoside | kaempferol | |
| | quercetin-7-O-glycoside | kaempferol-3-O-glycoside | |
| | apigenin-7-O-glycoside | quercetin-7-O-glycoside | |
| | kaempferol-7-O-glycoside | apigenin-7-O-glycoside | |
| | catechin-5-O-glycoside | kaempferol-7-O-glycoside | |
| | | catechin-5-O-glycoside | |
| Fruit | quercetin | quercetin | quercetin |
| | rutin | rutin | apigenin |
| | leuteolin | apigenin | catechin |
| | catechin | leuteolin | kaempferol |
| | kaempferol | catechin | |
| | kaempferol-3-O-glycoside | kaempferol | |
| | quercetin-7-O-glycoside | kamepferol-3-O-glycoside | |
| | apigenin-7-O-glycoside | quercetin-7-O-glycoside | |
| | | catechin-7-O-glycoside | |
| | | apigenin-7-O-glycoside | |
| | | catechin-5-O-glycoside | |

The HPLC chromatograms of standards and their retention times are shown in fig. 1 and table 2 respectively

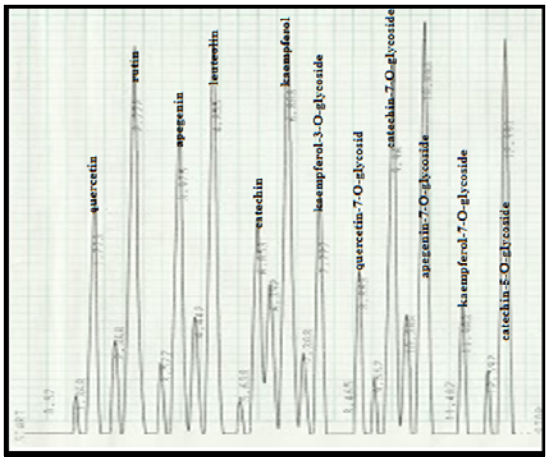



**Fig. 1: HPLC chromatogram showing the retention time of standard flavonoids**

**Table 2: Retention time in minutes of standard flavonoids**

| Standard materials | Retention time in minutes |
|---|---|
| Quercetin | 1.77 |
| Rutin | 2.77 |
| Apigenin | 3.97 |
| Leuteolin | 4.85 |
| Catechin | 6.05 |
| Kaempferol | 6.8 |
| Kaempferol-3-O-glycoside | 7.72 |
| Quercetin-7-O-glycoside | 8.86 |
| Catechin-7-O-glycoside | 9.96 |
| Apigenin-7-O-glycoside | 10.8 |
| Kaempferol-7-O-glycoside | 11.9 |
| Catechin-5-O-glycoside | 12.99 |

The HPLC chromatograms of ethyl acetate fractions of the leaves and fruits are shown in fig. 2 and 3 respectively. The retention times of these fractions are shown in table 3.

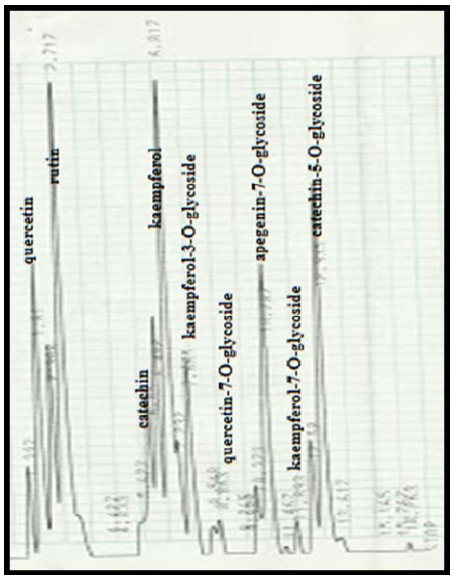



**Fig. 2: HPLC chromatogram of ethyl acetate fraction of the leaves**

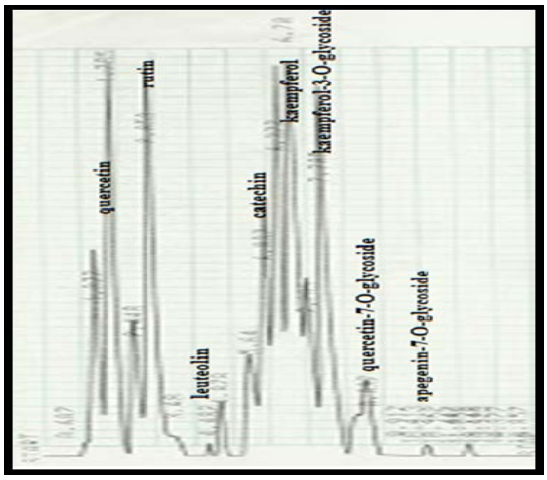



**Fig. 3: HPLC chromatogram of ethyl acetate fraction of the fruits**

The HPLC chromatograms of n-butanol fractions before hydrolysis of both leaves and fruits are shown in fig. 4 and 5 respectively and their retention time are shown in table 4.

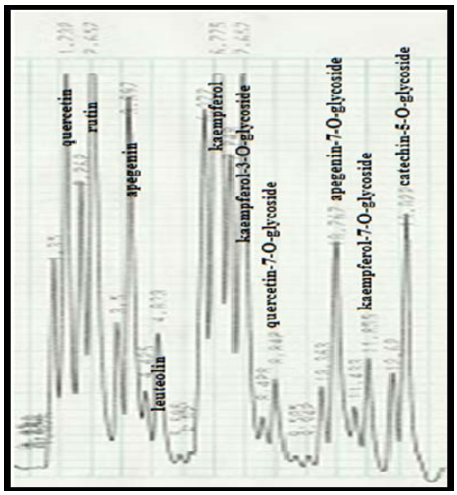



**Fig. 4: HPLC chromatogram of n-butanol fraction of leaves before hydrolysis**

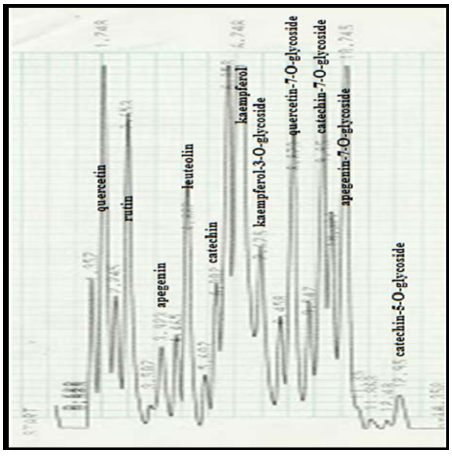



**Fig. 5: HPLC chromatogram of n-butanol fraction of fruits before hydrolysis**

**Table 3: Retention time in minutes of flavonoids of ethyl acetate fraction of leaves and fruits**

| Flavonoid | Retention time of standards | Retention time for flavonoids in ethyl acetate fraction of leaves | Retention time for flavonoides in ethyl acetate fraction of fruits |
|---|---|---|---|
| Quercetin | 1.77 | 1.81 | 1.78 |
| Rutin | 2.77 | 2.717 | 2.85 |
| Apigenin | 3.97 | ------ | ------ |
| Ieuteolin | 4.85 | ------ | 4.878 |
| Catechines | 6.05 | 6.06 | 6.03 |
| Kaempferol | 6.80 | 6.817 | 6.78 |
| Kaempferol-3-O-glycoside | 7.72 | 7.665 | 7.705 |
| Quercetin-7-O-glycoside | 8.86 | 8.863 | 8.837 |
| Catechin-7-O-glycoside | 9.96 | ------ | ----- |
| Apigenin-7-O-glycoside | 10.80 | 10.787 | 10.962 |
| Kaempferol-7-O-glycoside | 11.90 | 11.893 | ------ |
| Catechin-5-O-glycoside | 12.99 | 12.935 | ------ |

**Table 4: Retention time in minutes of flavonoids of n-butanol fractions before hydrolysis of leaves and fruits**

| Flavonoids | Rention time of standards | Retention time for flavonoids in normal butanol fraction before hydrolysis of leaves | Retention time for flavonoids in normal butanol fraction before hydrolysis of fruits |
|---|---|---|---|
| Quercetin | 1.77 | 1.73 | 1.748 |
| Rutin | 2.77 | 2.657 | 2.653 |
| Apigenin | 3.97 | 3.897 | 3.923 |
| Leuteolin | 4.85 | 4.833 | 4.882 |
| Catechine | 6.05 | ----- | 6.002 |
| Kaempferol | 6.80 | 6.775 | 6.748 |
| Kaempferol-3-O-glycoside | 7.72 | 7.657 | 7.675 |
| Quercetin-7-O-glycoside | 8.86 | 8.842 | 8.873 |
| Catechin-7-O-glycoside | 9.96 | ------ | 9.95 |
| Apigenin-7-O-glycoside | 10.80 | 10.767 | 10.745 |
| Kaempferol-7-O-glycoside | 11.90 | 11.855 | ------ |
| Catechin-5-O-glycoside | 12.99 | 13.022 | 12.95 |

The HPLC chromatograms for flavonoids of n-butanol fractions of leaves and fruits after hydrolysis are shown in fig. 6 and 7 respectively and their retention times in table 5.

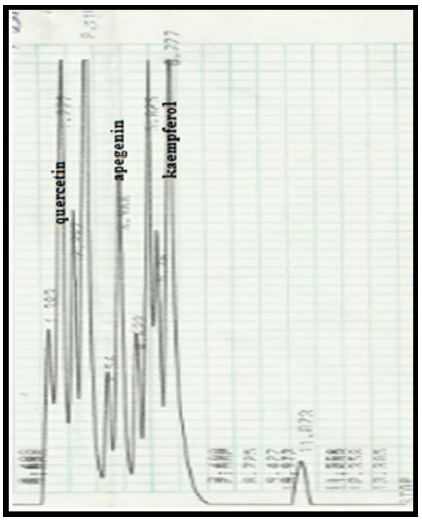



**Fig. 6: HPLC chromatogram of n-butanol fraction of leave after hydrolysis**

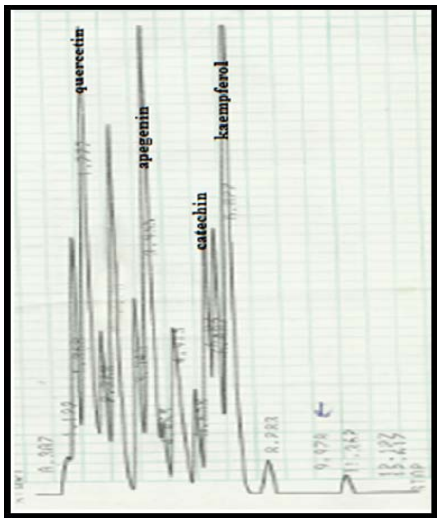



**Fig. 7: HPLC chromatogram of n-butanol fraction of fruit after hydrolysis**

**Table 5: Retention times in minutes for flavonoids of n-butanol fraction of leaves and fruits after hydrolysis**

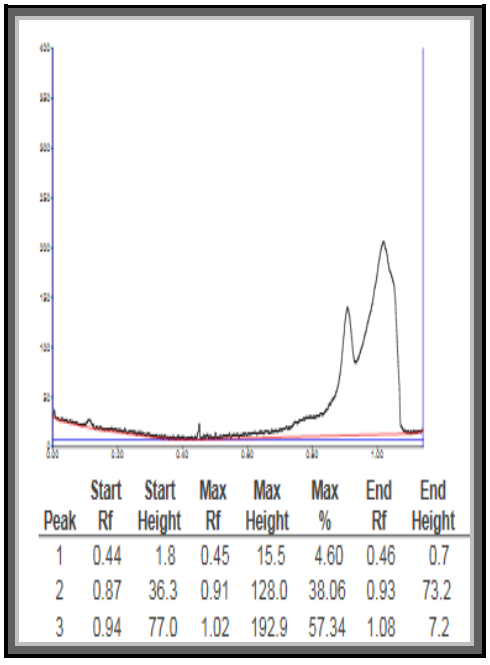
| Flavonoids | Rention time of standards | Retention time for flavonoids in normal butanol fraction after hydrolysis of leaves | Retention time for flavonoids in normal butanol fraction after hydrolysis of fruits |
|---|---|---|---|
| Quercetin | 1.77 | 1.777 | 1.777 |
| Rutin | 2.77 | ----- | ----- |
| Apigenin | 3.97 | 3.986 | 3.955 |
| Leuteolin | 4.85 | ----- | ----- |
| Catechines | 6.05 | ----- | 6.07 |
| Kaempferol | 6.80 | 6.777 | 6.827 |
| Kaempferol-3-O-glycoside | 7.72 | ----- | ----- |
| Quercetin-7-O-glycoside | 8.86 | ----- | ----- |
| Catechin-7-O-glycoside | 9.96 | ----- | ----- |
| Apigenin-7-O-glycoside | 10.80 | ----- | ----- |
| Kamepferol-7-O-glycoside | 11.90 | ----- | ----- |
| Catechin-5-O-glycoside | 12.99 | ----- | ----- |

HPTLC analysis results of both leaves and fruits different extracts contents of flavonoids and phenolic acids as compared with standards are shown in table 6.

**Table 6: The flavonoids and phenolic acids contents of different extracts of leaves and fruits**

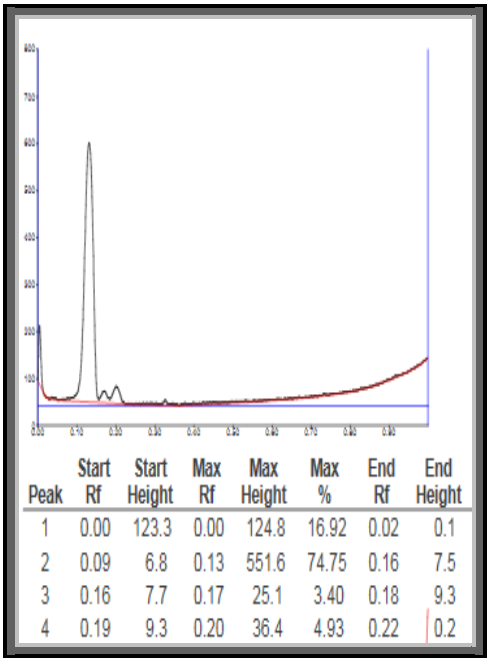
| Sample | Ethyl acetate fraction | N-butanol fraction before hydrolysis | N-butanol fraction after hydrolysis |
|---|---|---|---|
| Leaves | rutin<br>chlorogenic acid<br>kamepferol<br>quercetin | Rutin<br>chlorogenic acid<br>kamepferol | Quercetin<br>caffeic acid<br>kamepferol |
| Fruits | rutin<br>chlorogenic acid<br>kamepferol<br>quercetin | Rutin<br>chlorogenic acid<br>kamepferol | Quercetin<br>caffeic acid<br>kamepferol |

HPTLC results of standard flavonoids, phenolic acids and the analyzed fractions are shown in fig. 8 and fig. 9.



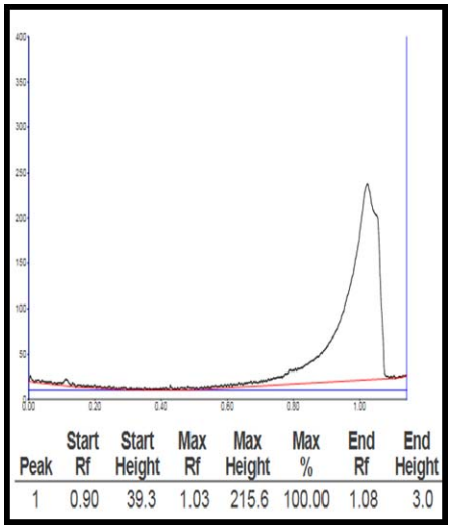
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height |
|---|---|---|---|---|---|---|---|
| 1 | 0.44 | 1.8 | 0.45 | 15.5 | 4.60 | 0.46 | 0.7 |
| 2 | 0.87 | 36.3 | 0.91 | 128.0 | 38.06 | 0.93 | 73.2 |
| 3 | 0.94 | 77.0 | 1.02 | 192.9 | 57.34 | 1.08 | 7.2 |

**Track 1: Quercetin standard**



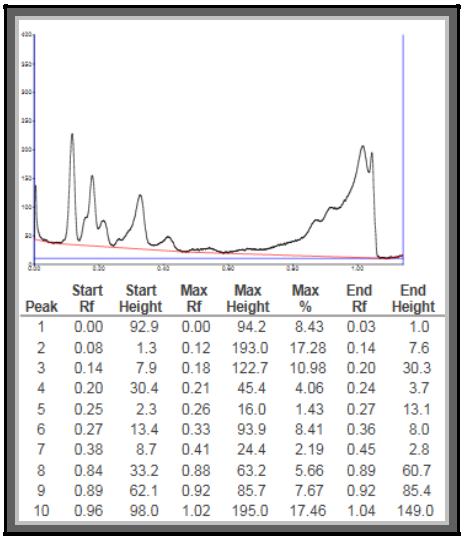
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height |
|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 123.3 | 0.00 | 124.8 | 16.92 | 0.02 | 0.1 |
| 2 | 0.09 | 6.8 | 0.13 | 551.6 | 74.75 | 0.16 | 7.5 |
| 3 | 0.16 | 7.7 | 0.17 | 25.1 | 3.40 | 0.18 | 9.3 |
| 4 | 0.19 | 9.3 | 0.20 | 36.4 | 4.93 | 0.22 | 0.2 |

**Track 2: Rutin standard**



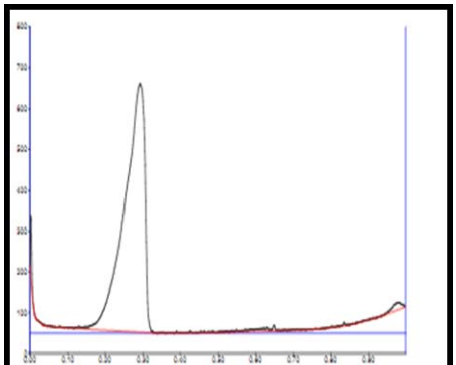
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height |
|---|---|---|---|---|---|---|---|
| 1 | 0.90 | 39.3 | 1.03 | 215.6 | 100.00 | 1.08 | 3.0 |

**Track 3: Kaempferol standard**



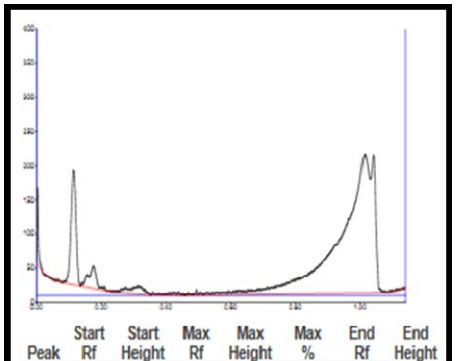
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height |
|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 92.9 | 0.00 | 94.2 | 8.43 | 0.03 | 1.0 |
| 2 | 0.08 | 1.3 | 0.12 | 193.0 | 17.28 | 0.14 | 7.6 |
| 3 | 0.14 | 7.9 | 0.18 | 122.7 | 10.98 | 0.20 | 30.3 |
| 4 | 0.20 | 30.4 | 0.21 | 45.4 | 4.06 | 0.24 | 3.7 |
| 5 | 0.25 | 2.3 | 0.26 | 16.0 | 1.43 | 0.27 | 13.1 |
| 6 | 0.27 | 13.4 | 0.33 | 93.9 | 8.41 | 0.36 | 8.0 |
| 7 | 0.38 | 8.7 | 0.41 | 24.4 | 2.19 | 0.45 | 2.8 |
| 8 | 0.84 | 33.2 | 0.88 | 63.2 | 5.66 | 0.89 | 60.7 |
| 9 | 0.89 | 62.1 | 0.92 | 85.7 | 7.67 | 0.92 | 85.4 |
| 10 | 0.96 | 98.0 | 1.02 | 195.0 | 17.46 | 1.04 | 149.0 |

**Track 6: Ethyl acetate fraction of leaves**



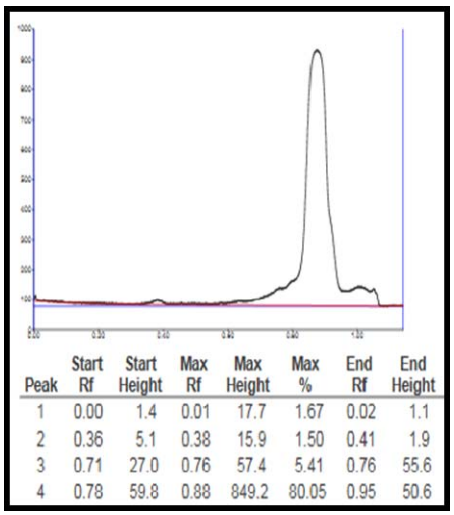
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height |
|---|---|---|---|---|---|---|---|
| 1 | 0.16 | 7.6 | 0.29 | 608.7 | 95.02 | 0.33 | 0.6 |
| 2 | 0.64 | 2.4 | 0.65 | 14.4 | 2.25 | 0.65 | 1.3 |
| 3 | 0.94 | 1.5 | 0.98 | 17.5 | 2.73 | 1.00 | 4.0 |

**Track 4: Chlorogenic acid standard**



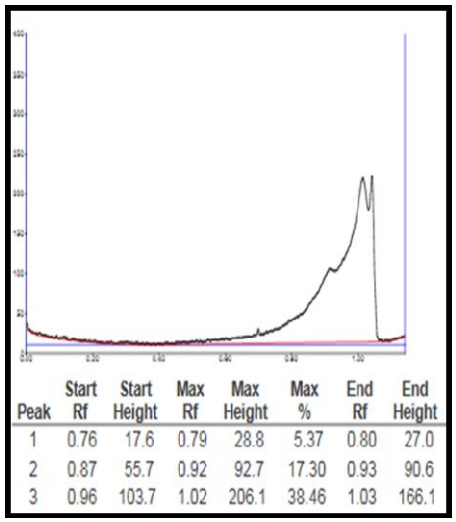
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height |
|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 110.9 | 0.00 | 110.9 | 14.94 | 0.02 | 0.2 |
| 2 | 0.09 | 3.4 | 0.12 | 167.4 | 22.55 | 0.14 | 0.3 |
| 3 | 0.14 | 3.9 | 0.16 | 17.9 | 2.41 | 0.17 | 15.7 |
| 4 | 0.17 | 15.9 | 0.18 | 32.7 | 4.40 | 0.20 | 2.9 |
| 5 | 0.32 | 10.8 | 0.32 | 10.8 | 1.45 | 0.34 | 3.5 |
| 6 | 0.90 | 56.0 | 1.02 | 202.1 | 27.22 | 1.03 | 165.7 |

**Track 7: N-butanol fraction of leaves before hydrolysis**



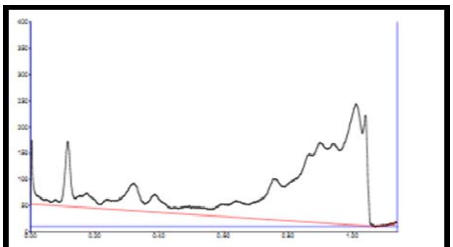
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height |
|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 1.4 | 0.01 | 17.7 | 1.67 | 0.02 | 1.1 |
| 2 | 0.36 | 5.1 | 0.38 | 15.9 | 1.50 | 0.41 | 1.9 |
| 3 | 0.71 | 27.0 | 0.76 | 57.4 | 5.41 | 0.76 | 55.6 |
| 4 | 0.78 | 59.8 | 0.88 | 849.2 | 80.05 | 0.95 | 50.6 |

**Track 5: Caffeic acid standard**



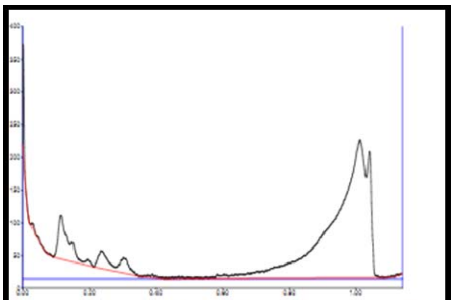
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height |
|---|---|---|---|---|---|---|---|
| 1 | 0.76 | 17.6 | 0.79 | 28.8 | 5.37 | 0.80 | 27.0 |
| 2 | 0.87 | 55.7 | 0.92 | 92.7 | 17.30 | 0.93 | 90.6 |
| 3 | 0.96 | 103.7 | 1.02 | 206.1 | 38.46 | 1.03 | 166.1 |

**Track 8: N-butanol fraction of leave after hydrolysis**



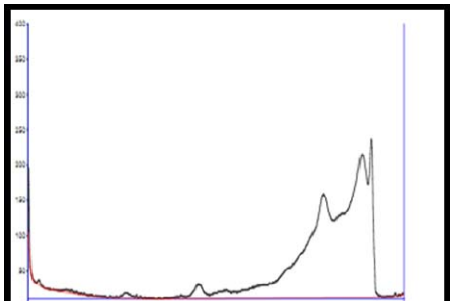
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height |
|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 121.9 | 0.00 | 121.9 | 9.01 | 0.03 | 12.6 |
| 2 | 0.09 | 6.4 | 0.12 | 123.5 | 9.12 | 0.14 | 15.5 |
| 3 | 0.16 | 20.9 | 0.18 | 27.3 | 2.02 | 0.20 | 17.3 |
| 4 | 0.22 | 7.4 | 0.24 | 18.0 | 1.33 | 0.24 | 17.0 |
| 5 | 0.28 | 20.2 | 0.32 | 51.9 | 3.83 | 0.35 | 19.2 |
| 6 | 0.37 | 17.2 | 0.39 | 33.3 | 2.46 | 0.42 | 15.2 |
| 7 | 0.57 | 13.6 | 0.60 | 23.9 | 1.77 | 0.60 | 22.0 |
| 8 | 0.72 | 36.2 | 0.75 | 77.8 | 5.75 | 0.76 | 76.7 |
| 9 | 0.83 | 80.9 | 0.87 | 129.1 | 9.54 | 0.88 | 127.5 |
| 10 | 0.88 | 127.6 | 0.90 | 152.4 | 11.26 | 0.92 | 141.7 |
| 11 | 0.93 | 142.2 | 0.94 | 152.1 | 11.24 | 0.96 | 140.2 |
| 12 | 0.96 | 140.4 | 1.01 | 231.3 | 17.09 | 1.03 | 175.7 |

**Track 9: Ethyl acetate fraction of fruits**



| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height |
|---|---|---|---|---|---|---|---|
| 1 | 0.09 | 0.2 | 0.12 | 66.3 | 11.32 | 0.14 | 25.1 |
| 2 | 0.14 | 25.5 | 0.15 | 29.5 | 5.04 | 0.17 | 6.0 |
| 3 | 0.22 | 0.7 | 0.24 | 27.3 | 4.66 | 0.26 | 11.8 |
| 4 | 0.28 | 7.9 | 0.30 | 23.7 | 4.04 | 0.32 | 8.0 |
| 5 | 0.82 | 25.0 | 0.85 | 35.2 | 6.00 | 0.85 | 34.1 |
| 6 | 0.90 | 67.8 | 1.01 | 210.5 | 35.94 | 1.03 | 154.3 |

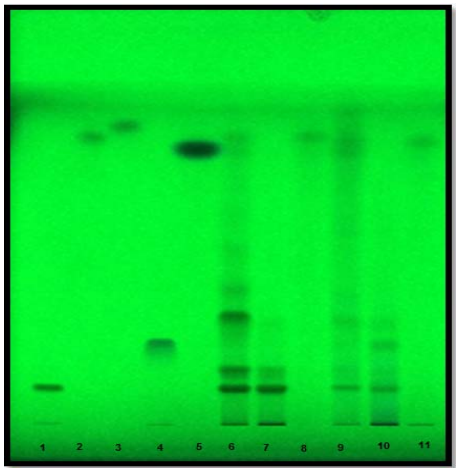
**Track 10: N-butanol fraction of fruit before hydrolysis**



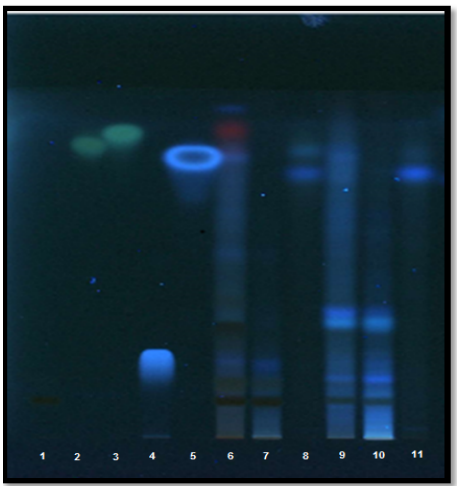
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height |
|---|---|---|---|---|---|---|---|
| 1 | 0.49 | 3.4 | 0.52 | 21.0 | 2.95 | 0.54 | 7.8 |
| 2 | 0.81 | 47.3 | 0.90 | 146.1 | 20.52 | 0.92 | 109.7 |
| 3 | 0.93 | 108.6 | 0.96 | 119.0 | 16.71 | 0.96 | 118.5 |
| 4 | 0.96 | 118.8 | 1.01 | 202.2 | 28.39 | 1.03 | 159.4 |

**Track 11: N-butanol fraction of fruit after hydrolysis**

**Fig. 8: HPTLC chromatograms showing end retardation factor values of standard flavonoids, phenolic acids and analyzed fractions**




**(a) HPTLC chromatogram at 254 nm**




**(b) HPTLC chromatogram at 366 nm**

**Fig. 9: (a and b) HPTLC plates of analyzed fractions with reference standards, detection under UV light at 254 nm and 366 nm (1: rutin, 2: quercetin, 3: kamepferol, 4: chlorogenic acid, 5: caffeic acid, 6: ethyl acetate fraction of leaves, 7: n-butanol fraction of leaves before hydrolysis, 8: n-butanol fraction of leaves after hydrolysis, 9: ethyl acetate fraction of fruits, 10: n-butanol fraction of fruit before hydrolysis, 11: n-butanol fraction of fruits after hydrolysis)**

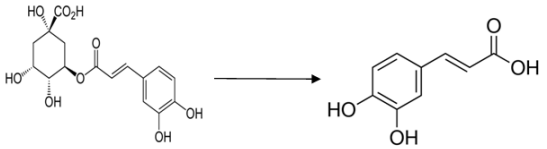
## DISCUSSION

The HPLC results revealed few differences between the flavonoids contents between the leaves and the fruits in both forms as aglycones and as glycosides. The n-butanol fraction revealed the presence catechin-7-O-glycoside in fruit only. Catechin had been detected in the fruits of *Melia azedarach* by HPLC but not the Iraqi species. [7] Kaempferol-7-O-glycoside was found in the leaves only, catechin was found as aglycone in the ethyl acetate fraction and in two forms of glycosides in an n-butanol fraction of fruits i.e. 5 and 7-O-glycoside, while in ethyl acetate fraction of leaves it was found as aglycone and in one type of glycoside which 5-O-glycoside only. From the above-mentioned results, we can conclude that catechin and its glycosides are more abundant in the fruits than in the leaves.

The HPTLC results revealed that kaempferol are present in all fractions of leaves and fruits which indicate the presence of kaempferol as aglycone and as glycosides, this explains the presence of kaempferol in the n-butanol after hydrolysis fraction. Kaempferol, Kaempferol-3-O-robinobioside, Kaempferol-3-O-rutinoside had been

isolated from leaves of *Melia azedarach* by column chromatography, [31,33] and also detected in fruits by HPLC but not the Iraqi species. [7] Quercetin detection in n. butanol after hydrolysis fraction could be due to hydrolysis of rutin (quercetin-3-rutinoside) and other quercetin glycosides. Chlorogenic acid is found in both leaves and fruits. caffeic acid, chlorogenic acid, rutin; quercetin were detected in leave by HPLC [30]

Caffeic acid detected in n. butanol after hydrolysis since chlorogenic acid is an ester of caffeic acid with quinic acid, so it has been formed after the hydrolysis of chlorogenic acid [34].




**Chlorogenic acid** **Caffeic acid**

These differences could be attributed to the differences in the biochemical reactions which are affected by the availability of enzymes, cofactors and other biochemical factors required.

This is the first phytochemical investigation done in Iraq concerning *Melia azedarach* and also the first study was done concerning the comparison between flavonoids and phenolic acids contents of leaves and fruits of this plant. Further steps are needed to be done to isolate the different chemical constituents detected.

## CONCLUSION

No major differences were found between the flavonoids and phenolic acids contents of the leaves and fruits of *Melia azedarach.* The main differences found are the presence catechin-7-O-glycoside in fruit only, while kaempferol-7-O-glycoside is found in the leaves only, and catechin and its glycosides are more abundant in the fruits than in the leaves.

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## CONFLICT OF INTERESTS

Declared none

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