Extraction and Detection of Some Flavonoids from *Tamarix aphylla* (F. Tamaricaceae) Grown in Iraq

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Abstract:

Objective: In the present study, screening for some flavonoids (aglycone and glycoside) in tamarix aphylla by using thin layer chromatography and high-performance liquid chromatography. Methods: The leaves of Tamarix.aphylla was extracted by soxhlet with Ethylacetate and then with the ethanol /water 90%. The flavonoids were detected by Analyticl thin layer chromatography using standards and by HPLC. Results:Three flavonoid aglycones were extracted with ethylacetate (Kaempferol .Quercetin , Isorhamnetin) and one flavonoid glycoside (Rutin) was detected in the ethanol /water 90% extract. Conclusion: From this study can be conclude that tamarix aphylla is a promising plant for many flavonoid compounds that have many pharmacological actions

Key words: Tamarix aphylla, flavonoids, HPLC, Tamaricaceae .

استخلاص وتشخيص الفلافونيدات من نبات الطرفة (العائلة:تامراكاسيا) النامي في العراق

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الخلاصة:

الهدف من الد راسة : البحث عن بعض الفلافونويدات في نبات الطرفة باستخدام تي ال سي التحليلي وباستخدام تقنية كروماتوغرافيا السائل عالي الاداء.الطرق: تم استخلاص اوراق نبات الطرفة بجهاز السوكليت باستخدام ايثل اسيتيت وبعد ذلك استخدام ايثانول /ماء .النتائج: تم استخلاص كامفيرول ,كوارستين,ازورمنتين من مستخلص الايثايل اسيتيت و ال روتين من مستخلص الايثانول/ماء. الخلاصة:من هذه الدراسة ممكن ان نستنتج بان نبات الطرفة نبات واعد للعديد من مركبات الفلافونويد التي لها العديد الاستخدامات الدوائية .

الكلمات المفتاحية: نبات الطرفة، الفلافينويدات، HPLC ، تامراكاسيا

Introduction

Tamaricaceae has four genera, one of them is *Tamarix* and one hundred and wenty species one of them is *aphylla*. *Tamarix aphylla* is a wild plant of important medicinal properties, that is naturally and widely distributed in Iraqi land, without any intervention by human, [1]. It is spread in the desert region and on roadside of Iraq especially in Baghdad, Anbar, karbalaa, muthana, Nasyria, Kut. It is natively found in Asia India, North Africa and Southeastern and introduced to US, Canada, Mexico and Australia for its ornamentals, windbreaks and stabilize stream banks [2].

Tamarix species are evergreen, ornamental bushes or trees, feathery leaves with pink or white blossoms**.** They are mostly long-lived and halophyte plants that endure a wide range of abiotic conditions such as high temperature, salt, and drought stresses [3]. *Tamarix* genus withstands saline soils byregulating its salt balance by excretion the excess salts through foliar glands and consuming large quantities of water, from underground sources [4]**.** It has It has anti-inflammatory [5], antioxidant [6], antifungal [7] activity cytotoxic [8] and antibacterial activity [9]**.**

The medicinal value of *Tamarix aphylla* is related in their phytochemical components which produce definite physiological actions on human body[10] Several flavonoids had been reported to found in the leaves of *T.aphylla* such as (kaempferol, quercetin, isorhamnetin, rutin, apigenin and Tamarixetin)[11] Chemically ,all flavonoids share the same basic structural feature (2-phenyl-benzo-α –pyrane) , which is characterized by C6-C3-C6 carbon-skeleton, and consisting of two aromatic C6 rings (A) and(B) and non-aromatic ;heterocyclic ring (C) [12] as shown in Figure (1).

In the current study objective was screening for some flavonoids (aglycone and glycoside) in *Tamarix*. *aphylla* by using thin layer chromatography and high-performance liquid chromatography

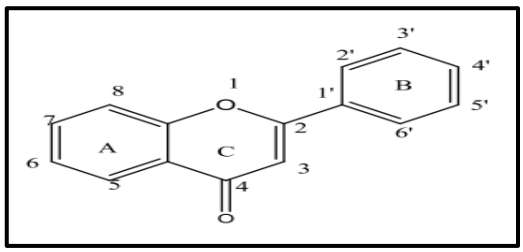


Figure (1): Basic structure of flavonoid

Materials and Methods

Plant material: Leaves of *T. aphylla* were collected from the garden of medicinal plants at the college of pharmacy / Mustansiriaya University. Authentication of the plant carried out by Dr.Sukaina Abbas in collage of science / university of Baghdad. The plant material was collected during September and dried at room temperature in the shade, then, the leaves grinded as powder and weighed.

Extraction of flavonoids:

The powdered leaves part of *T.aphylla* (26 gm) were defatted with hexane (600 ml).the defatted plant material was further extracted with ethyl acetate (600 ml) using soxhlet extractor. The ethyl acetate extract was concentrated by evaporation under reduced pressure using rotary evaporator Then the marc was extracted again with (750mL) ethanol: water (90%, 675:75) by soxhlet till exhaustion. The ethanolic extract was concentrated by evaporation under reduced pressure using rotary evaporator. Ethyl acetate and ethanol water extracts were analyzed for presence of flavonoids by using TLC and confirmed by HPLC analysis.

Detection of flavonoids by TLC

Small amount of concentrated ethyl acetate extract was suspended in absolute methanol in beaker then applied on a readymade analytical TLC plate pre - coated with silica gel GF_{254}, and developed in the following mobile phases [13]:

- S1. Chloroform: methanol (9:1)
- S2. Chloroform: acetone: formic acid (75:16:8)
- S3. Toluene: chloroform: acetone (40:25:35)
- The R_f value of flavonoids contents of these extracts were compared with R_f value of standards Kaempferol, isorhamnetin and quercetin.

While, used Small amount of concentrated ethanol/water extract to investigate the presence of Rutin and applied on a readymade analytical TLC plate precoated with silica gel (GF_{254}) and developed in the following mobile phases [14]:

- S4. Methanol: water: formic acid (40: 57: 3) (v/v/v)
- S5. Ethyl acetate: glacial acetic acid: formic acid: H2O (100:11:11:25)

The R_f value of Rutin contents of this extract was compared with R_f value of standards Rutin.

High Performance Liquid Chromatography (HPLC):

The ethyl acetate of *Tamarix.aphylla* was analyzed by HPLC to investigate the presence of flavonoids. HPLC analysis was achieved on a reverse-phase C18 column (5 µm, 4.6 mm × 250 mm) the mobile phase was a linear gradient with O-phosphoric acid 0.25% (A)- acetonitrile (B) for 42 min starting with A:B (95:5) for 2 min, changing to A:B (90:10) for 5 min, A:B (85:15) for 3 min, A:B (80:20) for 13 min, A:B (70:30) for 5 min, A:B (50:50) for 4 min with equilibrating for 10 min. The flow rate was 1 ml/min. The injection volume for all samples and standard solutions was 10 all [15].

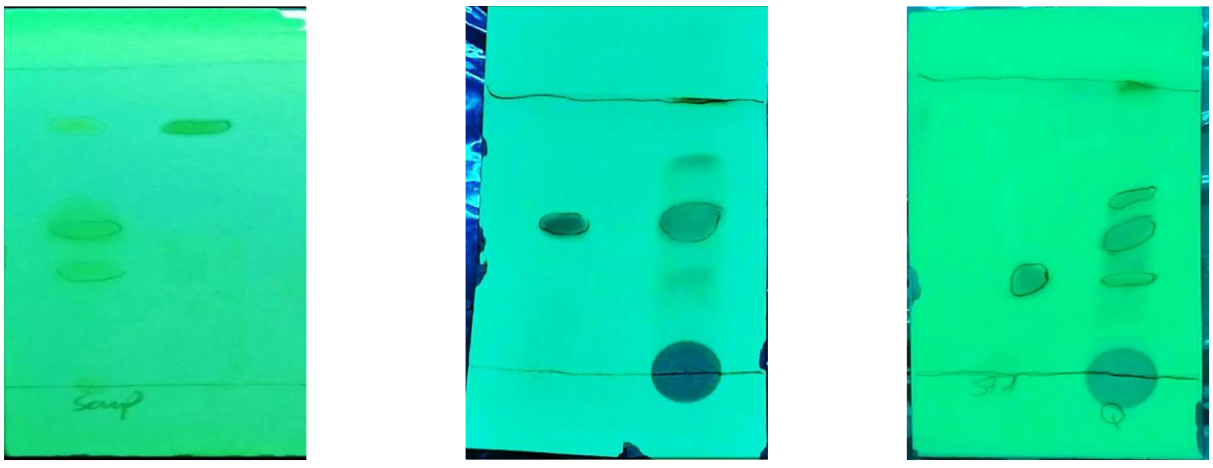
In addition, ethanol extracts of *Tamarix.aphylla* was also analyzed by HPLC to investigate the presence of rutin. A gradient elution was carried out in reverse-C18 column (100 A°, 100 mm × 4.6 mm, 2.6 µm pore size). The mobile phase consists of two different solutions 0.5% acetic acid and acetonitrile which act as solution A and solution B. The gradient elution initial conditions were 18% of eluent B with linear gradient to 18.5% from 0.01 to 7 min, followed by linear gradient to 35% of eluent B at 9 min and this proportion being maintained for 2 min. The mobile phase composition returned to the initial condition at 10 min and allowed to run for another 5 min before the injection of another sample. The total runtime of each sample is 15 min. The flow rate was 1.0 ml/min and the sample injection volume were 5 µl [16].

Results and Discussion:

The yields of the extraction of leaves were:

- Ethylacetate = 0.5 gm
- Ethanol /water = 4.6 gm

Analytical TLC of ethyl acetate extract confirmed the presence of kaempferol (kaemp.), isorhamnetin (isorh.), quercetin (quer.), these compounds appears as spot in three different mobile phases against kaempferol, isorhamnetin and quercetin reference standards the spots detected by UV at 254nm as shown in figure (2) and Rf values summarized in table (1).



(A) **(B)** **(C)**

Figure (2): Analytical TLC of. (A) Isorhamnetin, (B) Kaempferol, (C) Quercetin standard and Tamarix aphylla extracts using solvent S1 and S3.

Table (1): Rf values of standard quercetin, kempferol and isorhamnetin compared with plant extract in three mobile phases

Mobile Phase No.	Quer.	Quer. std.	Kaemp.	Kaemp std.	Isorh.	Isorh. std.
S1	0.62	0.64	0.75	0.78	0.88	0.89
S2	0.26	0.28	0.48	0.49	0.65	0.65
S3	0.32	0.32	0.54	0.52	0.8	0.81

On the other hand, the ethanol extract was analyzed for the presence of rutin by thin-layer chromatography (TLC) in comparison with standard as shown in Figure (3), Rf values are reported in Table (2)**.**

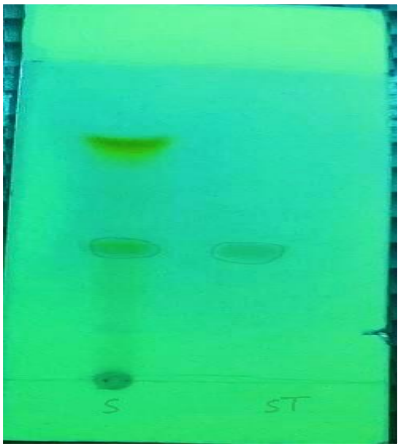


Figure (3): Analytical TLC showing ethanol extract against rutin standard in solvent S5

Table (2): Rf values of standard rutin compared with investigated ethanol / water extract in two mobile phases.

Mobile phase No.	Solvent System	Rf value of standard rutin	Rf value of rutin
S4	Methanol: water: formic acid 40: 57: 3 (v/v/v)	0.16	0.14
S5	Ethyl acetate: glacial acetic acid: formic acid: H2O (100:11:11:25)	0.4	0.42

Identifications by HPLC

In HPLC, qualitative identifications have been made by comparison of the retention times obtained at identical chromatographic conditions of analyzed samples with the authenticated reference standards**.**

The HPLC analysis for flavonoids in ethyl acetate and ethanol extracts revealed the presence of kaempferol together with quercetin and isorhamnetin in ethyl acetate extract and presence of rutin in ethanol extract in comparison with retention time of standard flavonoids as shown in figures (4,5) and table (3).

Table (3): Retention Time of Standards against Ethyl Acetate and ethanol / water Extracts.

sample	*Retention time of standard/min.*	*Retention time of sample/min.*
Quercetin	9.455	9.682
Kaempferol	32.017	32.237
Isorhamnetin	33.178	31.009
Rutin	3.761	3.810

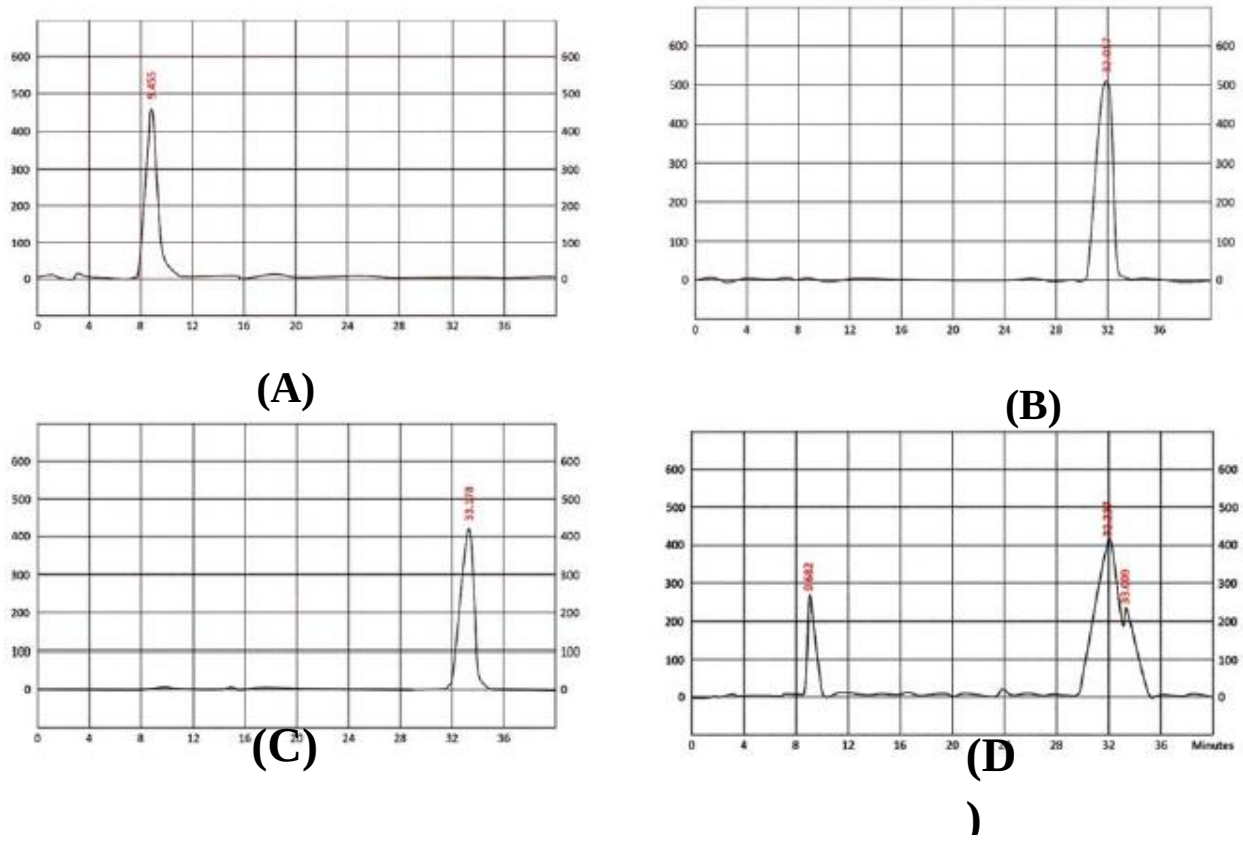


Figure (4): HPLC Analysis of flavonoids

(A): standard Quercetin
(B): standard Kaempferol
(C): standard Isorhamnetin
(D): Ethylacetate extract.

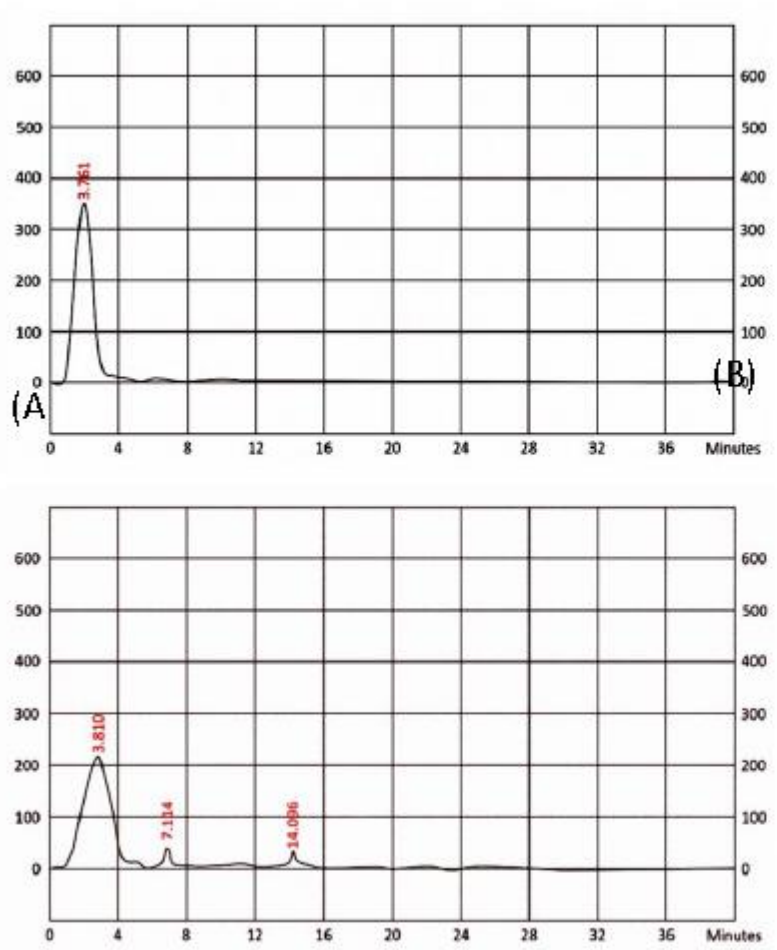


Figure (5): HPLC Analysis of rutin

(A): standard Rutin
(B): ethanol/water extract

Conclusion:

From this study can be conclude that *tamarix aphylla* is a promising plant for many flavonoid compounds that have many pharmacological actions.

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