Antimicrobial Activity And Phytochemical Investigation Of Tamarix Macrocarpa (Ehrenb.)Bge Wildly Grown In Iraq

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ABSTRACT

Ethanolic extract of Tamarix macrocarpa was tested for its, in vitro, antimicrobial activity by an agar dilution -streak method against eleven microorganisms. The results showed that it is active against Staphylococcus aureus , Bacillus subtilis at the level of 1000 mcg / ml. and against Candida albicans at the level of 500 mcg / ml. Phytochemical investigation indicated that this plant is rich with phenolic and Polyphenolic compounds, among which four compounds were identified by both Thin Layer Chromatography and High Pressure Liquid Chromatography.

The identified compounds were: Gallic acid, Isoferulic acid, Kampferol and Quercetin.

This is one of the most significant studies concerning the phytochemical investigation of this fractionation and characterization of the antimicrobial components**.**

الخلاصـــة

ان نبات الطرفة من النباتات الطبية التي تنمو بصورة طبيعية في اماكن متعددة من العراق.

ان الدراسات السابقة التي اجريت على فصائل مختلفة من الجنس Tamarix اثبتت احتواء النبات على العديد من المواد الفينولية ولكن لاتوجد اي دراسة تناولت هذه الفصيلة ال Macrocarpa من النبات ولاتوجد ايضا اي دراسة سابقة تناولت فعاليتها ضد المايكروبات والجراثيم، سوى بحث شامل واحد اشار الى فعاليتها ضد انواع من المايكروبات والجراثيم. لذا وجد من الضروري دراسة مكوناته بصورة كيمياوية مفصلة. لقد تم استخدام الكحول الاثيلي كمذيب عضوي. ولقد اثبتت لنا نتائج فحص المستخلص الكحولي بطريقة كروماتوغرافيا الطبقة الرقيقة عن وجود مالايقل عن 13 مركب بعضها مركبات فينولية كما اعطى المستخلص الكحولي فعالية ضد , Candida albican, Bacillus subtilis, Staphylococcus auerus بتركيز (1000 مايكروغرام/مل) فقمنا بتتبع المركب الذي يعطي هذه الفعالية ومحاولة فصله عن بقية المركبات الكثيرة الموجودة في هذه النبتة باستخدام مذيبات عضوية عديدة مثل الايثر، اثل استيت، مزيج من الماء والايثانول، واستطعنا حصر المركب الذي يعطي الفعالية ضد المليكروبات في جزء معين، ولكننا لم نستطع فصل هذا المركب لان المزيج كان سريع التاكسد لذا تقرر حفظه في الوقت الحاضر لغرض دراسته بصورة اكثر تفصيلا بالمستقبل.

استطعنا ايضا من خلال دراسة هذه النبتة تعيين بعض المركبات الموجودة فيها وذلك من خلال مقارنة قيمة RF لهذه المركبات مع قيمة RF للـ Standards الذي كان متوفرا لدينا ومن خلال تحليل الـ HPLC ايضا و هذه المركبات هي:

1- Gallic acid 2-Isoferulic acid 3-Kampferol 4-Quercetin

INTRODUCTION

Tamarix macrocarpa (Ehernb) Bge (Tamaricaceae) is an indigenous plant, widly distributed in Iraq. It is one of eleven species found in Iraq [(1)].

Tamarix macrocarpa (TARFA طرفة, arabic and Colloquial name) is a shrub or small tree with brown to blackish. Brown bark, found in saline swamps, flats along margin of sand dumes, salty west lands. It is distributed in the desert region of Iraq. especially on the irrigated alluvial plain near Hawija, Samawa, near Baghdad, Diwania, and Basra [(1)]. It's also distributed in Syria, Palestine, Jordan, Sinai, Egypt, Kuwait, Iran, West Pakistan and Libya [(1)].

Literature survey on different Tamarix species revealed a number of publications which mostly report the presence of phenolic and polyphenolic compounds, together with their esters and glycosides derivatives [(2-9)].

In concern of Tamarix macrocarpa, only one general screening study [(10)] reported that an ethanolic extract of the plant have a wide range of antimicrobial activities.

Since no further phytochemical studies were done on this plant, this study deals with the phytochemical investigation as well as fractionation and characterization of the active components**.**

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EXPERIMENTAL

Plant Material:

The plant material (aerial part) of Tamarix macrocarpa (Ehrenb) Bge was collected at the blooming stage during the months of February, March and April from local fields about 5 Km. West of Faluja (35 Km. West of Baghdad).

The plant material was identified by the Department of Pharmacognosy College of Pharmacy, University of Baghdad. And authenticated by the National Iraqi Herbarium, Botany Directorate at Abu-Ghraib.

Extraction:

Three Kilograms of the plant material, in moderately coarse powder, were extracted with 4 liters of petroleum ether (b.p. 60-80 ° c) at room temperature for 48 hours. The petroleum ether extract was filtered and evaporated to dryness under reduced pressure at a temperature not exceeding 40°c (leaving a yellowish — white viscous residue (15gm.).

The plant material was then percolated with 6 liters of ethanol 95%, collecting the percolate every 24 hours, until complete exhaustion. The alcoholic extract was evaporated to dryness, to give a dark green residue (95 gm), was designated as (F-l). F-l then was macerated with enough quantities of ether. The ether soluble fraction was separated, reduced to 300 ml. by evaporation, and then partitioned with equal volume of 5% sodium hydroxide solution (three times), in a separatory funnel.

The basic solution -was separated and then acidified with hydrochloric acid (35%) to PH2, using hydroion paper as indicator. A precipitate was formed which was extracted with 300 ml. Of ether (two times) by a separatory funnel.

The ether layer then separated, dried with unhydrous sodium sulphate, filtered and evaporated to dryness to leave a brownish residue about (9.5 gm.) designated as (F-2).

Maceration of the alcoholic extract (left after ether maceration) was continued with 300 ml. of ethyl acetate and filtered. The filtrate was partitioned (three times) with equal volume of 5% sodium hydroxide solution, in a separatory funnel. The basic aqueous solution was separated and acidified with hydrochloric acid (35%) to PH_2 .

A precipitate was formed, which was extracted with ethyl acetate (200ml) by a separatory funnel. The ethyl acetate layer then was separated, dried with anhydrous sodium sulphate, filtered and evaporated to dryness to leave a blackish residue about (13 gm) designated as (F-3).

Maceration of the original alcoholic extract (which was insoluble in ethyl acetate) was continued using 300 ml. of a mixture of methanol and water (50% $^{v}/v$): the insoluble fraction then was separated by filtration leaving a residue of about (20 gm) designated as (F-4). The filtrate was evaporated to dryness to give a dark residue about (50 gm) designated as (F-5).

Maceration of (F-5) with enough quantity of chloroform gave a white residue which upon separation by filtration turn dark brown in colour (35 gm) designated as (F-6). The soluble fraction was evaporated to dryness to give a dark brown residue (15 gm) designated as (F-7).

Antimicrobial Testins:

A general test for antimicrobial activity was carried for all crude fractions (F-l - F-7) obtained by the fractionation method.

They were tested by an agar dilution — streak method [11] at a concentration of 1000 mcgm /ml against eleven

microorganisms representing gram positive and Gram negative bacteria as well as yeast and fungi (table 1).

A positive and negative control were used in each test series. The two positive control plates: one contain 1000 mcgm / ml of Streptomycin as an antibiotic, and the other 1000 mcgm/ ml Nystatin as an antimycotic drug. While the negative control plate contain nothing but the organism with the agar medium.

Table -1 Microorganisms used in this study

MICROORGANISM	CULTURE TYPE	CLASSIFICATION
Bacillus subtilis	ATCC 10231	Gram Positive bacteria
Micrococcus latus	ATCC 9341	Gram Positive bacteria
Staphylococcus aureus	ATCC 6536	GramPositive bacteria
Escherichia coli	ATCC 25922	GramNegative Bacteria
Klebsiella pneumonia	Hospital isolate	Gram Negative bacteria
Pseudomonus aeruginosa	ATCC 9027	Gram Negative bacteria
Proteus vulgaris	Hospital isolate	Gram Negative bacteria
Aspergillus niger	ATCC 16404	Fungi
Microsporum gypseum	Hospital isolate	Fungi
Trichophyton mentagrophate	Hospital isolate	Fungi
Candida albicans	ATCC 10231	Yeast

Thin Layer Chromatography and High Pressure Liquid Chromatography

The components of all of the designated fractions (F-l —» F-7) were examined by Thin Layer Chromatography using the following system:

Silica gel GF 254 as a stationary phase with two solvent systems: Chloroform: Methanol (90:10 ^{v}/v) and Benzen, Acetone (95:5^{v}/v). Ferric chloride T.S. solution was used as a spraying reagent (12). Some of the identified components by Thin Layer Chromatography were furthes approved by High Pressure Liquid Chromatography as shown in (Fig-1, 2, 3).

The HPLC apparatus consist of Lc-6A liquid chromatography (Koyota, Japan), equipped with Reodyne 7125, 20 ml injector (USA). A Shimadzu Spd-6A UV/visible, detector set at 280 nm, 0.8 a. u.f.s. Column C-18 ODS (250x4.6 mm D): flow rate 1.5ml/ min, mobile phase, methanol: water (7.5: 92.5 ^{v}/v):

Wavelength 254 nm: Pressure 150: temperature25-30°

RESULTS and DISCUSSION

Literature survey revealed that all of the reported compounds isolated from different species of tamarix were phenolic and polyphenolic and their derivative compounds of different polarities.

Since most of the isolated antimicrobial and antifunal compounds of plant origin were phenolic and polyphenolic compounds our work was emphasized on the study of the these compounds. For this reason our experimental procedure dealt with the fractionation of the polyphenolic compounds of different polarities. So the alcoholic extract fraction (F-l) was fractionated using solvents of ascending polarities, starting with ether (F-2),ethyl acetate (F-3), and then methanol (F-7)

Each fraction was tested with Fecl 3 test solution for the presence of phenolic compounds, and then tested for antimicrobial activities at the level of 1000 mcg/ml. by an agar dilution -streak method.

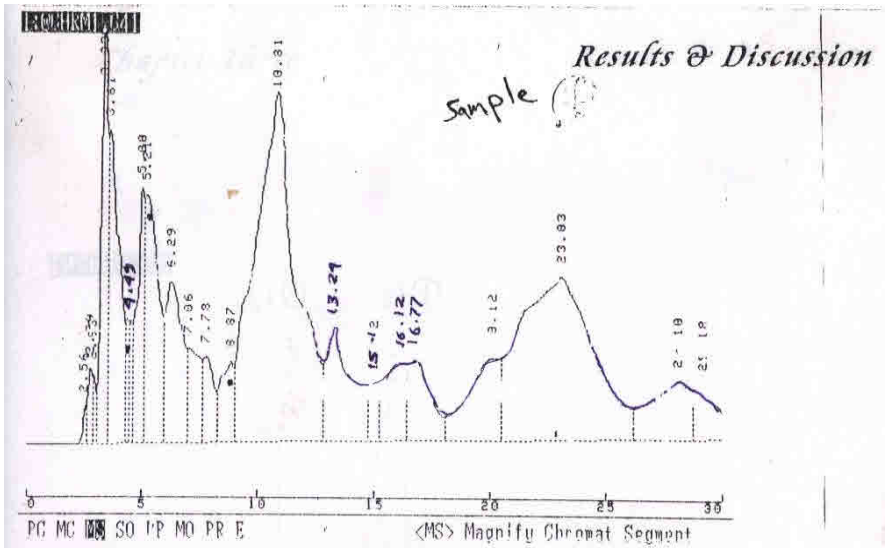
Although all of the fractions (F-l –F-7) showed a number of positive phenolic spots on TLC, they were all having no antimicrobial activities , with the exception of the alcoholic fraction (F-l), which showed activity at the level of 1000 mcg/ml against Staphvlococcus aureus, Bacillus subtilis and Candida albicans , and (F-7) which contains the highly polar phenolic compounds. This fraction (F-7) showed activities against Staphvlococcus aureus and Bacillus subtilis at the level of 1000 mcg/ml

And showed anti fungal activity against Candida albicans at the level of 500 mcg/ml.

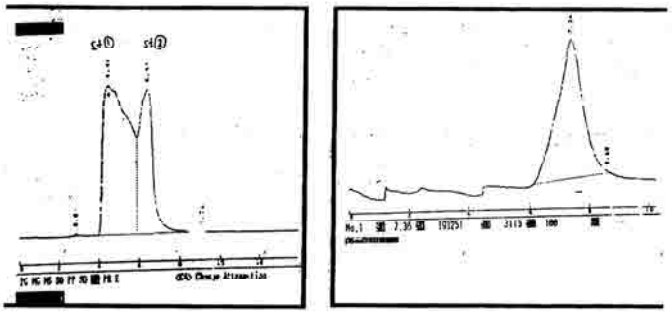
Attempts to obtain pure antimicrobial component "were not successful due to its highly oxidzable properties and their instability in the solution, as the colour of the solution was changing during the procedure from brown, to blackish - brown then to black in colour.

Phytochemical investigation of the alcoholic extract (F-l) by TLC indicated the presence of not less than 15 different phenolic compounds. Of these four of them were identified. They are Gallic acid, Isoferulic acid, Kampferol and Quercetin.

Identity of these compounds with authentic samples were confirmed by Thin Layer Chromatography using two different solvent systems. Further more they were confirmed by High Pressure Liquid Chromatography as shown in (Fig-1, 2,3)

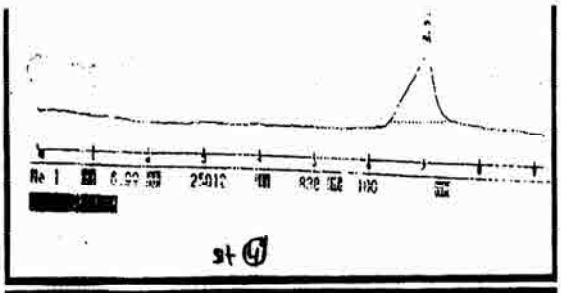


HPLC of the original alcoholic extract of *T. macrocarpa.*

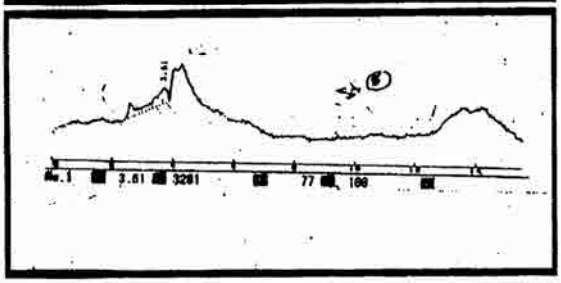


HPLC for the standard references

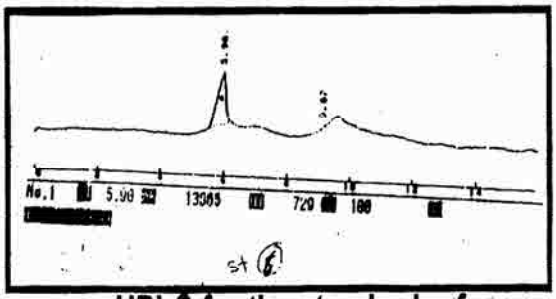
1. Gallic acid **2. Rutoside** **3. Quercetol.**



4. Isofeulic acid



5. Kamferol



6. Quercetin

HPLC for the standard references

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