Antioxidant activity of tannin from*Tamarix aphylla L.* leaves

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SUMMARY

Tannins of *T. aphylla* L. leaves have been extracted, then number of preliminary qualitative chemical tests were carried out on the extract. The results revealed that the extract consist of four tannin compounds. In an attempt to assess the possible antioxidant activity for tannin extract of *T. aphylla* L. leaves, two assays were conducted. It was found that tannin extract possesses strong antioxidant activity (80.1%) as well as strong reducing power (increases as the extract concentration increases).

Introduction

It is by now commonly accepted that under situations of oxidative stress, reactive species such as superoxide anion ($O_2^{\cdot -}$), hydroxyl ($OH^{\cdot}$) and peroxyl ($^{\cdot}OOH$, $ROO^{\cdot}$) radicals are generated (9). These reactive oxygen species play an important role in degenerative or pathological processes, such as aging, cancer, coronary heart, Alzheimer's disease (13), neurodegenerative disorders, atherosclerosis, cataracts and inflammation (3).The use of traditional medicine is widespread, and plants are still large source of natural antioxidants that might serve as leads for the development of novel drugs (21). In searching for novel natural antioxidants, some plants have been extensively studied in the past few years for their antioxidant and radical scavenging components (22). Phytochemicals from natural products are also important in that they provide further protection against oxidative damage from free radicals (6). Numerous natural antioxidants have been studied in fruits, vegetables and herbs (23). Antioxidants such as tocopherols, carotenoids, terpenoids, ascorbic acid and phenolic compounds, including tannins and flavonoids are introduced into the human body in a form of food components (4).

The present work aims to isolate the tannins compounds from *Tamarix aphylla L.* and assess their possible antioxidant activity.

Materials and Methods

2-1 : Chemicals

All chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO), and solvents were from E.Merck (Darmstadt, Germany). All of the reagents were prepared in deionized distilled water (ddH_2O) to eliminate the contamination of metal ions.

2-2 : Plant Material

Fresh leaves of plant were collected during the month of April 2010 from the premise of Science College, University of Basrah. The plant was botanically authenticated and voucher specimens were deposited in the Herbarium of Basrah (Iraq, Basrah, College of Science, University of Basrah). The plant leaves were air-dried under shade at room temperature for 3 days, after which it was grinded to a uniform powder.

2-3 : Preparation of extracts

2-3-1 : Aqueous extract of *Tamarix aphylla L.* leaves

The Aqueous extract was prepared by soaking 10 g of the dried powder plant leaves in 50 mL of ddH_2O, then heat at 50-60 °C for 3 hours. The residue was removed by filtration and the filtrate was concentrated under vacum in a rotary evaporator to afford 1.23g (1).

2-3-2: Tannin extract of *Tamarix aphylla L.* leaves

100g of dried powder leaves were refluxed with 400 mL of 70% acetone for 8 hours. The residue was removed by filtration in Buchner funnel, and the filtrate was concentrated under vacuum in a rotary evaporator. The brown viscous material was dissolved in 30 mL of deionized distilled water. Tannin was extracted from the water solution by ethyl acetate (3×30 mL). The ethyl acetate layer was evaporated under vacuum to afford 4.3g (16) .

2-4: Qualitative analysis

2-4-1: Preliminary phytochemicals analysis

This was carried out according to the methods described by Trease & Evans (18).

Tannins: (200 mg aqueous extract + 10 mL ddH_2O + filtered) 2mL filtrate + 2mL $FeCl_3$ → Blue or Black precipitate indicate the presence of Tannins & Phenols.

Alkaloids: (200 mg aqueous extract + 10 mL Methanol + filtered) 2mL filtrate + 1% HCl + steam 1mL filtrate + 6 drops Mayer's reagent or Wagner's reagent or Dragendroff's reagent → Creamish/ Brown/ Red/ Orange precipitate indicate the presence of alkaloids.

Saponins: (200 mg aqueous extract + 10 mL ddH_2O + filtered) 0.5mL filtrate + 5mL ddH_2O → frothing persistence indicate the presence of Saponins.

Terpenoids: (200 mg aqueous extract + 10 mL ddH_2O + filtered) 2mL filtrate + 2mL Acetic anhydride + conc. H_2SO_4 → Blue or Green ring indicate the presence of terpenoids.

Cardiac glycosides: (Keller Kiliani test): (200 mg aqueous extract + 10 mL ddH_2O + filtered) 2mL filtrate + 1mL Glacial acetic acid + $FeCl_3$ + conc. H_2SO_4 → Blue or Green precipitate indicate the presence of Cardiac glycosides .

Steroids:(Leibermann Burchard reaction): (200 mg aqueous extract + 10 mL Chloroform + filtered) 2mL filtrate + 2mL Acetic anhydride + conc. H_2SO_4 → Blue or Green ring indicate the presence of Steroids.

Flavonoids: (200 mg aqueous extract + 10 mL Ethanol + filtered) 2mL filtrate + 2mL Acetic anhydride + conc. H_2SO_4 + Magnesium ribbon → Pink or Red colour indicate the presence of flavonoids.

2-4-2:Thin layer chromatography (TLC):

The foregoing prepared tannin extract of *Tamarix aphylla L.* leaves were tested for Thin layer chromatography (TLC). A known volume (two drops) of this extract (1mg/1mL) were spotted on TLC silica gel plate (2 × 10 cm) using Toluene **:** Acetone **:** Formic acid (6**:**6**:**1) as a solvent system. After 30 second, the plates were dried using hair drier and examined under long wavelength 366 nm ultraviolet (UV). The plates were also stained with p-anisaldehyde/sulfuric acid (7). The plates were also stained with ferric chloride (5% in 0.5N HCl), Folin-Denis, Tollen's reagent and vanillin (1% in sulfuric acid) (19).

2-5 : Determination of antioxidant activity

2-5-1: The β-carotene bleaching method

Antioxidant activity of tannin extract of *Tamarix aphylla L.* leaves was determined according to the β-carotene bleaching method developed by Karadeniz *et al.* (8). A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 mL of chloroform, 1 mL of this solution was then pipetted into a round-bottom rotary flask containing 20 mg of linoleic acid and 0.2g of Tween 20. After removing the chloroform under vacum using a rotary evaporator at 30°C, 50 mL of aerated distilled water was added to the flask with manual shaking. Aliquots (5 mL) of this prepared emulsion were transferred into tubes containing 0.2 mL of extract (50, 100 and 150mg/L) or butylated hydroxyl toluene (BHT, 50mg/L) which was used for comparative purposes. The control consisted of 0.2 mL of 80% methanol instead of the extract. As soon as the emulsion was added

to each tube, the zero time absorbance was read at 470 nm. The samples were then subjected to thermal autoxidation at 50°C in a water bath. Subsequent absorbance readings were recorded at 15 min intervals until the color of the β-carotene in the control sample had disappeared (105 min). The extent of inhibition of the absorbance is related to the concentration of antioxidant compounds. The sample was assayed in triplicate. The degradation rate of extracts was calculated according to zero order reaction kinetics. Antioxidant activity (AA) was calculated as percent of inhibition relative to the control using the following equation:

$$\mathbf{AA} = [\ 1 - (\ Ai - At\ ) / (\ Ái - Át\ )\ ] \times 100$$

Where :
Ai = Measured absorbance value of sample at zero time .
At = Measured absorbance value of sample after incubation (105 min) at 50 °C.
Ái = Measured absorbance value of control at zero time.
Át = Measured absorbance value of control after incubation (105 min) at 50 °C.

2-5-2: Measurement of reducing power

The reductive capability of the extract was quantified by the method of Yen and Chen (10). The methanol extract (5, 10 and 15 mg/mL) or butylated hydroxyl toluene (BHT) was mixed with an equal volume of 0.2M phosphate buffer, pH 6.6, and 1% potassium ferricyanide, then incubated at 50°C for 20 min. An equal volume of 1% trichloroacetic acid was added to the mixture and centrifuged at 3000 Xg for 10 min at room temperature. The upper layer of the solution was mixed with ddH_2O and 0.1% $FeCl_3$ with a ratio of 1 : 1: 2 , and the absorbance at 700 nm was measured. This assay was done in triplicate. Increased absorbance indicated increased reducing power.

Statistical analysis

The results are expressed as mean values ± SD and tested with analysis of variance followed by Student's *t*-test. P-values < 0.05, < 0.01 were considered to be statistically significant.

Results and Discussion

3-1: Qualitative analysis for *Tamarix aphylla L.* leaves

Table (3-1) indicate the preliminary phytochemicals analysis of aqueous extract of *Tamarix aphylla L.* leaves. The results showed the presence of alkaloids, glycosides, tannins, phenolic compounds and saponins. The most phytochemical classified as secondary metabolites are produce mainly by the shoot part of the plant, often their functional in the plant is unknown but some phytochemicals are known to have structural, functional and general defense against plant pathogens (5). Plant extract is the best source of phytochemical, which used as medical treatment but their uses as well as other alternative form of phyto-treatment (20). The results presented in Tables (3-2) and Figure (3-1) show that the tannin extract of *Tamarix aphylla L.* leaves contains five components. These components are relate to the tannin family because they give a positive test with ferric chloride (5% in 0.5N HCl), Florin-Denis ,Tollen's reagent and vanillin (1% in sulfuric acid) reagents. Also, the results revealed that all the components have sugar compounds as glycosides because they indicate a positive test with p-anisaldehyde reagent.

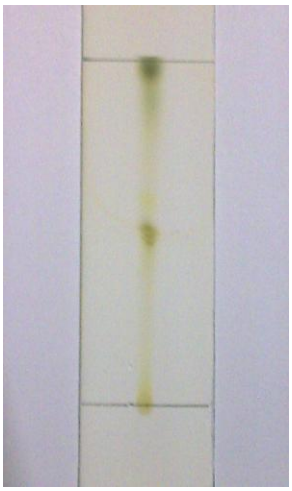

Figure (3-1): Thin layer chromatography for tannin extract of *Tamarix aphylla L.* leaves

Table (3-1): Qualitative analysis for aqueous extract of *Tamarix aphylla L. leaves*

Test	Reagent	Observation
Alkaloids	Dragendroff, Wagner, Mayer	+
Tannins	1%$FeCl_3$	+
Flavonoids	Magnesium turning	+
Cardiac glycosides	Keller Mililani test	-
Saponins	shaking	+
Phenols	1%$FeCl_3$	+
Steroids	Liebermann Burchard	-
Glycosides	Benedict	+
Terpenoids	Salkowski	-

Table (3-2): Thin layer chromatography for tannin extract of *Tamarix aphylla L*. leaves

UV 366 nm	p-anisaldehyde / sulfuric acid	Ferric chloride (5% in HCl)	Folin-Denis	Tollen's reagent	vanillin (1% in sulfuric acid)
0.58	0.96 0.91 0.48 0.01	0.96 0.91 0.48 0.01	0.96 0.91 0.48 0.01	0.96 0.91 0.48 0.01	0.96 0.91 0.48 0.01
Conjugated test	Glycoside test	Tannin test	Tannin test	Tannin test	Tannin test

3-2: Determination of antioxidant activity

The results of this study for antioxidant and reducing power tests are presented in Figure 3-2 and 3-3, respectively.

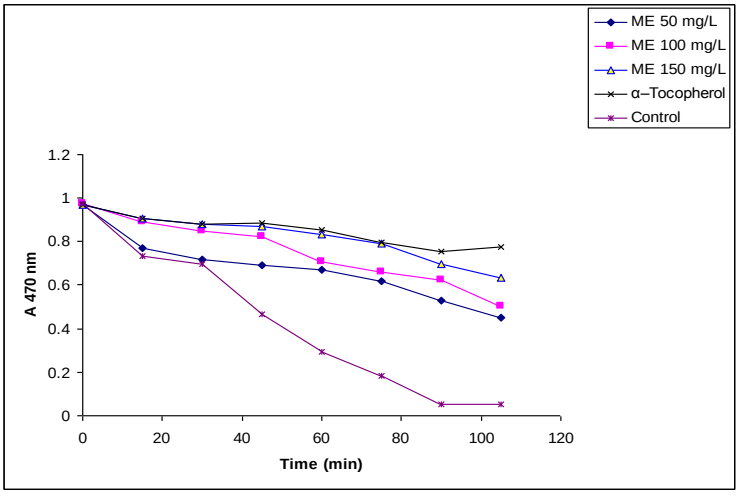

Figure 3-2. Antioxidant activity of the methanol extract in comparison with α-Tocopherol and control .

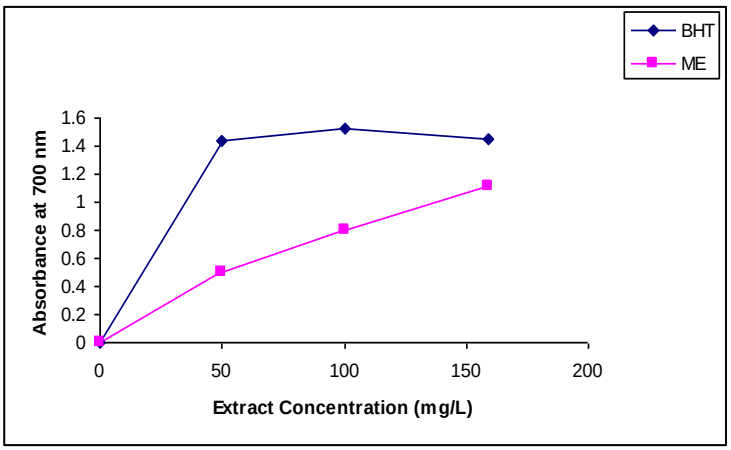

Figure 3-3: Reducing power of the methanol extract in comparison with BHT.

The results revealed that the inhibition of peroxidation was progressively increased by raising the extract concentration and reached a plateau (about 80.1% inhibition) when the concentration exceeded 150mg/L ($P < 0.01$). The material extracts had overall good antioxidant activity. Tannins are polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity (14). The phenolic compounds may contribute directly to antioxidative action (15). The antioxidative activities observed can be ascribed both to the different mechanisms exerted by different phenolic compounds and to the synergistic effects of different compounds. The antioxidant assay used in

this study measures the oxidation products at the early final stages of oxidation (12). The antioxidants have different functional properties, such as reactive oxygen species scavenging, inhibition of generation of free radicals and chain-breaking activity and metal chelation (11). A direct correlation between antioxidant capacity and reducing power of the extract has been reported. The reducing power of extract of *T. aphylla* L. leaves was very potent and the reducing power of the extract was increased with quantity of sample. The plant extract could reduce the most Fe^{+3} ions, which had a lesser reductive activity than the standard of BHT. The reducing properties are generally associated with the presence of reductions, which have been show to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (17). Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals (2). Further investgation on isolation and identification of tannin extract components in the plant may lead to chemical entities with potential for clinical and industrile use as antioxidant, which might be helpful in preventing or slowing the progress of various oxidative stress–related reactions.

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مجلة البصرة للعلوم الزراعية ، المجلد ٢٤ ، العدد (١) ،٢٠١١

التأثير المضاد للاكسده للتانينات المعزولة من أوراق نبات *Tamarix aphylla L.*

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ألخلاصه

استخلصت التانينات من أوراق نبات ألأثل *T. aphylla L.* وشخصت هويتها ألكيميائيه باستخدام عدد من الاختبارات الكيميائية النوعية الاوليه وقد أظهرت النتائج احتواء ألخلاصه على أربعة مركبات عائده إلى التانينات. كما تم دراسة الفعالية المضادة للأكسدة لمستخلص التانينات لأوراق نبات ألأثل باستخدام اختبارين، وقد أظهر المستخلص فعالية عاليه كمضادة لأكسدة حامض اللينولينك(80.1%) وقابلية اختزال قويه في النظام النموذجي (تزداد مع زيادة تركيز الخلاصة) .