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HPLC-UV Analysis and Antioxidant Potential of Phenolic Compounds from Endemic Shrub of Arid Environment *Tamarix pauciovulata* J. Gay

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Abstract: This research presents complete phenolic compounds and biological activity of *Tamarix pauciovulata* J. Gay, an endemic Saharan species. The antioxidant assays revealed that crude extract showed strong DPPH scavenging activity (IC50 = 49.357 µg/mL) but in reducing power and hydrogen peroxide scavenging activity, butanolic and ethyl acetate fractions have a potent ferrous ion-chelating ability in particularly the butanolic fraction (63.18% reduced power at 50 µg/mL) and a powerful scavenging activity on hydrogen peroxide in particularly ethyl acetate fraction (IC50 = 0.205 µg/mL). The phenolic compounds of *Tamarix pauciovulata* leaves were analyzed by HPLC-UV. The major phenolic of leaf extracts are syringic acid (1.07 g/100g), quercetin (34.1 mg/100g), kaempferol (5.77 mg/100g), isorhamnetin (5 mg/100g). Others phenols were identified such as isoquercetin, catechin, epicatechin and its derivatives. Results indicated that the leaves extracts from *Tamarix pauciovulata* have great capacities to prevent diseases caused by the overproduction of radicals and can become important source of dietary compounds with health protective potential.

Key words: *Tamarisk pauciovulata*, biological activity, polyphenols, syringic acid, quercetin.

1. Introduction

Tamarix species (Tamaricaceae Family) are bushes or trees, mostly evergreen and pink or white blossoms. They are relatively long-lived plants that can tolerate a wide range of environmental conditions and resist abiotic stresses [1]. *Tamarix* species, commonly known as tamarisk or salt cedar are widely distributed on a large area from Morocco to India. It is an introduced tree in southwestern United States [2]. Tolerant of alkaline and saline soils [3, 4], *Tamarix* withstands saline soils by regulating its salt balance via excretion of excess salts through foliar glands and consuming large quantities of water from underground sources [1]. *Tamarix* species are looked upon with favor as a check to erosion, as a windbreak and are also employed in traditional medicine as astringent, aperitif, stimulus of perspiration and diuretic [5], their fruit and leaves good astringents, are used for the treatment of dysentery and chronic diarrhea and are also considered to be effective in the treatment of leucoderma [6]. In traditional Egyptian medicine, extracts of *Tamarix* have been used especially as antiseptic agents, and also used for tanning and dyeing purposes [7]; they are useful again in spleen trouble and eye diseases [8]. Furthermore, several researches proved antioxidant and antimicrobial activities of some *Tamarix* species such as *Tamarix ramosissima* [9], *Tamarix hispida* [10], *Tamarix aphylla* [11]. Usually, *Tamarix* wood is used for fuel because it produces a fragrant odor when burned [12].

Chemopreventive activity of *Tamarix* species was

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due to their content on secondary metabolites, in particularly on their phenolic content, such as flavonoids, which are known to possess potent antioxidant properties. The reduction activity of phenolic and flavonoids compounds depends on the number of free hydroxyl groups in the molecular structure [13]. Several studies show that phenolic can directly scavenge molecular species of active oxygen, regulate nitric oxide, stabilize membranes by decreasing membrane fluidity and inhibit lipid peroxidation by trapping the lipid alkoxyl radical. Phenolic compounds, especially flavonoids, possess ideal structural chemistry for this activity and have shown to be more effective in vitro than vitamins E and C [14]. For example, The ortho 3',4'-dihydroxy structure in the B ring is the important determinant for the antioxidant potential of quercetin and catechin [15] and appears to cause many beneficial effects on human health. As antioxidants, phenolic compounds may protect cell constituents against oxidative damage and limit the risk of various degenerative disease associated to oxidative stress [16]. Their antioxidant capacity confers a therapeutic potential in cardiovascular diseases, gastric or duodenal ulcers, cancer or hepatic pathologies. Also important are their antiviral and their antiallergic actions, antithrombotic and anti-inflammatory properties [17].

Tamarix pauciovulata J. Gay is endemic to south area of Algeria, is a Saharan shrub, recommended to treat internal hematomas and inflammation. *Tamarix pauciovulata* has not been studied and no report in literature has done phytochemical and biological activity. For this reason, the aim of this work was to study several antioxidant activities and to analyze the phenolic constituents of *Tamarix pauciovulata* leaves.

2. Material and Methods

2.1 Source of Plant

Tamarix pauciovulata J. Gay collected from *Adrar* (arid area in south Algeria). The leaves was dried and ground finely to a powder in an electric mill and stored at 4 °C until use.

2.2 Extraction of Bioactive Compounds

About 100 g of the powdered leaves was extracted for 48 h with 70% methanol at room temperature. The mixture was filtered through whatman paper N°1 and filter paper of 0.45 µm porosity, then filtrate was dried using Laborota 4000 rotary evaporator at 50 °C. The residue was dissolved in sterile distilled water and freeze-dried using CHRIST-ALPHA 1-4 Lyophilizator.

For antioxidant activity, a part of the extract (CME) was dissolved in distilled water and partitioned with ethyl acetate and butanol successively to afford a butanol soluble fraction (BF), ethyl acetate fraction (EAF) and an aqueous fraction (QF).

2.3 Phytochemical Analysis

To detect the presence of various chemical constituents, The crude extract was qualitatively analyzed for the presence of alkaloids, saponin, flavonoids, tannins, cardiac glycosides, steroids and triterpenes, reducing sugars and cyanogen compounds [18-22] and each of the tests was qualitatively expressed as negative (–) or positive (+). The phenolic constituents of the leaves were analyzed by method CATHPLC: HPLC-UV (method Cofrac, Agrobio, *Rennes*).

2.4 Reducing Power Activity

The ferric reducing power assay was carried out by the method of Oyaizu [23]. Each concentration of aliquot of extract (1 mL) was mixed with 2.5 mL sodium phosphate buffer (200 mM; pH 6.6) and 2.5 mL potassium ferricyanide (1%). After incubation at 50 °C for 20 min; 2.5 mL TCA (10%) was added to the mixture and then centrifuged at 3000 rpm for 30 min. A 2.5 mL of upper layer was mixed with 2.5 mL distilled water and 0.5 mL ferric chloride (0.1%) was added. The green color developed was measured at 700 nm with UV-visible spectrophotometer

(Shimadzu UV mini 1240). A higher absorbance indicated the higher reducing power.

2.5 Scavenging of Hydrogen Peroxide

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1 mL of the extracts or standards in methanol were added to 2 mL of hydrogen peroxide solution in PBS. The absorbance was measured at 230 nm after 10 min against a blank solution contained extracts in PBS without hydrogen peroxide [24]. The percentage inhibition was calculated by using the following formula:

$$\text{Scavenging activity (\%)} = [(\text{Abs control} - \text{Abs sample})/\text{Abs control}] * 100 \quad (1)$$

IC50 value is the concentration of the sample required to scavenge 50% free radical. Experiments were performed in triplicate.

2.6 DPPH Antioxidant Activity

The free radical scavenging activity was determined by the method described by Brand-Williams et al. [25]. Leaves extract (0.1 mL) at different concentration was added to 3.9 mL of a 6×10^{-5} M DPPH solution in methanol. Absorbance at 515 nm was determined after 30 min and the percentage inhibition activity was calculated from {[(Ac-At/Ac] × 100}, where Ac, absorbance of the negative control (solution of DPPH without extract) and At is the absorbance of the extract.

2.7 Statistical Analysis

All the experimental results were mean ± SD of three measurements and data were evaluated by using student's *t*-test. $P < 0.05$ were regarded as significant.

3. Results and Discussion

3.1 Phytochemical and Phenolic Compounds of Tamarix Pauciovulata

The percentage yield of crude extract and fractions was found to be 285.2 mg/g leaves powder for crude methanolic extract (CME), 94.05 mg/g CME for ethyl acetate fraction, 67.857 mg/g CME for butanolic fraction and 534.528 mg/g CME for aqueous fraction.

Preliminary phytochemical screening of crude methanolic leaves extract of *Tamarix pauciovulata* revealed the presence of the following classes of chemical compounds such as saponins, favonoids, condensed and hydrolysable tanins, steroids and tritterpenes, cardiotonic glycosids and reducing glucids. We note that alkaloids and cyanogenic components were absents.

Phenolic composition, and quantification analysis with HPLC-UV showed that syringic acid was the major component (1.07 g/100g), naturally occurring 4-hydroxy-3,5-dimethoxybenzoic acid, a type of phenol acid C6-C1. Four flavonols (C6-C3-C6) were identified and quantified, quercetin, isoquercetin, kaempferol and isorhamnetin (Table 1). Plants need

Table 1 HPLC-UV analysis: chemical composition and quantification of *Tamarix pauciovulata* leaves.

Analysis method	mg/100g Leaves powder
Phenolic acids: HPLC-UV	
Syringic acid	= 1070
Flavonols: HPLC-UV	
Quercetin	= 34.1
Isoquercetin	< 5
Kaempferol	= 5.77
Isorhamnetin	= 5.00
Total	≤ 49.9
Catechins: CATHPLC: HPLC-UV	
(+)-Catechin	< 0.1
(−)-Epicatechin	< 0.1
(−)-Epicatechin gallate	< 0.1
(−)-Epigallocatechin	< 0.1
(−)-Epigallocatechin gallate	< 0.1

phenolic compounds for pigmentation, growth, reproduction, resistance to pathogens and for many other functions [26]. Plants are exposed to ambient solar ultraviolet-B (UV-B) radiation (280-320 nm) that is an environmental challenge negatively affecting DNA, proteins and membranes, thus leading to altered metabolism through the generation of reactive oxygen species (ROS). Plants protect themselves from this harmful radiation by synthesizing phenolic compounds [27, 28]. It has been proposed that flavonoids with their high absorptivity at 250-270 and 335-360 nm act as good UV screens [29]. Quantitatively, the important component of these flavonoids identified is quercetin: 34.1 mg/100g. Quercetin is one of the most active antioxidants found in medicinal plant. In human, it has known anti-inflammatory effects and it also helps to prevent cancer, prostatitis, heart disease, cataracts, allergies, bronchitis, and asthma.

3.2 Reducing Power Assay

The reducing power assay serves as a significant indicator of potential antioxidant proposed for the antioxidant activity such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, and prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [30]. Crude extract and fractions showed concentration dependent reductive effects.

The data in Fig. 1(A, B, C, D, E) show the reducing power (as indicated by the absorbance at 700 nm) of the crude extract and its derived fractions compared to ascorbic acid (e), their ranking order (as indicated by EC50) was as follows: butanolic fraction (0.037 mg/mL ± 0.007) > ethyl acetate fraction (0.098 mg/mL ± 0.009) > aqueous fraction (0.580 mg/mL ± 0.08) > ascorbic acid (0.812 mg/mL ± 0.062 ± 0.072) > crude extract (16.458 mg/mL ± 1.005).

The reducing power of three fractions of *Tamarix* was remarkably stronger than that of pure antioxidant standard ascorbic acid. The highest reducing activity was again observed for butanolic fraction. The reducing properties are generally associated with the presence of different reductants [31]. The antioxidant action of reductants is based on the breaking of the free radical chain by donating an hydrogen atom. Reductones also react with certain precursors of peroxide, thus preventing peroxide formation [32].

3.3 Hydrogen Peroxide Assay

Hydrogen peroxide can cross cell membrane [33]. It is not very reactive for itself, but sometimes is toxic to cell because it may give rise to hydroxyl radical in the cells [34] and can inactive a few enzymes directly, usually by oxidation of essential thiol (-SH) groups [33].

The scavenging abilities of crude extract and fractions with hydrogen peroxide were shown in Fig. 2(A, B, C, D) and compared with ascorbic acid (E). It was noticed that all the samples were able to scavenge hydrogen peroxide. In order the IC50, the hydrogen peroxide scavenging activity were: ethyl acetate fraction (0.20 ± 0.00161 µg/mL), > butanolic fraction (1.51 ± 0.0181 µg/mL) > ascorbic acid (4.77 ± 0.12056 µg/mL) > aqueous fraction (13.83 ± 0.1775 µg/mL > crude extract (40.057 ± 2.5866 µg/mL). According to these results, we can note that ethyl acetate fraction from *Tamarix pauciovulata* showed stronger hydrogen peroxide inhibition than the standard ascorbic acid.

3.4 DPPH Antioxidant Activity

Radical scavenging activities of crude extract, fractions and standard (Ascorbic acid) at different concentrations were tested by the DPPH method and the results are shown in Fig. 3(A, B, C, D, E). DPPH is relatively stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule [35], when DPPH expose to antioxidant compounds, its purple color changes to yellow. Antioxidant reacts with DPPH,

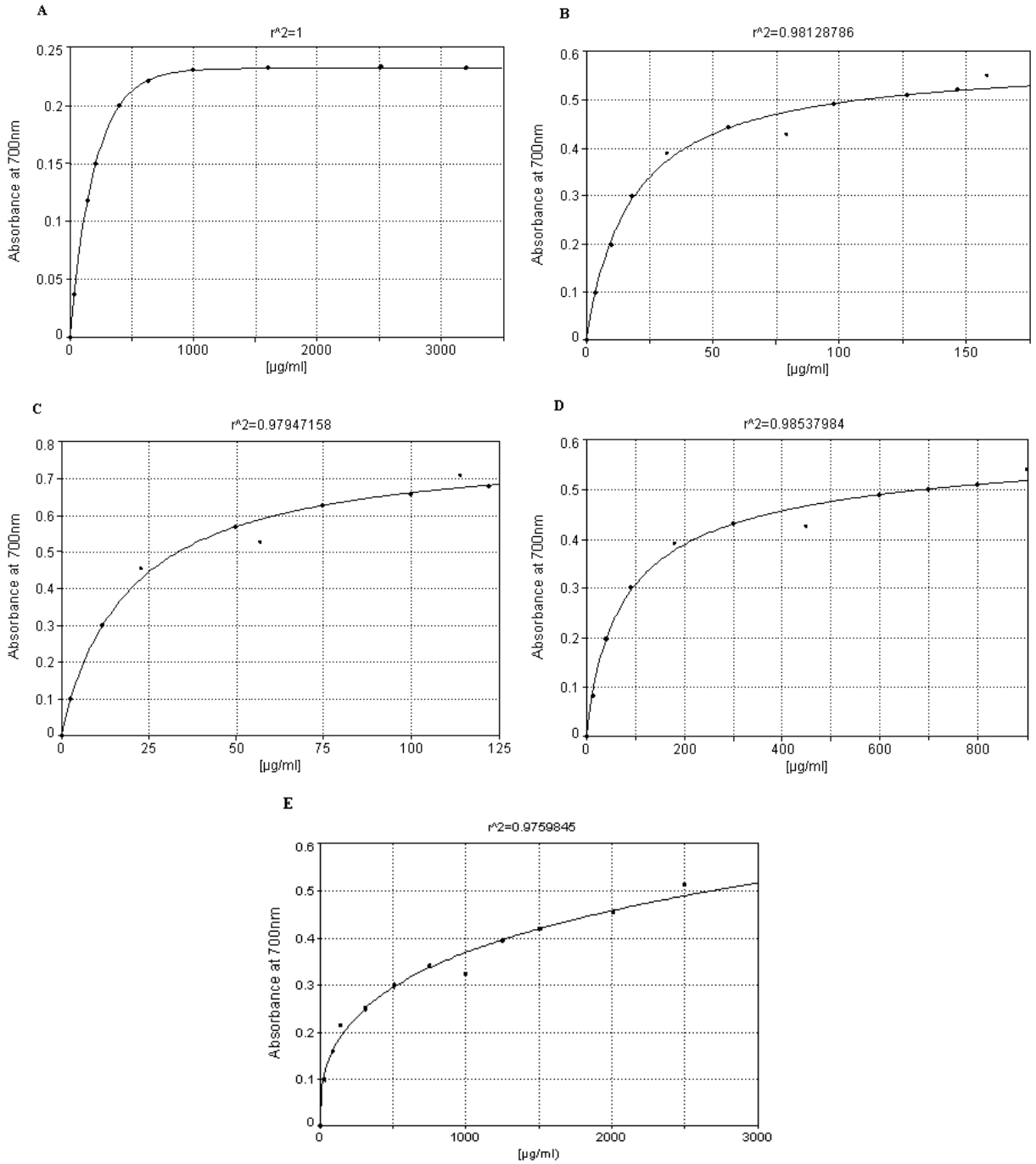

Fig. 1 Reducing power of crude extract and fractions compared to antioxidant standard; ascorbic acid.

which is a stable free radical and converts it to α-α-diphenyl-β-picrylhydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant extract [36]. From result, it may be postulated that crude extract and different fractions have hydrogen donors, thus scavenge free radical DPPH but the best DPPH scavenging activity was exerted by crude methanolic extract and ascorbic acid as reference.

The quality of the antioxidants in the extracts was determined by the IC50 values, a low IC50 value indicates strong antioxidant activity. In the present study, the radical scavenging activity of crude

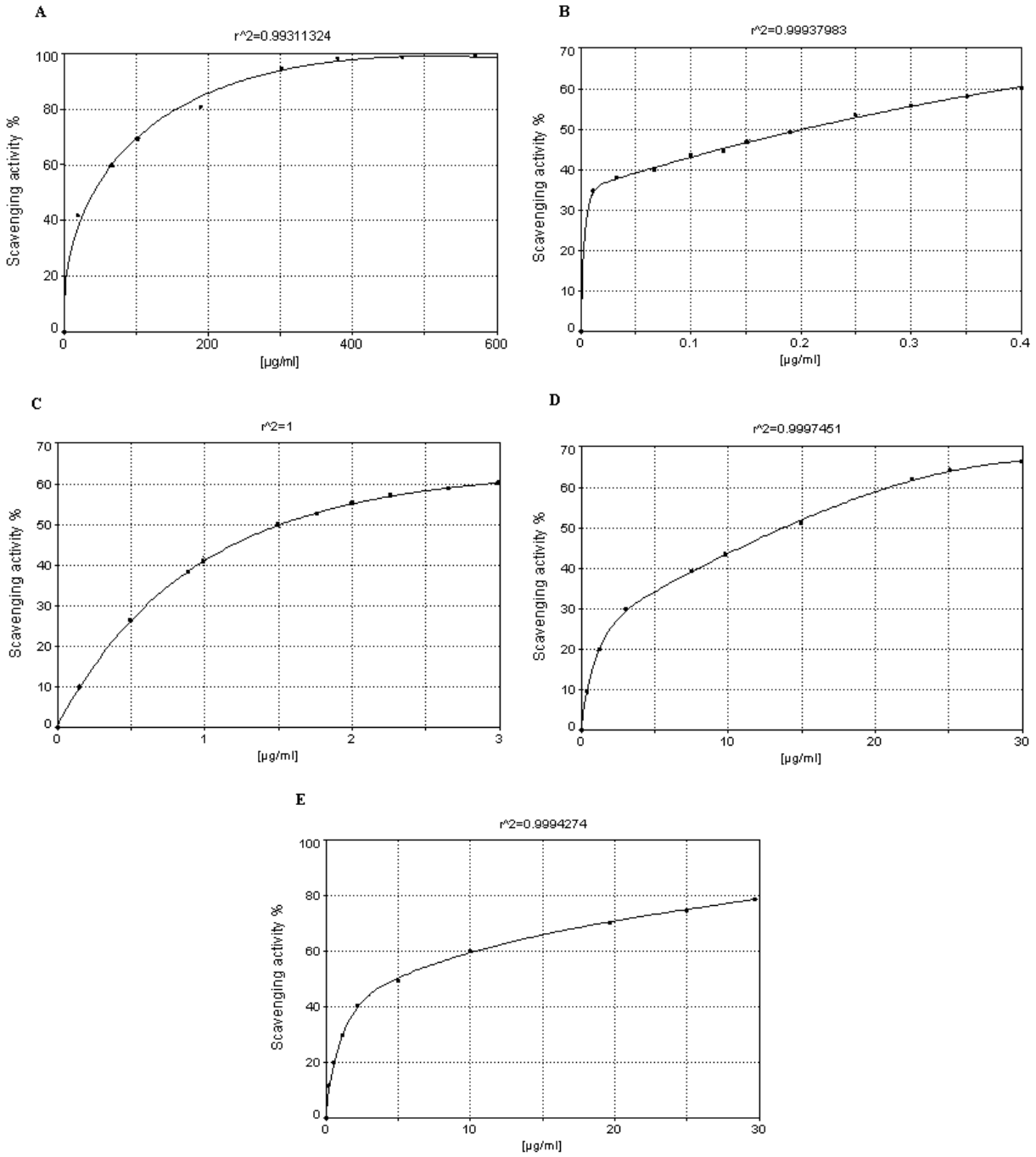

Fig. 2 Hydrogen peroxide scavenging activity of crude extract, fractions and ascorbic acid.

methanolic extract was more than that of the tree fractions. It means that the IC50 of crude methanolic extract was less than those of others but the standard have a high antioxidant power. IC50 for ascorbic acid, crude extract, aqueous fraction, butanolic fraction and ethyl acetate fraction were 4.498 ± 0.75095 µg/mL, 49.357 ± 2.42679 µg/mL, 892.03 ± 11.60786 µg/mL, 1.002 ± 0.07071 mg/mL, 2.653 ± 0.40164 mg/mL respectively.

It has been mentioned that the antioxidant activity of plants might be due to their phenolic compounds [37] and correlated with the content of this groups of compounds [38]. The leaves of *Tamarix pauciovulata* are rich on phenolic compounds and present a good

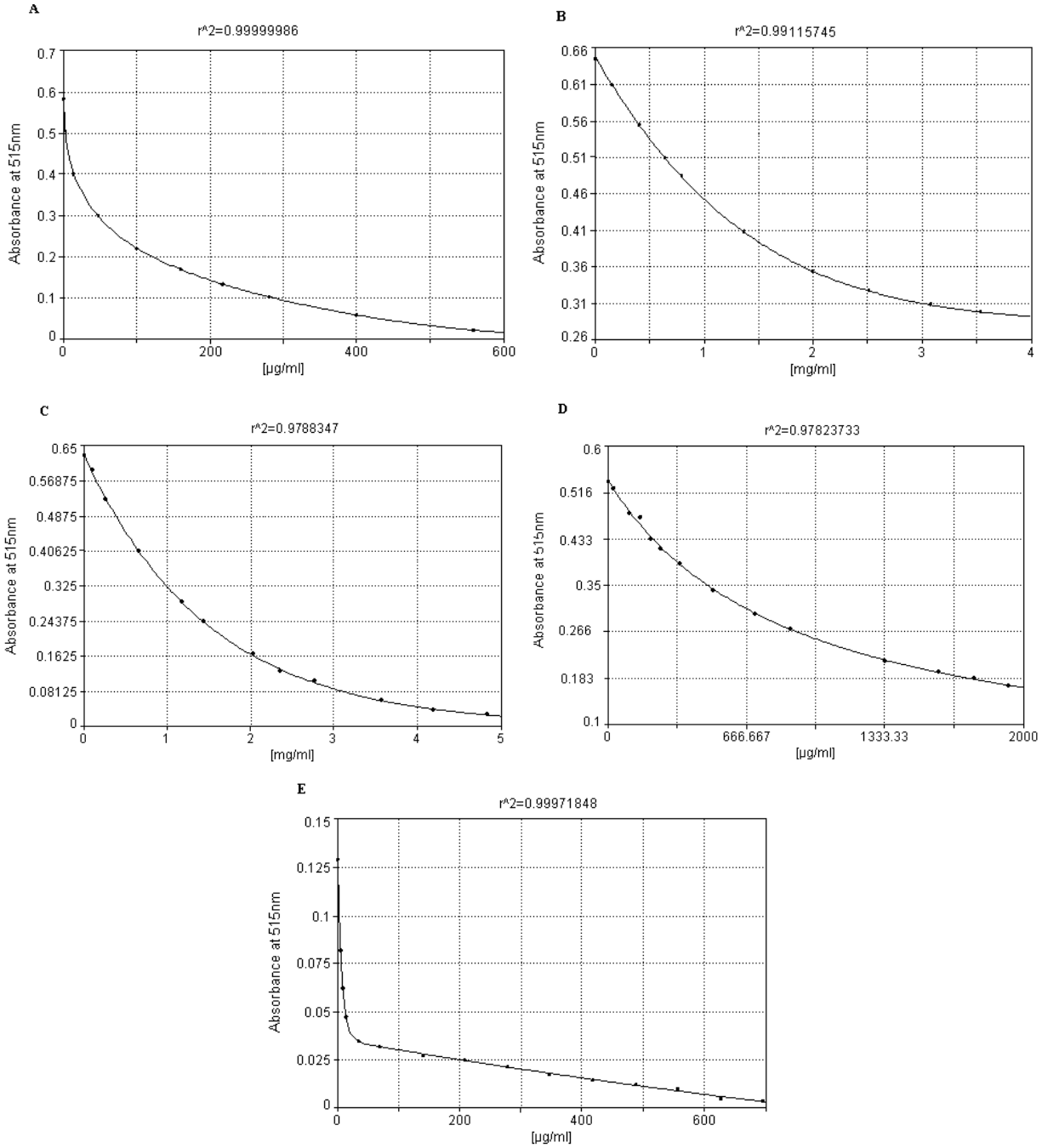

Fig. 3 DPPH scavenging activity of crude extract, fractions and ascorbic acid.

source of natural antioxidants. Free radicals cause autoxidation of unsaturated lipids in food [39] and Antioxidants cease the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups. Therefore formed stable end-product does not permit further oxidation of the lipid [40]. *In vitro* antioxidant activity of quercetin was tested for DPPH free radical, superoxide anions, hydrogen peroxide and hydroxyl radical. It scavenges oxygen radicals, inhibits xanthine oxidase, protects against lipid peroxidation, chelates metal ions and forms inert complexes that can't take part in the conversion of superoxide radicals and hydrogen peroxide into hydroxyl radicals [41].

4. Conclusion

In conclusion, the leaves extract and fractions showed antioxidant activity, by measuring their capacity to scavenge the DPPH and the hydrogen peroxide and to reduce iron (III) to iron (II). The results of the present study indicate that leaves from *Tamarix pauciovulata* can be used as easily accessible source of natural antioxidants. The high antioxidant activity showed may be directly correlated to the high phenolic contents of the leaves in particularly of a high content on synergic acid and quercetin.

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