HPLC-UV Analysis and Antioxidant Activities of Phenolic Compounds from Bark of *Tamarix articulata* vahl Shrubs Grown in Mosul Province.

N.A. S. Dawood1*, A. C. Al-Daody2 and T. K. AL-Takay3

1Employer in directorate of forest and rangeland/Duhok governance/Kurdistan regional/Iraq.
2Education College for girls, University of Mosul, Iraq.
3College of agriculture and forest, University of Mosul, Iraq.

*Corresponding author's e-mail: Zawi_nasser@yahoo.com

**Abstract.** Analytical trial was carried out to investigate phenolic compounds of *Tamarix articulata* (vahl.) Karst shrubs were extracted from dry powdered bark with different solvents: Ethyl acetate; Industrial methylated spirits (IMS) and distilled water, and their antioxidant activity were assayed via in vitro radical scavenging activity using DPPH assay. HPLC analysis showed that there were at least 6 remarkable phenolic compounds identified of bark, which included: (Apigenin, Caffeic acid, Chlorogenic acid, kaempferol, Quercetin and Rutin). Furthermore, both of crude extracts IMS and distilled water was better than crude ethyl acetate extract for extracting the bioactive compounds particularly the number and concentration of phenolic compounds identified in bark. The crude extract of IMS achieved the highest total concentration of 0.471 (mg g⁻¹), followed by the crude extract for hot water was 0.226 (mg g⁻¹) and the lowest crude extract for ethyl acetate was 0.031 (mg g⁻¹). However, all the phenolic compounds separated from the crude extracts were free radical inhibitors of DPPH but both the IMS crude extract and ethyl acetate at the same concentration of 200 (µ ml⁻¹), and at rates of (96.7% and 96) (%), respectively, were significantly superior, on the phenolic compounds separated from the aqueous crude extract. Additionally, it’s superior on the standard sample.

**1. Introduction**

Phenolic compounds are natural compounds most explored due to their potential curing properties as shown in several studies [1]. These compounds are referred as refer to "phenolic compounds", "phenols" or "polyphenols", More than 8,000 compounds have been identified in the plant kingdom, according to [2]. These phenolic compounds have one or more aromatic rings with one or more hydroxyl groups, and have been categorized into flavonoids and non-flavonoids [3, 4].These compounds are from the production of secondary metabolites by the shikimic acid pathway [4, 5]. Phenolic compounds regulate various metabolic processes including structure, growth, pigments and resistance to various pathogens in plants [6]. Free radicals are produced in the body in small amounts, the excess of these radicals is called "oxidative stress" [7]. This stress
is involved in many diseases such as cancer, diabetes, Alzheimer, rheumatism and cardiovascular [8]. Therefore, antioxidants, such as flavonoids and other phenols have gained more attention in recent years as potential agents for preventing and treating a number of chronic diseases associated with oxidative stress [9-14].

The Tamarix belong to family (Tamaricaceae) is considered halophytes and include more than 60 species grown in nearly all parts of the world [15]. There is one genus of this family in Iraq table (1), which is the genus Tamarix L, and it is called al-Athl or al-Tarfa and located within this genus Tamarix articulata vahl. It is also named is Tamarix aphylla. The Tamarix plant (Tarfa) is used in traditional medicine as an active compound used in part to refer to bark and gall that are used in various diseases such as tonic and aphrodisiac, astringent because it contains the main active compound which is tannin and is traditionally used to treat skin diseases such as eczema and is also used for hepatitis and syphilis and scaly skin diseases [16]. The aerial parts of T. articulata are used in folk medicine to treat many diseases such as diuretics, diabetes, anti-varicose, anti-worms, and hemorrhoids, gingivitis, as antibiotics, jaundice and for treatment of arthritis diarrhea [17-19]. Numerous previous studies on the pharmacological and biological activities of all parts of the tamarix plant indicated that it has antioxidant, antimicrobial, anti-inflammatory, cytotoxic, antifungal antipyretic, analgesic, anti-Alzheimer, anti-hyperglycemic and hepatic protective effects [16,20]. Different formulations of phenolic compounds of T. articulata have also been reported in previous scientific sources, all previous research about phytochemical on bark has been shown contains of Polyphenols, Flavonoids, Tannins, Saponin, Cumarins, Triterpenes and Alkaloids [21-24], While [25] found that the bark of T. articulata contains triterpeneD-friedoolean-14-en-3α,28-diol (isomyricadiol), its 3β isomer (myricadiol) and its 3-ketone. HPLC analysis of methanol extracts from T. articulata growing in southern Algeria also showed that there are at least eight phenolic compounds noticeable in the leave extract, the most important of which are vanillic acid, vanillic, naringin, caffeic acid, rutin, chlorogenic acid, p-coumaric acid and gallic acid [26]. According to the results of [27], analysis of leave and stem extracts of T. articulata of polyphenols, performed by HPLC-UV / DAD, HPLC-ESI-MS and MS², showed the presence of the following compounds: gallic acid, Caffeic acid, p-coumaric acid, ellagic acid, syringic acid isomer, luteolin, apigenin, quercetin, kaempferol, isorhamnetin, tamarixetin, quercetin dimethyl-ether, tamarixin, kaempferide and kaempferol-7,4’-dimethyl ether, and the quantitative analysis showed that the largest amount of total polyphenols was in the leaves compared to the stem. The aims of the present work are: (i) to identify the phenolic compounds of bark extracts from T. articulata, (ii) to estimate the antioxidant activities of phenolic compounds separated from bark extracts.

2. Materials and Methods

2.1 Taxonomy of Tamarix articulata

Table (1) Taxonomy of Tamarix articulata (vahl.) is given below [26, 28].

| Kingdom                     | Plantae            |
|-----------------------------|--------------------|
| Subkingdom:                 | Tracheobionta      |
| Division:                   | Magnoliophyta      |
| Class:                      | Dicotyledons       |
| Subphylum:                  | angiosperms        |
| Branching:                  | spermatophytes     |
| Genus:                      | Tamarix            |
| Subclass:                   | Archichlamydes     |
| Order:                      | Parlétales         |
| Species:                    | articulata Vahl.   |
| Synonym:                    | aphylla (L.)Krast  |
2.2 Experiment material and site
One shrub was chosen from the ideal healthy and without defects at the age of (14) years growing within the campus of the University of Mosul located at the northern end of the city of Mosul on the eastern side of the Tigris River, and after determining the northern direction of the shrub, it was dropped on the 20th of December of the year (2019). Were taken as tablets, 20 cm thick, at height (1.3 m) of the shrub.

2.3 Preparation of samples
Samples were taken as disc with a thickness of (20 cm) at height level (1.3 m), and the bark was removed from the wood, then the bark were cut into small pieces and left to air drying, after which grinded and screened bark, then the minutes that passed through the sieve (30 mesh) and settled on the sieve (50 mesh) were collected.

2.4 Preparation of extract
Plant extracts were prepared according to the method mentioned by [29], which depends on the nature of the active ingredients separated from the plants and the type of solvent used in the separation process, and by using the successive solvent system. Three solvents of different polarity were used in the extraction process: ethyl acetate, Industrial methylated spirits (IMS), which is a solvent system, namely (95% ethanol with 5% methanol), and hot aqueous extract. Extraction process was carried out depending on the boiling point of each solvent. (25) grams of dry powder of bark were placed well in the Batch in the Soxhlet continuous extraction apparatus, and 400 ml of solvent was added and the batch was soaked with solvent for 48 hours, after which the extraction continues at a rate of 7 hours per day until the solvent used in the apparatus becomes colorless and then the second solvent is applied, and so the process is repeated on the rest of the solvents and on the same plant model, and then the extract is concentrated by the rotary evaporator rotary vacuum evaporator at 40°C Then, Crudes extracts are placed in opaque glass bottles and closed tightly and placed in the refrigerator until use [30].

2.5 Phenols separation and purification using acid hydrolysis
(5) ml of crude extract of each of ethyl acetate, IMS and hot water were taken separately and 25 ml of HCl (1N) acid were added to it, after which the reflux was performed at a temperature of 100 °C for a period of one hour, then the solution was cooled down and placed in a separating funnel and 50 ml of ethyl acetate was added to it twice with continues shaking., then two upper layers (organic layer) of ethyl acetate and a bottom layer were obtained; The top layer was taken and 3 g of MgSO₄ was added to it. The samples were kept in sealed and opaque bottles and placed in the refrigerator until they were identified by the HPLC device [31] and their antioxidant efficacy was studied. Then the concentration of the separated phenolic compound was calculated by applying the equation according to [32]. Then the batch was taken and the plant material was soaked in baker with 400 ml distilled water and placed on the magnetic stirrer at a temperature of 60 °C to obtain the hot aqueous extract [33].

2.6 Phenolic identification using HPLC-UV
The identification of phenolic compounds was carried out in the laboratories of the Ministry of Science and Technology / Department of Environment and Water after conducting the acid hydrolysis process, According to the method presented before [34], By using high performance liquid chromatography device (HPLC) Type (SYKAMN) Of German origin, with a flow rate of
1.3 (ml min\(^{-1}\)). The mobile phase is (A), which include (Methanol: D.W.: Formic acid) (70:25:5), with the column (18-ODS) has dimensions (25cm * 4.6mm) and the responses were detected at the UV-280nm wavelength.

2.7 Antioxidant assays
For studying the activity of the phenolic compounds separated from the crude extracts of bark as an antioxidant to know the effect of their activity against free radicals, the method of (DPPH) was used, which is a common abbreviation for the organic chemical compound (2, 2-diphenyl-1-picrylhydrazil), which is a free fixed radical, Where a weight of 15.8 mg was taken and dissolved in 200 ml of methanol to obtain 200 mm. Different concentrations of phenolic compounds separated from crude extracts ranged from 50, 100, 150, 200 (µ ml-1), and ascorbic acid was used as a control sample, after which 1 ml of (DPPH) solution was added to each concentration besides the control sample, after which the samples were incubated at room temperature for 30 minutes in the dark, and each sample was measured at a wavelength of 517 nanometers by a (Jenway-UV-6705) Spectrophotometer of British origin, unicellular and then applied the following equation to find out the ratio Percentage for inhibiting free radicals [35-37]: \( \% = \frac{A_{BB} - A_{BS}}{A_{BB}} \times 100 \) Where: \( \% \) is an inhibition percentage of DPPH, \( A_{BB} \) is the absorbance of the control at 30 min, \( A_{BS} \) is the absorbance of the sample at 30 min, The analysis of all the samples were done in triplicate. A high percentage (\( \% \)) value showed the higher antioxidant activity of the plant extract.

2.8 Statistical Analysis
Data of the experiment were analyzed using Completely Randomized Design (C.R.D.) and using the electronic computer according to the SAS system programs (2002) in factorial experiments.

3. Results and Discussion:
3.1 Identification of a number of phenolic compounds with HPLC technology of study plants, both quality and quantity:
The chromatographic analysis charts were obtained, in which the retention time of each compound was determined for the study sample and the standard samples, Figures (1-3) which included Apigenin with a retention time of 7.020 minutes, Caffeic acid with a retention time of 6.560 minutes, Chlorogenic acid with a retention time of 2.703 minutes, kaempferol with a retention time. 4.460 minutes, Quercetin has 5.196 minutes, and Rutin has 5.617 minutes. The identification showed that the phenolic compounds separated into the study sample (bark) agree to the standard phenolic compounds Table (2), Table (3) and figures (1-9), which included:

3.2 Chlorogenic acid
It is evident from the results that the phenolic compound Chlorogenic acid was present in all the crude extracts under study for the bark for both the crude extracts of IMS and the hot water at a retention time of 2.703 minutes, with the exception of the crude extract of ethyl acetate, the compound did not prove its presence in the bark, Table 2 and figures (1-3), it appeared in the crude extract of IMS with the highest concentration compared to the aqueous crude extract of (0.107 and 0.043) (mg g\(^{-1}\)), respectively.

3.3 Kaempferol
Chromatographic results indicate to the appearance of the compound keampferol in both the crude extracts of IMS and hot water for the bark, at a retention time of 4.577 minutes and at a concentration of (0.003 and 0.001) (mg g\(^{-1}\)), respectively, while the compound was not proven to be present in the crude extract of ethyl acetate, Table 2 and Figures (1-3).

Quercetin The phenolic compound Quercetin appeared in the crude extracts of IMS and hot water for the bark figures (1-3), with the exception of the crude extract of ethyl acetate, the compound
did not prove its presence in the bark, as it was present at a retention time of 5.393 minutes and at a higher concentration in the crude extract of IMS compared to the aqueous crude extract, It was 0.107 and 0.042 (mg g⁻¹), respectively, Table 2.

Rutin Chromatographic identification by HPLC technology proved that the phenolic compound Rutin did not appear in the bark, except in the crude extract of ethyl acetate only at a concentration of 0.0006 (mg g⁻¹) and with a retention time of 5,913 minutes, Figures (1-3), and it did not appear in the rest of the other extracts under study, This indicates the lack of the compound and its scarcity in the bark, Table 2.

3.4 Caffeic acid

Chromatographic results indicated that caffeic acid was found in all crude extracts of ethyl acetate, IMS, and hot water for bark figures (1-3), at a retention time of 6.730 minutes, with the highest concentration in the crude extract of IMS amounting to 0.030 (mg g⁻¹), followed by The aqueous crude extract reached 0.013 (mg g⁻¹), and the lowest concentration of the ethyl acetate crude extract was 0.002 (mg g⁻¹), Table 2.

3.5 Apigenin

The chromatographic identification showed the presence of the phenolic compound Apigenin in each of the crude extracts of ethyl acetate, IMS and hot water for bark table 2 and Figures (1-3),The compound appeared in all the crude extracts at a retention time of 7.307 minutes and at a high concentration in the crude extract of IMS amounted to 0.224 (mg g⁻¹), followed by the aqueous crude extract at a concentration of 0.127 (mg g⁻¹) and the lowest concentration in the crude extract of ethyl acetate was 0.029 (mg g⁻¹).

It is evident from table 2 that the phenolic compounds identified with HPLC technology for *T. articulata* varied in the number, quality and concentrations of the phenolic compounds separated from the crude extracts under study, depending on the number of phenolic compounds identified, so the total number was (6) compounds, The total number varied according to the crude extracts used in the study. In the crude extract of ethyl acetate only (3) phenolic compounds were identified, while the number of the separated compounds in the crude extracts of IMS and hot water were (5) phenolic compounds, and based on the total concentration of the extract in the bark, The crude extract of IMS achieved the highest total concentration of 0.471 (mg g⁻¹), followed by the total concentration of the crude extract for hot water was 0.226 (mg g⁻¹), and the lowest total concentration of the crude extract for ethyl acetate was 0.031 (mg g⁻¹).

And based on the quality of the phenolic compounds identified in the bark for each crude extract under study Table 2, the phenolic compound Apigenin was formed with the highest concentration in the crude extracts of IMS and hot water, which was (0.224 and 0.127) (mg g⁻¹), respectively, followed by Chlorogenic acid and the Quercetin in IMS crude extract at a concentration of 0.107 (mg g⁻¹) compared to the rest of the other phenolic compounds identified, while the lowest concentration of crude ethyl acetate extract was 0.0006 (mg g⁻¹) compared with the concentration of other phenolic compounds.

The results show that the use of consecutive polar solvents in separating the phenolic compounds, namely ethyl acetate, IMS and hot water, resulted in the separation of the largest amount of phenolic compounds with varying efficiencies, Because the separation system of compounds is subject to the type of polarity and force of separation of the compound, and because the compounds are more familiar to one solvent than another [38-39]. In ethyl acetate solvent: it led to the separation of a small number of phenolic compounds at low concentrations, and this is due to the fact that it is the lowest polar (semi-polar solvent), while in the IMS solvent it obtained a higher share of the concentrations compared to hot water, which is higher polar and collected a larger quantity, although that the number of separated compounds was equal in the both of them, and this is due to the fact that phenols dissolve normally in polar organic solvents (unless they are bounded to a linkage) [40], and ethanol is an effective solvent for dissolving
phenols [41]. It is one of the most effective [39, 42]. Since the IMS solvent one of a mixture of two solvents, which is (95%) ethanol and (5%) methanol, this led to an increase in its polarization efficiency as well as preceded hot water in extraction.

Phenolic compounds play an important role in the normal growth and development of the plant as they work to protect it from external pressures and influences and defend it against infection and pathological injuries, and they also play an important role in the biological representation of the plant and regulating the transport of auxins in it [24], as are prolin and organic acids. The other is important for the plant, as it improves plant growth and modifies its response to drought in order to maintain plant cellular functions [43].

The bark of *T. articulata* shrub (Tarfa) differed among themselves in relation to the type of compounds separated, as well as their quantity and concentration, as a result of a number of factors that lead to the variation of the compounds in terms of their quality and quantity in the plant parts, including the age of the tree as the age of the tree affects the extracts vary in their quantity, content and quality, as nutrients accumulate over time due to an increase in crown size, which leads to an increase in the amount of nutrients produced and thus the amount of stored materials will increase with age [44, 45], as indicated by [46] Indicates that there are changes in the total content of phenols with age, and it was found that the content of organic compounds, whether in whole or individually, changes according to the age of the tree and the diversity of the compounds [47].

The abiotic natural environmental conditions also have an effect on the chemical content of the plant, such as soil fertility, temperature, humidity, carbon dioxide ratio, ozone and other factors that lead to change in the content of chemical compounds in the plant [48]. In compounds that dissolve in water, as it reduces its concentration, especially in the bark [40], and that the genetic factor has an effect on the content of compounds in the plant as its effect is stronger than that of the environmental factor [49].

Table (2) Phenolic compounds identified using HLPC technology separated from the crude extracts of ethyl acetate and IMS and hot water for bark of *T. articulata*.

| Seq | Standard phenolic compounds | Ethyl acetate | IMS | Hot water |
|-----|----------------------------|---------------|-----|-----------|
|     |                            | Conc (mg g⁻¹) | R.T (min) | Conc (mg g⁻¹) | R.T (min) | Conc (mg g⁻¹) | R.T (min) |
| 1   | Chlorogenic acid            | 2.980         | ---       | 0.107       | 2.703     | 0.043       | 2.703     |
| 2   | Kaempferol                  | 4.460         | ---       | 0.003       | 4.577     | 0.001       | 4.577     |
| 3   | Quercetin                   | 5.196         | ---       | 0.107       | 5.393     | 0.042       | 5.393     |
| 4   | Rutin                       | 5.617         | 0.006     | 5.913       | ---       | ---         | ---       |
| 5   | Caffeic acid                | 6.560         | 0.002     | 6.730       | 0.030     | 6.730       | 0.013     | 6.730     |
| 6   | Apigenin                    | 7.020         | 0.029     | 7.307       | 0.224     | 7.307       | 0.127     | 7.307     |
|     | The total concentration of the extract | 0.031 | 0.471 | 0.226 |

---: The compound did not appear.
IMS: meaning the solvent is a mixture of two solvents, namely (95% ethanol with 5% methanol).
R.T: meaning retention time.
Conc: concentration.

Table (3) Detection of phenolic compound based on (Retention time, area and area %) for the crude extracts of ethyl acetate, IMS and aqueous for bark of *T. articulata* via HLPC technology.

| Seq | Phenolic compounds | Ethyl acetate | IMS | Aqueous |
|-----|-------------------|---------------|-----|---------|
|     |                    | R.T | Area | Area % | R.T | Area | Area % | R.T | Area | Area % |
| 1   | Chlorogenic acid  | 2.70 | 16323.51 | 24.5 | 2.703 | 6600.055 | 23.2 |
| 2   | Kaempferol        | 4.57 | 3401.820 | 5.1  | 4.577 | 1566.446 | 5.5  |
|   | Phenolic Compound  | R.T (min) | Area (mAU) | Retention Time (min) | Area 1000 | Area 10000 | Area 100000 |
|---|-------------------|-----------|------------|---------------------|------------|------------|-------------|
| 3 | Quercetin         | 5.39      | 35297.96   | 53                  | 14014.34   | 49.2       |
| 4 | Rutin             | 5.913     | 305.825    | 17.8                | ---        | ---        | ---         |
| 5 | Caffeic acid      | 6.730     | 179.993    | 10.4                | 2111.703   | 6.73       | 954.548     |
| 6 | Apigenin          | 7.307     | 1237.09    | 71.8                | 9456.376   | 14.2       | 5372.069    |

---: The compound did not appear.
IMS: meaning the solvent is a mixture of two solvents, namely (95% ethanol with 5% methanol).
R.T: meaning retention time.

---

Figure (1) Standard curve of phenolic compounds identified for the crude extract of ethyl acetate for bark *T. articulata*.

---

Figure (2) Standard curve of phenolic compounds identified for the crude extract of IMS for bark *T. articulata*. 
Figure (3) Standard curve of phenolic compounds identified for the crude extract of Hot water for bark *T. articulata*.

Figure (4) Standard curve of Chlorogenic acid.

| Reten. Time [min] | Area [mV*μL] | Height [μV] | Area [%] | Height [%] | WRS [min] | Compound Name |
|-------------------|--------------|-------------|----------|------------|-----------|---------------|
| 1                 | 2,900        | 758.564     | 175.516  | 100.0      | 100.0     | F_{ch} chlorogenic acid 10 ppm - Detector 2 |
| Total             | 758.564      | 175.516     | 100.0    | 100.0      | 0.08      |               |
Figure (5) Standard curve of kaempferol.

Figure (6) Standard curve of Quercetin.

Figure (7) Standard curve of Rutin.
3.6 Phenolic compounds as antioxidants

The results of Table (4) indicated that the percentages of scavenging to free radicals by the phenolic compounds separated from the bark of *T. articulata*, the highest levels of inhibition were for the phenolic compounds separated from the crude extracts of IMS and ethyl acetate at the same concentration of 200 (µ ml<sup>-1</sup>), at rates of (96.7 and 96) (%), respectively, which had a significant agreement among them and were significantly higher in inhibiting free radicals of DPPH, compared to the phenolic compounds separated from aqueous crude extracts, in addition to the standard sample, Figures (10). As it can be seen from the table (4), the higher the concentration of the extract, the more effective it is in inhibiting, and this is what was indicated by [50], where they used different concentrations of the leaf extract of *T. articulata*, ranging between (10% to 400%) (µ ml<sup>-1</sup>), in order to scavenging free radicals with the use of (DPPH), their results show that the extract has a maximum active radical scavenging at 400 (µ ml<sup>-1</sup>). The results also indicated that the phenolic compounds separated from the aqueous crude extract of bark were less effective as antioxidants in scavenging free radicals, reaching (81.6%), compared with the rest of the other phenolic compounds (Figure 10).
These results are in agreement with what was indicated by [51] that the total content of phenols separated from crude extracts of (methanol, ethanol, acetone and distilled water) of \textit{T. articulata} leaves had a role in all the extracts as inhibitors of free radicals of DPPH. The results of the study are also in agreement with what [52] stated that the Tunisian \textit{T. articulata} extract has antioxidant activity according to the total phenolic content in the extract and also the results of the study are in agreement with what was found [53] that antioxidant molecules such as polyphenols and flavonoids reduced the free radical capacity of DPPH.

From the above results, it is evident that the processes of scavenging free radicals by natural phenolic compounds and reducing their risk are of great importance, by giving the hydrogen atom to the free radical DPPH and stabilizing it, and the reason for its strong effectiveness is the number of phenolic compounds in each sample and what it contains each of the hydroxyl groups is in line with what [54] reported that the activity of phenolic antioxidants is mainly due to its oxidation and reduction properties, and its ability to donate hydrogen, inhibiting the free radical oxygen reaction as well as stopping it to generate new free radicals, thus inhibiting oxidation. Lipids, proteins and DNA, by inhibiting the enzymes involved in generating free radicals [55].

Table (4) Concentration and percentage of the standard sample and the separated phenolic compounds from bark of \textit{T. articulata} as an antioxidant measured in (µ ml⁻¹).

| Phenolic compounds | Bark | Conc (µ ml⁻¹) | Ethyl acetate | IMS | Hot water | Standard sample |
|--------------------|------|--------------|---------------|-----|----------|-----------------|
|                    |      | 50           | 92.8 c        | 88.4 fg | 81.6 j  | 81.2 j          |
|                    |      | 100          | 93.4 bc       | 90.1 de | 87.6 g  | 82.8 i          |
|                    |      | 150          | 93.9 b        | 94.5 b  | 89.3 ef  | 85.4 h          |
|                    |      | 200          | 96a           | 96.7a   | 90.7 d  | 85.6 h          |

The different letters mean that there are significant differences at the 0.0001 probability level according to the Duncan Multiple Range Test.

Fig (10) The effectiveness of the phenolic compounds separated from the bark of \textit{T. articulata} shrub as antioxidants compared with ascorbic acid as a standard sample according to the DPPH method.

4. Conclusion
Identification phenolic compounds separated from bark extracts of \textit{T.articulata} shrub by HPLC analysis, and in vitro antioxidant activities assayed have been examined. With a powerful analytical HPLC technique, the identification and quantification of (6) phenolic compounds were achieved for bark of \textit{T. articulata}. The richness of the extracts with the phenolic and flavonoid
content was remarkable, and might be considered as a source of these compounds, which can be used as anti-radical drugs. Furthermore, in vitro assays showed high antioxidant activity, sufficient ability to inhibit the DPPH radical, and next prevent diseases caused by the overproduction of free radicals and can become important source of dietary compounds with health protective potential. Further work on phytochemical constituents and purification of individual groups of bioactive components can reveal the exact potential of the plant to inhibit free radicals and several pathogenic microbes.

References

[1] Del Rio,D, Costa, L G, Lean,M E J, and Crozier, A 2010, Polyphenols and health: What compounds are involved? Nutrition, Metabolism and Cardiovascular Diseases, 20(1), 1–6.
[2] AlJaber,N, and Allehaib,L 2017, The Pharmacological activity of some tamaricaceae plants. Natural Products Chemistry and Research, 5, 262.
[3] Ho,C T 1992, Phenolic compounds in food an overview. acs symposium series. American Chemical Society. Washington, DC. Vol. 507.
[4] Cartea,M E, Francisco,M, Soengas,P, and Velasco, P 2010, Phenolic Compounds in Brassica Vegetables. Molecules, 16(1), 251–280.
[5] Talaputra,S K, and Talaputra,B 2015, Shikimic acid pathway. In: chemistry of plant natural products: stereochemistry, Conformation, Synthesis, Biology and Medicine. pp. 625–678 Springer Berlin Heidelberg. DOI.org/10.1007/978-3-642-45410-3.13.
[6] Naumovski,N 2015, Bioactive composition of plants and plant foods. pp. 81–115. In: C. J. Scarlett and Q. V. Vuong (eds), plant bioactive compounds for pancreatic cancer prevention and treatment. NOVA Publishers, New York.
[7] Favier,A 2003, The oxidative stress: Concept and experimental interest to understand diseases mechanisms and therapeutic approaches. Journal of Actualite Chimique. 108-115.
[8] Bidie,A P, N’guessan, B B, Yapo,A F, N’guessan, J D, and Djaman,A, J 2011, Activités antioxydantes de dix plantes medicinales de la pharmacopée ivoirienne. Sciences and Nature, 8, N°1: 1 - 11.
[9] Rice-Evans,C A, Miller,N J, and Paganga,G 1996, Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine, 20(7), 933–956. DOI: 10.1016/0891-5849(95)02227-9.
[10] Stanner,S, Hughes,J, Kelly,C, and Butteriss,J 2004, A review of the epidemiological evidence for the “antioxidant hypothesis.” Public Health Nutrition, 7(03). DOI: 10.1079/phn2003543.
[11] Dimitrios,B 2006, Sources of natural phenolic antioxidants. Trends in Food Science and Technology, 17(9), 505–512.
[12] Fu,L, Xu,B T, Xu, X R, Qin, X S, Gan, R Y, and Li, H B 2010, Antioxidant capacities and total phenolic contents of 56 Wild Fruits from China. Molecules, 15(12), 8602–8617. DOI: 10.3390/molecules15128602.
[13] Galleano,M, Verstraeten,S V, Oteiza,P I, and Fraga,C G 2010, Antioxidant actions of flavonoids: Thermodynamic and kinetic analysis. Archives of Biochemistry and Biophysics, 501(1), 23–30. DOI: 10.1016/j.abb.2010.04.005.
[14] Gharekhani,M, Ghrbani,M, and Rasoulnejad,N 2012, Microwave-assisted extraction of phenol and flavonoid compounds from Eucalyptus camaldulensis Dehn leaves as compared with ultrasound-assisted extraction. Latin American Applied Research. 42, 305–310. https://www.researchgate.net/publication/259452213.
[15] Samadi,N, Ghaffari, S M, and Akhani,H 2013, Meiotic behaviour, karyotype analyses and pollen viability in species of Tamarix (Tamaricaceae). Willdenowia, 43(1), 195-203. DOI: http://dx.doi.org/10.3372/wi.43.43121.
[16] Jasiem, T M, Nasser, N M, and Albazaz, H 2019, Tamarix aphylla L.: A Review. Research Journal of Pharmacy and Technology, 12(7), 3219-3222. DOI: 10.5958/0974-360X.2019.00541.9.

[17] Sultanova, N, Makhmoor, T, Abilov, Z, Parween, Z, Omurkamzinova, V, ur-Rahman, A, and Choudhary, M I 2001, Antioxidant and antimicrobial activities of Tamarix ramosissima. Journal of Ethnopharmacology, 78(2-3), 201–205.

[18] Sultanova, N, Makhmoor, T, Yasin, A, Abilov, Z A, Omurkamzinova, V B, Rahman, A U, and Choudhary, M I 2004, Isotamarixen - A New Antioxidant and Prolyl Endopeptidase-Inhibiting Triterpenoid from Tamarix hispida . 2004. Planta Medica, 70(1), 65–67. DOI: 10.1055/s-2004-815458.

[19] Marwat, S K, Rehman, F, Khan, M A, and Ahmad, M 2011, Medicinal folk recipes used as traditional phyto therapies in district Dera Ismail Khan, KPK, Pakistan. Pakistan Journal of Botany, 43(3), 1453-1462.

[20] Bahramsoltani, R, Kalkhorani, M, Abbas Zaidi, S M, Farzaei, M H, and Rahimi, R 2019, The genus Tamarix: Traditional uses, phytochemistry, and pharmacology. Journal of Ethnopharmacology, 112245. Doi:10.1016/j.jep.2019.112245.

[21] Sultanova, N A, Abilov, ZA, Omurkamzinova, V B, and Chaudri, I M 2002, Flavonoids of Tamarix hispida. Chemistry of Natural Compounds. 38, 98-99. https://doi.org/10.1023/A:1015706520986.

[22] Gaskin, J F, and Shafroth, P B 2005, Hybridization of Tamarix ramosissima and T. chinensis (Saltcedars) with T. aphylla (Athel) (Tamaricaceae) in the Southwestern USA Determined from DNA Sequence Data. Madroño. Madroño, 52(1), 1–10. DOI: 10.3120/0024-9637.

[23] Saidana, D, Mahjoub, M A, Boussaada, O, Chriaa, J, Chérif, I, Daami, M, and Helal, A N 2008, Chemical composition and antimicrobial activity of volatile compounds of Tamarix boveana (Tamaricaceae). Microbiological Research, 163(4), 445–455.

[24] Ksouri, R, Falleh, H, Megdiche, W, Trabelsi, N, Mhamdi, B, Chaieb, K, and Abdelly, C 2009, Antioxidant and antimicrobial activities of the edible medicinal halophyte Tamarix gallica L. and related polyphenolic constituents. Food and Chemical Toxicology, 47(8), 2083–2091. DOI: 10.1016.

[25] Tabet, A, Boukhari, A, and Noudjem, Y 2018, Phenolic content, HPLC analysis and Antioxidant activity extract from Tamarix articulata. Journal of Advanced Pharmacy Education and Research; 8(4), 1-8.

[26] Mahfoudhi, A, Prencipe, F P, Mighri, Z, and Pellati, F 2014, Metabolite profiling of polyphenols in the Tunisian plant Tamarix aphylla (L.) Karst. Journal of Pharmaceutical and Biomedical Analysis, 99, 97–105. DOI: 10.1016/j.jpba.2014.07.013.

[27] Tariq, M, Wazir, M A, Hussain, W, Safaie, S, Sial, A S, Shehzadi, K, and Usman, A 2019, “Phytochemical and Biological Screening of Tamarix aphylla (L.) (Tamaricaceae)”. Acta Scientific Pharmaceutical Sciences 3(10) 77-84. DOI: 10.31080/ASPS.2019.03.0408.

[28] Romero, C Y, and Vargas, M 2005, Extraction of oil from the neem seed (Azadirachta indica). Scientific Journal from the Experimental Faculty of Sciences. Ciencia, 3(3), 464-474. https://www.researchgate.net/publication/242525258.

[29] Al-Daody, A Ch 1998, Chemical Study on Some Iraqi Plants. PhD. Thesis, University of Mosul/ Iraq.

[30] Harborne, J B 1998, Phytochemical methods. 3rd ed., Chapman and Hall. https://books.google.iq/books?hl=ar&lr=&id=2ywqRtE8CwC&printsec=frontcover&source=gbs_ge_summary_r&cad=0#v=onepage&q=%5B31%5D%09Harborne%2C%2BJ.B.%2B1998.%2BPhytochemical%2BMethods.%3B3rd%2Bed.%2C%2BChapman%2B%26
[32] Behbahani, M, Shanehsazzadeh, M, and Hessami, M J 2011, Optimization of callus and cell suspension cultures of Barringtonia racemosa (Lecythidaceae family) for lycopene production. *Sci. Agric.* (Piracicaba, Braz) 68(1), 69-76.

[33] Harborne, J B 1984, Phytochemical Methods. 2nd ed., Chapman and Hall. https://books.google.iq/books?hl=ar&lr=&id=2yvqeRtE8CwC&printsec=frontcover&q=%5B31%5D%09Harborne,+J.B.+1998.+Phytochemical+Methods.+3rd+ed.,+Chapman+%26+Hall&redir_esc=y#v=onepage&q=&f=false.

[34] Mradu, G, Saumyakanti, S, Sohini, M, and Arup, M 2012, HPLC profiles of standard phenolic compounds present in medical plants. *International journal of pharmacognosy and photochemical research.* 4(3), 162-167. Available online on www.ijppr.com.

[35] Sumathy, R T P, and Kumuthakalavalli, R 2013, DPPH Free Radical Scavenging Activity and Total Phenolic Content of Three Species of Oyster Mushrooms. *Indian Journal of Applied Research* 3(10) 1-3. DOI: 10.15373/2249555X/OCT2013/9.

[36] Sahu, R K, Kar, M, and Routray, R 2013, DPPH free Radical scavenging activity of some leafy vegetable used by tribals of odisha, *India. Journal of medicinal plants studies*, 1(4), 21-27. Online Available at www.plantsjournal.com

[37] Mahfoudhi, A, Grosso, C, Gonçalves, R F, Khelifi, E, Hammani, S, Achour, S, Trabelsi-Ayadi, M, Valentão, P, Andrade, P B, and Mighri, Z 2016, “Evaluation of antioxidant, anti-cholinesterase, and antidiabetic potential of dry leaves and stems in Tamarix aphylla growing wild in Tunisia,” *Chemistry Biodiversity*, 13(12), 1747-1755. DOI: 10.1002/cbdv.201600157.

[38] Castillo-Sanchez, L E, Jimenez-Osornio, J J, and Delgado-Herrera, M A 2010, Secondary metabolites of the Annonaceae, Solanaceae and Meliaceae families used as Biological control of insects. *Tropical and subtropical Agroecosystems*, 12,445-462: https://www.researchgate.net/publication/48186912.

[39] Nahak, G, and Sahu, R K 2010, In vitro antioxidative acitivity of Azadirachta indica and Melia azedarachLeaves by DPPH scavenging assay. *Nature and Science*; 8(4), 22-28. http://www.sciencepub.net.

[40] Lattanzio, V, and Lattanzio, V M T, Cardinali, A 2006, Role of Phenolics in the Resistance Mechanisms of Plants against Fungal Pathogens and Insects. *Research Signpost* 37,661 (2) Fort P.O., Trivandrum-695 023, Kerala, India. https://www.researchgate.net/publication/303270594.

[41] Siddhuraju, P, and Becker, K 2003, Antioxidant properties of various extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (Moringa oleifera Lam.) leaves. *Journal of Agriculture and Food Chemistry*, 51(8), 2144-2155.DOI: 10.1021/jf020444+.

[42] Ashraf, H, and javaid, A 2007, Evaluation of antifungal activity of Meliaceae family against Macrophomina phaseolina. *Mycopath*, 5(2), 81-84.

[43] Farooq, M, Wahid, A, Kobayashi, N, Fujita, D, and Basra, S M A 2009, Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development, Springer*. 29(1), pp.185-212. DOI: 10.1051/agro: 2008021.

[44] Gierlinger, N 2003, Chemistry, Color and Brown-rot Decay Resistance of Larch Heart Wood and FT_NIR Bead Predication Models. *Dissertation, Universität für Bodenkultur Wien Institut für Botanik.*

[45] Monica, E k, Gellerstedt, G, and Henriksen, G 2009, Pulp and Paper Chemistry and Technology Volume 1, Wood Chemistry and Wood Biotechnology. *Walter de Gruyer GmbH and Co. KG, 10785 Berlin.* P.145-151. DOI: https://doi.org/10.1515/9783110213409
[46] Johnson, J D, and Kim, Y 2005, The role of leaf chemistry in Melampsora medusae infection of hybrid poplar: effects of leaf development and fungicide treatment. *Canadian Journal of Forest Research*, 35(4), 763–771.

[47] Al-Hashumi, F H, Al-Khero, A N, and Al-daody, A C 2018, Investigation of some Carboxylic Acids and Phenolic Compounds of Ailanthus altissima Leaves and their effect on Italian Cupressus Seedlings Root Roz Fungi. *Rafidain Journal of Science*, 27(4), 8-18. DOI: 10.33899/rjs.2018.159366.

[48] Kaushik, N, Singh, B G, Tomar, U, Naik, S N, Vir, S, Bisla, S, Sharma, K K, Banerjee, S, and Thakkar, P 2007, Regional and habitat variability in azadirachtin content of Indian neem (Azadirachta indica A. Jusieu). *Current Science*, 92, 1400-1406.

[49] Nagy, N E, Fossdal, C G, Krokene, P, Krekling, T, Lonneborg, A, and Solheim, H 2004, Induced responses to pathogen infection in Norway spruce phloem: changes in polyphenolic parenchyma cells, chalcone synthase transcript levels and peroxidase activity. *Tree Physiology*, 24(5), 505–515.

[50] Yusufoglu, H S, and Alqasoumi, S, I 2011, Anti-inflammatory and Wound Healing Activities of Herbal Gel Containing an Antioxidant Tamarix aphylla Leaf Extract. *International Journal of Pharmacology*, 7, 829-835.

[51] Said, O, Kalill, K, and Azaizeh, H 2002, Ethnopharmacological survey of medicinal plants in Israel, the Golan Heights and the West Bank Region, *Journal of Ethnopharmacology*, 83(3), 251-265. DOI: 10.1016/S0378-8741(02)00253-2.

[52] Barnes, P, L 2003, Reproductive and population characteristics of Tamarix aphylla at Lake Mead National Recreation Area, Nevada. MS thesis, *University of Nevada Las Vegas, NV*.

[53] Upadhyay, N K, Yogendra Kumar, M S, and Gupta, A 2010, Antioxidant, cytoprotective and antibacterial effects of Sea buckthorn (Hippophae rhamnoides L.) leaves. *Food and Chemical Toxicology*, 48(12), 3443–3448. DOI:10.1016/j.fct.2010.09.019.

[54] Nagulendran, K R, Velavan, S, Mahesh, R, and Begum, V H 2007, In vitro Antioxidant Activity and Total Polyphenolic Content of Cyperus rotundus Rhizomes. *E-Journal of Chemistry*, 4(3), 440–449.

[55] Blainski, A, Lopes, G, and de Mello, J 2013, Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from Limonium Brasiliense L. *Molecules*, 18(6), 6852–6865.