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Estimation of Total Phenolic Content of the Algerian Plant *Tamarix gallica*

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ABSTRACT

In the current study, the composition of polyphenols of Tamarix Gallica, including phenolic acids and flavonoids, was fully examined. Colorimetric methods were applied to assess the total phenolic and flavonoid contents; the individual polyphenols were determined in various phenolic groups through HPLC analysis, and their quantities were measured. In this plant, a total of five phenolic compounds were identified for the first time. The main peaks which were removed at 18.53 min, were considered as vanillic acid. There were two other main peaks which were eluted at 35.78 and 17.27 min that were considered as naringin and caffeic acid respectively. In these compounds, three components with retention times of 23.46, 25.81 min were determined. A practical tool for the metabolite characterization of Tamarix gallica can be applied in the developed technique to demonstrate a potential source of bioactive compounds to be used in phytotherapy.

Keywords: *Tamarix Gallica, Flavonoids, Phenolic acids, Extraction, HPLC.*

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1. INTRODUCTION

Recently, studies have paid vast attention on vegetable crop considering the halophilic vegetation of arid and humid areas (Pyankov et al, 2001; Khan et al, 2006). Ellagitannins have been found to have various important medicinal effects including antioxidant, antitumor, antiviral, antimicrobial, immunomodulatory, and hepatoprotective activities (Quideau et al, 2008; Feldman, 2005; Miyamoto et al 2008).

Among the medicinal plants, those belonging to the Tamarix (Tamaricaceae) are rich in ellagitannins (Bolous, 1999). The genus Tamarix naturally grows in Western Europe and the Mediterranean to North Africa (Baum, 1978).

14 species of this plant have been identified in Europe including African tamarisk (Tamarix africana), Bove's tamarisk (Tamarix boveana), Canary Islands tamarisk (Tamarix canariensis), Dalmatian tamarisk (Tamarix dalmatica), French tamarisk (Tamarix gallica) (Brotherson et al, 1986; Allred, 2002; González 2004; Heywood et al, 2007). Halophyte aptitude to endure salt-triggered oxidative stress has been based on some factors, as well as the production of antioxidant molecules, such as phenolic acids and flavonoids (Bravo, 1998). In recent years, this exciting feature of halophytes as a potential source of natural antioxidants has aroused the interest of many researchers. In spite of the large use of Tamarix gallica preparations as natural remedies to treat several diseases, there is no detailed investigation on the photochemical analysis of this plant (Figure 01). The aim of this examination was to develop a right and reliable extraction with the maceration procedure, combined with HPLC method, to study the phenolic compositions of Tamarix gallica leaves in detail for the first time. The method has been completely validated and practical for the qualitative and quantitative analysis of phenolics in Tamarix gallica leaves, providing the first metabolite profiling of this plant material.

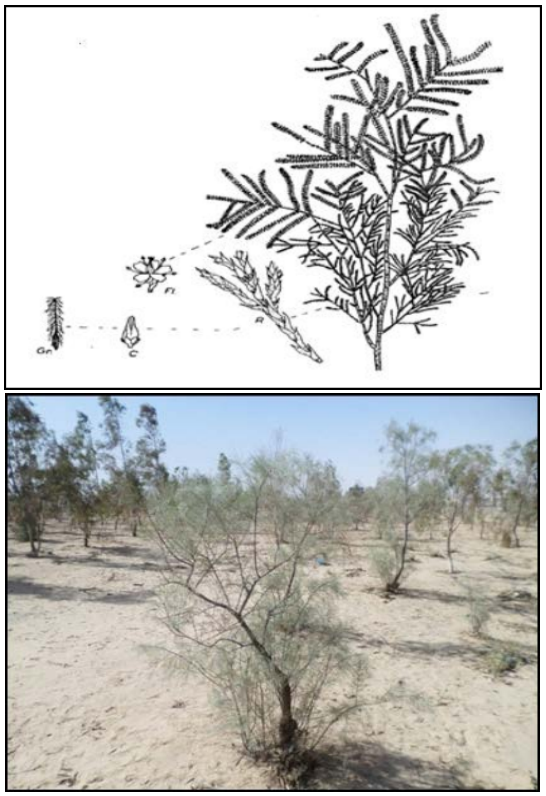


Figure 01. The morphology of Tamarix gallica.

2. MATERIALS AND METHODS

2.1. Plant materials

The leaves from Tamarix gallica were collected in the southeast of Algeria, province of El Oued in September 2016. Then, they were taken apart from each other, washed and dried at room temperature. A basic electric grinder was used to grind the leaves into powder; then, the powder was stored in the dark at room temperature before being used. Then, the powder was put in a hot air oven at 60 °C until complete drying for 48 h (Falleh et al, 2008).

2.2. Maceration or Classical extraction

In order to prepare the extract, 100 g of the plant material was macerated by methanol (500 ml) for 24 h at room temperature. After the filtration, the extract was rotary evaporated under vacuum at 45 ◦C, and then, it was stored at +4 °C.

2.3. Total phenolic content

Based on the method of Folin-Ciocalteu for the quantification of polyphenols (Raid et al, 2014), 0.1 ml of two extracts and gallic acid as standards were added to 0.25 ml of 1 N Folin-Ciocalteu reagent. The reaction was made up of 2.5 ml of water. After 5 min, 2.5 ml of Na2CO3 solution (2%, w/v) was mixed with the above reaction and the mixture was placed at room temperature for 30 min, and polyphenols were estimated to be 765 nm using a UV-visible spectrophotometer. Based on the standards, gallic acid equivalent (GAE) per g of dry weight and the following equation of calibration curve were applied:

Y= 0.00778x, R2 = 0.991,
X was the absorbance and Y was the gallic acid equivalent.

2.4. Total flavonoids content

The flavonoids were quantified based on the colorimetric assay (Hmidene et al, 2017). 1 ml of extract was mixed with 4 ml of distilled water. After that, 0.3 ml (5%) of the NaNO2 solution was added to the above reaction containing 0.3 ml of AlCl3 solution (10%). The reaction was incubated at ambient temperature for 5 min, and then 2 ml of 1 M NaOH were added to the mixture. Instantly, the above reaction was completed to 10 ml of distilled water. The mixture was thoroughly vortexed, and the absorbance was measured at 510 nm. A calibration curve was made with catechin, and the findings were represented as mg catechin equivalents (CE)/g of dry weight.

2.5. HPLC analysis

The identification of polyphenols of the extract was obtained using high-performance liquid chromatography (HPLC) based on the method represented by Tevfik 2010. The Shimadzu (LC 20, Japan), the system included an LC-10AD pump, a CTO-10A column oven, an SPD-10A UV-DAD detector, a CBM-10A interface; and an LC-10 Workstation was utilized. LC-18 column with characteristics of (250 mm x 4 mm i.d. x 5 mm) was employed. 20 µl of Tamarix gallica extract was injected, and the constituting elements of the mixture were taken apart by gradient elution at 30 °C. The mobile phases were: A, 98:2 (v/v) acetic acid and B acetonitrile. Acetic acid and the elution gradient were: 0–5 min, 95% B; 10 min, 90% A; 11 min, 80% A; 30 min 60% A; 40 min 50% A; 50 min 20% A. The stream was 0.8 ml/min and the absorbance of detection was 268 nm. Phenolic compound standards were: chlorogenic acid, rutin, gallic acid, caffeic acid, vanillic acid, vanillin, p-coumaric acid, and naringin dissolved in methanol, were used to identify polyphenols existing in the two extracts of Tamarix Gallica. In order to determine the peaks in HPLC, the retention times of reference standards were compared. The peak areas of the reference compounds were considered to determine the concentration of individual phenolic compounds in the extracts, and they were reported as mg/g of the extract.

3. RESULTS AND DISCUSSION

3.1. Total phenolic and flavonoids contents

The maceration method was used to determine the total polyphenol content of the methanolic leaves extract of Tamarix gallica, and it was represented as gallic acid existing in each gram of dry weight. The eatable leaves of Tamarix gallica were found to be rich in polyphenols; and a high concentration of phenolic content was found in Tamarix gallica 370.29 ± 6.31 mg of GAE/g of dry weight. Similar results were found for the total flavonoid content, the results showed that extracts from Tamarix gallica contained 175.22 ± 4.18 mg of catechin/g of dry weight. Table 1 has summarized the results obtained from these experiments. The presented results were consistent with the previous studies representing Tamarix Gallica as an available source of natural phenolic compounds (Hmidene et al, 2017; Bettaib et al, 2017).

Table 1. Phenolic content and flavonoids content of Tamarix gallica leaves extract.

	Tamarix gallica
Phenolic content (mg GAE /g DW)	370.29 ± 6.31
Total flavonoids (mg CE/g DW)	175.22 ± 4.18

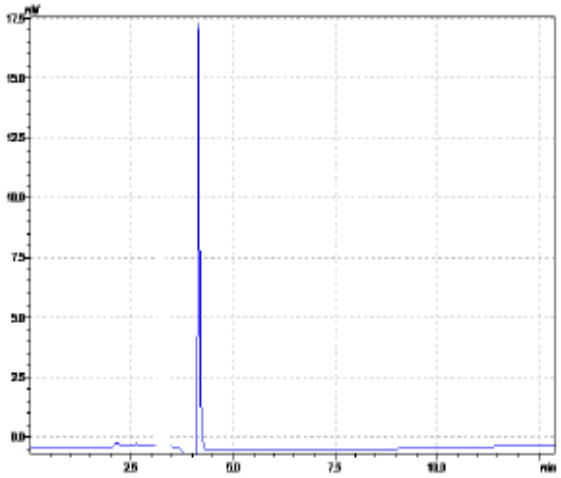
3.2. HPLC analysis

For precisely quantifying phenolic composition in methanolic leaves extracts, the HPLC assay was used. The obtained findings were in good consistency with those assessed by spectrophotometric determination of the examined extract. Figure 3 represents the chromatograms of leaves extract. The standard compounds of gallic acid, chlorogenic acid, vanillic acid, caffeic acid, vanillin, p-Coumaric acid, rutin, and naringin have been described in figure 2. The calibration curves described by Laouini et al. (2015) were considered to identify the contents of these components in various extracts. Where y was the peak area and x was the concentration of the compound (0–80 µg/ml). The quantitative results have been summarized in Table 2. For Tamarix gallica, the major peaks eluted at 18.53 min, were identified as vanillic acid. Two other main peaks which were eluted at 35.78 and 17.27 min were identified as naringin and caffeic acid; respectively. These compounds were determined through using authentic standards. Unfortunately, there were three components with retention times of 23.46, 25.81 min. This study has been the one which investigated the HPLC profile of Tamarix gallica leaves extract for the first time.

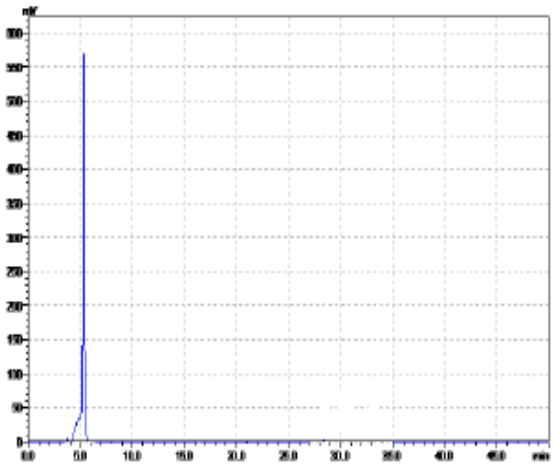
Table 2. Quantification of identified individual phenolic compounds of Tamarix gallica extract at 268 nm using HPLC system.

Identified compound	RetentionTime (min)	Equation curve	Area	Area (%) in fraction	Height (uV)	Quantity (mg/g DW)
Gallic acid	5.29	y=54681x	231429	1.019	10609	3.123±0.01
Chlorogenic Acid	13.39	y=21665x	108997	0.480	5856	5.531±0.02
Vanillic Acid	18.53	y=65077x	531407	2.340	8786	9.264±0.03
Caffeic Acid	17.27	y=84066x	525925	2.315	1883	7.596±0.02
Vanillin	23.46	y=58930x	-	-	-	ND
p-Coumaric Acid	25.81	y=49495x	-	-	-	ND
Rutin	28.37	y=28144x	-	-	-	ND
Naringin	35.78	y=19377x	151609	0.667	5599	8.124±0.02
Quercetin	45.05	y=55378x	159174	0.701	6891	3.364±0.01

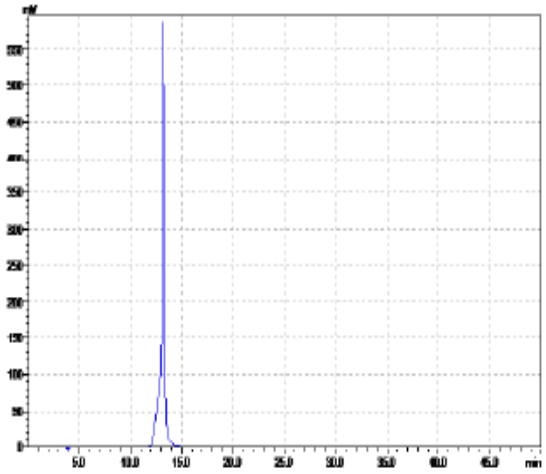
ND: Not detected



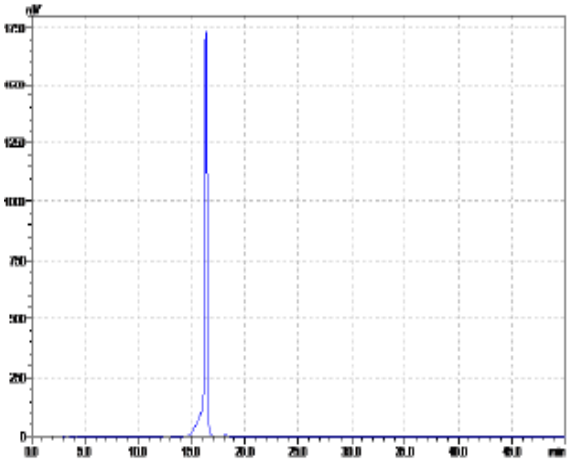
A- Chromatogram of ascorbic acid (solution standard)



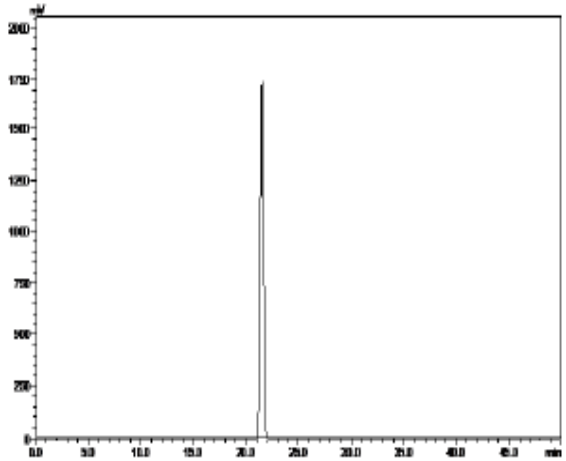
B- Chromatogram of gallic acid (solution standard)



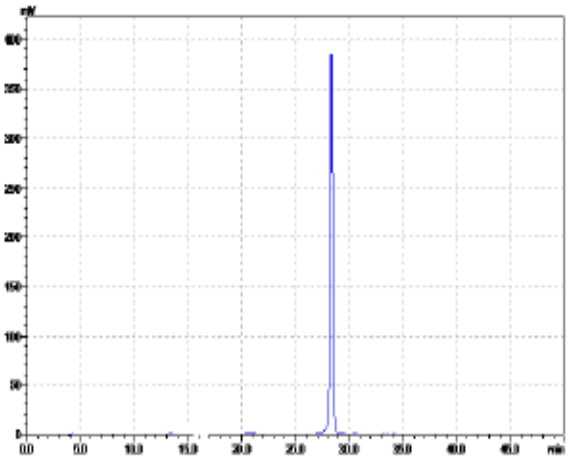
C- Chromatogram chloregénic acid (solution standard)



D- Chromatogram of Caffeic acid (solution standard)



E- Chromatogram of vanillin (solution standard)



F- Chromatogram of rutin (solution standard)

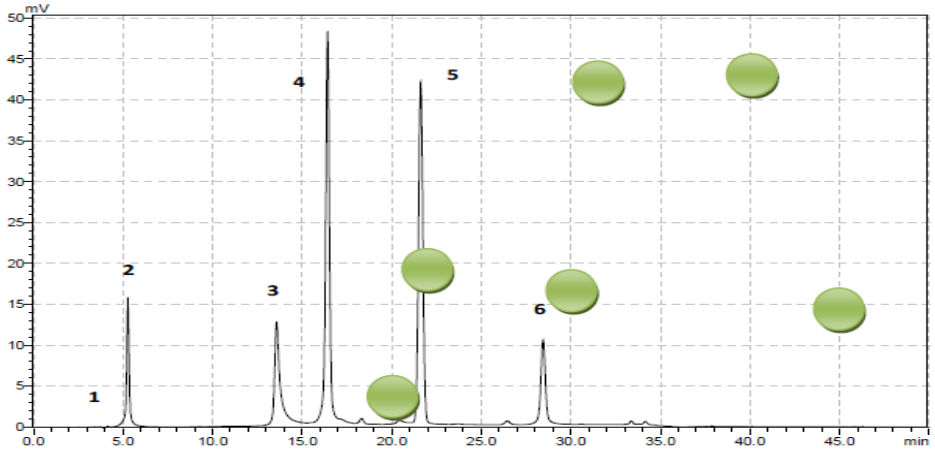


Figure 2. Chromatogramme Profile of melange etalons including
A: ascorbic Acid; B: Gallic acid; C: Chlorogenic Acid;
D: Caffeic Acid; E: vanilline; F: Rutin

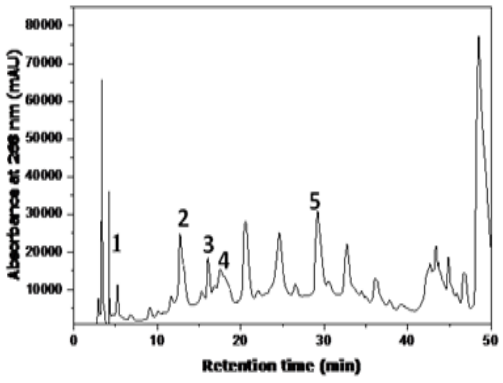


Figure 3. The HPLC chromatogram of methanolic leaves extract from Tamarix gallica at 268 nm.
1: Gallic acid; 2: Chlorogenic Acid; 3: Vanillic Acid; 4: Caffeic Acid; 5: Naringin.

4. CONCLUSION

In the present phytochemical investigation, the maceration technique was used for HPLC analysis of methanolic leaves extract of Tamarix Gallica. There was a significant abundance of the phenolic and flavonoid content in the extract indicating it to be a rich source of these compounds. Additionally, a powerful analytical HPLC technique was applied to determine and quantify five phenolic compounds in Tamarix Gallica which can be administered as an antiradical drug. In the current study, the presented data suggested that Tamarix Gallica could be a new source of polyphenols, which is a challenge for new medicaments. The current assays were conducted to assess the in vivo antibacterial, antimutagenic and anti-inflammatory potential as well as their toxicity, and also detect the bioactive molecules through mass spectroscopy and nuclear magnetic resonance of H1 and C13.

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