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Phenolic Acid Profiling and Antioxidant Activity of *Tamarix dioica* Leaves, Stem and Flowers

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Abstract. The aim of this study was to determine the phenolic acid profile and antioxidant activity of leaves, stem and flowers of *Tamarix dioica*. Nine compounds were identified by high performance liquid chromatography (HPLC). Overall results showed that the high content of gallic acid was detected in leaves and flowers and caffeic acid in stem. Antioxidant activity was evaluated using DPPH assay. A high antioxidant activity was observed, which is associated with very high phenolic content, indicating the potential benefit of using this species as a source of biochemical compounds.

Keywords: *Tamarix dioica*, phenolic acids, antioxidant activity, HPLC-DAD, UV/VIS

Introduction

All medicinal plants are essential components of the universe and widely used for the affection of various human diseases (Kaur and Mondal, 2014; Sultana *et al*., 2014). These plants contain a large variety of secondary metabolites of which phenolic compounds are observed the most essential non-nutritive natural compounds (Khan *et al*., 2014; Phoboo *et al*., 2014) Phenolic compounds (secondary metabolites) occupy a massive position in the plant kingdom, approximately 8,000 (eight thousand) phenolic structures known by (Robbins, 2003). The phenolic compounds have one or more than one aromatic rings attached with one or more than one hydroxyl groups (-OH) directly to an aromatic hydrocarbon group and play an important role in the human diet (Dai and Mumper, 2010). Phenolic compounds derived from plants are responsible for antioxidant and free radical scavenging activities (Halliwell, 2001). Phenolic compounds have significant and massive application in the food and pharmaceutical industries due to their physically powerful antioxidant actions that have a positive impact on human fitness. (Pandey *et al*., 2012; Ghasemzadeh *et al*., 2011). These compounds contain a defensive effect as opposed to oxidative reactions such as metal chelating properties, reducing activity and a hydrogen provider function. Therefore, these compounds turn out to be antioxidants (Diaz *et al*., 2012)

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Tamarix dioica. Roxb. ex Roth (family Tamaricaceae) commonly known as Lai that grows from 1 to 18 meters of height includes 60 different species and sized as small tree or shrub which retains green throughout the year. It consists of vagina leaves, rosy bark and fair violet flowers. The *Tamarix* genus is composed of about 250 different species of invertebrates and cattle (Bughio *et al*., 2018), while *Tamarix* seems to be a small sized tree, it has depth root that can extend about 30 meters down from the top. The tree is found abundantly in Pakistan, Nepal, India (Bharat), Afghanistan, Bhutan, Iran, Kashmir, Bangladesh and Myanmar. In Pakistan in Sindh and Khyber Pakhtun Khwa (KPK) (Khan *et al*., 2004). The leaves of *T. dioica* are applicable for splenic inflammation (enlarged spleen) cure liver disease, diuretic (increased production of urin) and carminative. Additionally, plant is also applicable for an astringent for vaginal secretions (Khan *et al*., 2013). *Tamarix dioica* has demonstrated antifungal (prevents the growth of yeasts and other fungal organisms) activity against three micro-organisms, such as *Candida glabrata, Trichophyton rubrum* and *Aspergillus fumigates.* This plant also shows antibacterial activity against *Klebsiella* (gram-negative) and *Pseudomonas aeruginosa* (gram-negative) (Samejo *et al*., 2013).

Literature survey revealed that, there is no study about phenolic acids and antioxidant activity of *T. dioica* different parts. Therefore, this present study was designed to evaluate the phenolic acids and antioxidant activity of *T. dioica* in the Jamshoro area, for the first time.

Phenolic compounds from *Tamarix dioica* in leaves, stem and flowers determined by using HPLC, whereas radical scavenging activity (RSA) and total phenolic content (TPC) was determined by UV-visible spectrophotometry.

Materials and Methods

The plant collection. The *T. dioica* parts of the plant in August, 2019 were collected from the mountainous region of Jamshoro, near the University of Sindh (UoS), Jamshoro. The fresh parts of the whole plant were sent to the herbarium of the Institute of Plant Sciences, Sindh University, Jamshoro. The plant dossier was made by taxonomist and confirmed the botanical name of the selected plant species. The taxonomist filed the coupon specimen as 2671317.

Chemicals and standards. All chemicals (Sigma-Aldrich Chemical Company) that were used for biological activity and analysis of phenolic acids in the *T. dioica* plant were issued at the Chemical Store of the Institute of Chemistry (Dr. M. A Kazi), UoS, Jamshoro. The purity of each chemical and solvent was checked before use.

Phenolic acids extraction. Ultrasonic assisted extraction (UAE) technique was performed for the extraction (removal) of phenolic acids from *T. dioica*. Dried powdered 0.5 g of each part (leaves, stem and flower) were extracted with methanol: water (80:20 v/v; amount 10 mL) in sonicator bath. Sonication was performed at approximately 50 °C for 25 min. The sample was removed from the sonicator bath and kept it at room temperature. The mixtures were filtered (0.45 µm Swinney syringe filter) for phenolic acids analysis by HPLC. These same extracts were also used for the estimation of scavenging capacity by 2,2-diphenyl-1-picrylhydrazyl (DPPH).

HPLC-DAD profiling of phenolic acids. Analysis of phenolic acid was performed using a spectral system (SCM 1000 (Thermo Finnigan, California, USA) liquid chromatography connected with a DAD (Diode Array Detector). The partition of acids was carried out by means of the use of reverse phase Hypersil Gold C-18(4.6 mm × 250 mm, 5µm) column (Thermo Corporation, USA) (Memon *et al*., 2013). The mobile phase was designed as 0.1% formic acid in water (A) and methanol (B) and the flow rate was 1mL/min. linear gradient was 5% (B) to 30% (B) for 25 min, followed by 30% (B) for 10 min was used. After 52 min, bring the mobile phase concentration back to 5% (B) and keep it for 08 min to reach the column equilibrium. UV detection was performed at 270, 310 and 325 nm. Interpretation was done by using Chromquest (version 4.2) software. The structure of each phenolic acid was made by matching its UV spectra with standards and retention time.

Assessment of total phenolic contents (TPC). TPC of each methanolic extract measured by Folin-Ciocalteu method (Boakye *et al*., 2015; Memon *et al*., 2012). 800 µL of fresh Folin-Ciocalteu reagent, 2 mL of sodium carbonate (saturated solution 75g/L) and 200 µL of diluted extracts were mixed and then added deionized water upto reached the total volume level at 7 mL. The mixture was kept at 25°C for 2.5 h in the darkness.The absorbance was determined at 765 nm by UV-vis spectrophotometer (Perkin Elmer lambda 35). Calculated the phenol concentration according to the standard curve of the gallic acid standard solution (mg gallic acid equivalents (GAE) /g) and the extraction yield of phenols is calculated as follows:

$$\text{Extraction yield \%} = \frac{\text{Phenolic compounds weight (g)}}{\text{dry plant powder weight (g)}} \times 100$$

Radical scavenging activity (RSA). DPPH radical scavenging activity of methonal extract containing the phenolic acids was calculated using reported method (Amron and Konsue, 2018; Memon *et al*., 2013). 2 mL of 0.1 mmol/L DPPH radical solutions was mixed to 2 mL of each *T. dioica* extract sample. Shake the mixture and leave it in the dark for thirty min. The mixture absorbance (UV/VIS spectrophotometer, Perkin Elmer lambda 35) was determined at 517 nm. DPPH radical scavenging effect calculated as follow:

$$\text{Scavenging (\%)} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100$$

Results and Discussion

The phenolic acids standards. Twenty phenolic acids in total were selected as a standard and injected onto the chromatographic column to determine phenolic acids in the leaves, stem and flower of *T. dioica*. The names of these standards are mentioned in Table 1 with the determination coefficient (R^2), the retention time (t_R) and the wavelength (λ_{max}).

Table 1. Name of phenolic acids standards

Standards	tR (min)	R^2	Regression equation	max (nm)
Gallic acid	7.89	0.999	y = 305726x - 249684	227, 272
2,4,6-THBA	9.51	0.998	y = 49119x + 29082	216, 255, 292
Protocatechuic acid	13.16	0.997	y = 530511x + 112990	228, 259,294
Pyrogallol aldehyde	14.18	0.999	y = 337860x + 147020	234, 291
Protocatechuic aldehyde	14.35	0.998	y = 548015x + 303632	234, 281
Gentisic acid	14.92	0.999	y = 13444x - 1829.4	232, 327
Sinapic acid	16.26	0.991	y = 643555x – 1E+06	255,294
β-resorcinolic acid	18.99	0.998	y = 200138x + 46398	255, 294
Hypogallic acid	19.61	0.998	y = 82657x - 14787	232, 314
Vanilline	20.13	0.999	Y=626260x-138097	233, 281, 307
Vanillic acid	25.33	0.999	y = 289390x - 82077	223, 260, 294
Caffeic acid	28.32	0.995	y = 169059x - 140031	233, 323
Chlorogenic acid	29.34	0.998	y = 97008x - 33773	217,233, 327
Syringic acid	32.22	0.999	y = 214749x - 72422	225, 275
PHBA	35.18	0.999	y = 88856x - 14995	234, 308
p-Coumaric acid	40.32	0.995	y = 213962x - 333316	232, 309
Ferullic acid	46.77	0.998	y = 174006x + 127640	235, 322
m-Coumaric acid	47.59	0.999	y = 533000x + 78590	216, 232, 278
o-Coumaric acid	48.75	0.999	y = 7E+06x - 2E+06	232, 277, 330
Cinnamic acid	49.05	0.992	y = 568487x + 305505	230, 280, 330

Determination of phenolic acids from *T. dioica* leaves, stem and flowers extracts. The HPLC chromatogram of leaves, stem and flower is shown in Fig 1-3 respectively. The result of each chromatogram represents that the eight phenolic acids were identified in leaves, six in stems and five in flowers. The names of each part of the phenolic acids are mentioned in Table 2. Gallic acid as the main compound was found in both leaves and flowers, while caffeic acid was found as a main component in the stem. The comparative result showed that the amount (43.93 mg/g) of phenolic acids was

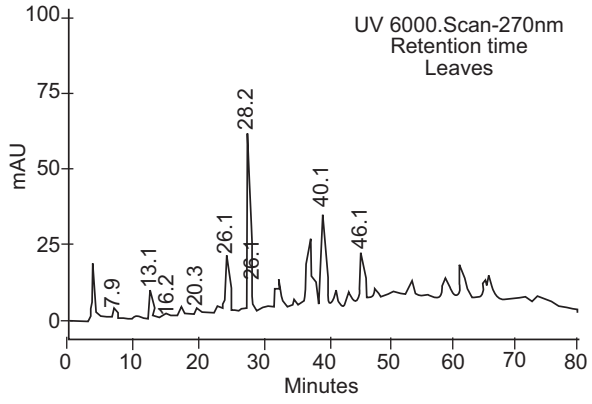

Fig. 1. HPLC chromatogram of phenolic compounds of *T. dioica* leaves.

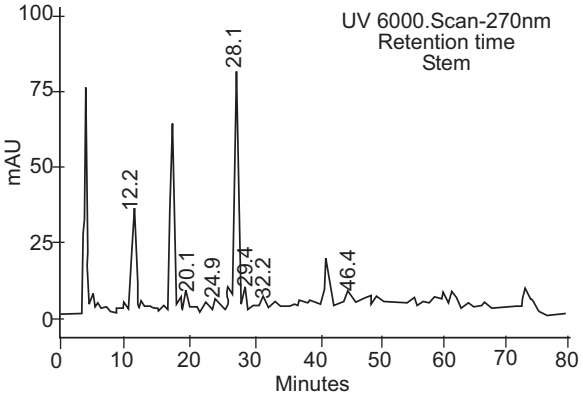

Fig. 2. HPLC chromatogram of phenolic compounds of *T. dioica* stem.

higher in leaves than in stems and flowers. The structure of each phenolic acid is identified shown in Fig 4.

It can be noted that gallic acid is higher in our selected plant (*T. dioica*) and the study of the literature revealed that gallic acid and its derivatives are commonly distributed in plants and fruits. Gallic acid is used as food stuff and preservatives. Gallic acid used as anti-inflammatory, antimicrobial, antiangiogenic, antimutagenic and anticancer agents. It is also used for serious illnesses such as microbial infections, depression, lipid related illnesses and cancer (Choubey *et al*., 2015). The selection of methanol as a solvent for extraction

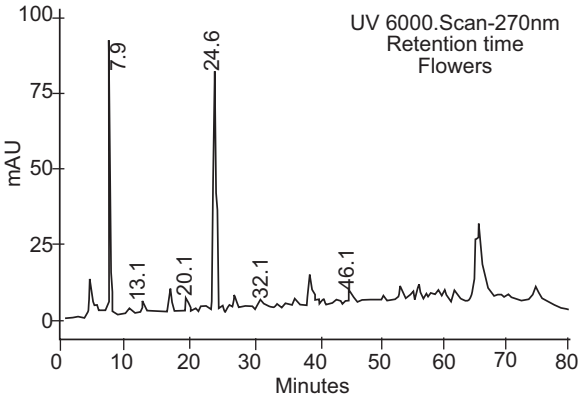

Fig. 3. HPLC chromatogram of phenolic compounds of *T. dioica* flowers.

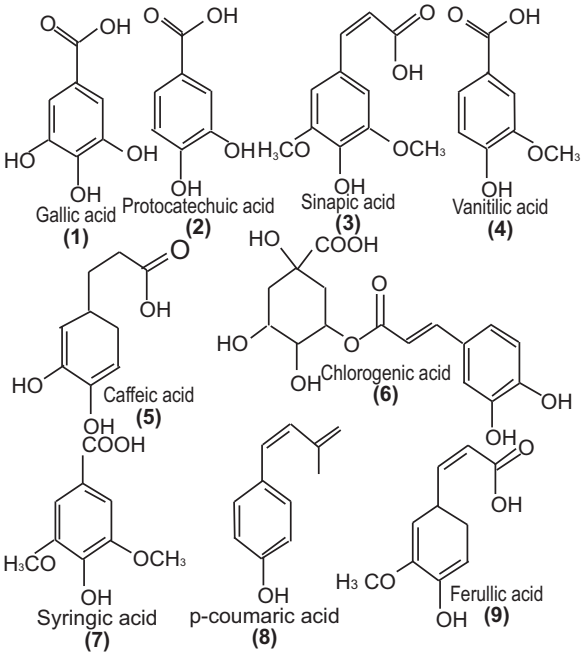

Fig. 4. Structurs of different phenolic acid.

due to its selectivity for phenolic acid and caution is also required for extraction of phenolic acids because in the presence of sunlight they can isomerizes (Samejo *et al*., 2017).

Determination of total phenol content (TPC), total phenolic acid (TPA) and antioxidant activity (AA). TPC in the extract was estimated by FC method. The celebration curve created from the analysis of the standard (gallic acid) was linear R^2=0.994. TPC, TPA and RSA were in range of 49.00 to 75.78 mg gallic acid equivalents (GAE) /g equivalent and 30.14 to 44.58 mg/g and 32.32 to 177.01 mmol/QE100g dry weights

Table 2. The content of some phenolic acids from *T. dioica* leaves, stem and flowers extracts.

Phenolic acids	$tR_{(min)}$	Areal parts of *Tamarix dioica*		
		Leaves (mg/g)	Stem (mg/g)	Flowers (mg/g)
Gallic acid	7.89	21.86	ND	18.37
Protocatechuic acid	13.16	2.27	3.00	0.40
Sinapic acid	16.26	7.08	ND	ND
Vanillin	25.33	3.58	2.55	2.68
Caffeic acid	28.32	1.45	11.77	ND
Chlorogenic acid	29.34	ND	6.19	ND
Syringic acid	32.22	1.56	3.04	3.88
p-coumaric acid	40.32	2.94	ND	ND
Ferullic acid	46.77	3.10	0.92	7.50
Total		43.93	27.14	32.83

Table 3. Total phenolic content, total phenolic acids and antioxidant activity assays.

Sample	Total phenolic content (TPC) as gallic acid eq. (mg/g)	Total phenolic acids (TPA) (mg/g)	DPPH radical scavenging activity (RSA) as Quercetin eq. (mmol/100g)
Leaves	75.78	44.85	177.01
Stem	49.00	30.14	32.23
Flowers	57.77	32.97	102.74

of *T. dioica* stem and leaves respectively shown in Table 3.

Conclusion

Present study shows that leave and flowers extract of *T. dioica* contained a high range of total phenolic contents, as well as radical scavenging activity as compare to stem extract, suggesting that leaves and flowers may be a better source of phenolic acids as compare to stem extract. Similarly the antioxidant activity of leaves and flowers extract of methanol were measured by DPPH assay. This suggests that leaves and flowers extract of methanol extract may give better protective result against free radical oxidative damage as compare to stem extract of methanol.

Conflict of Interest. The authors declare they have no conflict of interest.

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