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Geographic Conditions as Key Determinants of Polyphenols in *Tamarix Gallica* L. growing in Algeria

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ABSTRACT

The current study aimed to evaluate how climatic conditions impact both the quality and quantity of phenolics in the aerial parts of Tamarix Gallica. Plant samples were gathered from three distinct regions in Algeria, each representing different climatic zones: humid, semi-arid, and arid. Water and ethyl acetate extracts were assessed for total phenolic content, with values ranging from 16.51 to 20.35 mg EGA/g and 36.6 to 103.14 mg EGA/g, respectively. Similarly, flavonoid levels were determined, showing a range of 16.51 to 20.35 mg EQ/g for water extracts and 36.6 to 103.14 mg EQ/g for ethyl acetate extracts. Notably, the predominant phenolic compounds identified were Hesperidin and Rosmarinic acid, with their concentrations varying significantly based on the collection site and the solvent used for extraction.

Keywords: Tamarix gallica; Geographic factors; flavonoids; phenolics

1. Introduction

Plant secondary metabolites play a crucial role as the primary source for pharmaceuticals, food additives, perfumes, flavors, and various other key compounds utilized in chemistry and biochemistry, significantly impacting everyday life. Their significance remains a topic of debate. Unlike primary metabolites, which regulate fundamental physiological processes essential for plant growth and development, secondary metabolites are vital for the plant's survival within its environment. They form an integral part of the arsenal employed by plants to ensure their survival and proliferation. Their functions may include defense against predators (such as herbivores), pathogens, or competitors, assistance in pollination or seed dispersal, protection against or adaptation to external abiotic factors, coping with stress conditions, or a combination of these roles (Maqsood et al., 2024; Muthusamy & Lee, 2024; Sokouti, 2024). Environmental factors such as soil composition, temperature, latitude, humidity, light intensity, rainfall, evaporation, minerals, and CO2 levels exert significant influence on plant growth and the accumulation of secondary metabolites. These factors can directly impact the concentrations of secondary metabolites, thereby affecting the quality of the plant for medicinal use and therapeutic effectiveness. Plants possess the capability to adapt to biological, physical, chemical, and ecological stresses by adjusting the production of secondary metabolites. Therefore, research focusing on the effects of ecological factors on the accumulation of secondary metabolites in medicinal plants is essential for understanding the underlying reasons for geoherbalism. (Guo et al., 2013; WANG et al., 2023)

Algeria boasts a diverse climate, featuring a mild Mediterranean climate along the coast, a transitional climate in the northern hills and mountains characterized by moderate rainfall, and a desert climate dominating the vast Sahara region. The varied landscapes, ranging from deserts to mountains, valleys, plateaus, and basins, provide habitats for thousands of floral species, many of which are unique to Algeria. This rich biodiversity has inspired us to conduct a comparative study investigating the influence of climatic conditions on the phenolic content of both aqueous and ethyl acetate extracts of Tamarix gallica sourced from three distinct geographic regions: arid, semi-arid, and humid.

Tamarix gallica was reported to contain saponosides, steroids, terpenes, and unsaturated sterols besides the strong presence of polyphenols especially flavonoids and tannins (Mechaala et al., 2022). Tamarix gallica has a long history of traditional use in treating various ailments such as leucoderma, spleen issues, eye diseases, rheumatism, and gingivitis. The plant is rich in phytochemical compounds including tamarixin, tamarixetin, troupin, 4-methylcoumarin, 3,3’-di-O-methylellagic acid, and quercetol (methyllic ester). Pharmacological studies have indicated a range of potential therapeutic activities associated with Tamarix gallica, including anti-malarial, laxative, expectorant, antidiarrheal, anthelmintic, antihaemorrhoid, astringent, nephrolithiasis inhibition, diuretic, hepatoprotective, antioxidant, antihyperlipidemic, antinociceptive, antidiarrhoeal, anticancer, antimicrobial, and liver carcinogenesis inhibition properties. (Ateeq & Firdose, 2024; Bahramsoltani et al., 2020; Lefahal et al., 2021; Nisar et al., 2023).

2. Material and methods

2.1. Soil and climatic data

For geographic areas covered in this study: namely Oum El Bouaghi (site 1), el Taref (site 2) and Ouargla (site 3). The geographical and soil characteristics were given in table 1.

Table 1: Geographical and Soil Characteristics of the three Sites

parameters		**Oum El Bouaghi (site 1)**	**El Taref (site 2)**	**Ouargla(site 3)**
Latitude and Longitude		35°52′31″ N, 7°06′48″ E	36°46′01″ N, 8°18′49″ E	31°56′57″ N, 5°19′30″ E
Altitude (m a.s.l)		925	24	138
Soil	Clay %	27.94	19.82	00.83
	Silt %	48.36	33.51	25.94
	Sand %	23.7	46.66	73.23
	Texture	clay loam	clay loam	Silty loam
	Electric conductivity	0.54	0.34	13.73
	pH	7.81	7.06	7.64
	Total $CaCO_3$ %	23.22	0.00	3.44
	Active $CaCO_3$ %	16.38	0.00	0.00
	Organic matter	2.53	4.65	2.11

2.2. Plant material

The aerial parts of *Tamarix gallica* were collected from Oum El Bouaghi (site 1: semi arid), El Taref (site 2: humid) and Ouargla (site 3: arid). The plant was identified by Pr. A. zellagui, Oum El Bouaghi University, Algeria. Voucher specimens (1, 2, and 3 for the 3 sites respectively) were deposited in the Laboratory of Natural Resources and Management of Sensitive Environments, University of Oum El Bouaghi, Algeria

2.3. Water extract:

500 mL of boiling distilled water were added to 100 g of finely ground dry plant material of each sample. After 60 min the aqueous extract was filtered and dried under vacuum, weighed and prepared for HPLC analysis.

2.4. **Ethyl acetate extract**:

100 g of dry plant material of each sample was subjected to overnight extraction using ethyl acetate. After separation, the organic phase was evaporated and the crude extract was weighed and prepared for further analysis.

2.5. Determination of total phenolic contents

The concentration of phenolics in plant extracts was determined using spectrophotometric method (Pérez et al., 2023). Methanol solution of the extract in the concentration of 1 mg/mL was used in the analysis. The reaction mixture was prepared by mixing 0.5 mL of methanol solution of extract, 2.5 mL of 10% Folin-Ciocalteu's rEGAent dissolved in water and 2.5 mL 7.5% $NaHCO_3$. Blank was concomitantly prepared**,** containing 0.5 mL methanol. 2.5 mL 10% Folin-Ciocalteu's rEGAent dissolved in water and 2.5 mL of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45 °C for 45 min. The absorbance was determined using spectrophotometer at λmax = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the

measured absorbance, the concentration of phenolics was read (mg/mL) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GAE/g of extract).

2.6. Determination of total flavonoids

The content of flavonoids in the examined plant extracts was determined using spectroscopic method (Shraim et al., 2021). The sample contained 1 mL of methanol solution of the extract at a concentration of 1 mg/mL and 1 mL of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λ_{max} = 430 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of quercetin and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/mL) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of quercetin equivalent (mg of QE /g of extract).

2.7. HPLC-TOF/MS analysis

Phenolic content of the plant extracts was determined using Agilent Technology of 1260 Infinity. HPLC System was coupled with 6210 Time of Flight (TOF) LC/MS detector and ZORBAX SB-C18 (4.6 x100mm, 3.5µm) column. Mobile phases A and B were ultra-pure water with 0.1% formic acid and acetonitrile respectively. Flow rate was 0.6 mL min^{-1} and column temperature was 35°C. Injection volume was 10 µL. The solvent program was as follow: 0-1 min 10% B; 1-20.min 50% B; 20-23.min 80% B; 23-25.min 10% B; 25-30. min 10 % B. Ionization mode of HPLC-TOF/MS instrument was negative and operated with a nitrogen gas temperature of 325 °C. Nitrogen gas flow was 10.0 L min^{-1}, nebulizer of 40 psi, capillary voltage of 4000 V and finally, fragmentor voltage of 175 V. For sample analysis, dried crude extracts (200 ppm) were dissolved in methanol at room temperature. Samples were filtered passing through a PTFE (0.45 µm) filter by an injector to remove particulates.

3. Results And Discussion

3.1. Total phenolics content

The content of the total phenolics is determined from a calibration curve of gallic acid taken as standard with a correlation coefficient ($y = 0.0113X + 0.0686$, $R^2 = 0.9984$).

The amount of total polyphenols is reported in microgram equivalents of the standard used per milligram of dry weight extract (µg EGA/mg) as shown in table 2.

Table 2: Total phenolics (µg EGA/mg)

	water extract	**ethyl acetate extract**
Site 1	39.32±0.15	238.46±0.16
Site 2	133.84±0.22	348.56±0.11
Site 3	16.14±0.01	296.16±0.14

The results revealed important fluctuations in total phenolics. Site 2 (humid region) exhibits significant phenolic contents 133.84 to 348.56 mg GAE/g for *T. gallica***)**. This is certainly due to environmental factors especially water and nutrients favorable for the biosynthesis of such elements. Site 3, in turn, displays less content especially in water extracts (16.14 mg GAE/g).

3.2. Flavonoids content

The flavonoid content, expressed in micrograms equivalent of quercetin per milligram of dry

weight extract (µg EQ/mg), was determined from the regression curve whose equation is: $y = 0.0299 X + 0.0979$, $R^2 = 0.9746$.

Table 3: Total flavonoids (µg EQ / mg)

	water extract	**ethyl acetate extract**
Site 1	16.51±0.18	62.18±0.01
Site 2	20.35±0.13	36.6±0.10
Site 3	17.20±0.05	103.14±0.06

The flavonoids contents seem to be less location-dependent for the case of water. Nonetheless for ethyl acetate extracts, important oscillations were observed especially in site3 (103.14 µg EQ/mg).

3.3. **High performance liquid chromatography analysis**

To identify the active principles responsible for activity, recognition and quantification of phenolic compounds were carried out using analytical HPLC-TOF-MS and the result are shown in table 4 expressed as mg of phenol per kg of dry weight of plant material.

Table 4: phenolic compositions of *T. gallica* for the 3 sites as mg/ kg of dry weight of plant material (W: water extract, and EA: EtOAc extract)

compound	**W1**	**W2**	**W3**	**EA1**	**EA2**	**EA3**
Gallic Acid					**2966.460**	
Gentisic Acid		5.451	3.082			
Chlorogenic Acid		136.058	55.251			
4-Hydroxy Benzoic Acid		57.027	43.791			
Protocatechuic Acid	16.898	16.627	16.454	1.363	2.414	0.624
Caffeic Acid	8.343	21.355	9.575			
Vanilic Acid		25.723		9.007	3.699	2.429
Rutin		14.080	0.371			
P-Coumaric Acid				11.176	29.721	70.3052
Chcoric Acid				1.687	1.636	0.576
Ferulik Acid		8.998	2.042	0.692	6.788	0.452
Hesperidin	**3752.284**	**6369.896**	**11194.851**	**0.257**		**2.369**
Apigenin-7-Glucoside				18.638	75.923	66.395
Rosmarinic Acid	**8.298**	**1778.545**	**1943.1755**			**0.919**
Protocatechuic Acid Ethyl Ester				7.884		
Resveratrol				24.961		
quercetin						13.846
kaempferol				0.744	7.053	0.150
Cinnamic Acid	70.272	66.999	191.415		1.403	2.431

The phenolic contents of *Tamarix gallica* are well documented. Previous reports stated clearly a strong dependence on the environmental factors. Total phenolic contents and flavonoid from an extract of Tamarix gallica were found high against Tamarix articulata, 334.19 ± 8.47, 395.62 ± 6.23 mg GAE /g DW for phenolic content and 159.73 ± 6.28, 117.47 ± 4.04 mg

CE/g DW respectively. The HPLC analysis showed that at least 6 considerable phenolic compounds of leaves extract exist for the two extracts, the major ones being vanillic acid, naringin and caffeic acid (Said et al., 2018). Other studies revealed big diversities and fluctuations (Al-Othman et al., 2020; Bencherif et al., 2019; Bencherif et al., 2020; Boulaaba et al., 2015; Elamin, 2016; Lefahal et al., 2010; Najjaa et al., 2020; Nisar et al., 2023; Tabet & Boukhari, 2019; Zar Kalai et al., 2023)

4. Conclusion

The increased demand of natural products as substituent of synthetic drugs has rehabilitated concern in large-scale production. Environmental factors have impact on availability of active principles and affect remarkably the secondary metabolites contents in higher medicinal plants hence, therapeutic value also get influenced. It is well apparent that climate change, soil nature, aridity and other factors considerably influence water availability, salinity and several unfavorable circumstances having direct attitude on secondary metabolites yields and qualities. The present work, in the same context, highlights the effect of some abiotic aspects on polyphenolic contents in *Tamarix Gallica.* Nevertheless, further studies are required to deepen the knowledge towards the establishment of a relationship leading to the use of such stresses as tools to increase the health-related properties of plant material.

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