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OCCURRENCE, COMPARATIVE GROWTH AND COMPOSITION OF *TRIBULUS TERRESTRIS* L. UNDER VARIABLE *IN-SITU* WATER STRESS

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Abstract

Medicinal plants are widely used in the present-day economy, and therefore, their study is of considerable interest both to botanists and to pharmacologists. Till date, few studies have been carried out on the economic valuation of medicinal plants growing under drought-stressed and unstressed natural in-situ conditions. In the present study, *T. terrestris* L. plants, which have not been investigated with respect to the above objective, were collected from different geographical and climatic conditions during 2015—2016, and their occurrence, comparative growth, and chemical compositions were determined. The results of the study indicated heterogeneous distribution of *T. terrestris* L. across the study area. The plant growth parameters including the number of stems plant^{-1}, average length of stems, number of leaves plant^{-1}, number of flowers plant^{-1}, fresh biomass plant^{-1} and dry biomass plant^{-1} were found to decrease because of drought stress. However, the chemical composition, including total phenolic content, antioxidant activity, ferric-reducing ability of plasma assay (μM $FeSO4$ g^{-1}), and free radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl inhibition (%), and saponin content increased with drought stress. Mineral composition of plants was found to decrease with drought stress. Thus, the findings of this study are expected to be beneficial to researchers and medicinal plant businesses.

Key words: Growth, Phenolics, Saponin, Macro elements, Micro elements.

Introduction

Tribulus terrestris L. plant is valued for their natural remedies, as an alternative treatment for erectile dysfunction and for stimulating the immune system. The medicinal properties of the plants have been related to the phenolic and saponin components of the plants (Rehman *et al*., 2017). Like many other medicinal plants, it is collected from nature. However, there is an important query related to the plants collected from nature, as it is believed that plants in unstressed conditions have lower phenolic content than those growing in stressed conditions. Thus, the present study was focused on verifying the effect of drought on the saponin content of *T.terrestris* L. However, along with saponins, other important aspects such as occurrence, plant growth, and chemical composition were also considered important for evaluation, as no earlier studies were carried out on these traits in the study area.

The metabolic responses of various food crops to drought stress have been studied, but such studies lack or have very little information on medicinal plants. Sometimes, drought might lead to biochemical disorders, especially biosynthesis of primary and secondary metabolites, which make individual plant study essential, as reported by Rebey *et al.* (2012), Javed *et al.* (2013), and Klein (2015). The importance of this plant has been widely recognized owing to previous studies on its protective effect on lipid profile and oxidative stress (Sailaja *et al*., 2013), antitumor activity, and sperm quality enhancement (Oliveira *et al*., 2015). The biomass and chemical composition of the plants are important factors; however, there is no study to confirm the effect of drought on these factors, especially its chemical composition. Previous studies were focused on individual phenolic and saponin compounds; such as dioscin, trillarin, gracillin, protodioscin, protogracillin, quercetin-3-O-rhamnoglucoside rutin, quercetin glycosides, kaempferol-3-O-rhamnoglucoside, kaempferol glycosides, and caffeoil. Since the study on individual compounds is an expensive and difficult procedure, therefore it seemed more meaningful and economical to study the total antioxidant power because of the cooperative action of the phytochemicals. For example, Jana & Shekhawat (2010) reported that crude extracts of medicinal plants were more biologically active than isolated compounds, owing to their synergistic effects. For this reason, the current trend preferred by many researchers (Dai & Mumper, 2010; López-Alarcón & Denicola, 2013), use assays for quick overall quantification and estimate for the effectiveness of phenolic and saponin compounds. Moreover, the study of minerals in the soil and in *T. terrestris L.* plants in the study area was an important concern, as it has not been previously appraised.

With respect to the above considerations, we attempted to test how the plant growth, dry matter, total phenol and total saponin contents, and mineral composition differed under in-situ water-stressed and unstressed conditions. Plants were collected from different ecoregions in Saudi Arabia (Jeddah, Makkah, Al Ta'if and Al Baha) and Pakistan (D.I. Khan, Bannu, Abbottabad and Mansehra) to compare the effect of natural water stress. In the present study, various attributes of the plant, such as occurrence, growth, and chemical composition were compared using standard procedures. The findings of the present study are expected to provide a baseline for pricing of the crop biomass, and its visualization may encourage field production of the plant.

Materials and Methods

Details of the in-situ sites: *T. terrestris* L. plants growing under in-situ natural conditions were collected from Jeddah, Makkah, Al Ta'if and Al Baha regions of Saudi Arabia and D.I. Khan, Bannu, Abbottabad, and Mansehra regions of Pakistan. The geographical and climatic conditions for all the aforementioned regions are listed in Table 1. Furthermore, soil samples were also collected at the time of plant collection from all the in-situ sites, and their properties are presented in Table 2.

Procedure for study of plant occurrence: The study was performed from September 2015—April 2016 during active plant growth period. Data on the distribution of Tribulus *terrestris* L. plants was recorded using the technique of transect walks carried out in the areas following Baskaran *et al*. (2012). A different transect (1–2 km long, 6 m wide) was followed each time to count and record the number of plants. The number of plants from various visits to each site was averaged using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Species was classified on the basis of their occurrences into frequent (>50 plants km^{-2}), occasional (30 to 50 plants km^{-2}) and rare species (20 to 30 plants km^{-2}) using the method of Anon., (2001).

Plant growth: At least 10 plants were collected in-situ at full blossom stage. The number of stems $plant^{-1}$, average length of stems (cm), number of leaves $plant^{-1}$, number of flowers $plant^{-1}$, and fresh biomass $plant^{-1}$ were determined. Subsequently, the plants were washed and then dried at room temperature (25°C) for dry biomass and chemical analysis.

Table 1. Geographical and climatic conditions of the study area (in-situ sites).

	In-situ sites	Climate*	Latitude, N	Longitude, E	Altitude (m)	Av. Temp. (°C)	Av. precipitation (mm)
Saudi Arabia	Jeddah	desert / arid	21.496	39.244	15	28.4	61.0
	Makkah	desert / arid	21.331	39.949	277	30.8	22.4
	Al Ta'if	desert / arid	21.432	40.491	1518	23.04	179.3
	Al Baha	desert / arid	20.179	41.635	1680	22.9	138.1
Pakistan	D.I. Khan	desert / arid	31.842	70. 895	165	24.2	268.8
	Bannu	local steppe	32.989	70.603	379	23.1	401.2
	Abbottabad	humid subtropical	34.168	73.221	1250	19.2	1269
	Mansehra	humid subtropical	34.333	73.201	1088	18.5	1445

*According toKöppen climate classification

Table 2. Initial soil properties (0-30 cm) of the in-situ sites.

Soil properties	Jeddah	Makkah	In-sito sites					
			Al Ta'if	Al Baha	D.I. Khan	Bannu	Abbottabad	Mansehra
Bulk density (g cm^{-3})	1.50	1.52	1.44	1.48	1.42	1.39	1.19	1.10
pH	7.93	7.76	7.90	7.86	8.06	7.80	7.38	7.35
Organic matter (%)	0.63	0.53	0.83	0.79	0.49	0.75	2.17	2.12
CEC ($cmol_c$ kg^{-1})	14.37	14.30	14.28	14.41	16.64	14.33	13.12	14.28
EC (*dS/m)*	1.99	1.92	1.30	1.24	1.79	1.55	0.42	0.40
Soil texture	LS	LS	LS	LS	LS	CLS	CLS	CLS
	Quantity (g kg^{-1})							
N	0.450	0.381	0.864	0.792	0.740	0.611	1.043	1.168
P	0.054	0.050	0.049	0.052	0.062	0.076	0.071	0.073
K	0.328	0.316	0.171	0.288	0.379	0.312	0.168	0.309
Ca	4.234	4.249	4.139	4.195	4.109	4.201	4.143	4.286
Mg	0.258	0.273	0.268	0.319	0.273	0.225	0.296	0.310
S	0.063	0.063	0.065	0.071	0.065	0.064	0.074	0.068
	Quantity (mg kg^{-1})							
Mn	11.352	9.127	10.424	14.634	16.868	18.114	22.742	26.313
Fe	16.875	14.711	19.123	18.750	21.127	20.650	46.347	46.216
Cu	2.034	2.749	3.528	3.721	4.239	6.839	8.167	10.724
Zn	3.055	3.553	4.831	5.441	5.294	4.165	12.502	13.545
Cd	ND	ND	ND	ND	ND	ND	ND	ND
Ni	0.043	0.031	ND	ND	ND	0.068	ND	ND
Pb	0.062	0.040	ND	ND	ND	0.096	ND	ND

Averages soil conditions for the respective sites. LS = Loamy soil

Plant composition: To ascertain and compare the plant composition, the following chemical analysis was performed in triplicate for each sample of powdered air-dried plant material.

Total phenolics and antioxidant activities: The total phenolic content and antioxidant activities were measured following the procedure described by Daur (2015). Accordingly, 1 g of each sample of powdered air-dried plant material was subjected to extraction in 50 mL methanol (70%) by swirling for 1 h at room temperature in an orbital shaker. The extracts were then filtered through Whatman filter paper (No.1) and stored at 4°C for phenolic and antioxidant analysis. The amount of total phenolics in the extracts was measured at 750 nm by using a spectrophotometer (U-2001, model 121-0032 Hitachi, Tokyo, Japan) and were calculated by comparing with a gallic acid calibration curve, and the results were expressed as milligram gallic acid equivalent g^{-1} [GAE (mg g^{-1})] of air-dried plant material. Similarly, antioxidant activities of the extracts were determined by using Ferric-reducing ability of plasma (FRAP) assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, according to the method of Daur (2015). The results for FRAP assay were expressed as μM $FeSO_4$ g^{-1} of dry plant material, and the results for DPPH radical scavenging assay were expressed as the percentage inhibition of the DPPH radical.

Total saponin content: The measurement of total saponins was performed according to the method of Makkar *et al*. (2007). Accordingly, for each sample, a mixture of 10 g of powdered air-dried plant material was subjected to extraction in 100 mL of 50% aqueous methanol in a 250-mL flask and was shaken overnight at room temperature using a magnetic stirrer. The content was then centrifuged at 3000 × *g* for 10 min, and the supernatant was collected. It was then filtered through Whatman filter paper. Methanol was evaporated from the filtrate under vacuum at 42°C by using a rotary evaporator. Then, the aqueous phase was centrifuged at 3000 × g for 10 min to remove the water insoluble materials. After that, the aqueous phase was extracted with equal volume of chloroform (three times) in a separating funnel to remove the pigments. Finally, the concentrated saponins were extracted in the aqueous solution with equal volume of n-butanol (twice). The n-butanol was later evaporated under vacuum at 44°C. The dried fraction containing saponins was dissolved in 10 mL of distilled water and was placed in a pre-weight container. It was gently immersed at the speed of 0.5 cm min^{-1} in a liquid nitrogen bath to obtain freeze-dried saponin fractions. Consequently, it was calculated as percent recovery of saponins.

Soil and plant elemental analysis: Elemental analyses of plant samples were performed as follows. Nitrogen (N) content was quantified using a Perkin-Elmer CHNS/O Analyzer (Model 2400), following the manufacturer's instructions (PerkinElmer, Inc., USA). Phosphorus (P) was determined colorimetrically, following the protocol described by Ryan *et al*. (2001), whereas all other elements (K, Ca, Mg, Fe, Cu, Zn, Cd, Cr, Ni, Pb, and Mn) were determined using Varian inductively coupled plasma-optical emission spectroscopy (ICP-OES) according to manufacturer's instructions.

Result and Discussion

Plant occurrence: According to the scale given in Materials and Methods under "Procedure for study of plant occurrence," *T. terrestris* L. plants were found to occur randomly in the study area (Table 3); for example, Jeddah and Makkah had almost similar environmental conditions, but the plants were occasionally found in Jeddah and rarely in Makkah. Al Ta'if and Al Baha had similar environmental conditions, but the plants were found frequently in Al Ta'if and rarely in Al Baha. It was randomly found in the other sites with similar geographical and environmental conditions. Thus, we can say that it is found heterogeneously across the study area, and its distribution is not related to environmental conditions. The finding for heterogeneous distributions is also supported by Gaston (2000) and Xie *et al*. (2015), who reported that the heterogeneous distribution of the plant species was increasingly well documented, and understanding their existence constitutes one of the most significant research challenges to both ecologists and biogeographers. However, the present study confirmed that *T. terrestris* L. grows in extremely dry and harsh climatic conditions of Jeddah and Makkah, where only few other plants can survive. Our findings in this regard are supported by El-Ghareeb (1999), Akhter & Arshad (2006), and Shaheen *et al*. (2014).

Table 3. Occurrence and growth of *Tribulus terrestris* L. under variable natural in-situ water stress.

In-situ sites		Occurrence*	Number of stems plant^{-1}	Average length of stems (cm)	Number of leaves plant^{-1}	Number of flower plant^{-1}	Fresh biomass plant^{-1} (g)	Dry biomass plant^{-1} (g)
Saudi Arabia	Jeddah	Occasional	5.58^{c}	13.75^{d}	29.08^{f}	6.60^{d}	8.79^{e}	4.27^{c}
	Makkah	Rare	5.24^{c}	12.33^{d}	31.09^{e}	5.29^{e}	8.42^{e}	4.31^{c}
	Al Ta'if	Frequent	8.18^{b}	18.81^{c}	59.78^{c}	9.02^{c}	12.57^{d}	5.45^{bc}
	Al Baha	Rare	8.04^{b}	18.03^{c}	59.49^{cd}	9.02^{c}	15.75^{bc}	5.82^{ab}
Pakistan	D.I. Khan	Rare	8.11^{b}	17.79^{c}	58.15^{d}	7.18^{d}	12.86^{d}	4.57^{bc}
	Bannu	Frequent	9.04^{ab}	18.87^{c}	60.26^{bc}	10.19^{b}	14.63^{c}	5.41^{bc}
	Abbottabad	Occasional	10.10^{a}	20.99^{b}	64.50^{b}	8.86^{c}	16.30^{b}	5.46^{bc}
	Mansehra	Rare	10.11^{a}	27.18^{a}	66.60^{a}	11.62^{a}	19.63^{a}	6.99^{a}

***Occurrence key:** frequent (>50 plants km^{-2}), occasional (20—50 plants km^{-2}), and rare species (5—20 plants km^{-2})

Table 4. Total phenolic content, antioxidant and free radical scavenging activity, and saponins content of *Tribulus terrestris* L. under variable in-situ drought stress.

	In-situ sites	Total phenolic content GAE (mg/g)	Antioxidant activity FRAP (µM FeSO4/g)	Free radical scavenging activity [DPPH inhibition (%)]	Saponins content (%)
Saudi Arabia	Jeddah	49.08^{a}	1766.2^{a}	33.15^{a}	4.18^{a}
	Makkah	47.83^{a}	1710.8^{b}	33.52^{a}	4.02^{ab}
	Al Ta'if	39.972^{b}	1438.2^{c}	26.00^{b}	4.10^{ab}
	Al Baha	39.02^{b}	1433.0^{c}	25.39^{b}	4.10^{ab}
Pakistan	D.I. Khan	39.62^{b}	1452.9^{c}	25.61^{b}	2.94^{abc}
	Bannu	40.32^{b}	1461.5^{c}	26.55^{b}	3.08^{abc}
	Abbottabad	39.89^{b}	1350.20^{d}	22.77^{bc}	2.63^{bc}
	Mansehra	37.31^{b}	1278.4^{e}	20.14^{c}	2.14^{c}

Table 5. Macro and micro elemental contents of *Tribulus terrestris* L. under variable in-situ drought stress.

	In-situ sites	Macro-elements (g kg-1 DM)						
		N	P	K	Ca	Mg	S	Na
Saudi Arabia	Jeddah	15.37^{bc}	2.57^{c}	22.66^{d}	3.32^{bc}	13.01^{c}	2.42	2.38^{ab}
	Makkah	15.27^{c}	2.50^{c}	23.09^{d}	3.06^{c}	12.75^{c}	2.41	2.26^{abc}
	Al Ta'if	15.73^{abc}	2.92^{a}	25.84^{bc}	3.68^{a}	15.42^{b}	2.55	2.10^{c}
	Al Baha	15.56^{abc}	2.90^{ab}	24.90^{c}	3.53^{ab}	14.99^{b}	2.43	2.14^{bc}
Pakistan	D.I. Khan	15.79^{abc}	2.60^{bc}	25.63^{bc}	3.29^{bc}	13.70^{c}	2.50	2.48^{a}
	Bannu	16.84^{a}	2.67^{abc}	26.69^{b}	3.14^{c}	15.46^{b}	2.60	2.27^{abc}
	Abbottabad	16.62^{abc}	2.93^{a}	28.69^{a}	3.33^{bc}	18.74^{a}	2.45	2.28^{abc}
	Mansehra	16.77^{ab}	2.90^{ab}	29.09^{a}	3.67^{a}	19.38^{a}	2.53	2.11^{bc}
	In-situ sites	Micro-elements (mg kg-1 DM)						
		Mn	Fe	Cu	Zn	Cd	Ni	Pb
Saudi Arabia	Jeddah	13.98^{d}	7.30^{de}	1.27^{d}	4.36^{d}	0.0	0.02	0.01
	Makkah	9.60^{e}	7.14^{e}	1.35^{d}	4.60^{d}	0.0	0.01	0.01
	Al Ta'if	17.06^{bc}	7.60^{cde}	2.45^{d}	5.94^{bc}	0.0	0.00	0.00
	Al Baha	15.38^{bcd}	8.27^{bcd}	2.08^{d}	6.76^{b}	0.0	0.00	0.00
Pakistan	D.I. Khan	14.86^{cd}	8.65^{b}	4.70^{c}	4.99^{cd}	0.0	0.00	0.00
	Bannu	17.96^{b}	8.50^{bc}	6.46^{b}	4.56^{d}	0.0	0.03	0.02
	Abbottabad	23.88^{a}	25.80^{a}	7.61^{ab}	14.00^{a}	0.0	0.00	0.00
	Mansehra	24.90^{a}	26.09^{a}	8.26^{a}	14.80^{a}	0.0	0.00	0.00

Plant growth*:* The results of the study on various growth parameters of Tribulus *terrestris* L. are provided in Table 3, which shows the maximum values for number of stems plant^{-1} (10.11), average length of stems (27.18 cm), number of leaves plant^{-1} (66.60), number of flowers plant^{-1} (11.62), fresh biomass plant^{-1} (19.63 g) and dry biomass plant^{-1} (6.99 g) recorded at the Mansehra site. Minimum values for number of stems plant^{-1} (5.24-5.58), average length of stems (12.33-13.75 cm), number of leaves plant^{-1} (29.08-31.09), number of flowers plant^{-1} (5.29-6.60), fresh biomass plant^{-1} (8.42-8.79 g) and dry biomass plant^{-1} (4.27-4.31 g) were recorded either at Makkah or at Jeddah, whereas those for all the other sites the values for the fore mentioned parameters were observed between the minimum and maximum.

Water can affect plant growth both positively and negatively, because the amount of water required for optimal plant functioning varies according to the type of plant. The results of the present study demonstrate that *T. terrestris* L., though considered as a highly drought-tolerant plant, but grows best with a constant supply of water. Although we have no literature and data related to the exact water/or rainfall requirements of *T. terrestris* L., Lambers & Poorter (1992) and Al-Kaisi *et al*. (2013) support our findings on the differences in growth parameters due to rainfall, climatic conditions, or topographic conditions.

Plant composition: Theresults for plant composition, including total phenolics, FRAP and DPPH activities, and total saponin contents (Table 4), were found to be in contrast to growth parameters, as it indicated an increase in total phenolics, FRAP and DPPH activities, and total saponin content with increasing drought. The maximum values for total phenolic content [47.83-49.08 GAE (mg g^{-1})], antioxidant FRAP measurement [1710.8-1766.2 (µM FeSO4 g^{-1})], free radical scavenging activity (33.15-33.52% DPPH inhibition) and saponin content (4.02-4.18%) were recorded either at Makkah or at Jeddah. At the Mansehra site, minimum values for total phenolic content [37.31 GAE (mg g^{-1})], antioxidant FRAP measurement [1278.4 (µM FeSO4 g^{-1})], free radical scavenging activity

(20.14% DPPH inhibition) and saponin content (2.14%) were recorded. Thus, our results suggest a positive effect of drought on the studied chemical analysis of *T. terrestris* L. Similarly, positive effects of limited water supply on peanut had also been reported earlier, but were linked to the ability of plant types to survive under constraining conditions (Aninbon *et al.*, 2016).

The results for ***plant macro elements*** (Table 5) appeared to be variable by denoting mixed results; for example, N was highest (16.84 g kg^{-1}) in Bannu and lowest (15.27 g kg^{-1}) in Makkah; P was highest (2.93 g kg^{-1}) in Abbottabad and lowest (2.50 g kg^{-1}) in Makkah; K was highest (29.09 g kg^{-1}) in Mansehra and lowest (23.09 g kg^{-1}) in Jeddah; Ca was highest (3.68 g kg^{-1}) in Al Ta'if and lowest (3.06 g kg^{-1}) in Makkah; Mg was highest (19.38 g kg^{-1}) in Mansehra and lowest (12.75 g kg^{-1}) in Makkah; Na was highest (2.48 g kg^{-1}) in D.I. Khan and lowest (2.10 g kg^{-1}) in Al Ta'if. Sulfur (S) showed non-significant differences among locations. Overall, the results of plant macro-element analysis indicated statistical similarity among the locations; thus, it can be concluded that it is more related to water stress, and to some extent, to the soil factors (Table 2). Nutrient uptake is a complex process affected by various stresses, balance of different nutrients, root growth, and various other factors. However, our results are supported by those of Daur *et al.* (2011) and Winner & Pell (2012), who reported that the plant nutrient composition depended on availability of nutrient and its uptake by plants. The results of ***micro-element contents*** (Table 5) also indicated relation to water stress as in Mansehra Mn (24.90 mg kg^{-1}), Fe (26.09 mg kg^{-1}), Cu (8.26 mg kg^{-1}) and Zn (14.80 mg kg^{-1}) were found to be higher than those at other locations. However, close observation indicates that micro elements are much affected by their availability in the soil, as supported by Fageria *et al*. (2010) and Shaheen *et al*. (2013), who reports that micro-nutrients are affected by both water stress and soil nutrient contents. Thus, keeping in consideration the results of micro-elements (Table 5) and soil factors (Table 2) indicates that it is more related to soil factors than to water stress.

Conclusion

The study indicated that *T. terrestris* L. occurs variably in the study area and is not related to climatic conditions. Moreover, drought significantly reduced the growth and biomass of *T. terrestris* L., whereas total phenolics, and FRAP and DPPH activities, and total saponin content increased with increasing drought. Mineral elements were found to be related to both stress conditions and availability. Based on the results of the present study, *T. terrestris* L. plants collected from areas with drought-stress conditions might be considered better than those collected from areas with non-drought conditions.

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