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Seasonal Physiological & Biochemical Responses of Three Medicinal Halophytes From Karachi University Campus

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Abstract: Three medicinally important halophytes: *Cyperus rotundus* L., *Dactyloctenium scindicum* Boiss. and *Lasiurus scindicus* Forssk., were chosen from Karachi University campus to explore seasonal physiological and biochemical responses. Seasonal and diurnal water potentials (Ψ) showed a great variation among species. Pre-dawn water potential (Ψ_{PD}) was higher before and after rains in all test species than the mid-day water potential (Ψ_{MD}) corresponding to the soil matric potential (Ψsoil), electrical conductivity (EC) as well as % soil moisture (SM). *Lasiurus scindicus* had lowest Ψ_{PD} & Ψ_{MD} in comparison to *C. rotundus* and *D. scindicum* before rainfall. Similar trends were observed for osmotic potential (Ψ_S) of all species. Both Ψ_{MD} & Ψ_s substantially increased in *D. scindicum* and *L. scindicus* after rainfall, whereas, *C. rotundus* showed no significant change and this species also maintained highest turgor (Ψ_P). Leaf stomatal conductance (*gS*) was higher in *C. rotundus* while proline (*PRO*) in *D. scindicum* before rain. Leaf *PRO* decreased while *gS* increased after rains and this increase was 3 fold in *D. scindicum*. Sodium (Na^+) and chloride (Cl^-) were higher than potassium (K^+) in all species before rain with maximum values in *D. scindicum*. In general Na^+ and Cl^- decreased while K^+ increased in all species after rain. Total soluble sugars (*TSS*) were consistently higher in *C. rotundus* while *D. scindicum* maintained higher Na^+ and Cl^- even after rains compared to other species. The differences in ecophysiological responses owing to differences in life forms suggest the existence of plant species in different habitats.

Keywords: Biochemical, Diurnal, Grass, Physiological, Seasonal, Sedge, water relations, Proline

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Introduction:

A wide variety of the edible plants consumed as vegetables, condiments and spicing agents are known to have medicinal value with antibacterial, antiviral and antioxidant properties [1]. They are largely being cultivated for human consumption since long. However, overexploitation of existing resources and poor irrigation techniques have resulted in land degradation and decreased production of medicinal and edible crops [2]. Global rise in population has resulted in food scarcity, famine and disease outbursts particularly in the third world countries. Poor management techniques e.g., strenuous cultivation of annual plants over the years has led to inappropriate land utilization [3]. In view of expanding population pressure sustainable ways are needed to find non-conventional plants in nature which could be grown in field as medicinal herbs besides providing staple food or fodder [4]. Farming of perennial halophytes in heterogeneous environment seems plausible choice for the above mentioned purpose.

Halophytes are represented by a number of plants which could grow in variable saline environments [5]. Recent focus on eco-physiological studies has revealed that a number of perennial halophytes could be used as medicines, fodder and forage [4, 6]. Pragmatic utilization of marginal lands, requires recognition of the vegetation per se and its relationship with environmental conditions particularly in semi-arid conditions. Seasonal physiological and biochemical screening of plants is the first step for sustainable utilization of such plants [7, 8].

A vast variety of perennial grasses (Poaceae) and sedges (Cyperaceae) are found in semi-arid conditions of Karachi having both medicinal and forage properties [5]. Vegetative propagation of such plants usually increases after monsoon rains owing to some changes in their physiological and biochemical processes [8, 9]. In Karachi and its vicinity, low lying flooded areas are represented by hydro-halophytes, salt flats by xerohalophytes and dry sandy soil by psammophytes [5]. In this study each of three monocotyledonous group of perennial halophytes were chosen (viz: *Cyperus rotundus* L., *Lasiurus scindicus* Forssk., and *Dactyloctenium scindicum* Boiss.) for seasonal physiological & biochemical responses. *Cyperus rotundus* is a hydrohalophyte sedge (family *Cyperaceae*) which grows on moist alkaline soils. This species has great medicinal value for its antidiuretic, analgesic, antispasmodic and antibacterial activities [10] besides being consumed by local animals as forage in India [11]. *Lasiurus scindicus* is a psammophyte grass (family Poaceae) which grows on sandy tracts in Karachi. Leaf extracts of this plant species are known to have antibacterial activities against several microbes [12] and this fodder grass is relished by cattle, camels and sheep. *Dactyloctenium scindicum* is a Xero-halophyte grass (family Poaceae) with several medicinal uses for its antioxidant, antibacterial and anti-fungal activities [4] and it is also used as fodder grass [13].

Marginal lands with poor soil where cost efficient crop production is not possible could be used for non-conventional agriculture [14]. Eco-physiological investigations are needed for non-conventional plants with different life forms in nature for sustainable use and land management. Two main questions were addressed in this study 1) Do the physiological and biochemical responses of medicinally important halophytes before and after rains differ due to their life forms? 2) Based on their seasonal water status (before and after rains) which habitat would suit their growth?

Materials & Methods

Study Area:

The study was conducted at Karachi University campus (ca 15 miles away from the Karachi Coast). Karachi city (latitude 24º 48' N, longitude 65º 55' E) is characterized by subtropical desert conditions with an average of 220 mm precipitation, the major part of which is received during monsoon. Five plants each of all test species were randomly selected and properly tagged for the study. Soil samples (n = 5) were taken with the help of soil augur (from 6 -12 inches depth near plant roots). Leaf and soil samples were collected from mid of May to June (before rains) and midst of August and September (after rains) in plastic bags, stored in ice box containing dry ice and brought back to the laboratory for analysis.

Soil Physiochemical Characteristics:

Soil moisture content was calculated using the difference of immediately taken fresh and oven dried weights. Soil pH and Electrical conductivity was estimated on 1:5 soil-water extracts with the help of pH and Conductivity meter (Ion-85 Radiometer). Soil matric potential (Ψsoil) was measured with the help of pre-calibrated soil probe (PST-55-30-SF), connected to a dew point micro-voltmeter (HR 33-T, Wescor Inc., Logan, Utah-USA).

Water relations:

Diurnal (pre-dawn at 5:00 H and 12:00 H at mid-day) water potential (Ψ_{PD} and Ψ_{MD}) on leaves were estimated with the help of Dew point microvoltmeter (HR-33 T, Wescor Inc., Logan, Utah-USA) attached to a L-51 sample chamber before and after rains. Osmotic potential (Ψs) was determined on freeze killed samples of same leaves brought to the laboratory. A pre-calibrated A-P4 Porometer (Delta-T Devices, U.K) was used on abaxial surface of leaves to determine stomatal conductance (*gS*).

Physiological and Biochemical Tests:

Five hundred mg finely ground oven dried leaf samples were boiled in 100 ml distilled water for 2 H to prepare hot water extracts. Different dilutions of the extract were used for biochemical analysis. Proline was determined by the method of Bates et al., [15], total soluble carbohydrates by the method of Yam & Willis [16], cations (Na^+ & K^+) by using flame photometer and Cl^- with the help of chloride meter (Ion 85, Radiometer Ion Analyzer).

Results

Soil EC was generally higher in the root zones of all species before rains (Table 1). Soil EC in the root zone of *Dactyloctenium scindicum* was higher and lower in *C. rotundus* (Table 2). A significant decrease ($p < 0.05$) in EC was observed after monsoon rains but the trend remained similar (Table 2). Soil pH ranged between alkaline levels of 8 to 8.5 with lower values after monsoon rains but no significant differences were observed before and after rains (Table 1). Soil moisture content increased after rains with highest values near the root zones of *C. rotundus* corresponding to higher soil matric potential (Ψsoil) and lowest in *D. scindicum* (Table 1).

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Table: 1. Physio-chemical characteristics of the Soil matric potential (Soil Ψ in -Mpa), pH, soil moisture (SM in % dry weight) and soil electrical Conductivity (EC in mS cm $^{-1}$).

Species	Soil Ψ BR	AR	pH BR	AR	SM BR	AR	EC BR	AR
C. rotundus	-1.9 + 0.3^a	-1.7 + 0.1 ^{a}	8.3 + 0.3^a	7.9 + 0.3 ^{a}	29 + 2.2 ^{b}	38 + 2.4 ^{b}	34 + 1.3^a	27 + 2.1 ^{a}
D. scindicum	-2.5 + 0.2 ^{b}	-2.1 + 0.2 ^{b}	8.4 + 0.2 ^{a}	8.2 + 0.2 ^{a}	11 + 1.1 ^{a}	17 + 1.8 ^{a}	47 + 1.1 ^{b}	33 + 2.3^b
L. sindicus	-2.4 + 0.3 ^{b}	-2.0 + 0.3 ^{b}	8.5 + 0.3 ^{a}	8.1 + 0.3 ^{a}	13 + 4.8 ^{a}	19 + 4.7 ^{a}	37 + 1.3 ^{ab}	29 + 1.7 ^{a}

Mean ± S.E before (BR) and after (AR) rains with different letters among species are significantly different at p<0.05 (Bonferroni's test).

Table: 2. Results of two Way ANOVA of characteristics in leaves by plant species (P) and Seasons (S) and their interactions (P x S).

Independent variable	P	S	P X S
Water Potential	47.7*	66.1**	21.5*
Osmotic potential	37.7**	51.2*	42.1*
Sodium	28.6*	34.2*	19.3*
Potassium	13.7*	19.5*	21.2$^{n.s}$
Chloride	31.3*	57.8*	27.4*
Stomatal conductance	27.3*	67.8**	48.4**

Numbers represent F-values * = p<0.01; * = p<0.001**; n.s = non significant

Seasonal and diurnal trend of leaf water relations is illustrated in Fig. 1. Pre-dawn water potential (Ψ_{PD}) was generally higher in all species compared to the mid-day (Ψ_{MD}) with lowest (more negative) values in *D. scindicum* before rains (Fig. 1). Similar trend was observed for leaf osmotic potential (Ψs) in all test species (Fig. 1). However, after monsoon rains leaf Ψ_S was lowest in *L. scindicus* (Fig. 1). A two way ANOVA detected significant differences ($p < 0.001$) between plant species (P), seasons (S) and their interactions (PxS) ($p < 0.01$; Table 2). Both seasonal and diurnal turgor potentials (Ψ_P) were higher in *C. rotundus* (Fig. 1). Lowest Ψ_P in both seasons was observed in *D. scindicum* (Fig. 1).

Results for proline (*PRO*) and stomatal conductance (*gS*) are illustrated in Fig. 2. Leaf *gS* was highest in *C. rotundus* followed by *L. scindicus* before rains (Fig. 2). Leaf *gS* generally increased in all species after rains but this increase was 3 fold in *D. scindicum* (Fig. 2). *Lasiurus scindicus* had higher *gS* values than *D. scindicum* before rains but lowest among all test species after rains (Fig. 2). A two way ANOVA revealed significant effect of species (P) = $p < 0.01$, seasons (S) = $p < 0.001$ and their interactions (P x S) = $p < 0.001$ (Table 2). Among organic solutes *PRO* was higher before rains with maximum values in *D. scindicum* (Fig. 2). A 2-3 fold decrease in *PRO* was observed after rains with *D. scindicum* inexorably retaining highest values (Fig. 2). *Cyperus rotundus* accumulated highest amount of total soluble carbohydrates (*TSS*) than any other test species both before and after rains (Table 3). In general *TSS* increased in all species after rains. Lowest amount of *TSS* was found in *D. scindicum* (Table 3). Ionic content of the test species indicate that both Na^+ and Cl^- were higher in *D. scindicum* while K^+ in *C. rotundus* before rains (Table 3). Accumulation of Na^+ and Cl^- in leaf tissues decreased after rains while K^+ accumulation increased (Table 3). A two way ANOVA detected significant changes in leaf ionic content ($p < 0.01$) in species (P) and seasons (S) as well as their interactions (P x S), however, two way interaction (P x S) for K^+ remained non-significant (Table 1). Values for Na/K ratio were higher in *D. scindicum* (15.9), compared to *L. scindicus* (10.9) and *C. rotundus* (8) before rains. After rains Na/K ratio was substantially decreased in all test species with lowest values in *C. rotundus* (5.3), followed by *L. scindicus* (6.7) and *D. scindicum* (8.17).

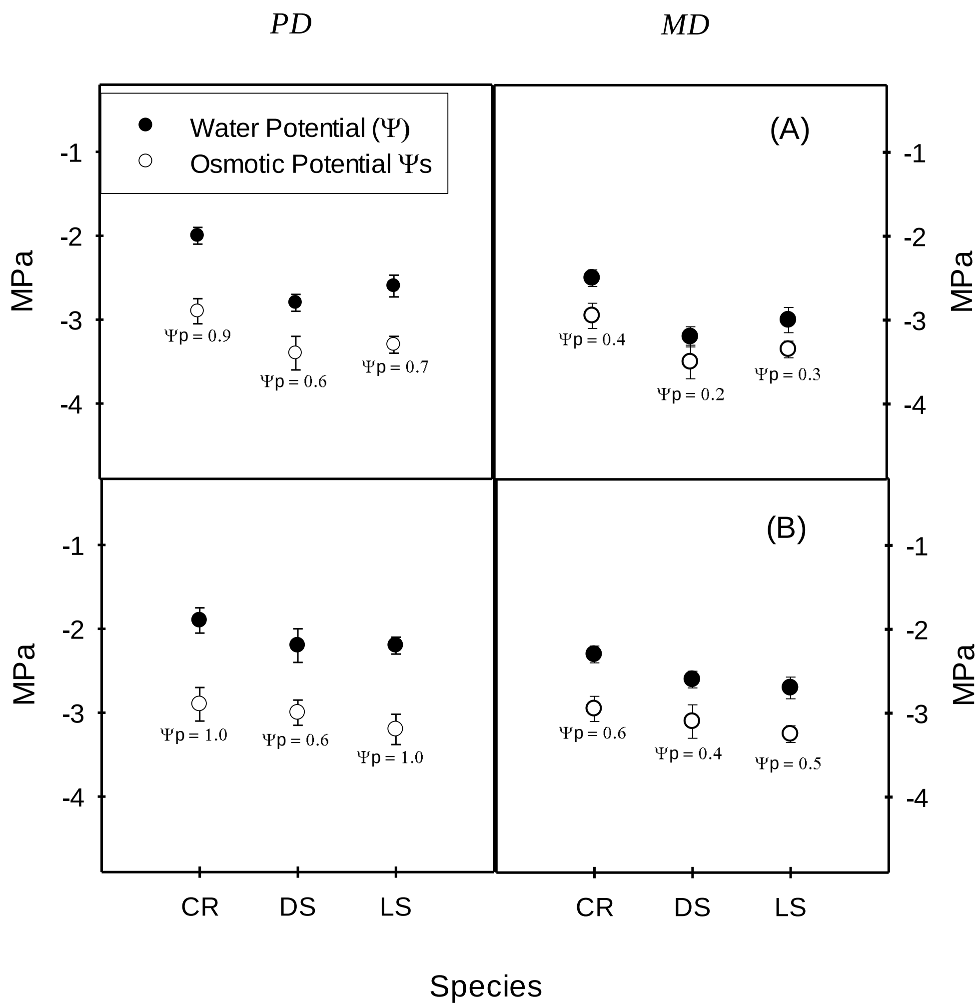

Fig. 1. Plant water relations in megapascals (-MPa). Black shaded circles represents water potential (Ψ) and open circles represents osmotic potential (Ψs) in different species (CR= Cyperus rotundus; DS= Dactyloctenium scindicum and LS= Lasiurus scindicus) before and after rains. Pre-dawn water potential (*PD*) are indicated on left while mid-day water potential (*MD*) on right side. (A) represent values before and (B) after rains. Turgor potentials (Ψ_P) are directly represented below circles

Table: 3. Leaf ionic content (Na^+, K^+ Cl^-) and Total soluble sugars (TSS) in mg g^{-1} dry weight before (BR) and after (AR) monsoon rains.

Species	Na^+		K^+		Cl^-		TSS	
	BR	AR	BR	AR	BR	AR	BR	AR
C. rotundus	469 + 11.3^a	395 + 9.8^a	57 + 5.6^b	74 + 4.3^b	391 + 12.1^a	278 + 9.2^a	340 + 12.1^a	371 + 9.2^a
D. scindicum	590 + 13.2^b	425 + 7.5^b	37 + 3.8^a	52 + 2.8^a	483 + 14.1^b	377 + 4.7^b	217 + 14.1^b	277 + 4.7^b
L. sindicus	535 + 14.1^a	388 +9.9^b	49 + 4.8^b	58 + 3.7^b	461 + 15.3^b	353 + 5.1^b	315 + 15.3^b	333 + 3.7^b

(Mean values having different letters among species are significantly different at $p < 0.05$ by the Bonferroni test).

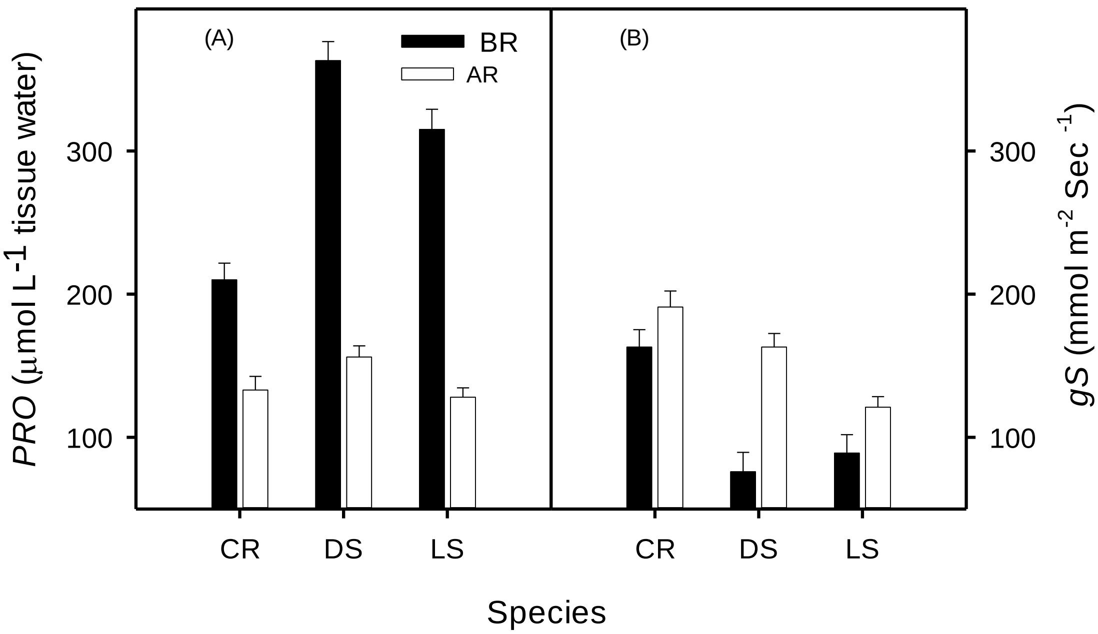

Fig. 2. (A) Proline (*PRO*) and (B) Stomatal Conductance (*gS*) in leaves Before (BR) and after rains (AR) in plant species (CR = *Cyperus rotundus*; DS = *Dactyloctenium scindicum*; LS = *Lasiurus scindicus*)

Discussion

Halophytes provide a number of benefits to the mankind [17] and a wide variety of these plants are of high medicinal value [4]. Halophytes in nature could be grown on marginal / degraded lands where conventional crops fail to grow. They are well adapted to endure environmental stresses with different physiological and biochemical mechanisms which require detailed analyses [6, 17].

Soil EC is an important parameter for evaluating plant water status under salt stress [18]. Higher soil EC near root zone of *D. scindicum* corresponds to the lower soil moisture and soil matric potential (Ψsoil) indicating the ability of plants to resist salt stress. By contrast, root zone of *Cyperus rotundus* showed lower soil EC with higher Ψsoil and moisture. Soil pH in root zone of all plants was alkaline (8 to 8.5) in both rainy and dry seasons which is typical of saline soils in arid regions [19]. Values of Ψsoil were comparable to the leaf Ψ_{PD} both before and after rains. Leaf Ψ_{PD} was generally higher than the Ψ_{MD} when Ψ_{P} was also low. Lowest Ψ_{P} in *D. scindicum* could be attributed to more negative values of Ψs, though Ψ_{P} in this specie was slightly increased after rains. *Cyperus rotundus* which prefers more mesic to hydric conditions for growth consistently maintained higher Ψ_{P} before and after rains with minor seasonal and diurnal changes in water relations which indicates its water spending strategy [20]. Interestingly, *L. scindicus* had lower Ψ_{P} with greater variation in Ψ_{PD} and Ψ_{MD} while *D. scindicum* had lowest Ψ_{P} both before and after rains with greater oscillations in diurnal water potential (Ψ_{PD} and Ψ_{MD}). Hence, *D. scindicum* appeared to follow water conserving strategy [20]. Stomatal conductance (*gS*) and accumulation of *PRO* in this study also correlates well with plant water relations. All test species showed an increase in *gS* after rains however, this increase was 3 fold higher in *D. scindicum* indicating temporal respite from salt stress. *Lasiurus scindicus* had higher *gS* values than *D. scindicum* before rains but lower *gS* after rains. Greater oscillations in diurnal water potential (Ψ_{PD} and Ψ_{MD}) and a substantial increase in *gS* after rains suggest better salt and drought tolerance of *D. scindicum* and lowest Ψ_{P} values indicates water conserving strategy [20]. Among organic solutes highest amount of *PRO* was accumulated in *D. scindicum* before rains. Levels of *PRO* were lowest in *C. rotundus* followed by *L. scindicus* both before and after rains. Plants in nature accumulate higher amount of *PRO* during water stress which is lowered as soon as the stress is relieved [21]. In addition to osmotic adjustment *PRO* is also known to be involved in ROS scavenging to protect metabolic machinery under stress conditions [22]. *Cyperus rotundus* accumulated highest amount of *TSS* compared to lower amount of *PRO* both before and after rains. On the contrary, *D. scindicum* accumulated higher *PRO* and lower *TSS* than any other species. Interestingly this species had highest Na^{+} and Cl^{-} in its leaf tissues followed by *L. scindicus* which accumulated comparatively higher *TSS* both before and after rains.

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Leaf K^+ increased in all species after rains thereby improving Na/K ratio which may be related to higher *gS* in plants after rains. Our results indicate that *L. scindicus* acquire osmotic adjustment by increasing Na^+ and Cl^- and increased amount of *PRO* provides osmo-protection during the dry and saline period (before rains). Rise in *TSS* with lower amount of Na^+, Cl^- and *PRO* indicates an improved osmotic adjustment after rains. *Dactyloctenium scindicum* appeared to resist saline conditions before rains by accumulating higher leaf ions and *PRO* compared to other test species for its osmo-protection. Although *TSS* increased significantly in *D. scindicum* after rains, ionic content remained comparatively higher, indicating their possible use for efficient osmotic adjustment [23]. Although ionic content in *Cyperus rotundus* decreased after rains, consistently higher amounts of *TSS* and lower amount of *PRO* before and after rains with higher *gS* and Ψ_{MD} indicates water spending strategy of this specie [20]. Higher Ψ_{MD} and *gS* are known to exist in plants with higher water use efficiency [24].
Based on the findings of this study, it may be concluded that physiological and biochemical responses of the species differ due to their life forms. Among test species *C. rotundus* (a hydro-halophyte) displayed lesser seasonal and diurnal fluctuations in water potential, turgor and low ionic content (Na^+ and Cl^-) with water spending strategy, whereas, *L. scindicus* (a psammophyte) showed higher fluctuations in diurnal water relations. Higher water use efficiency of *L. scindicus* depends upon the availability of moisture after rains. *Dactyloctenium scindicum* (a Xero-halophyte) showed greater seasonal and diurnal oscillations in water relation parameters with lowest turgor. Higher Na^+ and Cl^- in leaf tissues for osmotic adjustment and use of *PRO* as an osmo-protectant along with lowering of water and osmotic potentials indicate its water conserving strategy. Based on water status of plants it may be concluded that *C. rotundus* could be grown in areas with high soil moisture and lower fluctuations in salinity regimes whereas, *L. scindicus* on saline / sandy tracts, occasionally flushed with water. *Dactyloctenium scindicum* could be grown in areas with higher fluctuations in salinity and low amount of water requirements. However, laboratory trials for the salt and drought resistance are needed for all test species. Future lab studies would help in optimizing growth of these plants for yielding chemical constituents.

Conclusion

Physiological and biochemical aspects in field grown species showed promising results to be used as non-conventional medicinal plants. These plants of the wild could be grown on marginal / degraded lands with variable soil moisture and salinity. However, detailed laboratory trials for their growth, physiology and biochemistry are needed in future to maximize their growth in suitable environments. Further detailed analysis of chemical constituents of the above mentioned species will provide benefit for yielding medicines from halophytes.

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References

[1] Qasim M, Abideen Z, Adnan MY, Ansari R, Gul B, Khan MA. Traditional ethno-botanical uses of medicinal plants from coastal areas of Pakistan. J Coast Life Med 2014; 2: 22-30
[2] Pimentel D, Burgess M. Soil erosion threatens food production. Agriculture 2013; 3: 443-63
[3] Bouman BAM, Peng S, Castanˇ eda AR, Visperas RM. Yield and water use of irrigated tropical aerobic rice systems. Agric Water Manag 2005; 74: 87-105
[4] Qasim M, Aziz I, Rasheed M, Gul B, Khan MA. Effect of extraction solvents on polyphenols and antioxidant activity of medicinal halophytes. Pak J Bot 2016; 48: 621-7.
[5] Khan MA, Qaiser M. Halophytes of Pakistan: Characteristics, Distribution and potential economic usages. In: Khan MA, Kust GH, Barth H.-J, Böer B Eds. Sabkha Ecosystems II The Netherlands: Springer 2006; pp. 129-53.
[6] Qasim M, Fujii Y, Ahmed MZ, Aziz I, Watanabe KN, Khan MA. Phytotoxic analysis of coastal medicinal plants and quantification of phenolic compounds using HPLC. 1126-3504. Plant Biosys. 2018 Oct 29; Available from: http://www.tandfonline.com/action/journalInformation?journalCode=tplb20)
[7] Aziz I, Gulzar S, Noor M, Khan MA. Seasonal variation in water relations of *Halopyrum mucronatum* (L.) Stapf., growing near Sandspit, Karachi. Pak J Bot 2005; 37: 141-8
[8] Aziz I, Gul B, Gulzar S, Khan MA. 2011. Seasonal variations in plant water status of four desert halophytes from semi-arid region of Karachi. Pak J Bot 2011; 23: 587-94
[9] Aziz I, Khan MA. Proline and water status of some desert shrubs before and after rains. Pak J Bot 2003; 35: 911-5
[10] Sivapalan SR. Medicinal uses and pharmacological activities of *Cyperus rotundus* Linn. - A Review. Int J Sci Res Pub 2013; 3: 1-8

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[11] Cook CDK. Aquatic and Wetland Plants of India. New York: Oxford University press Inc. 1996.
[12] Ara I, Bukhari NA, Solaiman D, Bakir MA. Antimicrobial effect of local medicinal plant extracts in the Kingdom of Saudi Arabia and search for their metabolites by gas chromatography-mass spectrometric (GC-MS) analysis. J Med Plant Res 2012; 6(Pt 45): 5688-94
[13] Gul B, Ansari, R, Ali H, Adnan MY, Weber DJ, Nielsen BL, *et al*. The sustainable utilization of saline resources for livestock feed production in arid and semi-arid regions: A model from Pakistan. Emir J Food Agric 2014; 26 (Pt 12): 1032-45
[14] Carlsson G, Artensson LMM, Prade T, Svensson, S-E, Jensen ES. Perennial species mixtures for multifunctional production of biomass on marginal land. GCB Bioen 2017; 9: 191-201
[15] Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies. Plant and Soil 1973; 39: 205-7
[16] Yemm EW, Willis AJ. The estimation of carbohydrates in Plant extracts by Anthrone. Biochem J 1954; 57: 50-514
[17] Rasool SG, Siddiqui H, Hameed A, Hussain T, Aziz I, Khan MA, Gulzar, S. Diurnal and seasonal changes in water and ion relations of coastal halophytes. In: Gul B et al., .Eds. Sabkha Ecosystems, Tasks for Vegetation Science VI: Asia/Pacific. Switzerland: Springer Nature AG 2019; pp. 447-58
[18] Corwin DL, Lesch SM. Application of Soil Electrical Conductivity to Precision Agriculture. Agron J 2003; 95: 455-71
[19] Läuchli A. Grattan SR. Plant responses to saline and sodic conditions. In: Wallender WW, Tanji KK, Eds. Agricultural Salinity Assessment and Management. ASCE manuals and reports on engineering practice No 71 2nd ed. New York: American Society of Civil Engineers 2011.
[20] Shoukat E, Aziz I, Ahmed MZ, Abideen Z, Khan MA. Growth patterns of *Phragmites karka* under saline conditions depend on the bulk elastic modulus. Crop Pasture Sci 2018; 69 535-45
[21] Khan F, Adnan MY, Aziz I. Metabolic implications of salt induced osmolyte accumulation in *Avicennia marina* Pak J Bot 2016; 48: 29-36
[22] Ben Rejeb K, Abdelly C, Savoure´ A. How reactive oxygen species and proline face stress together. Plant Physio Biochem 2014; 80: 278-84
[23] Munns R, Passioura JB, Colmer TD, Byrt CS. Osmotic adjustment and energy limitations to plant growth in saline soil. New Phytol. Tansley Insight. Available from: (www.newphytologist.com)
[24] Williams LE, Baeza P, Vughan P. Midday measurements of leaf water potential and stomatal conductance are highly correlated with daily water use of Thompson Seedless grapevines. Irrig Sci 2012; 30: 201-12