Pak. J. Bot., 48(2): 629-636, 2016.

SECONDARY METABOLITES AS ANTI-NUTRITIONAL FACTORS IN LOCALLY USED HALOPHYTIC FORAGE/FODDER

SAMAN EHSEN[1], MUHAMMAD QASIM[1], ZAINUL ABIDEEN[1], R. FATIMA RIZVI[1], BILQUEES GUL[1*], RAZIUDDIN ANSARI[1] AND M. AJMAL KHAN[2]

[1]Institute of Sustainable Halophyte Utilization, University of Karachi, Karachi-75270, Pakistan
[2]Centre for Sustainable Development, College of Arts and Sciences, Qatar University, PO Box 2713, Doha, Qatar
[*]Corresponding author e-mail: bilqueesgul@uok.edu.pk, Phone office+9221-32044350, Fax +9221-34820258

Abstract

Rampant salinity coupled with population explosion necessitates search for suitable alternatives to conventional sources of food both for human and animal consumption. While it may be difficult to change our culinary preferences, training animals to adopt a changed diet of nonconventional salt tolerant plants is easier. Using these wild plants however, requires estimation of undesirable secondary metabolites (SMs) produced during stressful conditions, which may be harmful for health of animals. Some of these anti-nutritional components (total phenols, flavonoids, tannins, nitrates, saponins and oxalates) were determined in 22 halophytes locally used as fodder/forage. Most of the species were perennial shrubs and herbs of an area where environmental conditions like high mean annual temperature (~35 ^{o}C), low rainfall (< 250mm) with soil mostly dry (average 2% moisture) and saline (average EC 13 dSm^{-1}) supported the growth of halophytes and xerophytes. Values of SMs in the studied plants ranged from 0.13-4.05% for total phenols, 0.38-6.99% for tannins, 0.15-1.50% for flavonoids, 0.10-1.15% for nitrates, 0.45-8.68% for saponins and 0.36-2.34% for oxalates. Most of the species (19) contained low to moderate amount of individual as well as total SMs which were within the non-toxic ranges. However, three species distributed in coastal habitats where average soil salinity (27.67 dSm^{-1}) was considerably higher than inland ones (7.09 dSm^{-1}) had SMs contents above the safe limits. It is evident from these results that most of these plants contained moderate to low levels of anti-nutritional factors, which lies under the safe limits and hence, could be used as a potential feed source to raise animals, particularly in arid/semiarid areas. Additionally, these plants represents a viable choice as they can be grown without encroaching on agricultural lands and fresh water resources and could promote livestock production which may improve socio-economic conditions of poor farmers in a sustainable and eco-friendly manner.

Key words: Animal feed, Coastal plants, Salt tolerant species, Saline agriculture, Toxicity.

Introduction

Pakistan is a country of arid and semiarid region having an extensive network of irrigation canals. Agriculture is the main source of livelihood of a predominantly rural population, but almost every household also raises some cattle head. Mismanaged agricultural practices and other anthropogenic activities have rendered vast areas which decrease plant productivity and increase competition between crops of human and animal consumption for land and water resources. Rapid increase in human population is a further impediment for food sufficiency especially in the low income, less developed countries. Exploration of new plant species tolerant to harsh conditions as an alternative to the conventional animal feed is consequently gaining popularity to meet the challenge of population pressure on inadequate food resources (Al Sherif, 2009). Halophytes with diverse economic usages like food, fodder, fuel wood, oilseed, medicines, chemicals, landscaping, C-sequestration (Khan *et al*., 2009; Weber *et al*., 2007; Abideen *et al*., 2015a; Qasim *et al*., 2011) can supplement the basic needs of ever-growing population, particularly in third world countries.

The use of halophytes is receiving attention for past few decades (Abideen *et al*., 2012; Gul *et al*., 2013; Koyro *et al*., 2013; Qasim *et al*., 2014) and has been found promising for animal feed in several trials (Glenn *et al*., 1999; Swingle *et al*., 1996; Khan *et al*., 2009). However, chemical composition of the foliage and/or seeds of halophytes need careful consideration before recommending as animal feed. This is important because halophytes may accumulate large quantities of salt from which increase the thirst of the animals, consuming such material and may have other adverse effects on animal health. Moreover, plants that grow in inhospitable environments (soil salinity in the case of halophytes) accumulate secondary compounds, some of which may have anti-nutrient properties, with negative effects on the productivity of livestock, feeding on such vegetation (D'Mello, 2000).

Anti-nutrients can be defined as toxic substances in the diet of human and animals which negatively influence their health by disturbing normal physiological functions. The significant characteristic of anti-nutrients can be predicted by the animal performance, behavioral pattern and adaptation to feed. The major damages of anti-nutrients include reduced immune-competence and negative impact on growth and reproduction, leading to morbidity and mortality (D'Mello, 2000; Panhwar, 2005). These chemicals can limit the digestibility of essential nutrients by impairing normal metabolism, causing various disorders that reduce growth rate, decrease palatability and their excessive quantities may be fatal (Panhwar, 2005). As a consequence, potential weight loss or less weight gain of animal fed on such diet brings economic loss to the owner. However, some ruminants have ability to adapt and neutralize the possible damages (Estell, 2010).

Little or no information is available about chemical composition of wild plants, particularly from our region that are being used by local people to raise their animals. Therefore, for assessing suitability of common halophytes as animal feed, a detailed analysis of their anti-nutritional

components needs to be carried out. Present study was conducted to quantify plant secondary metabolites of anti-nutritional importance i.e. phenols, tannins, flavonoids, nitrates, saponins and oxalates in some halophytic plants commonly used as animal feed.

Materials and Methods

Collection of plant material: Selection of plant species was done on the basis of their traditional use as fodder/forage by local communities (Khan & Qaiser, 2006; Qasim *et al*., 2010). Plants were collected during 2011 from their natural habitats along the coastal and nearby areas of Karachi. Perennial plants were collected during their vegetative growth period (March-June) and annuals were collected after monsoon (September-October). Environmental data of study area is presented in figure 1, showing maximum/minimum temperatures, precipitation and humidity during the year of sampling. The area is semiarid where temperature is high with long summer and short winter. Rainfall is less (< 250 mm) and unpredictable usually during monsoon leaving soil dry for long periods.

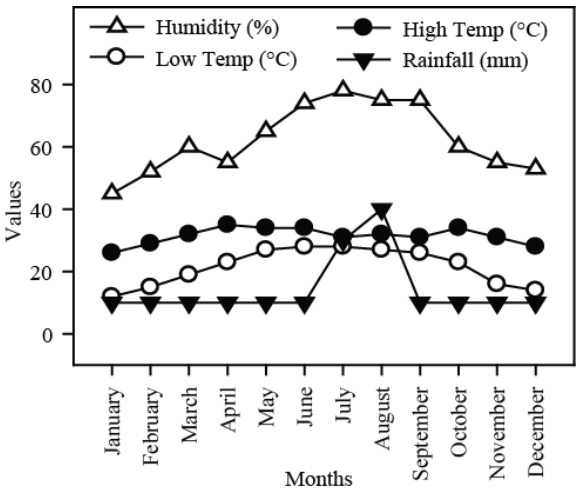

Fig. 1. Environmental data of the study area comprising mean annual temperatures, rainfall and humidity of the year of plant collection (Pakistan metrological department).

Determination of phenols: Total phenolic content was estimated using the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). Plant samples were extracted in 80% methanol (Abideen *et al*., 2015b) and extracts were oxidized with 0.2 N Folin-Ciocalteu reagent. The reaction was neutralized with saturated sodium carbonate (75 g/L) and mixture was left for 90 min at room temperature. The absorbance of the resulting blue color was measured at 760 nm with a spectrophotometer (DU530 Beckman Coulter UV/Vis) and results are expressed as mg Gallic acid equivalent per gram dry weight.

Determination of flavonoids: The aluminum chloride colorimetric method was used (Chang *et al*., 2002) to quantify flavonoids. Plant extract (in 80% methanol) was mixed with 1.5 mL of 95% alcohol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer using Quercetin as standard and results were expressed in mg Quercetin equivalent per gram dry weight.

Determination of tannins: Method described by Pearson (1976) was used to determine total tannins. Aliquots of the hot water extract (1 ml) were mixed with 1 ml of Folin-Denis reagent. Saturated sodium carbonate (2.5 ml) was added, the solutions were made up to 10 ml with distilled water and incubated at room temperature for 30 min. Absorbance of resulting solution was recorded at 760 nm by spectrophotometer and results were expressed as mg Tannic acid equivalent per gram dry weight.

Determination of nitrates: Total nitrates were analysed by the method of Cataldo (1975). Ground samples were suspended in 10 mL distilled water and left for 30 minutes at 45°C. An aliquot of 0.1 mL extract was mixed with 0.4 mL salicylic acid solution. After 20 min at room temperature, 9.5 mL 2N NaOH solution was slowly added. Absorbance was measured at 410 nm using a spectrophotometer. Potassium nitrate was used as standard and results were expressed in mg of Potassium nitrate per gram dry weight.

Determination of saponins: Total saponin content was determined by using method of Hiai *et al*. (1976) with some modifications (Makkar *et al*., 2007). The aliquots of 0.25 ml extracts (defatted samples extracted in 80% methanol) were mixed with 0.25 ml vanillin reagent and 2.5 ml of sulfuric acid (72%). The solution were transferred to a water bath (GFL model 1092) at 60°C for 10 min followed by cooling in ice-cold water for a few min. Absorbance was measured at 544 nm against reagent blank using spectrophotometer. Results are expressed in mg Diosgenin equivalent per gram dry weight.

Determination of oxalates: Oxalates were determined according to Karimi & Ungar (1986). Hot water plant extracts (5ml) were precipitated twice with 1 mL of precipitating reagent (a mixture of 96.5 g anhydrous sodium acetate in 250 mL water and 18g anhydrous calcium acetate in 250 mL of 50% acetic acid) over night at 4^{o}C. Precipitate was dissolved in 5 mL of washing reagent (240 mL 96% ethanol in 125 mL ammonium hydroxide) and oven-dried at 100^{o}C. The dried extract was re-dissolved in 5 mL of 2N sulfuric acid and heated in boiling water bath. Mixture was titrated with 0.02N potassium permanganate and total oxalates were calculated using formula:

Total Oxalates (%) = titration volume (mL) x 1.8001

Soil analysis: Soil samples (0-12 cm) were collected from root zone of studied plants. Soil analysis including moisture, electrical conductivity (EC) and pH was done by the methods described in Anon. (2005).

Table 1. Taxonomic description of local fodder species used in this study.

Families and species name	Life form	Habit	Distribution	Plant type	Flowering period
Acanthaceae					
Avicennia marina (Forssk.) Vierh	Perennial	Tree	Coastal	Hydrohalophyte	February-June
Aizoaceae					
Zaleya pentandra (L.) Jeffrey	Perennial	Herb	Both	Xerohalophyte	June-September
Amaranthaceae					
Atriplex stocksii Boiss	Perennial	Shrub	Both	Halophyte	December-January
Chenopodium album (L.) Boiss	Annual	Herb	Both	Xerohalophyte	July-September
Haloxylon stocksii (Boiss.) Benth. & Hook	Perennial	Shrub	Inland	Halophyte	October-December
Salsola imbricata Forssk.	Perennial	Shrub	Both	Xerohalophyte	August-October
Asteraceae					
Launaea resedifolia (L.) Kuntze	Perennial	Herb	Inland	Xerophyte	April-May
Boraginaceae					
Heliotropium bacciferum Forssk.	Perennial	Shrub	Both	Xerohalophyte	July-September
Combretaceae					
Conocarpus erectus L.	Perennial	Shrub	Both	Hydrohalophyte	July-September
Convolvulaceae					
Convolvulus arvensis L.	Perennial	Herb	Inland	Xerophyte	Year round
Cressa cretica L.	Annual	Herb	Both	Hydrohalophyte	Year round
Ipomoea pes-caprae (L.)	Perennial	Herb	Coastal	Psammophyte	July-September
Fabacae					
Acacia nilotica (L.) Delile	Perennial	Tree	Inland	Xerophyte	March-August
Indigofera cordifolia Heyne ex Roth	Perennial	Herb	Both	Xerophyte	August-October
Indigofera oblongifolia Forssk	Perennial	Shrub	Both	Xerophyte	September-November
Prosopis cineraria (L.) Druce	Perennial	Shrub	Inland	Xerophyte	December-March
Prosopis glandulosa Torr.	Perennial	Shrub	Inland	Xerohalophyte	March-September
Prosopis juliflora (Swartz) DC.	Perennial	Shrub	Both	Xerophyte	March-June
Gentianaceae					
Enicostemma hyssopifolium (Wild). Verdoo	Perennial	Herb	Both	Psammophyte	July-September
Lamiaceae					
Leucas urticifolia (Vahl) R.Br	Annual	Herb	Both	Xerophyte	July-October
Malvaceae					
Thespesia populnea (L.) Sol. ex Corr.	Perennial	Tree	Coastal	Hydrohalophyte	Year round
Salvadoraceae					
Salvadora oleoides Decne	Perennial	Shrub	Inland	Xerophyte	March-June

Results and Discussion

The work aims at survey and quantification of plant Secondary Metabolite (SM) as anti-nutritional components of non-grass halophytes growing on the seacoast and nearby areas of Karachi for assessing their potential use as animal feed. A total of 22 plant species from 12 families have been included in this study (Table 1). The most dominant family was Fabaceae (6 species) followed by Amaranthaceae (4species), Convolvulaceae (3 species) and one species each from the rest (Table 1). Plants were largely perennial shrubs and herbs of either halophytes or xerophytes which reflects the condition of study area where rainfall is less (Fig. 1) or unpredictable, soil is mostly saline to variable extent and dry with pH around 7 (Table 2). Soil salinity was generally higher nearer to coast than inland (Fig. 2). Although, some halophytic species have appreciable amounts of essential nutrients and many among them are well comparable with conventional glycophytic fodder (e.g *Panicum turgidum*, Khan *et al*., 2009; Ali *et al*., 2014; Gul *et al*., 2014), adequate information about their anti-nutritional composition is not available which needs to be accounted for before recommending their commercial use in animal husbandry. In the present study, some major anti-nutritive compounds (total phenols, tannins, flavonoids, nitrates, saponins, oxalates) were analyzed in local non-grass halophytic forage/fodder species.

Polyphenols are biologically active molecules with several beneficial properties; however their high quantities may create problems in animals. High doses of phenols may be associated with reduced bone mineralization (Mudzwiri, 2007) and cholesterol or estrogen imbalance (Francis *et al*., 2002; Duke, 2000). Flavonoids, due to their metal chelating properties may bind with iron and make it less available and also disturb acid/base balance (Sood *et al*., 2002). High tannins (4-10%) in diet reduce digestion and absorption of proteins and essential amino acids which depress voluntary feed intake (Terriell *et al*., 1992; Barry & McNabb, 1999). In this study, polyphenolic analysis showed considerable variation in total phenol (0.13-4.05%), tannin (0.38-6.99%) and flavonoid (0.15-1.50%) contents (Table 3). Among all species tested, the highest polyphenols

(phenols, tannins and flavonoids) were found in *Ipomoea pes-caprae* whereas lowest were found in *Salsola imbricata* except for total phenols (*Prosopis glandulosa* contained the least amount). Most of the species had polyphenols below the toxic levels which may contribute to other potential advantages. For instance, phenols and flavonoids have a wide range of hormonal and non-hormonal health benefits in animals including anti-oxidative, anti-inflammatory, anti-allergy, anti-diabetic, anti-microbial and gastro or hepato-protective activities (Yao *et al*., 2004). Tannins in low doses (~4.5%) were reported to increase animal feed intake however, its higher amount (~9%) depressed food consumption in lambs (Villalba *et al*., 2002). Barry & McNabb (1999) recommended 0.5 to 3% of tannins for animal feed as it improved protein digestion and productivity of grazing ruminants however, higher amounts (7-10%) were harmful. Authors also observed that above mentioned concentrations of tannins could improve the digestion efficiency, wool growth, milk production and ovulation rate in animals.

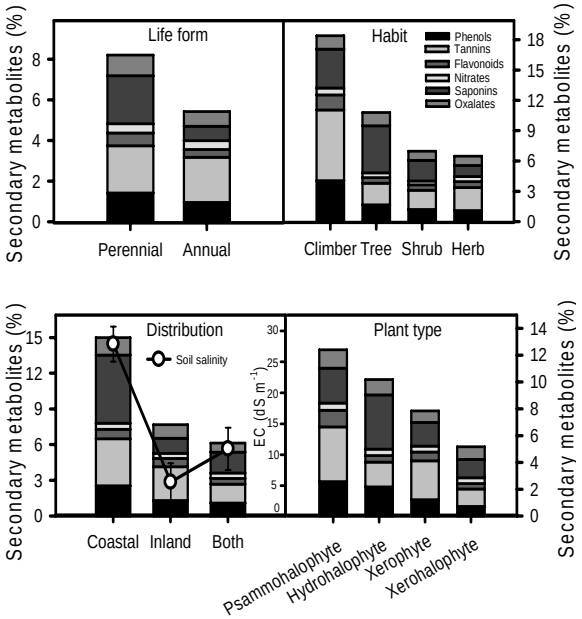

Fig. 2. Accumulation of secondary metabolites in studied plants on the basis of their life form, habit, distribution and plant type.

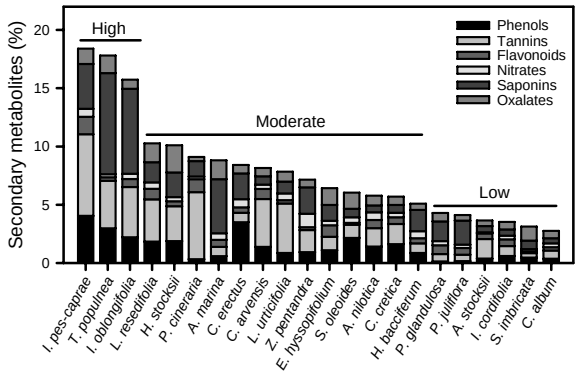

Fig. 3. Characterization of halophytes on the basis of total secondary metabolite accumulation.

Checking nitrate content in plants is important because high level of nitrates in animal diet can convert nitrate into nitrites which cause anoxia and increased pulse and respiration rates by converting hemoglobin to methemoglobin (Launchbaugh, *et al.,* 2001). Nitrate content of most of the species of this study (Table 3) was comparable with other 17 different wild edible and fodder plants (up to 0.05-0.6%; Guil *et al*., 1997) except for *Zaleya pentandra* which showed slightly higher value (1.15%). Villalba *et al*. (2002) observed that dietary intake of protein rich food was not affected when lambs were provided with low (~0.6%) or high (~1.2%) amount of nitrates however; nitrates reduced the dietary intake of energy rich food. In another experiment, feed intake in lambs was reduced when diet containing high (~1.5%) nitrates was fed (Burritt & Provenza, 2000).

Saponins characterized by their hemolytic and foaming properties, are also responsible for bitter taste and astringency. They have the ability to produce pores in membranes (Francis *et al*., 2002) and affect intestinal absorption by inhibiting various digestive enzymes (Shi *et al*., 2004). In the present study, levels of saponins varied greatly between species tested (Table 3), ranging from 0.45% (*Chenopodium album*) to 8.68% (*Thespesia populnea*) whereas, most of the conventional legumes and beans contain 2-6% saponins (Khokhar & Chauhan, 1986; Shi *et al*., 2004). No harmful effects were observed when calves were fed on lucerne containing 2.6% saponins (Howarth, 1988) however, above 3% saponins in diet was harmful (Burns, 1978). It is also noteworthy that many recent experimental studies have shown beneficial effects of saponins in low doses. For instance, Shi *et al*. (2004) reported that saponins help to reduce blood cholesterol, improve immune defense system and fight against cancer. Diet containing 1.0-1.2% saponins reduced plasma and liver cholesterol in rabbits (Shi *et al*., 2004). Bosler *et al*. (1997) found a daily weight gain among lambs when fed low amount (upto 40 mg/kg body weight) of saponins. Adjuvant activities of saponins in rabbits, sheep, cattle, mice and other animals have also been reported (Kensil, 1996).

Oxalates are another important class of anti-nutritional factors, which are widely distributed in plants. Oxalates are known to interfere with calcium absorption by forming insoluble calcium salts which may increase the risk of renal calcium oxalate formation, leading to kidney stones (Bhandari & Kawabata, 2004). Almost all species in this study showed low levels of oxalates (Table 3) ranging from 0.36% (*Prosopis cineraria*) to 2.34% (*Haloxylon stocksii*). These values are within the acceptable limit (2%, Njidda, 2010), except for *H. stocksii.* Although, *H. stocksii* showed relatively higher amount of oxalates, this value is much less than the common halophytic fodder used in Egypt and Near East region (3-5%; El Shaer, 2010). Some common edible and fodder species of Africa including *Amaranthus* species also contained higher oxalates (3.3-4.3%, Mziray *et al*., 2001). The amounts of oxalates present in studied plants are also lower than in many other local (14-29%; Khan *et al*., 2009) and halophytic or non-conventional fodder species (3.3 to 6.6%; Malcolm *et al*., 1988).

Table 2. Analysis of soil collected from the root zones of studied species.

Species	Month of collection	EC (dS m^{-1})	pH	Moisture (%)
Atriplex stocksii	April	28.04 ± 2.341	7.785 ± 0.015	0.723 ± 0.032
Acacia nilotica	April	1.341 ± 0.051	7.331 ± 0.280	0.541 ± 0.012
Avicennia marina	March	32.22 ± 1.761	6.873 ± 0.108	13.69 ± 0.254
Chenopodium album	August	6.371 ± 0.541	7.163 ± 0.271	1.211 ± 0.321
Conocarpus erectus	July	5.251 ± 0.461	7.163 ± 0.157	2.145 ± 0.321
Convolvulus arvensis	September	3.531 ± 0.081	8.051 ± 0.085	0.854 ± 0.042
Cressa cretica	March	28.12 ± 2.671	6.727 ± 0.087	3.214 ± 0.542
Enicostemma hyssopifolium	September	2.531 ± 0.171	7.537 ± 0.011	0.267 ± 0.587
Haloxylon stocksii	March	23.01 ± 2.111	6.841 ± 0.226	0.825 ± 0.235
Heliotropium bacciferum	March	22.85 ± 1.431	6.871 ± 0.233	2.384 ± 0.065
Indigofera cordifolia	October	2.051 ± 0.211	6.741 ± 0.921	3.225 ± 0.762
Indigofera oblongifolia	October	5.341 ± 0.441	6.785 ± 0.050	0.215 ± 0.050
Ipomoea pes-caprae	April	23.52 ± 4.511	8.616 ± 0.133	0.459 ± 0.226
Launaea resedifolia	July	2.531 ± 0.151	7.551 ± 0.105	4.127 ± 0.531
Leucas urticifolia	September	3.171 ± 0.251	8.081 ± 0.284	3.887 ± 0.283
Prosopis cineraria	August	5.581 ± 0.321	6.831 ± 0.133	0.861 ± 0.032
Prosopis glandulosa	August	6.311 ± 1.171	6.961 ± 0.143	0.748 ± 0.284
Prosopis juliflora	August	12.42 ± 0.861	7.081 ± 0.156	0.651 ± 0.043
Salsola imbricata	March	27.03 ± 3.351	7.411 ± 0.135	1.036 ± 0.156
Salvadora oleoides	March	8.531 ± 1.211	8.251 ± 0.166	0.872 ± 0.031
Thespesia populnea	March	28.35 ± 2.111	6.881 ± 0.141	4.327 ± 0.846
Zaleya pentandra	September	4.431 ± 0.551	6.805 ± 0.108	0.471 ± 0.297

Table 3. Analysis of secondary metabolites (%) as anti-nutritional factors in leaves of local fodder species.

Species	Phenols	Tannins	Flavonoids	Nitrates	Saponins	Oxalates	Total
Atriplex stocksii	1.418 ± 0.036	1.571 ± 0.004	0.705 ± 0.007	0.659 ± 0.023	0.589 ± 0.015	0.841 ± 0.241	5.781
Acacia nilotica	0.386 ± 0.006	1.663 ± 0.181	0.487± 0.003	0.102 ± 0.013	0.531 ± 0.025	0.481 ± 0.061	3.649
Avicennia marina	0.588 ± 0.035	0.795 ± 0.001	0.604 ± 0.001	0.567 ± 0.051	4.638 ± 0.086	1.621 ± 0.181	8.812
Chenopodium album	0.367 ± 0.007	0.711 ± 0.005	0.281 ± 0.002	0.321 ± 0.046	0.447 ± 0.016	0.631 ± 0.091	2.435
Conocarpus erectus	3.491 ± 0.246	0.821 ± 0.005	0.472 ± 0.003	0.691 ± 0.039	2.221 ± 0.069	0.721 ± 0.131	8.414
Convolvulus arvensis	1.381 ± 0.048	4.114 ± 0.122	0.859 ± 0.019	0.379 ± 0.058	0.702 ± 0.031	0.721 ± 0.121	8.154
Cressa cretica	1.612 ± 0.012	1.735 ± 0.153	0.572 ± 0.007	0.393 ± 0.191	0.665 ± 0.039	0.721 ± 0.141	5.697
Enicostemma hyssopifolium	1.091 ± 0.009	1.155 ± 0.079	0.991 ± 0.004	0.392 ± 0.057	1.351 ± 0.039	1.441 ± 0.207	6.417
Haloxylon stocksii	1.876 ± 0.041	3.003 ± 0.021	0.427 ± 0.007	0.345 ± 0.016	2.112 ± 0.039	2.341 ± 0.519	10.103
Heliotropium bacciferum	0.865 ± 0.154	0.815 ± 0.008	0.455 ± 0.003	0.587 ± 0.035	1.825 ± 0.058	0.541 ± 0.103	4.222
Indigofera cordifolia	0.599 ± 0.022	0.864 ± 0.009	0.563 ± 0.008	0.318 ± 0.009	0.536 ± 0.023	0.641 ± 0.110	2.881
Indigofera oblongifolia	2.204 ± 0.089	4.316 ± 0.021	0.683 ± 0.044	0.458 ± 0.023	7.284 ± 0.242	0.781 ± 0.061	15.725
Ipomoea pes-caprae	4.053 ± 0.111	6.996 ± 0.011	1.491 ± 0.051	0.691 ± 0.039	3.849 ± 0.036	1.321 ± 0.261	18.399
Launaea resedifolia	1.844 ± 0.224	3.621 ± 0.026	0.899 ± 0.001	0.557 ± 0.059	1.723 ± 0.032	1.621 ± 0.181	10.263
Leucas urticifolia	0.862 ± 0.024	4.239 ± 0.302	0.302 ± 0.009	0.577 ± 0.023	1.011 ± 0.017	0.841 ± 0.121	7.831
Prosopis cineraria	0.331 ± 0.021	5.751 ± 0.074	1.113 ± 0.058	0.224 ± 0.016	1.324 ± 0.054	0.361 ± 0.111	7.447
Prosopis glandulosa	0.127 ± 0.003	0.646 ± 0.013	0.755 ± 0.011	0.356 ± 0.024	1.693 ± 0.035	0.721 ± 0.121	4.297
Prosopis juliflora	0.159 ± 0.004	0.551 ± 0.001	0.579 ± 0.014	0.282 ± 0.018	2.069 ± 0.268	0.481 ± 0.121	4.121
Salsola imbricata	0.473 ± 0.009	0.376 ± 0.021	0.147 ± 0.001	0.197 ± 0.017	0.732 ± 0.052	1.211 ± 0.121	3.125
Salvadora oleoides	2.152 ± 0.321	1.132 ± 0.054	0.164 ± 0.002	0.481 ± 0.021	0.726 ± 0.013	1.381 ± 0.261	3.882
Thespesia populnea	2.986 ± 0.481	4.066 ± 0.059	0.305 ± 0.005	0.256 ± 0.018	8.684 ± 0.031	1.511 ± 0.261	17.797
Zaleya pentandra	0.925 ± 0.011	1.892 ± 0.034	0.253 ± 0.004	1.152 ± 0.021	2.268 ± 0.015	0.661 ± 0.061	7.151

Based on SM accumulation, plants are divided into three anti-nutritive categories i.e. high (> 10%), moderate (5-10%) and low (< 5%). Figure 3, shows that most of the species (13) lies in moderate category followed by low (6) and high (3). Plants from low and moderate categories are within the acceptable limits for animal consumption. Although, species of high category contained lesser SMs than some other fodder species (e.g. 8 different browse plants of Nigeria; Njidda, 2010), these plant are however, not recommended. It is also noteworthy that intake of diverse SMs could lead to toxin inactivation due to possible interactions between various anti-nutrients which neutralize their inhibitory effects. Makkar *et al*. (1995) reported that saponins interact with other anti-nutrients in such a manner that nullify the toxic effects of both substances. Consumption of saponin-tannin combination reduced their individual toxicity in rat (Freeland *et al*., 1985). Increased dietary intake in lambs was observed when they were offered food containing mixture of saponins, tannins and oxalates than those with one or two of these SMs and their intake was comparable to feeds without SMs (Villalba & Provenz, 2009). This may be due to reactions between SMs leading to formation of chemical complexes which inactivate their individual biological effect (Villalba *et al*., 2004).

It was also observed that perennials accumulated more anti-nutrients than annuals (Fig. 2), which may be a function of the former remaining in field longer than the later species. It also reflects the strategy of perennials investing more energy in their chemical defense than annuals (De Jong, 1995). In terms of accumulation of secondary metabolites (SM), climbers accumulated high amount of SM followed by trees, however, shrubs and herbs had lesser SMs (Fig. 2). These results are in line with the use of local fodder plants where majority of shrubs and herbs have been used to raise house hold animals (Ajaib *et al*., 2010; Badshah & Hussain, 2011).

In terms of distribution, most of the species with wide distribution (coastal and inland) contained low amount of anti-nutrients (Fig. 2) and were used commonly as animal fodder, whereas species of restricted distribution (particularly coastal) having higher anti-nutrients were less in use. This gets further support from SM accumulation, based on plant type (Fig. 2), where coastal species (particularly, psammohalophytes and hydrohalophytes) showed higher SM content than other ones. High fluctuations in abiotic stresses, particularly soil salinity, stimulates the production of SM among coastal plants (Bandaranayake, 2002). Results of soil analysis showed that coastal plants were growing in more saline habitats than inland ones (Table 2). The average soil salinity of coastal plants (27.67 dSm^{-1}) was around three times higher than those which were found in inland habitats (7.09 dSm^{-1}), whereas plants which can grow in both situations were distributed in intermediately saline soils (12.04; Fig. 2). Under those hostile conditions, increased production of SMs is considered to protect plants from damaging effects of environmental stresses to ensure their survival but which also renders such vegetation unsuitable as feed due to the harmful consequences on animal health.

Conclusions

Proper maintenance of plants' vital functions to survive under harsh environmental conditions, like those faced by halophytes, demands protective measures accordingly. Production of secondary metabolites is plants' strategy but their high amounts in animal feed, may disrupt normal metabolism and hinder animal growth, especially when supplied over a long period. This study identified halophytes of moderately saline soils that contained SMs within the acceptable ranges, hence presenting a potential source as animal fodder. However, ecophysiological investigations of these plants under different stress regimes and its effect on their chemical constituents is recommended to utilize halophytes on saline degraded lands for commercial purposes.

Acknowledgments

Part of Ph.D. research of S. Ehsen supported by the Higher Education Commission of Pakistan (HEC) under their Indigenous Ph.D. Fellowship Program.

References

Abideen, Z., R. Ansari, B. Gul and M.A. Khan. 2012. The place of halophytes in Pakistan's biofuel industry. *Biofuels*, 3(2): 211-220.

Abideen, Z., M. Qasim, R.F. Rizvi, B., Gul, R. Ansari and M.A. Khan. 2015a. Oilseed halophytes: a potential source of biodiesel using saline degraded lands. *Biofuels*, 6(5-6): 241-248.

Abideen, Z., M. Qasim, A. Rasheed, M.Y. Adnan, B. Gul and M.A. Khan. 2015b. Antioxidant activity and polyphenolic content of *Phragmites karka* under saline conditions. *Pak. J. Bot*., 47(3): 813-818.

Ajaib, M., Z.U.D. Khan, N. Khan and M. Wahab. 2010. Ethnobotanical studies on useful shrubs of district Kotli, Azad Jammu & Kashmir, Pakistan. *Pak. J. Bot.*, 42(3): 1407-1415.

Al Sherif, E.A. 2009. *Melilotus indicus* (L.) All. A salt tolerant wild leguminous herb with high potential for use as a forage crop in salt- affected soil. *Flora-Morphol. Distr. Funct. Ecol. Plant.*, 204: 737-746.

Ali, H., B. Gul, M.Y. Adnan, M.Z. Ahmed, I. Aziz, S. Gulzar and M.A. Khan. 2014. NPK mediated improvement in biomass production, photosynthesis and Na^+ regulation in *Panicum antidotale* under saline conditions. *Pak. J. Bot.*, 46(6): 1975-1979.

Anonymous. 2005. *Association of Official Analytical Chemists*, (18^{th} Ed). AOAC Official Methods of Analysis, Gaithersburg, MD.

Badshah, L. and F. Hussain. 2011. Farmers preferences and use of local fodder flora in Tank District, Pakistan. *Afr. J. Biotechnol.*, 10(32): 6062-6071.

Bandaranayake W.M. 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetl. Ecol. Manag.*, 10: 421-452.

Barry, T.N. and W.C. McNabb. 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *Br. J. Nutr*., 81(4): 263-272.

Bhandari, M.R and J. Kawabata. 2004. Assessment of antinutritional factors and bioavailability of calcium and zinc in wild yam (*Dioscorea* spp.) tubers of Nepal. *Food Chem.*, 85(2): 281-287.

Bosler, D.A., M. Blu¨mmel, P. Bullerdieck, H.P.S. Makkar and K. Becker. 1997. Influence of a saponin containing feed additive on mass development and carcass evaluation of growing lambs. *Proc. Soc. Nutr. Physiol*., 6: 46.

Burns, J.C. 1978. Antiquality factors as related to forage quality. *J. Dairy Sci.*, 61(12): 1809-1820.

Burritt, E.A and F.D. Provenza. 2000. Role of toxins in intake of varied diets by sheep. *J. Chem. Ecol.*, 26: 1991-2005.

Cataldo, D.A., M. Maroon, L.E. Schrader and V.L. Youngs. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid 1. *Commun. Soil Sci. Plant Anal*., 6(1): 71-80.

Chang, C., M. Yang, H. Wen and J. Chern. 2002. Estimation of total flavonoids content in *Propolis* by two complementary colorimetric methods. *J. Food Drug Anal*., 10: 178-182.

De Jong, T.J. 1995. Why fast-growing plants do not bother about defence. *Oikos*, 74: 545-548.

D'Mello, J.F. 2000. *Farm animal metabolism and nutrition*. Cabi Publishing. UK.

Duke, J. 2000. Phytochemical and ethnobotanical databases. Available from http://www.ars-grin.gov./duke/.

El Shaer, H.M. 2010. Halophytes and salt-tolerant plants as potential forage for ruminants in the Near East region. *Small Ruminant Res*., 91(1): 3-12.

Estell, R.E. 2010. Coping with shrub secondary metabolites by ruminants. *Small Ruminant Res.*, 94(1): 1-9.

Francis, G., Z. Kerem, H.P. Makkar and K. Becker. 2002. The biological action of saponins in animal systems: a review. *Br. J. Nutr.*, 88(6): 587-605.

Freeland, W.J., P.H. Calcott and D.P. Geiss. 1985. Allelochemicals, minerals and herbivore population size. *Biochem. Syst. Ecol.*, 13(2): 195-206.

Glenn, E.P., J.J. Brown and E. Blumwald. 1999. Salt tolerance and crop potential of halophytes. *Crit. Rev. Plant Sci.*, 18: 227-255.

Guil, J.L., I. Rodríguez-Garcí and E. Torija. 1997. Nutritional and toxic factors in selected wild edible plants. *Plant Food Hum. Nutr.*, 51(2): 99-107.

Gul, B., Z. Abideen, R. Ansari and M.A. Khan. 2013. Halophytic biofuels revisited. *Biofuels*, 4(6): 575-577.

Gul, B., R. Ansari, H. Ali, M.Y. Adnan, D.J. Weber, B.L. Nielsen and M.A. Khan. 2014. The sustainable utilization of saline resources for livestock feed production in arid and semi-arid regions: A model from Pakistan. *Emir. J. Food Agric*., 26(12): 1032-1045.

Hiai, S., H. Oura and T.Nakajima. 1976. Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. *Planta Med.*, 29(02): 116-122.

Howarth, R.E. 1988. Antiquality factors and non-nutritive chemical components. In: (Eds.): Hanson, A.A., D.K. Barnes, R.R. Hill Jr. *Alfalfa and Alfalfa Improvement*. ASA Inc., CSSA Inc., SSSA Inc. Publishers, Madison, USA. pp. 493-514.

Karimi, S.H. and I.A. Ungar. 1986. Oxalate and inorganic ion concentrations in *Atriplex triangularis* Willd. organs in response to salinity, light level, and aeration. *Bot. Gaz*., 65-70.

Kensil, C.R. 1996. Saponins as vaccine adjuvants. *Crit. Rev. Ther. Drug. Carrier Syst.*, 13: 1-55.

Khan, M.A. and M. Qaiser. 2006. Halophytes of Pakistan: characteristics, distribution and potential economic usages. In: *Sabkha ecosystems, Tasks for Vegetation Science Volume 42*, (Eds.): Khan, M.A., B. Benno, G.S. Kust and H.J Barth. Springer Netherlands. pp. 129-153.

Khan, M.A., R. Ansari, H. Ali, B. Gul and B.L. Nielsen. 2009. *Panicum turgidum*, a potentially sustainable cattle feed alternative to maize for saline areas. *Agric. Ecosys. Environ*., 129: 542-546.

Khokhar, Santosh and B.M. Chauhan. 1986. Antinutritional factors in moth bean (*Vigna aconitifolia*): varietal differences and effects of methods of domestic processing and cooking. *J. Food Sci*., 51(3): 591-594.

Koyro, H.W., T. Hussain, B. Huchzermeyer and M.A. Khan. 2013. Photosynthetic and growth responses of a perennial halophytic grass *Panicum turgidum* to increasing NaCl concentrations. *Environ. Exp. Bot*., 91: 22-29.

Launchbaugh, K.L., F.D. Provenza and J.A. Pfister. 2001. Herbivore response to anti-quality factors in forages. *J. Range Manag*., 431-440.

Makkar, H.P.S., M. Blümmel and K. Becker. 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in *In vitro* techniques. *Br. J. Nutr.*, 73: 897-913.

Makkar, H.P., P. Siddhuraju and K. Becker. 2007. *Plant secondary metabolites*. Humana Press.

Malcolm, C.V., A.J. Clarke, M.F. D'Antuono and T.C. Swann. 1988. Effect of plant spacing and soil conditions on the growth of five Atriplex species. *Agric. Ecosys. Environ*., 21: 265-279.

Mudzwiri, M. 2007. Evaluation of traditional South African leafy plants for their safety in human consumption. Doctoral dissertation, Durban University of Technology, Durban, South Africa.

Mziray, R.S., J.K. Imungi and E.G. Karuri. 2001. Nutrient and antinutrient in contents of raw and cooked *Amaranthus hybridus*. *Ecol. Food Nutr*., 40(1): 53-65.

Njidda, A.A. 2010. Chemical composition, fibre fraction and anti-nutritional substances of semi-arid browse forages of North-Eastern Nigeria. *Nig. J. Basic Appl. Sci.*, 18(2): 181-188.

Panhwar, F. 2005. Anti-nutritional factors in oil seeds as aflatoxin in ground nut. *Digitalverlag gmbh, Germany*, 1-7.

Pearson, D. 1976. *The Chemical Analysis of Food*. (4th Ed) Churchill Living Edudiburgh, London. pp. 78-80.

Qasim, M., S. Gulzar, Z. Shinwari, I. Aziz and M. Khan. 2010. Traditional ethnobotanical uses of halophytes from hub, Balochistan. *Pak. J. Bot*., 42(3): 1543-1551

Qasim, M., S. Gulzar and M.A. Khan 2011. Halophytes as medicinal plants. In: *Urbanisation, Land Use, Land Degradation and Environment.* (Eds.): Ozturk, M., A.R. Mermut and A. Celik. Daya Publishing House, Dehli, India, pp. 330-343.

Qasim, M., Z. Abideen, M.Y. Adnan, R. Ansari, B. Gul and M.A. Khan. 2014. Traditional ethnobotanical uses of medicinal plants from coastal areas of Pakistan. *J. Coast. Life Med.*, 2(1): 22-30.

Shi, J., K. Arunasalam, D. Yeung, Y. Kakuda, G. Mittal and Y. Jiang. 2004. Saponins from edible legumes: chemistry, processing, and health benefits. *J. Med. Food*, 7(1): 67-78.

Singleton, V.L. and J.A. Rossi. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vit.*, 16: 144-158.

Sood, P., R. Modgil, M. Sood and P.K. Chuhan. 2012. Anti-nutrient profile of different *Chenopodium* cultivars leaves. *Annals. Food Sci. Techol.*, 13(1): 68-74.

Swingle, R.S., E.P. Glenn and V. Squires. 1996. Growth performance of lambs fed mixed diets containing halophyte ingredients. *Anim. Feed Sci. Technol.*, 63(1): 137-148.

Terrill, T., A.M. Rowan, G.B. Douglas and T.N. Barry. 1992. Determination of extractable and bound tannin concentrations in forage plants, protein concentrate meals and cereal grains. *J. Sci. Food Agric*., 58: 321-329

Villalba, J.J., F.D. Provenza and J.P. Bryant. 2002. Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: benefits or detriments for plants? *Oikos*, 97(2): 282-292.

Villalba, J.J., F.D. Provenza and H. Guo-dong. 2004. Experience influences diet mixing by herbivores: implications for plant biochemical diversity. *Oikos*., 107(1): 100-109.

Villalba, J.J. and F.D. Provenza. 2009. Learning and dietary choice in herbivores. *Rangeland Ecol. Manag*., 62(5): 399-406.

Weber, D.J., R. Ansari, B. Gul and M.A. Khan. 2007. Potential of halophytes as source of edible oil. *J. Arid Environ.*, 68(2): 315-321.

Yao, L.H., Y.M. Jiang, J. Shi, F.A. Tomas-Barberan, N. Datta, R. Singanusong and S.S. Chen. 2004. Flavonoids in food and their health benefits. *Plant Food Hum. Nutr*., 59(3): 113-122.

(Received for publication 16 February 2015)