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DISTRIBUTION OF SECONDARY METABOLITES IN PLANTS OF QUETTA-BALOCHISTAN

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Abstract

In the present study, 20 plant species from Hazarganji Chiltan National Park Quetta and 17 plant species from Wali Tangi Area, Quetta were collected. The shoots of all plant species and the roots of 7 species were oven dried and investigated for the detection and estimation of alkaloids, saponins, tannins and quantification of total phenolic contents. Out of 37, only four plant species viz., *Caragana ambigua*, *Clematis graveolens*, *Juniperous excelsa* and *Pistacia khinjak* contained all three secondary metabolites and their total phenolic contents were 440, 120, 176 and 230 µg g^{-1}, respectively. While, 6 plant species viz., *Chrysopogon aucheri*, *Ferula oopoda*, *Fraxinus xanthoxyloides*, *Pennisetum orientale*, *Saccharum griffithii* and *Verbascum erianthum* lack all three secondary metabolites and their total phenolic contents were between 80-500 µg g^{-1}. However, among the remaining 26 plant species, only *Alhaji maurorum*, *Ampelopsis vitifolia*, *Ephedra intermedia*, *Prunus microcarpa*, *Rosa lacerans* and *Salix acmophylla* contained two out of three metabolites. Results also deciphered that *Euphorbia osyridea* contained only saponin and *Cymbopogon jwarancusa* contain medium concentration of alkaloids. Whereas, 18 plants species contained only traces of alkaloids, and their phenollic contents are in the range of 120-524 µg g^{-1}. Moreover, roots analysis of 7 plant species viz., *Erodium cicutarium*, *Ferula oopoda*, *Lactuca serriola*, *Nepeta preaetervisa*, *Peganum hermala*, *Saccharum griffithii* and *Serriphedium quettensis* of Hazarganji Chiltan National Park Quetta, exhibited the absence of all three metabolites, and their total phenolic contents were very low (<113 µg g^{-1}).

Introduction

Secondary metabolites are chemicals produced by means of secondary reactions resulting from primary carbohydrates, amino acids and lipids (Ting, 1982).Their direct role in plant metabolism is not yet well documented. However, their ecological role (Dey & Harborne, 1997) and particularly in plant herbivore interaction (Feeny, 1976; Swain, 1979) and chemotaxonomy (Gibbs,1974) has been well established. Plants containing secondary metabolites particularly alkaloids, saponins and tannins are generally avoided by grazing animals and leaf feeding insects. Their presence in plants and intake at high level reduces the nutrient utilization, feed efficiency, animal productivity and in some cases death of animals (Makkar & Goodchild, 1996). Thus grazing potential of rangeland plants depends upon the presence and level of aforesaid metabolites. Recently, Wahid & Ghazanfar (2004) and Wahid & Babu (2005) reported that high level of secondary metabolites can enhance salt tolerance in sugarcane and wheat, respectively.

Study pertaining to distribution of secondary metabolites is a pre-requisite for rangeland management point of view. But unfortunately no work has been done on this aspect in Pakistan. Balochistan's 93% area is rangeland. The main objective of the present work was to study the level of secondary metabolites having anti-nutritional factors and phenolic contents in plants of Hazar Ganji Chiltan National Park and Walitangi Area of Balochistan, Pakistan.

Materials and Methods

Hazar Ganji Chiltan National Park is located approximately 20 km south west of Quetta, the provincial capital of Balochistan. Position of park is within the middle east and south asian sub-region, approximately 29º 59´- 30º 07´ N 66º 24´- 66º 54´ E (Anon.,1998). Walitangi is located on the 67º 11´ longitudes and 30º 17´ latitudes. According to Holdridge's (1947) Bioclimatic System, the study area fall under temperate desert bush type of bioclimate (Qadir, 1968) the climate of the area indicates Mediterranean trend (Qadir & Ahmad, 1989).

Study areas i.e., Hazar Ganji Chiltan National Park and Wali Tangi, Quetta were visited in the months of June and July 2004, respectively. Thirty seven plant species were collected, brought to the laboratory and identified. Nomenclature followed was that of Nasir & Ali (1971-1995) and Ali & Qaiser (1995-2004). All plants were oven dried at 70 ^{0}C for 72 hours and their leaves were separated and grinded to fine powder, which was used for detection and estimation of alkaloid, saponin and tannin following the procedure adopted by Makkar & Goodchild (1996). The details of the procedure are given below:-

Alkaloids: Plant powdered material 500mg, was taken and it was extracted with 3ml of methanol containing 10% acetic acid. Ammonium hydroxide was added in extracted material drop wise, formation of precipitate was taken as an indication of presence of alkaloid.

Saponins: Oven dried plant powdered material 500mg, was extracted in 50% aqueous methanol. Extracted material was transferred into a test tube and it was well hand shaken. Formation of persistent foam on the surface was taken as an indication of presence of saponin.

Tannin: Plant material of 100mg, was taken in test tube and 3ml of butanol-HCl reagent (95ml of n-butanol and 5ml of concentrated HCl) was added to it. Test tube was plugged with cotton and was heated at 70° on water bath for an hour. Appearance of pink color was taken as presence of tannin.

Determination of total phenolic contents: The procedure of Folin & Denis (1912) and Waterman & Mole (1994) were followed.

Plant powdered material 50mg was extracted with 100ml of MeOH-H_2O (80:20) and heated at 70ºC on water bath for three hours. The suspension of water extraction was filtered and aqueous solutions were used for total phenolic contents determination. Extract 0.5ml was taken in test tube and its final volume was made 17ml by addition of 16.5ml distilled water. Then 1ml of Folin Reagent and 2ml of saturated solution of Sodium Carbonate was added. After 30 minutes its absorbance was measured at 760nm by Shimadzu UV-Visible Recording Spectrophotometer (UV 160) with 10mm-matched quartz cells. Aqueous solutions of Tannic acid were used as standards for plotting working curve (Ranganna, 1986). The data of phenolic contents of plant species of given locality was subjected to ANOVA (Completely Randomized Design with equal replication) as per procedure of Gomez & Gomez (1984).

Results and Discussion

Distribution of alkaloids, saponins and tannins: The shoot of 20 plant species, belonging to 17 families of Hazarganji Chiltan National Park and 17 plant species belonging to 14 families of Wali Tangi Area Quetta were studied (Table 1 & 2). Results depicted that *C. ambigua, C. graneolens, J. excelsa* and *P. khinjak* contained alkaloid, saponin and tannins. Animals do not prefer these plants. Perhaps this is why *P. khinjak* is a dominant tree species of Hazar Ganji Chiltan National Park and *C. ambigua* and *J. excelsa* are dominant plant species of Wali Tangi.On the basis of phytosociological data Qadir & Ahmad, (1989) concluded that *P.khinjak* is only tree species likely to maintain its dominance in Hazar Ganji area. Results also showed that *A. maurorum, A. vitifolia, E. intermedia, P. microcarpa, R. lacerans* and *S. acmophylla* are plant species containing two out of three antinutritional factors studied during the present work. Results further demonstrates that out of 20 plants, collected from Hazar Ganji Chiltan National Park, shoots of 11 plant species contained only traces or low conentration of alkaloids (Table 1). These plant species are *A. stocksii, B. crispa, C. leiocalycinus, D. mucronata, E. cicutarium, E. osyridea, L. serriola, N. praetervisa, P. hermala, S. cabulica* and *S. quettensis.* Whereas 8 plant species viz., *B. crispa, C. intybus, C. iberica, C. graveolens, H. acutifolium, M. minima, M. longifolia, T. minima* of Wali Tangi area also contained only traces of alkaloid (Table 2). However, *C. jwarancusa* contained medium concentration of Alkaloids while *E. osyridea* contained only tannin. Data also revealed that shoots of *C. aucheri, F. oopoda, F. xanthoxyloides, P. orientale, S. griffithii* and *V. erianthum* are antinutritional factors free. Present data also revealed that roots of 7 plant species viz., *E. cicutarium, F. oopoda, L. serriola, N. praetervisa, P. hermala, S. griffithii* and *S. quettensis* lack all three (alkaloids, saponin and tannin) antinutritional factors (Table 1-3).

Quantification of total phenolic contents: Results pertaining to phenolic contents (exhibited that phenolic contents of shoots and roots varied significantly ($p<0.05$) from species to species (Table 1-3). Four plant species viz., *C. intybus, C. ambigua, F. xanthoxyloides* and *R. lacerans* contained phenolic contents in the range of 440-524 µg g^{-1} while *O. hispidum* and *C. jwarancusa* contained negligible amounts of total phenolic contents. Twelve plant species viz., *A. maurorum, A. vitifolia, B. crispa, C. leiocalycinus, D. mucronata, E. intermwdia, E. osyridea, M. longifolia, N. praetervisa, P. khinjak, S. acmophylla* and *S. cabulica* showed their phenolic cintents between 200-400 µg g^{-1}. Whereas the remaining 18 plant species viz., *A. stocksii, C. aucheri, C. iberica, C. graveolens, E. cicutarium, E. osyridea, F. oopoda, H. acutifolium, J. excelsa, L. serriola, M. minima, P. hermala, P. microcarpa, P. orientale, S. griffithii, S. quettensis, T. minima* and *V. erianthum* showed their total phenolic contents in the range of 80-200 µg g^{-1} (Table 1-2). Results further suggest that the six plants which are free of alkaloids, saponins and tannins, have appreciable amount of total phenolic contents (80-500 µg g^{-1}). This increased level of total phenolics might be due to the presence of phenolic compounds like coumarins, flavonoids, lignans, neolignans, lignins, phenylpropenes in the afore said plants.

The quantitative analysis of roots of 7 plant species of Hazarganji Chiltan National Park also showed that roots have total phenolic contents lesser than phenolic contents of shoots (Table 1-3).

Table 1. Distribution of secondary metabolites in shoot of plants of Hazarganji Chiltan National Park, Quetta.

S. No.	Plant species	Families	Total phenolic contents (μ g^{-1})	Alkaloids			Saponins			Tannins		
				+	++	+++	+	++	+++	+	++	+++
1.	*Ampelopsis vitifolia* (Boiss)Planch	Vitaceae	252^{d}	–	√	–	–	–	–	–	√	–
2.	*Astragalus stocksii* Bunge	Papilionaceae	124^{kl}	√	–	–	–	–	–	–	–	–
3.	*Buddleja crispa* Bth	Loganiaceae	204^{g}	√	–	–	–	–	–	–	–	–
4.	*Chrysopogon aucheri* (Boiss) stapf	Poaceae	160^{j}	–	–	–	–	–	–	–	–	–
5.	*Convolvulus leiocalycinus* Boiss	Convolvulaceae	210^{g}	√	–	–	–	–	–	–	–	–
6.	*Daphne mucronata* Royle	Thymelaeceae	240^{e}	√	–	–	–	–	–	–	–	–
7.	*Ephedra intermedia* Schrenk	Ephedraceae	268^{c}	–	–	√	–	–	–	–	–	√
8.	*Erodium cicutarium* (L) L`Herit.ex.Ait	Geraniaceae	180^{i}	√	–	–	–	–	–	–	–	–
9.	*Euphorbia osyridea* Boiss.	Euphorbiaceae	380^{b}	√	–	–	–	–	–	–	–	–
10.	*Ferula oopoda* Boiss.	Umbelliferae	120^{l}	–	–	–	–	–	–	–	–	–
11.	*Fraxinus xanthoxyloides*(Wall ex G.Don) Dc.	Oleaceae	500^{a}	–	–	–	–	–	–	–	–	–
12.	*Lactuca serriola* L.	Asteraceae	128^{k}	√	–	–	–	–	–	–	–	–
13.	*Nepeta praetervisa* Boiss	Labiateae	244^{e}	√	–	–	–	–	–	–	–	–
14.	*Peganum hermala* L.	Zygophylaceae	164^{j}	√	–	–	–	–	–	–	–	–
15.	*Pennisetem orientale* L.C.Rich	Poaceae	96^{m}	–	–	–	–	–	–	–	–	–
16.	*Pistacia khinjak* Stocks	Anacardiaceae	230^{f}	–	√	–	–	–	√	–	–	√
17.	*Prunus microcarpa* Brukill non C.A.Mey	Rosaceae	190^{h}	–	√	–	–	–	–	–	–	√
18.	*Saccharum griffithii* Munro ex Boiss	Poaceae	80^{n}	–	–	–	–	–	–	–	–	–
19.	*Salvia cabulica* Bth.	Labiateae	376_{b}	√	–	–	–	–	–	–	–	–
20.	*Serriphedium quettensis* (Podlech) ling	Asteraceae	180^{t}	√	–	–	–	–	–	–	–	–

Mean values within columns with different letters are significantly different ($p< 0.05$) Explanation of symbols: √ metabolites present, – metabolites absent,+ low concentration; ++ medium concentration and +++ high concentration.

Table 2. Distribution of secondary metabolites in shoots of plants of Walitangi Area.

S. No.	Plant species	Families	Total phenolic contents (μ g^{-1})	Alkaloids			Saponins			Tannins		
				+	++	+++	+	++	+++	+	++	+++
1.	*Alhaji maurorum* Medic.	Papilionaceae	280[d]	–	√	–	–	–	√	–	–	–
2.	*Buddleja crispa* Bth.	Loganiaceae	232[f]	√	–	–	–	–	–	–	–	–
3.	*Caragana ambigua* Stocks.	Papilionaceae	440[b]	–	–	√	–	√	–	–	–	√
4.	*Cichorium intybus* L.	Asteraceae	524[a]	√	–	–	–	–	–	–	–	–
5.	*Centaurea iberica* Trev.ex spring	Asteraceae	136[i]	√	–	–	–	–	–	–	–	–
6.	*Clematis graveolens* Lindly.	Rununculaceae	120[j]	√	–	–	–	√	–	–	√	–
7.	*Cymbopogan jwarancusa* (Jones) schutt.	Poaceae	63[k]	–	√	–	–	–	–	–	–	–
8.	*Euphorbia osyridea* Boiss.	Euphorbiaceae	168[h]	–	–	–	√	–	–	–	–	–
9.	*Haplophyllum acutifolium* (DC) G.Don.	Rutaceae	196[g]	√	–	–	–	–	–	–	√	–
10.	*Juniperous excelsa* M.B.	Cupressaceae	176[h]	√	–	–	–	√	–	–	–	–
11.	*Mentha longifolia* (L.) Huds.ss.longifolia.	Labiateae	292[c]	√	–	–	–	–	–	–	–	–
12.	*Medicago minima* (L.) Grufb.	Papilionaceae	128[ij]	√	–	–	–	–	–	–	–	–
13.	*Onosma hispidum* Wall.ex.G.Don.	Boraginaceae	62[k]	–	–	–	–	–	–	–	–	–
14.	*Rosa lacerans* Boiss & Bushes.	Rosaceae	524[a]	√	–	–	–	√	–	–	–	–
15.	*Salix acmophylla* Boiss	Salicaceae	264[e]	√	–	–	–	–	–	–	√	–
16.	*Typha minima*	Typhaceae	120[j]	√	–	–	–	–	–	–	–	–
17.	*Verbascum erianthum* Bth.	Scrophulariaceae	196[g]	–	–	–	–	–	–	–	–	–

Mean values within columns with different letters are significantly different ($p < 0.05$). For symbol explanation, see Table 1.

Table 3. Distribution of secondary metabolites in roots of seven plants of Hazar Ganji Chiltan National Park, Quetta.

S. No.	Plant species	Families	Total phenolic contents (μ g^{-1})	Alkaloids			Saponins			Tannins		
				+	++	+++	+	++	+++	+	++	+++
1.	*Erodium cicutarium* (L) L`Herit.ex.Ait	Geraniaceae	72[b]	–	–	–	–	–	–	–	–	–
2.	*Ferula oopoda* Boiss.	Umbelliferae	48[c]	–	–	–	–	–	–	–	–	–
3.	*Lactuca serriola* L.	Asteraceae	112[a]	–	–	–	–	–	–	–	–	–
4.	*Nepeta praetervisa* Boiss	Labiateae	72[b]	–	–	–	–	–	–	–	–	–
5.	*Peganum hermala* L.	Zygophylaceae	48[c]	–	–	–	–	–	–	–	–	–
6.	*Saccharum griffithii* Munro ex Boiss	Poaceae	56[c]	–	–	–	–	–	–	–	–	–
7.	*Serriphedium quettensis* (Podlech) ling	Asteraceae	37[d]	–	–	–	–	–	–	–	–	–

Mean values within columns with different letters are significantly different ($p < 0.05$). For symbol explanation, see Table 1.

Higher amounts of phenolics in shoots in comparison to that of roots may be attributed to the presence or absence of light that affects the phenolic contents of plant organs. There is a well-established positive relationship between the intensity of solar radiation and quantity of phenolics produced by plants. Generally there is a rise in total phenolics in plants grown in the sunny situations relative to the shady ones, but it can be seen at the intra-individual level by comparing plant parts, exposed to different amounts of light (Mole & Waterman, 1987). Several studies have shown that many secondary metabolites are released in to the environment, either as exudation from living plant tissues or by decomposition of plant material under certain conditions (Rice, 1984; Putnam, 1988; Einhellig, 1995). These chemicals, like phenolics, terpenoids and alkaloids and their derivatives are potential inhibitors of germination, seedling growth (Macias *et al*., 1992; Siddiqui, *et al*., 1999; Siddiqui & Zaman, 2004), enzymes activity (Borua & Das, 2000; Cremer & Eichner, 2000). Most of the researchers also found that secondary products differ from primary metabolites in having a restricted distribution in plant kingdom. That is found in only one plant species or a taxonomically related group of species, whereas the basic primary metabolites are found throughout the plant kingdom (Taiz & Zeiger, 1991).

From the results of present studies it can be safely concluded that the presence of appreciable amount of antinutritional factors and total phenolic contents in the plants of both the areas might be one factor for the presence of these plants in the study area having high grazing pressure and arid environment.

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