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RESPONSE OF PLANT PARTS AND AGE ON THE DISTRIBUTION OF SECONDARY METABOLITES ON PLANTS FOUND IN QUETTA

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Abstract

Eight plant species belonging to 7 different families were collected from Botanical Garden, University of Balochistan, Quetta. Their leaves and stem was separately oven dried, powdered and analyzed for the arbitrary level of alkaloids, saponins, tannins and total phenolic contents in their young and old plant parts of leaves and stem. Results showed that leaves and stem of all plant species contained alkaloids. Their level in leaves was comparatively greater over the stem of the same plant species. Generally young plant parts contained greater level of alkaloids as compared to old parts. Results also showed that most of the plants are either lacking saponin, or if present are in a least quantity. However, it was noted that the level of saponin were comparatively found greater in stem over their respective leaves. Aged plant parts usually contained greater level of saponins. Results further revealed that except of *Tamarex aphylla,* all other remaining plant species are generally lacking tannins. The quantitative analysis of total phenolic contents also revealed that old leaves contained high level of phenolic contents as compared to old stem, while young leaves and stem showed mix trend towards the total phenolic contents. Minimum quantity of total phenolic contents (120 µg/g) was found in young leaves of *Nerium* oleander, while young leaves and stem of *Rhododendron sp.,* contained maximum phenolic contents (340 µg/g) among all test plants. Whereas, all other remaining plant species produced 123-308 µg/g phenolic contents. These secondary metabolites (chemical defenses) are likely to have come about as a consequence of natural selection, as only that can protect themselves from predators are likely to survive to breed. The present findings might be useful to optimize the processing methodology of wild-harvested material to obtain increased concentration of these secondary metabolites. This then could be used as a natural source of pesticides.

Introduction

Secondary metabolites are those chemical substances which are not directly involved in the growth and development of plants. They lead to the biosynthesis of simple to complex metabolites, utilizing the intermediates from the primary metabolites through specific pathways. Important secondary metabolites include alkaloids, terpenes, phenolics, vitamins etc. Most of these metabolites have no direct roles in plant growth and development and are important to those plants, which show their biosynthesis (Taiz & Zeiger, 2006). Most secondary metabolites are toxic and therefore play defensive roles against biotic factors like protection from attack of pathogens and herbivores, allelopathy etc., (Athanasiadou & Kyriazakis, 2004; Khan & Singh, 2008).

The available literature revealed that terpenes and phenolics are more important to abiotic stress (light, temperature, drought, salinity) tolerance than the others owing to structural properties (Harborne & Williams, 2000). Phenols were selected as stress indicator as it is known that exposure to toxic chemicals and various stresses lead to elevated total phenols in plants (Reid *et al.,* 1992; Siddiqui & Arif-uz-Zaman, 2004).

Plant phenolics are characterized as aromatic compounds, which possess one or more acidic hydroxyl group attached to phenyl ring. Phenolics perform a broad range of physiological roles in plants, which may be growth inhibitory and promotory. Upon being oxidized, they change color (usually turn brown), generating the products that form complex with protein and inhibit enzyme activity (Ohno, 2001). At the same time they play a profound role in enhancing plant growth, development, reproduction and also act as a line of defense against a variety of biotic and abiotic stresses. The significance of latter roles of phenolics has been greatly emphasized during previous decade (Buchanan *et al.,* 2000). They have specific taste and smell due to which the animals, insects and humans do not eat the plants containing phenolic components for their food that is why these are also called as anti-grazing factors. Sometimes these are toxic to fauna and sometimes these are auto toxic (Strack, 1997).

Many researchers reported that the concentration of secondary metabolites are varying from plant to plant species and even in the different parts of the same species. Kowalski & Wolski (2006) reported that *Silphium perfoliatum* contained total phenolics of 20.9, 23.7 mg 100^{-1} g, and caffeic acid 19.0 mg 100^{-1} g in leaves, inflorescence and rhizome, respectively. Whereas, Hyder *et al.,* (2002) reported that the leaves, green stem and root of *Larrea tridentate* contained total phenolics of 36.2, 40.8 and 28.6 mg 100^{-1} g dry weight, respectively. Similar trend of results in case of *Lespedeza capitata* have also been achieved by Springer *et al.,* (2002). Higher amounts of phenolics in leaflets in comparison to that of roots may be attributed to the presence or absence of light that affects the phenolic contents of organs. 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; Mole *et al.,* 1988).

Grazing animals and leaf feeding insects generally avoid the plants containing secondary metabolites particularly alkaloids, saponins and tannins. Because their intake at high level reduces the nutrient utilization, feed efficiency, animal productivity and in some cases death of animal (Makkar & Goodchild, 1996). While Wahid & Babu (2005) and Wahid & Ghazanfar (2006) reported that wheat and sugarcane showed enhanced salt tolerance at high level of secondary metabolites. A very little is known about the distribution of secondary metabolites in term of plant age or developmental stage. Therefore, the present study was mainly aimed to chalk out those local plants that possess high level of anti-nutritional factors, which then could be used as a natural source of chemical defense for crop plants against their predators. The study was also aimed to point out differences between the levels of the same metabolites with respect to plant parts and age.

Materials and Methods

Quetta, provincial capital of Balochistan, lies between 30°-03 and 3°-27° N and 66°-44°, and 67°-44°, and 67°-18° E and at an altitude of 1700 meters. The total geographical area of Quetta district is 26531 km^2 (Anon, 1998). Ahmad (1951) classified the climate of Quetta under arid subtropical continental high land. The old Botanical Garden University of Balochistan Quetta was established in 1981. It is situated in front of the university towards west, across Sariab road Quetta. The total area of the botanical garden is 4 hectare. It consists of a large number of local indigenous and exotic plant species belonging to gymnosperms and angiosperms. Study area was visited on Sunday, 5th August 2004 for sampling. Eight plant species were selected for present study as test plants. These were brought to the laboratory and identified. Nomenclature followed was that of Nasir & Ali (1971-1995), and Ali & Qaiser (1995-2004). All plants belonging to seven different families were dried at 70^{o}C in oven for 72 hours and young and old leaves and stem were carefully separated and finely grinded for each test plant and their powder was then used for the determination of arbitrary level of alkaloids, saponins, tannins following the procedure adopted by Makkar & Goodchild (1996) .

Alkaloids: A 500 mg oven dried plant powder was extracted with 3 ml of methanol containing 10% acetic acid. Ammonium hydroxide was added to it drop wise, formation of precipitates indicated the presence of alkaloids. Then the arbitrary level were given to alkaloids in plant samples and arranged as absent (x); traces (y); low level (+); slightly high level (++); high level (+++) and very high level (++++).

Saponins: A 500 mg plant powder was extracted in 50% aqueous methanol. Extract was transferred into a test tube and was well hand shaken. Formation of persistent foam on the liquid surface indicates the presence of saponins. Then the arbitrary level of saponins in plant samples were arranged as absent (x); traces (y); low level (+); slightly high level (++); high level (+++) and very high level (++++).

Tannins: A 100 mg plant powder was taken into a test tube and 3 ml of butanol-HCl reagent (95 ml of n-butanol and 5 ml of concentrated HCl) was added to it. Test tube was plugged with cotton and was heated at 70^{o}C on water bath for one hour. The formation of pink color confirmed the presence of tannins. Again arbitrary level of tannins in plant samples were arranged as absent (x); traces (y); low level (+); slightly high level (++); high level (+++) and very high level (++++).

Quantification of total phenolic contents: The procedures represented by Folin & Denis (1912) and Waterman & Mole (1994) were followed. A 50 mg plant powder was extracted with 100 ml of MeOH:H_2O ratio (80:20) and heated at 70°C on water bath for three hours. Suspension was filtered and aqueous solutions were used for the determination of total phenolic contents. A 0.5 ml extract was taken in test tube and diluted up to 17 ml by adding 16.5 ml deionized distilled water. Then 1.0 ml Folin reagent and 2.0 ml saturated solution of sodium carbonate were also added to it. After 30 minutes, its absorbance was measured at 760 nm by using UV-Visible Spectrophotometer (Model 160, Shimadzu, Japan) with 10 mm-matched quartz cells. Aqueous solutions of tannic acid were used as standards for plotting working curve (Ranganna, 1986). Data obtained were arranged in a Completely Randomized Design (CRD), and then a statistical test of Gomez & Gomez (1984) was applied to the data for their significance.

Results and Discussion

Alkaloids: Results showed that leaves and stem of all plant species contained alkaloids. While the level of alkaloids in leaves were comparatively greater over the stem of the same plant species (Table 1). Similar trend of results has been also reported by Kayani *et al.,* (2007) and Çirak *et al.,* (2008). However, a maximum significant level of alkaloids by them is recorded in full opened flowers. Results also showed that except of *Olea europea* and *Tamarex aphylla,* the young plant parts contained greater level of alkaloids over old parts. These findings are also in line with those of Suzuki & Waller (2006). They resulted that alkaloids in the leaves and stem of tea plant were significantly decreased as the plant ages progressively. Among eight plant species, the leaves of *Berberis vulgaris* & *Prosopis glandulosa* and *O. europea* & *Melia azedarach* contained high to very high level of alkaloids, respectively. These plant species could use alkaloids to protect themselves against herbivores. Because of the sessile lifestyle plants are unable to avoid their predators. Therefore, producer plants could use alkaloid as a chemical defense against all their enemies. They could also be used as a natural source of insecticides and fungicides. The present findings might be useful to optimize the processing methodology of wild-harvested material and obtained increased concentration of alkaloids. Researchers also revealed that alkaloids help biologically in storage of waste nitrogen, cationic balancing and protection against parasites (Ting, 1982). The principal action of alkaloids is on the nervous system (Cordell, 1981) that's why its high concentrations in plants secure them from grazing animals. The *B. vulgaris* was at one time considered a common horticultural hedge but is now out of fashion due to the high incidence of poisoning in young children attracted to its bright red berries (Hopkins & Hűner, 2004).

Saponins: Results indicated that most of the plants are either lacking saponin, or if present in a least quantity. It was also noted that the level of saponin contents were comparatively found greater in stem over the leaves of the same plant species (Table 1). Similar results are also obtained by Tao *et al.,* (2007) and Kayani *et al.,* (2007). They stated that the levels of total saponins in individual plant parts can vary considerably and the concentration of each kind of saponin is also significantly different. Results further exhibited that aged stem produced greater amount of saponin over their respective young stem. A high arbitrary level of saponin is obtained in aged stem of *O. eurpea, O. ferruginea* and *P. glandulos.* Research revealed that leaf age affected the concentrations of secondary metabolites. Plants that were 5 weeks older had higher levels of iridoid glycosides (IGs) than plants harvested 5 weeks earlier. Therefore, our findings in term of age factor are strongly in accordance with the results obtained by Stamp & Bowers (1994). As explained by Hopkins & Hűner (2004) that the principal roles of saponin in plants appear to be as a performed defense against attack by their enemies. Therefore, the aforementioned plant species might use their saponins as defense mechanisms against predation by microorganisms, insects and herbivores. These plant parts could also be exploited as a natural mean of herbicides as well as fungicides.

Tannins: Results pertaining to distribution of tannins showed that except of *T. aphylla*, other remaining plant species are either lacking it in their leaves (specially) or in stem (generally). However, the old stem of *M. azedarach, B. vulgaris, P. gladulosa* and *T. aphylla* contained a low to very high level of tannins, respectively (Table 1). Kayani *et al.,* (2007) also found that out of 37, only four plant species viz., *Caragana ambigua, Clematis graveolens, Juniperus excelsa* and *Pistacia khinjak* contained tannins. Research revealed that tannins deter feeding by herbivores (Taiz & Zeiger, 1991), and they are naturally occurring polyphenol compounds found in all plant parts. In leaves they serve to reduce palatability and thus protect against predators. After plant cell break down and death they act and have metabolic effects on plant predator or pathogen thus to be considered a plant molecular defender strategy against herbivores, granivores, insects and bacterial, fungal and viral infections (Buttler, 1989; King *et al.,* 2000). In present study, a very high level of tannins found in *T. aphylla* could be one major reason that such plant parts are not preferred by ruminants especially sheep and goats.

Table 1. Arbitrary level of secondary metabolites in young (YL) and old (OL) leaves, young (YS) and old (OS) stem of eight plant species found in Quetta.

Plant species	Alkaloids				Saponins				Tannins			
	YL	OL	YS	OS	YL	OL	YS	OS	YL	OL	YS	OS
Berbaris vulgaris	+++	+++	++	y	x	+	x	++	x	x	++	++
Melia azedarach	++++	++	++	+	y	+++	y	+	x	x	x	+
Nerium olender	++	y	++	y	+	+	x	x	x	x	x	x
Olea europea	++++	++++	+	y	+	++	+	+++	x	x	x	x
O. ferruginea	+++	+	++	y	x	y	x	+++	x	x	x	x
Prosopis glandulosa	+++	+	++	y	x	x	y	+++	x	x	x	+++
Rhododendron sp.	y	+	++	y	++++	++++	++	y	x	x	x	x
Tamarex aphylla	++	+	y	++	x	x	y	y	+++	+++	++++	++++

Description of scale used for level of secondary metabolites: x = absent; y = traces; + = low level; ++ = slightly high level; +++ = high level and ++++ = very high level.

Table 2. Total phenolic content (µg/g) of young and old leaves and stem of eight plant species grown in Quetta.

S. no.	Plant species	Young leaves (µg/g)	Old Leaves (µg/g)	Young stem (µg/g)	Old stem (µg/g)
1.	*Berberis vulgaris*	167 cd	184	177 bc	156 b
2.	*Melia azedarach*	123 e	204	151 c	189 b
3.	*Nerium olender*	120 e	273	225 b	185 b
4.	*Olea europea*	191 bc	161	172 bc	141 b
5.	*Olea* sp.	300 a	300	201 bc	174 b
6.	*Prosopis glandulosa*	152 de	131	166 bc	157 b
7.	*Rhododendron* sp.	206 b	340	340 a	308 a
8.	*Tamarix aphylla*	187 bcd	145	166 bc	161 b
	Average values	180.75	217.25	199.75	183.88

Data is the mean of three readings, and mean values sharing the same letter(s) within a column are statistically non-significant at $p<0.05$.

Quantitative determination of total phenolic contents: Data showed that leaves and stem of all plants contained phenolic contents. Statistically a significant variation is recorded among various plant species (except in old leaves). Data also showed that young stem contained higher level of phenolic contents over young leaves (except in *Olea* and *Tamarex* species), whereas reverse is true for old stem and leaves except in *P. gladulosa* and *T. aphylla*. Results based on average values exhibited that old leaves produced 36.50 µg/g increased phenolic contents over their young leaves while, young stem produced 15.87 µg/g increased phenolic contents over their old stem (Table 2). Results further indicated that the young stem of *Rhododendron sp.,* have significantly highest level of total phenolic contents (340 µg/g) among all test plants and *Nerium olender* is the plant specie with lowest level of phenolic contents (120 µg/g). Variation in the concentration of various phenolic contents in plants during its phenological cycle is also reported by Çirak *et al.,* (2007ab). They stated that among different tissues, full opened flowers were found to be superior to stems, leaves and the other reproductive parts. Therefore, the present findings might be useful to optimize the processing methodology of wild harvested plant material and obtained increased concentration of phenolic contents.

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