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Seasonal Variations in Tannin Profile of Tree Leaves

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ABSTRACT : Forest tree leaves (12 different species) of semi hilly arid region of Punjab State were collected at 30-day interval throughout the year to assess the seasonal variations in tannin profile. Tannins were extracted and fractionated from fat free samples and data were analyzed statistically by 12×12 factorial design. The leaves of *Anogeissus latifolia* had the highest ($p<0.05$) concentration of total phenols (17.4%), net (15.9%) and hydrolysable (16.9%) tannins, followed by leaves of *Acacia nilotica*. Majority of the tree leaves selected had moderate levels (2-5%) of net tannins. Leaves of *Carrisa* had the highest ($p<0.05$) concentration of condensed tannins (CT), whereas the leaves of *Anogeissus* had the lowest ($p<0.05$) concentration of condensed tannins. The protein precipitable phenols (PPP) corresponded well with the net tannin content present in different tree leaves. Seasonal variation data revealed that in summer, net tannins and PPP decline in leaves of *Bauhinia* and *Zizyphus* whereas the net tannin content of *Anogeissus* and that of *Carrisa* increased during summer. The CT and PPP content in the leaves of *Pheonix, Leucaena, Zizyphus and Ougenia* increased in winter till spring season. Tree leaves generally had higher concentration of HT during summer months. It was concluded that leaves of leaves of *A. nilotica, A. latifolia* and *L. leucocephala* could serve as an excellent alternate feed stuffs for ruminants. However, leaves of *Phoenix, Carrisa, Bauhinia* and *Dodonea* should be avoided. (**Key Words :** Tannin Profile, Tree Leaves, Seasonal Variation, Semi-hilly Arid Region)

INTRODUCTION

Acute shortage of conventional feedstuffs, for feeding to livestock, in the developing countries has forced nutritionists to look for unconventional feed resources like forest tree leaves. Some of the forest tree leaves of semi-hilly arid region like *Morus alba, Ehretia, Grewea* and *Leucaena* were found to be rich in protein, soluble carbohydrates minerals and showed great potential as an alternate feed resource (Bakshi and Wadhwa, 2004). However, animals refuse to eat certain tree leaves in a particular season. The logical explanation, which comes in mind, is the presence of high concentration of a particular anti-metabolite especially tannins. Tannins are polyphenolic substances with various molecular weights of variable complexity and are classified into two classes: hydrolysable and condensed tannins. They have both adverse and beneficial effects depending on their concentration and nature besides other factors such as composition of the diet, physiological status and species of the animal. Makkar (2003) also reported that trees and shrub foliage are generally rich in tannins, which vary widely and largely unpredictably, and their effects on animals range from beneficial to toxicity and death. The astringent effect and slow degradation rate (resulting in high rumen fill) in tannin rich feeds may restrict feed intake (Makkar et al., 1995). The purpose was to exploit the real potential of forest tree leaves, on the basis of seasonal variations in different types of tannins.

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MATERIALS AND METHODS

Plant material

Leaves of twelve forest trees, commonly fed to the livestock in the semi-hilly arid region of Punjab State, viz., *Acacia nilotica, Toona ciliate, Bauhinia variegata, Phoenix acaulis, Kango, Anogeissus latifolia, Carrisa* spinarum, *Ougenia oojeiuealis, Leucaena leucocephala, Zizyphus mauritiana, Zizyphus xylopyrus* and *Dodonea viscosa* were collected at 30 day interval throughout the year. The leaves were dried in a forced air oven at 60°C for 48 h, ground (1 mm) in a Willey mill and stored in the dark, till analyzed in duplicate.

Extraction of tannins

The fat and pigments were removed by extracting the ground tree leaves with petroleum ether containing 1%

Table 1. Tannin profile of tree leaves, irrespective of season (% DM basis)

Leaves of	Total phenols	Simple phenols	Net tannins	Condensed tannins	Hydrolysable tannins
Acacia nilotica	16.2^{d}	1.6^{cd}	14.6^{e}	1.1^{bc}	15.1^{e}
Toona cililate	3.8^{a}	1.5^{cd}	2.3^{ab}	0.9^{ab}	2.9^{bcd}
Bauhinia variegata	4.8^{bc}	1.1^{ab}	3.7^{bcd}	3.4^{e}	1.4^{a}
Phoenix acaulis	5.8^{bc}	0.9^{a}	4.8^{d}	4.3^{f}	1.5^{ab}
Kango	4.8^{bc}	2.4^{g}	2.4^{ab}	1.6^{c}	3.1^{cd}
Anogeissus latifolia	17.4^{d}	1.5^{cd}	15.9^{e}	0.4^{a}	16.9^{f}
Carrisa spinarum	6.6^{c}	2.1^{fg}	4.5^{d}	4.6^{f}	2.0^{abc}
Ougenia oojeiuealis	4.2^{ab}	1.3^{bc}	2.9^{abc}	2.6^{d}	1.6^{ab}
Leucaena leucocephala	4.9^{bc}	2.8^{h}	2.1^{a}	0.8^{ab}	4.1^{d}
Zizyphus mauritiana	4.9^{bc}	2.0^{ef}	2.9^{abc}	2.6^{d}	2.3^{abc}
Zizyphus xylopyrus	3.9^{ab}	1.4^{bc}	2.6^{ab}	2.4^{d}	1.6^{ab}
Dodonea viscosa	5.8^{bc}	1.8^{de}	4.1^{cd}	3.3^{e}	2.6^{abc}
Pooled SE	0.47	0.10	0.51	0.23	0.47

Figures with different superscripts in a column differ significantly, $p<0.05$; n = 3.

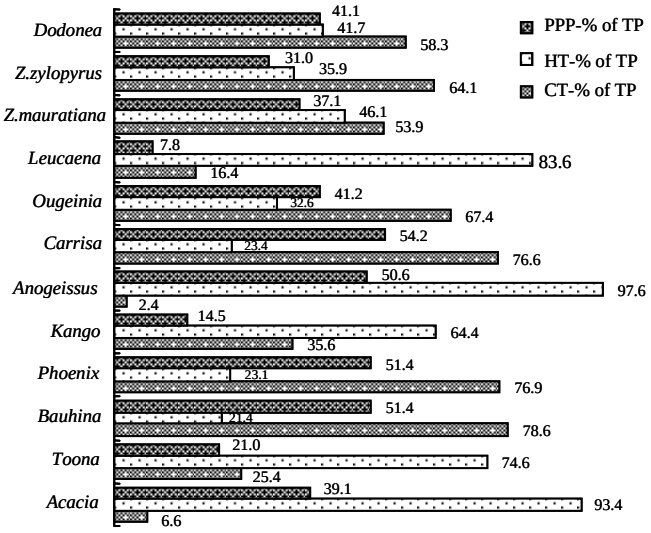

Figure 1. Relative proportion of tannins in different tree leaves (mg % DM basis).

acetic acid using Soxhlet apparatus (AOAC, 1995). Tannins were extracted, from fat free samples, using 70 per cent aqueous acetone. The contents were centrifuged (3,000 g at 4°C for 10 min) and the supernatant was taken for determination of tannins. Total phenols and simple phenols were estimated using Folin-Ciocalteu reagent using tannic acid as a standard (Makkar et al., 1993) and net tannins were calculated by difference between total phenols and simple phenols. Condensed tannins were determined by using Butanol-HCl (Porter et al., 1986), protein precipitable phenols (PPP) by BSA precipitation method (Makkar et al., 1988) and expressed as % of total phenols. The hydrolysable tannins were calculated by subtracting the condensed tannins from total tannins.

Statistical analysis

The data were analyzed statistically in a 12×12 factorial manner (Snedecor and Cochran, 1994) by using STATGRAPHICS version 5.0. The means were compared for statistical significance by using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The total phenols (Table 1) and net tannins varied from 3.8% (*Toona*) to 17.4% (*Anogeissus)* and 2.1% (*Leucaena*) to 15.9% (*Anogeissus)*, respectively. Leaves of *Acacia nilotica* followed the trend of *Anogeissus* both for TP and NT (16.2 and 14.6%) contents. The leaves of *Zizyphus xylopyrus* and *Ougeinia* had the contents similar to *Toona* leaves. *Bauhinia variegata*, *Phoenix acaulis*, *Carrisa spinarum* and *Dodonea viscosa* leaves showed moderate levels of TP (4-7%) and NT (4-5%) content. Low level of net tannin content (2-3%) was observed in *Toona ciliate*, *Kango*, *Ougeinia oojeivealis*, *Leucaena*, *Zizyphus mauritiana* and *Zizyphus xylopyrus* species. Moderate levels of tannins (less than 4%) in forage legumes can have beneficial responses in ruminants, resulting in higher growth rates and milk yield, however, even in ruminants, levels of tannins exceeding 6% of the diet resulted in negative affect on growth rate and milk yield (Makkar, 2003). Reed et al. (1990) showed that tannins could exhibit both negative and positive effects on nutritive value depending upon their net tannin content in the forages. Tanner et al. (1990) also reported high levels of tannin content in *Acacia nilotica*, which reduced growth rate of animals due to lower feed intake and protein digestibility. The positive effects of moderate tannin content on nutritive value include protein protection from microbial enzymes by formation of tannin protein complex, which bypass the rumen. These complexes are unstable at the acid pH of the abomasum and the protein thus becomes available for digestion (Barry and Manley, 1984). Ally and Kunjikutty (2003) have also stated that a level of 5.5 per cent of total tannins exerted no deleterious effects on crude protein

Table 2. Seasonal variation in the tannin profile, irrespective of tree leaves sp. (% DM basis)

Month	Total phenols	Simple phenols	Net tannins	Condensed tannins	Hydrolysable tannins
October	7.7^{ef}	1.8^{de}	5.9^{cdef}	3.2^{de}	4.4^{cd}
November	4.6^{a}	1.1^{a}	3.5^{a}	2.0^{c}	2.6^{a}
December	8.3^{f}	1.6^{bcd}	6.7^{f}	3.1^{de}	5.2^{cdef}
January	5.7^{ab}	1.4^{b}	4.3^{ab}	1.9^{bc}	3.9^{bc}
February	6.7^{bcde}	1.9^{ef}	5.4^{bcdef}	3.0^{d}	4.3^{bc}
March	7.2^{cdef}	1.9^{ef}	5.3^{bcde}	2.9^{d}	4.3^{bc}
April	7.5^{def}	2.1^{f}	4.0^{ab}	3.2^{de}	3.0^{ab}
May	8.2^{f}	1.8^{de}	6.4^{def}	3.7^{e}	4.5^{cd}
June	6.4^{bcd}	1.5^{bc}	4.9^{abc}	0.7^{a}	5.7^{def}
July	6.8^{bcde}	1.7^{cde}	5.1^{bcd}	1.0^{a}	5.8^{ef}
August	6.0^{bc}	1.5^{bc}	4.5^{abc}	1.3^{ab}	4.7^{cde}
September	8.4^{f}	1.9^{def}	6.5^{ef}	1.8^{bc}	6.5^{f}
Pooled SE	0.46	0.10	0.51	0.23	0.47

Figures with different superscripts in a column differ significantly, $p<0.05$.

utilization of animals. Small ruminants have been known to relish the leaves of *A. nilotica*, *A. latifolia* and *Leucaena*, under field conditions.

The condensed tannin (CT) profile showed that leaves of *C. spinarum* and *P. acaulis* had higher ($p<0.05$) levels (4.6 and 4.3%), respectively, (Table 1) amongst the selected tree leaves and constituted almost 76-79% (Figure 1) of the total phenols. The level was observed to be lowest in *A. latifolia* (0.4%) followed by that in *Leucaena*, *Toona*, *Acacia* and *Kango* species. The presence of higher levels of condensed tannins, in the diet, is toxic to the animal as they affect the mucosa of the digestive tract, which could decrease absorption of methionine and lysine. Decreased methionine availability could increase the toxicity of other plant compounds such as cyanogenic glycosides, because methionine is involved in the detoxification of cyanide (Reed, 1995). At low to moderate levels, CT increases the quantity of dietary protein, especially essential amino acids, flowing to the small intestine. Bakshi and Wadhwa (2004) reported the presence of various types of tannins and adverse effects of condensed tannins on the digestibility of nutrients (correlation coefficient for DM, NDF and CP was -0.71, -0.79 and -0.64, respectively), whereas hydrolysable tannins showed no such adverse affect (correlation coefficient for DM, NDF and CP were, 0.56, 0.46 and 0.4, respectively).

The hydrolysable tannin content (Table 1) was highest ($p<0.05$) in *Anogeissus sp.* (16.9%) and *Acacia sp.* (15.1%). The level of hydrolysable tannins was higher than the condensed tannins in *Acacia*, *Toona*, *Kango*, *Anogeissus* and *Leucaena* leaves. The hydrolysable tannins constituted more than 93% of total phenols in *Anogeissus* and in *Acacia* leaves (Figure 1). Leaves of *Bauhinia variegata* had lowest (1.4%) concentration of HT, but comparable to that in *Phoenix*, *Carrisa*, *Ougenia*, *Zizyphus mauritiana*, *Zizyphus xylopyrus* and *Dodonea viscosa*. The leaves of *Toona*, *Kango* and *Leucena* had moderate (2-4%) concentration of HT. Tannin toxicity from HTs may occur in animals fed oak (*Quercus spp.*) and several tropical tree legumes (e.g. *Terminalia oblongata* and *Clidema hirta*) containing more than 20% HTs. Protein precipitable phenolics (PPP) were low (8%) in *Leucaena leucocephala* leaves and high (more than 50%) in *Anogeissus latifolia*, *Carrisa spinarum*, *Bauhinia variegata* and *Phoenix acaulis* leaves (Figure 1). The level of PPP corresponded well with the content of net tannins present in the different tree leaves. Bakshi and Wadhwa (2004) reported that the levels of PPP adversely affect the digestibility of nutrients, as indicated by their negative correlations with DMD (-0.50), NDFD (-0.62) and CPD (-0.54). Kumar and Vaithiyanathan (1990) reported that both the percentage of protein precipitable phenolics and the protein precipitating capacity of the tannin fractions increase with the increase in the degree of polymerization of tannins.

Season showed significant impact on the TP and NT content, irrespective of tree leaves (Table 2). Moderate and comparable concentration of TP and NT was observed from February through August. The highest concentration was observed in May, September and December, whereas lowest in November. During summer, the level of net tannin content decreased in *Bauhinia variegata*, *Phoenix acaulis*, *Leucaena*, *Zizyphus mauritiana* and *Zizyphus xylopyrus* but increased in *Anogeissus latifolia* and *Carrisa spinarum* species. Vaithiyanathan and Singh (1989) reported that various species of tree leaves responded differently to seasonal changes, with respect to their tannin content. Some species showed an increase in tannin content in summer and some showed an increase in winter. Gupta et al. (1992) also reported an increase in tannin content in summer season. *Toona ciliate* and *Phoenix acaulis* showed an increase in net tannin content during winter season. Salaj and Karmutak (1995) have also reported such increase in net tannin

content, in winter season. The levels of net tannin content in leaves of *Kango*, *Ougenia* and *Dodonea viscosa*, remained unchanged and low, around the clock.

Seasonal variations in the concentration of CT, irrespective of tree leaves sp. were quite significant. The highest ($p<0.05$) concentration of CT was observed in May. The lowest concentration was observed in hot humid period (June-August). The condensed tannins of *Phoenix acaulis* increased from December onwards (during winter season) and reached to the maximum level in April and May. Leaves of *Carrisa spinarum* also showed increasing levels of condensed tannins from September to April. Small ruminants do not consume these leaves specifically during winter months. *Ougeinia oojeivealis*, *Zizyphus xylopyrus* and *Leucaena leucocephala*, though had lower content of condensed tannins but seasonal variations followed the same trend i.e. high during winter and spring season. Macauley and Fox (1980) also observed higher contents of total phenols and condensed tannins, particularly, in winter season. Gomes (1990) reported that condensed tannins are synthesized mainly during winter and early spring season. Subba et al. (1996) also observed higher tannin content, during spring season, in fodder trees. The condensation of tannins, during winter season, is an adaptive process of frost resistant mesophyll cells, so as to avoid any injury during unfavourable temperate conditions (Salaj and Karmutak, 1995) and to help the newly emerging leaves against attacks by herbivorous insects.

The pattern of hydrolysable tannins showed that concentration was high during summer and autumn season. Though contents were low, the levels were comparatively higher during May to September in *Dodonea*, *Pheonix* and *Kango* species. Leaves of *Bauhinia* species also showed a slight increase from May to September. Higher levels of tannins during summer season (July to August) was also reported by Gupta et al. (1992).

Seasonal variations in the levels of PPP showed a trend similar to that of condensed tannins. The leaves of *Phoenix*, *Carrisa*, *Leucaena*, *Zizyphus xylopyrus* and *Ougenia* species showed an increase in PPP concentration from winter to spring season. During summer, the contents decreased to undetectable values in the leaves of *Leucaena*, *Ougenia* and *Zizyphus xylopyrus* species. Leaves of other cultivars, e.g. *Toona*, *Bauhinia* and *Zizyphus mauritiana* also showed lower or negligible content of PPP as per cent of total phenols in the summer season. PPP content of *Anogeissus* and *Kango* species was almost constant throughout the year.

Based on tannin profile, it was concluded that leaves of *A. nilotica*, *A. latilfolia* and *L. leucocephala* could serve as an excellent alternate feed stuffs for ruminants. However, leaves of *Phoenix*, *Carrisa*, *Bauhinia* and *Dodonea* should be avoided.

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