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# Determination of Condensed Tannin Contents from Different Plants of Kherimurat Rangeland (Attock, Pakistan)

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## ABSTRACT

In this study, the tannin (Proanthocyanidins) contents of various plants/forages of Kherimurat rangeland (District Attock, Pakistan) were determined as to their suitability for feeding to animals. To this end, the leaves of 20 commonly grazed plants by the animals were collected from Kherimurat rangeland and processed for determination of condensed tannins (CT). The CT ranged from 0.008 to 1.43% of dry matter in different plants. All the plants were found to contain CT within safe limits to exert beneficial effects in the ruminants. Thus, plants included in this study may provide suitable alternative to scarcity in feed resources of the country/rangelands. 

**Key Words:** Condensed tannins; Rangeland; Pakistan; Sorghum; University cattle feed; Kenya

## INTRODUCTION

Rangelands play a pivotal role in the farming systems throughout the world. There are a number of plants in the rangelands, which are of high nutritive value owing to their chemical composition. Besides, proteins and other nutritional components, the most common phyto–chemicals reported from such plants are the polyphenolic compounds. The beneficial or harmful/toxic effects of plants are mainly attributed to the polyphenols depending on the amount ingested by the animals. Often, these compounds exhibit healthy effects on lower doses; whereas, toxic symptoms on higher doses.

Tannins are phenolic compounds capable of forming strong complexes with proteins and other macromolecules (Mangan, 1988), Since tannins combine with many proteins, therefore, their negative effect occurs, because the proteins of the diet are less available to the animal (Zucker, 1983; Robbins *et al.,* 1987a; Mole *et al.,* 1993). Evidence, however, also indicates that some animals can tolerate (Robbins *et al.,* 1987b) and even require the presence of tannins in their diets (Bernays, 1978, 1981; Haslam, 1988). Condensed tannins (CT) interfere with the digestion of protein via their ability to form reversible complexes with protein (Mangan, 1988; Spencer *et al.,* 1988; Perez–Maldonado *et al.,* 1995). In the rumen, CTs are expected to bind strongly to protein and protect them from degradation by rumen microbes. Protection of dietary protein from rumen degradation can increase protein availability in the small intestine (Coop & Kyriazakis, 1999).

Tanniniferous trees and shrubs are of importance in animal production, because they can provide significant protein supplements but unfortunately the amounts of tannins that they contain vary widely and unpredictably (Jackson *et al.,* 1996; Foo *et al.,* 1996, 1997). Their effects on animals range from beneficial to toxic, including death. The toxic or anti-nutritional effects may be exacerbated in times of stress when a very large proportion of the diet is tanniniferous (Makkar, 2000). With a better understanding of tannin properties, the mechanism of tannin action and proper management of forages, browses could become an invaluable source of protein for strategic supplementation. As the demand for food rises, tanniniferous plants and agro-industrial by-products must play an increasingly important part in the diet of animals.

The present study was, therefore, undertaken to determine the CT contents in some plants/forages of Kherimurat rangeland in district of Attock in Pakistan as to their suitability for feeding to animals.

## MATERIALS AND METHODS

**Plant materials:** The leaves of 20 plants (Table I) were collected from Kherimurat rangeland (District Attock, Pakistan) and brought to the Department of Veterinary Parasitology, University of Agriculture, Faisalabad for determination of CT. The samples were dried in an oven at 50–52°C. The samples were stored in a cool, dark and dry place. The dried samples (500 g each) were ground first to pass a 2 mm screen. All the ground material including those parts remaining inside the mill taken, mixed well and approximately 100 g of sample again ground to pass through a 0.5 mm screen. The ground samples were stored in separate plastic containers for determination of tannins.

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**Extraction of tannins:** The samples were extracted to quantitatively diffuse the phenolics present in the materials to liquid phase. For the extraction process, aqueous acetone (70%) was used. Each of the dried (finely ground) sample (200 mg) was taken in a glass beaker of approximately 25 mL capacity. Ten mL of aqueous acetone (70%) was added and the beaker was suspended in an ultrasonic water bath (Branson 3210) and subjected to ultrasonic treatment for 20 min at room temperature. The contents of the beaker was then transferred to centrifuge tubes and subjected to centrifugation for 10 min at approximately 3000 *g* at 4°C using a refrigerated centrifuge. The supernatant was collected and kept on ice. The pellet left in the tube was transferred to the beaker using two portions of 5 mL each of 70% aqueous acetone and again subjected the contents to ultrasonic treatment for 20 min. The supernatant was again collected as described above.

**Determination of condensed tannins (Proanthocyanidins):** The method described by Porter *et al.* (1986) was followed for the determination of condensed tannins in the extracts. Briefly, Butanol–HCl reagent (butanol–HCI 95:5 v/v) was prepared by mixing 950 mL of n–butanol with 50 mL concentrated HCl (37%). Ferric reagent (2% ferric ammonium sulfate in 2N HCl) was prepared by dissolving 2.0 g of ferric ammonium sulfate in 2N HCl (16.6 mL of concentrated HCl was made up to 100 mL with distilled water to make 2N HCl). The reagents were stored in dark bottles.

In a 100 mm x 12 mm glass test tube, 0.5 mL of the tannin extract diluted with 70% acetone was pipetted. The quantity of acetone was large enough to prevent the absorbance (550 nm) in the assay from exceeding 0.6. Three mL of the butanol–HCl reagent and 0.1 mL of the ferric reagent was added to the tubes. The tubes capped with a glass marble were shaken using a Vortex and then placed on a heating block adjusted at 97 to 100°C for 60 min. After cooling the tubes, absorbance was recorded at 550 nm. Absorbance of the unheated mixture (considered as a suitable blank) was subtracted from the absorbance of heated mixture, which was actual reading at 550 nm to be used for calculation of condensed tannins. Development of pink color without heating the sample indicates presence of flavan–4–ols. If this happened, one heated blank for each sample, comprising 0.5 mL of the extract, 3 mL of butanol and 0.1 mL of the ferric reagent was used. Condensed tannins (% in dry matter) as leucocyanidin equivalent were calculated by the formula:

(A 550 nm x 78.26 x Dilution factor)/(% dry matter)

This formula assumes that the effective El%, 1 cm, 550 nm of leucocyanidin is 460 (Porter *et al.*, 1986). Here, the dilution factor is equal to 1 if no 70% acetone was added and the extract was made from 200 mg sample in 10 mL solvent. Where 70% acetone is added (for example to prevent the absorbance from exceeding 0.6) the dilution factor is: 0.5 mL/(volume of extract taken). In the current study, dilution factor was 1 as 0.5 mL extract was taken.

**Determination of dry matter:** Sample (5 g) was placed in hot air oven maintained at 100–105°C. The sample was dried to a constant weight. Weight of the dried sample was recorded after cooling the sample to room temperature in the desiccator. Dry matter content was calculated by the following formula:

$$\text{Dry matter (\%)} = W_2/W_1 \times 100$$

Where, $W_2$ is the mass (g) of the sample before drying. $W_1$ is the mass (g) of the sample after drying.

## RESULTS AND DISCUSSION

The CT and dry matter (%) contents in different forages of Kherimurat rangeland have been presented in Table I. The range of CT of the subjected plants is from 0.008 to 1.43% of dry matter. Tannins are secondary metabolites that occur naturally in variety of plants. For example, *Schinopsis* spp. and *Acacia* spp. (Howes 1953; Endres *et al.*, 1962; Adewoye & Rao, 1977; Seigler *et al.*, 1986; Seigler & Hernandez, 1989) and *Prosopis juliflora* (Theresa *et al.*, 1977). The condensed content in Cassava ranged from 0.083 to 0.134 mg% of the DM in different cuts of the plant (Wanapat *et al.*, 1997). The tannin content in different species of plants was, *Lotus corniculatus* (birdfoot trefoil) 48 g/kg DM, *Lotus pedunculatus* (big trefoil) 77 g/kg DM, *Onobrychis viciifolia* (sainfoin) 29 g/kg DM, *O. arenaria* 29–38 g/kg DM, *Hedysarum cornarium* (sulla) 51–84 g/kg DM, *Medicago sativa* (lucerne) 0.5 g/kg DM, *Lespedeza cuneata* 46 g/kg DM, *Leucanea diversifolia* 96 g/kg DM, *Desmodium ovalifolium* 232 g/kg DM, *Lolium perenne* (perennial ryegrass) 1.8 g/kg DM and *Chicorium intybus* (chicory) 3.1 g/kg DM (Jackson *et al.*, 1996; Foo *et al.*, 1996, 1997).

There is abundant evidence to confirm that the tannins produced by different species or by the same species in different parts or at different times vary in their capability to precipitate proteins. The production of tannins seems to depend to a considerable extent on extrinsic factors, most notably soil conditions and light intensity. The impact of light can be quite extraordinary at the intraplant level so that the foliage in different parts of a shrub or tree can vary by several percentage points in its tannin content (Waterman & Mole, 1989). The underlying mechanisms by which extrinsic factors, notably light, influence tannin levels has been speculated upon but remains in need of hard experimental data performed under conditions where as many as possible of the potential variables are controlled.

Rangelands have been focused for research throughout the world to identify the useful plants, which could be used for feeding animals. One of such plants containing tannins is *Leucaena leucocephala*, which when added to the basal sugarcane/urea, was found to improve the availability of amino acids and long–chain fatty acids, with energy becoming the limiting factor in dairy cows (Kebreab *et al.*,

**Table I: Percentage of Dry matter contents of Condensed tannins from twenty forages of Kherimurat Rangeland of district Attock, Pakistan**

| Local Name of forage | Botanical Name | Tannin (% of DM) | DM (%) |
|---|---|---|---|
| Iple Iple | *Leucaena leucocephala* | 0.64 | 94.2 |
| Amla | *Phyllanthus emblica* | 0.10 | 94.8 |
| Kachnar | *Bauhinia veriegata* | 1.07 | 94.2 |
| Dheela | *Cyperus rotundus* | 1.11 | 94.6 |
| Snatha | *Dodonea Viscosa* | 1.35 | 95.4 |
| Desi beri | *Zizyphus jujube* | 1.24 | 93.6 |
| Jaman | *Fugena Jambolana* | 1.09 | 94.2 |
| Shehtoot | *Morus indica* | 0.12 | 92.4 |
| Shreen | *Albezia lebbek* | 0.03 | 94 |
| Amal tass | *Cassia fistula* | 1.24 | 95.2 |
| Kahuw | *Olea cuspidata* | 0.21 | 96.4 |
| Kandair | *Cassisu spomerum* | 1.45 | 94.6 |
| Dhaman | *Penisetum eiliari* | 0.25 | 94.6 |
| Dodh Tibel | NA | 0.09 | 96 |
| Kahawe | *Stuntedolea didata* | 0.06 | 94.4 |
| Waree (Grow on trees) | NA | 0.06 | 94.6 |
| Waree (Grow on beri) | NA | 0.008 | 95 |
| Junglee Beri | *Zizyphus nummularia* | 1.43 | 92 |
| Junglee Swank | *Echinocloa colonum* | 0.85 | 95 |
| Bhkair | NA | 0.04 | 93.5 |

NA = Not available

2001). Leucaena has been reported as a good source of quality food for sheep and goats (Jabbar *et al.*, 1997).

Results of the present study revealed that tannin content in the plants collected from Kherimurat rangeland is good enough for its beneficial activity and is lower than the toxic levels reported in many other plants reported in literature. Therefore, a number of plants used in the present study provide suitable alternative to scarcity in feed resources of the country/rangelands.

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