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Condensed tannin and hydrolysable tannin contents of commonly available forages for ruminants

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Abstract
Condensed tannin (CT) and hydrolysable tannin (HT) content of commonly available forages in Tamil Nadu for ruminants feeding were estimated. Six samples each of sixteen commonly available forages were collected, dried and stored in airtight containers for analysis of condensed tannin and hydrolysable tannin contents. The levels of CT were not different between grasses/ shrubs and tree fodders. The levels of CT among grasses/ shrubs varied from 0.03 to 0.88% which was the highest in *Stylosanthus scabra a*nd lowest in *Brachiaria mutica* and *Medicago sativa*. Among tree fodders, the maximum CT was found in *Acacia nilotica* (1.07%) and the minimum CT was in *Moringa olifera*. Hydrolysable tannin contents were significantly (P<0.01) different between grasses/shrubs and tree fodders. *Acacia nilotica* (9.05%) had maximum HT and *Gliricidia sepium* (0.11%) had the minimum HT among tree fodders. In grass/ shrubs, the maximum and minimum content of HT was present in *Sorghum vulgare* (2.9%) *and Pannicum maximum* (0.02%) respectively. It was concluded that CT did not differ between grass/shrubs and tree fodders, whereas HT contents were higher in tree fodders.

Keywords: Condensed tannin, Forages, Hydrolysable tannin, Ruminants

Tannins are the secondary plant metabolites responsible for natural defence mechanism against bacteria, insects, fungi and grazing animals and therefore tannins assist in the survival of the plants (Kumar and Singh, 1984). They are complex polyphenolic compounds and have a variable effect in decreasing digestibility of proteins in ruminant animals. The most important property of tannins is their strong affinity for enzymes of feed protein, but even this varies depending on species; for instance, *Prosopis cineraria* tannin has been observed to have a very high protein precipitating capacity, comparatively higher than tannins from other tree foliages (Patra and Saxena, 2011). The total tannin (TT) contents of tree fodders had higher values than grasses/shrubs (Bharathidhasan *et al*., 2013). Therefore, tannin content potentially alters the use and value of tree foliages and may at times be responsible for the poor utilization of such forages by ruminant livestock. It protects the protein on degradation in rumen and improves nitrogen metabolism. Tannins in higher concentration have the ability to bind and inhibit the digestive activities (Kumar and Singh, 1984) and affect the microbial enzyme production (Makkar *et al*., 1989), whereas a low concentration of tannin can improve the nutrition for ruminant animals by reducing protein degradation in the rumen and increasing the flow of amino acids to the intestine (Mc Nabb *et al*., 1996). Concentration of tannins less than 4% of the ration was found beneficial by promoting by pass proteins and bloat suppression in ruminant animals (Aganga and Tshwenyane, 2003). Tannin concentration at more than 5% adversely affected the forage intake and digestibility (Silanikove *et al*., 1996) in ruminant animals. The dietary tannin at 2-3% had favourable effect against bloat (Waghorn and Jones, 1989) and increased the by-pass protein for host benefit in dairy animals (Wang *et al*., 1994).

Tannins are broadly classified into hydrolysable tannin (HT) and condensed tannins (CT). Condensed tannins affects nutrient supply to the animal by making complex with digestive enzymes, and dietary and endogenous proteins in ruminants. By contrast the HT is smaller molecules and may be hydrolyzed in the digestive tract (Getachew *et al*., 2008). The tannins like tannic acid (HT) and *Quebracho* tannin (CT) showed a strong protein protection from ruminal degradation indicating the similarity in biological effect of both condensed and hydrolysable tannins (Getachew *et al*., 2008). The tannin either in the form of CT or HT was found beneficial for the ruminant animals not only for protecting proteins but also reducing the methane emission by modulating the rumen microbial population and their activities. Hence, the present study was conducted to find out the condensed

tannin and hydrolysable tannin contents of the commonly available forages for ruminant animals in Tamil Nadu.

Six samples each of sixteen commonly available forages in Tamil Nadu used for feeding ruminant animals were collected (approximately 2 kg of each sample) as per the standard procedure. The collected forage samples were dried in a hot air oven at a temperature of 55-65 °C to constant weight and ground to pass through 1 mm sieve and stored in airtight containers for analysis. The plant extract was prepared and estimated for total tannin content as per Makkar *et al*. (1993). The condensed tannin present in extracts was estimated as per the method of Porter *et al*. (1986), wherein 0.5 ml of plant extract was taken in test tube and to it was added 3 ml of butanol HCl and 0.1 ml of ferric reagent. The test tubes were vortex mixed to ensure proper mixing. The mouth of the tubes was covered with glass marble and contents were boiled for 60 minutes. The tubes were cooled to room temperature and optical density was read at 550 nm using spectrophotometer. Condensed tannin as leucocyanidine equivalent was calculated by using the following formula.

Percentage of condensed tannin = (Absorbance at 550 nm x 78.26 x dilution factor) / (Percentage of DM)

The hydrolysable tannin was then calculated by subtracting condensed tannins from total tannins (Singh *et al*., 2005). The data were analysed through the procedure of statistical analysis system (IBM SPSS® Version 20.0 for Windows®) as per the Snedecor and Cochran (1989).

There was no significant differences between the CT content of grasses/ shrubs and tree fodders, however a significant (P<0.01) difference in HT content was observed between grasses/shrubs and tree fodders (Table 1). Among the grasses/shrubs, the highest percentage of CT was found in *Stylosanthus scabra* (0.88%) and lowest percentage of CT was found in *Brachiaria mutica* (0.03%) and *Medicago sativa* (0.03%), respectively. The other grasses/shrubs like *Vigna unguiculata, Sorghum vulgare* and *Pennisetum typhoides* x *Pennisetum purpureum* had 0.82%, 0.28% and 0.07 % CT, respectively. *Pannicum maximum, Cyanodon dactylon* and *Saccharum officinarum* had similar values of CT (0.06 %).

Baloyi *et al*. (2001) reported that the CT of natural pasture was 2.14% which was higher than the CT content observed in the present study for *Pannicum maximum, Cyanodon dactylon, Pennisetum typhoides x Pennis-*

Table 1. Percentage of CT and HT content of commonly available forages for ruminants on DM basis (Mean# ± S.E)

Forages	Condensed tannin (%)	Hydrolysable tannin (%)
Grasses/shrubs		
Pannicum maximum	0.06 ± 0.02	0.02 ± 0.08
Cyanodon dactylon	0.06 ± 0.02	0.11 ± 0.08
Pennisetum typhoides x *Pennisetum purpureum*	0.07 ± 0.02	0.09 ± 0.05
Brachiaria mutica	0.03 ± 0.01	0.11 ± 0.08
Sorghum vulgare	0.28 ± 0.01	2.90 ± 0.19
Medicago sativa	0.03 ± 0.01	0.28 ± 0.02
Vigna unguiculata	0.82 ± 0.06	0.19 ± 0.11
Stylosanthus scabra	0.88 ± 0.11	0.50 ± 0.11
Saccharum officinarum	0.06 ± 0.02	0.07 ± 0.03
Tree fodders		
Gliricidia sepium	0.04 ± 0.01	0.11 ± 0.04
Leucaena leucocephala	0.40 ± 0.02	0.88 ± 0.07
Sesbania grandiflora	0.09 ± 0.01	1.55 ± 0.06
Acacia nilotica	1.07 ± 0.04	9.05 ± 0.52
Albezia lebback	0.06 ± 0.01	1.10 ± 0.04
Azadirachta indica	0.19 ± 0.01	0.45 ± 0.08
Moringa oleifera	0.03 ± 0.01	3.24 ± 0.22
Grasses/Shrubs (Mean ± S.E)*	0.30 ± 0.05	0.47 ± 0.13[a]
Tree fodders (Mean ± S.E)*	0.26 ± 0.05	2.22 ± 0.54[b]
Test of significance	NS	P<0.01

#Mean of six observations; *Means bearing different superscripts in the same column differ significantly

-etum purpureum and Brachiaria mutica. *Sorghum vulgare* had higher level of total CT in this study than that earlier reported by Gwanzura and Ngambi (2012). The CT content of *Vigna unguiculata* (0.82%) was higher (0.03%) than earlier reported value by Gwanzura and Ngambi (2012) and lower (1.8%) than that reported by Baloyi *et al*.(2001). The finding of CT in *Medicago sativa* was similar with the earlier findings of Ramirez-Restrepo and Barry (2005) and Tavendale *et al.* (2005). The CT content of the *Stylosanthus scabra* (0.88%) was also lower than the earlier reported value (8.09%) by Baloyi *et al*. (2001). In tree fodders the maximum and minimum CT was fond in *Acacia nilotica* (1.07%) and *Moringa oleifera* (0.03%) respectively. Among the tree fodders, the CT levels of *Gliricidia sepium, Leucaena leucocephala, Moringa oleifera* were higher when compared to the values reported earlier (Jackson *et al*., 1996; Alexander *et al*., 2008). The CT content of tree leaves, commonly used for livestock feeding in low (1000-1500 metres) and high (2000-2500 metres) altitudes of temperate sub-Himalayas in northern India were 0.02 to 5.82% and varied widely (Sahoo *et al*., 2016) when compared with the present study. Nag *et al*. (2017) also reported that the higher CT content of legumes with threshold limit of 5% and in non-legumes had 6-7 % CT.

The HT content was maximum in *Sorghum vulgare* (2.9%) and minimum in *Pannicum maximum* (0.02%) among shrubs/grasses. In tree fodders which had higher HT compared to grasses or shrubs, *Acacia nilotica* (9.05%) had maximum hydrolysable tannin and *Gliricidia sepium* (0.11%) had the minimum HT. The earlier reports on HT for *Sorghum vulgare* (11.7%) and *Vigna unguiculata* (8.13%) were higher compared to the present study (2.9 %) but the observed value of Hydrolysable Tannin in *Moringa oleifera* (3.24%) was higher than the earlier reported values (1.1%) by Alexander *et al.* (2008). Tree leaves commonly used for livestock in low and high altitudes of temperate sub Himalayas in northern India contained 0.5-9.2% of HT tannin, which corroborated the values obtained in the present study (Sahoo *et al*., 2016). Thus, the variation in CT and HT values might also be due to the changes in the environmental factors like the soil, harvest stage, irrigation interval and time etc.

It was concluded that CT contents did not differ between grass/shrubs and tree fodders, whereas HT contents were higher in tree fodders. The *Acacia nilotica* had highest value of CT (1.07%) and HT (9.05%) among forages. The feeding of CT and HT through forages could be considered to the maximum extent of 4-5% of dry matter intake for ruminants to improve nitrogen metabolism and reduce methane emission without affecting other rumen fermentation characteristics.

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