



Journal of Biological Sciences

ISSN 1727-3048

science
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Concentration of Soluble Condensed Tannins and Neutral Detergent Fibre-bound Tannins in Fodder Trees and Forage Crops in Botswana

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Abstract: This study was conducted to determine soluble and fibre-bound condensed tannins in fodder trees and forages used in ruminant feeding in Botswana. Samples of *Acacia nilotica* and *A. robusta* were obtained from paddocks at Content Farm while *Lablab purpureus* (Lablab) was obtained from dried hay bales and *Ipomoea batatas* (sweet potato) was sourced from Department of Agricultural Research. Acacia and sweet potato vine samples were dried under shade before been ground and analysed for soluble tannins and fibre-bound tannins. It was found that amounts of soluble and fibre-bound tannins were different between plant species. There was a highly significant ($p < 0.001$) plant effect on soluble tannins. *A. nilotica* had the highest levels while *Ipomoea batatas* had the least amounts. The fibre-bound tannins was significantly different ($p < 0.01$) between plant species. Once again *A. nilotica* had high levels while sweet potato had the least. However *A. robusta* and Lablab had similar ($p > 0.05$) amounts of fibre-bound tannins. The level of soluble tannins in acacia species may interfere with animal performance since they exceed 50 g kg^{-1} . However, goats may be able to utilise these plants as no sign of toxicity was found in goats consuming $10\text{-}23 \text{ g kg}^{-1}$ of tannin-rich leaves in Israel.

Key words: Acacia, tannins, forages, sweet potato, Lablab

INTRODUCTION

Inadequate amounts and low feed quality result in reduced livestock productivity in tropical countries (Odenyo *et al.*, 1997). However, the feed base can be improved by using perennial legume fodders, particularly those with multipurpose function that are more attractive to the resource limited farmers in these countries (Odenyo *et al.*, 1999) and forage crops with higher crude protein content. Indigenous multipurpose fodder trees that are common include Acacia species. During drought and extended dry period, livestock are fed on these plants and in some instances pods are collected and traded to livestock owners. Madibela *et al.* (2004) recorded crude protein levels of acacia browse trees in Botswana which ranged from 114.7 to 213.3 g kg^{-1} DM. In Botswana, *Lablab purpureus* (*Dolichus lablab*) has been adapted and is commonly used by small scale small ruminants and dairy farmers. *Ipomoea batatas* (sweet potatoes) have high dry matter yield and digestible protein (Madibela *et al.*, 1999). According to Odenyo *et al.* (1999) fodder trees often contain anti-nutritional factors (ANFs) and a common ANF in fodder trees is tannin. Condensed Tannin (CT) of four mistletoes plants associated with multipurpose trees in Botswana

was found to be 75 , 31 , 65 and 56 g kg^{-1} DM for *Viscum verrucosum*, *V. rotundifolium*, *Erianthenum ngamicum* and *Tapinanthus lugardii*, respectively (Madibela *et al.*, 2002). Tannins are water-soluble polyphenolics that precipitate proteins from solution (Nelson *et al.*, 1995) and this can have both detrimental and beneficial effects to livestock consuming CT containing fodder trees. At high levels, tannin cause over protection of protein resulting in low utilisation of nitrogen (Silanikove *et al.*, 1997) while at quantities of less than 50 g kg^{-1} DM, condensed tannins may increase supply of protein to the small intestine (Miller *et al.*, 1995). *A. nilotica* is used by livestock but in Botswana *A. tortilis* is the most preferred browse plant by goats. These, may be an indication of differences in the nature (chemical structure) and quantities of polyphenolic substances between these plants. *Dolichus Lablab* is highly digestible and this may be a sign of low presence of tannins. Though Madibela *et al.* (1999) found that sweet potato vine have high dry matter digestibility, dark-leaved varieties may indicate presence of tannins (Dr. Karachi, 1996, Personal communication). The absence of information on levels of tannins in *Dolichus lablab* and sweet potato lead to the investigation of tannins levels in these plants. Fodder trees were used for comparison since there are known for their high level of condensed tannins.

MATERIALS AND METHODS

Study site: Botswana College of Agriculture (BCA) is situated between latitude of 24°33' S and longitude of 25°57' E at an altitude of 994 m asl. BCA is situated about 10km from Gaborone City centre in Botswana. The vegetation type is a mixture of Acacia savanna with broadleaved middle layer trees. The soil type of the area is classified as moderately deep to very deep, imperfectly to moderately well drained dark brown to red sandy clay loams to clays (De Wit and Nachtergaele, 1990). The station receives an average annual rainfall of about 500 mm. Monthly averages minimum and maximum temperatures are 12.8 and 28.6°C, respectively.

Samples and analysis: Samples of *Acacia nilotica* and *A. tortilis* were obtained from Botswana College of Agriculture farm in 2004. Samples were handpicked from the terminal shoots. *Dolichus lablab* samples were obtained from hay stacks at dairy farm. *Dolichus lablab* was harvested at 50% flowering stage, cut and left for a week to wilt and then baled. The bales from which the samples were obtained were under a shed. Samples of sweet potato vines (Variety; Kenya) were obtained from Department of Agricultural Research Horticulture Programme. The Acacia samples and sweet potato samples were dried under the shade to avoid oxidative polymerization of the tannins. They were milled through a 1 mm screen before analysis.

Soluble tannins extraction and analysis: Soluble condensed tannins were extracted from finely ground samples (200 g) of each plant in duplicate with 10 mL of aqueous acetone (70:30; v/v) according to Makkar (1995). Condensed tannins were determined according to Makkar (1995) with slight modification. Briefly, the tubes were centrifuged and the supernatant was collected. A 0.50 mL of extracted tannin from each sample was diluted with 70% acetone before being poured into test tubes. Butanol-HCL reagent (3.0 mL) and 0.1 mL ferric reagent (2% ferric ammonium sulphate in 2 N HCL) were added into the duplicate tubes and the tubes were vortexed. Each tube was covered with a glass marble and placed in a heating block adjusted at 97 to 100°C for 60 min. Then the tubes were cooled and absorbance at 550 nm was read using a single beam spectrophotometer. Blank samples containing unheated mixture were used for correction. Amount of condensed tannins (g/kg DM) as leucocyanidin equivalent was calculated using the following formula; (A 550 nm x 78.26 x dilution factor)/(g/kg dry matter).

Analysis for fibre-bound condensed tannins: Fibre-bound condensed tannins were determined in the Neutral Detergent Fibre (NDF) fraction of forages after extraction of NDF. NDF was determined according to AOAC (1996). The NDF residue samples were weighed (200 g) into duplicate screw cap test tubes and 5 mL of butanol-HCL reagent was added to each tube. The extraction was done according to Makkar (1995) but a preheated water bath (100°C) was used instead of dry block heater. The supernatant was decanted into vials and absorbance was read at 550 nm using a single beam spectrophotometer. Tannins were calculated as for soluble condensed tannins.

Statistical analysis: Analysis for effects of plant species was performed using the General Linear Models (GLM) procedure (SAS, 1990). Differences between plant species were tested for significance by Least Significant Difference (LSD). Means are reported as least square means with standard error.

RESULTS AND DISCUSSION

There was variation of both soluble and fibre-bound condensed tannins between the plant species. There was a highly significant plant effect ($p < 0.001$) on soluble condensed tannins and *Acacia nilotica* had the highest content and *Ipomoea batatas* had the least amount of soluble condensed tannins. The fibre-bound condensed tannins level was significantly different ($p < 0.01$) between plant species. *A. nilotica* had the highest levels while *I. batatas* had the lowest level. However, *A. robusta* and *Lablab purpureus* had similar ($p > 0.05$) amounts of fibre-bound condensed tannins (Table 1). According to Boitumelo (2000) browse plants generally have condensed tannins. This is because tannins are part of anti-herbivory strategy employed by plants. Typically, browse plants are important source of forage in the tropics. This is because during extended dry period or droughts browse plants provide green forage which has high levels of protein, minerals and vitamin. However, due to binding of tannins to protein, the uses of many browse species as supplements have limited impact on increasing the productivity of animals (Getachew *et al.*, 2001) Among the two browse plants tested in the present study, *A. nilotica* was found to have the highest soluble and fibre-bound condensed tannins. However, Kahiya *et al.* (2003) found that in Zimbabwe, *Acacia karoo* had higher levels of soluble condensed tannins (1.30 versus 0.07 absorbance units) than *A. nilotica*. Similar trend was observed for fibre-bound condensed tannins.

Table 1: Amount of soluble and fibre-bound condensed tannins (g/kg DM) in *Acacia* species and forage crops

Plants	Soluble condensed tannins	Fibre-bound condensed tannins
<i>Acacia nilotica</i>	90.0 ^a	55.6 ^a
<i>Acacia robusta</i>	75.9 ^b	47.0 ^b
<i>Ipomoea batatas</i>	67.7 ^c	30.9 ^c
<i>Lablab purpureus</i>	57.1 ^d	39.9 ^b
Standard Error	1.2	1.9
Mean	72.7	43.4
Significant level	**	*

* = p<0.01; ** = p<0.001

Getachew *et al.* (2001) reported values of 100 and 79 g kg⁻¹ condensed tannins for *Acacia cyanophylla* and *A. albida*, respectively. Levels of condensed tannins in *A. cyanophylla* are higher than levels of CT observed in any of the plants tested in the present study but those of *A. albida* were lower than those found in *A. nilotica* in the present study. Mupangwa *et al.* (2000) recorded a value of 3.7 g kg⁻¹ condensed tannins and 0.6 g kg⁻¹ fibre-bound tannins in *Lablab purpureus* and this values are lower than those found in the present study. Mupangwa *et al.* (2000) attributed variation in tannins content of the forage they tested to different reactions of tannins or other compounds present in the forage legumes. This may be the case in the present study. For instance, Kahiya *et al.* (2003) found that although *A. nilotica* had less amounts of soluble condensed tannins than *A. karoo*, it nevertheless had higher levels of phenolics. *A. nilotica* has been reported to have high levels of phenolic compounds (Dr. Pell, 2001, Personal Communication). In Botswana the most preferred browse legume by goats is *A. tortilis*, this is despite the fact that on dry matter basis *A. robusta* has thicker leaves bundled together at a node than in the case with *A. tortilis*. However, *A. tortilis* have been found to have high protein level than *A. robusta* (Madibela *et al.*, 2004) and this may contribute for it been preferred by goats. According to Kahiya *et al.* (2003) CT may reduce rumen digestibility of dry matter and fibre in ruminants, causing a depression in metabolisable energy intake. This may explains the low weight gain often reported when high amount of condensed tannins forage is fed. However, Silamkove *et al.* (1996) found that metabolic indices indicative of liver damage and kidneys in goats were within the normal range despite the fact that the animals consumed 10-23 g kg⁻¹ of tannin-rich leaves. It therefore appears that goats are a species of choice in utilisation rangeland resource such as browse plants rich in tannins. Both *Lablab purpureus* and *Ipomoea batatas* have lower CT than browse plants and therefore can be used as supplements without much detrimental effects on ruminants.

ACKNOWLEDGMENTS

The first author was supported by Botswana College of Agriculture to carry out this study. Mr I. Boko of DAR is acknowledged for supplying the sweet potato vines samples.

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