

## Quantification and characterisation of condensed tannin of selected indigenous browse tree species leaves of north-western Tanzania

Chrispinus D.K. Rubanza<sup>1,3</sup>, Martin N. Shem<sup>2</sup>, Toshiyoshi Ichinohe<sup>3</sup> and Tsutomu Fujihara<sup>3\*</sup>

<sup>1</sup>Tanzania Forestry Research Institute (TAFORI), P.O Box 1257, Shinyanga, Tanzania. <sup>2</sup>Department of Animal Science, Sokoine University of Agriculture, P.O Box 3004, Morogoro, Tanzania. <sup>3</sup>Laboratory of Animal Science, Shimane University, Matsue, Shimane 690-8504, Japan. \*e-mail: fujihara@life.shimane-u.ac.jp, ckrubanza@yahoo.co.uk

Received 18 January 2008, accepted 20 March 2008.

### Abstract

Browse tree foliages (leaves, twigs and fruits) represent available feed resources that could be used for improved livestock productivity in the tropics due to their high contents of protein, although their utilisation could be limited by their high contents of anti-nutritional factors such as phenolics and tannins. Browse tree leaves from seven species including three Acacias (*A. nilotica*, *A. polyacantha* and *A. tortilis*), *Dichrostachys cinerea*, *Flueggea virosa*, *Harrisonia abyssinica* and *Piliostigma thorningii* were screened to quantify levels of extractable total phenolics (TEP), extractable tannin (TET) and condensed tannin (CT). The CT in the leaf samples was assayed for soluble, protein-bound and fibre-bound CT using a modified butanol/HCl technique through improved extraction of tannin in leaf samples with aqueous sodium dodecyl sulphate (SDS)- $\beta$ -mercaptoethanol solution. The species had variable levels of TEP, TET and TCT ranging 104-281, 93-256 and 52.8-98.3 g kg<sup>-1</sup> DM, respectively. Most of CT was bound to protein (22.2 to 50.5 g kg<sup>-1</sup>DM). Soluble and fibre-bound CT fractions varied ( $P < 0.05$ ) among the species from 14.5 to 22.9 g kg<sup>-1</sup> DM and from 13.0 to 28.6 g kg<sup>-1</sup> DM, respectively. The species had detectable and variable ( $P < 0.05$ ) levels of delphinidins, cyanidins and pelargonidins. Structural elucidation of the assayed proanthocyanidins revealed flavan-3-ols, flavan-3,4-diols or a mixture of the two flavonoids. Presence of different flavonoids denotes variable proanthocyanins' stereochemistry that implies variation in tannin activity and thus reactivity that could be attributed to variable feed anti-nutritive activity. High levels of phenolics and tannins in these species could limit utilisation of browse foliages in ruminants through impaired feed digestibility and nutrient utilisation. There is a need to establish both safety levels of inclusion in the diets of ruminants and tannin structure-activity relationship *in vivo*.

**Key words:** Acacia, browse, phenolics and tannins, bound tannins, proanthocyanidins, livestock nutrition.

### Introduction

Browse fodder tree and shrub foliages (i.e., leaves, twigs, pods, fruits and barks) represent important feed resources as cheap crude protein (CP) and mineral supplements for ruminants in the tropics. However, optimal utilisation of browse tree foliages in ruminant feeding could be limited by presence of plant secondary compounds such as polyphenolics (e.g., phenolics and tannins). Tannins refer to high molecular weight (i.e., >3000 Daltons) soluble phenolic compounds that possess enough hydroxyl groups that bind to proteins to form complexes which precipitate dietary feed nutrients such as carbohydrates, proteins, minerals and vitamins<sup>1</sup>. Tannins are classified into hydrolysable and condensed tannins (CT). Hydrolysable tannins (HT) represent polyesters of gallic acids (gallotannins) and hexahydroxydiphenic acids (ellagitannins)<sup>2</sup>. Condensed tannins (CT) or proanthocyanidins (PA) refer to complex of oligomers and polymers of flavonoid units<sup>2,3</sup> (Fig. 1). Deleterious effects of phenolics and tannins include inhibition of digestive enzymes, toxic effects on rumen microbes<sup>4</sup> and toxic effects on intestinal mucosa<sup>1</sup>. In order to optimise animal production responses, there is a need to establish levels of tannins and structural elucidation of CT molecule of common in browse tree fodder species' foliages.

Screening of browse fodder for phenolic and tannin contents is limited by inconsistent analytical techniques used for quantification of tannins<sup>3</sup>. Little is known about the chemistry,

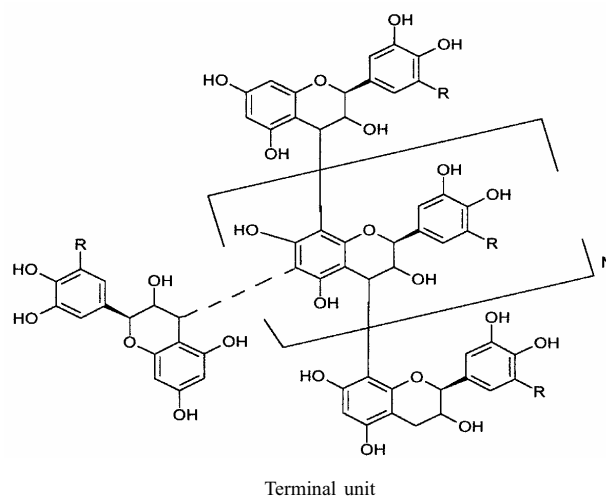


Figure 1. Generic structure of polymerised condensed tannin molecule.

tannin activity and tannin structure-biological activity relationship in different species of *Acacia*, and other tropical browse foliage. As a result, there is little utilisation of foliage from *Acacia* spp. and other browse trees and shrubs<sup>5</sup>. A study was therefore conducted to screen tannin composition of browse tree leaves from three species of *Acacia* and four key fodder species native to western Tanzania to: 1) quantify levels of extractable phenolics and tannins; 2) determine soluble/extractable CT, protein-bound and fibre-bound CT fractions; 3) elucidate proanthocyanidins' structure and composition of selected species' leaves.

### Materials and Methods

**Study area:** Browse tree leaves from seven species: three *Acacia* (*A. nilotica*, *A. polyacantha* and *A. tortilis*), *Dichrostachys cinerea*, *Flueggea virosa*, *Harrisonia abyssinica* and *Piliostigma thorningii* were collected from the Shinyanga region (1000-1300m above sea level) in north-western Tanzania (2-3°S; 31-31.5°E). The region receives low unimodal annual rainfall of 600 to 800 mm between November and mid May. Minimum and maximum temperatures vary from 16.7 to 28.9°C, respectively. Common vegetation in the study area includes short grasses and scattered shrubs and trees, mainly dominated by *Acacia* spp.

**Forage sample collection and processing:** Samples of leaves and soft twigs were hand plucked from 8-10 trees from each species in established four sub-plots of 70 m x 70 m in four grazing lands in six districts of Shinyanga region. The samples were dried at 50°C in a forced air oven for 48 h to constant weight and ground to pass a 1.0 mm sieve.

**Extraction and determination of total phenolics and tannins:** Approximately 200 mg (DM) of finely ground sample was extracted in 10 ml of aqueous acetone (7:3 v/v) in water<sup>6</sup>. The supernatants were centrifuged at 1,670 x g (4°C) for 20 min. The aqueous aliquots were assayed for total extractable phenolics (TEP) and extractable tannins (TET) and the residue discarded. Content of TEP was assayed using the Folin-Ciocalteu's reagent based on the tannic acid standard<sup>7</sup>. Content of TET was estimated gravimetrically as the difference of phenolics remaining from total phenolics after binding tannins with polyvinyl polypyrrolidone (PVPP)<sup>8</sup>. The concentrations of TEP and TET were expressed as the tannic acid equivalent.

### Soluble and bound condensed tannins assay

**Extraction and determination of soluble/free-bound condensed tannins:** An approximate 0.1 g sample was extracted thrice with a mixture of 4 ml of acetone/water (7:3 v/v), containing 1 g/l ascorbic acid and 2 ml of dichloromethane<sup>9</sup>. The tubes were vortexed and centrifuged thrice at 1,670 x g for 15 min., while saving the aqueous fraction. The soluble CT fraction was determined using the butanol/HCl assay<sup>10</sup>, by adding 0.25 ml of the aqueous extract to 6 ml of *n*-butanol/HCl (95:5 v/v)<sup>11,12</sup>. The absorbance of the red anthocyanidin products (*i.e.*, CT) was measured at 550 nm.

**Extraction and determination of protein-bound condensed tannins:** The solid residues obtained following extraction of free-bound CT were dried in a stream of N<sub>2</sub> gas at room temperature to eliminate residual volatile solvents. The residues were heated at 95°C on a metal block for 45 min. with 3 ml of 10 g l<sup>-1</sup> aqueous

sodium dodecyl sulphate (SDS) containing 50 g l<sup>-1</sup> 2-β-mercaptoethanol (SDS solution)<sup>11</sup>. The protein-bound CT fraction was estimated by the butanol/HCl/Fe<sup>3+</sup> assay<sup>10</sup>. The SDS extract, 0.5 ml, was added to 6 ml of *n*-butanol/HCl (95:5 v/v); vortexed and heated for 1 h at 95°C. Absorbance of the red anthocyanidin products (*i.e.*, CT) was measured at 550 nm.

**Extraction and determination of fibre-bound condensed tannins:** Fibre-bound CT was estimated similar to protein-bound CT with 6 ml of butanol/HCl/Fe<sup>3+</sup> plus 0.5 ml of SDS solution, added directly to the solid residues. The mixture was heated at 100°C on a metal block for 1 h. The samples were then cooled and centrifuged at 1,670 x g for 15 min. before the absorbance of the supernatant was measured at 550 nm. The absorbance values for soluble/free-bound, protein-bound and fibre-bound CT for each species were compared with the respective species blank values. Blank samples constituted plant extracts that were extracted in a similar procedure with butanol/H<sub>2</sub>O (95:5 v/v) replacing the butanol/HCl assay<sup>12</sup>. Absorbance values for soluble, protein-bound and fibre-bound CT were converted to CT concentrations by including the authentic tannin standard purified from *A. nilotica* at known concentrations in each of the three runs<sup>12,13</sup>.

**Characterisation of condensed tannin flavonoids:** The CTs in leaves were assayed into proanthocyanidins (PAs) or leucoanthocyanidin flavonoid composition using high performance liquid chromatography (HPLC)<sup>9,14</sup>. A 1.00 g DM (1.00 mm) sample was extracted for 30 min. in an ultrasonic water bath with 4.0 ml aqueous acetone (7:3 v/v) containing 1 g l<sup>-1</sup> ascorbic acid and then centrifuged at 3 000 rpm (15 min.). A 0.5 ml supernatant sample was then vortexed with 0.25 ml of dichloromethane and centrifuged again. A 50 µl aliquot sample of the aqueous upper layer was combined with 3.0 ml of butanol/HCl (95:5, v/v) and heated at 95°C for 1 h. Butanol/HCl was evaporated to dryness under N<sub>2</sub> stream with the tubes kept at 50°C. The residue was re-dissolved in 0.5 ml of methanol/HCl (99:1, v/v) and filtered through a 0.02 polytetrafluoroethylene membrane, followed by injection of 10.0 µl aliquot into Inertsil ODS-80A (C 18) column, 150 mm x 4.6 mm (Shimadzu Co., Kyoto, Japan). Water/acetic acid (96:4, v/v; solvent A) and methanol (solvent B) were used for gradient elution at 2 ml/min. The gradient profile was 5-40% B (0-5 min.); 40-50% B (6-10 min.), 50-100% B (11-15 min.) and 100-5% B (16-20 min.). The absorbance at 525 nm was recorded using a LC-10AT HPLC system (Shimadzu Co., Kyoto, Japan) fitted with a CR-6A data processor (Shimadzu Co., Kyoto, Japan) and a SPD-10A variable wavelength detector (Shimadzu Co., Kyoto, Japan). The peaks were identified using cyanidin chloride, delphinidin chloride and pelargonidin chloride (Extrasynthese Co., Genay, France), which had retention times of 7.48, 10.24 and 12.64 min., respectively.

**Statistical analysis:** Data on tannin composition and their fractions were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure<sup>15</sup>. The data were analysed based on the statistical model:  $Y_{ij} = \mu_{ij} + S_i + e_j$ , where,  $Y_{ij}$  is the general observation on chemical composition and tannin estimates,  $\mu_{ij}$  is the general mean common for each parameter under investigation,  $S_i$  is the  $i^{\text{th}}$  effect of browse species on the observed parameters, and  $e_j$  is the standard error term.

## Results

**Content of phenolics and tannins:** All species had tannin contents higher than 50 g kg<sup>-1</sup> DM (Table 1). *Acacia nilotica* had higher ( $P<0.05$ ) contents of TEP and TET than the rest of the species' leaves. *Dichrostachys cinerea* and *P. thorningii* had comparatively low contents of TEP and TET. The TCT varied among the species from 52.8 (*A. nilotica*) to 98.3 g kg<sup>-1</sup> DM (*A. polyacantha* and *P. thorningii*). *Flueggea virosa* and *H. abyssinica* had low contents of TCT.

**Extractable, protein-bound and fibre-bound condensed tannins:** Fractionation of condensed tannins revealed variable levels of CT fractions among the species (Table 1). Soluble or free CT fraction varied from 14.5 (*H. abyssinica*) to 22.9 mg g<sup>-1</sup> DM (*D. cinerea*). Protein-bound CT fraction varied from 22.5 (*F. virosa* and *A. nilotica*) to 50.5 g kg<sup>-1</sup> DM (*A. polyacantha* and *P. thorningii*). The species had variable levels of fibre-bound CT that ranged from 13.0 to 28.6 g kg<sup>-1</sup> DM in *A. polyacantha* and *P. thorningii*, respectively.

**Flavonoid composition and structural elucidation:** Flavonoid composition of the selected species is shown in Table 2. The species had variable levels of proanthocyanidins (PAs) or leucoanthocyanidins. Delphinidins varied among the species from 0.062 g kg<sup>-1</sup> DM (*A. nilotica*) to 5.288 g kg<sup>-1</sup> DM (*A. tortilis*). *Acacia tortilis* had the lowest content of cyanidins compared to *A. polyacantha*, which had the highest concentration of cyanidins (Table 2). Content of pelargonidins varied among the species ( $P<0.05$ ) from 0.004 g kg<sup>-1</sup> DM (*F. virosa*) to 4.392 g kg<sup>-1</sup> DM (*D. cinerea*). There was no difference ( $P>0.05$ ) in pelargonidins among all the species (Table 2). The ratio of delphinidins: cyanidins varied from 0.11 (*A. polyacantha*) to 28.13 (*A. polyacantha*).

**Table 1.** Content<sup>§</sup> of TEP, TET and TCT and soluble and bound CT.

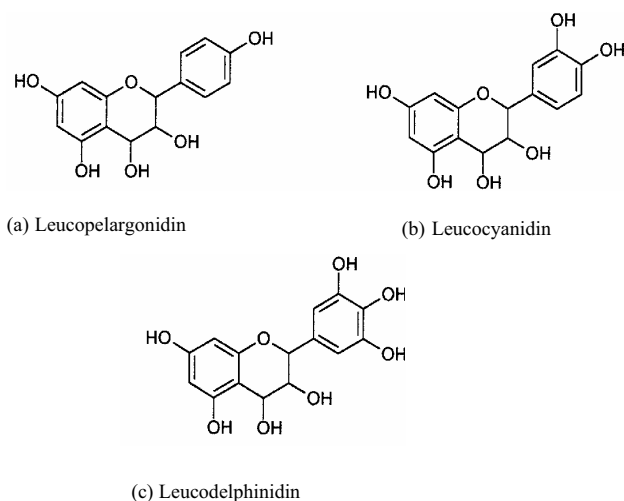
Species	TEP <sup>†</sup>	TET <sup>†</sup>	TCT <sup>‡</sup>	Soluble CT	Protein-bound CT	Fibre-bound CT
<i>A. nilotica</i>	281	256	52.8	17.6	22.2	13.0
<i>A. polyacantha</i>	104	93	98.3	19.2	50.5	28.6
<i>A. tortilis</i>	241	226	77.8	18.9	37.5	21.5
<i>D. cinerea</i>	114	96	75.4	22.9	35.4	17.1
<i>F. villosa</i>	234	220	53.2	17.6	22.2	13.4
<i>H. abyssinica</i>	156	139	54.3	14.5	25.0	14.8
<i>P. thorningii</i>	112	95	98.3	19.2	50.5	28.6
Mean	177.4	160.7	72.9	18.6	34.8	19.6
Effect of species	***	***	***	***	***	***

<sup>§</sup>Content of polyphenolics expressed in g kg<sup>-1</sup> DM, <sup>†</sup>TEP and TET are expressed as mg g<sup>-1</sup> tannic acid equivalent, <sup>‡</sup>TCT and CT are expressed as mg g<sup>-1</sup> DM *Acacia nilotica* tannin equivalent, \*\*\* $P<0.001$ .

**Table 2.** Proanthocyanidin flavonoid composition<sup>†</sup> of selected browse tree species leaves.

Species	Delphinidins	Cyanidins	Pelargonidins	Delphinidins: Cyanidins ratio
<i>A. nilotica</i>	0.062 <sup>a</sup>	0.243 <sup>a</sup>	0.009 <sup>a</sup>	0.26
<i>A. polyacantha</i>	0.454 <sup>b</sup>	4.179 <sup>b</sup>	0.098 <sup>a</sup>	0.11
<i>A. tortilis</i>	5.288 <sup>c</sup>	0.188 <sup>c</sup>	0.018 <sup>a</sup>	28.13
<i>D. cinerea</i>	1.630 <sup>d</sup>	1.424 <sup>d</sup>	4.392 <sup>b</sup>	1.14
<i>F. villosa</i>	0.461 <sup>b</sup>	0.537 <sup>c</sup>	0.004 <sup>a</sup>	0.86
<i>H. abyssinica</i>	0.384 <sup>b</sup>	0.619 <sup>c</sup>	0.017 <sup>a</sup>	0.62
<i>P. thorningii</i>	0.191 <sup>c</sup>	0.311 <sup>c</sup>	0.009 <sup>b</sup>	0.61
Mean	1.510	1.324	0.705	0.81
Effect of species	**	**	**	

<sup>†</sup>Flavonoid composition expressed in g kg<sup>-1</sup> DM, <sup>a,b,c,d,e</sup> different superscript letters in the same column indicate significant differences ( $P<0.05$ ), \*\* $P<0.01$ .



**Figure 2.** Stereochemistry of the assayed proanthocyanidin flavonoids.

Stereochemistry of the assayed proanthocyanidin flavonoids (delphinidin, cyanidin and pelargonidin) is shown in Fig. 2.

## Discussion

**Content of phenolics and tannins in *Acacia browse leaves:*** Optimal utilisation of crude protein (CP) supplements from these species leaves to ruminants could be limited by high levels of phenolics and tannins (e.g., >80 g kg<sup>-1</sup> DM) and even high levels of polymerised condensed tannins (e.g., >50 g kg<sup>-1</sup> DM). Such high levels of tannins could impair utilisation of CP from browse supplements by ruminants<sup>16</sup> through chemical complexes formation with dietary nutrients<sup>1</sup>. High contents of phenolics and tannins have similarly been reported in browse tree leaves<sup>17, 18</sup>. Slight variations between observed and literature values in phenolic and tannin contents could be due to the nature of assays used, nature of tannin in different fodder species, stage of growth and the proportion of leaf sample harvested and also, the influence of soil and climatic factors on accumulation of polyphenolic compounds in plants<sup>2</sup>. Variation in levels of phenolics and tannins among species of *Acacia* leaves could be explained by differences in genotypic factors that control biosynthesis and accumulation of polyphenolic compounds in *Acacia*<sup>2</sup>. Alternatively, a narrow difference between total extractable phenolics and tannins may suggest the presence of high molecular weight phenolics (tannins) in these species' foliage vs. lower molecular weight phenolic compounds such as ellagitannins and gallotannins.

**Extractable, protein-bound and fibre-bound condensed tannins:** Relatively high soluble CT fraction in *D. cinerea*, *A. tortilis*, *A. polyacantha* and *P. thorningii* could result in depressed palatability and intake of these feeds, as soluble CT has been associated with reduced palatability and feed intake in ruminants<sup>20</sup>. Adverse effects due to high proportions of CT bound to protein would be through reduced CP digestibility, mainly by formation

of protein-tannin complexes<sup>16</sup>. Negative effects of the fibre-bound CT fraction on feed digestibility would be mainly through formation of complexes with dietary carbohydrates<sup>20</sup>. The high levels of CT in these species could result in depressed digestibility. Variable CT fractions among the species could be related to variable tannin activity, the close relationship between proanthocyanidin composition and distribution of CT fractions, as well as tannin stereochemistry<sup>21</sup>, as well as the influence of tannin structure on tannin biological activity<sup>2</sup>.

**Characterisation of condensed tannins:** Structural elucidation of the detected delphinidin, cyanidin and pelargonidin flavonoids (Fig. 2) reveals the type and nature of condensed tannin molecule and provide an inference on the influence of PA on the tannin biological activity. These results suggest that the polymerised CT of browse species under study constitute flavan-3-ols, flavan-3,4-diols or a mixture of the two flavonoids. The flavan-3-ols possess two asymmetrical carbon atoms at C-2 and C-3 positions (Fig. 3), thus four isomers would exist, namely (+) and (-)-catechin in which 2-phenyl and 3-hydroxy groups, respectively, are trans<sup>22</sup>. Other isomers would include (-)-epicatechin and (+)-epigallocatechin<sup>23</sup>. The majority of proanthocyanidins or leucoanthocyanidins has flavan-3-ols as chain-terminating units with (+)-catechin being the most popular terminating unit among flavan-ols although variations among forage species exist<sup>22</sup>. Flavan-3,4-diols anthocyanidins possess asymmetric carbon at C-2, C-3 and C-4; a structure that suggests presence of a maximum of eight stereo-isomers<sup>23</sup>.

Variation in flavonoid classes among the species in this study could be due to differences in genetic and biochemical processes that control biosynthesis and accumulation of flavonoids in plant tissues<sup>23</sup>, environmental factors such as light intensity, nutrient status and interactions between the environment and genetic/biochemical processes<sup>16,19</sup>. Some important genetically controlled biochemical processes include overall flavonoid production, specific flavonoid synthesis and distribution of flavonoids in different plant tissues<sup>19</sup>.

The nomenclature of the assayed flavonoids (pelargonidins, cyanidins and delphinidins) is based on hydroxylation pattern. Pelargonidin flavonoids constitute 3,4',5,7-OH; procyanidins (3,3',4',5,7-OH); and prodelfinidins (3,3',4',5,5',7-OH) at the respective positions in the typical pyran ring<sup>22</sup> (Fig. 3). Variations in proanthocyanidin flavonoids could be due to simple structural modifications probably due to biochemical processes such as hydroxylation, methylation and glycosylation<sup>2,22</sup>.

The hydroxylation pattern in ring A (Fig. 3) bears an implication on the PA interflavan linkages. Most PAs are linked between C-4 of the preceding unit and C-8 or C-6 of the next flavan A-ring<sup>3,22</sup>.

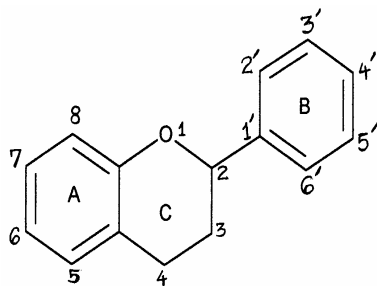


Figure 3. Generic structure of condensed tannin flavonoids.

The 4→8 and 4→6 linkages represent common bonding type in most PA molecules in plants<sup>3,22</sup> although the 4→8 linkages are the most predominant bonding type in procyanidins and prodelfinidins in forages and consist higher ratios of prodelfinidin/procyanidin (3:1), as compared to the 4→6 linkages<sup>22</sup>. The bonding type is directly related to the degree of polymerisation and polymer strength and therefore tannin reactivity<sup>3,24</sup>. Some propelargonidins and procyanidins could have additional (2β→O→7) interflavan ether linkages, which are categorised as class A-proanthocyanidins although are generally rare<sup>22</sup>. The class A-proanthocyanidins are characterised by high degree of conformational stability, and therefore lack the dynamic rotational isomerism in contrast to the B-type proanthocyanidins, which are linked via a single bond (i.e., they lack ether linkages).

Tannin anti-nutritive activity and reactivity would therefore be enhanced by type, stereochemistry at the three chiral centres (i.e., C-2, C-3 and C-4) (Fig. 3), increased concentration and molecular weight of PA as well as increased prodelfinidin/procyanidin ratio in the PA molecule<sup>3,16</sup>. Variable levels of pelargonidin, cyanidin and delphinidin flavonoids among the species foliages would have an implication on the polymerised PA chemical structure and tannin reactivity, and thus biological activity. These results suggest that the condensed tannin in *A. nilotica*, *A. polyacantha*, *A. tortilis*, *D. cinerea*, *F. virosa*, *H. abyssinica* and *P. thorningii* could have different biological activity and tannin anti-nutritive activity *in vivo* when fed to animals due to their variable chemical structures.

## Conclusions

Utilisation of N-supplements of browse fodder species in this study as supplements to ruminants could be limited by their high contents of phenolics and tannins. The species had higher levels of phenolic and tannin ANFs than the lower beneficial level of tannin (50 g kg<sup>-1</sup> DM) in ruminant diets. High proportions of total CT were bound to protein compared to soluble CT and fibre-bound CT fractions. The species' leaves contained detectable proanthocyanidins that constituted flavan-3-ols and flavan-3,4-diols that elucidate variable flavonoids' stereochemistry. Further studies are needed to elucidate the stereochemistry of the polymerised CT molecule tannin anti-nutritive activity and tannin structure-biological activity relationship.

## Acknowledgements

The first author expresses sincere gratitude to the Japanese Government for financial support from through scholarship (Mombukagakusho) award. We are very grateful to the International Centre for Research in Agroforestry (ICRAF)/Tanzania Agroforestry Project for various supports.

## References

- Mangan, J. 1988. Nutritional effects of tannins in animal feeds. *Nutr. Res. Rev.* 1:209-231.
- Haslam, E. 1998. *Practical Polyphenolics: From Structure to Molecular Recognition and Physiological Action*. Cambridge University Press, Cambridge, UK.
- Schofield, P., Mbugua, D.M. and Pell, A.N. 2001. Analysis of condensed tannins: A review. *Anim. Feed Sci. Technol.* 91:21-40.
- Brooker, J.D., O'Donovan, L.A., Skene, I., Clarke, K., Blackall, L. and Musklera, P. 1994. *Streptococcus caprinus* sp. nov, a tannin-resistant ruminal bacterium from feral goats. *Letters in Appl. Microbiol.* 17:224-227.

- <sup>5</sup>Makkar, H.P.S. 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effect of feeding tannin-rich feeds. *Small Rum. Res.* **49**:241-256.
- <sup>6</sup>Makkar, H.P.S. 2000. Quantification of tannins in tree foliage. A Laboratory Manual for the FAO/IAEA Co-ordinated Research Project on 'Use of Nuclear and Related Techniques to Develop Simple Tannin Assays for Predicting and Improving the Safety and Efficiency of Feeding Ruminants on Tanniniferous Tree Foliage. Joint FAO/IAEA working Document, IAEA Vienna, Austria, 40 p.
- <sup>7</sup>Julkunen-Tiitto, R. 1985. Phenolic constituents in the leaves of northern willows: Methods for the analysis of certain phenolics. *J. Agric. Food Chem.* **33**:213-217.
- <sup>8</sup>Makkar, H.P.S., Blümmel, M., Borrowy, N.K. and Becker, K. 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *J. Sci. Food Agric.* **6**:161-165.
- <sup>9</sup>Stewart, J., Mould, F. and Mueller-Harvey, I. 2000. The effect of drying treatment on fodder quality and tannin content of two provenances of *Calliandra calothyrsus* Meissner. *J. Sci. Food Agric.* **80**:1461-1468.
- <sup>10</sup>Porter, L.J., Hrstich, L.N. and Chan, B.G. 1986. The conversion of proanthocyanidins and prodelphinidins to cyanidins and delphinidin. *Phytochem.* **25**:223-230.
- <sup>11</sup>Terrill, T.H., Rowman, A.M., Douglas, G.B. and Barry, T.N. 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *J. Sci. Food Agric.* **58**:321-329.
- <sup>12</sup>Jackson, F.S., Barry, T.N., Lascano, C. and Palmer, B. 1996. The extractable and bound condensed tannins of leaves from tropical tree, shrub and forage legumes. *J. Sci. Food Agric.* **71**:103-110.
- <sup>13</sup>Waterman, P.G. and Mole, S. 1994. Analysis of Phenolic Plant Metabolites. Blackwell Scientific Pub., Victoria, Australia.
- <sup>14</sup>Hedqvist, H., Mueller-Harvey, I., Reed, J.D., Kruger, C.G. and Murphy, M. 2000. Characterisation of tannins and *in vitro* protein digestibility of several *Lotus corniculatus* varieties. *Anim. Feed Sci. Technol.* **87**:41-56.
- <sup>15</sup>SAS/ Statview 1999. Using Statview. Statistical Analytical System (SAS) Inc., 3<sup>rd</sup> edn. SAS Inc., Cary, NC, USA.
- <sup>16</sup>Aerts, R.J., Barry, T.N. and McNabb, W.C. 1999. Polyphenols and agriculture: Beneficial effects of proanthocyanidins in forages. *Agric. Ecosyst. Environ.* **75**:1-12.
- <sup>17</sup>Abdulrazak, S.A., Fujihara, T., Ondiek, T. and Ørskov, E.R. 2000. Nutritive evaluation of some Acacia from Kenya. *Anim. Feed Sci. Technol.* **85**:89-98.
- <sup>18</sup>Rubanza, C.D.K., Shem, M.N., Otsyina, E.R., Ichinohe, I. and Fujihara, T. 2003. Content of phenolics and tannins in leaves and pods of some *Acacia* and *Dichrostachys* species and effects on *in vitro* digestibility. *Anim. Feed Sci.* **12**:645-663.
- <sup>19</sup>Wong, E. 1973. Plant Phenolics. In Buttler, G.W. and Bailey, R.W. (eds). *Chemistry and Biochemistry of Herbage*. Vol. 1. Academic Press, Inc., London, UK, pp. 265-322.
- <sup>20</sup>Muhammed, S., Stewart, C.S. and Acamovic, T. 1994. Effects of tannic acid on cellulose degradation, adhesion and enzymatic activity of rumen microorganisms. *Proc. Soc. Nutr. Physiol.* **3**:174.
- <sup>21</sup>Waterman, P.G. 2000. The tannins - An overview. In Brooker, J.D. (ed.). *Tannins in Livestock and Human Nutrition*. Proceedings of an International Workshop, Adelaide, Australia, pp. 10-13.
- <sup>22</sup>De Bruyne, T., Pieters, L., Deelstra, H. and Vlietinck, A. 1999. Condensed tannins: Biodiversity in structure and biological activities. *Biochem. Systemat. Ecol.* **27**:445-459.
- <sup>23</sup>Chung, K.T., Wei, C.I. and Johnson, M.G. 1998. Are tannins a double-edged sword in biology and health? *Trends of Food Sci. Technol.* **9**:168-175.
- <sup>24</sup>Dalzell, S.A. and Kerven, G.L. 1998. A rapid method for the measurement of *Leucaena* spp. proanthocyanidins by the proanthocyanidin (butanol/HCl) assay. *J. Sci. Food Agric.* **78**:405-416.