Research Article

Condensed Tannins in Some Tropical Legumes Residue

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Abstract

The objective of this study was to compare condensed tannin (CT) and nutritive values of two edible legumes residue (leaf and stem) i.e. *Vigna unguiculata*, 'KVC7' cultivar (KVC7) and *Arachis hypogaea*, 'KKU 60' cultivar (KKU60) with two tropical legumes i.e. *Stylosanthes guianensis* CIAT 184 cv. Tha pra (Tha pra stylo) and *Centrosema pascuorum* cv. Cavalcade (Cavalcade). The Randomized Completely Block Design (RCBD) with four replications was conducted in this study, and data were analyzed by the analysis of variance (ANOVA). The results showed that condensed tannin (CT) (total CT, soluble CT, and bound CT) and percentage of either soluble CT or bound CT - varied with legume species. The residue of KKU60 had highest total CT (70.5 g/kg DM) and soluble CT (68.9 g/kg DM), while Cavalcade had the lowest total CT (8.4 g/kg DM). All legumes were found to possess 8.4-70.5 g/kg of dry matter (DM) of total CT. The residue of KVC7 cultivar had lowest DM (24.0%) and had relatively lower values in other traits, such as neutral detergent fiber (NDF, 33.5%), acid detergent fiber (ADF, 20.0%), and acid detergent lignin (ADL, 4.2%). The residue of KVC7 had high CP content (14.4%) similar to the residue of KKU60 (15.3%) but higher than Tha pra stylo (11.2%) and Cavalcade (11.5%). KVC7 and KKU60 had lower fiber and higher CP, comparing with Tha pra stylo and Cavalcade. The vegetative parts of KVC7 and KKU60 were recommended to use as feed stuff for the ruminants.

Key Words: Condensed tannin; Ruminant feed; Tropical legumes; Crop residue

Introduction

The scarcity of pastures for animal grazing in a dry season (Peters et al., 1997; Suttie, 2000; Reiber et al., 2010) has prompted researcher to conduct study to seek an alternative feeds for animal production (Norris and Thomas, 1982; Kemp and Culvenor, 1994). These research included using forage legumes either fed fresh, hay or silage or in improved pastures (Harricharan et al., 1988; Shelton et al., 1991; Argel et al., 2000), applying residue supplemented with leguminous forage (Hendricksen and Minson, 1985; Poppi and McLennan, 1995), and supplementing feed with tree leaf, flower and pod (not leguminous) for animal production (Everist, 1986; Lowry and Wilson, 1999; Kennedy et al., 2000).

Rice straw has been used for feeding ruminant animals because it is a major crop residue which is

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abundant in Thailand (Devendra, 1980; FAO, 1981). However, rice straw is a bulky material which is low in protein (Milford and Minson, 1968; Jackson, 1977; Tinnimit, 1983). To solve this problem, rice straw has been supplemented with green forage material such as kenaf leaves, cassava leaves, and legumes straw, making it more nutritious for animal consumption (Tawinprawat et al., 1971; Devendra, 1976; Intramongkol et al., 1978; Devendra, 1982). Cowpea (*Vigna unguiculata* L.) and peanut (*Arachis hypogaea* L.) are widely grown in Asia (Barrett, 1990) and Thailand (Sukharomana and Dobkuntod, 2003). The residue of these crops may be used for feeding the ruminant animals all year round.

Although the nutritive value of the supplements in ruminant feed has been extensively studied (Evaldson, 1970; Preston and Leng, 1987; Heuzé et al, 2013), the investigation with respect to the determination of condensed tannin was comparatively limited. Condensed tannin is a polyphenolic secondary metabolite which is found in forage crops and the level of this compound at 3-4% dry weight will be beneficial in determining a nutritional status of the roughage (Robbins et al., 1998; Khanbabaee and van Ree, 2001). At high levels of condensed tannin in forage and fodder, the usefulness of this roughage will be decreased (Mueller-Harvey and McAllan, 1992; Robbins et al., 1998).

This study aimed to determine the values of three types of condensed tannins (soluble, bound, and total condensed tannin) and other nutritional values in legume residues after edible seeds were harvested in cowpea and groundnut in rainy seasons.

Materials and Methods

Plant materials, seed supplying, growing conditions

In this study, the vegetative biomass of four legume species planted in plots was harvested for determination in laboratory. Four legume species included two well-known of tropical forage legume species [1) *Stylosanthes guianensis* CIAT 184 cv. Tha pra (Tha pra stylo); 2) *Centrosema pascuorum* cv. Cavalcade (Cavalcade)] and crop residue of two edible legumes [3) *Vigna unguiculata*, 'KVC7' cultivar (KVC7); 4) *Arachis hypogaea*, 'KKU 60' cultivar (KKU60)]. Forage seeds were bought from the Department of Livestock, Thailand. Two edible legumes were acquired from the researcher of the Faculty of Agriculture, Khon Kaen University, Thailand.

The study was carried out from June to September (rainy season) in 2009, when an average rainfall, temperature, and duration of sunshine were 41.9-127.0 mm, 23.3-35.7°C, and 4.74 h, respectively. These plant species were cultivated at Phetchaburi Animal Nutrition Research Center, Phetchaburi. The plot location has an elevation approximately 0.4 meter above sea level at 12°39′10″ N, 99° 52′ 18″ E.

Growing practice and harvesting

Two seedlings of each legume species were planted per hill at 50 cm between rows and 20 cm between hills in 2 × 5 m^2 plot size. Four blocks were set-up and each block was further divided in four plots for four legume species, in which the plants were randomized in each plot.

The agronomic practice in the field included irrigation with sprinkler throughout the growing seasons, from sowing the seed to the emerging of a true leaf. This was followed by irrigating twice until the plant samples were collected for chemical analysis. For KVC7, it was harvested 55 d after planting. Both Cavalcade and Tha pra stylo were harvested 90 d after planting, while KKU60 was harvested 107 d after planting. All of these plant samples were cut 10 cm above the ground for determination in laboratory. The harvested sample was oven-dried at 60°C for 72 h and subsequently was sieved through a mesh (with 1 mm^2 pore size). The sample was then stored in the bag at 4°C prior to analysis.

Proximate analysis

After harvesting these legume species, the nutritive values were determined. Dry matter (DM), organic matter (OM), and ash contents were calculated according to standardized methods (AOAC, 1995), and crude protein (CP) was measured by the Kjeldahl method (AOAC, 1980). Fiber components, both neutral detergent fiber (NDF) and acid detergent fiber (ADF), were determined by a method reported by Van Soest et al. (1991). Acid detergent residue was treated with 72% H_2SO_4 for acid detergent lignin (ADL).

Extraction of condensed tannin (CT)

The condensed tannin purified from *Stylosanthes guianensis* CIAT 184 cv. Tha pra was used as standard (Berard et al., 2011). Fresh tissue sample of *Stylosanthes guianensis* CIAT 184 cv. Tha pra was extracted with acetone/H_2O (70:30, v/v) containing 0.1% ascorbic acid in which the acetone was subsequently removed by rotary-evaporation, followed by three extractions with methylene chloride to remove all chlorophyll and lipids. The aqueous defatted extracts were then evaporated and solubilized in 1:1 (v/v) methanol/H_2O. The mixture was subsequently purified chromatographically on a Sephadex LH-20 (Jackson, 1996; Berard et al., 2011). Purified CT was rotary-evaporated and stored at -20 °C in the dark until used as standard. The standard solutions of CT were prepared by dissolving 1 mg of previously purified CT in 1 mL of distilled water and serially diluted further four times to obtain five standard solutions (0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL).

Determination of condensed tannin

Soluble condensed tannin (soluble CT) were measured in triplicate for each legumes sample (Terrill et al., 1992; Guimarães-Beelen et al., 2006). In each replication, 50 mg of oven-dried, ground plant material was extracted with a 2.5 mL of 7:3 (v/v) acetone/water containing 0.1% ascorbic acid and 2.5 mL diethyl ether. The mixture was thoroughly mixed and allowed to settle until the aqueous and organic phase was visibly separated. The upper (organic) phase was removed and discarded. The remaining lower (aqueous) phase was adjusted to 5 mL with distilled water. The solution was centrifuged at 3000 × g for 10 min, after which the supernatant was used to determine soluble CT and the residue was subject to bound CT analysis. Either 1 mL of supernatant of the CT or standard solutions of CT in a tube was mixed with 6 mL of butanol-HCl reagent [95% butanol: 5% HCl (36%) (v/v)]. The mixtures were incubated in a water bath at 95 °C for 70 min. When the solution developed to deep-pink color, the tubes were taken out from a water bath and put in the box containing ice. Light absorbance was measured at 550 nm with a spectrophotometer (Biochrom Libra S22, Cambridge, UK). The 6 mL of butanol-HCl reagent mixed with 1 mL of distilled water was used as a blank concentration of CT.

Bound condensed tannin (bound CT) were determined from the residue after extraction of soluble CT by adding 1 mL of distilled water and 6 mL of butanol-HCl reagent in dry residue (Guimarães-Beelen et al., 2006). The mixtures were incubated in a boiling water bath at 95 °C for 70 min. The tubes were subsequently cooled and centrifuged at 3000 × g for 10 min. The supernatant was decanted into vial and absorbance was measured at 550 nm using a spectrophotometer. Concentration of condensed tannin in the legume samples were converted to g/kg DM from the purified *S.guianensis* CT regression equation: $y = 1.5315x + 0.0216$ ($R^2 = 0.9974$). The total condensed tannin (total CT) was obtained by the addition of the soluble fraction and the fraction bound to the residue.

Experimental design

The Randomized Completely Block Design (RCBD) was conducted in this study, with four replicated. Data were analysed by the analysis of variance (ANOVA). Means among treatment were compared with the Duncan's New Multiple Range Test (DMRT).

Results

The results of condensed tannin contents of four legume species, including Tha pra stylo, Cavalcade, KVC7 and KKU60 in rainy reasons were shown in Table 1. Different legume species had varied values of CT contents (total CT, soluble CT, and bound CT) and the percentages of soluble CT and bound CT in total CT in rainy season.

Both soluble CT and bound CT values were highly significant difference among legume species. KKU60 had highest value of soluble CT at 68.9 g/kg DM, but had lower value of bound CT at 1.6 g/kg DM, comparing with other legume species [excepted KVC7 (0.4 g/kg DM) on bound CT] (Table 1). On the other hand, Tha pra stylo had the highest value of bound CT at 12.4 g/kg DM.

For percentage of total CT, there was a highly significant difference among legume species in the soluble (ranged between 38.2-97.8%) and bound fraction (ranged between 2.2-61.8%) in legumes (Table 1).

For soluble CT percentage, KKU60 and KVC7 possessed the highest contents (97.8% and 97.2%, respectively) (Table 1). In contrast, both legumes (KKU60 and KVC7) inherited lower values of bound CT percentage (2.2% and 2.8%, respectively). Tha pra stylo and Cavalcade, having lower values of soluble CT (65.3% and 38.2%, respectively), had higher values of bound CT (34.8% and 61.8%, respectively).

The seven traits of nutritive values of different legume species were shown in Table 2. The result showed highly significant different among legume species on all nutritional values, including DM, OM, ash, NDF, ADF, ADL and CP.

The Cavalcade and Tha pra stylo had values of DM (36.7% and 32.6%, respectively) and OM traits (92.9% and 92.6%, respectively) (Table 2). These legumes (Cavalcade and Tha pra stylo) also had high values in other characteristics, such as NDF(60.1% and 55.7%, respectively) and ADF(41.5% and 40.0%, respectively). KVC7 and KKU60 had values of ash (13.3% and 10.4%, respectively) and CP traits (14.4% and 15.3%, respectively) (Table 2).

Discussion

All values of CT (total, soluble, and bound CT) were different in various legume species (Table 1), indicating the effect of genetic control of the CT concentration characteristic as reported by Terrill et al. (1992). The different in these values provide a platform for utilizing these materials as animal feed for ruminant production.

Thra pra stylo, Cavalcade and KVC7 possessed the total CT value between 5-55 g/kg DM which was reported to have some benefits to the production of ruminant, including preventing bloat (Li et al., 1996), producing plant protein from microbial degradation (Aerts et al., 1999) and improving egg count and ovulation, weight gain, milk and wool yield (Wang et al., 1996; Min et al., 2000; Min and Hart, 2003).

KKU60 had highest value of total CT (at 70.5 g/kg DM) among these legume species. It was thus an appealing material used for feed supplement because the total CT value as reported above 80-90 g/kg DM would affect feed intake and animal production due to the decreased palatability and the digestibility of feed (Kumar, 1983; Kumar and Singh, 1984; Marten and Ehle, 1984; Reed, 1995). High tannin content was reported to induce the

Table 1 Means of chemical values and percent of total condensed tannin of various legumes species

Varieties	Condensed Tannin (g/kg DM)			Percent of Total CT	
	Total CT	Soluble CT	Bound CT	Soluble CT	Bound CT
Stylosanthes guianensis CIAT 184 cv. Tha pra (Tha pra stylo)	35.7 ± 5.7 [b]	23.3 ± 4.0 [b]	12.4 ± 2.1 [a]	65.3 ± 2.6 [c]	34.7 ± 2.6 [b]
Centrosema pascuorum cv. Cavalcade	8.4 ± 2.5 [c]	3.3 ± 1.4 [d]	5.1 ± 1.2 [b]	38.2 ± 4.5 [d]	61.8 ± 4.5 [a]
Vigna unguiculata, 'KVC7' cultivar	13.5 ± 3.9 [c]	13.1 ±3.7 [c]	0.4 ± 0.2 [c]	97.2 ± 1.4 [b]	2.8 ± 1.4 [c]
Arachis hypogaea, 'KKU 60' cultivar	70.5 ± 15.5 [a]	68.9 ± 15.0 [a]	1.6 ± 0.5 [c]	97.8 ± 0.4 [a]	2.2 ± 0.4 [d]
F-test	**	**	**	**	**
CV(%)	17.1	14.5	28.1	0.0	0.0

** Significant at the 0.01 level of probability.

binding and precipitation of protein, carbohydrates and other molecules in ruminants (Barry and Duncan, 1984; Mueller-Harvey and McAllan, 1992).

For KKU60, which possessed highest CT content, had relatively severe damage to its vegetative part as a result of insects and herbivores more than other plant species. Pest infestation was reported to affect CT concentration in plant (Häring et al., 2008; Dixon et al., 2012). The synthesis of CT was postulated to play a role in defending against herbivore attack in plant (Bate-Smith, 1973).

The value of CT was also reported to be affected by plant developmental stage (McMahon et al., 2000). KKU 60 reached maturity stage (at 107 days after planting). KVC7 matured quite early (at 55 days after planting), contributing to its lower CT content. The total CT of KVC7 (at 13.5 g/kg DM) was lower than that of other tropical legume species, such as *Lablab purpureus* (lablab) (at 16.9 g/kg DM) (Mupangwa et al., 2000), *Arachis pintoi, Centrosema* spp. and *Desmodium* spp. (at CT content from 22.2-23.7 g/kg DM) (Jackson et al., 1996).

The CT was reported to interact with proteins, forming both soluble and insoluble (bound) structures (Mupangwa et al., 2000) and tannin-containing feed was reported to use as feed for animal production (Kumar and Singh, 1984; Martin-Tanguy et al., 1977). In this study, Cavalcade had highest CT in the bound fraction (at 61.8% of Total CT). Mupangwa et al. (2000) also reported that bound fraction (especially in protein-bound CT) constituted 56.3% of total CT in tropical herbaceous forage legumes.

Tha pra stylo, KVC7 and KKU60 had soluble CT fraction (between 65.3-97.8% of Total CT).The percentage of soluble CT constituted more than 50 percent in these legumes (Tha pra stylo, KVC7 and KKU60). This result was similar to the study conducted in tropical legumes by Jackson et al. (1996) which reported that soluble CT was at 70-95%. The values of both soluble CT and bound CT were reported to be dependent upon many factors, including the legumes species, stage of maturity, climate, nutrient and the interaction between legume species and growing conditions (Mansfield et al., 1999; Veteli et al., 2007; Naumann et al., 2013).

The ratio between a soluble and a bound CT fraction may affect the biological activity of CT in animal rumen (Naumann et al., 2013) by influencing the amount of dry matter intake and digestion of protein and fiber (Makkar, 2003; Guimarães -Beelen et al., 2006). Bound CT was reported to affect the digestion of fiber in the animal rumen by reducing the activity of hemicellulase and cellulolytic enzymes produced by the ruminant microorganisms (Muhammed et al., 1994). The relatively higher soluble CT in the material means that this plant

Table 2 Percent of some nutritive values include as dry matter (DM), organic matter (OM), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and crude protein (CP) of various legumes species

Varieties	Percent (%)						
	DM	OM	ash	NDF	ADF	ADL	CP
	DM basis						
Stylosanthes guianensis CIAT 184 cv. Tha pra (Tha pra stylo)	32.6 ± 1.6 [b]	92.6 ± 0.9 [a]	7.4 ± 0.9 [c]	55.7 ± 2.7 [b]	40.0 ± 0.1 [b]	9.1 ± 0.4 [b]	11.2 ± 1.4 [b]
Centrosema pascuorum cv. Cavalcade	36.7 ± 1.0 [a]	92.9 ± 0.9 [a]	7.1 ± 0.9 [c]	60.1 ± 1.8 [a]	41.5 ± 0.4 [a]	11.4 ± 0.1 [a]	11.5 ± 1.0 [b]
Vigna unguiculata, 'KVC7' cultivar	24.0 ± 1.6 [d]	86.2 ± 1.5 [c]	13.3 ± 1.3 [a]	33.5 ± 3.1 [d]	20.0 ± 0.4 [c]	4.2 ± 0.5 [c]	14.4 ± 0.5 [a]
Arachis hypogaea, 'KKU 60' cultivar	29.7 ± 1.0 [c]	89.2 ± 0.9 [b]	10.4 ± 0.9 [b]	48.2 ± 2.9 [c]	40.4 ± 0.3 [b]	11.4 ± 0.1 [a]	15.3 ± 0.4 [a]
F-test	**	**	**	**	**	**	**
CV(%)	4.6	1.1	9.2	3.4	0.8	2.2	5.7

** Significant at the 0.01 level of probability.

material should be more readily digestible and the nutrients are more efficiently absorbed in animal rumen (Terrill et al., 1992; Muhammed et al., 1994)

Although Cavalcade and Tha pra stylo had comparatively high value of DM, both species had high fibrous characteristics, such as NDF, ADF and ADL as well (Table 2). The increased values of ADF (representing cellulose and lignin) and NDF (representing fiber) in these plants may make them undesirable to use as supplements in feed for animal production as they may cause reduction in dry matter digestibility (Van Soet, 1994; Amiri and Mohamed Shariff, 2012).

CP was reported to be a good indicator to determine the quality of forage (Amiri and Mohamed Shariff, 2012). For example, high level of CP has been associated with high quality forage (Heitschmidt et al. 1982). The KVC7 and KKU 60 possessed CP content higher than Tha pra stylo and Cavalcade (Table 3). The decreasing CP value corresponded with plant growth from seed emergent to maturation (Griffin and Jung, 1983). Leaf defoliation naturally occurred at the maturation stage of plant development. NDF and lignin values in stem were higher than those in leaves (Griffin and Jung, 1983), contributing to the increased value of NDF and decreased value of CP when the plant sample was collected at the maturation stage. Leaves of KVC7 and KKU60 were retained during growth, attributing to the comparatively higher value of CP comparing with that of Cavalcade and Tha pra stylo.

KVC7 (14.4%) and KKU60 (15.3%) possessed CP content lower than cassava hay (25%). The cassava hay was considered to be a candidate to use for forage instead of rice straw (Intramongkol et al., 1978; Reed et al., 1982; Wanapat et al., 1997). The high level of CT in cassava hay may have a negative effect because this compound was found to bind with NDF (Reed et al., 1982). As a result, both KVC7 and KKU60 which contained soluble CT lower than cassava hay should be recommended for use as they may reduce the degradation of protein in the rumen and enhance the level of by-pass protein.

Conclusions

The values of a total CT of KVC7 and KKU60 were different. In the rainy season, 2009, the percentage of soluble CT was higher than bound CT in Tha pra stylo, KVC7 and KKU60.

KVC7 and KKU60 had CP content higher than Tha pra stylo and Cavalcade. KVC7 and KKU60 had lower fiber but higher CP, comparing with Tha pra stylo and Cavalcade. The vegetative parts of KVC7 and KKU60 were recommended to use as feed for the ruminants.

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