African Journal of Basic & Applied Sciences 5 (6): 261-267, 2013
ISSN 2079-2034

DOI: 10.5829/idosi.ajbas.2013.5.6.13462

Effects of Species, Storage Conditions and Dosage of Extraction Solvents on Condensed Tannin Concentration

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Abstract: The study was carried out to evaluate the effects of leaves tropical tannin rich fodder tree species, storage conditions and extraction solvents on condensed tannin concentrations (CT). The studied species were *Albizia gummifera, Carissa edulis, Ficus ovata, Maytenus obscura* and *Rhus glutinosa*. Non-dried fresh leaf extracts, dried and ground as well as dried and ground leaves preserved for 1.5 years at room temperatures (avg. 20°C) were used. Aqueous acetone and ethanol, each having 50 and 70% v/v dose, were incorporated for the extraction of CT. Interspecies variations were significant (P<0.001) for CT concentration across the storage conditions. The lowest CT value (P<0.001) was determined for the fresh leaf extracts of all species compared to other storage conditions. Forages containing CT could offer a nutritionally and ecologically sustainable system for feeding livestock.

Key words: Acetone · Ethanol · Tannin · Tannin rich plants

INTRODUCTION

Curently smallholder farmers are increasingly relying on various multipurpose fodder trees and shrubs (MPTS) to supplement their herbivore livestock [1-2]. Multipurpose fodder trees and shrubs (MPTS) are underutilized potential feed resources rich in protiens and other essential nutrients [3]. However, browse plants often contain bioactive secondary plant metabolites such as condensed tannins (CTs), which indicate that CTs commonly found in MPTS can affect intake, growth, digestibility, onset of puberty and reproductive functions via direct toxicity, interference in the metabolic process, reduction of nutrient availability or a combination of these pathways [4, 5], but also represent potential in terms of anthelmintics [6].

There are several factors that can influence the extraction efficiency of tannins from tanniferous feed resources, including extraction method, solvent type and dosage, particle size of plant materials, extraction time and temperature, solvent to solid ratio and extraction pH [7]. Still now different laboratories in different countries are using a number of organic solvents for extracting tannins from various feed resources. There is no official procedure developed by Association of Official Analytical chemists (AOAC) for quantification of tannins. The study was planned to evaluate the effects of tannin rich tree leaves, storage conditions, methods and doses of extraction solvents on concentration of extractable condensed tannins

MATERIALS AND METHODS

Study Area: The quantification of extractable CTs was carried out at Jimma University in animal nutrition laboratory, south western Ethiopia, located at 7°40'N and 36°50'E. Jimma is situated at an average altitude of 1780 m (5,840 ft).

Collection of Plant Materials and Tannin Extraction Protocol: The plant species such as *Albizia gummifera, Carissa edulis, Ficus ovata, Maytenus obscura* and *Rhus glutinosa* were collected from their natural habitat, Omo Nada district of Jimma zone southwestern Ethiopia. The fresh leaf extracts, leaves dried at 55°C and ground immediately as well as the dried (55°C) and ground leaf

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samples that were preserved for 1.5 years at room temperature (avg. 20°C) were used for this particular study. The fresh leaf samples were transported to the laboratory in fresh state with a plastic bag kept on ice and transported under dark conditions. Immediately after arriving to the laboratory the fresh leaf samples chopped and sieved with 1mm whereas the non-chopped leaf samples were dried to constant weight at 55°C and ground to 1mm screen. Fresh, dried and finely ground leaf material (200 mg) was taken in a glass beaker of approximately 25 ml capacity. For each samples 10 ml of aqueous ethanol (50%) was added and the beaker was suspended in an ultrasonic water bath and subjected to ultrasonic treatment for 20 min at room temperature. Then, the content of the beaker was transferred to centrifuge tubes, cooling by keep on ice and subject to centrifugation for 15 minute at 2000 rpm. The extractives were stored at 4°C until used. The same procedure was applied on an aqueous ethanol, (70%) and for aqueous acetone (50%, 70%). The complete extraction process was followed FAO/IAEA [8].

Quantification of Extractable Condensed Tannins: The determination of extractable condensed tannin (CT) concentration was based on the oxidative depolymerization of plant extracts in butanol-HCl reagent using 2% ferric ammonium sulfate catalyst in 2N HCl [9].

Statistical Analysis: A variance analysis model with four fixed factors was used following general linear model (GLM) procedure of statistical analysis system [10]: plant species (*P*; 5 levels), storage conditions (*S*; 3 levels), extraction solvents (*M*; 2 levels) and dosage of extraction solvents (*D*; 2 levels) in 5 × 3 × 2 × 2 factorial arrangement. The GLM model used was:

$$Y_{ijkl} = \mu + P_i + S_j + M_k + D_l + (P\times S)_{ij} + (P\times M)_{ik} + (P\times D)_{il} + (S\times M)_{jk} + (S\times D)_{jl} + (M\times D)_{kl} + (P\times S\times M\text{ÍD})_{ijkl} + \epsilon_{ijkl}$$

Where: Y_{ijkl}, total observation; μ, population mean; P_i, ith plant species effect; S_j, jth effect of storage condition; M_k, k^{th} extraction solvents; D_l, l^{th} effect of dosage of extraction solvents; $(P\times S)_{ij}$, interaction effect between plant species and storage conditions; $(P\times M)_{ik}$, interaction effect between plant species and extraction solvents; $(P\times D)_{il}$, interaction effect between plant species and dosage of extraction solvents; $(S\times M)_{jk}$, interaction effect between storage condition and extraction solvents; $(S\times D)_{jl}$, interaction effect between storage condition and dosage of extraction solvents; $(M\times D)_{kl}$, interaction effect between extraction solvents and their dosages; $(P\times S\times M\times D)_{ijkl}$, interaction effect of all fixed factors and e_{ijkl}, residual error. The overall interactions were used to interpret the analyses. Differences between means were tested using least significance difference with significances declared at $P < 0.05$.

RESULTS AND DISCUSSIONS

The Effects of Storage Conditions and Dosages of Acetone on Condensed Tannin Concentrations: The concentration of CT obtained for 50% acetone extraction in *A. gummifera* and *M. obscura* leaves showed similar value at fresh condition (6%); however, the CT values were decreased (P<0.001) from dried to dried-preserved conditions (18.8 to 15.9% in *A. gummifera* and 18.6 to 13.9% in *M. obscura*) (Table 1). On the contrary, statistically significant difference was not observed (P>0.001) for CT value in the leaves of *C. edulis* and *R. glutinosa* at dried and dried-preserved conditions. The CT concentration of *A. gummifera* and *F. ovata* leaves showed nearly similar value at fresh state (5%) (P>0.001). On the contrary, CT value was increased from dried (12.8 and 9.5%) to dry-preserved condition (19 and 13.6%), respectively (Table 1, *see acetone 70% extraction*). Similar to 50% acetone extraction, fresh leaves had the smallest CT concentration (P<0.001) across the plant species compared to the dried leaves.

The increasing trends of CT concentration as advances in storage time and drying conditions might be indication of formation of a new molecular bond between CTs and new chemical substance produced during heating and storage time. Variations in CT concentration athwart plants and storage conditions might be associated to differences in the length of storage as well as differences in plant species. The highest value of CT both in dried and dried-preserved conditions across plant species compared to fresh plant material might be due to oxidation and/or polymerization of CTs with other chemical constituents during drying and length of storage after drying. According to Ferreira *et al* [11], tannin quantification has to be done immediately after harvesting crops to avoid formation of protein complexes or polymerization. Lin *et al* [12] also reported rapid loss of total phenolics and extractable CT from pericarps of tanniferous plants during dry storage. According to Lin *et al* [12], during the dry storage condition most of CT of hypocotyls formed c omplexes with multiple phenolic hydroxyl groups and may form complexes with proteins, metal ions, amino acids and polysaccharides.

Table 1: Least square means of ct determined for different plant species extracted by acetone 50 and 70% at different storage conditions

Plant species	Extraction Solvent	Storage condition	CT	SE	*P*-value
Albizia gummifera	Acetone 50%	F	6.0^{c}	0.52	<0.001
		D	18.8^{a}		
		DP	15.9^{b}		
Carissa edulis		F	5.1^{b}	0.71	<0.001
		D	15.1^{a}		
		DP	15.4^{a}		
Ficus ovata		F	6.0^{c}		<0.001
		D	12.0^{b}	0.78	
		DP	16.3^{a}		
Maytenus obscura		F	6.0^{c}		<0.001
		D	18.6^{a}	0.67	
		DP	13.9^{b}		
Rhus glutinosa		F	6.8^{b}		<0.001
		D	16.0^{a}	0.35	
		DP	16.6^{a}		
Albizia gummifera		F	4.9^{c}		
		D	12.8^{b}	0.66	<0.001
		DP	19.0^{a}		
Carissa edulis	Acetone 70%	F	5.8^{b}		
		D	9.1^{ab}	0.61	<0.001
		DP	12^{a}		
Ficus ovata		F	4.6^{c}		
		D	9.5^{b}	0.61	<0.001
		DP	13.6^{a}		
Maytenus obscura		F	6.8^{b}		
		D	15.3^{a}	1.57	<0.001
		DP	13.6^{a}		
Rhus glutinosa		F	5.6^{b}		
		D	18.5^{a}	0.60	<0.001
		DP	19.6^{a}		

F, fresh; D, dried to constant weight at 55°C; dried to constant weight at 55°C and preserved for 1.5 years at room temperature; CT, extractable condensed tannin; SE, standard error of means; ***P<0.001

Boudhrioua *et al* [13] also has reported polymerisation effects of drying tanniferous feedstuffs on the concentration of CT.

The Effects of Storage Conditions and Dosages of Ethanol on Condensed Tannin Concentrations: The effect of plant species and storage conditions on the concentration of CTs extracted with 50% and 70% ethanol is presented in Table 2. Similar to acetone 50 and 70% extraction, the least values of CT (P<0.001) was determined for the fresh plant leaf samples compared to CT values determined both in dried and dried-preserved conditions. These differences could be associated with differences in DM content of plant species. Haslam [14] reported the chemical complexity and heterogeneity of plant tannins. Kelman and Tanner [15], Ayres *et al*. [16] and Ozturk *et al.* [17] also reported major difference in tannin structure between plant species. On the contrary to acetone extraction (Table 1), significant difference (P>0.001) was not observed between dried and dried-preserved samples with ethanol 50% extraction This variation might be due to differences in the tannin removing ability of the solvents from the same plant species. Makkar [7], Liyana-Patthirana and Shahidi [18] and Nobre *et al*. [19] reported different tannin removing abilities of organic solvents such as acetone, methanol and ethanol that would contribute in influencing the rate of extraction and quality of extracted bioactive phenolic compounds. On top of plant species variation, the present study also investigated the influences of storage condition on concentration of CTs. According to Ferreira *et al*. [20], different storage times showed significant differences for phenolic substances, indicating that tannin quantification should be done immediately after harvesting plants to avoid formation of protein complexes or polymerization which leaded to exaggerated results. Mueller - Harvey [21] reported the wide range of different tannin structures between plant species, varieties and even within plant parts. Makkar and

Table 2: Least square means of CT determined for different plant species extracted by ethanol 50 and 70% at different storage condition

Plant species	Extraction solvent	Storage condition	CT	SE	*P-value*
Albizia gummnifera	Ethanol 50%	F	5.6[b]	1.1	<0.001
		D	15.5[a]		
		DP	16.0[a]		
Carissa edulis		F	6.9[b]	0.90	<0.001
		D	12.8[a]		
		DP	14.9[a]		
Ficus ovata		F	6.0[b]	0.90	<0.001
		D	13.0[a]		
		DP	13.4[a]		
Maytenus obscura		F	7.2[b]	1.2	<0.001
		D	16.0[a]		
		DP	16.2[a]		
Rhus glutinosa		F	7.2[b]	0.34	<0.001
		D	15.3[a]		
		DP	15.3[a]		
Albizia gummnifera		F	2.5[c]	0.51	<0.001
		D	10.2[a]		
		DP	8.2[b]		
Carissa edulis	Ethanol 70%	F	4.2[c]	1.47	<0.001
		D	14.0[a]		
		DP	7.8[b]		
Ficus ovata		F	3.2[c]	0.34	<0.001
		D	9.3[a]		
		DP	6.5[b]		
Maytenus obscura		F	5.8[b]	1.0	<0.001
		D	11.8[a]		
		DP	11.7[a]		
Rhus glutinosa		F	5.1[b]	0.48	<0.001
		D	8.1[a]		
		DP	6.5[ab]		

F, fresh; D, dried to constant weight at 55°C; dried to constant weight at 55°C and preserved for 1.5 years; CT, extractable condensed tannin; SE, standard error of means; ***P<0.001

Singh [22-23] also confirmed the relative degree of polymerization of tannin in stored leaves. Makkar [7] reported absences of decreasing tannin inactivation either by steaming or autoclaving plant leaves.

Ethanol 70% extracted more condensed tannin than 50% (P<0.01). *A. gummifera, C. edulis* and *F. ovata* leaves had a highly significant difference (*P*<0.001) for CT concentration in each storage conditions. The CT concentration didn't vary (*P*>0.001) both in dried and dried-preserved storage conditions for *M. obscura* and *R. glutinosa* leaves. The smallest concentration of CT in fresh leaf extracts compared to the dried and dried-preserved storage conditions might be due to variation in plant species as well as the dry matter content of the plant species. The high concentration of tannins in dried and dry preserved plants compared to fresh plants indicated the presence of polymerization of tannins with by-products following heating feedstuffs. Also tannin concentration varies with variations in plant species and extraction solvents, the literature sources cited for the previous table (Table 1) can also be considered for comparison of the results indicated in the Table 2.

The Interaction Effects of Plant Species, Dosages of Extraction Solvents and Storage Conditions on CT Concentrations: The combined effect of plant species and storage time on CT concentration compared for doses of extraction solvents is presented in Table 3. For each extraction solvent, the interspecies as well as variations in storage time had a significant effect (*P*<0.001) on CT concentration. For each plant species and extraction solvent, the least concentration of CT (*P*<0.001) was observed in fresh leaf samples compared to the CT values recorded in other storage conditions. In general, it was observed that the concentration of CT in fresh leaves for all plants across the extraction solvents was not more that 7.2%. Further, the CT content was found to be the highest (18.8%) in *A. gummifera* that was extracted with acetone

Table 3: Least square means for the effects of plant species, extraction solvents and storage time on concentration of extractable CT

	Albizia Gummifera			*Carissa Edulis*			*Ficus Ovata*			*Maytenus obscura*			*Rhus glutinosa*				
ES	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	SE	*P*
A 50%	6^{j}	18.8^{a}	15.9^{e}	5.1^{k}	15.5^{f}	15.4^{f}	6.0^{j}	12.0^{h}	16.3^{d}	6.0^{j}	18.6^{b}	13.9^{g}	6.8^{i}	16.0^{e}	16.6^{c}	0.05	***
A 70%	4.9^{n}	12.8^{f}	19.0^{b}	5.8^{k}	9.1^{i}	12.0^{f}	4.6^{n}	9.5^{h}	13.6^{e}	6.7^{j}	15.3^{d}	13.6^{e}	5.6^{l}	19.6^{a}	18.5^{c}	0.05	***
E 50%	5.5^{k}	15.5^{c}	16.0^{b}	6.9^{i}	12.8^{g}	14.9^{e}	6.0^{j}	12.9^{g}	13.4^{f}	7.2^{h}	16.0^{b}	16.2^{a}	7.2^{h}	15.3^{d}	15.3^{d}	0.05	***
E 70%	2.4^{m}	10.2^{c}	8.2^{e}	4.2^{k}	14.0^{a}	7.8^{g}	3.2^{l}	9.3^{d}	6.5^{h}	5.8^{j}	11.9^{b}	11.9^{b}	5.9^{i}	8.1^{f}	6.5^{h}	0.05	***

F, fresh leaves; FD, dried; DP, dried-preserved; ES. extraction solvent; ST, storage time; A, acetone; E, ethanol; SE, standard error of mean;***P<0.001

Table 4: Least square means for the combined effects of plant species and extraction solvents on concentration of extractable CT compared for each storage type

	Albizia Gummifera			*Carissa Edulis*			*Ficus Ovata*			*Maytenus obscura*			*Rhus glutinosa*		
ES	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP	F	FD	DP
A50%	6^{a}	18.8^{a}	15.9^{b}	5.1^{c}	15.5^{a}	15.4^{a}	6.0^{a}	12.0^{b}	16.3^{a}	6.0^{c}	18.6^{a}	13.9^{b}	6.8^{b}	16.0^{b}	16.6^{b}
A70%	4.9^{c}	12.8^{c}	19.0^{a}	5.8^{b}	9.1^{d}	12.0^{c}	4.6^{b}	9.5^{c}	13.6^{b}	6.7^{b}	15.3^{c}	13.6^{c}	5.6^{d}	19.6^{a}	18.5^{a}
E50%	5.5^{b}	15.5^{b}	16.0^{b}	6.9^{a}	12.8^{c}	14.9^{c}	6.0^{a}	12.9^{a}	13.4^{c}	7.2^{a}	16.0^{b}	16.2^{a}	7.2^{a}	15.3^{c}	15.3^{c}
E70%	2.4^{c}	10.2^{d}	8.2^{c}	4.2^{d}	14.0^{b}	7.8^{d}	3.2^{c}	9.3^{d}	6.5^{d}	5.8^{d}	11.9^{d}	11.9^{d}	5.9^{c}	8.1^{d}	6.5^{d}
SE	0.10	0.04	0.08	0.05	0.03	0.05	0.04	0.05	0.06	0.05	0.05	0.05	0.02	0.05	0.07
P	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***

F,fresh leaves; FD, fresh dried; DP, dried & preserved; PS, plant species; ES, extraction solvents; ST, storage time; A, acetone; E, ethanol; SE, standard error of mean;***P<0.001

50% at dried condition whereas the lowest CT value (5.1%) was observed in *C. edulis* at fresh leaf samples (*P*<0.001). In contrast with 70% acetone extraction, the highest and lowest values of CT were recorded in *R. glutinosa* (19.6% at fresh dried condition) and *A. gummifera* (4.9%) or *F. ovata* (4.6%) leaves determined in fresh condition, respectively (*P*<0.001). The dried and preserved leaves of *M. obscura* had the highest CT concentration (16.2%) with ethanol 50% extraction compared to the rest of plants in various storage conditions. On the contrary, among the plant species included in this study, the CT concentration in fresh leaves of *A. gummifera* extracted both with ethanol 50 and 70% levels showed the least value (5.5 and 2.4% CT, respectively) (*P*<0.001) compared to the rest of plant species and storage conditions.

The combined effects of plant species and extraction solvents on concentration of CT compared for each storage condition is presented in Table 4. For each storage condition, differences in plant species and extraction solvents had a significant variation (*P*<0.001) on the concentration of CT. The tannin extracting ability of different doses of acetone and ethanol varied with variations in plant species and storage condition (*P*<0.001). Ethanol 50% showed the better ability of extracting CT in fresh samples than the rest of extraction solvents (*P*<0.001). In general, it was observed that the concentration of CT extracted by 70% ethanol showed the least value for all plants and for all storage conditions. This might be due to differences in the biological activity of the plant species, the molecular mass and the type of phenolic compounds of the solvent. According to Downey and Hanlin [24], acetone extracts more CT than ethanol from tanniferous grape skin; however, in the present study we observed variations in tannin extracting capacity of acetone and ethanol which have been tending to vary with storage time and plant species. Shu-Dong *et al.* [25] reported that the acetone-water (1:1, v/v) was more effective solvent for extracting total phenolics and extractable CTs from *Machilus pauhoi* leaves than methanol, ethanol, acetone, water, methanol water (1:1, v/v) and ethanol-water (1:1, v/v). However, in the present study the tannin extracting ability of the various solvents varied with variations in the storage conditions and plant species.

CONCLUSION

The high concentration of CTs in dried and dry preserved plant leaves compared to fresh plants indicated the occurrence of oxidation and/or polymerization of tannins due to drying of the feedstuffs. In addition, tannin concentration varied with variations in doses of extraction solvents. As it might be known that the toxic or antinutritional effects tend to occur in times of stress when a very large proportion of the diet is tanniferous. With a better understanding of tannin properties and proper management, they could become an invaluable source of protein for strategic supplementation.

ACKNOWLEDGEMENTS

Authors wish to thank the VLIR-UOS Institutional University Cooperation project (IUC-JU) for the financial support.

REFERENCES

1. Arigbede, O.M., U.Y Anele, K.H. Südekum, J. Hummel, A.O. Oni, J.A. Olanite and A.O. Isah, 2012. Effects of species and season on chemical composition and ruminal crude protein and organic matter degradability of some multi-purpose tree species by West African dwarf rams. J. Anim. Physiol. Anim. Nutr., 96(2): 250-259.
2. Yisehak, K., A. Becker, J.M. Rothman, E.S. Dierenfeld, B. Marescau, G. Bosch, W. Hendriks and G.P.J. Janssens, 2012. Aminoacidic profile of salivary proteins and plasmatic trace mineral response to dietary condensed tannins in free-ranging zebu cattle (*Bos indicus*) as a marker of habitat degradation. Livestock Science, 144(3): 275-280.
3. Yisehak, K. and G.P.J. Janssens, 2013. Evaluation of nutritive value of leaves of tropical tanniferous trees and shrubs. Livestock Research for Rural Development (25)28. http://www.lrrd. org/ lrrd 25/02/ yise 25028.htm
4. Kaitho, R.J., 1997. Nutritive value of browses as protein supplement(s) to poor quality roughages. PhD Theses, Department of Animal Nutrition, Wageningen Agricultural University, Wageningen, The Netherlands.
5. Norton, B.W., 2000.The significance of tannins in tropical agriculture. In: Brooker, J.O. (Ed). Tannins in Livestock and Human Nutrition. Proceedings of an International Work shop, Adelaide, Australia, May 31-June 2, 1999. Canberra. ACIAR (Australian Centre for International Agricultural Research) Proceedings, 92: 14-23.
6. Athanasiadou, S., I. Kyriazakis, F. Jackson and R.L. Coop, 2000. Consequences of long term feeding with condensed tannins on sheep parasitized with *Trichostrongylus colubriformes*. J. Parasitol., 30: 1025-33.
7. Makkar, H.P.S., 2003. Effects and fate tannins in ruminant animals, adaptation to tannins and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Ruminant Research, 49: 241-256.
8. FAO/IAEA Working Document. 2000. Quantification of Tannins in Tree Forliag IAEA, VIENNA.
9. Porter, L.J., L.N. Hrstich and B.G. Chan, 1988. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry, 25: 223-230.
10. SAS (statistical analysis system), 2010. Statistical analysis system (SAS/STAT program, version 9.3). SAS institute Inc, carry, NC, USA.
11. Ferreira, D., J.P.J. Marais and D. Slade, 2003. Phytochemistry of the mopane, Colophosperum mopane. Phytochemistry, 64: 31-51.
12. Lin, Y.M., J.W. Liu, P. Xiang, P. Lin, G.F. Ye and L.D.S.L. Sternberg, 2006. Tannin dynamics of propagules and leaves of *Kandelia candel* and *Bruguiera gymnorrhiza* in the Jiulong River Estuary, Fujian, China. Biogeochemistry, 78: 343-359.
13. Boudhrioua, N., N. Bahloul, I. Ben Slimen and N. Kechaou, 2009. Comparison on the total phenol contents and the color of fresh and infrared dried olive leaves. Journal of Industrial crops and Products, 29: 412-419.
14. Haslam, E., 1998. Practical polyphenolics: From structure to molecular recognition & physiological action. Cambridge University Press, Cambridge, UK.
15. Kelman, W.M. and G.J. Tanner, 1990. Foliar condensed tannin levels in lotus speces growing on limed and unlimed soils in south Eastern New Zealand Association, 52: 51-54.
16. Ayres, M.P., M.P. Clausen, E.F. McLean, Jr. A.M. Redman and P.B. Reichardt, 1997. Diversity of structure and antihervibore activity in condensed tannins. Ecology, 78: 1696-1712.
17. Ozturk, D., C.O. Ozkan, A.I. Atalay and A. Kamalak, 2006. The effect of species and site on the condensed tannin content of shrub and tree leaves. Res. J. Anim. Vet. Adv., 1: 41-44.
18. Liyana-Pathirana, C. and F. Shahidi, 2005. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chemistry, 93(1): 47-56.
19. Nobre, C.P., F.N. Raffin and T.F. Moura, 2005. Standardization of extracts from Momordica charantia L. (Cucurbitaceae) by total flavonoids content determination. Acta Farm. Bonaerense, 24(4): 526-566.
20. Ferreira, E.C., A. Rita, A. Nogueira, G.B. Souza and L.A.R. Batista, 2004. Effect of drying method and length of storage on tannin and total phenol concentrations in Pigeon pea seeds. Food Chemistry, 86: 17.
21. Mueller-Harvey, I., 2006. Unraveling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric., 86: 2010-2037.

22. Makkar, H.P.S. and B. Singh, 1991. Distribution of condensed tannins (proanthocyanidins) in various fraction of young and mature leaves of some oak species. Animal Feed Science and Technology, 32: 253-260.
23. Makkar, H.P.S. and B. Singh, 1993. Effects of storage and urea addition on detannification and *in sacco* dry matter digestibility of mature oak (*Quercus incana*) leaves. Animal Feed Science and Technology, 41: 247-259.
24. Downey, M.O. and R.L. Hanlin, 2010. Comparison of Ethanol and Acetone Mixtures for Extraction of Condensed Tannin from Grape Skin. Department of Primary Industries Victoria, PO Box 905, Mildura, VIC 3502, Australia. major trichostrongylid parasites of sheep. Veterinary Parasitology, 142: 1-15.
25. Shu-Dong, Wei1, Rui-Yan Chen, Meng-Meng Liao, Nian-Wan Tu, Hai-Chao Zhou and Yi-Ming Lin 2011. Antioxidant condensed tannins from *Machilus pauhoi* leaves. Journal of Medicinal Plants Research 5(5): 796-804, 4 March, 2011. http://www. academicjournals. org/JMPR