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Qualitative Assessment of Secondary Metabolites and In vitro Gas Production of Ficus species

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Abstract

Five different species of Ficus leaves (FL) were assessed for their chemical compositions and presence of secondary metabolites. Residues from the extracted and unextracted FL were further subjected to in vitro gas production to evaluate the effect of secondary metabolites on gas production, Metabolisable Energy (ME), Organic Matter Digestibility (OMD), Short Chain Fatty Acids (SCFA) and Methane (CH_4). Crude protein for the unextracted FL ranged from 9.41% (*F. benjamina*) to 14.4% *(F. abutilifolia)* while the extracted FL varied from 10.8 to 15.8%. All the FL contained condensed tannin and saponin. Removal of secondary metabolites enhanced fermentation in extracted FL as against the unextracted FL as depicted on gas volume. The estimated values of ME, OMD, SCFA and CH_4 of all the extracted FL were not significantly different ($p>0.05$). Significant ($p<0.05$) differences were observed among the values of unextracted FL for ME, OMD and SCFA. Highest values was observed in unextracted *F.polita* (3.66MJKg/DM, 26.18% and 0.12µmol, respectively) but lowest in *F.vogeli* (3.00MJ/Kg DM, 21.58% and 0.03µmol, respectively).The results from this study suggest that Ficus leaves has the potential to serve as feed resource of by-pass protein and for control of methanogens in small ruminants

Keywords: Ficus species; Chemical composition; Secondary metabolites; in vitro gas; extracted and unextracted Ficus leaves.

Introduction

The improvement of animal and feed supplies is a panacea to low livestock productivity, particularly that of ruminants is a major challenge in Nigeria. In the tropics, ruminants obtain the bulk of their feed from herbage growing on unimproved native pasture. The availability and quality of these forages depends on season which often affect preference and growth performance of grazing animals (Mellado *et al*., 2006).To overcome the challenges of seasonal variation in feed supply, poor quality grasses, roughages and crop residues, smallholder farmers embrace the utilization of leaves of tree fodders commonly referred to as browse plants to feed their livestock, especially, small ruminants, purposely to supply protein. These protein sources are digested in the rumen to provide ammonia and amino acids for microbial protein synthesis and more importantly during the dry season periods when low quality roughages abound.

Browse plants play a major role as feed for ruminants especially in arid and semi-arid region (Kibon and Orskov, 1993) because of their resistance to drought, extreme heat, drifting sand, salinity, grazing and repeated cutting (Fagg and Stewart, 1994). Their tender shoots, twigs, leaves and sometimes fruits and pods are good forage resources which grazing or browsing animals can utilize without competition from monogastrics and man.

In Nigeria, Ficus plants have become well recognized by many households as ornamental trees commonly used as shade

trees and row hedges. They are evergreen trees and sometimes utilized for live fencing but their leaves are rarely harvested by small holder farmers to feed their animals during the dry season periods when most grassland pasture are deficient in nutrients. Ficus species belong to the family Moraceae, and are often distinguished by their characteristic root growing from the branches (Keay *et al.,*1964). Ficus have increased level of acceptability among sheep and goats, and feeding Ficus-Panicum maximum mixtures have been reported to improve their weight gain, dry matter and organic matter intake (Bamikole *et al.*, 2004). However, as a multipurpose tree fodder, the presence of phenolic compounds in the foliage could also present constraints to livestock performance and nutritive value of forages vis-à-vis, inactivation of some nutrients, interference with digestive process or metabolic utilization of feed, availability and utilization of nutrient in the animal body which exert effects contrary to optimum nutrition (Norton, 2003; Adesogan *et al.*, 2006).

The in vitro gas production method is a tool widely used for quick assessment of nutritional values and potential deleterious activity of anti-nutritional compound present in feed (Getachew *et al*.,1998) and also remains one of the reliable means to measure the quality of feeds (Fievez et al.,2005). In view of the above revelations and since the inclusion of Ficus plants as feed supplement for ruminants is becoming acceptable, this study was carried out to qualitatively determine their secondary metabolites, chemical composition and in vitro gas production to ascertain their potential as feed resources in Ruminant nutrition.

Materials and Methods

Leave Collection

Leaves were harvested from mature Ficus trees namely, *Ficus abutilifolia, Ficus vogeli, Ficus polita, Ficus thornningii* and *Ficus benjamina*, all within the University of Ibadan campus, Nigeria, which is located at 7°27'N and 3°45'E with an altitude of 200 - 300 m above sea level and average annual rainfall of about 1250mm, mean temperature of 25-29°C and average humidity of about 84% during the rainy season and 76.6% during the dry season. Leaves were harvested at about twelve weeks old from different locations and ensuring that both mature and young leaves were plucked for the study. Identification and authentication of the species was carried out at the Department of Forest Resources and Management (University of Ibadan) and Forestry Research Institute of Nigeria (FRIN), Ibadan.

Chemical Analysis

Samples were dried to constant weight in an oven at 65°C for dry matter determination. Dried samples were ground in a Restch mill to pass a 1mm sieve and stored in airtight bottle for subsequent determination of chemical composition, secondary metabolites (Saponin, Phenols and Steriods) and in vitro gas production. Crude protein, ether extract, and ash were determined according to AOAC (1995). Neutral Detergent Fibre (NDF), Acid detergent fibre (ADF) and Acid Detergent Lignin (ADL) were also determined (Van Soest et al., 1991).

Qualitative determination of saponin, phenols and steroids

Saponin, phenols and steroids were determined as described (Babayemi *et al*.

,2004). 2g of each milled sample (*F. abutilifolia, F.vogeli, F. polita, F. thornningii* and *F. benjamina)* were extracted with 30ml petroleum ether (PE) and 25ml methanol-water (MW, 9/1, v/v). The mixture was agitated at 250 revolution per minute for 90 minutes, filtered and separated using a funnel. The lower methanol-water (MW) and upper petroleum ether (PE) layers were emptied into 50ml volumetric flask each. From the MW fraction, 1.67ml was dispensed into 9ml distilled water. 1ml of it was pipette into caliberated test tubes. The test tube was agitated for 30 seconds and left to stand for 15 minutes. Saponin content was evaluated from the height of the foam layer as described (Babayemi *et al.*, 2004).Negative (<5mm), low (5-9mm), medium (10-14mm) and high (>15mm). For determination of phenols, 1m from the MW fraction was dispensed into 5 bottles with 1% Fe_2Cl_3 (w/v) added at different levels (0.2, 0.4, 0.6, 0.8 and 1ml) respectively. Phenols form complexes with ferric iron, resulting in a blue solution. A characteristic colour change or otherwise which indicate the presence of phenols was scored as; No phenol (No colour change), hydrolysable (dark blue) and condensed tannin (dark green). For steroids, 10mls of PE fraction was evaporated in a water bath at 45^0C after which 0.5ml chloroform, 0.25ml acetic anhydride and 0.125ml conc. H_2SO_4 were added. The mixture was agitated briefly and colour reaction was inferred as being steroids (blue or green) triterpenoids (Red, pink or purple) or saturated steroids (light green).

In vitro gas production

Rumen fluid was obtained from three West African Dwarf goats by means of stomach suction tube thrust directly into the rumen compartment via the oesophagus of the animals before the morning feed. Animals were fed Albiziasaman pod, *Gliricidias epium* foliage, *Panicum maximum* and water ad-libitum. Incubation was carried out as described (Menke and Steingass, 1988) using 100ml calibrated syringes in three batches at 39^oC.

200mg milled feed samples (extracted and unextracted Ficus species) were put in each syringe, and 30ml buffered rumen liquor was dispensed into each syringe. Three syringes containing 30ml inoculum served as blanks. All handling of buffered rumen liquor was under continuous flow with CO_2. Gas production was measured at 6, 12, 18, 24, 30 and 36 hours post incubation. After 36 hour post incubation, 4ml of NaOH (10M) was introduced to estimate the amount of methane (CH_4) produced. Average volume of gas produced from the blanks was deducted from the volume of gas produced per sample.

The volume of gas produced was plotted against the incubation time. Rates and extent of gas production were estimated for each substrate from the linear equation $Y = a+b (I – e^{-ct})$ described by Orskov and McDonald (1979).

Where; Y = Volume of gas produced at time; t

a = intercept (gas produced from the soluble fraction)

b= Potential gas production from the insoluble fraction

c= Gas production rate constant for the insoluble fraction (b)

t = Incubation time

The Metabolisable Energy (ME, MJ/kg DM) and Organic Matter Digestibility

(OMD%) were estimated from the volume (ml) of gas produced at 36 hours post incubation and the proportion of crude protein (CPg/100g DM) as established (Menke and Steingass,1988) and Short Chain Fatty Acids (SCFA) was calculated as reported (1999):ME (MJ/kg DM)

= 2.20+0.136GV+0.057CP

OMD(%

= 14.88+0.889GV+0.45CP+ 0.0651XA

SCFA (mmol) = - 0.00425 + 0.0222*GV

Where;
GV = Net gas production (ml/200mg DM)
CP = Crude protein
XA = Ash (%)

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using statistical analysis model (SAS, 1988) and means where significant were separated using Duncan's Multiple Range Test.

Results and Discussion

The proximate composition of the *Ficus species* (unextracted) and crude protein and ash of the extracted *Ficus specie* as represented in Tables 1 and 2.Values of dry matter in this study was highest in *F. benjamina* (32.3%) and lowest in *F. abutilifolia* (26.8%). The range (26.8 to 32.3% DM) obtained in this study was higher than those reported for *F. mucoso* and *F. religiosa* (Bamikole *et al.,* 2004). The crude protein content in the leaves ranged from 9.41% (*F. benjamina*) to 14.4% (*F.abutilifolia*), and falls within the range (10 to 15%) for some multipurpose trees in Nigeria (Arigbede *et al.,* 2004) which suggests a possibility of an increased intake when fed to ruminants. CP content in both extracted and unextracted *Ficus* leaves were higher than the critical level of 7.0g/100g DM below which feed intake of ruminant is depressed(Minson,1990). The CP values in this study were also adequate and above 8% recommended for ruminant's body maintenance and below which feeds will not provide the required levels of ammonia for optimum rumen microbial activity (Norton,2003).Neutral detergent fibre ranged between 49.5% in *F. polita* and 65.5% in *F.benjamina*, which implies that mean value (57.5g/100 DM) of NDF in this study was lower than the reported values for *Ficus religiosa* (Bamikole *et al.,*2004) but was within the range of 55-60g/100g DM that could increase feed intake (Meissner *et al.,*1991). Acid detergent fibre (ADF) ranged between 46.5% in *F. polita* and 61.0% in *F. vogeli.* However, the mean percentages of ADF (52.1g/100g DM) obtained were higher when compared to high quality roughages, which ruminants can effectively digest. The crude protein of the extracted *Ficus* leaves ranged between 10.8% (*F. benjamina*) and 15.8% *(F. abutilifolia).* Ash content was highest (22.0%) in *F. polita* and lowest (13.0%) in *F. vogeli*. Variations in the chemical composition of these *Ficus species* could be attributed to differences in ages of trees, stages of maturity of leaves, season and time of leaf harvesting and presence of secondary metabolites (Larbi *et al.,*1996).

The results showing the presence of saponin, phenols/tannins and steroids in the leaves of *Ficus* species is presented in Table 3. All the analyzed *Ficus species* in this study contained one form of secondary metabolites or the other. Saponin and tannins were present in all the leaves of *Ficus species.* This further confirmed Norton's (2003) report that virtually all

Table 1: Proximate composition and fibre fractions (g/100gDM) of Ficus species (Unextracted)

Ficus species	DM	CP	Ash	EE	NDF	ADF	ADL	Cell	Hem
F. abutilifolia	26.8	14.4	13.5	15.0	52.5	50.0	40.5	9.5	2.5
F. vogeli	31.6	10.4	10.5	10.0	64.5	61.0	47.3	13.7	3.5
F. polita	31.6	12.5	12.0	10.0	49.5	46.5	44.6	1.9	3.0
F. thornningii	31.2	12.5	15.5	10.0	56.5	50.5	40.2	10.3	6.0
F. benjamina	32.3	9.41	12.0	15.0	65.5	52.0	49.0	3.00	3.5

DM: Dry matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, ADL: Acid detergent lignin, Cell: Cellulose, Hem: Hemicellulose.

Table 2: Crude protein and Ash content (g/100gDM) of extracted Ficus species

Ficus species	Crude protein	Ash
F. abutilifolia	15.8	14.0
F. vogeli	13.6	13.0
F. polita	14.4	22.0
F. thornningii	15.3	16.0
F. benjamina	10.8	17.0

forage tree legumes and browse plants in the tropics contain anti- nutritional factors. The presence of saponin in these leaves suggests an important reason to feed *Ficus* to ruminants because saponin had been implicated to suppress rumen fermentation and methanogenesis (Hess *et al.,* 2003).This will enhance efficient utilization of dietary energy and protein by the animals and reduction of ruminal methane impact on global warming. The presence of condensed tannins in the leaves of *Ficus species*in this study was confirmed as reported (Bamikole *et al*., 2004) in *F. mucoso* and *F. religiosa*. The increase in crude protein (15.8%) and ash (22.0%) of extracted *Ficus* leaves in this study could be attributed to the precipitation of soluble proteins and minerals by the

Table 3: Qualitative content of secondary metabolites (Saponin, Phenols/tannin and Steroids) in leaves of Ficus species

Ficus spp.	Saponin Foam(mm)	Comment	Phenols/Tannin Comment	Steroids Colour change	Comment	Colour change
F. abutilifolia	1	Negative	Dark green	Con. Tannin	Pink	Triterpenoids
F. vogeli	1	Negative	Dark green	Con. Tannin	Green	Steroids
F. polita	0	Negative	Dark green	Con. Tannin	Green	Steroids
F. thornningii	1	Negative	Dark green	Con. Tannin	Pink	Triterpenoids
F. benjamina	0	Negative	Dark green	Con. Tannin	Pink	Triterperiods

Con. Tannin = Condensed Tannin.

Table 4: In vitro gas production characteristics for unextracted and extracted Ficus species

Parameters/Treatment	*F. abu.*	*F.vog.*	*F. pol.*	*F. thorn.*	*F. ben.*	SEM
(a) Unextracted	0.00b	0.00b	1.00a	1.00a	1.00a	0.000
Extracted	0.00b	0.00b	1.00a	1.00a	1.50ab	0.158
(b) Unextracted	1.50b	1.50b	4.50a	4.00a	3.50a	0.316
Extracted	5.00a	6.00a	4.00a	6.50a	5.00a	0.962
(a+b) Unextracted	1.50b	1.50b	5.50a	5.00a	4.50a	0.316
Extracted	5.00a	4.00a	7.00a	7.50a	5.50a	0.922
(c) Unextracted	0.000b	0.000b	0.0384a	0.0347a	0.0373a	0.005
Extracted	0.0433a	0.0438a	0.0294a	0.0316a	0.0231a	0.007
(t) Unextracted	24.00a	21.00a	30.00a	30.00a	24.00a	2.121
Extracted	24.00a	27.00ab	30.00a	27.00ab	30.00a	0.949
(Y) Unextracted	1.50b	1.50b	4.00a	3.50a	3.00a	0.274
Extracted	3.00b	2.75b	4.50a	4.00a	3.00b	0.177

a,b means within the same row with same superscripts are not significantly different under Duncan's Multiple Range Tests (DMRT's)
SEM = Standard error of mean
F. abu = Ficusabutilifolia, F. pol. = Ficuspolita, F. vog = Ficusvogeli
F.thor = Ficusthornningii, F.ben= Ficusbenjamina

Table 5: Metabolizable energy (ME), Organic matter digestibility (OMD), Short chain fatty acids (SCFA) and Methane (CH4) of extracted Ficus species.

Ficus species	ME (MJ/kg DM)	OMD (%)	SCFA (µmol)	CH4(mmol)
Ficus abutilifolia	3.79	27.35	0.11	58.59
Ficus vogeli	3.51	25.40	0.09	58.59
Ficus polita	3.98	29.02	0.15	106.92
Ficus thornningii	4.09	29.61	0.16	107.42
Ficus benjamina	3.57	25.79	0.12	78.12
Means	3.79	27.43	0.12	81.93
SEM	0.09	0.67	0.01	10.79

petroleum ether and methanol, which came in the residue (Makker and Becker,1996) as well as the removal of compounds, notably, tannin which bind and form complexes with protein (Mueller and Mc Allan 1992).

The values for *invitro* gas production parameters for unextracted and extracted *Ficus* species is presented in Table 4. The values of gas produced from the soluble fraction (a) between unextracted and extracted *Ficus* leaves were not significantly different. However, there were significant difference in all the unextracted *Ficus* leaves and among extracted *F. vogeli, F. polita* and *F. thornningii*. The rates and extent of gas production in unextracted and extracted *Ficus species* in this study was similar to the findings of Odenyo *et al.* (1997) who reported low volume of gas production at 12hours *invitro* fermentation of *Acacia angustissima* leaves and 24hours for *Leucaena leucocephala* and *Terminalia catappa* leaves(Babayemi,2007). Reduction in the rate and extent of gas production in the *Ficus* leaves could be due to the concentration of soluble phenolic, which has negative influences on degradability (Khazaal *et al.,*1994), nature of proteins, time of incubation and fibre bound condensed tannin (Longland *et al.,* 1995). *Ficus vogeli*, with ADF and NDF (61.0g/100g DM and 64.5g/100g DM) had low rate and extent of gas production compared to *Ficus polita* with ADF and NDF (46.5g/100g DM and 49.5g/100g DM), which had high rate and extent of gas production was also in agreement with Babayemi *et al.*(2004). Gas produced from the insoluble fraction in the substrates was highest in extracted Ficus species (4.0-6.50ml) and lowest for unextracted *Ficus* leaves (1.50ml-4.50ml).

Table 6: Metabolizable energy (ME), Organic matter digestibility (OMD), Short chain fatty acids (SCFA) and Methane (CH4) ofUnextractedFicus species

Ficus species	ME (MJ/KgDM)	OMD (%)	SCFA(µmol)	CH4(mmol)
Ficus abutilifolia	3.23b	23.58b	0.02b	0.00b
F*icus vogeli*	3.00c	21.58c	0.03b	0.00b
Ficus polita	3.66a	26.18a	0.12a	68.36a
Ficus thornningii	3.59a	25.96a	0.11a	58.59a
Ficus benjamina	3.35b	23.90b	0.10a	48.83a
Means	3.36	24.24	0.07	35.15
SEM	0.08	0.58	0.01	10.00

a,b,c means along the same column with similar superscript are not significantly (p>0.05) different. SEM, standard error of mean.

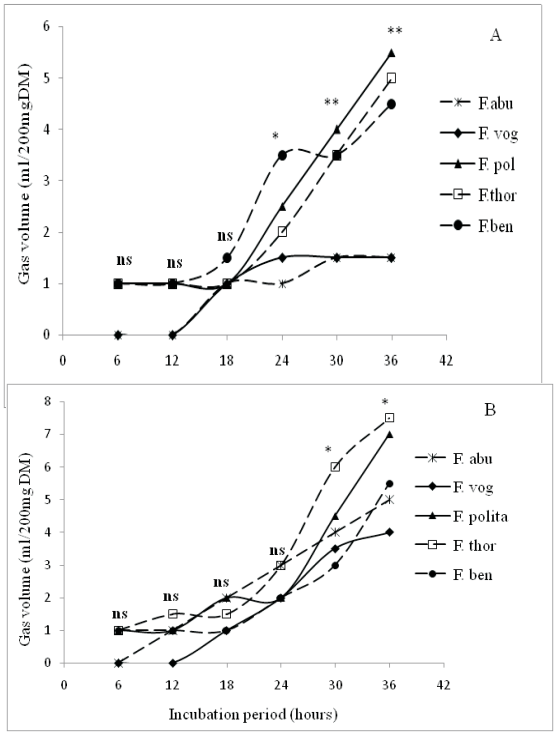

Fig. 1: Trend of in-vitro gas production in unextracted (A) and extracted (B) Ficus species incubated for 36 hours
ns: no significant difference among the treatments at each incubation period (hour).
*and ** indicate significant differences at P<0.05 and P<0.01 for unextracted and extracted Ficus leaves at each incubation time (hour).
F. abu:Ficus abutilifolia; F.vog:Ficus vogeli; F. pol: Ficus polita; F.thor: Ficus thornningi; F.ben:Ficus benjamina.

This suggests a low solubility of fermentable substrates in unextracted *Ficus* leaves in rumen fluid, which is similar with those reported for *Moringe peregrine* (Al-kahtani and Abou-Arab,1993). The trend of gas volume produced from 6 to 36hours post incubation for the unextracted and extracted *Ficus* leaves is depicted in Figures 1A and 1B. Volume of gas produced (unextracted *F.polita)* from 6 to 18hours, showed no significant difference but significantly different ($p<0.05$ and $p<0.01$) at 24,30 and 36hours respectively. On the other hand, gas volume produced in *F. thornningii* (extracted) was not significant from 6 to 24hours, but significantly different ($p<0.05$) at 30 and 36 hours. However, the trend of gas produced for unextracted and extracted *Ficus* leaves is an indication of increase in digestibility beyond 24hours incubation, particularly in *F. polita* and *F. thornningii.*

Results of metabolisable energy, ME(MJ/kg DM), organic matter digestibility, (OMD%) short chain fatty acids, SCFA (µmol) and methane, (CH_4) (ml) of extracted and unextracted *Ficus species* is presented in Table 5 and 6

respectively.

The ME, OMD, SCFA and CH_4 of all the extracted *Ficus species* were not significantly different. However, values for ME,OMD,SCFA and CH_4 ranged from 3.51 to 4.09MJ/kg DM, 25.40 to 29.61%, 0.09 to 0.16µmol and 58.59 to107.42 mmolin extracted *F. vogeli and F. thornningii* respectively. There were significant ($p<0.05$) differences among the unextracted *Ficus species* for ME, OMD, SCFA and CH_4.Estimated values for ME,OMD and SCFA was highest in unextracted *F. polita* (3.66 MJ/Kg DM, 26.18% and 0.12µmol) and lowest in *F.vogeli* (3.00MJ/Kg DM, 21.58% and 0.03µmol), while *F.polita* is not significantly different from *F. thornningii,* which is an indicator of their potential as ruminant feed. The values of ME and OMD (extracted and unextracted) obtained in this study were lower than those reported for *Tephrosia candida* and guinea grass mixtures (Babayemi and Bamikole, 2006) as feedstuffs containing anti nutritive factors had been implicated to be low in metabolisable energy and organic matter digestibility (Aregheore and Abdulrazak, 2005). Numerically, OMD was higher in all the extracted *Ficus species* compared to the unextracted leaves. The Least SCFA (0.03 and 0.09µmol) predicted in this study for extracted and unextracted *F. vogeli* compared to other *F .species* was attributed to a lower gas production at 36hour of incubation since gas production *in vitro,* for tannin-containing browses is closely related to SCFA production (Getachew *et al.,* 2002). Since gas production is a nutritionally wasteful product, it has a direct influence on the ME, OMD and SCFA values predicted for both extracted and unextracted *F. species.* This was due to removal of some secondary metabolites and fractions of lignin (Longland *et al.,* 1995) as ethanol extraction improved the bioavailability of nutrients, for rumen microbes to utilize during fermentation process, for production of useful volatile fatty acids (VFAs) as major source of energy for ruminants (Aregheore, 2002) and reduced end products such as methane gas, which is a loss of the gross energy in feed. In contrast, the presence of saponinin the unextracted leaves could have also reduced microbial population and activities during incubation while condensed tannin inhibited protein degradation (Min *et al.,*2002), hence, reduced metabolisable energy, organic matter digestibility, short chain fatty acids and methane gas. Methane gas measured were not significant in extracted *F. species* but with appreciable differences in values.However, there were significant ($p>0.05$) differences in unextracted ficus leaves. Though negligible in *F. vogeli* and *F. abutilifolia,*it is suggestive of the roles of some anti-nutritional factors in rumen manipulation to suppress methane production, maximize efficiency of feed utilization and increased ruminant productivity. However, variations in amount of methane gas produced could possibly be attributed to the nature of proteins, plant contents, how fermentable the plants are, and contribution, to methanogens (Nagaraja *et al.,*1997).

Conclusion

This study revealed the nutrient potentials and secondary metabolites of *Ficus species.* Lower volume of gas produced *in vitro,* is an indication of the proportion of low fermentable substrate and presence of secondary metabolites in all the *F. species*. Estimated ME, OMD and SCFA are

indicators of the nutrient potential that can be made available to the animals. In addition, the presence of condensed tannins and saponin is also suggestive of the nutritional quality that, when included in the diet of small ruminants could serve as feed resource of by-pass protein and rumen manipulation to reduce degradation of feedstuffs and suppression of rumen fermentation. Hence decreased methane emission and increased net energy to animals. Therefore, any of the *F. species* can serve as feed supplement to sustain small ruminants in the dry season.

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