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The impact of extraction methods on chemical composition and phytochemical constituents of common browse plants and selected tree species

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Target audience: Animal scientists; animal nutritionists; livestock farmers

Abstract

This study investigated the effects of different extraction process on chemical composition, in-vitro and methane gas production of selected browse plants and tree species (Albizzia lebbeck, Enterolobium cyplocarpum, Millettia grifoniana, Moringa oleifera and Pterocarpus santalinoides) which were collected from the vicinity of Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria during late dry season. Samples collected were air-dried for 14 days after which they were milled, ground and packaged for further analysis. Phytochemical constituents, In vitro gas production, fibre fractions and crude protein content were assessed using standard procedures. Data collected were subjected to ANOVA using SAS. Results revealed that Millettia grifoniana recorded the lowest gas production value while Moringa oleifera produced the highest value throughout 72 hours of incubation. Albizzia lebbeck had highest DM (891.7 g/kg) while Moringa oleifera had highest CP (161.7 g/kg), ash (81.7 g/kg) and EE (165.6 g/kg) contents. Diethyl-extracted Pterocarpus santalinoides had highest NDF (680.0 g/kg) and hemicellulose content (360.0 g/kg) while water-extracted Pterocarpus santalinoides had highest ADF (5520.0 g/kg) and cellulose content (366.7 g/kg). Highest ($p<0.05$) tannin contents were observed in Diethyl-extracted Albizzia lebbeck (0.08%) and Millettia grifoniana (0.08%) while water-extracted Moringa oleifera produced highest phenols. It was concluded that Moringa oleifera proves to be the best due to its high in vitro gas production, reduced methane production, high protein content and reduced fibre fraction. Furthermore, all plants investigated, except Pterocarpus santalinoides, can serve as supplement in ruminants feeding.

Keywords: Browse plants, Gas production, Albizia, Anti-nutrients, Fibre fractions

Description of Problem

In the tropics, the productivity and performance of ruminant animals have been reported to be lower than expected. Despite the geometric increase in the growing population, the contribution of

the ruminant livestock animals continue to be on the decrease. This poor performance is occasioned by low inputs, protracted rainy season followed by long period of drought (up to 5 months) low quality and quantity of animal feeds which resulted in the poor nutritional status (in terms of quality) of the animals (1, 2). Though, feeding on quality natural pastures, the availability of which is also subject to seasonal variability will cushion the challenges of nutritional quality being faced by the farmers. (3) reported that native pastures are the most widely available low cost feeds for ruminants in the tropics. Farm animals, though consume and do well on natural herbage, rice straw, crop residues, and crop by-products, the availability of these feeds poses a challenge to the farmers. This situation is further stressed during the dry season and under rain-fed conditions, when almost all crops cannot be grown, and natural pasture, grasses, and weeds become lignified and unpalatable feed. Farmers, either feed their animals with the low-quality hay of the stored crop residues or they travel long distances to gather green grasses or fodders. In order to avert this nutritional crisis, there are countless number of shrubs and fodder trees which are able to withstand the drought, stay green, available all year round and nutritious. There are many shrub and tree species in the tropics and subtropics that can serves as fodders, however, they must be used more efficiently in order to meet up with the nutritional requirement of the ruminant livestock. Browse and shrubby species of natural vegetation contribute to the sustainability of agricultural systems as they increase the recycling of nutrients, control erosion, improve the physical and biological conditions of the soil and are considered as elements of reforestation of the system (4). Browse tree (especially legume trees) leaves have a high protein contents (18-26% crude protein on average) and some of them have low rates of degradability in the rumen (5). This makes them an alternative source of by-pass protein to be evaluated as a supplement for ruminant production systems in the tropics. However, utilization of these plants are limited by the presence of anti-nutrients like saponins, cyanogens, mimosine, coumarins, etc which make other nutrient unavailable for use by the animals (6, 7). Secondary compounds have been known to be present in most browse plants and they form complexes with useful and important quality components of the forage thus making these useful components unavailable to animals and in most cases depress their intake by animals (7). Therefore, the study carried out to determine the effects of different extraction process on chemical composition, *in-vitro* and methane gas production.

Materials and Methods

Experimental Site

The experiment was carried out at the Pasture and Range Management (PRM) Laboratory, College of Animal Science and Livestock Production (COLANIM), Federal University of Agriculture, Alabata, Abeokuta, Ogun State, Nigeria.

Sample collection and preparation

Leave samples of 5 forage trees namely; *Albizzia lebbeck, Enterolobium cyplocarpum, Millettia grifoniana,*

Moringa oleifera and *Pterocarpus santalinoides* were collected from the vicinity of Federal University of Agriculture, Alabata, Abeokuta (FUNAAB), Ogun State, Nigeria. Samples collection were done in the late dry season, they were then air-dried for 14 days after which they were milled, ground and packaged for later use.

Phytochemicals extraction procedures

Water extraction

About 100 g of dried, ground plant materials were soaked in water for 5-7 days separately. The soaked material was stirred every 18 h using a sterilized glass rod. The final extracts were passed through Whatman filter paper No.1 (Whatman Ltd., England). The filtrates obtained were concentrated under vacuum on a rotary evaporator at 40°C and stored at 4°C for further analysis.

Preparation of fat free sample

Two grams (2 g) of the sample were defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 hours.

Tannin determination

500 mg of the fat free sample was weighed into 100 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out into a tube and mixed with 3 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120 nm wavelength, within 10 min. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured (8).

Determination of total phenols

For the extraction of the phenolic component, the fat free sample was boiled with 50 ml of ether for 15 min. 5 ml of the extract was pipette into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelengths (9, 10).

Saponin determination

Twenty grams (20 g) of each defatted samples were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated in percentage (10).

Flavonoid determination

Ten grams (10 g) of the plant samples

were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed (11).

The In Vitro Gas Production Techniques

Approximately 0.2 g of ground leaf samples were weighed into 100 ml calibrated syringes with pistons lubricated with vaseline. A buffered mineral solution was prepared consisting of $NaHCO_3$ + Na_2HPO_4 + KCl + NaCl + $MgSO_4$. $7H_2O$ + $CaCl_2$. $2H_2O$ (1:4, v/v) and stirred at 39 °C under continuous flushing with carbon dioxide (CO_2). Rumen liquor was collected directly from the rumen of slaughtered cattle at an abattoir, and was then filtered through three layers of cheesecloth and flushed with carbon dioxide. Thirty (30) ml of buffered rumen fluid were taken into syringes containing the ground leaf samples. The syringes were placed in an incubator at 39°C. Gas production rates were recorded at 3, 6, 12, 24, 36, 48, 60, and 72 hours of incubation and each syringe was gently swirled after reading. Rates and extent of gas production were then determined for each substrate by recording the corresponding increase in the 30ml volume of the prepared buffered rumen fluid in the glass syringes. Methane gas produced over the incubation period was determined by introducing 4ml of sodium hydroxide solution into the glass syringes. The difference obtained between the initial and final gas head of the syringes was then recorded as the amount of methane produced.

Fibre and Proximate Analysis

Ground samples of water extract) and ethanolic extract of *Albizzia lebbeck, Enterolobium cyplocarpum, Moringa oleifera, Pterocarpus santalinoides* and *Millettia grifoniana* were analysed for dry matter, crude protein content, ash, ether extract using the procedure of AOAC (15). Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were analysed using the method of (14).

Experimental design and Statistical analysis

Data collected during *In-vitro* experiment were subjected to One Way Analysis of Variance (16). For Extracted and Water extracted samples, data were subjected to Two-way Analysis of Variance (16) using a 5 x 2 factorial involving 5 browse species and 2 methods of processing. The significant differences were separated by Duncan's multiple range tests within the package.

Results

In Vitro sequential fermentation of Albizzia lebbeck, Enterolobium cyplocarpum, Millettia grifoniana, Moringa oleifera and Pterocarpus santalinoides.

The sequential gas production from 3hrs to 72 hours of fermentation for all the selected forage were statistically ($p<0.05$) differed as represented in Table 1. *Millettia grifoniana recorded* the lowest (0.67ml/200mgDM) gas production while *Moringa oleifera* produced the highest (4.67ml/200mgDM) at 3 hours of

incubation. Gas production followed the same trend for *Moringa oleifera* from 3 hours of incubation through 72 hours of incubation where it maintained the highest mean values for 6, 9, 12, 24, 36, 48, 60 and 72 hours compared to other forages. This is also buttress in graph (Figure 1) of sequential gas production where *Moringa oleifera* had the highest gas from 3 – 72 hours compared to other plants. Although, gas production increased in all selected plants but not as high as Moringa curve.

Methane production characteristics of the species

Moringa oleifera produced the lowest (4ml/200mgDM) volume of methane as shown in Figure 2. However, *Pterocarpus santalinoides* had highest methane gas production, followed by

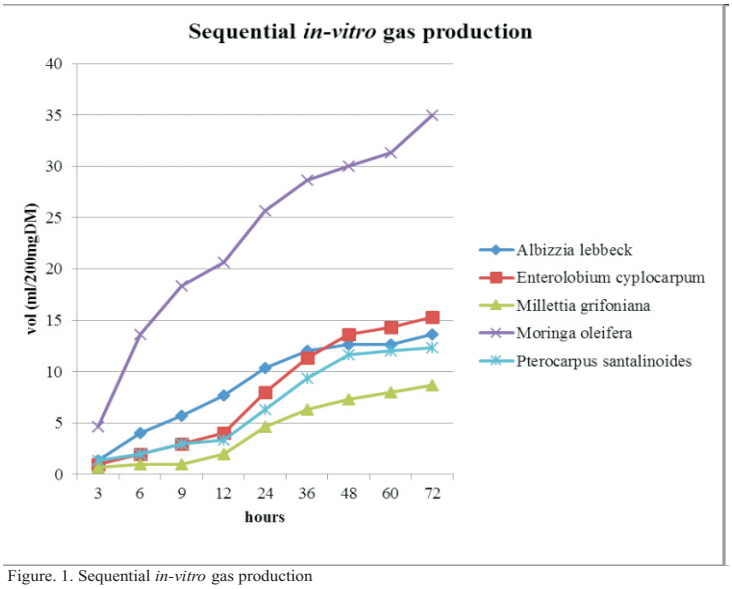

Figure. 1. Sequential *in-vitro* gas production

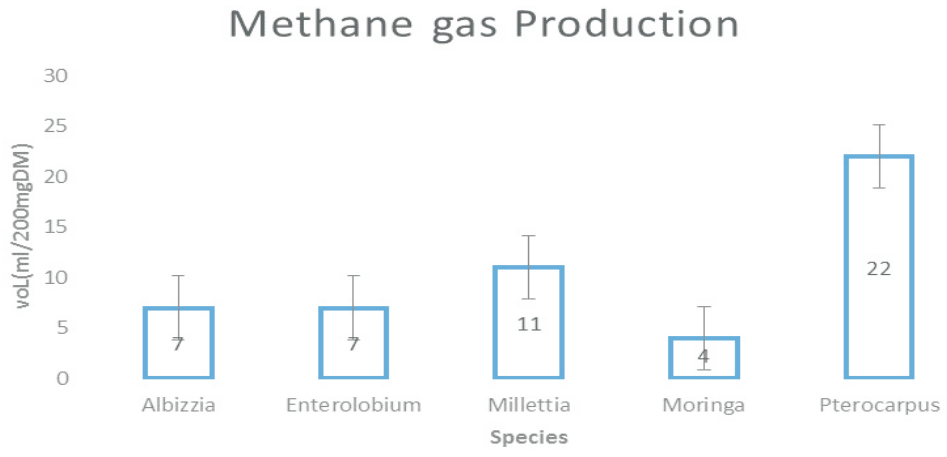

Figure. 2. Methane production characteristics of the species

Table 1: *In-Vitro* gas fermentation of *Albizzia lebbeck, Enterolobium cyplocarpum, Millettia grifoniana, Moringa oleifera* and *Pterocarpus santalinoides.*

Species	3hrs	6hrs	9hrs	12hrs	24hrs	36hrs	48hrs	60hrs	72hrs
Albizzia lebbeck	1.33^{ab}	4.00^{b}	5.67^{b}	7.67^{b}	10.33^{b}	12.00^{b}	12.67^{b}	12.67^{b}	13.67^{b}
Enterolobium cyplocarpum	1.00^{b}	2.00^{b}	3.00^{bc}	4.00^{bc}	8.00^{b}	11.33^{b}	13.67^{b}	14.33^{b}	15.33^{b}
Millettia grifoniana	0.67^{b}	1.00^{b}	1.00^{c}	2.00^{c}	4.67^{b}	6.33^{b}	7.33^{b}	8.00^{b}	8.67^{b}
Moringa oleifera	4.67^{a}	13.67^{a}	18.33^{a}	20.67^{a}	25.67^{a}	28.67^{a}	30.00^{a}	31.33^{a}	35.00^{a}
Pterocarpus santalinoides	1.33^{ab}	2.00^{b}	3.00^{bc}	3.33^{c}	6.33^{b}	9.33^{b}	11.67^{b}	12.00^{b}	12.33^{b}
SEM	0.56	1.29	1.72	1.89	2.14	2.24	2.27	2.38	2.78

^{abcd} = means in the same column with different superscript are significantly (P<0.05) different. SEM = Standard Error of Means.

Millettia grifoniana (11ml/200mgDM) and then by *Albizzia lebbeck,* and *Enterolobium cyplocarpum* with individual production of 7ml/200mgDM.

Main Effects of the Species and Processing methods on Chemical Composition of the selected forages

The chemical composition of the selected forage as affected by different species and extraction methods is presented in Table 2. *Albizzia lebbeck* had the highest mean value (891.7 g/kg) for DM content while lowest (846.7 g/kg) was observed for *Pterocarpus santalinoides*. The mean values of CP ranged from 90.0 g/kg in *Pterocarpus santalinoide* to 161.7 g/kg in *Moringa oleifera*. *Moringa oleifera* recorded the highest mean values (81.7 g/kg, and165.6 g/kg) for ash and EE content, respectively while *Millettia grifoniana* had the corresponding lowest mean values of (56.6 g/kg and 98.1 g/kg) for both, respectively. *Enterolobium cyplocarpum* recorded the highest value (266.2 g/kg) for NFC while *Pterocarpus santalinoides* had lowest value (97.3 g/kg).

The processing methods significantly ($p<0.05$) influenced the DM, CP and ash while the EE and NFC were not significantly ($p>0.05$) affected by the processing methods. The DM content varied from 822.7 g/kg in diethyl ether- to 924.0 g/kg in the water. The CP and ash content also followed the same trend in the diethyl-extracted and water-extracted, both recording the lowest values for the diethyl-extracted. The NFC had the higher value (159.8 g/kg) in the water and lower value (157.4 g/kg) in the diethyl.

Interactive effect of Species and Processing methods on Chemical Composition of the selected forages

Table 3 shows the interactive effect of Species and Processing methods on Chemical Composition of the selected forages. The interaction between the species and processing methods significantly influence CP, Ash, EE and NFC, however the DM content of the forages was not affected. Water extracted *Millettia grifoniana* recorded the highest value (176.7 g/kg) forCP. The ash and EE contents were also highest in water-extracted *Moringa oleifera* while ash was lowest (45.2 g/kg) in water-extracted *Millettia grifoniana*. The NFC

Table 2: Main Effects of the Species and Processing methods on Chemical composition of the selected forage

Factors	DM	CP	Ash	EE	NFC
			g/kg		
Species					
Albizzia lebbeck	891.7^{a}	131.7^{a}	72.4^{ab}	116.1^{b}	163.2^{b}
Enterolobium cyplocarpum	875.0^{ab}	148.3^{a}	70.5^{abc}	121.6^{b}	266.2^{a}
Millettia grifoniana	876.7^{ab}	136.7^{a}	56.6^{c}	98.1^{c}	92.0^{c}
Moringa oleifera	876.7^{ab}	161.7^{a}	81.7^{a}	165.6^{a}	219.3^{a}
Pterocarpus santalinoides	846.7^{b}	90.0^{b}	60.8^{bc}	116.9^{b}	52.3^{c}
SEM	25.40	10.32	6.77	6.34	19.82
Processing					
Diethyl ether	822.7^{b}	120.3^{b}	61.8^{b}	120.2	157.4
Water	924.0^{a}	146.7^{a}	75.0^{a}	127.2	159.8
SEM	7.43	9.50	3.68	6.92	24.29
Processing (main)	0.0001	0.0165	0.0060	0.1598	0.8946
Species (main)	0.2231	0.0026	0.0114	0.0001	0.0001
P*S (interaction)	0.7770	0.1447	0.3870	0.0174	0.3473

^{a,b,c} means in the same column with different superscript are significantly (P<0.05) different.
DM=Dry Matter, CP= Crude Protein, EE= Ether Extract, NFC= Non Fibre Content, SEM = Standard Error of Means.

Table 3: Interactive effects of Species and Processing methods on Chemical Composition of selected forages

Factors		DM	CP	Ash	EE	NFC
				g/kg		
Processing	Species					
Diethyl ether	*Albizzia lebbeck*	840.0	130.3^{abc}	63.5^{bcd}	109.5^{de}	130.4^{bcd}
	Enterolobium cyplocarpum	833.3	143.3^{ab}	72.0^{abc}	106.4^{de}	271.6^{a}
	Millettia grifoniana	823.3	96.7^{bc}	45.2^{d}	108.0^{de}	76.9^{de}
	Moringa oleifera	833.3	153.3^{a}	72.9^{abc}	155.0^{ab}	238.7^{a}
	Pterocarpus santalinoides	783.3	80.0^{c}	55.5^{cd}	121.9^{cd}	69.3^{d}
Water	Species					
	Albizzia lebbek	943.3	133.3^{ab}	81.3^{ab}	122.7^{cd}	196.0^{abc}
	Enterolobium cyplocarpum	916.7	153.3^{a}	69.1^{abc}	136.9^{bc}	260.7^{a}
	Millettia grifoniana	930.0	176.7^{a}	68.0^{bc}	88.3^{e}	107.1^{cde}
	Moringa oleifera	920.0	170.0^{a}	90.6^{a}	176.2^{a}	199.9^{ab}
	Pterocarpus santalinoides	910.0	100.0^{bc}	66.0^{bcd}	119.9^{de}	35.4^{c}
SEM		11.03	7.03	2.84	4.99	16.90

^{a,b,c,d,e} means in the same column with different superscripts are significantly (P<0.05) different
DM=Dry Matter, CP= Crude Protein, EE= Ether Extract, NFC= Non Fibre Content, SEM = Standard Error of Means.

content was lowest in water-extracted *Pterocarpus santalinoides* forage. The mean values of EE ranged from 106.4 g/kg to 155.0 g/kg in diethyl-extracted while it ranged from 88.3 g/kg to 176.2 g/kg in unextracted.

Main effect of species and processing methods on the fibre composition of the selected forage

There were significant ($p<0.05$) differences among the mean values obtained for Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL), hemicellulose and cellulose of *Albizzia lebbeck, Enterolobium cyplocarpum, Millettia grifoniana, Moringa oleifera* and *Pterocarpus santalinoides* as affected by species (Table 4). The least value (371.7 g/kg) NDF was observed for *Moringa oleifera* while the highest (736.6 g/kg) was recorded for *Pterocarpus santalinoides.* The ADF value ranged from 220.0 g/kg in *Moringa oleifera* to 506.7 g/kg in *Millettia grifoniana*. The highest mean values (200.0 g/kg) for ADL were recorded for *Millettia grifoniana* while *Enterolobium cyplocarpum* recorded the lowest mean value (115.0 g/kg). The highest value (263.3 g/kg) for hemicellulose was obtained for *Pterocarpus santalinoides* and the least (110.3 g/kg) in *Millettia grifoniana*. The least (83.3 g/kg) and highest (306.7 g/kg) cellulose content was obtained for *Moringa oleifera and Millettia grifoniana*, respectively. Processing methods had no significant ($p>0.05$) effect on most of the fibre fractions except the NDF and hemicellulose content. Diethyl-extracted forages had highest values (562.7 g/kg and 90.7 g/kg) for NDF and hemicellulose.

Table 4: Main Effect of the Species and Processing methods on Fibre Composition of the selected forages

Factors	NDF	ADF	ADL	HEM	CELL
			g/kg		
Species					
Albizzia lebbek	516.7^{c}	383.3^{c}	143.3^{b}	133.3^{b}	240.0^{b}
Enterolobium cyplocarpum	393.3^{d}	280.0^{d}	115.0^{b}	113.3^{b}	165.0^{c}
Millettia grifoniana	616.7^{b}	506.7^{a}	200.0^{a}	110.3^{b}	306.7^{a}
Moringa oleifera	371.7^{d}	220.0^{e}	136.7^{b}	151.7^{b}	83.3^{d}
Pterocarpus santalinoides	680.0^{a}	473.3^{b}	183.3^{a}	263.3^{a}	290.0^{a}
SEM	18.41	13.1	18.2	25.4	21.5
Processing					
Diethyl ether	562.7^{a}	372.3	148.0	190.7^{a}	224.0
Water	491.3^{b}	373.0	163.3	118.0^{b}	210.0
SEM	33.78	30.5	13.7	19.5	25.1
Processing (main)	0.0011	0.8728	0.1256	0.0003	0.2118
Species (main)	0.0001	0.0001	0.0001	0.0001	0.0001
P*S (interraction)	0.0200	0.0001	0.0001	0.0049	0.0001

$^{a, b, c, d}$ = means in the same column with different superscript are significantly ($P<0.05$) different.

NDF= Neutral Detergent Fibre, ADF= Acid Detergent Fibre, ADL=Acid Detergent Lignin, HEM= Hemicellulose, CELL= Cellulose, SEM = Standard Error of Means.

Interactive effect of species and processing methods on the fibre composition of the selected forages

Interactions between the species and processing methods significantly ($p<0.05$) influenced the fibre composition is presented in Table 5. For NDF, Diethyl-extracted *Pterocarpus santalinoides* had the highest mean value (680.0 g/kg) while the least mean value (363.3 g/kg) was recorded in water-extracted *Moringa oleifera.* It was also observed that diethyl-extracted *Moringa oleifera* recorded the lowest means of 213.3 g/kg for ADF while water-extracted *Pterocarpus santalinoides* had the highest mean value (5520.0 g/kg). The lowest value (46.7 g/kg) for hemicellulose content was obtained in water-extracted *Millettia grifoniana* while the highest mean value (360.0 g/kg) was recorded

Table 5: Effects of Interaction between the Species and Processing methods on Fibre Composition of the selected forages

Factors		NDF	ADF	ADL	HEM	CELL
				g/kg		
Processing	Species					
Diethyl ether	*Albizzia lebbeck*	566.7^{c}	420.0^{b}	180.0^{abc}	146.7^{b}	240.0^{cd}
	Enterolobium cyplocarpum	406.7^{de}	300.0^{d}	86.7^{e}	106.7^{bc}	213.3^{d}
	Millettia grifoniana	673.3^{b}	500.0^{a}	173.3^{bc}	173.3^{b}	326.7^{ab}
	Moringa oleifera	380.0^{e}	213.3^{f}	86.7^{e}	166.7^{b}	126.7^{e}
	Pterocarpus santalinoides	786.7^{a}	426.7^{b}	213.3^{ab}	360.0^{a}	213.3^{d}
Water	Species					
	Albizzia lebbek	466.7^{d}	346.7^{c}	106.7^{de}	120.0^{bc}	240.0^{cd}
	Enterolobium cyplocarpum	380.0^{e}	260.0^{e}	143.3^{cd}	120.0^{bc}	116.7^{e}
	Millettia grifoniana	560.0^{c}	513.3^{a}	226.7^{a}	46.7^{c}	286.7^{bc}
	Moringa oleifera	363.3^{e}	226.7^{ef}	186.7^{abc}	136.7^{b}	40.0^{f}
	Pterocarpus santalinoides	686.7^{b}	520.0^{a}	153.3^{cd}	166.7^{b}	366.7^{a}
SEM		23.92	21.30	9.63	15.91	18.18

$^{a, b, c, d e f}$ = means in the same column with different superscript are significantly ($P<0.05$) different.

NDF= Neutral Detergent Fibre, ADF= Acid Detergent Fibre, ADL=Acid Detergent Lignin, HEM= Hemicellulose, CELL= Cellulose, SEM = Standard Error of Means.

diethhyl-exracted. *Pterocarpus santalioides.* The cellulose content ranged from 40.0 g/kg in water-extracted *Moringa oleifera* to 366.7 g/kg in water-extracted *Pterocarpus santalinoides.*

Main Effects of Species and Processing methods on Phytochemical constituents of the selected forage

The phytochemical constituents of the selected forages varied ($p<0.05$) with different species (Table 6). The highest tannin content (0.05%) was recorded in *Millettia grifoniana and Moringa Oleifera* with others having comparable lowest tannin content. Highest saponins content (1.50 %) was in *Moringa oleifera* compared with other forages. The phenols ranged from 0. 85 % in *Albizzia lebbeck* to 1.39 % in *Moringa oleifera*. Flavonoids recorded the highest mean value (1.08 %) in *Enterolobium cyplocarpum* while the least (0.00%) was obtained in *Millettia*

Table 6: Main effects of the Species and Processing methods on Phytochemical Constituents of the selected forage

Factors	Tannin	Saponin	Phenols	Flavonoids
			%	
Species				
Albizzia lebbek	0.04[a]	0.02[b]	0.85[c]	0.73[d]
Enterolobium cyplocarpum	0.04[b]	0.03[b]	1.18[b]	1.08[a]
Millettia grifoniana	0.05[a]	0.00[b]	1.20[b]	0.00[e]
Moringa oleifera	0.05[a]	1.50[a]	1.39[a]	0.82[c]
Pterocarpus santalinoides	0.03[b]	0.01[b]	0.85[c]	0.92[b]
SEM	0.01	0.15	0.49	0.32
Processing				
Diethyl ether	0.07[a]	0.62[a]	0.02[b]	1.42[a]
Water	0.01[b]	0.00[b]	2.19[a]	0.00[b]
SEM	0.00	0.17	0.56	0.10
Processing (main)	0.0001	0.0001	0.0001	0.0001
Species (main)	0.0001	0.0001	0.0001	0.0001
P*S (interraction)	0.0554	0.0001	0.0001	0.0001

[a, b, c, d, e] = means in the same column with different superscript are significantly ($P<0.05$) different.
SEM = Standard Error of Means.

grifoniana.

The processing methods significantly ($p<0.05$) influenced the phytochemicals. Tannin, saponin and flavonoids were significantly ($p<0.05$) higher in diethyl-extracted forages while phenols was higher in water extracted forages.

Effects of Interaction between the Species and Processing methods on Phytochemical Constituents of the selected forages

The interaction between the species and processing methods significantly ($P<0.05$) influence the phytochemicals constituent of the forages (Table 7). Diethyl-extracted *Albizzia lebbeck* and *Millettia grifoniana* had highest tannin content (0.08 %) while the least (0.00 %) was observed for diethyl-extracted *Moringa oleifera* and water-extracted *Pterocarpus santalinoides*. Saponin ranged from 0.00 % - 3.00 % in the selected forages. Phenols was highest in water-extracted *Moringa oleifera* with all diethyl-extracted species recorded the lowest mean value. Flavonoids ranges from 0.00 % in all water-extracted species and *Millettia grifoniana* to 2.17 % in *Enterolobium cyplocarpum.*

Discussion

In vitro gas has been a helpful approach in evaluating the nutritive value of both conventional and unconventional feed resources. It has advantages of screening many feed resources, developing a supplementation strategy for locally available feed resources and in study of rumen modulators of methane with a good insight in plant bioactive compounds (17). The lower methane gas production observed in Moringa can

Table 7: Effects of Interaction between Species and Processing methods on Phytochemical Constituents of the selected forage.

Factors		Tannins	Saponins	Phenols	Flavonoids
				%	
Processing	Species				
Diethyl ether	*Albizzia lebbeck*	0.08^{a}	0.05^{b}	0.01^{e}	1.47^{d}
	Enterolobium cyplocarpum	0.06^{b}	0.06^{b}	0.02^{e}	2.17^{a}
	Millettia grifoniana	0.08^{a}	0.00^{b}	0.03^{e}	0.00^{e}
	Moringa oleifera	0.0^{e}	3.00^{a}	0.01^{e}	1.63^{c}
	Pterocarpus santalinoides	0.06^{b}	0.01^{b}	0.05^{e}	1.83^{b}
Water	Species				
	Albizzia lebbek	0.01^{d}	0.00^{c}	1.71^{d}	0.00^{e}
	Enterolobium cyplocarpum	0.01^{d}	0.00^{c}	2.35^{c}	0.00^{e}
	Millettia grifoniana	0.02^{c}	0.00^{c}	2.42^{b}	0.00^{e}
	Moringa oleifera	0.01^{d}	0.00^{c}	2.78^{a}	0.00^{e}
	Pterocarpus santalinoides	0.00^{e}	0.00^{c}	1.72^{d}	0.00^{e}
SEM		0.01	0.17	0.21	0.16

$^{a, b, c, d, e}$ = means in the same column with different superscript are significantly ($P<0.05$) different.
SEM = Standard Error of Means.

be as a result of its higher tannin, saponin and phenols content. The presence of tannin could have interfere with the activities of the rumen microbes.

The chemical composition among different species tree and browse species varies as a result of different in soil type (location), the plant part (leaf, stem, pod), age of leaf/plant and season (19). This can be attributed to the variations observed among chemical composition of the selected forages used in this present study. The difference in their crude protein content can also be due to their different natural ability to fix atmospheric nitrogen, extract and store nutrient from the soil (19). With reference to their crude protein content, all the selected forges can serves as supplemental feed for ruminant as their crude protein is higher than the minimum 7-8 % required for ideal rumen function and maintenance requirement for ruminant (20, 21) indicating relative potentials as fodder resources in ruminant nutrition. Our crude protein obtained for the selected forages is comparable to those of (18, 19). The significantly higher crude protein and ash observed for the water-extracted plants may be due to the fact that they are readily soluble in water. Also, polarity of solvents play a vital role in extraction process since with change in solvent polarity its ability to dissolve certain compounds decreased (22). This is further revealed in the interaction between the species and methods of extraction where plants extracted in water has higher chemical composition compared to ethyl-extracted forages. Suffice it to say, ethyl ether extraction is efficient when extracting compounds that has low affinity for water (23).

The least NDF (37.17 %) was observed for *Moringa oleifera* and the highest

(68.00 %) recorded for *Pterocarpus santalinoides* show that the some of the forages can serve the use as supplement especially during the dry season. Apart from *Pterocarpus santalinoides* which has the NDF values beyond the range of 24 – 61 % reported for tropical forages (24), all others tree forage sample i.e *Albizzia lebbeck, Enterolobium cyplocarpum, Moringa oleifera* had their NDF values within the range. According to (25), high NDF content of more than 65%, will limit Dry Matter intake of such forage. The NDF, ADF, ADL, and Cellulose obtained for *Albizzia lebbeck, Enterolobium cyplocarpum, Moringa oleifera* in this present study were within the ranges earlier reported (26, 27). The hemicellulose value (8.3 %) recorded for *Moringa oleifera* was lower than range reported by (27) while the range reported by these author was lower compare to those obtained for other forages. The significantly higher ethyl-extracted NDF was above that reported by (26, 27) while the water-extracted NDF value was within the reported range. The values obtained ethyl and water extracted hemicellulose were within the reported by (26). Athough, water is regarded as a universal solvent, the use of ethyl-ether and the corresponding higher NDF and hemicellulose can be attributed to different in the polarity of the solvents and its solubility with other compound present in the plant. It has been reported that if moisture is not completely removed from the solvent or sample, diethyl ether can react with either sugars or urea in the sample, thereby leading to the higher values obtained (28). However, most of the values were lower compared to earlier report on tropical grasses (18, 29).

Phytochemicals/anti-nutrients were present in all selected species. This in an intrinsic characteristics of most dicots, forbs, shrubs and tree leaves/plant (30). The variations observed can be attributed to species differences, soil type, maturity and other environmental factors (18). The tannin obtain in this study is lower compared to the recommended value (2.5 %) by (20, 31). (32) were also of the opinion that tannin level above 2-5 % in ruminant's diet will adversely affect feed digestibility. The saponin level in this present study is within the range reported by (26, 31, 33). The phenolics obtained in this study was lower to that reported by (19) while the flavonoids were also higher than that stated by (18). The presence of the phenolics and flavonoids indicated that the plant can serve as source of antioxidant for the ruminant animals, hence, preventing the animals from variety of environmental stresses. Higher phytochemicals obtained from the ethyl extracted plants justify the choice of other solvent (alcohol, methanol and ethyl ether) over water for extraction. (34) stated that plant extracts from most organic solvents have been found to give more consistent antimicrobial activity compared to water extract. Also water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics only important as antioxidant compound. This also justify the higher phytochemicals obtained

from most ethyl-extracted plant in the interaction between the species and processing methods

Conclusions and Applications

Based on the results of this study, it could be concluded *Moringa oleifera* proves to be the best due to its high *in vitro* gas production, reduced methane production, high protein content and reduced fibre fraction. In addition, all plants except *Pterocarpus santalinoides* can serve as supplement in ruminants' as they are highly nutritive (higher crude protein above the minimum requirement for ruminant) and their used will be based on their level of inclusion in the ruminant diet.

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