JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

NUTRITIONAL PROFILES OF PROCESSED *Spondias mombin* FOLIAGE AND PHYSIOLOGICAL RESPONSE OF RUMEN MICROORGANISMS TO THE EXTRACTS

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doi: 10.15414/jmbfs.2016.5.5.434-439

ARTICLE INFO

Received 28. 9. 2014
Revised 26. 11. 2015
Accepted 25. 12. 2015
Published 1. 4. 2016

Regular article

OPEN ACCESS

ABSTRACT

Spondias mombin foliage was processed into fresh (as control), air-dried and sun-dried samples and were analyzed for proximate, fibre, minerals, vitamins and antinutrients. Also, Identified rumen microorganisms were exposed to non-reconstituted ethanol extract of the foliage. The results showed that crude protein, crude fibre and gross energy were greatly improved by drying from 4.9% to 15.1%, 2.1% to 18.4% and 0.9 kcal/g to 2.9kcal/g in that order. Fibre constituents improved from 11.5% to 67.9%, 7.2% to 53.6% and 2.9% to 9.3% for neutral detergent fibre, acid detergent fibre and acid detergent lignin. Similarly, all the mineral components were improved from 0.083% to 0.21%, 0.193% to 0.533% and 0.073% to 0.23% for Calcium, Phosphorus and Sodium respectively by drying. Drying decreased the Ascorbic acid, Riboflavin and Niacin contents from 27.8mg/100g to 9.1mg/100g, 0.083mg/100g to 0.033mg/100g and 0.323mg/100g to 0.143mg/100g in that arrangement. Above all, it was observed that drying tremendously improved the nutritional value of *S. mombin* foliage by reducing tannin from 2.2% to 1.64%, oxalic acid (2.1% to 1.38%), phytic acid (1.15 to 0.45%), saponin (1.18% to 0.72%) and trypsin inhibitor from 39.74% to 16.57%. However, drying did not influence the toxic potential of the foliage as indicated by susceptibility of all the rumen microorganisms except the mould species. The nutritional quality potentials observed in the present study suggested that *S. mombin* foliage may be efficiently utilized by ruminants for optimal performance.

Keywords: Feed processing, novel feed resource, nutritional quality, rumen microorganisms susceptibility, ruminant nutrition

INTRODUCTION

Spondias mombin commonly called yellow mombin belongs to the *Anacardiaceae* family. It is well distributed across Mexico, South America and widely cultivated in Paleotropics and native range in West Indies. It is a multipurpose shrub of enormous economic values as food (i.e. the fruits), fence (i.e. boundary demarcation), environmental ornament and antitumour agent (**Idu *et al.*, 2002; Wiersema and Leon, 1999**). Yet, there is insufficient reports on its possible utilization as fodder in ruminant nutrition particularly in Nigeria, where it grows everywhere in the rainforest belt. Although it is a deciduous shrub, it has a very high aftermath capability indicating high biomass fodder availability at all times. Since grasses and agricultural waste have been speculated to be inadequate for optimum ruminant productivity, shrubs and trees with potential feeding values should be evaluated for diet supplementation, especially during off season when grasses and agro byproducts may not be readily available.

Several researches have shown that feed resource evaluation is an important tool that provides useful information necessary for diet formulation in order to optimize performance. In series of experimental studies, it was established that rumen microorganisms are responsible for feedstuff degradability, digestibility and utilization by the host ruminant. But the earliest research frontiers however dwelled so much on feedstuff quality testing using chemical constituent technique (**Midkiff, 1984**), gas production technique (**Menke *et al.*, 1979**) and cell-free fungal cellulose technique (**De-Boever *et al.*, 1986**). Also, nylon bags technique and feed resource digestion technique with unidentified/mixed rumen microorganisms were used to determine the nutritional quality of feedstuff meant for ruminants (**Mehrez and Orskov, 1977; Tilley and Terry, 1963**). Unfortunately, some of these techniques were somewhat not adopted in animal agriculture probably due to some forms of complexities. Above all, feed resource quality evaluation using a mixed culture of rumen microorganisms may not have actually indicated which rumen microorganism was actively involved in the gas production or the fermentation processes (**Makkar, 2004; Sullivan and Martin, 1999**). Consequently *in vitro* exposure of pure culture rumen microorganisms to feedstuff sample could be a novel and better technique of feed quality evaluation. Hence, the present study examined the nutritional profile and susceptibility of rumen microorganisms to ethanol extract of *S. mombin* foliage.

MATERIALS AND METHODS

Samples preparation

Fresh foliage of *S. mombin* was collected from the University of Ibadan Campus and authenticated at the Herbarium, Department of Botany and Microbiology at the same University. Two hundred and fifty grams (250g) of the foliage without leaf stalk was weighed and processed as fresh, air-dried and sun-dried samples. The fresh sample (which served as the control) was crushed in a mortar (Pyrex®) and stored in the freezer. The sample for air-drying (i.e. indoor drying) was spread on the bench in a well-ventilated laboratory at the Institute of Agricultural Research and Training, Ibadan, at a mean room temperature of 28.5°C for 48hrs. Sample for sun-drying (i.e. outdoor drying) was spread on a special drying platform at a mean temperature of 33.2°C for 2 days between 08.00 and 16:00hr GMT each day. The air-dried and sun-dried samples were crispy dried yet retained the green colour. Thereafter, they were ground using hammer mill (Arthur Thomas Co. USA) to a mesh size of 2mm and stored in a cool dry shelf in the laboratory. In a cold extraction, 1g of each sample was thoroughly mixed with 40ml of ethanol (80%). The mixture was left overnight and was filtered using Whitman No. 1 filter paper®. Although concentration of the bioactive ingredients of the filtrate was not determined, it was heated for 2mins to get rid of the ethanol while the pure extract which was not also quantified was stored in McCartney bottles kept in the refrigerator.

Nutrients and antinutrients determination

While the fresh *Spondias mombin* foliage samples were analyzed as wet basis (control treatment), the dried samples were analyzed on dry matter basis for proximate compositions according to AOAC (2000). The fibre constituents were determined following the description of van Soest *et al.* (1991). The mineral and vitamin contents were estimated adopting the methods of **Wiseman and Cole**

(1990) while the gross energy was measured with an adiabatic bomb calorimeter (IKA C7000, Staufen - Germany) standardised with benzoic acid as prescribed by **Witt (1987)**.

Haemagglutinin was estimated as described by **Liener (1955)**, mimosine (**Megarrity, 1978**), cyanide (**Bradbury, 1999**), trypsin inhibitor (**Smith *et al*., 1980**), Oxalic acid (**Bateman and Beer, 1965**) while the alkaloid content was evaluated according to **Henry (1973)**. The phytic acid was determined as demonstrated by **Vaintraub and Lapteva (1988)** and the total saponin and tannin contents were analyzed using the methods of **Makkar *et al*. (1993)**.

Isolation and identification of rumen microorganisms

Samples of rumen liquor were collected in sterile bottles from cattle and goats just slaughtered at Bodija abattoir, Ibadan. Meanwhile, what the animals were fed prior to liquor collection was not taken into cognizance. The samples were pooled and 1g was thoroughly mixed with 9ml of distilled water in a serial dilution procedure given by Black (1986). In an aseptical condition, 1ml of the serial dilution was mixed with 5ml of sterile Eosin Methylene Blue – EMB (idg Lab M^{TM}, UK) and Plate Count Agar – PCA (idg Lab M^{TM}, UK) for rumen bacteria isolation. The mixture was incubated using Temperaturregler (GmbH, Germany) at 37^{o}C for 48hrs under anaerobic condition recommended by **Levett (1990)**. Subculture of the growth observed was done by flaming and streaking followed by incubation at 37^{o}C overnight to obtain pure isolates.

Using flamed inoculating wire loop, samples were collected from the pure isolates in an Oxygen free atmosphere and further subcultured in a liquid substrate "Peptone Water Broth" (idg Lab M^{TM}, UK) and stored in an incubator at 35^{o}C. Similarly, 1ml of the serial dilution was mixed with 5ml of sterile Potato Dextrose Agar – PDA (DM215, Micro Master Laboratories®) and an antibiotic (Vanclox, Evans®) was added to prevent bacterial growth and then incubated for 4 days at 34^{o}C according to **Kudo *et al.* (1990)**. The rumen fungal isolates were purified and transferred into liquid broth (Malt Extract: Difco Laboratories, USA) stored in an incubator at 35^{o}C. The rumen bacteria pure isolates were subjected to morphological and biochemical tests as reported by Yokoyama and Johnson (1993). Meanwhile, the procedure was modified to include antibiosis (i.e. Ionophore and Avopacin) for detailed characterization of the rumen microbial strains. Originally, Avopacin and Ionophore (monesin, lasalocid) were used to depress certain rumen microbial strains in order to improve feed utilization efficiency (**Aderinboye and Onwuka, 2010; Yokoyama and Johnson, 1993**). The rumen fungi isolates were identified according to **Kudo *et al*. (1990)**. The identification features were modified to include some structural characteristics like reproductive stage, sporangiophore, mycelium and rhizoid for proper characterization of the rumen fungal strains. Although concentration of the *S. mombin* foliage extract was not estimated, susceptibility of the rumen microorganisms was examined by subjecting each of the identified rumen bacteria and fungi to the *S. mombin* foliage non-reconstituted pure extract. Each of the 12-punched-well agars was filled with the non-reconstituted foliage extract, streaked with each of the identified rumen microorganisms at a time and incubated overnight in the case of rumen bacteria and 4days for rumen fungi (**Levett, 1990**).

Experimental design, data collection and analysis

Randomized Completely Block Design where the feedstuff processing technique was blocked was adopted and all the parameters were determined in triplicates. Data were collected on proximate compositions, fibre components, mineral and vitamin contents as well as antinutritional constituents of the fresh, air-dried and sun-dried *S. mombin* foliage. Also, information on the suspected rumen microorganisms following morphological examination and biochemical tests were recorded. The growth pattern around each of the wells was observed and recorded as minimum inhibition concentration after overnight and 4 days incubation periods for the identified rumen bacteria and fungi respectively **(Black, 1986)**. The sets of data obtained were subjected to analysis of variance procedure of **SAS (1999)** and the means were separated as given by Duncan's multiple range test of the same software package.

RESULTS AND DISCUSSION

Nutritional compositions

The nutrient compositions of processed *S. mombin* foliage are given in Table 1. All the parameters measured were significantly improved ($P<0.05$) by air-drying and sun-drying compared to the control treatment. Meanwhile the sun-dried values were superior in all the cases except in gross energy and cellulose, where there were no statistical differences ($P<0.05$) in the air-dried and sun-dried values. The crude protein value ranged from 4.9% in control to 10.3% (air-dried) and 15.1% in sundried. Ether extract, crude fibre and ash values were 1.3%, 2.1% and 2.8% in control compared to 13.3%, 18.4% and 13.8% in sun-dried. The energy value varied from 2.5kcal/g in air-dried to 2.9kcal/g in sundried compared to as low as 0.9kcal/g in control. The neutral detergent fibre, acid detergent fibre and acid detergent lignin values which were as high as 67.9%, 53.6% and 9.3% respectively in sun-dried, was slightly followed by air-dried (43.8%, 21.9% and 6.8%) with the least values (11.5%, 7.2% and 2.9%) in control.

The observed nutritional values contradicted the report of Ikhimioya and Oriakhi (2004) where fresh *S. mombin* leaves had 10.06% crude protein and dry leaves 6.41%. Although the crude protein contents (4.9 to 15.1%) observed in the present study were less than 18.83% reported by Mecha and Adegbola (2006), they were within the reported values 3.6 – 22.5% **(ESGPIP, 2008)**, 5.08 – 10.06% (Ikhimioya and Oriakhi, 2004) and recommended range value of 1.61 – 7.76g per kilogram metabolic weight ($W^{0.75}$) (NRC, 1981) for ruminant nutrition. The ether extract, crude fibre and ash contents observed were within 0.7 – 9.3%, 7.8 – 40.7% and 1.0 – 12.5% respectively recorded in some forages (**Mecha and Adegbola, 2006**). Meanwhile, the energy value was less than 0.7 – 6.88Mcal recommended by **NRC (1981)** for optimal ruminant productivity. However, all the values were in agreement with the nutritional requirements recommended by **Givens *et al*. (2000)**. The range values of neutral detergent fibre (11.5 – 67.9%), acid detergent fibre (7.2 – 53.6%) and cellulose (4.3 – 44.3%) were within 18.31 – 68.87% recorded in some other ruminant feed resources (**Idahor *et al*., 2012; Rosiji and Iposu, 2002**).

Also, drying was observed to significantly improved ($P<0.05$) all the minerals determined. Calcium, Iron, Magnesium, Potassium and Phosphorus values were highest (0.21%, 0.003%, 0.353%, 0.463% and 0.533%) in sun-dried followed by air-dried (0.17%, 0.002%, 0.193%, 0.343% and 0.153%) and control (0.083%, 0.001%, 0.093%, 0.153% and 0.193%). Furthermore, Sodium, Zinc, Copper and Manganese values were highest (0.233%, 0.0034%, 0.0009% and 0.004% respectively) in sun-dried sample and slightly followed by air-dried sample (0.153%, 0.0029%, 0.0005% and 0.003%) with the least (0.73%, 0.002%, 0.0002% and 0.001%) in fresh sample (control). The Magnesium, Phosphorus, Potassium and Sodium values were within the ranges 0.04 – 0.25%, 0.16 – 0.4%, 0.5 – 0.8% and 0.06 – 0.18% respectively recommended for ruminant animals **(NRC 1981)**.

However, the Calcium level was less than 0.21 – 0.58% recommended for optimal ruminant productivity (**Church, 1993; NRC, 1981**).

In all the vitamins evaluated, it was observed that dry processing significantly depressed ($P<0.05$) the values compared to the control. The Ascorbic acid, Riboflavin and Niacin values varied from 9.1 to 15.4mg/100g, 0.033 to 0.053mg/100g and 0.143 to 0.213mg/100g) in sun- dried and air-dried samples but were quite high (27.8mg/100g, 0.083mg/100g and 0.323mg/100g) in control. The levels of Riboflavin and Niacin observed were less than 4.5 – 32mg/100kg BW and 26 – 182mg/100kg BW respectively recommended for ruminant animals. This observation concurred with the earlier report by **Wiseman and Cole (1990)** that vitamins were insufficient in processed feed hence their inclusion in ruminant nutrition is essential. The observed depression in the vitamin values could be largely due to their nature as biologically active biochemical compounds that are generally sensitive to their physical and chemical environments. According to **Coelho, (1999)**, several vitamins contain unsaturated carbon atoms or have double bonds that make them highly susceptible to oxidation. Although, feed processing tend to improve the distribution and digestibility of nutrients, it could be harmful to heat labile nutrients such as vitamins that can easily oxidized **(Gadient, 1986; Schneider, 1986)**.

It was observed that all the fresh sample values were seemingly lower, suggesting feed drying (particularly in hay form) superiority over fresh form (fodder). The observed distinct disparities in all the values recorded in the nutritional parameters, could be largely due to the moisture contents and possibly due to denaturation of heat-labile nutrients thereby enhancing the concentration of others. While, the disparities with other reported values could be due to the agro climatic differences, stage of growth, plant species and the laboratory protocols adopted.

Table 1 Effect of processing on nutritional profiles of *S. mombin* foliage

Parameters (††)	Processing techniques			
	Fresh	Air-dried	Sun-dried	SEM
Proximate				
Organic matter (%)	97.3^{b}	91.0^{ab}	86.2^{a}	0.01
Crude protein (%)	4.9^{a}	10.3^{b}	15.1^{c}	0.01
Ether extract (%)	1.3^{a}	7.5^{b}	13.3^{c}	0.01
Crude fibre (%)	2.1^{a}	8.8^{b}	18.4^{c}	0.01
Ash (%)	2.8^{a}	9.1^{b}	13.8^{c}	0.01
Gross energy (kcal/g)	0.9^{a}	2.5^{b}	2.9^{b}	0.07
Fibre constituents (%)				
Neutral detergent fibre	11.5^{a}	43.8^{b}	67.9^{c}	0.01
Acid detergent fibre	7.2^{a}	21.9^{b}	53.6^{c}	0.01
Acid detergent lignin	2.9^{a}	6.8^{b}	9.3^{c}	0.01
Cellulose	4.3^{a}	15.1^{b}	44.3^{c}	0.02
Hemicellulose	4.3^{a}	22.0^{b}	14.3^{c}	0.02
Minerals (%)				
Calcium	0.083^{a}	0.17^{b}	0.21^{b}	0.01
Iron	0.001^{a}	0.002^{ab}	0.003^{b}	0.02
Magnesium	0.093^{a}	0.193^{b}	0.353^{c}	0.01
Potassium	0.153^{a}	0.343^{b}	0.463^{c}	0.01
Phosphorus	0.193^{a}	0.343^{b}	0.533^{c}	0.01
Sodium	0.073^{a}	0.153^{b}	0.233^{b}	0.01
Zinc	0.002^{a}	0.0029^{b}	0.0034^{b}	0.02
Copper	0.0002^{a}	0.0005^{b}	0.0009^{c}	0.02
Manganese	0.001^{a}	0.003^{b}	0.004^{b}	0.02
Vitamins (mg/100g)				
Ascorbic acid	27.8^{c}	15.4^{b}	9.1^{a}	0.01
Riboflavin	0.083^{c}	0.053^{b}	0.033^{a}	0.01
Niacin	0.323^{c}	0.213^{b}	0.143^{a}	0.01

^{a,b,c}: Means along the row with different superscripts differ significantly at P<0.05; ^{SEM}: Standard error of means; ††: All values were expressed on DM basis except fresh sample on wet basis.

Toxic factor components

The antinutrients determined in *S. mombin* foliage are shown in Table 2. It was discovered that drying drastically reduced (P<0.05) all the antinutrients concentrations. The determined phytic acid, oxalic acid, tannin and saponin levels that were as high as 1.15%, 2.1%, 2.21% and 1.18% respectively in the fresh sample, were reduced to as low as 0.45 – 0.54%, 1.38 – 1.53%, 1.64 – 2.04% and 0.72 – 0.93% by drying. Also, the alkaloid, cyanide, mimosine, haemaglutinin and trypsin inhibitor concentrations declined from 2.37 %, 84.74mg/kg, 3.92mg/100g, 9.66HIU/mg protein and 39.74% accordingly in the fresh sample to as low as 1.1 – 1.55%, 36.04 – 66.92 mg/kg,1.55 – 2.13mg/100g, 4.14 – 7.69HIU/mg protein and 16.57 – 21.32% after drying.

Table 2 Effect of processing on antinutrients components of *S. mombin* foliage

Antinutrients (††)	Processing techniques			
	Fresh	Air-dried	Sun-dried	SEM
Phytic acid (%)	1.15^{b}	0.45^{a}	0.54^{a}	0.01
Oxalic acid (%)	2.1^{b}	1.53^{a}	1.38^{a}	0.02
Tannin (%)	2.21	2.04	1.64	0.01
Saponin (%)	1.18	0.93	0.72	0.01
Alkaloid (%)	2.37^{b}	1.55^{a}	1.1^{a}	0.01
Cyanide (mg/kg)	84.74^{c}	66.92^{b}	36.04^{a}	0.01
Mimosine (mg/100g)	3.92^{b}	2.13^{a}	1.55^{a}	0.02
Haemaglutinin(HIU/mg protein)	9.66^{c}	7.69^{b}	4.14^{a}	0.02
Trypsin inhibitor (%)	39.74^{c}	21.32^{b}	16.57^{a}	0.02

^{a,b,c}: Means along the row with different superscripts differ significantly at P<0.05; ^{SEM}: Standard error of means. ††: All values were expressed on DM basis except fresh sample on wet basis.

The phytic acid and saponin concentrations were less than 3.1% and 5.0% respectively reported in *Moringa oleifera* leaves which Makkar and Becker (1996) described as innocuous. However, the tannin concentration was higher than 0.62% detected in Sesbania (**Reed 1986**) and 2.05% found in Gliricidia (**Ahn *et al*., 1989**) but was lower than 3.0 – 14.0% reported in Leucaena (**D'Mello and Fraser 1981**). Also, the mimosine and saponin concentrations were observed to be less than 12.0% and 11.0% respectively discovered in Leucaena (**Tangendjaja *et al*., 1990**). Since **Barry and McNabb (1999)** reported that tannin concentration greater than 4.0% could depress feed intake and there are several reports that ruminants can tolerate and utilize some levels of antinutrients (**Hoskin *et al*., 1997**), *S. mombin* foliage could be a suitable feed resource in ruminant production.

More significantly, the oxalic acid, trypsin inhibitor, alkaloid, haemaglutinin and cyanide values were less than the threshold levels reported in livestock (**Olomu, 2011**). The relatively safe levels estimated in all the antinutrients determined, could be probably due to the part collected and maturity of the foliage. The observed supremacy of feedstuff drying technique could explain the need for proper feed processing prior to utilization in ruminant nutrition.

Susceptibility of the identified rumen microorganisms

The morphological examinations and biochemical tests of the rumen microorganisms are shown in Tables 3 and 4. The identified rumen bacteria were *Bacteroides ruminicola, B. succinogenes, Butyrivibrio fibrisolvens* and *Lactobacillus ruminus.* Others were *Ruminococcus albus, R. flavefaciens, Selenomonas ruminantium and Streptococcus bovis*. The rumen fungi strains were *Neocallimastix frontalis, Orpinomyces joyonii, Saccharomyces cerevisiae, Mucor species, Caecomyces communis and Rhizopus species*. The kinds of rumen microorganisms that were recorded in the rumen liquor could be possibly due to the nature of feed the hosts (cattle and goats) were fed and probably due to their age.

Table 3 Morphological and biochemical tests of the rumen bacterial isolates

Isolate code No.	Morph. Exams		Biochemical tests								Suspected microorganism
	Gram stain		Proteolysis		Amylolysis				Antibiosis		
	Gr	Shape	Gel	Cas	L.mil	Star	Cellu	Sucr	Iono	Avop	
PCA C_1^a	-	Cocci	-	-	+	-	-	-	R	R	*Selenomonas ruminantium*
PCA C_1^b	+	Cocci	-	-	-	+	+	+	S	S	*Ruminococcus albus*
PCA C_4	-	Rods	+	+	+	+	-	-	S	S	*Butyrivibrio fibrisolvens*
PCA C_5	+	Cocci	-	-	-	-	+	-	S	S	*Ruminococcus flavefaciens*
PCA C_7	+	Cocci	-	-	+	-	-	-	R	R	*Selenomonas ruminantium*
PCA C_8	+	Cocci	-	-	+	-	-	-	R	R	*Selenomonas ruminantium*
PCA C_9	+	Cocci	-	-	+	-	-	-	R	R	*Selenomonas ruminantium*
PCA C_{10}^a	+	Cocci	-	-	-	-	+	-	S	S	*Ruminococcus flavefaciens*
PCA C_{10}^b	+	Cocci	-	-	-	+	+	+	S	S	*Ruminococcus albus*
PCA G_1	-	Rods	+	+	+	+	+	+	R	R	*Bacteriodes ruminicola*
PCA G_2	-	Rods	+	+	+	+	+	+	R	R	*Bacteriodes ruminicola*
PCA G_3	+	Rods	+	+	+	+	-	-	S	S	*Butyrivibrio fibrisolvens*
PCA G_4	+	Rods	+	+	+	+	-	-	S	S	*Butyrivibrio fibrisolvens*
PCA G_6	-	Rods	-	-	-	+	+	+	R	S	*Bacteroides succinogenes*
PCA G_8	-	Rods	+	+	+	+	+	+	R	R	*Bacteroides ruminicola*
PCA G_9	-	Rods	+	+	+	+	+	+	R	R	*Bacteroides rumincola*
EMB C_1	-	Rods	+	+	+	+	+	+	R	R	*Bacteroides rumincola*
EMB C_2^a	-	Rods	+	+	+	+	+	+	R	R	*Bacteroides rumincola*
EMB C_2^b	+	Cocci	+	+	+	+	+	+	S	R	*Streptococcus bovis*
EMB C_6	+	Cocci	-	-	-	-	+	-	S	S	*Ruminococcus flavefaciens*
EMB C_{10}	-	Rods	-	-	-	+	+	+	R	S	*Bacteroides succinogenes*
EMB G_1	+	Cocci	-	-	-	+	+	+	S	S	*Ruminococcus albus*
EMB G_2^a	+	Rods	+	+	+	+	+	+	S	S	*Lactobacillus ruminus*
EMB G_2^b	-	Rods	-	-	-	+	+	+	R	S	*Bacteroides succinogenes*
EMB G_4^a	+	Cocci	-	-	-	+	+	+	S	S	*Ruminococcus albus*
EMB G_4^b	+	Rods	+	+	+	+	+	+	S	S	*Lactobacillus ruminus*
EMB G_7	-	Rods	-	-	-	+	+	+	R	S	*Bacteroides succinogenes*

EMB G_8	-	Rods	-	-	-	+	+	+	R	S	*Bacteroides succinogenes*
EMB G_9	+	Cocci	+	+	+	+	+	+	S	R	*Streptococcus bovis*
EMB G_{10}	+	Cocci	-	-	-	-	+	-	S	S	*Ruminococcus flavefaciens*

Morph. Exams = Morphological Examinations; EMB = Eosin methylene blue; PCA = Plate count agar; $C_1 – C_{10}$ = Isolates from cattle rumen liquor; $G_1 – G_{10}$ = Isolates from goats rumen liquor; Gel = Gelatin; Cas = Casein; L.mil = Litmus milk; Star = Starch; Cellu = Cellubiose; Sucr = Sucrose; Iono = Ionophore; Avop = Avopacin; Gr = Gram stain response; + = Positive response; - = Negative response; R = Resistant; S = Susceptible.

Table 4 Morphological and biochemical test of the rumen fungal isolates

Isolate code No.	Morphological examination						Biochemical Test						Identification
	Structure						Starch fermentation						Suspected microorganism
	Col	Cell	Myc	Rhi	Rep	Spo	Pec	Malt	Glu	Gal	Lac	Hcell	
PDA C_1^a	ND	Poly	ND	ND	ND	ND	+	+	+	-	+	+	*Orpinomyces joyonii*
PDA C_1^b	ND	Poly	ND	ND	ND	ND	+	+	+	-	+	+	*O. joyonii*
PDA C_3	ND	Mono	ND	ND	ND	ND	-	+	+	-	+	+	*Neocallimastix frontalis*
PDA C_4	ND	Mono	ND	ND	ND	ND	-	-	+	-	-	-	*Caecomyces communis*
PDA C_5^a	ND	ND	ND	ND	Bud	ND	+	+	+	+	+	+	*Saccharomyces cerevisiae*
PDA C_5^b	ND	ND	ND	ND	Bud	ND	+	+	+	+	+	+	*S. cerevisiae*
PDA C_6	ND	Mono	ND	ND	ND	ND	-	+	+	-	+	+	*N. frontalis*
PDA C_7^a	ND	Mono	ND	ND	ND	ND	-	+	+	-	+	+	*N. frontalis*
PDA C_7^b	White/brown/yellow	CAM	Asep	Abs	Hyp	Bran	ND	ND	ND	ND	ND	ND	*Mucor spp*
PDA C_7^c	White/brown/yellow	CAM	Asep	Abs	Hyp	Bran	ND	ND	ND	ND	ND	ND	*Mucor spp*
PDA C_7^d	White/gray	DCAM	Asp	Pre	Hyp	Unbra	ND	ND	ND	ND	ND	ND	*Rhizopus spp*
PDA C_8	ND	Poly	ND	ND	ND	ND	+	+	+	-	+	+	*O. joyonii*
PDA C_9	ND	Mono	ND	ND	ND	ND	-	+	+	-	+	+	*N. frontalis*
PDA C_{10}	ND	Poly	ND	ND	ND	ND	+	+	+	-	+	+	*O. joyonii*
PDA G_1	ND	Mono	ND	ND	ND	ND	-	-	+	-	-	-	*C. communis*
PDA G_2	ND	Mono	ND	ND	ND	ND	-	+	+	-	+	+	*N. frontalis*
PDA G_3	ND	Mono	ND	ND	ND	ND	-	-	+	-	-	-	*C. communis*
PDA C_4	ND	Mono	ND	ND	ND	ND	-	+	+	-	+	+	*N. frontalis*
PDA C_6^a	ND	Poly	ND	ND	ND	ND	+	+	+	-	+	+	*O. joyonii*
PDA C_6^b	White/brown/yellow	CAM	Asep	Abs	Hyp	Bran	ND	ND	ND	ND	ND	ND	*Mucor spp*
PDA C_7^a	ND	Poly	ND	ND	ND	ND	+	+	+	-	+	+	*O. joyonii*
PDA C_7^b	White/brown /yellow	CAM	Asep	Abs	Hyp	Bran	ND	ND	ND	ND	ND	ND	*Mucor spp*
PDA G_7^c	White/gray	DCAM	Asep	Pre	Hyp	Unbra	ND	ND	ND	ND	ND	ND	*Rhizopus spp*
PDA G_8	ND	ND	ND	ND	Bud	ND	+	+	+	+	+	+	*S. cerevisiae*
PDA G_9	ND	ND	ND	ND	Bud	ND	+	+	+	+	+	+	*S. cerevisiae*
PDA G_{10}^a	ND	Mono	ND	ND	ND	ND	-	+	+	-	+	+	*N. frontalis*
PDA G_{10}^b	White/gray	DCAM	Asep	Pre	Hyp	Unbra	ND	ND	ND	ND	ND	ND	*Rhizopus spp*

ND = Not determined; Col = Colour; Myc = Mycelium; Rhi = Rhizoid; $C_1 – C_{10}$ = Isolates from cattle rumen liquor; - = Negative response; $G_1 – G_{10}$ = Isolates from goats rumen liquor; + = Positive response; Rep = Reproductive stage; PDA = Potato Dextrose Agar; Spo = Sporangiophore; Pec = Pectin; Malt = Maltose; Glu = Glucose; Gal = Galactose; Lac = Lactose; Hcell = Hemicellulose; Poly = Polycentric; Mono = Monocentric; Hyp = Hyphae; Abs = Absent; Pre = Present; Asp = Aseptate; Unbra = Unbranched; Bran = Branched; CAM = Cottony aerial mycelium; DCAM = Dense cottony aerial mycelium.

The minimum inhibition concentration of *S. mombin* foliage extract on the rumen microorganisms is expressed in Table 5. All the treatments were observed to absolutely inhibited (2mm MIC) *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens*, *Bacteroides ruminicola*, *Streptococcus bovis* and *Lactobacillus ruminus* and mildly inhibited (1.60 – 1.62mm MIC) *Bacteroides succinogenes*. Air-dried and sun-dried samples were also observed to absolutely inhibited (2mm MIC) *Selenomonas ruminantium* and mildly inhibited (1.51 – 1.52mm MIC) *Ruminococcus albus*. Similarly, sun-dried and fresh samples absolutely inhibited (2mm MIC) *Caecomyces communis*, *Saccharomyces cerevisiae* and *Neocallimastix frontalis* but *Orpinomyces joyonii* was mildly inhibited (0.31 – 1.31mm MIC). On the other hand, it was discovered that *Mucor* and *Rhizopus species* were absolutely insusceptible (0.0mm MIC) to all the treatments.

It was shown that all the rumen microorganisms were susceptible to the processed *S. mombin* foliage pure extract except, *Mucor* and *Rhizopus species* that were absolutely not inhibited, indicating that dry processing technique may not improve rumen microbial degradation and subsequent nutrient utilization of feedstuff resource by the host as reported by some scientists **(Olomu, 2011; Hoskin *et al*., 1997; Makkar and Becker (1996**). The observed toxic potential of *S. Mombin* foliage to rumen microorganisms, agreed with the reports in extracts of *Vernonia amygdalina*, *Chamaecytisus palmensis*, *Sesbania sesban*, *Acacia angustissima* and *Leucaena leucocephala* that affected the growth of pure culture of *Cellulolytic bacteria* (**Osuji *et al*., 1995**). However, it contradicted the report of **Makkar and Becker (1996)** in *Moringa oleifera* leave extract where uninhibited microbial growth was recorded.

The cause of high degree susceptibility observed in the present study is somewhat not clear because the antinutrients concentrations in browse plants reported by several scientists to be a possible cause of rumen microbial toxicity vis-à-vis host ruminant were tremendously reduced by the feedstuff processing techniques evaluated in the present study. Meanwhile, it could be due to the growth media used, number of punched-well, foliage extract concentration and probably due to the medium of extraction. The absolute insusceptibility of the mould species could simply be due to their characteristics mode of growth.

Table 5 Physiological response of rumen microorganisms to ethanolic extract of *S. mombin* foliage

Rumen microorganisms	Minimum inhibition concentration (mm)			
	Fresh	Air-dried	Sun-dried	SEM
Bacteria				
S. ruminantium	1.04^a	2.00^b	2.00^b	0.35
R. albus	2.00^b	1.51^a	1.51^a	0.16
B. fibrisolvens	2.00	2.00	2.00	0.003
R. flavefaciens	2.00	2.00	2.00	0.003
B. ruminicola	2.00	2.00	2.00	0.003
B. succinogenes	1.61	1.62	1.60	0.006
S. bovis	2.00	2.00	2.00	0.003
L. ruminus	2.00	2.00	2.00	0.003
Fungi				
O. joyonii	1.31^b	0.31^a	1.03^b	0.29
N. frontalis	2.00^b	1.51^a	1.52^a	0.16
C. communis	1.31^a	1.30^a	2.00^b	0.24
S. cerevisiae	1.51^b	1.01^a	2.00^c	0.29
Mucor species	0.0	0.0	0.0	0.03
Rhizopus species	0.0	0.0	0.0	0.003

^{a,b,c}: Means along the row with different superscripts differ significantly at $P<0.05$; ^{SEM}: Standard error of means.

The approach used in this study where pure culture rumen microorganisms were exposed to feedstuff sample *in vitro* could be a novel and better technique of feed quality evaluation. Besides, it may be more economical compared to the digestion technique, nylon bags technique, gas production technique and cell-free fungal cellulose technique described by other scientists. This is because the outcome from the mixed rumen microbial culture as adopted in other techniques does not actually reflect which microorganism is actively involved in the fermentation and degradation processes in the feedstuffs as shown in **Sullivan and Martin (1999)**. More so, the experimental outcome takes lesser time, cannulation is not required and above all, many feed resources can be assessed at the same time. Although *in vitro* results may not conform to *in vivo* trial, the present study is targeted at encapsulating myriads of known rumen

microorganisms that would be made available in commercial quantity for feed resource quality rapid test anywhere in the world.

CONCLUSIONS

The results revealed that drying technique significantly improved the proximate, fibre and mineral compositions of *S. mombin* foliage. In contrast, all the vitamins determined were considerably reduced by drying. Similarly, the concentrations of all the antinutrients were significantly lowered by drying. As a result, drying of feed resources may be necessary prior to utilization in ruminant feeding. On the other hand, all the rumen microorganisms exposed to the *S. mombin* foliage extract were susceptible except the mould species. This apparently indicated that drying may not be proficient in reducing the toxic potential of *S. mombin* foliage. However, the observed nutritional quality potentials suggested that *S. mombin* foliage may be efficiently utilized by ruminants for optimal performance. Although, it has been established that rumen fermentation is the net result of different microorganisms interactions in the rumen ecosystem, the present findings may not conform to *in vivo* feedstuff trial. Thus, ruminant feeding trial to elucidate *S. mombin* foliage suitability for prompt adoption and utilization or otherwise is required.

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