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Nutritional value, fiber fractionation and secondary metabolite composition of selected agroforestry tree leaves as ruminant feed resources

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ABSTRACT

In tropical and subtropical ruminant production systems, seasonal feed scarcity and the high cost of conventional protein supplements remain major constraints. Agroforestry tree leaves represent sustainable, readily accessible forage resources capable of bridging these nutritional deficits. This study evaluated the proximate composition, detergent fiber fractions, and quantitative plant secondary metabolite (PSM) profiles of four widely distributed agroforestry browse species: *Leucaena leucocephala* (Subabul), *Gliricidia sepium* (Quickstick), *Moringa oleifera* (Drumstick tree), and *Azadirachta indica* (Neem). Samples of mature, edible foliage were hand-plucked and analyzed using AOAC standard procedures, the Van Soest fiber fractionation system, and established spectrophotometric assays for phenolics, tannins, and saponins. Significant differences ($P < 0.05$) were observed across all evaluated parameters. Crude protein (CP) content ranged from 17.65% dry matter (DM) in *A. indica* to 25.10% DM in *M. oleifera*. Neutral detergent fiber (NDF) varied between 32.40% DM (*M. oleifera*) and 46.85% DM (*A. indica*), while acid detergent fiber (ADF) ranged from 19.30% to 31.20% DM. *M. oleifera* exhibited superior dry matter digestibility (DMD: 76.45%) and the highest relative feed value (RFV: 188.62), coupled with low total condensed tannins (0.84% DM). *L. leucocephala* and *G. sepium* showed balanced protein-to-fiber profiles (23.40% and 21.80% CP; 38.60% and 42.15% NDF, respectively). Total extractable polyphenols and condensed tannins were highest in *A. indica* (5.42% and 3.12% DM, respectively). These findings indicate that while *M. oleifera* serves as an exceptional protein and energy supplement, *L. leucocephala* and *G. sepium* provide reliable nitrogen-rich basal supplements. Controlled inclusion of tannin-containing species like *A. indica* offers potential for ruminant by-pass-protein optimization and enteric methane mitigation.

Keywords: Agroforestry foliage, Ruminant nutrition, Detergent fiber, Secondary metabolites, Condensed tannins, Feed evaluation

1. Introduction

Ruminant livestock production across tropical and arid agro-ecosystems relies heavily on natural pastures, crop residues, and agro-industrial by-products. These basal feedstocks are predominantly characterized by low crude protein (often below the critical 7% DM threshold required for optimum microbial activity), elevated lignocellulosic content, and poor voluntary dry matter intake (Leng, 1990; Makkar, 2013) [1, 2]. While conventional oilseed meals and cereal concentrates can correct these deficits, their escalating costs and direct competition with human food systems limit their economic viability for smallholder and commercial producers alike (Devendra, 1995) [3].

Agroforestry tree species and multipurpose fodder trees have emerged as indispensable alternative forage and feed resources. Browse foliage provides green fodder during extended dry periods, when herbaceous vegetation senesces and depletes in essential nutrients (Patra et al., 2011; Fernandes et al., 2021) [5, 4]. Species such as *Leucaena leucocephala*, *Gliricidia sepium*, *Moringa oleifera*, and *Azadirachta indica* are well adapted to diverse edaphic and climatic conditions, displaying resilient biomass yield and high leaf nitrogen concentrations.

The nutritional efficacy of tree foliage is governed not merely by gross protein content, but fundamentally by its structural carbohydrate architecture and secondary metabolite profile. The classical proximate analysis often fails to describe the functional availability of energy in browse species; hence, the Van Soest detergent fiber fractionation method—delineating neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid

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detergent lignin (ADL)—is critical for predicting ruminal degradability, voluntary feed intake, and gut fill characteristics (Van Soest *et al.*, 1991) [6].

Furthermore, tropical browse species synthesize diverse plant secondary metabolites (PSMs), notably polyphenols, condensed tannins, hydrolysable tannins, and saponins (Min *et al.*, 2003; Patra *et al.*, 2011) [7, 5]. Historically viewed strictly as *anti*-nutritional factors due to their ability to bind dietary proteins and inhibit microbial enzymes, moderate levels of condensed tannins (20–40 g/kg DM) are now recognized for protecting dietary protein from excessive ruminal degradation, enhancing duodenal amino acid flux, reducing the incidence of pasture bloat, and suppressing enteric methanogenesis (Makkar, 2013; Waghorn, 2008) [2, 8].

Despite extensive regional work, comparative evaluations integrating modern fiber fractionation, derived energetic indices, and secondary metabolite profiling under standardized analytical conditions remain essential for formulating balanced ruminant rations. This study was undertaken to evaluate and compare the proximate chemical composition, detergent fiber fractions, derived nutritional parameters, and secondary metabolite profiles of four key agroforestry tree leaves (*L. leucocephala*, *G. sepium*, *M. oleifera*, and *A. indica*).

2. Materials and Methods

2.1 Study site and sample collection

Representative foliar samples were collected from mature stands of four agroforestry species:

1. *Leucaena leucocephala* (Lam.) de Wit
2. *Gliricidia sepium* (Jacq.) Kunth ex Walp.
3. *Moringa oleifera* Lam.
4. *Azadirachta indica* A. Juss.

Samples were harvested during the mid-vegetative stage, prior to pod formation and leaf senescence. From each species, representative samples (composite leaves comprising leaflets and petiolules) were hand-plucked from five distinct, healthy trees within the same agro-ecological zone. Subsamples were pooled to form five replicate composite samples per species ($n = 5$).

2.2 Sample preparation

Fresh foliage samples were immediately weighed to determine green weight, placed in aerated paper bags, and shade-dried for 48 hours to minimize thermal degradation of volatile phytochemicals and

polyphenols. Samples were subsequently dried in a forced-air oven at 55 °C until constant weight was achieved for analytical dry matter determination. The dried leaf material was ground using a Wiley mill equipped with a 1.0 mm stainless steel sieve and stored in sealed, opaque, airtight polypropylene containers at room temperature until laboratory analysis.

2.3 Proximate chemical analysis

Analytical dry matter (DM, Method 930.15), total ash (Method 942.05), and ether extract (EE, Method 920.39) were determined according to standard methods of the Association of Official Analytical Chemists (AOAC, 2005) [9]. Total nitrogen (N) was quantified using the standard micro-Kjeldahl digestion and distillation procedure (Method 984.13), and crude protein (CP) was calculated as:

$$\text{CP (\% DM)} = \text{N (\% DM)} \times 6.25$$

Organic matter (OM) was computed as $100 - \text{Ash (\% DM)}$. Nitrogen-free extract (NFE) was determined by difference:

$$\text{NFE (\% DM)} = \text{OM} - (\text{CP} + \text{EE} + \text{CF})$$

2.4 Detergent fiber fractionation and derived indices

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined according to the procedures described by Van Soest *et al.* (1991) [6]. Sodium sulfite and heat-stable alpha-amylase were omitted from the neutral detergent solution to prevent solubilization of browse phenolics. ADL was determined by solubilization of cellulose with 72% sulfuric acid (H_2SO_4). All fiber values are expressed inclusive of residual ash.

Cell wall carbohydrate fractions were calculated as follows:

- **Hemicellulose:** $\text{NDF} - \text{ADF}$
- **Cellulose:** $\text{ADF} - \text{ADL}$

Dry matter intake (DMI, % body weight), in vitro dry matter digestibility (DMD, %), and relative feed value (RFV) were estimated using empirical formulas established by Rohweder *et al.* (1978) [10] and the National Forage Testing Association:

$$\text{DMI (\% BW)} = \frac{120}{\text{NDF (\% DM)}}$$

$$\text{DMD (\%)} = 88.9 - (0.779 \times \text{ADF (\% DM)})$$

$$\text{RFV} = \frac{\text{DMD (\%)} \times \text{DMI (\% BW)}}{1.29}$$

2.5 Secondary metabolite quantitation

Extraction of plant secondary metabolites was performed using a 70% aqueous acetone solvent containing 1 g/L ascorbic acid via ultrasonication (3 cycles of 15 min at 4 °C), followed by centrifugation at $3,000 \times g$ for 15 minutes (Makkar, 2013) [2].

- **Total Extractable Phenols (TEP):** Measured using the Folin-Ciocalteu reagent method, with absorbance recorded at 725 nm against a reagent blank. Concentrations were expressed as tannic acid equivalents (TAE, % DM) from a standard calibration curve.

- **Total Condensed Tannins (CT / Proanthocyanidins):** Quantified using the butanol-HCl-iron assay (Porter *et al.*, 1986) [11]. The reaction mixture was incubated in a boiling water bath for 60 minutes, cooled, and absorbance was measured at 550 nm. Values were expressed as leucocyanidin equivalents (% DM).
- **Total Saponins:** Quantified by the vanillin-sulfuric acid spectrophotometric method (Hiai *et al.*, 1976) [12], using diosgenin as the external standard with absorbance measured at 544 nm, expressed as diosgenin equivalents (% DM).

2.6 Statistical analysis

Data were subjected to one-way Analysis of Variance (ANOVA) using the General Linear Models (GLM) procedure. The statistical model used was:

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

Where Y_{ij} is the dependent parameter, μ is the overall mean, T_i is the fixed effect of the tree species ($i = 1, \dots, 4$), and ϵ_{ij} is the random

residual error. Differences among treatment means were evaluated using Tukey's Honest Significant Difference (HSD) post-hoc test at a significance threshold of $P < 0.05$.

3. Results and discussion

3.1 Proximate composition

The proximate nutrient composition of the four agroforestry tree leaves is presented in Table 1. Substantial variation was observed across all species ($P < 0.05$).

Table 1. Proximate composition (% DM basis) of selected agroforestry tree leaves ($n = 5$)

Parameter (% DM)	<i>Leucaena leucocephala</i>	<i>Gliricidia sepium</i>	<i>Moringa oleifera</i>	<i>Azadirachta indica</i>	SEM	P-value
Dry Matter (Air-dry, %)	89.40 ^b	88.15 ^c	91.20 ^a	90.55 ^a	0.38	< 0.001
Organic Matter	91.25 ^a	89.80 ^b	90.10 ^b	92.40 ^a	0.31	0.004
Crude Protein	23.40 ^b	21.80 ^c	25.10 ^a	17.65 ^d	0.46	< 0.001
Ether Extract	4.15 ^c	3.60 ^d	6.85 ^a	5.20 ^b	0.18	< 0.001
Crude Fiber	14.80 ^b	15.90 ^b	10.45 ^c	18.25 ^a	0.42	< 0.001
Total Ash	8.75 ^b	10.20 ^a	9.90 ^a	7.60 ^c	0.25	< 0.001
Nitrogen-Free Extract	48.90 ^b	48.50 ^b	47.70 ^b	51.30 ^a	0.52	0.012

Values within the same row with different superscript letters differ significantly ($P < 0.05$). SEM = Standard error of the mean.

Crude protein content ranged from 17.65% DM in *A. indica* to 25.10% DM in *M. oleifera*. All species evaluated exceeded the 7–8% CP threshold required to sustain ruminal cellulolytic bacterial proliferation and maintain host maintenance requirements (Norton, 1994) [13]. *Moringa oleifera* possessed the highest CP (25.10%) and EE (6.85%), reinforcing its profile as a dual-purpose protein and lipid supplement for high-yielding dairy animals (Foidl *et al.*, 2001) [14]. The CP concentration of *L. leucocephala* (23.40%) and *G. sepium* (21.80%) aligns closely with values reported by Sanchez *et al.* (2006) [15], Devendra (1995) [3] and Castro-Montoya *et al.* (2020) [16],

demonstrating their suitability as leguminous nitrogen sources to supplement poor-quality straw and stover.

Total ash was highest in *G. sepium* (10.20%) and *M. oleifera* (9.90%), reflecting substantial macromineral density (notably calcium, phosphorus, and magnesium), which are often depleted in mature rangeland grasses.

3.2 Fiber fractionation and inferred digestibility indices

The Van Soest fiber fractions and associated energetic and intake indices are detailed in Table 2.

Values within the same row with different superscript letters differ significantly ($P < 0.05$). NDF = Neutral detergent fiber; ADF = Acid detergent fiber; ADL = Acid detergent lignin; DMI = Dry matter intake; DMD = Dry matter digestibility; RFV = Relative feed value.

Table 2. Detergent fiber fractions and calculated quality indices (% DM basis) of agroforestry foliage

Parameter	<i>Leucaena leucocephala</i>	<i>Gliricidia sepium</i>	<i>Moringa oleifera</i>	<i>Azadirachta indica</i>	SEM	P-value
NDF (% DM)	38.60 ^c	42.15 ^b	32.40 ^d	46.85 ^a	0.74	< 0.001
ADF (% DM)	22.45 ^c	26.30 ^b	19.30 ^d	31.20 ^a	0.58	< 0.001

(continued on next page)

Parameter	<i>Leucaena leucocephala</i>	<i>Gliricidia sepium</i>	<i>Moringa oleifera</i>	<i>Azadirachta indica</i>	SEM	P-value
ADL (% DM)	7.15 ^b	6.80 ^b	4.65 ^c	9.85 ^a	0.32	< 0.001
Hemicellulose (% DM)	16.15 ^a	15.85 ^a	13.10 ^b	15.65 ^a	0.41	0.008
Cellulose (% DM)	15.30 ^c	19.50 ^b	14.65 ^c	21.35 ^a	0.48	< 0.001
DMI (% BW)	3.11 ^b	2.85 ^c	3.70 ^a	2.56 ^d	0.06	< 0.001
DMD (%)	71.41 ^b	68.41 ^c	76.45 ^a	64.60 ^d	0.45	< 0.001
Relative Feed Value (RFV)	172.19 ^b	151.15 ^c	188.62 ^a	128.24 ^d	3.12	< 0.001

Neutral detergent fiber varied across species, with *M. oleifera* exhibiting the lowest concentration (32.40%) and *A. indica* the highest (46.85%). Low NDF content strongly correlates with rapid particle breakdown, faster rumen clearance, and greater voluntary dry matter intake (Van Soest, 1994) [17]. This relationship is reflected in the predicted DMI, which was significantly higher for *M. oleifera* (3.70% of body weight) compared to *A. indica* (2.56% BW).

Acid detergent lignin (ADL), which represents the completely indigestible structural barrier preventing enzymatic access to cell-wall polysaccharides, was significantly lowest in *M. oleifera* (4.65%) and highest in *A. indica* (9.85%). Consequently, the estimated in vitro dry

matter digestibility (DMD) was highest for *M. oleifera* (76.45%) and lowest for *A. indica* (64.60%).

The Relative Feed Value index—a composite score benchmarked against full-bloom alfalfa hay (RFV = 100)—categorized all four tree species as high-quality forage resources (RFV > 120). *Moringa oleifera* achieved a score of 188.62, indicative of prime forage status, followed by *L. leucocephala* (172.19), *G. sepium* (151.15), and *A. indica* (128.24).

3.3 Secondary metabolite profile

Quantitative concentrations of the primary plant secondary metabolites are summarized in Table 3.

Table 3. Phytochemical and secondary metabolite concentrations (% DM basis) in tree leaves

Species	Total Extractable Phenols (TAE %)	Condensed Tannins (Leucocyanidin Eq. %)	Total Saponins (Diosgenin Eq. %)
<i>Leucaena leucocephala</i>	3.25 ^b	1.85 ^b	0.42 ^c
<i>Gliricidia sepium</i>	2.10 ^c	0.65 ^c	1.15 ^b
<i>Moringa oleifera</i>	1.85 ^c	0.84 ^c	1.45 ^a
<i>Azadirachta indica</i>	5.42 ^a	3.12 ^a	0.88 ^b
SEM	0.18	0.12	0.08
P-value	< 0.001	< 0.001	< 0.001

Values within the same column with different superscript letters differ significantly ($P < 0.05$). TAE = Tannic acid equivalents.

Secondary metabolite concentrations varied widely among the four browses. *Azadirachta indica* foliage exhibited the highest total extractable phenols (5.42% DM) and condensed tannins (3.12% DM). Condensed tannins in concentrations exceeding 5.0% DM can severely depress dry matter intake, bind dietary proteins into insoluble complexes across the entire gastrointestinal tract, and suppress micro-

bial cellulolysis through direct enzyme inactivation (Min *et al.*, 2003; Makkar, 2013; Su *et al.*, 2020) [7, 2, 18]. However, moderate concentrations—such as those observed in *A. indica* (3.12%) and *L. leucocephala* (1.85%)—fall within the beneficial range (2.0–4.0% DM). In this window, tannins form reversible hydrogen-bonded complexes with proteins at neutral ruminal pH (6.5–7.0), protecting them from microbial deamination, and subsequently dissociate under the acidic conditions of the abomasum (pH < 3.0) to release amino acids for

post-ruminal absorption (Waghorn, 2008; Patra & Saxena, 2010; Kholif, 2015) [8, 19].

Furthermore, these phenolic fractions are known to shift the ruminal archaeal balance, reducing enteric methanogenesis by acting as alternative hydrogen sinks or by directly inhibiting methanogenic archaea and protozoa (Patra, 2010) [20].

Moringa oleifera and *G. sepium* showed modest condensed tannin contents (0.84% and 0.65%, respectively), suggesting minimal antinutritional binding risk and high ruminal protein degradability. Total saponin concentration was highest in *M. oleifera* (1.45% DM). Saponins interact with membrane cholesterol of ruminal ciliate protozoa, causing cell lysis (defaunation) and improving microbial nitrogen synthesis efficiency (Patra & Saxena, 2010).

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