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Nutritional evaluation of top foliages for livestock feeding in semi arid region of India

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ABSTRACT

Top foliages from 9 plants were evaluated for nutritional, anti-nutritional attributes and *in vitro* fermentation. Protein, NDF, ADF, cellulose and lignin contents varied. Protein fractions (P_A, P_{B1}, P_{B2}, P_{B3} and P_C) and carbohydrate fractions (C_A, C_{B1}, C_{B2} and C_C) varied among foliages. Foliages total carbohydrates, non-structural carbohydrates and structural carbohydrates were 69.53, 33.34 and 36.20%, respectively. Lignin bound/unavailable carbohydrate fraction (C_C) was lowest in *Securinega virosa* (13.58) and highest in *Cassia fistula* (53.44). Free condensed tannin (6.40), protein bound condensed tannin (9.34) and fibre bound condensed tannin (101.12 mg/g) differed. Tree foliages differed in total digestible nutrients (TDN), digestible energy (DE), metabolisable energy contents and net energy efficiency for maintenance (NE_M), lactation (NE_L) and growth (NE_G). Foliages differed in gas and CH_4 production with mean values of 205.75 and 31.8 ml/g DDM, respectively. Methane% of gas ranged 12.67–18.58, while loss of DE as CH_4 varied from 6.64–13.70. Partition factor, short chain fatty acids (SCFA), microbial protein and efficiency of microbial protein differed and their values were 5.15, 2.51, 292.5 and 0.55, respectively. Results revealed that *Moringa oleifera* and *Cnidoscolus aconitifolius* had more CP, low fibre, more energy, TDN and SCFA and less CH_4% of total gas.

Key words: Carbohydrate and protein fraction, Condensed tannins, Energy value, Gas, Methane, Tree leaves

Feeding of tree and shrub foliages to grazing ruminants has been practiced traditionally as multipurpose resources in many parts of world (Smith 1992). Leaves and other plant parts constitute cheaper and affordable supplements for ruminants of resource poor livestock keepers in several regions of the world (Yisehak *et al.* 2012, Yisehak and Janssens 2013). Efficient utilization of tree leaves as a low-cost CP and minerals supplement to poor-quality roughages based diets in the tropics, particularly during the prolonged feed scarcity periods (Pamo *et al.* 2007, Patra 2010). Tree leaves contain certain compounds which have been associated with negative nutritional effects (Barry 1989) while Piluzza *et al.* (2014) observed that feeding of locally available native plants might have several potential benefits for ruminant livestock nutrition, ruminal digestion and fermentation (Patra and Saxena 2011) as well as methane production (Puchala *et al.* 2005, Abberton *et al.* 2008). There is ample scope to increase fodder production/availability from top foliages as India has about 54.01 mha common property resources other than 10.90 mha under permanent pasture and grazing lands. In changing climate scenario where there is limited water availability for cultivated crops, top foliages from trees and shrubs are the better alternate for fodder production as shallow root grasses and herbs die off (Chen *et al.* 1991).

The utilization of plant foliages in ruminants ration primarily depends on their chemical composition and nutrient digestibility. Worldwide information is available on the nutritive value of top foliages in respect of chemical composition, digestibility, mineral contents, anti-nutritional factors, fermentation, gas and methane production potential (Negi *et al.* 2003, Singh *et al.* 2013, Theart *et al.* 2015, Aderinboye *et al.* 2016, Singh *et al.* 2017, Tefera and Mlambo 2017, Garcia *et al.* 2017, Ulger *et al.* 2017, Sisay *et al.* 2017) and as supplement of poor quality roughages-grasses, straws, stovers etc (Hove *et al.* 2001, Singh *et al.* 2013). These nutritional attributes of tree foliages varies with variety, maturity stage, season and fertilization, environment and location/region (Negi *et al.* 2003, Parissi *et al.* 2018). This makes essential to evaluate the native plants leaves of a particular region for their nutritive value to consider as potential feed resource for their optimum use in ruminants diets. Hence the present study was undertaken to determine the chemical composition including carbohydrate and proteins factions along with condensed tannin content, digestibility and fermentation pattern (gas and methane) pattern of selected top foliages

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available in semi arid region of India.

MATERIALS AND METHODS

Collection of top foliages: Foliages (leaves and small twigs) of 9 plants (Table 1) were collected from the research farm of the Institute. Leaves were harvested manually from different plants of each species. The samples from different plants of same species were pooled and initially dried at cemented floor followed by drying in hot air oven at 60°C. Dried samples were ground to pass though 1 mm sieve using electrically operated mill, stored in plastic containers and subsequently used for chemical components (proximate constituents and tannins) and biochemical analysis (*in vitro* fermentation).

Table 1. Family name, scientific name, common names and dry matter contents of tested tree foliages

Family name	Scientific name	Common name	DM%
Rutaceae	*Aegle marmelos*	Bael	33.2
Euphorbiaceae	*Securinega* virosa	Chakhedi/ Patala	29.8
Euphorbiaceae	*Jatropha curcas*	Jatropha	28.97
Fabaceae	*Pithecellobium dulce*	Jungle Jalebi	29.32
Sapotaceae	*Madhuca longifolia*	Mahua	32.8
Fabaceae	*Bauhinia variegata*	Kachnar	34.7
Fabaceae	*Cassia fistula*	Amaltas	38.74
Euphorbiaceae	*Cnidoscolus aconitifolius*	Chhaya	31.95
Moringaceae	*Moringa oleifera*	Sahjan	30.52

Proximate analysis of tree leaves: All chemical analyses were carried out in triplicate. Dry matter (DM), crude protein (CP), ether extracts (EE) and ash/organic matter were estimated as per AOAC (2005). Crude protein was calculated as N × 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the procedure of Van Soest *et al.* (1991).

Protein and carbohydrate fractionation: Top foliages CP contents were partitioned into 5 fractions according to the Cornell Net Carbohydrate and Protein System (CNCPS; Sniffen *et al.* 1992) as modified by Licitra *et al.* (1996). For soluble protein (SP) estimation, samples were treated in borate-phosphate buffer, pH 6.7–6.8, consisting of monosodium phosphate ($Na_2PO_4.H_2O$) 12.2 g/L, sodium tetraborate ($Na_2B_4O_7$.10H2O) 8.91 g/L and tertiary butyl alcohol 100 mL/L and freshly prepared 10% sodium azide solution (Krishnamoorthy *et al.* 1983). Protein fractions of evaluated tree foliages were calculated using following equations

P_A (%CP) = NPN (%SP). 0.01. SP (%CP)
P_{B1} (%CP) = SP (%CP) – P_A (%CP)
P_C (%) = ADIP (%CP)
P_{B3} (%CP) = NDIP (%CP) –ADIP (%CP)
P_{B2} (%CP) = 100-P_A (%CP)-P_{B1} (CP)-P_{B3} (%CP) - C_C (%CP)

where NPN, non–protein nitrogen; SOLP, soluble protein; NDIP, neutral detergent insoluble protein; ADIP, acid detergent insoluble protein; P_A, non-protein nitrogen; P_{B1}, rapidly degraded protein; P_{B2}, intermediately degraded protein; P_{B3}, slowly degraded protein; P_C, unavailable/ bound protein.

Estimation of carbohydrate fractions was carried out as per the Cornell Net Carbohydrate and Protein system (Sniffen *et al.* 1992). Total CHO (% DM) was determined by subtracting CP, EE and ash contents from 100. Structural carbohydrates (SC) were calculated as the difference between NDF and NDIP, and non-fibre carbohydrates were estimated as the difference between total CHO and SC (Caballero *et al.* 2001). For starch estimation, foliage samples were initially extracted with ethyl alcohol to solubilize free sugars, lipids, pigments and waxes. The residue rich in starch was solubilized with perchloric acid and the extract was treated with anthrone–sulfuric acid to determine glucose colorimetrically using standard glucose (Sastry *et al.* 1991).

tCHO (%DM) = 100 - (CP + EE+ Ash)
NSC (%DM) = 100 - [(NDF-NDIP) + protein + fat + ash)]
SC (% DM) = NDF - NDIP
C_C (%tCHO) = 100 × (NDF (%DM) × 0.01 × Lignin (%NDF) × 2.4)/CHO (%DM)
C_{B2} (%tCHO) = 100 × {NDF (%DM) – NDIP (%CP) × 0.01 × CP (%DM) – NDF (%DM) × 0.01 × Lignin (%NDF). 2.4/ CHO (%DM)
NSC (%tCHO) = 100 – C_{B2} (%CHO) – C_C (%CHO)
C_{B1} (%tCHO) = Starch (%NSC). {100 – C_{B2} (%CHO) – C_C (% CHO)}/100
C_A (%tCHO) = {100-Starch (%NSC)}. (100 – C_{B2} (%CHO) – C_C (%CHO)}/ 100

where tCHO, total carbohydrate; NSC, non structural carbohydrates; SC, structural carbohydrates; C_C, unavailable/ lignin bound carbohydrate fraction; C_{B2}, slowly degradable carbohydrate fraction; C_{B1}, intermediately degradable pectin and starch; C_A, rapidly degradable sugars.

Dry matter intake, digestibility and energy calculations: Digestible dry matter (DDM), total digestible nutrients (TDN) and net energy (NE) of selected top foliages for different animal functions, i.e. lactation (NE_L), gain (NE_G), and maintenance (NE_M) were estimated as per Undersander *et al.* (1993). Digestible energy (DE) and metabolizable energy (ME) values were calculated as per Fonnesbeck *et al.* (1984); and Khalil *et al.* (1986), respectively.

Determination of tannin fractions and flavonoids: Condensed tannins were determined by the butanol-HCl method (Porter *et al.* 1985). Top foliages were characterized for extractable condensed tannin (ECT), protein bound condensed tannin (PBCT) and fibre bound condensed tannin (FBCT) concentrations following colorimetric methods of Terrill *et al.* (1992). Total flavonoids content was measured by the aluminium chloride colorimetric assay (Zhishen *et al.* 1999) using quercetine as a reference compound. Total flavonoid content of top foliages was expressed as mg quercetine equivalents of dried tree leaf material.

In vitro *incubations: In vitro* gas production was determined according to the pressure transducer technique of Theodorou *et al.* (1994) using 0.5 g sample incubated for 24h in ruminal fluid collected from adult male Jalauni sheep maintained on berseem hay diet. Methane in total gas measured at 24 h from three bottles incubated for each of the tree leaves was analysed by gas chromatography equipped with a stainless steel column packed with Porapak-Q and a flame ionization detector. Methane measured was related to total gas to estimate its concentration (Tavendale *et al.* 2005). Short chain fatty acids (SCFA) were calculated using 24 h gas production (Getachew *et al.* 2002), while the partition factor (PF) and microbial mass (MBM) were estimated as described by Blummel *et al.* (1997). The metaboisable energy (ME) contents were calculated from 24 h gas values as ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057CP + 0.002859 EE and joules values were converted to calories by dividing joule values by 4.14.

Statistical analysis: Data from *in vitro* experiments were subjected to analysis of variance using SPSS 17.0 in a CRD using model

$$Y_{ijk} = \mu+s_i+\varepsilon_{ijk}$$

where Y_{ij}, individual observation; μ, population mean, s_i, top foliages effect (i =1–9) and εij residual error used to test the top foliages variability for different nutritional traits. Duncan's multiple range test was carried out to determine significant differences for chemical composition, carbohydrate and protein fractions, energy values, fermentation parameters of different top foliages at P<0.01 level. Correlation analysis was used to establish relationships between tannin fractions and fermentation parameters.

RESULTS AND DISCUSSION

Chemical composition: Foliages protein contents varied (P<0.01) and were highest in *Moringa oleifera* (23.95) and lowest in *Madhuca longifolia* (8.35%; Table 2). The NDF, ADF, cellulose and lignin contents differed (P<0.01) amongst the top foliages and their mean values were 37.23, 27.16, 17.88 and 10.05%, respectively. Ash and EE contents were highest (P<0.01) in *Aegle marmelos* (17.08) and *Moringa oleifera* (6.71) against lowest in *Madhuca longifolia* (7.02) and *Aegle marmelos* (2.05%), respectively. Except *Madhuca longifolia* (8.35%), CP contents were above 11.0% which are adequate to meet the maintenance requirement of ruminants. Globally many studies have reported CP, EE, NDF, ADF, cellulose and lignin contents of tree and shrub foliages (Khanal and Subba 2001, Negi *et al.* 2003, Theart 2015, Sisay *et al.* 2017, Garcia *et al.* 2017) which are more or less similar to our results. Variation in tree foliages protein and fibre contents for different studies may be attributed to growth stage, season, variety, fertilization, location etc.

Protein and carbohydrate fractions: In foliages, NDIP, NPN and SP contents differed (P<0.01) and values ranged between 28.78–60.32, 10.97–87.66 and 6.74–16.44% CP, respectively (Table 3). Reddy and Elanchezhian (2008) reported NDIP and ADIP contents of tree leaves in range of 33.06 to 57.81% and 10.40 to 18.97% CP, respectively. Protein fractions (P_A, P_{B1}, P_{B2}, P_{B3} and P_C) of evaluated foliages differed (P<0.01) and their mean values were 12.51, 10.66, 33.85, 20.39 and 22.59% CP, respectively. Lignin bound protein fraction (P_C) was highest in *Madhuca longifolia* (40.86) and lowest in *Bauhinia variegata* (9.78% CP). In the tree foliages mean contents of moderately degradable protein fraction (P_{B2}) were highest (33.85% CP). Protein fractions of foliages from tree leaves and shrubs reported by Bhadauria *et al.* (2002) are in partial agreement to our values.

Top foliages differed (P<0.01) in tCHO, NSC and SC contents and their mean values were 69.53, 33.34 and 36.20% DM, respectively (Table 4). Carbohydrate fractions, viz. C_A, C_{B1}, C_{B2} and C_C contents varied (P<0.01) in the top foliages. In foliages accumulation of rapidly degradable

Table 2. Chemical composition (% DM) of top foliages

Foliage	CP	NDF	ADF	Cellulose	Lignin	Ash	EE
Aegle marmelos	12.01^{cd}	26.96^{a}	19.04^{a}	12.46^{a}	6.72^{c}	17.08^{h}	2.05^{a}
Securinega virosa	11.38^{b}	29.29^{b}	21.41^{b}	16.97^{c}	4.27^{b}	10.06^{c}	3.11^{b}
Jatropha curcas	11.09^{b}	33.23^{c}	26.98^{d}	16.10^{b}	9.05^{d}	16.57^{g}	4.67^{cd}
Pithecellobium dulce	16.42^{e}	42.47^{d}	30.81^{e}	18.60^{d}	10.7^{e}	10.15^{cd}	5.82^{e}
Madhuca longifolia	8.35^{a}	52.09^{g}	34.41^{f}	18.43^{d}	14.3^{f}	7.02^{a}	4.37^{c}
Bauhinia variegata	11.86^{c}	45.56^{e}	30.70^{e}	20.61^{e}	10.35^{e}	12.68^{f}	4.40^{c}
Cassia fistula	12.49^{d}	48.5^{f}	36.43^{g}	19.14^{d}	16.55^{g}	8.97^{b}	4.16^{c}
Cnidoscolus aconitifolius	22.72^{f}	26.84^{a}	25.37^{c}	21.82^{f}	4.41^{b}	10.28^{d}	5.09^{d}
Moringa oleifera	23.95^{g}	30.06^{b}	19.28^{a}	16.84^{c}	3.68^{a}	10.95^{e}	6.71^{f}
Mean	14.47	37.23	27.16	17.88	10.05	11.52	4.49
SEM	0.055	0.102	0.114	0.083	0.143	0.014	0.054
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means in the same column with different superscript letters are significantly different (P<0.01). CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; EE, ether extract; SEM, standard error of means.

carbohydrate fraction (C_{B1}) was lowest (1.66) against highest of C_A (54.70% tCHO). Lignin bound carbohydrate fraction (C_C) contents were lowest in *Securinega virosa* (13.58) and highest in *Cassia fistula* (53.44% tCHO). Total carbohydrate contents in leaves of 50 indigenous trees and shrubs in range of 53.4–74.5% (Yisehak and Janssens 2013, Taku *et al.* 2019) are in agreement to our carbohydrate values (58.39–75.40%) except *Madhuca longifolia* (80.26%). Higher NSC in *Aegle marmelos*, *Securinega virosa*, *Cnidoscolus aconitifolius* and *Moringa oleifera* may be due to their lower NDF, ADF and lignin contents as recorded in the present study. Tree foliages had higher contents of C_A fraction (39.66–76.53%) and feeds higher in this fraction are considered good energy sources to stimulate rumen microorganism growth (Carvalho *et al.* 2007) and the synchronism between the protein and carbohydrate digestion rates, having an important effect on the end products of fermentation and on animal production (Nocek 1988). Higher unavailable carbohydrate fraction (C_C) in *Pithecellobium dulce*, *Madhuca longifolia* and *Cassia fistula* may be due to their higher lignin contents (10.7, 14.3 and 16.55%). This indigestible fraction with C_{B2} usually affects animal intake by the rumen fill, which can reduce animal performance (Mertens 1987). Bhadauria *et al.* (2002) reported wide variability in carbohydrate fractions in foliages of tree leaves and shrubs like present observations and our values lie more or less within their values of carbohydrate fractions.

Tannins and flavonoid contents: Top foliage's total condensed tannin (TCT) and flavonoid contents differed (P<0.01) and their values ranged from 5.5 (*Cassia fistula*) to 402.1 (*Pithecellobium dulce*), and 38.15 (*Cassia fistula*) to 102.9 mg/g (*Jatropha curcas*), respectively (Table 5). These values lies within the range of values of previous workers (Grainger *et al.* 2009, Bhatta *et al.* 2015, Gemeda and Hassan 2015, Garcia *et al.* 2017), except *Jatropha*

Table 3. Protein and its fractions (% CP) of top foliages

Foliage	NDIP	NPN	SP	P_A	P_{B1}	P_{B2}	P_{B3}	Pc
Aegle marmelos	28.78^{a}	75.65^{c}	9.39^{d}	12.10^{c}	9.65^{b}	49.48^{d}	10.62^{a}	18.14^{c}
Securinega virosa	34.62^{c}	87.66^{e}	9.47^{d}	14.02^{e}	2.75^{a}	48.00^{d}	19.19^{c}	15.43^{b}
Jatropha curcas	37.91^{c}	85.67^{de}	9.29^{cd}	13.71^{de}	2.59^{a}	45.80^{cd}	12.87^{ab}	25.04^{de}
Pithecellobium dulce	31.8^{a}	83.77^{d}	13.45^{e}	13.40^{d}	4.70^{a}	50.06^{d}	15.58^{bc}	16.25^{bc}
Madhuca longifolia	60.32^{f}	76.73^{c}	6.74^{a}	15.48^{f}	3.76^{a}	20.45^{b}	19.47^{c}	40.86^{g}
Bauhinia variegata	29.05^{a}	16.91^{b}	8.75^{b}	11.23^{b}	16.22^{c}	43.57^{c}	19.19^{c}	9.78^{a}
Cassia fistula	53.77^{d}	10.97^{a}	8.97^{c}	9.80^{a}	18.41^{d}	18.0^{ab}	30.7^{e}	23.06^{d}
Cnidoscolus aconitifolius	56.8^{ef}	10.97^{a}	16.44^{g}	10.97^{b}	16.04^{c}	16.20^{ab}	30.39^{e}	26.40^{ef}
Moringa oleifera	60.32^{f}	11.92^{a}	15.92^{f}	11.90^{c}	21.60^{e}	13.25^{a}	25.19^{d}	28.05^{f}
Mean	42.69	53.36	10.91	12.51	10.66	33.85	20.39	22.59
SEM	0.454	0.400	0.044	0.065	0.249	0.540	0.440	0.252
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means in the same column with different superscript letters are significantly different (P<0.01). NDIP, neutral detergent insoluble protein; NPN, non–protein nitrogen; SP, soluble protein; P_A, non-protein nitrogen; P_{B1}, rapidly degraded protein; P_{B2}, intermediately degraded protein, P_{B3}, slowly degraded protein; P_C, unavailable/bound protein.

Table 4. Total carbohydrate and its fractions of top foliages

Foliages	% DM			% tCHO			
	tCHO	NSC	SC	C_C	C_{B2}	C_{B1}	C_A
Aegle marmelos	68.85^{d}	42.45^{f}	26.40^{b}	23.44^{d}	10.68^{c}	2.24^{h}	63.64^{d}
Securinega virosa	75.46^{g}	46.79^{g}	28.66^{c}	13.58^{a}	20.01^{e}	1.61^{e}	64.80^{d}
Jatropha curcas	67.67^{c}	35.11^{d}	32.55^{d}	32.09^{e}	10.80^{c}	2.12^{g}	54.99^{c}
Pithecellobium dulce	67.61^{c}	25.98^{a}	41.63^{e}	38.02^{g}	17.07^{d}	2.75^{i}	42.16^{b}
Madhuca longifolia	80.26^{h}	28.98^{b}	51.28^{h}	42.83^{h}	15.81^{d}	1.71^{f}	39.66^{a}
Bauhinia variegata	71.05^{e}	26.2^{a}	44.90^{f}	34.77^{f}	24.34^{f}	1.06^{b}	39.83^{a}
Cassia fistula	74.37^{f}	27^{a}	47.42^{g}	53.44^{i}	2.75^{a}	1.30^{d}	42.55^{b}
Cnidoscolus aconitifolius	62.01^{b}	37.23^{e}	24.79^{a}	17.08^{c}	5.40^{b}	0.99^{a}	76.53^{f}
Moringa oleifera	58.39^{a}	30.28^{c}	28.13^{c}	15.11^{b}	15.57^{d}	1.13^{c}	68.17^{e}
Mean	69.53	33.34	36.20	30.04	13.60	1.66	54.70
SEM	0.085	0.127	0.099	2.19	1.11	0.10	2.25
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means in the same column with different superscript letters are significantly different (P<0.01). tCHO, total carbohydrates; NSC, non structural carbohydrates; SC, structural carbohydrates; C_C, unavailable/ lignin bound carbohydrate fraction; C_{B2}, slowly degradable carbohydrate fraction; C_{B1}, intermediately degradable pectin and starch; C_A, rapidly degradable sugars.

Table 5. Tannin fractions and flavonoids contents of foliages (mg/g)

Foliage	ECT	PBCT	FBCT	TCT	Flavonoids
Aegle marmelos	3.45	5.1	68.64	77.19	82.1
Securinega virosa	0.55	0.74	62.84	64.12	85.82
Jatropha curcas	16.88	3.28	338.5	358.65	102.9
Pithecellobium dulce	11.5	70.28	320.31	402.08	80.2
Madhuca longifolia	2.68	3.89	81.49	88.05	95.2
Bauhinia variegata	1.12	0.044	4.89	6.054	88.05
Cassia fistula	1.25	0.36	3.89	5.5	38.15
Cnidoscolus aconitifolius	8.27	0.28	9.77	18.32	–
Moringa oleifera	11.9	0.08	19.72	31.7	–
Mean	6.40	9.34	101.12	116.85	81.77
SEM	1.97	7.64	44.25	50.91	6.92
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means in the same column with different superscript letters are significantly different (P<0.01). ECT, Extractible condensed tannin; PBCT, protein-bound condensed tannin; FBCT, fibre-bound condensed tannin; TCT, total condensed tannins.

curcas and *Pithecellobium dulce* tree foliages. The variation in TCT contents of tree foliages may be attributed to differences in season of harvest (Negi *et al.* 2003). Free condensed tannin, PBCT and FBCT of evaluated foliages differed (P<0.01) and their mean values were 6.40, 9.34 and 101.12 mg/g, respectively. *Jatropha curcas* had the highest concentration of FCT, FBCT and flavonoids while *Pithecellobium dulce* contained highest PBCT and TCT. In leaves of *Cassia fistula* the concentration of TCT and flavonoids were lowest among the all. ECT and PBCT fractions of TCT from tree foliages recorded in present study were within the range of values reported by Garcia *et al.* (2017), while FBCT values were higher than these workers. Values of flavonoid content were in the range of earlier workers (Cetinkaya and Kulak 2016, Mahmoudi *et al.* 2016).

Energy and digestible nutrients: Top foliages differed (P<0.01) in energy contents, viz. DE, ME and NE efficiency for maintenance (NE_M), lactation (NE_L) and growth (NE_G). DE and ME contents were higher in *Aegle marmelos*, *Securinega virosa* and *Cassia fistula*, and lower in *Moringa oleifera*, *Madhuca longifolia* and *Pithecellobium dulce* (Table 6). Mean NE_M was higher (1.89) than NE_L (1.58) and NE_G (1.01 Kcal/g). Total digestible nutrients (TDN) were higher (P<0.01) for *Aegle marmelos* and *Moringa oleifera* (80.18 and 79.19), and lowest for *Cassia fistula* (57.56%). Truly digestible crude protein (tdCP) contents were higher (P<0.01) in *Cnidoscolus aconitifolius* and *Moringa oleifera*, while truly digestible non fibrous carbohydrate contends were higher in *Aegle marmelos* and *Securinega virosa*. The DE, ME, TDN and DCP contents of leaves from 50 indigenous tree and shrubs in range of 6–17 MJ/Kg DM, 5–15 MJ/Kg DM, 33.2–79.01% and 6–22%, respectively (Yisehak and Janssens 2013) are within the range of energy, TDN and DCP values of foliages evaluated in present study. Rodriguez *et al.* (2014) recorded ME values of tree foliages in range of 3.73–6.30 MJ/Kg DM was relatively lower than our values. Truly digestible NFC were lower (less than 30%) in *Pithecellobium dulce*, *Bauhinia variegata* and *Cassia fistula*, and higher (more than 40%) in *Aegle marmelos* and *Securinega virosa*, and this variation may be attributed to differences in NDF, ADF, cellulose and digestibility of foliages.

In vitro *gas, methane production and fermentation:* Total gas production at 24h of incubation from fermentation of top foliages varied (P<0.01) and mean values were 111.30 ml/g and 205.75 ml/g DDM (Table 7). Methane production was highest from *Cnidoscolus aconitifolius* (26.41 and 47.18) and lowest from *Madhuca longifolia* (6.42 ml/g and

Table 6. Energy value and truly digestible crude protein and non fibrous carbohydrate contents of top foliages

Tree leaves	DE (Kcal/g)	ME (Kcal/g)	TDN (%)	NE_L (Kcal/g)	NE_M (Kcal/g)	NE_G (Kcal/g)	tdCP (%)	tdNFC (%)
Aegle marmelos	3.54^g	2.91^g	80.18^g	1.84^g	2.20^g	1.32^g	9.64^c	44.00^d
Securinega virosa	3.40^f	2.79^f	77.10^f	1.77^f	2.11^f	1.23^f	9.02^b	48.18^e
Jatropha curcas	3.08^d	2.53^d	69.85^d	1.59^d	1.90^d	1.02^d	8.75^b	36.70^c
Pithecellobium dulce	2.86^c	2.35^c	64.86^c	1.47^c	1.75^c	0.87^c	13.99^e	27.58^a
Madhuca longifolia	2.65^b	2.18^b	60.17^b	1.36^b	1.62^b	0.74^b	6.09^a	30.55^b
Bauhinia variegata	3.18^e	2.60^e	65.10^c	1.48^c	1.77^c	0.87^c	9.47^c	28.06^a
Cassia fistula	3.59^g	2.90^g	57.56^a	1.29^a	1.54^a	0.66^a	10.12^d	28.31^a
Cnidoscolus aconitifolius	2.88^c	2.37^c	71.93^e	1.63^e	1.97^e	1.09^e	20.22^g	37.40^c
Moringa oleifera	2.54^a	2.09^a	79.91^g	1.83	2.20^g	1.31^g	21.46^h	30.70^b
Mean	3.08	2.52	69.63	1.58	1.89	1.01	12.08	34.61
SEM	0.06	0.05	1.34	0.03	0.04	0.04	0.86	1.20
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means in the same column with different superscript letters are significantly different (P<0.01). DE, digestible energy; ME, metabolisable energy; TDN, total digestible nutrients; NE_L, net energy for lactation; NE_M, net energy for maintenance; NE_G, net energy for gain; tdCP (truly digestible crude protein), CP x exp (–1.2 x (ADIP/CP)) CP*exp (–1.2 * (2.2/CP)) ; tdNFC (truly digestible non fibrous carbohydrates), 0.98 × (100 – ((NDF-NDICP) + CP + EE + Ash).

21.96 ml/g DDM). Aderinboye *et al.* (2016) recorded gas production in range of 107–168 ml/g at 24 h of fermentation of tree foliages in rumen inoculums of cattle, sheep and goats. These workers recorded highest gas production for *Moringa oleifera* (168 ml/g) at 24 h similar to gas production from *Moringa oleifera* (172.50 ml/g) evaluated in present study. Differences in gas production of tree foliages may be attributed to their different contents of CP, NDF and anti-nutritional factors. Higher gas production from *Moringa oleifera* and *Cnidoscolus aconitifolius* may be ascribed to their higher CP and low fibre particularly lignin as many studies have demonstrated that browse species CP contents are positively related to gas production (Aderinboye *et al.* 2016, Sisay *et al.* 2017). Lower gas production from *Jatropha curcas, Pithecellobium dulce* and *Madhuca longifolia* may be attributed to their higher total condensed tannins and lower digestibility as recorded in present study. Theart *et al.* (2015) reported that gas and CH_4 production varied between 44.75–154.3 and 1.58–63.75 ml/g for 10 trees, and 71.75–158.5 and 1.82–64.00 ml/g for 9 shrubs, respectively. These workers further reported that CH_4 production varied widely from 4.04–15.48 for trees and 2.16–18.38 g/kg IVOMD for shrubs, respectively. Rodriguez *et al.* (2014) reported *in vitro* gas production from different tree foliages in range of 31.20–135.31 ml/g OM at 24 h of incubation. Sisay *et al.* (2017) recorded significant differences in the *in vitro* gas and CH_4 production of browse leaves and their values varied from 19.94–46.84 and 4.18–9.05 ml/400 mg, respectively. Gas and CH_4 production from tree leaves of North-East Himalayan region in range of 111.1–612.3 ml/g DDM and 14.7–102.2 ml/g DDM (Taku *et al.* 2019), respectively supports our observations.

Methane% of total gas ranged from 12.67–18.58 across the evaluated top foliages, while loss of digestible energy (DE) as methane (CH_4%DE) on fermentation of foliages at 24 h varied ($P<0.01$) from 6.64 in *Securinega virosa* to 13.70 in *Cnidoscolus aconitifolius*, respectively. Methane% of total gas varied from 10.5–28.0% across the browse leaves (Sisay *et al.* 2017) are in agreement to our values (12.67–18.58%). Ulgar *et al.* (2017) reported that gas and methane production varied ($P<0.0001$) across the tree foliage with their values 21.72–31.54 and 2.62–4.41 ml/200 mg are within the range of our values. Values of CH_4% gas recorded by these workers (10.56–14.32%) of evaluated tree foliages are in agreement to our values. These workers further reported that CP was ($P<0.0001$) was positively correlated with gas and CH_4 production and also to ME and OMD. NDF and ADF were negatively correlated with gas and methane production. Jayanegara *et al.* (2011) reported that gas, methane and IVOMD of 27 tree leaves varied ($P<0.001$) and values ranged between 8.1–42.8, 0.84–7.36 ml/200 mg and 30.3–82.5%, respectively. Methane% of gas also varied ($P<0.01$) with their values of 9.3–18.5% across the 25 tree leaves.

Partition factor, SCFA, MBM and efficiency of microbial protein production (EMBM) of top foliages calculated for 24 h fermentation differed ($P<0.01$) and their mean values were 5.15, 2.51, 292.5 and 0.55, respectively (Table 8). The DMD of *Madhuca longifolia* was as low as 29.68 and *Moringa oleifera* as high as 75.95%, respectively. The ME contents calculated using gas volume (24 h) were relatively lower (2.00) than calculated using chemical constituents (2.52 Kcal/g) but the pattern of ME contents in tree foliages was almost on the similar pattern. Top foliages evaluated in present study had higher partition factor values than the theoretically possible maximum value (4.14); Blummel *et al.* 1997) except *Bauhinia variegata* and *Cassia fistula*. Higher partition factor values for *Jatropha curcas* (7.23) and *Madhuca longifolia* (6.21) could not translate to higher microbial mass probably due to their lower degradation and less gas production. Harnandez *et al.* (2015) reported partition factor and microbial protein production in range of 3.8–6.4 and 514–695 mg/g for eight non leguminous tree foliages at 24 h without PEG addition and our results are within the range of these values. Higher SCFA for *Cnidoscolus aconitifolius* and *Madhuca longifolia* maybe be attributed to their higher gas production as reported by Sisay *et al.* (2018) for *Acacia seyal*. Bayssa *et al.* (2016) reported SCFA (mmol/l) and partition factor in range of

Table 7. *In vitro* total gas production, methane production and digestible energy loss as methane from fermentation of top foliages

Tree leaves	Gas (ml/g)	Gas (ml/g DDM)	CH_4 (ml/g)	CH_4 (% gas)	CH_4 (%DE)	CH_4 (ml/g DDM)
Aegle marmelos	134.44^{d}	202.95^{bcd}	20.43^{d}	15.14^{ac}	8.02^{a}	30.69^{bc}
Securinega virosa	135.03^{d}	192.45^{bcd}	17.20^{cd}	12.67^{a}	6.64^{a}	24.45^{ab}
Jatropha curcas	60.15^{a}	139.04^{a}	10.25b	17.00^{abc}	7.06^{a}	23.54^{a}
Pithecellobium dulce	89.12^{bc}	210.43^{cd}	16.52^{c}	18.58^{c}	12.38^{c}	38.30^{d}
Madhuca longifolia	49.02^{a}	168.21^{ab}	6.42^{a}	13.59^{ab}	7.67^{a}	21.96^{a}
Bauhinia variegata	84.78^{b}	185.38^{bc}	14.65^{c}	17.40^{bc}	10.27^{b}	31.95^{cd}
Cassia fistula	98.93^{c}	208.67^{bcd}	17.03^{cd}	17.20^{bc}	13.09^{c}	35.92^{cd}
Cnidoscolus aconitifolius	177.68^{e}	317.48^{e}	26.41^{e}	14.86^{abc}	13.70^{c}	47.18^{e}
Moringa oleifera	172.50^{e}	227.13^{d}	24.03^{e}	13.93^{sb}	8.16^{a}	31.69^{cd}
Mean	111.30	205.75	16.99	15.60	9.67	31.80
SEM	7.48	8.70	1.05	0.51	0.49	0.748
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means in the same column with different superscript letters are significantly different ($P<0.01$).

Table 8. Partition factor, short chain fatty acids, microbial protein production, efficiency for microbial mass production and dry matter digestibility of tree leaves

Tree leaves	PF	SCFA (mmole/g DM)	MBM (mg/g)	EMBM	DMD (%)	ME (Kcal/g)
Aegle marmelos	4.94 ^{b}	3.00^{d}	371.11^{de}	0.55^{bcd}	66.41^{d}	2.92^{e}
Securinega virosa	5.23^{bc}	3.03^{d}	413.33^{e}	0.58^{bcd}	70.61^{de}	2.93^{e}
Jatropha curcas	7.23 ^{d}	1.34^{a}	304.60^{cd}	0.69^{e}	43.52 ^{b}	1.68^{c}
Pithecellobium dulce	4.89^{b}	1.99^{b}	240.22^{bc}	0.53^{bc}	43.37^{b}	2.24^{d}
Madhuca longifolia	6.21^{cd}	1.09^{a}	190.75^{ab}	0.63^{de}	29.68^{a}	1.46^{a}
Bauhinia variegata	3.16^{a}	1.93^{b}	282.52^{c}	0.59^{cd}	46.03^{b}	1.89^{d}
Cassia fistula	4.40^{b}	2.27^{c}	267.27^{c}	0.54^{bcd}	47.58^{b}	1.87^{d}
Cnidoscolus aconitifolius	5.48^{bc}	4.02^{e}	171.95^{a}	0.30^{a}	55.96^{c}	1.42^{a}
Moringa oleifera	4.82^{b}	3.94^{e}	391.10^{e}	0.50^{b}	75.95^{e}	1.54^{b}
Mean	5.15	2.51	292.5	0.55	53.19	2.00
SEM	0.125	0.17	15.38	0.02	0.844	0.09
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means in the same column with different superscript letters are significantly different (P<0.01). PF, Partition factor (gas ml/mg DM); SCFA, short chain fatty acids; MBM, microbial mass; EMBM, efficiency for microbial mass production; DMD, dry matter degradability.

Table 9. Correlation between tannin contents and fermentation parameters

Parameter	ECT	PBCT	FBCT	TCT	Gas (ml/g)	Gas (ml/g DDM)	CH_4 (ml/g)	CH_4 (ml/g DDM)	DMD
ECT	1								
PBCT	0.329	1							
FBCT	0.698*	0.650	1						
TCT	0.694*	0.728*	0.994**	1					
Gas (ml/g)	0.018	–0.214	-0.504	–0.469	1				
Gas (ml/DDM)	–0.013	-0.003	–0.454	–0.395	0.795*	1			
CH_4 (ml/g)	0.079	-0.066	–0.426	–0.377	0.958**	0.844**	1		
CH_4 (ml/g DDM)	0.094	0.265	–0.215	–0.144	0.556	0.877**	0.734*	1	
DMD	–0.011	–0.265	-0.371	–0.362	0.846**	0.359	0.754*	0.124	1

*Significant at P<0.05; **Significant at P<0.01.

0.51–1.34 and 2.18–5.07, respectively for 10 browse species. Lower SCFA from *Jatropha curcas, Pithecellobium dulce* and *Madhuca longifolia* may be attributed to their higher total condensed tannins as tannins reduced the SCFA concentration (Makkar *et al.* 1995). The partition factor values for *Eucalyptus citriodora* fresh (6.1 mg/ml) leaves or residue leaves (5.7 mg/ml) incubated after oil extraction at 24 h of incubation in sheep inoculum (Sallam *et al.* 2010) are closer to our values.

The microbial protein production was lowest (171.95) and methane production was highest (47.18 ml/g DDM) from *Cnidoscolus aconitifolius* which is consistent with observations of McCrab *et al.* (1997). Partition factor and MPP values of top foliages recorded in our study are within the values of partition factor (6.2–7.5) and microbial protein production (426.7–514.7) reported by Salem (2012) and Taku *et al.* (2019) for tree leaves.

Aderinboye *et al.* (2016) reported IVDMD of different tree leaves in range of 50.7–61.8% in rumen fluid of cattle, sheep and goats. Theart *et al.* (2015) recorded IVDMD of tree leaves and shrubs in range of 23.0–64.75 and 30.9–81.1%, respectively. Dry matter degradability of tree foliages varied possibly due to differences in their fibre contents. Higher condensed tannins in tree foliages of *Jatropha curcas, Pithecellobium dulce* and *Madhuca longifolia* reduced their dry matter degradability. Rodriguez *et al.* (2014) reported DMD of different tree foliages in range of 37.18–79.16%. *Moringa oleifera* had the highest DMD like our DMD values for MO (75.95%). Parissi *et al.* (2018) reported IVDMD of five browse leaves in the range of 31.9–81.1% and our DMD values lie within this range.

Correlation between tannin contents and methane production: Nonsignificant negative correlation was observed between tannin and its fraction with gas production, methane production and DMD (Table 9). Similar to our findings Jayanegara *et al.* (2008) recorded nonsignificant negative correlation between CT and methane production, while statistically significant and negative relationships were observed between total phenols (TP), total tannins (TT) or tannin activity and methane production. Among the CT fractions, FBCT had higher negative correlation values for gas, methane and DMD than other fractions of condensed tannins and this may be due to the fact that more foliage dry matter contains more proportion of the fibre contents.

It is evident form results that tree foliages differed ($P<0.01$) in protein, fibre, carbohydrate and protein fractions, energy, digestibility and fermentation characteristics (gas and methane production, digestibility, partition factor, SCFA and microbial protein production). Higher contents of condensed tannins influenced the *in vitro* fermentation pattern (reduced gas production, short chain fatty acids and dry matter degradability) of *Jatropha curcas, Pithecellobium dulce* and *Madhuca longifolia* tree foliages. *Moringa oleifera and Cnidoscolus aconitifolius* had more CP, low fibre, more energy, TDN and short chain fatty acids and less CH_4% of total gas and can be used as potential feed resource for environment friendly livestock production.

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