Nutritive assessment of four local herbal plants as animal feed supplements

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Abstract

Many local herbal plants are generally rich in secondary metabolites and contain high amount of essential nutrients.. A study was conducted to evaluate the antioxidant content and antimicrobial activities of 4 selected herbal plants: *Andrographis paniculata* (Hempedu Bumi), *Orthosiphon stamineus* (Misai Kucing), *Euphorbia hirta* (Ara Tanah) and *Boreria latifolia* (Boreria) that are widely available in Malaysia. Proximate analysis, phyto-chemical determination and *in vitro* technique were used to evaluate nutritive value of the herbal plants. Fatty acid profile and 2,2-diphenyl-1-picrylhydrazyl (DDPH) free radical scavenging activity were also explored. *A. paniculata* had the highest content of crude protein (18.13±0.18%), calcium (11.92±1.66%), saponin (18.73±1.13%) and flavonoids (1.25±0.21%). while, *E. hirta* contained highest tannin (0.24±0.007%), phenol (0.02±0.004%) and antioxidant content (9.22±0.02%). For antimicrobial activity, *E. hirta, A. paniculata* and *O. stamineus* methanol extracts at 500 mg/ml concentration showed moderate antimicrobial activities. The methanol extracts of all herbal plants exhibited stronger antimicrobial activities against the test pathogens compared to the herbal water extracts. Among the 4 local herbal plants examined, *A. paniculata* contained the lowest total saturated fatty acids (26.53±0.19 g/100g FAME) and highest unsaturated fatty acids (73.47±0.19 g/100g FAME) and *E. hirta* had the highest total gas production (49.10±8.97 ml), rate of gas production (2.05±0.37 ml/h). All herbal plants studied have their own potential as animal feed supplements.

Keywords: Chemical composition, Antioxidant properties, Antimicrobial properties, *In vitro* technique

Introduction

Malaysia is identified as one of the world's 12 mega diversity areas with extremely rich biological resources. One example of its biological resources is the various herbal plant species. Several herbal plants such as *Andrographis paniculata* (Hempedu Bumi), *Orthosiphon stamineus* (Misai Kucing), *Euphorbia hirta* (Ara Tanah) and *Boreria latifolia* (Boreria) are examples of local herbal plants that are widely available in Malaysia. These herbal plants have been utilized for folk medicine for decades and also are taken as dietary supplements. The antimicrobial and antioxidant properties of these herbal plants are said to be due to various phytochemicals such as flavonoids, phenols, tannins and tocopherols present in them (Akowuah *et al*., 2004). Herbal plants are still the mainstay of common people in the developing countries for cure and improving general health purposes. Worldwide, herbal plants have become mainstream alternative medicine in late 20th century due to their widespread

acceptance as remedies for common diseases which form the integration of derivatives from natural sources in pharmaceutical products. These herbal plants generally contain high amount of essential nutrients, like fatty acids, minerals, vitamins and plant proteins (Gafar and Itodo, 2011), thus contributing to the intake of essential nutrients.

However not much information on the chemical composition of these plants, especially in terms of phytochemical, antioxidant and antimicrobial content, *in vitro* dry matter and organic matter digestibility, volatile fatty acid and also fatty acid profiles is available. Chemical composition, rumen fermentation and digestibility can be used as proxies for nutritive value of herbal plants. Chemical composition parameters include crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), phytochemical substances such as tannin, saponin and phenol. Medicinal value of the herbal plants lies on the chemically active substances present in these herbal plants. The future of using these herbal plants in human and animal nutrition will in great measure be dependent on the knowledge of their chemical composition and characteristics. Hence, the objectives of this study were to determine the chemical and nutrient content of the local herbal plants with the intention of using them as feed supplement for livestock.

Materials and Methods

Plants collection and extract preparation

Four herbal plants: *Andrographis paniculata* or Hempedu Bumi (Family: *Acanthaceae*), *Orthosiphon stamineus* or Misai Kucing (Family: *Lamiaceae*), *Euphorbia hirta* or Ara Tanah (Family: *Euphorbiaceae*) and *Boreria latifolia* or Boreria *(*Family*: Rubiaceae)* were selected for this study. Samples of the arial parts of these local herbal plants were collected in Universiti Putra Malaysia campus in Serdang Selangor (2.99917°N 101.70778°E). These plants have been botanically and taxonomically identified by Universiti Putra Malaysia.

Extract preparation

Fresh arial parts of plants (7cm from shoot of plant) were thoroughly washed using tap water and rinsed with distilled water. The herbal plant were dried for 3 days in an oven at 30ºC and then pulverized to a fine powder with the aid of blender to pass through 2-mm sieve. Two solvent were used for preparation of the extracts namely distilled water and methanol 60% concentration. The aqueous extract was prepared by weighing out 20 g of the milled powdered axial part of herbal plants were soaked in 100 ml of distilled water in a conical flask and stirring vigorously with glass road every 6 hour with glass rod for proper extraction. The mixture was allowed to settle down for 48 hours. The mouth of conical flask was covered by aluminium foil. For the methanolic extract, 20 g of the milled powdered axial part of herbal plants were soaked in 100 ml of 60% concentration for 48 hours also. The extracts then were then filtered using Whatman no.1 filter paper. All filtrate were air dried at 28ºC and the poured in amber bottle and keep in the refrigerator at 4ºC for next analysis.

Chemical compounds determination

The proximate analyses (moisture, ash, crude proteins, and ether extract content) of the plant samples were analyzed following the methods of Association of Official Analytical Chemists (AOAC), (2012). Meanwhile for content of crude fibre

including Acid detergent fiber (ADF), Neutral detergent fiber (NDF) and Acid detergent lignin (ADL) was analyzed following Van Soest detergent fiber analysis system (Van Soest *et al*., 1963). While gross energy content was determined using Fully Automatic IKA® Adiabatic Bomb Calorimeter C2000 and calcium and phosphorus content were determined by Atomic Absorption Spectrophotometer (Emission flame photometry: PSS-AVR-Model SS 103). The phytochemical compounds which included alkaloid, flavonoid, phenol, tannin, saponin, and hydrogen cyanide were determined based method described by Mbagwu *et al.* (2010).

Determination of antioxidant using DPPH radical-scavenging activity

The antioxidant content in the herbal plants was determined according to the method described by Sreeramulu and Raghunath (2011), with slight modification using gallic acid as standard solution. An aliquot of 2.9 ml of 0.1mM DPPH radical in methanol was mixed with 0.1 ml of methanolic or aqueous extract of the sample. After incubation in the dark for 30 min at 28°C and then the absorbance of the curvette was read at 517 nm using Thermo spectronic GENESYS 20 spectrophotometer. The DPPH radical-scavenging activity in the extracts was expressed as percentage of inhibition (%) of gallic acid.

Disk diffusion method to determine antimicrobial sensitivity

The antibacterial potential of the selected herbal plant extracts was assessed from their reaction against 2 bacterial cultures using the Kirby-Bauer disc diffusion method (Bauer *et al*., 1966; Zaidan *et al.*, 2005). Pure cultures of *Escherichia coli* and *Salmonella enterica* bacteria were provided by Bacteriology Laboratory, Faculty of Veterinary Medicine, UPM. The bacterial culture (108 CFU/ml) was spread on nutrient agar petri dish before putting the paper disks Sterile 6.0 mm diameter filter paper disk (Whatman No.1) on impregnated petri dishes seeded with dilution of each of herbal plant extracts 50,000 ppm or 500 mg/ml. Chloramphenicol (commercial antibiotic, 20 µg/ml) (Oxoid, UK) was served as a positive control whereas sterile distilled water and methanol were employed as a negative control. Thereafter, petri dishes were incubated at 37ºC for 24 h. After that antibacterial activity was determined by measuring the diameter of the inhibition zone formed in the disk. The inhibition zone bigger than 15 mm was considered as strong activity, the zone from 10 to 15 mm as moderate activity and the zone smaller than 10 mm as weak activity.

Determination of fatty acid composition

Fatty acid composition of the plants was determined following the methods of Peterson *et al.* (2011) with slight modification using Agilent 6890 N gas chromatography (GC) equipped with an injector and a FID detector and a 30 m x 0.32 mm, 0.2 µm thickness, Supelco 123-2332 capillary column (Supelco, Inc., Bellefonte, PA, USA). Boron trifluoride at 20 % concentration in a complex methanol solution from Merck® was used to convert the fatty acids in a complex lipid to fatty acid methyl esters (FAMEs). An internal standard (C21:0, 100 µL, Sigma Aldrich) was added to each sample. Analysis was performed at a range of 40˚C to 250˚C at a rate of 1 ml/ min constant flow with the linear velocity of 26 CNM/s, and hydrogen as the carrier gas. Fatty acid samples were identified by comparing their retention times with fatty acid standard (Supelco® 37 component FAME Mix) that had been analyzed prior to, during, and at the end of the sample analysis to compensate for

shifts in retention times. Results were expressed as percentages of total fatty acids.

In vitro studies of selected medicinal plant

In vitro digestibility of the selected herbal plants was determined following the procedure of Menke and Steingass (1988) to estimate the extent to which they affected fermentation via digestibility and biohydrogenation in the laboratory. Meanwhile, procedures for ammonia determination and gas production were those of Parsons *et al.*, (1984) and of Tilley and Terry (1963) respectively.

Statistical analysis

All the experiments were done in triplicate and the data generated were analyzed using descriptive statistics, analysis of variances and correlation analysis using SPSS version 17 (StatSoft Inc., Tulsa, OK).

Results and Discussion

Table 2 shows proximate composition, gross energy content and phosphorus and calcium content of 4 selected local herbal plants. Nutrient composition varied several folds among the herbal plants. *E. hirta* had significantly higher fresh dry matter compared to *B. latifolia* and *O. staminieus* at 36.56±1.39%. However percentage of dry matter content of *E. hirta* and *A. paniculata* did not differ significantly.

A. paniculata had lower ADF content (18.80±0.26%), but higher protein content at 18.13±0.18%, while *B. latifolia* had the highest DM content and highly indigestible part (ADF) of forage, but had the lowest gross energy content and protein content which were 97.11±0.09%, 35.23±0.37% and 16.02±0.15 MJ/kg and 9.49±0.07%, respectively. On the other hand, *O. stamineus* had quite a high percentage of NDF, which was 43.8±0.92% compared to the other herbal plants. The lignin content in *O. stamineus* was higher than the other herbal plants (22.27 ± 2.69%). Lignin has no nutritive value, except it is a bulk factor. However, at a high level, it reduces digestibility of other nutrients in a ration. On the contrary, *E. hirta* had the lowest value for ash and crude protein content compared to the other herbal plants at 8.22±0.08 % and 8.84±0.07%, respectively. In spite of having the lowest protein content, *E. hirta* had the highest crude fat content at 2.98±0.65% compared with other herbal plants.

Different from *O. stamineus* and *E. hirta*, *A. paniculata* had the lowest lignin and crude fat content, however, this herbal plant contained high protein and the highest energy content at 18.13±0.18% and 16.68 MJ/kg, respectively. At the same time, *A. paniculata* had the highest ash percentage representative of inorganic compounds and minerals. *A. paniculata* has the highest calcium content at 11.92±1.66mg/L, two and three times more than the other herbal plants. Meanwhile, O. stamineus had the highest phosphorus content (9.69±0.54 mg/L) compared to other herbal plants. On contrary *B. latifolia* contained the lowest calcium and *E. hirta* contained the lowest phosphorus content at 2.83±0.21 mg/L and 5.12±0.36 mg/L, respectively. The calcium concentration in the herbal plants varied from 2.83 mg/L to 11.92 mg/L and the mean of calcium in these plants was 5.89±2.06 mg/L.

Phytochemical compound content in the herbal plants studied are presented in Table 3 indicating the presence of alkaloids, saponin, tannins, flavonoids, phenols and hydrogen cyanide at various levels. *A. paniculata* had higher flavonoid content compared with the others at 1.25±0.21%. It also had the highest saponin content compared to the other herbal plants at 18.73±1.13%. Meanwhile, *E. hirta* had the highest value of tannin and phenol content at 0.24±0.007% and 0.02±0.001,

Tannin content also showed a highly significant positive correlation with phenol (r=0.981, P<0.001).

Figure 1 shows the antioxidant activities of four herbal plants extracted with different solvents were assessed by determining DPPH free radical scavenging activities of the extract in different solvents in terms of % inhibition Gallic acid equivalent (GAE). Among the herbal plants, the methanolic extract of *E. hirta* showed the highest percentage of antioxidant GAE at 9.22% as compared with the other herbs and significantly higher than the water solvent extract. The water extract exhibited significantly lower DPPH scavenging activity as compared with methanol extract of each herbal plant tested.

Table 4 shows the antimicrobial activity of the 4 local herbal plants. In general, all herbal plant extracts in methanol solvent exhibited at least some degrees of bacterial growth inhibition. Among the treatments and control, 20 μg/disc of Chloramphenicol (positive control) showed the strongest anti-bacterial effect with *E. coli* and S. *enterica.* Meanwhile among the plant extracts, the methanolic extract of *E. hirta* and *A. paniculata* had the same effect on both bacteria tested at 500 mg/disc followed by *O. stamineus* that also had the same effect on *E. coli* but was unable to inhibit the growth of *S. enterica* to more than 10 mm diameter. On the other hand, *B. latifolia* did not inhibit the growth of both bacteria tested to more than 10 mm diameter.

Table 1: Proximate composition and gross energy content of four local herbal plants

Chemical composition	Herbal plants (Mean ± SEM)			
	A. paniculata	*O. stamineus*	*B. latifolia*	*E. hirta*
Fresh DM (%)	34.56±0.73^{bc}	32.22±0.83^{b}	23.11±0.59^{a}	36.56±1.39^{c}
DM (%)	93.77±0.01^{a}	92.93±0.84^{a}	97.11±0.09^{b}	93.34±0.05^{a}
Ash (%)	14.93±0.07^{b}	13.99±0.12^{c}	13.11±0.13^{d}	8.22±0.08^{a}
CP (%)	18.13±0.18^{b}	17.28±0.19^{c}	9.49±0.07^{a}	8.84±0.07^{a}
NDF (%)	32.00±0.15^{b}	43.80±0.92^{cd}	42.87±1.19^{c}	47.17±0.29^{d}
ADF (%)	18.80±0.26^{a}	30.83±2.14^{c}	35.23±0.37^{b}	34.9±0.51^{b}
ADL (%)	7.17±0.09^{ab}	22.27±2.69^{c}	9.87±1.13^{ab}	12.10±0.87^{b}
Ether Extract (%)	0.51±0.13^{b}	1.05±0.35^{d}	0.65±0.12^{c}	2.98±0.65^{a}
GE (MJ/kg)	16.68±0.07^{a}	16.31±0.09^{a}	16.02±0.15^{a}	16.96±0.15^{a}
Phosphorus mg/L)	5.52±0.22^{b}	9.69±0.54^{d}	6.06±1.08^{c}	5.12±0.36^{a}
Calcium (mg/L)	11.92±1.66^{b}	3.82±0.11^{a}	2.83±0.21^{a}	5.00±0.36^{a}

^{abcd}Means with the same superscript in the same row are not statistically different (P≤0.05).
GE-Gross Energy

Table 3: Phytochemical compound concentration in four local herbal plants

Phytochemical	Herbal plants (Mean ± SEM)			
	A. paniculata	*O. stamineus*	*B. latifolia*	*E. hirta*
Alkaloids (%)	8.50±0.390[b]	8.61±0.900[b]	4.27±0.740[a]	4.79±0.440[a]
Saponin (%)	18.73±1.130[b]	9.61±0.320[a]	8.18±0.340[a]	8.38±1.380[a]
Tannins (%)	0.09±0.001[a]	0.19±0.005[c]	0.06±0.001[a]	0.24±0.007[d]
Flavonoid (%)	1.25±0.210[a]	1.23±0.720[a]	0.29±0.040[a]	0.59±0.030[a]
Phenol (%)	0.01±0.001[a]	0.02±0.003[c]	0.01±0.001[a]	0.02±0.004[d]
HCN (%)	0.05±0.003[b]	0.02±0.001[a]	0.05±0.002[b]	0.05±0.001[b]

[abcd]Means with the same superscript in the same row are not statically difference at P≤0.05.
HCN- Hydrogen cyanide

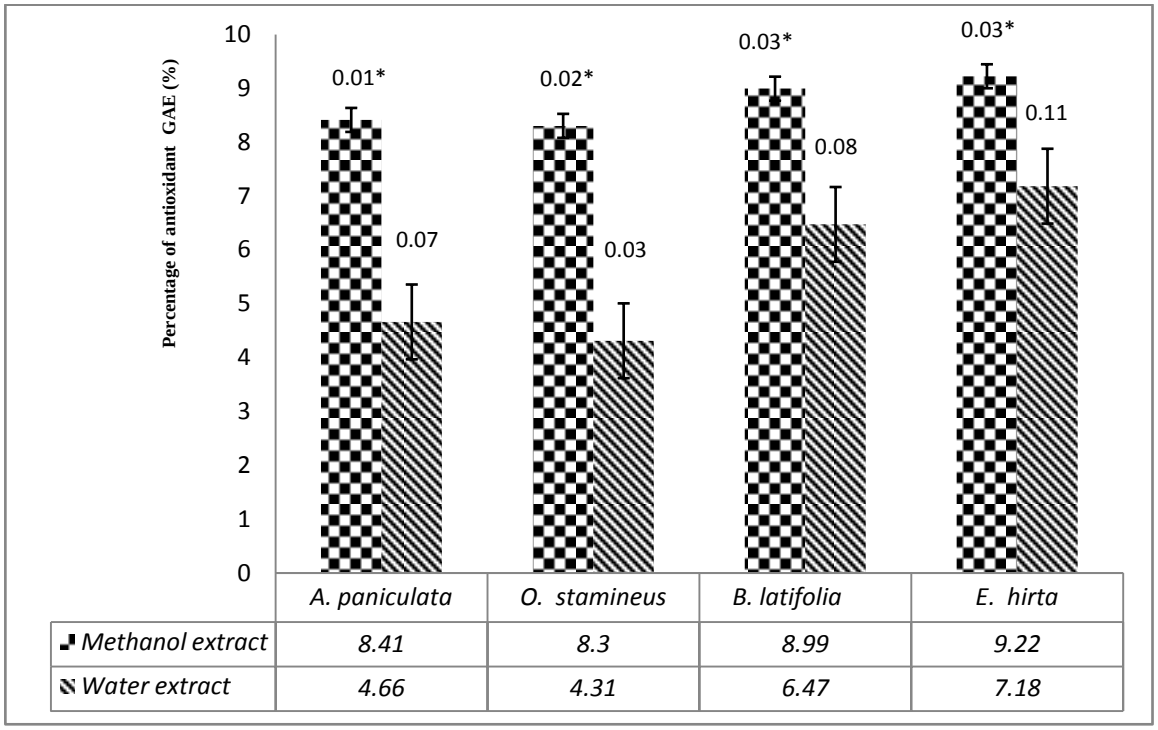

Figure 1: Antioxidant activity of methanol and water extracts of four local herbal plants

Table 4: Antimicrobial activity of four local herbal plants

		Microorganism	
Herbs (500mg/ml)	Extract	*E. coli*	*S. enterica*
A. paniculata	Water	-	-
	Methanol	**	**
O. stamineus	Water	-	-
	Methanol	**	*
B. latifolia	Water	-	-
	Methanol	*	*
E. hirta	Water	*	*
	Methanol	**	**
CP (positive control)	20µg/disc	***	***
Water (negative control)	20µl	-	-
Methanol (negative control)	20µl	-	-

IZ Inhibition zone (mm), - Lack of IZ, * IZ >10mm, ** 10mm<IZ <30mm, *** IZ >30mm CP Chloramphenicol

Table 5: Fatty acid profile of four local herbal plants

Fatty acid g/100g FAME	Herbal plants			
	A. paniculata	*O. stamineus*	*B. latifolia*	*E. hirta*
Lauric (C12:0)	4.16 ± 0.06^{b}	4.25 ± 0.01^{b}	7.01 ± 0.16^{c}	2.57 ± 0.25^{a}
Myristic (C14:0)	0.47 ± 0.09^{a}	2.43 ± 0.05^{b}	3.54 ± 0.15^{c}	2.37 ± 0.19^{b}
Myristoleic (C14:1)	1.88 ± 0.06^{b}	$1.36\pm0.23^{a,b}$	1.72 ± 0.16^{b}	0.88 ± 0.21^{a}
Palmitic (C16:0)	19.97 ± 0.15^{a}	40.56 ± 0.19^{c}	29.19 ± 0.39^{b}	39.81 ± 0.69^{c}
Stearic (C18:0)	1.93 ± 0.06^{b}	1.31 ± 0.01^{a}	1.85 ± 0.14^{b}	3.64 ± 0.19^{c}
Oleic (C18:1)	3.52 ± 0.06^{a}	12.14 ± 0.11^{c}	8.56 ± 0.15^{b}	12.54 ± 0.24^{c}
Linoleic (C18:2, n-6)	28.93 ± 0.23^{b}	33.18 ± 0.24^{c}	3.55 ± 0.15^{a}	34.84 ± 0.89^{c}
Linolenic (C18:3, n-3)	36.89 ± 0.30^{b}	2.45 ± 0.02^{a}	42.68 ± 0.91^{c}	2.03 ± 0.24^{a}
Ratio n-6:n-3	0.78 ± 0.01^{a}	13.57 ± 0.17^{b}	0.08 ± 0.01^{a}	19.49 ± 2.64^{c}
Erucic (C22:1 n-9)	2.26 ± 0.06^{b}	2.33 ± 0.12^{b}	$1.89\pm0.15^{a,b}$	1.34 ± 0.31^{a}
Total SFA	26.53 ± 0.19^{a}	48.54 ± 0.17^{c}	41.59 ± 0.47^{b}	48.38 ± 0.39^{c}
Total USFA	73.47 ± 0.19^{c}	51.46 ± 0.17^{a}	58.41 ± 0.47^{b}	51.62 ± 0.39^{a}

[abcd] Means with the same superscript in the same row are not statically different at $P\leq0.05$.
SFA- saturated fatty acid; USFA-unsaturated fatty acid

A. paniculata contained the lowest value of Myristic (C14:0), Palmitic (C16:0) and Oleic (C18:1) acids at 0.47±0.09, 19.97±0.15 and 3.52±0.06 g/100g FAME, respectively. Otherwise, *A. paniculata* contained the highest concentration of Linolenic acid (C18:3n-3) at 36.89±0.30 g/100g FAME compared to the other herbal plants. In addition, *O. stamineus* contained the lowest Stearic acid (C18:0) concentration at 1.31 ±0.01g/100g FAME and the highest Palmitic acid (C16:0) and SFA at 40.56 ±0.19 and 48.54±0.17 g/ 100 g FAME, respectively. As a matter of facts, ratio of Linoleic (C18:2 n-6) to Linolenic (C18:3 n-3) acid (n6:n3 ratio) of A. paniculata herbal plant was almost 1 (0.78±0.01). There was significantly no difference among the herbal plants with respect to the proportion of n-6 FA and n-3 FA.

Additionally, *A. paniculata* herbal plant was characterized by its highest concentration of unsaturated fatty acid (USFA) at 73.47±0.19 g/100g FAME and followed by *B. latifolia* at 58.41±0.47 g/100g FAME. In contrast to USFA, *A. paniculata* had the lowest saturated fatty acid (SFA) content (26.53±0.19 g/100g FAME), followed by *B. latifolia* at 41.59±0.47 g/100g FAME. Subsequently, *E. hirta* and *O. stamineus* had significantly high SFA compared to *A. paniculata* and *B. latifolia.* The range of unsaturated fatty acid and saturated fatty acid of the herbal plants was 51.46-73.47 g/100g FAME and 26.53-48.54 g/100g FAME, respectively.

Table 6 shows the values for *in vitro* gas production, *in vitro* digestibility and ammonia concentration and volatile fatty acid profile of the four herbal plants. *In vitro* gas production can be used to predict plant digestibility. Gas production of *E. hirta* was the highest (49.10 ml) and the fastest (2.05 ml/h) compared to the other herbal plants. Meanwhile, the slowest and lowest gas volume produced per h in 48 h was *A. paniculata* which was 0.9 ml/h and 21.48 ml, respectively. *A. paniculata* had significantly high percentage of IVDMD (67.73±5.89%) compared to *E. hirta* (51.38±2.89%) (Table 6). However, IVDMD of *A. paniculata* did not significantly differ from *O. stamineus* and *B. latifolia*. Meanwhile, *O. stamineus* had significantly higher IVOMD at 94.25±0.87% compared *B. latifolia* and *E. hirta*. On the other hand, the lowest IVOMD content was of *E. hirta* at 83.07% despite its highest and the fastest IVGP compared the other herbal plants.

Table 6: *In vitro* studies of four local herbal plants

	Herbal plants			
Parameters	*A. paniculata*	*O. stamineus*	*B. latifolia*	*E. hirta*
Total GP (mL)	21.48±5.35[a]	25.76±1.45[a]	35.41±2.79[b]	49.10±8.97[c]
Rate GP (mL/hr)	0.98±0.22[a]	1.07±0.06[a]	1.48±0.12[b]	2.05±0.37[c]
IVDMD (%)	67.73±5.89[a]	66.36±0.54[a]	66.72±6.14[a]	51.38±2.89[b]
IVOMD(%)	93.69±2.30[a]	94.25±0.87[a]	85.51±3.16[b]	83.07±0.62[c]
Ammonia (ppm)	1.88±0.17[b]	1.77±0.19[a]	1.88±0.17[b]	1.82±0.25[b]
Total VFA	75.15±6.81[a]	89.10±8.43[b]	67.71±11.90[c]	72.10±15.34[a]
Parameters	*A. paniculata*	*O. stamineus*	*B. latifolia*	*E. hirta*
Acetic : Propionate	2.83±0.17[a]	3.04±0.06[b]	2.73±0.13[a]	2.94±0.11[a]
Acetic (mg/L)	46.50±3.68[a]	55.06±4.50[b]	40.41±7.04[a]	45.69±10.22[a]
Propionate (mg/L)	16.70±2.24[a]	18.13±1.73[a]	14.92±2.71[b]	15.55±3.30[b]
Butyric (mg/L)	7.02±0.54[a]	8.89±1.07[b]	6.69±1.19[a]	6.04±0.93[a]

[abcd] Means with the same superscript in the same row are not statically different at P≤0.05.
GP-Gas production; IVDMD- *in vitro* dry matter digestibility; IVOMD- *In vitro* organic matter digestibility; VFA- Volatile fatty acid

In the present study, concentration of ammonia was significantly higher in rumen liquor containing *B. latifolia* (1.88 ± 0.17 ppm), *A. paniculata* (1.88±0.17 ppm) and *E. hirta* compared to *O. stamineus* (1.77±0.19 ppm). However, concentration of ammonia in *E. hirta* did not differ significantly with *B. latifolia* and *A. paniculata. O. stamineus* contained the highest of total VFA content which was 94.25±0.87 mg/L compared to other herbal plants. Meanwhile *B. latifolia* contained the lowest total VFA which was at 67.71±11.90 mg/L. Total VFA content for *A. paniculata* and *E. hirta* were not significantly different. Ammonia content had a significant positive correlation (r =0.661) with propionate. Both total IVGP and rate of GP were significantly correlated with ADF (r=0.613) and NDF (r=0.614) but had negative correlation with CP (r=-0.733), ash (r=-0.763) and IVOMD (r =-0.721).

The dry matter content of herbal plants is a very important parameter to estimate dry matter yield of the herbal plants. Herbal plants of high water content will have lower percentage of dry matter. Dry matter also acts as an indicator of the amount of nutrients that are available in a particular feed. Based on Yvette Fofie *et al.* (2015), the moisture content of *E. hirta* was 7.73% ± 0.00% and total ash 7.48 ± 0.03%. This result is in agreement with the results of the current study that *E. hirta* had 93.34± 0.03% dry matter. The total ash of E. *hirta* after drying in 660ºC oven for 6 h was 8.22±0.08%. According to Mokoboki *et al*. (2005), low levels of the neutral detergent fiber were associated with high voluntary DM intake in ruminants. The higher the ADF content, the lower the digestibility or available energy of these herbal plants. Apart from that, forages with low crude protein and energy feedstuff

also will affect feeding acceptability, beside high tannin and ADF content will also reduce digestibility.

Generally, the optimum level of calcium in plants ranges from 0.31 to 1.98% (Minison, 1990; Skerman and Riveros, 1990), making it sufficient to fulfill the maintenance and production requirements of most animals of 0.17 to 0.42% (Sultan *et al*., 2009). If the level of calcium in plants was above 1.0%, it is considered to have high calcium content (Khan *et al.,* 2007). *A. paniculata* has the highest calcium content at 11.92 mg/L (1.19%), two and three times more than the other herbs. Based on Burgos *et al.* (2000), *A. paniculata* has the ability to select block voltage operated calcium channels and hence able to inhibit the calcium influx.

Apart from that, *A. paniculata* had the highest saponin content at 18.73% compared to the other herbal plants. The effect of feeding plants with high saponin content would enable producers to increase animal production comparable to feeding antibiotic and other synthetic chemicals (Sultana *et al.*, 2012). These results indicate that saponin has strong antiprotozoal activity and might be able to serve as an alternative to antibiotics in feed or growth hormone that can used for ruminants. On the other hand, *E. hirta* had the highest level of tannin content at 0.24% compared the other herbal plants. Based on Kariuk and Norton (2008), higher level of tannin of more than 0.2% can impact negatively on digestibility by its ability to bind to protein and carbohydrate modifying the rate and extent of their digestion. Little or moderate level of tannin may possibly reduce animal protein breakdowns and increase duodenal protein flow. *E. hirta* and *A. paniculata* also have the high level of phenol and flavonoid at 0.02% and 1.25%, respectively. Cook and Samman (1996) mentioned that the antioxidant activity of plants might be due to their phenolic compounds such as phenol and flavonoid. According to Wong et al. (2006), the antioxidant activity of the plant extracts basically depended on many factors such as the composition of extract, natural antioxidant, type of solvent used for extraction process (hydrophobic or hydrophilic), method of extraction, temperature and condition of test system.

Several studies have reported that there was a high positive linear relationship between antioxidant activity, antibacterial activity and total phenolic content in herbal plants (Shan e*t al*., 2007). The results on antioxidant and antimicrobial activities showed that methanolic extract is significantly higher than water extract. This finding also is consistent with several reports on the effectiveness of methanolic extraction in deriving bioactive compounds of the herbal plants due to the discrepancies in the level of bioactive compounds in all medicinal plant extracts which could be related to polarity and the composition of bioactive compounds (Akhowuah *et al*., 2004; Malahubban et al., 2013).

Quantifying fatty acid concentration and profile in the herbal plants could help in designing management strategies to increase precursors for beneficial fatty acids in animal feed. Based on Dewhurst et al. (2003) study, leaf content is very important in determining fatty acid content. The application of fertilizer would be able to increase palmatic, linoleic and linolenic acids in herbage and then causing an overall increase in fatty acid concentration. Based on Elgersma *et al*. (2006), lipid in plants is not a static entity. It is said to be due to lipid degradation which is a normal process in the living plants. In addition, it also due to lipase that is normally present. Usually under normal growing condition, this will not have an important influence on the fatty acid composition of the lipid fraction in plants. However, there is probability at least three times when the lipid

fraction in plants may significantly modify, being senescence, after detachment process such as grazing or cutting and during storage.

In vitro gas production can also be used to predict plant digestibility (Getachew *et al*., 1998). This is due to its high association with digestibility (Khazaal *et al.*, 1993). Low fibre content would result in higher digestibility. This was proven by the current result stating that ADF had a significant negative correlation with in vitro digestibility. In addition, tannin has an inverse relationship with IVDMD but positive relationship with IVGP. Getachew *et al.* (2000) reported similar finding between tannin and IVGP but had a negative relationship between tannin and digestibility ((Mokoboki *et al.*, 2005)). The high IVDMD in *A. paniculata* was presumably due to low acid and neutral detergent fibre and tannin. But IVGP was the lowest compared to the other herbal plants and may due to moderate level of tannin since tannin will interfere with plant digestibility. Tannin had greater influence on digestibility and fermentation than acid and neutral detergent fibers.

Conclusion

These nutrient analyses suggest that all herbal plants have their own advantages. Thus all four herbal plants could be utilized as a cheap source of nutrients.

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