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Research Article

Nutritive Assessment of Four Local Herbal Plants in Malaysia as Animal Feed Supplements: Chemical Composition, Antioxidant Properties, Antimicrobial Activity, Fatty Acid Profile and *In Vitro* Studies

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Abstract

Local herbal plants were the herbaceous plant that can be found locally and are generally rich with secondary metabolites, low cost and contain high amount of essential nutrients especially for health purpose. Proximate analysis, phytochemical determination and *in vitro* technique were used to evaluate nutritive value in term of chemical composition of those herbal plants. Meanwhile, fatty acid profile, *in vitro* studies, and 2,2-diphenyl-1-picrylhydrazyl (DDPH) free radical scavenging activity was carried out to evaluate the antioxidant content yet disk diffusion method was used for antimicrobial activity the four selected herbal plant, *Andrographis paniculata* (*Hempedu Bumi*), *Orthosiphon stamineus* (*Misai Kucing*), *Euphorbia hirta* (*Ara Tanah*) and *Borreria latifolia* (*Borreria*) that widely available in Malaysia. *A. paniculata* had the highest content of protein (18.13 ± 0.18%), calcium (11.92 ± 1.66%), Saponin (18.73 ± 1.13%) and flavonoid (1.25 ± 0.21%). while, *E. hirta* contain 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 extract at a concentration of 500 mg/ml showed moderate antimicrobial activities. The methanol extracts of all herbal plants exhibited stronger antimicrobial activities against the tested pathogens as compared to the herbal water extracts. *A. paniculata* contains lowest of total saturated fatty acids (26.53 ± 0.19 g/100g FAME) and highest unsaturated fatty acids (73.47 ± 0.19 g/100g FAME). On the other hand, *E. hirta* had the highest total gas production (49.10 ± 8.97ml), rate of gas production (2.05 ± 0.37 ml/hour). All this herbal plant has their own potential as choice for animal feed supplement.

Keywords: *Chemical Composition; Antioxidant Properties; Antimicrobial Properties; In Vitro Technique*

Introduction

Malaysia is identifying as one of the world's twelve mega diversity area with extremely rich biological resources. The examples of biological resources are its various herbal plants species. Several herbal plants such as *Andrographis paniculata* (*Hempedu Bumi*), *Orthosiphon stamineus* (*Misai Kucing*), *Euphorbia hirta* (*Ara Tanah*) and *Borreria latifolia* (*Borreria*) were examples of local herbal plants that widely available in Malaysia. These herbal plants had been utilized for folk medicine and also had taken as dietary supplementation. The antimicrobial and antioxidant characteristics of these herbal plants were due to the presence of various phytochemical such as flavonoid, phenol, tannins and tocopherol [1]. Herbal plants are still the mainstay of people in the world, mostly in the developing countries, for cure and improving general health purpose. Worldwide, herbal plant have become mainstream in late of 20th century due to widespread acceptance of importance of local herbal plants remedies an also integration of derivatives from natural source in pharmaceutical products. These herbal plants generally contain high amount of essential nutrients, like fatty acid, minerals, vitamins and plant protein [2]. These herbal plants could contribute to the intake of essential nutrients and toxic metal but not much information on the

chemical composition of these medicinal plants especially in term of phytochemical, antioxidant and antimicrobial content, *in vitro* dry matter and organic matter digestibility, volatile fatty acid and also fatty acid profile is available. Hence, the objectives of this study were to determine the nutrient content of the local herbal plants that will achieve from their use in marginal communities. Chemical composition, rumen fermentation and digestibility can be used as proxies for nutritive value of herbal plant. Chemical composition parameters include crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), phytochemical substances such as tannin, saponin, phenol and etc. Medicinal value of the herbal plants lies on the chemical active substances that present in those herbal plants. Future of using these herbal plants in human and animal nutrition will in great measure depend on the knowledge of the chemical composition, their value and characteristic of practical herbs or their extract.

Materials and Methods

Plants collection

Four local herbal plants were collected in Universiti Putra Malaysia 2.99917°N 101.70778°E. These plants were botanically and taxonomically identified by department of botany of the UPM. The part used are Arial part. The details of each plant species, their local names were elaborated in table 1.

Species name	Family name	Local name
Andrographis paniculata	*Acanthaceae*	Hempedu Bumi
Orthosiphon stamineus	*Lamiaceae*	Misai Kucing
Euphorbia hirta	*Euphorbiaceae*	Ara Tanah
Boreria latifolia	*Rubiaceae*	Boreria

***Table 1**: Experimental herbal plants.*

Chemical compounds determination

The proximate analyses (moisture, ash, crude proteins, and ether extract content of samples were analyzed according the methods of Association of Official Analytical Chemists (AOAC) [3]. Meanwhile for content for crude fibre that composed of Acid detergent fiber (ADF), Neutral detergent fiber (NDF) and Acid detergent lignin (ADL) was analyzed following Van Soest detergent fiber analysis system [4]. While gross energy content was determined using Fully Automatic IKA® Adiabatic Bomb Calorimeter C2000 and calcium and phosphorus content (mg/L) were determined by Atomic Absorption Spectrophotometer (Emission flame photometry: PSS-AVR-Model SS 103). The phytochemical compounds include alkaloid, flavonoid, phenol, tannin, saponin, and hydrogen cyanide were determined based method in Mbagwu., *et al* [5].

Determination of antioxidant using DPPH radical-scavenging activity

The antioxidant content in herbal plants was determined according Sreeramulu and Raghunath [6], with slight modification using gallic acid as standard solution. An aliquot of 2.9 ml of 0.1 mM DPPH radical in methanol was mixed with 0.1 ml of methanolic or aqueous extract of the sample. After incubation in dark for 30 minutes at 28°C and then the absorbance of the cuvette was reading at 517 nm using Thermo spectronic GENESYS 20 spectrophotometer. The DPPH radical-scavenging activity in extracts was express out as percentage of inhibition (%) of gallic acid.

Disk diffusion method to determine antimicrobial sensitivity

The antibacterial potential of selected herbal plants extracts was assessed from their reaction against two bacterial cultures using the Kirby-Bauer disc diffusion method [7,8]. Pure cultures of Escherichia coli and Salmonella bacteria were provided by Bacteriology laboratory, Faculty of Veterinary Medicine, UPM. The bacterial culture (10^8 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 24h. After that antibacterial activity was determined by measuring the diameter of the inhibition zone formed around the disk. The inhibition zone bigger than 15 mm was considered as strong activity, the zone from 10 to 15 mm was considered as moderate activity and the zone smaller than 10 mm as weak activity.

Determination of fatty acid composition

Fatty acid composition of medicinal plant was determined following the methods of Peterson., *et al*. (2011) with slight modification. Fatty acid compositional analysis of lipids of this medicinal plant was carried out using Agilent 6890N gas chromatography (GC) equipped with an injector and an FID detector and using a 30m x 0.32 mm, 0.2 µm thickness, Supelco 123-2332 capillary column (Supelco, Inc., Bellefonte, PA, USA). Twenty percent of boron trifluoride 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 with a temperature programmed from 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 selected herbal plants was tested following the procedure of Menke and Steingass [9] to determine the extent to which they affect fermentation via digestibility and biohydrogenation in the laboratory. Meanwhile, procedure for Ammonia determination and gas production was determined as modified from Parsons., *et al*. (1984) and of Tilley and Terry [10] respectively.

Statistical analysis

All the experiments were done in triplicate and the data generate were analyze using descriptive statistic, analysis of Variances and correlation analysis with SPSS 17 (StatSoft Inc., Tulsa, OK).

Results

Table 2 show proximate composition, gross energy content and phosphorus and calcium content of four selected local herbal plants. Nutrient composition was varied several fold among herbal plants. *Euphorbia hirta* had significantly higher fresh dry matter compares to *Borreria latifolia* and *Orthosiphon stamineus* at 36.56 ± 1.39%. However, percentage of dry matter content of *Euphorbia hirta* and *Andrographis paniculata* (34.56 ± 0.73%) do not different significantly at ($p < 0.05$).

Chemical	Herbal plant (Mean ± SEM)			
Composition	***A. paniculata***	***O. stamineus***	***B. latifolia***	***E. hirta***
Fresh DM (%)	$34.56 \pm 0.73^{b,c}$	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}	$43.8 \pm 0.92^{c, d}$	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 (%)	$7.17 \pm 0.09^{a,b}$	22.27 ± 2.69^{c}	$9.87 \pm 1.13^{a,b}$	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}

Table 2: *Proximate composition and gross energy content of four local herbal plants.*

$^{a-d}$: Mean with the same superscript in the same row is not statically difference at $P \leq 0.05$. GE-Gross Energy.

A. paniculata have lower ADF content (18.80 ± 0.26%) but have higher protein content which was at 18.13 ± 0.18%. Meanwhile *B. latifolia* have highest DM content and highly indigestible part (ADF) of forage but has 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 ± 0.07a% respectively. On the other hand, *O. stamineus* have quite high percentages of NDF, which was 43.8 ± 0.92% compared to other herbal plant. The lignin content in *O. stamineus* was higher than other herbal plants (22.27 ± 2.69%). Lignin has no nutritive value, except it as a bulk factor. However, at a high level, it reduces digestibility of other nutrients in a ration. On contrary, *E. hirta* has lowest in ash and crude protein content compared to other herbal plants which 8.22 ± 0.08% and 8.84 ± 0.07% respectively. In spite of having lowest protein content, it has highest crude fat content at 2.98 ± 0.65% if compared with other herbal plants.

Different from *O. stamineus* and *E. hirta, A. paniculata* have the lowest lignin and crude fat content, however, this herbal plant contains high protein and the highest energy content at 18.13 ± 0.18% and 16.68 MJ/kg respectively. At same time, *A. paniculata* had the highest ash percentages that representative of inorganic compound or mineral. Mineral was another chemical element. The examples of macro mineral were calcium and phosphorus. *A. paniculata* has the highest calcium content which was 11.92 ± 1.66 mg/L, two and three time more than other herbal plants. Meanwhile, *O. stamineus* have highest phosphorus content (9.69 ± 0.54 mg/L) compared to other herbal plants. On contrary *B. latifolia* contains the lowest calcium and *E. hirta* contain the lowest phosphorus content which were at 2.83 ± 0.21 mg/L and 5.12 ± 0.36 mg/L respectively. The calcium concentration in herbal plants was varied from 2.83 mg/L to 11.92 mg/L and the mean of calcium in plant was 5.89 ± 2.06 mg/L.

Analysis of phytochemical compound content in herbal plants in table 3 indicate the present of alkaloids, saponin, tannins, flavonoids, phenols and hydrogen cyanide at various level. *A. paniculata* have higher flavonoid content compared other at 1.25 ± 0.21%. It also has the highest saponin content compared to other herbal plants at 18.73 ± 1.13%. Meanwhile, *E. hirta* have the highest value of tannin and phenol content at 0.24 ± 0.007% and 0.02 ± 0.001% respectively. Tannin content also has a highly significant positive correlation (r = 0.981) at P < 0.001 with phenol. This may explain why *E. hirta* has a high percentage in both tannin and phenol content.

Phytochemical	Herbal plants (Mean ± SEM)			
Content	*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 ± 1.13^{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}

Table 3: *Phytochemical compound determination of four local Herbs.*

$^{a-d}$: Mean with the same superscript in the same row is not statically difference at P ≤ 0.05. HCN- Hydrogen cyanide.

Figure 1 show 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 solvent in term of % inhibition Gallic acid equivalent (GAE). Among the herbal plants solvent, the methanolic extract herbal plant, *E. hirta* show highest percentage of antioxidant Gallic acid equivalent (GAE) at 9.22% as compared with the other herbs attempted and significantly higher than water solvent. The water extract exhibited significantly lower DPPH scavenging activity as compared with methanol extract from each herbal plant tested.

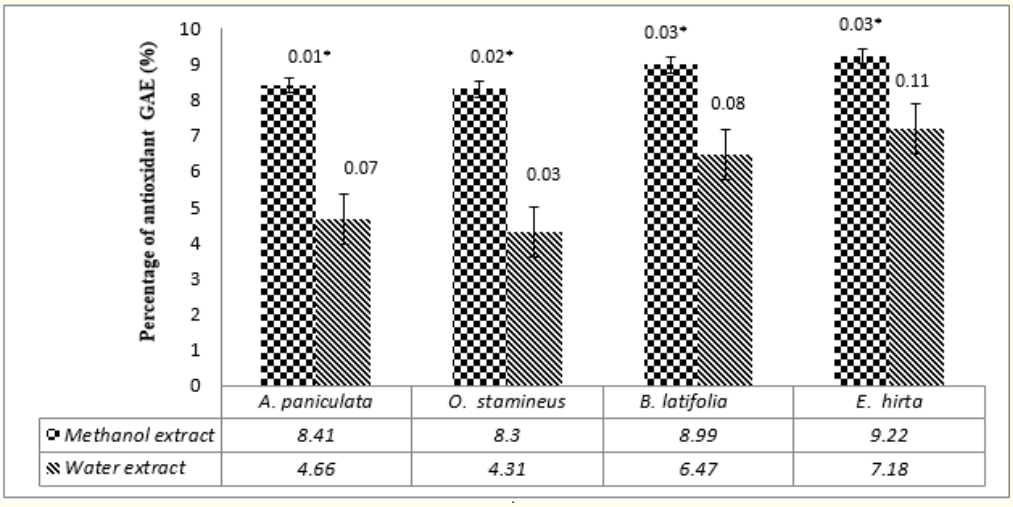

Figure 1: *Antioxidant activity of four local herbal plants.*

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

Table 4: *Antimicrobial activity of four local herbal plants.*

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

Table 5 show the antimicrobial activity of four local herbal plants. In general, all herbal plants extract in methanol solvent exhibit at least some degree of bacterial growth inhibition. Among the treatment 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 extract, the methanolic extract of *E. hirta* and *A. paniculata* have same effect on both bacteria tested at 500 mg/disc and followed by *O. stamineus* that also have same effect on *E. coli* but unable inhibit the growth of *S. enterica* to more than 10 mm diameter. On the other hand, *B. latifolia* unable inhibited the growth of both bacteria tested to more than 10 mm diameter.

Fatty acid g/100g FAME	Herbal plant			
	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 ± 0.23^{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 ± 0.15^{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}

Table 5: *Fatty acid profile of herbal plants.*

$^{a-d}$: Mean with the same superscript in the same row is not statically difference at P≤0.05. SFA- saturated fatty acid; USFA-unsaturated fatty acid.

A. paniculata contain the lowest 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* contain the highest of Linolenic acid (C18:3n-3) at 36.89 ± 0.30 g/100g FAME compared to other herbal plants. In addition, *O. stamineus* contain lowest Stearic acid (C18:0) at 1.31 ± 0.01g/100g FAME and contain the highest Palmitic acid (C16:0) and SFA at 40.56 ± 0.19 and 48.54 ± 0.17 g/ 100 g FAME respectively. As matter of facts, ratio Linoleic (C18:2 n-6) to Linolenic (C18:3 n-3) acid (n6:n3 ratio) of *A. paniculata* herbal plants was almost 1 which was 0.78 ± 0.01. There was significantly difference between 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 the highest contribution of unsaturated fatty acid (USFA) (73.47 ± 0.19 g/100g FAME) and followed by *B. latifolia* at 58.41 ± 0.47 g/100g FAME. In contrast with USFA, *A. paniculata* had lowest saturated fatty acid (SFA) content (26.53 ± 0.19 g/100g FAME) and 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 are around 51.46 - 73.47 g/100g FAME and 26.53 - 48.54 g/100g FAME respectively.

Table 6 show the value of *in vitro* gas production, *in vitro* digestibility, ammonia concentration and volatile fatty acid profile of 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/hour) compared to other herbal plants. Meanwhile, result shows that, the slowest and lowest of gas produced per hour in 48 hours was *A. paniculata* which is that 0.9 ml/hour and 21.48 ml respectively Based on table 6, *A. paniculata* have significantly high percentages of IVDMD (67.73 ± 5.89%) compared to *E. hirta* (51.38 ± 2.89%). However, IVDMD of *A. paniculata* do not significantly different to IVDMD of both *O. stamineus* and *B. latifolia*. Meanwhile, *O. stamineus* have significantly high IVOMD at 94.25 ± 0.87% compared *B. latifolia* and *E. hirta*. On the other hand, the lowest IVOMD content was *E. hirta* at 83.07% despites had the highest and the fastest IVGP compared other herbal plants. In current study, concentration of ammonia was significantly higher in rumen liquor that 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* does not different significantly with *B. latifolia* and *A. paniculata*. *O. stamineus* contain the highest of total VFA content which was 94.25 ± 0.87 mg/L compared to other herbal plants. Meanwhile *B. latifolia* contain the lowest total VFA which was at 67.71 ± 11.90 mg/L. Total

VFA content for *A. paniculata* and *E. hirta* was not significantly different to each other. Ammonia have significant positive correlation (r = 0.661) with propionate at $p < 0.05$. Both total IVGP and rate of GP was significantly have positive correlation with ADF (r = 0.613) and NDF (r = 0.614) at $p < 0.05$ and significantly have negative correlation with CP (r = -0.733), ash (r = -0.763) and IVOMD (r = -0.721) at $p < 0.001$.

Parameter	Herbal plants			
	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)	0.9 ± 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]
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]

Table 6*: In vitro studies of herbal plants.*

Discussion

The dry matter content of herbal plants were very important parameter to estimate the dry matter yield of the herbal plants. Herbal plants that had high water content will have lower percentages of dry matter. Dry matter also as an indicator of the amount of nutrients that were available to the animal 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 was almost agree with current study that show that *E. hirta* powder have 93.34 ± 0.03% dry matter that equivalent to 6.66% of moisture. The total ash of *E. hirta* in 660ºC for 6 hour was 8.22 ± 0.08% and the total ash of *E. hirta* in current study (550ºC for 3 hour) was 7.48%. In addition, the presence of high fibre level in diet is good. However, if too much, it can caused intestinal irritation, lower digestibility and also overall decreased nutrient utilization. Based on Mokoboki., *et al*. [11] low levels of the detergent fiber are associated with high voluntary DM intakes in ruminants. The higher of the ADF content, the lower the digestibility or available energy of those herbal plants. Apart from that, forages with low crude protein and energy feedstuff also will affect feeding acceptability In addition, high tannin and ADF content also will reduce digestibility.

Mineral was another chemical element. The examples of macro mineral were calcium and phosphorus. The variation of calcium level in plant was varies. Generally, the optimum level of calcium in plants ranged from 0.40 to 0.60% or ranging from 0.31 to 1.98% (Minison, 1990; Skerman and Riveros 1990.). If the level of calcium in plant was above than 1.0%, it was considered to have high calcium content [12]. The calcium level requirement for livestock is within the range of 0.17 to 0.42%. It was enough to fulfill its maintenance and production requirements [13]. *A. paniculata* has highest calcium content which was 11.92 mg/L (1.19%), two and three times more than other herbs. Based on Burgos., *et al*. [14] *A. paniculata* has ability to select block voltage operated calcium channels and hence able to inhibit the calcium influx.

Apart from that, *A. paniculata* have the highest Saponin content compared to other herbal plants at 18.73%. The effect of feeding plant that containing high Saponin able to increase animal production that was comparable to feeding antibiotic and other synthetic chemical [15]. These results have indicated that saponin have strong antiprotozoal activity and might able to serve as an alternative to antibiotics in feed or growth hormone that can used for ruminants. On the other hand, *E. hirta* have highest level of tannin content at 0.24% compared other herbal plants. Based on Kariuk and Norton (2008), higher level of tannin more than 0.2% can impact negatively on digestibility by their ability to bind to protein and carbohydrates modifying the rate and extent their digestion. Meanwhile little or moderate level of

tannin may possible reduce animal protein break down and increases duodenal protein flow. *E. hirta* and *A. paniculata* also has the highest level of phenol and flavonoid at 0.02% and 1.25% respectively. Cook and Samman [16] been 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 depends on many factors such as the composition of extract, nature antioxidant, type of solvent used for extraction process (hydrophobic or hydrophilic), method of extraction, temperature and condition of test system. Some studies have reported that, there was highly positive linear relationship between antioxidant activity, antibacterial activity and total phenolic content in herbal plants (Shan., *et al*. 2007). The result for antioxidant and antimicrobial activity show that methanolic extract is significantly higher than water extract. This finding also was consistent with several reports on the effectiveness of methanolic extraction in extraction of bioactive compounds of the herbal plants due to the discrepancies in the level of bioactive compound in the all medicinal plant extract were could be related to polarity and the composition of bioactive compounds [1,17].

Quantifying fatty acid concentration and profile in the herbal plants could help in design of management strategies to increase precursors for beneficial fatty acid in animal feed product. Based on Dewhurst., *et al.* (2003) study, leaf content is very important in determining fatty acid content. Meanwhile in other study stated that application of fertilizer able to increase of palmatic, linoleic and linolenic acid in herbage and then caused overall increase in fatty acid concentration. Based on Elgersma., *et al*. (2006), lipid in plant was not static entities. It was due to lipid degradation which is one of a normal process in the living plant. 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 modified, 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 [18]. This is due to this parameter was proved to be positively associated with digestibility [19]. Low fibre content result in higher digestibility. This was prove by current result which is ADF have significantly negative correlation with *in vitro* digestibility at $p < 0.05$. In addition, tannin has inverse relationship with both IVDMD but positive relationship with IVGP. Other researcher reported similar finding between tannin and IVGP [20] but oppositely for relationship between tannin and digestibility [11]. 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 other herbal plants and may due to moderate value of tannin since tannin will interfere with plant digestibility. Tannin had greater influence on digestibility and fermentation than acid and neutral detergent fibre [21].

Conclusion

These nutrient analyses suggest that all herbs have their advantageous. In view of present study, all these herbs could be utilized as a cheap source of nutrient but high in advantageous. Further studies on isolation of the antifungal agents and toxological evaluation of plant extract and also pharmacological advantages of the herbal plants were recommended.

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