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Nutritional Values and Amino Acid Profiles of *Clinacanthus nutans* (Belalai Gajah/ Sabah Snake Grass) from Two Farms in Negeri Sembilan, Malaysia.

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ABSTRACT

The objective of the study was to determine the content of moisture, ash, protein, fat, carbohydrate, crude fibre, total sugar and amino acid profile for the medicinal herb *Clinacanthus nutans*. Three-month-old Sabah Snake Grass/Belalai Gajah (*C. nutans*) was collected from You Dun Chao Herb Farm (YDC) and Yik Poh Ling Herb Farm (YPL) in Negeri Sembilan, Malaysia. All the experiments were conducted in triplicate. Total crude fibre was found significantly higher in the stem samples. A comparison of non-shaded and shaded samples from YDC revealed higher ($p<0.05$) moisture, protein, ash, total crude fibre and total sugar content in the shaded samples for both leaves and the stem. Total fat was higher ($p<0.05$) in the shaded leaves than in the non-shaded leaves but it was the opposite for the stem. In comparing non-shaded samples from the two different farms for moisture, protein, ash, fat and total sugar content, the YPL leaves and stems showed significantly higher amounts than the YDC samples. The leaves of *C. nutans* contained more amounts of all essential and non-essential amino acids than the stem. Aspartic acid exhibited significantly higher amounts in both leaves (3.48, 1.08 and 2.13% of dry weight sample for YPL, YDC non-shaded and shaded samples, respectively) and stem (2.17, 0.95 and 1.96% of dry weight sample for YPL, YDC non-shaded and shaded samples, respectively) than the other amino acids. Geographical factors and planting conditions revealed different nutritive composition.

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INTRODUCTION

Clinacanthus nutans (Burm.f.) Lindau is a native medicinal herb that grows in tropical

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climate, and is mainly found in Malaysia. *C. nutans* is commonly called Sabah Snake Grass or 'Belalai Gajah'. However, there is limited information on this plant and its benefits have been under-cultivated. *C. nutans* has been listed as a research target in NKEA Malaysian Herbal Monograph 2012 and EPP#1 Research Grant Scheme. *C. nutans* belongs to the domain *Eukaryote*, kingdom *Plantae*, sub-kingdom *Viridaeplantae*, phylum *Tracheophyta*, subphylum *Euphyllophytin*a, infraphyllum *Radiatopses*, class *Magnoliopsida*, subclass *Asteridae*, superorder *Lamianae* and order *Lamiales*, family *Acanthaceae* and genus *Clinacanthus* with the specific epithet *nutans*. It is a shrub plant, which can be grown by stem propagation method. *C. nutans* is used in home decoration, teas and the bath (Siew et al., 2014).

The washed leaves of *C. nutans* can be freshly eaten or blended with apple and drunk as fruit juice. *C. nutans* is also used to treat skin affections, insect and snakebites and swellings due to a fall or boils (Chiwapreecha, Janprasert, & Kongpakdee, 2014). The phytochemical compounds that can be found in *C. nutans* (Burm.f.) Lindau plants are flavonoids, betulin, phytosterols such as stigmasterol, lupeol and β-sitosterol, saponin and diterpenes, which contribute to antimicrobial and anti-inflammatory properties (Sakdarat, Shuyprom, Pientong, Ekalaksananan, & Thongchai, 2009; Yang, Peng, Madhavan, Shukkoor, & Akowuah, 2013). The phenolic acids and flavonoids that are found in the *C. nutans* contribute to antioxidant activities. Petroleum ether extract of *C. nutans* has the radical scavenging activity of 82.0±0.02%, compared with ascorbic acid and α-tocopherol corresponding values of 88.7±0.0 and 86.6±0.0%, respectively (Arullappan, Rajamanickam, Thevar, & Kodimani, 2014). Chloroform leaf extract of *C. nutans* shows good antioxidant activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and galvinoxyl radicals, but is less effective in negating nitric oxide and hydrogen peroxide radicals (Yang et al., 2013, pp. 349–355). No study was found covering the nutritive aspects of *C. nutans* such as moisture, ash, protein, fat, carbohydrate, crude fibre, total sugar and amino acid profile. The objective of this study was to identify the quantity of nutrients in *C. nutans* in two farms in Malaysia, You Dun Chao Herb Farm (YDC; shaded and non-shaded samples) and Yik Poh Ling Herb Farm (YPL; non-shaded samples) in Negeri Sembilan.

MATERIALS AND METHOD

Three-month-old Sabah Snake Grass/ Belalai Gajah (*C. nutans*) was collected from You Dun Chao Herb Farm (YDC) and Yik Poh Ling Herb Farm (YPL) located in Negeri Sembilan, Malaysia. YDC farm had planted *C. nutans* in two conditions, shaded and non-shaded, while YPL farm had planted *C. nutans* only under the non-shaded condition. The YPL farm was located in a hilly area, while the YDC farm was situated on flat land. The samples were collected in triplicate. The leaves and stem were washed with water to remove sand and

dust particles. The leaves and stem were then freeze-dried using the ALPHA freeze dryer (Hampshire UK) and homogenised to 0.5 mm using the Universal cutting mill (FRITSCH, Germany) before analysis. All the chemicals used were from Sigma-Aldrich, USA. All the experiments were conducted in triplicate.

Determination of Moisture Content

About 5 g of dried leaves and stem of *C. nutans* samples were weighed using an aluminium dish and placed overnight in an oven at 105°C. Then, the samples were cooled down in a dessicator. The samples were weighed after they had attained room temperature and then re-weighed. The process was repeated until the difference in two successive weighings was less than 1 mg (Nielsen, 1994). The percentage of moisture was calculated using the formula below:

$$\text{Percentage of moisture (\%)} = \frac{(W1 - W2) \times 100}{S}$$

where
W1 = sample weigh (g) before drying
W2 = sample weigh (g) after drying
S = sample weigh (g)

Determination of Ash Content

About 5 g of dried leaves and stem of *C. nutans* samples was weighed using tared silica. The samples were heated on an electric heating mantle (Favorit®, Malaysia) in the fume hood till fumes were no longer produced. The sample was transferred onto a muffle furnace (Nabertherm, Germany) and the temperature was set at 550°C until the ash was free of carbon (overnight). The sample was cooled down in a dessicator and weighed. The process was repeated until the difference between two successive weighings was less than 1 mg (Nielsen, 1994). The formula below was used to determine the ash content of the sample:

$$\text{Total ash (g)} = \text{Weight of tared silica with ash} - \text{Weight of tared silica}$$

$$\text{Percentage of total ash (\%)} = \frac{\text{Weight of ash} \times 100}{\text{Weight of sample}}$$

Determination of Protein Content

About 0.5 g of dried leaves and stem of *C. nutans* samples was placed in a digestion tube (Favorit®, Malaysia). About 3 to 5 g of catalyst (mixture of potassium sulphate and copper sulphate in 10:1 ratio) and about 12 to 13 ml of concentrated sulphuric acid was added into the digestion tube. Then, the digestion tube was shaken gently and placed in a Tecator™ Digestor (FOSS, Denmark). The sample was digested until the colour green was observed. The homogenised sample was distilled using the Kjeltec™ 8200 Auto Distillation Unit (FOSS, Denmark). The green coloured receiver solution was titrated with 0.096N of hydrochloric acid, HCl, until the colour changed to red. The volume of used hydrochloric acid was noted (AOAC, 1990). The percentage of nitrogen present in the sample was calculated using the formula below:

$$\text{Percentage of nitrogen (\%)} = \frac{0.1 \times (S - B) \times 14 \times 100}{W \times 1000}$$

where

S = Volume of used hydrochloric acid for sample

B = Volume of used hydrochloric acid for blank

W = Weight of sample

Percentage of crude protein (%) = percentage of nitrogen X 6.25

Determination of Fat Content

About 1 to 2 g of the dried leaves and stem of *C. nutans* samples (W1) was weighed using Whatman filter paper wrapped neatly and placed in a thimble. The thimble was placed on a thimble holder. A thin layer of cotton was placed on the top of the sample. The thimble was moved to a thimble support. The pre-dried extraction cup was weighed (W2). About 50 ml of hexane was poured into an extraction cup using a measuring cylinder, after which the extraction cup was placed on a cup holder that was attached to the Soxtec™ 2043 Extraction Unit (FOSS, Denmark). After that, the extraction cup was transferred to an oven and dried at 105°C for 1 hr. The extraction cup was cooled down in a dessicator until it reached room temperature. The extraction cup with fat was weighed (W3) (AOAC, 1997). The percentage of crude fat in the sample was calculated using the formula below:

$$\text{Percentage of crude fat (\%)} = \frac{W3 - W2 \times 100}{W1}$$

Determination of Carbohydrates

The percentage of carbohydrates in the sample was calculated using the formula below:

Total carbohydrates = 100 − (moisture + proteins + fat + ash)

where moisture, proteins, fat and ash stand for their masses, respectively, expressed in units of 1 g.

Determination of Total Crude Fibre

The capsule cap and lid were weighed (*W1*). Next, 1 g of the sample was weighed (W2) into the capsule and sealed. One empty capsule was sealed with no sample due to correction (W4). The sealed capsule was placed on a hot plate FiberCap 2022 (FOSS, Tecator™ Technology, Denmark) and 350 mL of 1.25% sulfuric acid (H_2SO_4) was added. The heating temperature was set to 100°C. After 30 min, the capsule was re-washed in hot distilled water several times and 350 mL of 1.25% sodium hydroxide (NaOH) was added. The temperature was set to 100°C and the reaction time was set to 30 min. Afterwards, the samples were re-washed using hot distilled water and 350 mL of 1% hydrochloride acid (HCl). The heating temperature was set to 100°C. After 30 min, the capsule was re-washed in hot distilled water several times. Subsequently, the samples were kept in an oven at 105°C for 5 h and after cooling in the desiccator, weighed (W3). Finally, all the capsules were burnt in a muffle furnace at 550°C for 5.5 h and after cooling in the desiccator,

they were weighed (*W5*). The crude fibre content was calculated as follows (AOAC, 1997):

$$\% \text{ Crude fibre} = \frac{W3 - (W1 \times C) - (W5 - W4 - D) \times 100}{W2}$$

where
W1 = Initial capsule weight (g)
W2 = Sample weight (g)
W3 = Capsule + residue weight (g)
W4 = Crucible weight (g)
W5 = Total ash + crucible (g)
C = Blank correction for capsule solubility
D = Blank capsule ash (g)

Determination of Total Sugar

A total of 5 g of dried leaves and stem of *C. nutans* samples was refluxed with 20 ml of 80% ethanol for 1-2 h. Then, the ethanol was removed by evaporation using a rotary evaporator (Heidoplph, America) and the aqueous portion was transferred to a 100-ml volumetric flask and diluted to volume with distilled water and filtered with filter paper. The standard glucose stock solution prepared was of 1 mg/ml concentration (100 mg of glucose with 100 ml of distilled water). The volumes of standard glucose solution (0, 200, 400, 600, 800 and 1000 µl) were pipetted into test tubes and distilled water was added to make up a volume of 1000µl each. A 1000-µl of the sample was added into the test tubes. Each test tube had 1000 µl phenol and 5 ml concentrated sulphuric acid added to it. The test tubes were left for 15-30 min and then the optical density was measured spectrophotometrically (SpectrOstar^{Nano}, Germany) at 490 nm (AOAC, 1997).

$$\text{Total sugar (µg/ g)} = R \times (TV/SV) \times (DF/Wt)$$

where
R = Sugar content from standard curve (µg)
TV = Sample volume from extraction step
SV = Volume of sample used for spectrophotometric measurement
Wt = Sample weight
DF = Dilution factor

Determination of Amino Acid Profile

Acid hydrolysis (6 N HCl) of the freeze-dried sample was performed according to the AOAC (1995) method. Approximately 0.3 g of sample was weighed onto a glass-toppered test tube and hydrolysed with 5 mL of 6 N HCl at 110°C for 24 h. Samples were cooled to room temperature before they were filtered using filter paper (Sartorius grade 292) into a 100-mL volumetric flask. Mobile phase A (AccQ Taq Eluent A) was prepared by diluting 100 mL of AccQ Taq Eluent A with 1000 mL deionised water and filtered via a nylon cellulose membrane (size 0.45 µm). The diluted mobile phase A was later digested in an ultrasonic bath for 15 min. Mobile phase B was prepared by diluting 600 mL HPLC grade acetonitrile with 400 mL of deionised water and then filtered via a nylon cellulose membrane (size 0.45 µm). The internal standard (400 µL) (50 µmoL mL-1 α-Aminobutyric Acid (AABA) in 0.1 M HCl) was added and

made up to 100 mL with distilled water. The aliquot was filtered through 0.20 mm polytetrafluoroethylene microfilter (Merck, Germany). As for derivatisation, 10 µL of filtered hydrolysated samples or standard was transferred to a 1.5 mL glass vial and 70 µL of borate buffer solution was added to it and mixed well. Then, 20 µL of AccQ Flour Reagent (3 mg/mL in acetonitrile) was added to the mixture and thoroughly mixed through vortex for several seconds. After that, amino acid analysis was performed on high performance liquid chromatography (Shimadzu LC-10 AD, Shimadzu Corporation, Japan) and the samples were analysed on AccQ Tag type column (3.9 x 150 mm) at a constant flow rate of 1 ml/min. The amino acids from the samples were derivatised with *AccQ Flour Reagents* detected using a fluorescent detector and the data were integrated using an integrator model C-R7A (Shimadzu chromatopac data processor), where two channels were used simultaneously at a wavelength of 250 nm, a bandwidth of 5 nm and another wavelength of 395 nm.

Statistical Analysis

The data obtained were statistically analysed using SPSS Version 22 (Chicago, Inc.) and one-way ANOVA followed by Duncan's Multiple Range test. A p value of <0.05 was considered statistically significant for all the statistical tests conducted.

RESULTS AND DISCUSSION

For YPL samples and YDC samples, the leaves of *C. nutans* exhibited higher moisture, ash, protein, fat content and total sugar than the stem (Table 1 & 2). The total protein of *C. nutans* leaves for YPL and YDC non-shaded and shaded samples was 16.14%, 8.79% and 15.76% respectively. The value of protein content in all the samples was lower than the protein content found in the leaves of *Jatropha curcas* (26.00±0.47%) (Asuk, Agiang, Dasofunjo, & Willie, 2015). Comparing *C. nutans* with date fruit, *Phoenix dactylifera* (dried/*tamar* stage), date fruit revealed significantly higher moisture (15-21%) than *C. nutans*. However, *C. nutans* contained significantly higher ash content and protein than date fruit (ash content: 1.0-2.0%; protein: 1.8-8.0%) (Al-Harrasi et al., 2014).

In YPL samples, the content of ash in the leaves and stem were 12.71% and 5.39%, respectively. For YDC samples, the content of ash in non-shaded leaves, non-shaded stem, shaded leaves and shaded stem were 6.33%, 4.57%, 10.25% and 5.82%, respectively. All the tested samples exhibited higher amounts of the substance than cooked whole eggs (3.7%), okra leaf (2.44%), young fruit (1.38%) and mature fruit (1.17%) (Kassis, Beamer, Matak, Tou, & Jaczynski et al., 2010; Nwachukwu, Nulit, & Go, 2014). Compared with a previous study on saffron, *Crocus sativus*, some of the samples contained lower ash content than the saffron whole flower (7.39±0.12%) (Serrano-Díaz, Sánchez, Martínez-Tomé, Winterhalter, & Alonso, 2013).

The total fat in YPL leaves and stem were 4.82 and 0.85%, respectively. For YDC samples, the content of fat in non-shaded leaves, non-shaded stem, shaded

leaves and shaded stem were 2.08%, 0.84%, 3.53% and 0.52%, respectively. All the tested samples were more than 10 times lower in fat than cooked whole eggs (42.3%) and lower than saffron stigma (8.76±0.16%) and stamen (10.73±0.38%) (Serrano-Díaz et al., 2013, pp. 101–108).

The total crude fibre analysis comprised acid and alkaline hydrolysis. Acid hydrolysis was performed using sulfuric acid for the extract of sugars and starch from the samples, while alkaline hydrolysis was done using sodium hydroxide to remove the proteins, hemi-cellulose and lignin. Total crude fibre was found significantly higher in both leaves and stem of YDC shaded samples, with a value of 13.65% and 43.35%, respectively compared with the other treatments. Stem samples comprised more total crude fibre than the leaf samples in all areas. Total crude fibre was ranged between 10.72-13.65% in the leaf samples and between 32.80% and 43.35% for stem samples. The current results showed much higher fibre than recorded in previous studies done on flakes (made up of commercial white wheat: 1.68%; Dickopf wheat: 2.09%; red wheat: 1.87%; Kamut: 1.77%; Spelta: 1.24%) and muesli (made up of commercial whiter wheat: 4.76%; Dickopf wheat: 5.72%; red wheat: 5.40%; Kamut: 5.35%; Spelta: 3.62%) (Sumczynski, Bubelova, Sneyd, Erb-Weber, & Micek, 2015). Moreover, the *C.nutans* leaves and stem contained more total crude fibre compared to what was discovered in previous studies done on Sri Lankan rice varieties (Pokkali: 0.9%; Kalu heenati: 0.9%; Kahawanu: 1.0%; Sudu murunga: 1.1%; Unakola Samba: 0.9%; Gurusinghe wee: 1.0%) (Kariyawasam, Godakumbura, Prashantha, & Premakumara, 2016).

In comparing non-shaded and shaded samples of YDC, higher ($p<0.05$) moisture, protein, ash, total crude fibre and total sugar content was observed in the shaded samples for both leaves and stem. Total fat was higher ($p<0.05$) in the shaded leaves than in the non-shaded leaves but not so for the stem. Comparing the content of the leaves and stem from the two different farms for the non-shaded samples, it was found that the moisture, protein, ash, fat and total sugar content of YPL leaves and stem samples was significantly higher than that of the YDC samples.

Table 1
Percentage of moisture, ash, protein, fat, carbohydrate, crude fibre content and total sugar in leaves of Clinacanthus nutans

Nutritional parameter	**YPL Non-Shaded** Mean ± SD	**YDC Non-Shaded** Mean ± SD	**YDC Shaded** Mean ± SD
Moisture (%)	7.91 ± 0.01^{a}	6.72 ± 0.19^{c}	7.64 ± 0.38^{b}
Ash (%)	12.71 ± 0.00^{a}	6.33 ± 0.25^{c}	10.25 ± 0.14^{b}
Protein (%)	16.14 ± 0.04^{a}	8.79 ± 0.02^{c}	15.76 ± 0.02^{b}
Fat (%)	4.82 ± 0.10^{a}	2.08 ± 0.09^{c}	3.53 ± 0.49^{b}
Carbohydrate (%)	58.42 ± 0.12^{c}	76.08 ± 0.02^{a}	62.82 ± 0.04^{b}
Crude fibre (%)	11.35 ± 0.26^{b}	10.72 ± 0.02^{c}	13.65 ± 0.81^{a}
Total sugar (mg/g)	34.23 ± 0.17^{c}	52.31 ± 0.13^{a}	42.31 ± 0.02^{b}

± = Standard deviation of triplicate samples (n=3)

$^{a-c}$: Values with different superscripts in the same row are significantly different at $p<0.05$.

Table 2
Percentage of moisture, ash, protein, fat, carbohydrate, crude fibre content and total sugar in stems of Clinacanthus nutans

Nutritional parameter	**YPL Non-Shaded** Mean ± SD	**YDC Non-Shaded** Mean ± SD	**YDC Shaded** Mean ± SD
Moisture (%)	6.28 ± 0.33^{a}	5.06 ± 0.09^{c}	5.72 ± 0.74^{b}
Ash (%)	5.39 ± 0.17^{b}	4.57 ± 0.15^{c}	5.82 ± 0.29^{a}
Protein (%)	7.58 ± 0.12^{b}	5.36 ± 0.02^{c}	10.40 ± 0.02^{a}
Fat (%)	0.85 ± 0.02^{a}	0.84 ± 0.12^{b}	0.52 ± 0.08^{c}
Carbohydrate (%)	79.90 ± 0.23^{b}	84.17 ± 0.15^{a}	77.54 ± 0.13^{c}
Crude fibre (%)	32.80 ± 0.13^{c}	36.23 ± 1.23^{b}	43.35 ± 0.31^{a}
Total sugar (mg/g)	24.12 ± 0.67^{b}	33.43 ± 0.15^{a}	23.69 ± 0.14^{c}

± = Standard deviation of triplicate samples (n=3)
$^{a-c}$: Values with different superscripts in the same row are significantly different at $p<0.05$.

The leaves of *C. nutans* contained higher amounts of all essential and non-essential amino acids than its stem. Aspartic acid exhibited a significantly higher amount in both leaves (3.48%, 1.08% and 2.13% of dry weight sample for YPL, YDC non-shaded shaded samples, respectively) and stem (2.17%, 0.95% and 1.96% of dry weight sample for YPL, YDC non-shaded and shaded samples, respectively) than other amino acids (Table 3 & 4). However, the value was about three times less than the content of aspartic acid in cooked whole eggs (9.04%) (Kassis et al., 2010). The content of aspartic acid in all samples in the current study was higher compared to that in wild and cultivated *Panax* ginseng, with a value of 6.40 mg/g or 0.64% and 5.24mg/g or 0.52% (Sun et al., 2016). Furthermore, the current result revealed significantly higher amounts than in genuine Dong Chong Xia Cao (corpus: 1.70%; fruiting part: 1.84%), fermented preparations of *Cordyceps sinensis* (mycelium: 1.05%; supernatant of broth: 0.31%) and counterfeit Dong Chong Xia Cao (corpus: 0.62%; fruiting body: 0.82%) (Hsu,Shiao, Hsieh, & Chang, 2002), while methionine exhibited the lowest amount in both leaves (0.17%, 0.13% and 0.19% of dry weight sample for YPL, YDC non-shaded and shaded samples, respectively) and stem (0.08%, 0.08% and 0.04% of dry weight sample for YPL, YDC non-shaded and shaded samples, respectively). The content of methionine in all the samples except for YDC shaded stem samples exhibited a higher amount than in wild and cultivated *Panax* ginseng with a value of 0.71 mg/g or 0.07% and 0.55 mg/g or 0.05% (Sun et al., 2016). Glutamine and aspartate are major metabolic fuels for the small intestine. They regulate neurological functions along with glycine. Leucine activates mammalian targets of rapamycin to stimulate protein synthesis and inhibits proteolysis, while tryptophan

modulates neurological and immunological functions through multiple metabolites, including serotonin and melatonin (Wu, 2010). Dietary supplementation with one or a mixture of amino acids may be beneficial for ameliorating health problems at various stages of the life cycle such as fetal growth restriction, neonatal morbidity and mortality, weaning-associated intestinal dysfunction and wasting syndrome, obesity, diabetes, cardiovascular disease, metabolic syndrome and infertility. In addition, dietary supplementation optimises efficiency of metabolic transformations to enhance muscle growth, milk production, egg and meat quality and athletic performance, while preventing excess fat deposition and reducing adiposity (Wu, 2009). Glycine, histidine, isoleucine, leusine, lysine phenylalanine, threonine, valine, serine, glutamic acid, arginine, alanine and proline exhibited higher concentrations in all the samples compared to cultivated *Panax* ginseng (3.29 mg/g or 0.33% for glycine; 0.94 mg/g or 0.09% for histidine; 1.41 mg/g or 0.14% for isoleucine; 2.68 mg/g or 0.27% for leucine; 2.13 mg/g or 0.21% for lysine; 1.48 mg/g or 0.15% for phenylalanine; 1.88 mg/g or 0.19% for threonine; 1.92 mg/g or 0.19% for valine; 2.13 mg/g or 0.21% for serine; 8.09 mg/g or 0.81% for glutamic acid; 10.87 mg/g or 1.09% for arginine; 2.66 mg/g or 0.27% for alanine; 2.15 mg/g or 0.22% for proline) (Sun et al., 2016).

Table 3
Amino acid percentage in dry leaves of Clinacanthus nutans

Essential Amino Acid (% of dry weight sample)	**YPL Non-Shaded**	**YDC Non-Shaded**	**YDC Shaded**
	Mean ± SD	Mean ± SD	Mean ± SD
His	0.36 ± 0.01^{o}	0.32 ± 0.01^{l}	0.35 ± 0.01^{m}
Ile	0.74 ± 0.01^{l}	0.61 ± 0.01^{f}	0.73 ± 0.03^{k}
Leu	1.45 ± 0.01^{c}	1.05 ± 0.02^{b}	1.32 ± 0.06^{c}
Lys	1.15 ± 0.10^{e}	0.95 ± 0.01^{c}	1.03 ± 0.02^{f}
Met	0.17 ± 0.01^{p}	0.13 ± 0.01^{n}	0.19 ± 0.01^{n}
Phe	0.93 ± 0.01^{i}	0.72 ± 0.01^{e}	1.10 ± 0.24^{d}
Thr	0.82 ± 0.01^{j}	0.61 ± 0.01^{f}	0.73 ± 0.03^{k}
Val	0.98 ± 0.01^{g}	0.82 ± 0.01^{d}	0.94 ± 0.04^{h}
Non-Essential Amino Acid (% of dry weight)			
Ala	1.03 ± 0.01^{f}	0.34 ± 0.03^{j}	1.00 ± 0.08^{g}
Arg	0.47 ± 0.72^{n}	0.23 ± 0.03^{m}	0.85 ± 0.01^{i}
Asp	3.48 ± 0.08^{a}	1.08 ± 0.08^{a}	2.13 ± 0.08^{a}
Glu	2.29 ± 0.03^{b}	0.56 ± 0.07^{g}	1.84 ± 0.07^{b}
Gly	0.95 ± 0.01^{h}	0.35 ± 0.04^{i}	1.00 ± 0.07^{g}
Pro	1.18 ± 0.01^{d}	0.51 ± 0.02^{h}	1.06 ± 0.07^{e}
Ser	0.81 ± 0.02^{k}	0.33 ± 0.04^{k}	0.78 ± 0.05^{j}
Tyr	0.65 ± 0.01^{m}	0.13 ± 0.02^{n}	0.66 ± 0.01^{l}

± = Standard deviation of triplicate samples (n=3)
$^{a-p}$: Values with different superscripts in the same column are significantly different at $p<0.05$.

Table 4
Amino acid percentage in dry stem of Clinacanthus nutans

Essential Amino Acid (% of dry weight)	**YPL Non-Shaded**	**YDC Non-Shaded**	**YDC Shaded**
	Mean ± SD	Mean ± SD	Mean ± SD
His	0.18 ± 0.01^{n}	0.11 ± 0.01^{n}	0.14 ± 0.02^{m}
Ile	0.34 ± 0.04^{l}	0.25 ± 0.01^{l}	0.22 ± 0.03^{l}
Leu	0.56 ± 0.06^{f}	0.44 ± 0.01^{d}	0.39 ± 0.05^{i}
Lys	0.58 ± 0.02^{e}	0.34 ± 0.02^{g}	0.39 ± 0.04^{i}
Met	0.08 ± 0.01^{p}	0.08 ± 0.01^{o}	0.04 ± 0.01^{n}
Phe	0.35 ± 0.04^{k}	0.29 ± 0.01^{i}	0.25 ± 0.03^{k}
Thr	0.40 ± 0.07^{j}	0.26 ± 0.01^{k}	0.25 ± 0.03^{k}
Val	0.31 ± 0.16^{m}	0.35 ± 0.01^{f}	0.31 ± 0.04^{j}
Non-Essential Amino Acid (% of dry weight)			
Ala	0.60 ± 0.06^{c}	0.34 ± 0.01^{g}	0.90 ± 0.02^{d}
Arg	0.42 ± 0.19^{i}	0.27 ± 0.02^{j}	0.71 ± 0.02^{f}
Asp	2.17 ± 0.36^{a}	0.95 ± 0.02^{a}	1.96 ± 0.16^{a}
Glu	1.12 ± 0.15^{b}	0.67 ± 0.01^{b}	1.58 ± 0.01^{b}
Gly	0.46 ± 0.08^{g}	0.36 ± 0.01^{e}	0.82 ± 0.01^{e}
Pro	0.59 ± 0.12^{d}	0.48 ± 0.04^{c}	1.46 ± 0.44^{c}
Ser	0.45 ± 0.07^{h}	0.32 ± 0.01^{h}	0.67 ± 0.01^{g}
Tyr	0.15 ± 0.01^{o}	0.17 ± 0.01^{m}	0.55 ± 0.03^{h}

± = Standard deviation of triplicate samples (n=3)
$^{a-p}$: Values with different superscripts in the same column are significantly different at $p<0.05$.

CONCLUSION

The leaves of *C. nutans* exhibited more moisture, ash, protein, fat content and total sugar than its stem in all the tested samples. However, total crude fibre was found significantly higher in the stem samples. In comparing the non-shaded and shaded samples of YDC, higher ($p<0.05$) moisture, protein, ash, total crude fibre and total sugar content was observed in the shaded samples for both leaves and stem. Total fat was higher ($p<0.05$) in the shaded leaves than in the non-shaded leaves but this was not so for the stem. Comparing the non-shaded samples of the two different farms for moisture, protein, ash, fat and total sugar content showed that YPL leaves and stem had significantly ($p<0.05$) higher amounts of these substances than the YDC samples. The leaves of *C. nutans* contained more amounts of all essential and non-essential amino acids than the stem. Aspartic acid exhibited a significantly ($p<0.05$) higher amount in both leaves (3.48%, 1.08% and 2.13% of dry weight sample for YPL, YDC non-shaded and shaded samples, respectively) and stem (2.17%, 0.95% and 1.96% of dry

weight sample for YPL, YDC non-shaded and shaded samples, respectively) than other amino acids. Geographical factors and planting conditions revealed different nutritive composition.

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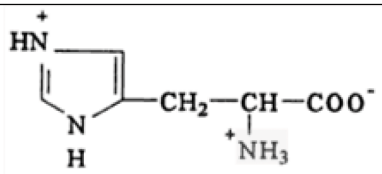
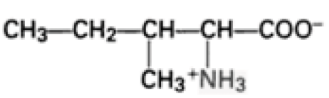
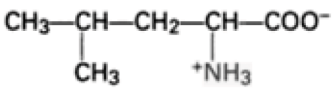
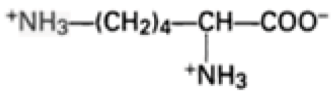
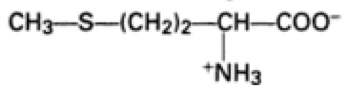
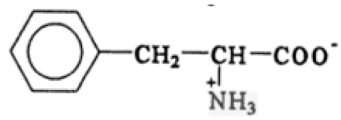
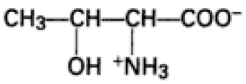
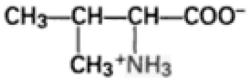
ABBREVIATIONS

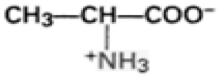
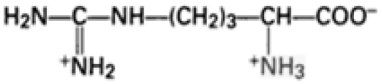
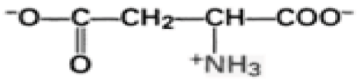
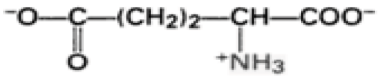
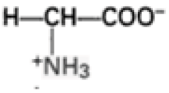
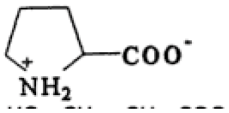
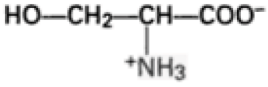
Supplementary 1

Essential amino acids (Fennema, 1996)

Name	Symbol	Structure at neutral pH
Histidine	His	
Isoleucine	Ile	
Leucine	Leu	
Lysine	Lys	
Methionine	Met	
Phenylalanine	Phe	
Threonine	Thr	
Valine	Val	

Supplementary 2

Non-essential amino acids (Fennema, 1996)

Name	Symbol	Structure at neutral Ph
Alanine	Ala	
Arginine	Arg	
Aspartic acid	Asp	
Glutamic acid	Glu	
Glycine	Gly	
Proline	Pro	
Serine	Ser	
Tyrosine	Tyr