ASM Sc. J., 12, 2019
https://doi.org/10.32802/asmscj.2019.87

Qualitative and Quantitative Phytochemical Analysis and Antioxidant Properties of Leaves and Stems of *Clinacanthus nutans* (Burm. f.) Lindau from Two Herbal Farms of Negeri Sembilan, Malaysia

H.S. Kong[1], K.H. Musa[2], Z. Mohd Kasim[1] and N. Abdullah Sani*[1]

[1]*Centre for Biotechnology and Functional Food, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM, Bangi Selangor, Malaysia*

[2]*College of Agriculture and Veterinary Medicine, Qassim University, KSA*

The objective of the study is to analyse the phytochemical content by quantitative and qualitative methods and to investigate the antioxidant properties on *Clinacanthus nutans* leaves and stems from different areas: *Yik Poh Ling* (YPL) un-shaded sample (exposed to direct sunlight) and *You Dun Chao* (YDC) un-shaded and shaded (with black shade netting) samples. Extracts were prepared by dissolving fine powder of *C. nutans* in 1:10 solvent using ultrasonic extraction for an hour. Preliminary screening and quantitative estimation of phytochemical (alkaloids, flavonoids, cardiac glycosides, saponins, steroids, terpenoids, phenols and tannins) analysis were conducted. Then, determination of antioxidant properties (total phenolic content, TPC, ferric reducing/ antioxidant power, FRAP and radical-scavenging activities, DPPH) were conducted. All the samples contained alkaloids, flavonoids, cardiac glycosides, saponins, steroids, terpenoids, phenols and tannins. Un-shaded leaves of *C. nutans* from YDC exhibited significantly higher result in antioxidant properties (966.00mg GAE/g of dried sample in TPC, 20.44mg TE/g of dried sample in FRAP and 11.14mg TE/g of dried sample in DPPH) than the other samples ($p < 0.05$). As a conclusion, *C. nutans* has a good potential to be an alternative antioxidant source.

Keywords: *Clinacanthus nutans*, Sabah Snake Grass, phytochemical, antioxidant, shaded and un-shaded

I. INTRODUCTION

Clinacanthus nutans is a native medicinal herb that grows in a tropical climate that mainly can be found in Malaysia and Thailand. It is a shrub green plant, which can be grown by stem propagation method. The *C. nutans* has been utilised for its benefits and functions according to folklore, especially in the Southeast Asia region. It was believed can treating skin rashes, gout and diabetes. The previous study showed the presence of different phytochemicals compound such as flavonoids and terpenoids in the plant. These phytochemical compounds contributed to its antioxidant properties of plants. Furthermore, it contributes to good health and disease prevention. The presence of phytochemical compounds also contributed to antibacterial and antifungal properties.

Glycosides involve enzyme hydrolysis and heart disease treatment. Saponins are plant-derived anti-inflammatory compounds, which lower blood cholesterol, prevent heart disease and cancers. Abaoba and Efuwape (2001) and Mohanta *et al.* (2007) claimed that saponins have antifungal properties. Steroids may decrease postoperative atrial fibrillation and inhibiting the inflammatory process post cardiopulmonary bypass, decrease capillary wall permeability, preventing and migration of inflammatory mediators into the systemic circulation (Kristeller *et al.*, 2014). Terpenoids act as biological antioxidants to protect

*Corresponding author's e-mail: norrasani@ukm.edu.my

cells or tissues from the damaging effect of free radicals (Prakash *et al.*, 2004).

Tannins are potential metal ion chelators, protein precipitating agents, and biological antioxidants, which contribute to antitumor effects and astringent activity. Tannins also improve wound healing through protein precipitation (Okuda *et al.*, 1995). De-Ruiz *et al.* (2001) and Elegani *et al.* (2002) stated that tannins (found in medicinal plants) are responsible in antiviral and antibacterial activities. Flavonoids were detected in all leaves extracts of the herbal plant (Siew *et al.*, 2014; Sakdarat *et al.*, 2009). Flavonoids are potent antioxidants agents and regulating activities of various enzyme systems due to their interaction with various biomolecules (Maldonado *et al.*, 2003). Flavonoids are renowned for their free radical scavenging potency, which underline their antibacterial, anti-inflammatory, anti-thrombotic and vasodilatory activities (Yamamoto and Gaynor, 2002). The presence of flavonoids and saponins are contributing to traditional treatment (Othira *et al.*, 2009; Zwadyk, 1992). Alkaloids is important as constitute potent therapeutic agents, possess anti-inflammatory, and antibacterial properties (Gao *et al.*, 2015; Liu *et al.*, 2015).

Antioxidant activity can be tested using total phenolic content (TPC), ferric reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) tests. The previous study showed that the radical scavenging activity of petroleum ether extracts of *C. nutans* was 82.00 ± 0.02 % (Arullappan *et al.*, 2014). Besides, the chloroform extract of *C. nutans* leaves was a good antioxidant agent against galvinoxyl radicals and 2, 2-diphenyl-1-picrylhydrazyl (DPPH). However, it was less effective in negating hydrogen peroxide radicals and nitric oxide (Yang *et al.*, 2013).

Moreover, higher ferric reducing antioxidant power (FRAP) in six-month-old buds of *C. nutans* than one-month-old buds were recorded with the denomination of 488µM of Fe(II)/g and 453µM of Fe(II)/g respectively (Fong *et al.*, 2014). Medicinal plants contain plenty of chemical compounds (bioactive secondary metabolites such as terpenes, phenolics and alkaloids, from secondary plant metabolism), which is exhibiting different biological and pharmacological activities such as antimicrobial and antioxidant (Stefanović *et al.*, 2015).

The previous study was focused more on leaves extract of *C. nutans*, and only certain phytochemical compounds were investigated such as flavonoids, alkaloids, phenol and saponin. This study focused on determining the presence of phytochemical via qualitative method, analysing the phytochemical content by quantitative methods and learning the antioxidant properties on *C. nutans* leaves and stems from two herbal farms of Negeri Sembilan, Malaysia: *Yik Poh Ling* (YPL) un-shaded samples ; *You Dun Chao* (YDC) un-shaded and shaded samples.

II. MATERIALS AND METHODS

A. Plant material preparation

Three-month-old fresh and healthy leaves and stems of Sabah snake grass (*C. nutans*) were obtained from *Yik Poh Ling* (YPL, in Senawang) and *You Dun Chao* (YDC, in Sendayan) Herbal Farms, in the state of Negeri Sembilan, Malaysia (Figure 1 and 2). The YPL had planted the *C. nutans* in un-shaded condition (exposed to direct sunlight) while YDC had planted *C. nutans* in shaded (with black shade netting) and un-shaded conditions (Figure 3). About 10kg of the plant materials were collected from the farms. The leaves and stems were washed with water to remove sand and dust particles. The leaves and stems were then freeze-dried using ALPHA freeze dryer (ALPHA, Hampshire UK), homogenised into 0.5mm size using Universal cutting mill (FRITSCH, Idar-Oberstein, Germany) before further analyses were conducted. The freeze-dried samples were stored in a freezer (ALPHA, Hampshire, UK) (-20^{o}C) for further use.

B. Extraction of plant materials

The method of Hassan *et al.* (2015) was used for the preparation of the plant materials of *C. nutans* (leaves and stems) and extracts with minor modifications. The 70% acetone (Merck, Germany) extracts were prepared by dissolving fine powder of *C. nutans* leaves and stems in 1:10 solvent using ultrasonic extraction for an hour. The extracts were then filtered with the aid of a Bucker funnel and Whatman filter paper #1. The extracts were preserved in airtight bottles at -40^{o}C for further use.

Figure 1. Leaves of *Clinacanthus nutans*

Figure 2. Stems of *Clinacanthus nutans*

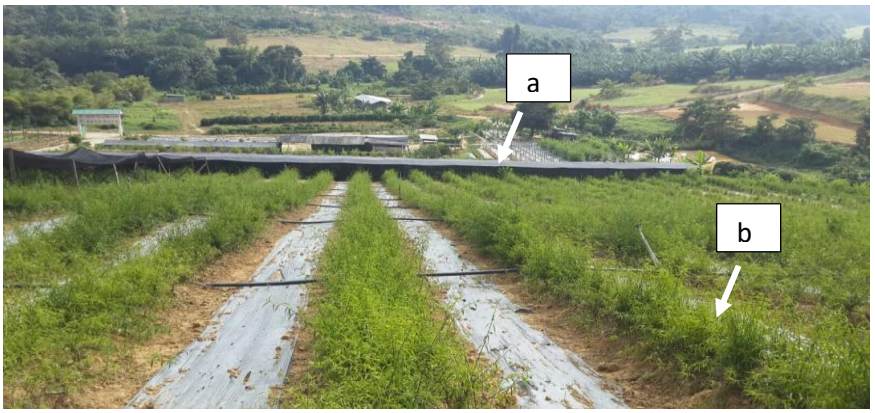

Figure 3. Shaded (a) and un-shaded (b) samples of *Clinacanthus nutans*

C. Preliminary qualitative phytochemical analysis of C. nutans leaves and stems

The qualitative phytochemical screening of the *C. nutans* leaves and stems extracts was performed using methods suggested by Sofowora (1993), Harborne (1984) and Trease and Evans (2002).

1. Alkaloid test

2mL of potassium iodide (*Sigma-Aldrich*, US) was mixed with 10mL of the leaves and stems crude extracts, which forms brown precipitation with alkaloid solutions.

2. Flavonoid test

2mL of sodium hydroxide (2%) (*Sigma-Aldrich*, US) was added to 10mL of the leaves and stems crude extracts, followed by a few drops of diluted sulphuric acid (10%) (*Sigma-Aldrich*, US). The transformation from an intense red-orange colour to colourless indicated the presence of flavonoid.

3. Cardiac glycoside test

2mL of glacial acetic acid (*Sigma-Aldrich*, US) and one or two drops of 2% ferric chloride (*Sigma-Aldrich*, US) were mixed with 10 mL of the leaves and stem crude extracts. The mixtures were poured carefully into separate test tubes with 2 mL of concentrated sulphuric acid (*Sigma-Aldrich*, US). Cardiac glycoside was determined by the presence of a brown ring inter-phase in the mixtures.

4. Phenol and tannin tests

2mL of ferric chloride solution (2%) (*Sigma-Aldrich*, US) was mixed with 10mL of the leaves and stem crude extracts. The presence of phenol and tannin were determined by the changing of the mixture colour from blue-green to black.

5. Saponin test

5mL of distilled water was added to 10mL of dilute the leaves and stems crude extracts (100µL extract in 5mL distilled water). The mixture was vigorously shaken, and the formation of stable foam determined the presence of saponin.

6. Steroid test

2mL of acetic anhydride (*Sigma-Aldrich*, US) and sulphuric acid (*Sigma-Aldrich*, US) were added to 10mL of the leaves and stems crude extracts on the sidewise of the test tube. The presence of steroid was determined when the samples colour change from violet to blue-green was observed.

7. Terpenoid test

2mL of chloroform (*Sigma-Aldrich*, US) was used to dissolve 10mL of the leaves and stems crude extracts and evaporated to dryness using a water bath (Memmert, Germany) (Boiling point for chloroform = 62°C). 2mL of concentrated sulphuric acid (*Sigma-Aldrich*, US) was added and heated for about 2 mins. The presence of terpenoid was determined when the mixture appeared in red.

D. Quantitative phytochemical analysis of C. nutans leaves and stems

1. Total alkaloid determination

The method of Harborne (1973) was used for this analysis. 5g each of leaves or stems of *C. nutans* powder was mixed in a test tube with 200mL of ethanol (*Sigma-Aldrich*, US) and 10% acetic acid (*Sigma-Aldrich*, US). The test tube was capped and left for 4 hours in room temperature. The mixture sample was filtered with filter paper Whatman No. 42 (125mm), and the volume was reduced to a quarter of its original volume using a water bath (Memmert, Germany). 5mL of concentrated ammonium hydroxide solution (*Sigma-Aldrich*, US) was added into the reduced mixture sample drop-wise until precipitation occurred. After filtration (using filter paper Whatman No. 42) and drying in an oven (Memmert, Germany) at 40°C, the precipitate was collected and weighed. The percentage of the total alkaloid content was calculated as below:

$$Percentage\ of\ the\ total\ alkaloid\ (\%) = \frac{Weight\ of\ residue}{Weight\ of\ the\ sample\ taken} \times 100$$

2. Cardiac glycoside determination

The method of El-Olemy, Al-Muhtadi dan Affi (1994) was adopted. About one g of the dried leaves or stems of *C. nutans* was weighed separately into different test tubes, and 10mL of ethanol (70%) (*Sigma-Aldrich*, US) was placed into each test tube. The test tube was covered and placed in a shaker (Intertech, Taipei, Taiwan) and was shaken at 300rpm for 6 hours at room temperature (25°C). The mixture was filtered with Whatman No. 42 filter paper. The filtrate was treated with 5mL distilled water, followed by 1mL of 12.5% lead acetate (*Sigma-Aldrich*, US) to precipitate tannins, resins and pigments. Distilled water was added until the volume was 8mL and shaken in a shaker set (Intertech, Taipei, Taiwan) at 300rpm for 10 mins. 2mL of 4.77% disodium hydrogen phosphate (Na_2HPO_4) solution (*Sigma-Aldrich*, US) was added to precipitate the excess phosphorus ions. The resultant solution was filtered with Whatman No. 42 filter paper to give a clear filtrate. The filtrate was then evaporated to dryness in an oven (Memmert, Germany) at 40°C. The percentage of cardiac glycoside content was calculated as below:

$$Percentage\ of\ cardiac\ glycoside\ (\%) = \frac{Weight\ of\ residue}{Weight\ of\ the\ sample\ taken} \times 100$$

3. Total flavonoid determination

The method of Boham and Kocipai-Abyazan (1994) was used for the analysis of the flavonoids in *C. nutans* leaves and stems. About 10 g each of the dried leaves or stems was extracted with 100mL of aqueous methanol (80%) (*Sigma-Aldrich*, US) at room temperature. The extraction was repeated thrice. The solution was filtered with Whatman No. 42 filter paper (125mm). The filtrate was heated to dry condition using a water bath (Memmert, Germany) (65°C) until constant weight was obtained. The percentage of total flavonoid content was calculated as below:

$$Percentage\ of\ total\ flavonoid\ (\%) = \frac{Weight\ of\ residue}{Weight\ of\ the\ sample\ taken} \times 100$$

4. Total terpenoid determination

The method of Fergusan (1956) was used for the determination of total terpenoids in the *C. nutans* leaves and stems. About two g each of the leaves or stems powders were mixed with 50mL of ethanol (95%) (*Sigma-Aldrich*, US) for 24 hours. The mixture was collected after being filtered using Whatman No. 42 filter paper. The filtrate was extracted with petroleum ether (*Sigma-Aldrich*, US) at a temperature range of 60 - 80°C and was dried using a water bath (Memmert, Germany) (65°C). The percentage of total terpenoid content was calculated as below:

$$Percentage\ of\ total\ terpenoid\ (\%) = \frac{Weight\ of\ residue}{Weight\ of\ the\ sample\ taken} \times 100$$

5. Total saponin determination

The total saponin content was determined following the method of Makkat *et al.* (2007) based on vanillin-sulphuric acid colourimetric reaction. About 50µL of the *C. nutans* leaves or stems extract was mixed with 250µL of distilled water. Then, 250µL of vanillin reagent (*Sigma-Aldrich*, US) was added into the mixture. A 2.5mL of sulphuric acid (72%) (*Sigma-Aldrich*, US) was added into the mixture and mixed well. The solution mixture was kept in a water bath at 60 ± 5°C for 10 mins before it was cooled down. The absorbance was determined at 544nm wavelength using spectrophotometer (*SPECTROstar^{Nano};* Offenburg, Germany). The saponin value was indicated as diosgenin equivalents (mg DE/g of dried sample) derived from a standard curve. Standard was prepared by dissolving 0.1g of diosgenin in 10mL of 95% ethanol (*Sigma-Aldrich*, US).

6. Steroid determination

The method of Devanaboyina *et al.* (2013) was adopted for the determination of steroids content in the *C. nutans* leaves and stems extracts. 100µL of the extract was mixed with 2mL of sulphuric acid (4N; 2M H_2SO_4) (*Sigma-Aldrich*, US) and 2mL of iron (III) chloride (0.5% w/v) (*Sigma-Aldrich*, US), followed by 0.5mL of potassium hexacyanoferrate (III) solution (0.5% w/v) (*Sigma-Aldrich*, US). The solution mixture was kept in a water bath (Memmert, Germany) at 70 ± 5°C for 30 mins. The absorbance was read at 780nm wavelength using spectrophotometer (*SPECTROstar^{Nano};* Offenburg, Germany). The value was expressed as cycloartenol equivalents (mg CA/g of dried sample) derived from a standard curve. Standard was prepared by dissolving 1 g of cycloartenol in 10mL of 95% ethanol (*Sigma-Aldrich*, US).

7. Tannin determination

The method of Siddhuraj and Manian (2007) was adopted to determine the tannin content in the *C. nutans* leaves and stems extracts. A total of 500µL of the extract was placed in the test tube separately. Then, 100mg of polyvinylpolypyrrolidone (*Sigma-Aldrich*, US) and 500µL of distilled water was added to the extract. The mixture was vortexed using *IKA®VORTEX 3* and incubated at 4°C for 15 mins. Then, the sample mixture was centrifuged (Kubota, Japan) at 5000rpm for 5 mins. The supernatant was collected and kept in a vial before conducting the experiment. Only simple phenolic free of tannins was found in the supernatant (the tannins have been precipitated with the polyvinylpolypyrrolidone). The phenolic content of the supernatant was determined at 765nm wavelength using spectrophotometer (*SPECTROstar^{Nano};* Offenburg, Germany). The result was indicated as the content of free phenolic or non-tannin phenolic on a dry matter basis. The tannin content of the extracts was calculated as below:

Tannin (mg GAE/g sample) = total phenolics (mg GAE/g sample) – free phenolics (mg GAE/g sample).

E. Determination of antioxidant properties in C. nutans leaves and stems

1. Determination of Folin–Ciocalteu Index (Total Phenolic Content, TPC*)*

The method of Slinkard and Singleton (1977) was used for the TPC determination in *C. nutans* leaves and stems. A 0.1mL of the leaves or stems extracts and gallic acid (standard curve) (*Sigma-Aldrich*, US) were added in a 96 deep well block. Then, 0.5mL of diluted Folin–Ciocalteu reagent (*Sigma-Aldrich*, US) was added into mixture content in the 96 deep well block. The mixture was covered

and kept at room temperature for 5 mins. 1mL of sodium carbonate (7.5%; w/v) (*Sigma-Aldrich*, US) was mixed with the mixture. A blue colour mixture was kept for 2 hours at room temperature. The absorbance was read at 765nm wavelength using a spectrophotometer (*SPECTROstar*^{Nano}, Offenburg, Germany). The results were indicated as milligram of gallic acid equivalents to per gram of dried leaves and stem samples (mg GAE/ g of dried sample). Food additives for antioxidant such as 0.1g / 10mL of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (*Sigma-Aldrich*, US) were used as a comparison with the plant extract mixtures. The same procedure was adopted for standard preparation.

2. Determination of Ferric reducing/ antioxidant Power (FRAP)

The method of Benzie and Strain (1996) was adopted to determine the FRAP content in *C. nutans* leaves and stems. FRAP reagent was ready using 300mM acetate buffer (*Sigma-Aldrich*, US)), pH3.6, 10mM TPTZ (2,4,6-tri (2-pyridyl)-triazine) (*Sigma-Aldrich*, US) in 10mL of 40mM hydrochloric acid (HCl) (*Sigma-Aldrich*, US), and 20mM $FeCl_3.6H_2O$ (*Sigma-Aldrich*, US) in the ratio of 10:1:1 to serve as a working reagent. FRAP reagent (light brown colour) with the volume of 1mL was prepared freshly and mixed with 100µL of *C. nutans* leave and stem samples or standards as a blank reagent. After 30 mins, the absorbance was determined at 595nm wavelength using spectrophotometer (*SPECTROstar*^{Nano}, Offenburg, Germany). The result was indicated as mg of Trolox equivalents per gram of dried sample (mg TE/g of dried sample). Standard was prepared by dissolving 2mg of Trolox in 10mL of ethanol (*Sigma-Aldrich*, US). Food additives for antioxidant such as 0.1g / 10mL of BHA and BHT were used as the comparison with the plant extract mixtures.

3. Determination of Radical-Scavenging Activity (DPPH)

The method of Musa *et al.* (2011) was adopted. The decrease of the absorption at 516nm wavelength of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution (*Sigma-Aldrich*, US) after the addition of the blank or sample extracts was determined via a spectrophotometer (*SPECTROstar*^{Nano}, Offenburg, Germany). 1mL of methanolic DPPH solution (24mg/L) (purple colour) was added to 100µL of the sample solution (10mg/mL). Standard was prepared by dissolving 2mg of Trolox in 10mL of ethanol (*Sigma-Aldrich*, US). Food additives for antioxidant such as 0.1g / 10mL of BHA and BHT were used for comparison with the plant extract mixtures. The percentage of DPPH was calculated as below:

$$Radical\ scavenging\ activity = \frac{[Asb\ 516nm\ (t=0) - Abs\ 516nm(t=t') \times 10]}{Abs\ 516nm\ (t=0)}$$

F. Statistical Analysis

The experiments were conducted in six replications. The results obtained were analysed using SPSS Version 22 (Chicago, Inc.) one-way ANOVA, Duncan's Multiple Range test with $P < 0.05$ and Pearson's correlation coefficient (r).

III. RESULT AND DISCUSSION

A. Preliminary qualitative phytochemical analysis of C. nutans leaves and stems

Phytochemical studies were carried out on *C. nutans* leaves and stem that indicated the presence of alkaloids, flavonoids, glycosides, saponins, steroids, terpenoids, phenols and tannins in all samples. Ismail *et al.* (2017) stated that the environmental factors such as light intensity, temperature and soil characteristics (pH of soil and type of planting soil) were influencing the presence and quantity of phenolic, flavonoids and antioxidant properties. The previous study on ethanol (50%) leaves extract of *C. nutans* showed the presence of alkaloids, flavonoids, terpenoids, phenols and tannins (Aslam *et al.*, 2016). However, methanol leaves extract of *C. nutans* only showed the presence of saponins, phenol, and flavonoids (Yang *et al.*, 2013). The herbs plant *Leucas indica* (L) var. nagalapuramiana had the same medicinal functions with *C. nutans*; there were treating a snake bite, fever and swelling. Acetone extract of whole aerial part of these plants only contained alkaloids, phenols, flavonoids, steroids, saponins and tannins (Pranoothi *et al.*,

2014). Acetone leaves extract of lemongrass showed the presence of flavonoids and saponins (Geetha and Geetha, 2014). The methanol extract of medicinal plant *Abrus precatorius*, *Dalbergia sissoo*, *Millettia pinnata* and *Tephrosia purpurea* contained alkaloids, flavonoids, phenols, saponin, steroids and tannins (Gnanaraja *et al.*, 2014). Ethanol leaves extract of medicinal plant *Phyllanthus amarus*, *Euphorbia heterophylla*, *Senna occidentalis*, *Piper nigrum* and *Ageratum conyzoides* contained alkaloids, flavonoids, phenols, saponins and tannins (Ajuru *et al.*, 2017).

Table 1. Total alkaloid, cardiac glycosides, flavonoid and terpenoid of various areas on *Clinacanthus nutans* leaves and stems

Sample	Area	Leaves (%)				Stems (%)			
		Alkaloid	**Cardiac glycoside**	**Flavonoid**	**Terpenoid**	**Alkaloid**	**Cardiac glycoside**	**Flavonoid**	**Terpenoid**
YPL	Un-shaded	8.40 ± 2.11 ^{b}	8.98 ± 0.83 ^{b}	5.88 ± 0.78 ^{b}	2.41 ± 0.17 ^{b}	4.83 ± 2.27 ^{b}	11.13 ± 1.78 ^{b}	7.17 ± 3.16 ^{b}	1.14 ± 0.09 ^{b}
YDC	Un-Shaded	10.14 ± 2.40 ^{a}	10.24 ± 0.73 ^{a}	6.16 ± 2.37 ^{a}	2.87 ± 0.68 ^{a}	6.79 ± 0.38 ^{a}	13.84 ± 0.66 ^{a}	9.03 ± 2.28 ^{a}	1.77 ± 0.10 ^{a}
	Shaded	4.81 ± 2.56 ^{c}	9.76 ± 1.25 ^{ab}	3.86 ± 1.30 ^{c}	2.30 ± 0.70 ^{c}	2.53 ± 1.54 ^{c}	12.88 ± 0.96 ^{ab}	6.32 ± 2.71 ^{c}	0.98 ± 0.22 ^{c}

$^{a-c}$ mean values with different superscripts in the same column are significantly different at $p < 0.05$

B. Quantitative phytochemical analysis of C. nutans leaves and stems

1. Total alkaloid determination

Total alkaloid contents in leaves and stems parts of *C. nutans* exhibited higher contents in YDC un-shaded area with values of 10.14 and 6.79%. The alkaloids content of leaves and stems samples were ranged between 4.81 – 10.14% and 2.53 – 6.79%, respectively (Table 1). Ethanol leaves extract of medicinal plant *P. amarus*, *E. heterophylla*, *S. occidentalis*, *P. nigrum* and *A. conyzoides* contained alkaloids lower than the leaves sample of YDC un-shaded area (1.56, 7.15, 2.95, 0.67 and 9.40% respectively) (Ajuru *et al.*, 2017).

2. Cardiac glycoside determination

The cardiac glycoside contents of both leaves and stems parts of *C. nutans* were ranging between 8.98 and 13.84%. The cardiac glycoside was high in stems samples than leaves samples. The YDC un-shaded stems contained the significant higher cardiac glycosides than others (Table 1). *Chrysophyllum albidum* was used in traditional medicine in treating diabetics, cancer and coronary heart disease and its seed kernel contained 1.88g / 100g or 1.88% cardiac glycosides, which is lower than current results (Muhammad and Abubakar, 2016).

3. Total flavonoid determination

The total flavonoid content was high in YDC un-shaded stems sample with a value of 9.03% followed with YPL un-shaded, and YDC shaded stems samples with values of 7.17 and 6.32%. The flavonoids content of leaves samples were ranging between 3.86 – 6.16% (Table 1). All leaves sample of *C. nutans* contained higher flavonoids than ethanol leaves extract of medicinal plant *P. amarus*, *E. heterophylla*, *S. occidentalis* and *P. nigrum* (1.62, 0.28, 1.16 and 0.57% respectively) (Ajuru *et al.*, 2017).

Table 2. Saponin, steroid and tannin contents of various areas on *Clinacanthus nutans* leaves and stems

Sample	Area	Leaves			Stems		
		Saponin (mg DE/ g)	**Steroid (mg CA/g)**	**Tannin (mg GAE/g)**	**Saponin (mg DE/g)**	**Steroid (mg CA/g)**	**Tannin (mg GAE/g)**
YPL	Un-shaded	67.12 ± 0.60 [b]	542.00 ± 0.48 [c]	453.49 ± 1.05 [b]	63.09 ± 0.54 [c]	158.75 ± 2.16 [c]	191.83 ± 0.71 [b]
YDC	Un-Shaded	75.93 ± 1.57 [a]	634.72 ± 0.90 [b]	496.81 ± 0.56 [a]	65.10 ± 2.11 [b]	213.84 ± 0.97 [b]	204.35 ± 1.57 [a]
	Shaded	82.72 ± 0.71 [a]	833.32 ± 1.43 [a]	340.12 ± 2.53 [c]	71.90 ± 1.89 [a]	247.35 ± 1.38 [a]	166.50 ± 1.24 [c]

DE: diosgenin; CA: cycloartenol; GAE: gallic acid;

[a-c] mean values with different superscripts in the same column are significantly different at $p < 0.05$

4. Total terpenoid determination

The total terpenoid contents of both leaves and stems of *C. nutans* ranged between 0.98 and 2.87%. The total terpenoid content was high in the leaves samples than in the stems samples. YDC unshaded leaves contained the highest terpenoid content than the other samples (Table 1). Terpenoids played an important role in treating type 2 diabetes and cardiovascular diseases (Goto *et al.*, 2010).

5. Saponin determination

The saponin content of *C. nutans* leaves and stems ranged between 67.12 – 82.72mg DE/g of dried leaves sample and 63.09 – 71.90mg DE/g of dried stems sample respectively. The YDC shaded leave and stem samples exhibited the highest saponin content (Table 2). The previous study on medicinal plant *T. purpurea* and *D. sissoo* contained saponins lower than the current result (0.70 and 0.12mg/g of dry weight) (Gnanaraja *et al.*, 2014).

6. Steroid determination

The steroid content of both leaves and stems of *C. nutans* ranged between 542.00 – 833.32mg CA/g of dried sample and 158.75 – 247.35mg CA/g of the dried sample respectively. The steroid content was high in the leave samples than in the stem samples. YDC shaded leaves contained significantly higher steroid content than the other samples (Table 2). The previous study on medicinal plant *T. purpurea*, *Delonix regia* and *D. sissoo* contained steroids lower than the current result (0.18, 0.17 and 0.11mg/g of dry weight) (Gnanaraja *et al.*, 2014).

7. Tannin determination

The tannin content of *C. nutans* leaves and stems ranged between 340.12 – 496.81mg GAE/g of dried samples and 166.50 – 204.35mg GAE/g of the dried sample, respectively. Un-shaded leaves samples from YDC showed a high amount of tannin (Table 2). The current result is higher than the previous study on lemongrass (3mg rutin/g of dried sample) (Geetha and Geetha, 2014). The previous study on medicinal plant *T. purpurea*, *D. regia* and *D. sissoo* contained tannins lower than the current result (0.77, 1.59 and 0.52mg /g of dry weight) (Gnanaraja *et al.*, 2014).

C. Determination of antioxidant properties in C. nutans leaves and stems

Ali *et al.* (2008) claimed that natural anti-oxidants mainly present in the form of phenolic compounds such as flavonoids and phenolic acids from the plants. Un-shaded leaves sample of *C. nutans* from YDC exhibited significantly ($p < 0.05$) higher result on antioxidant properties (966mg GAE/g of dried sample in TPC, 20.44mg TE/g of dried sample in FRAP and 11.14mg TE/g of dried sample in DPPH) than the others (Table 3). The results were below the result of BHA and BHT (Table 4). The current TPC results on all leaves and stems samples were higher than the previous

Table 3. Effect of different areas on the antioxidant activities of *Clinacanthus nutans* leaves and stems

Sample	Area	Leaves			Stems		
		TPC (mg GAE/g)	**FRAP (mg TE/g)**	**DPPH (mg TE/g)**	**TPC (mg GAE/g)**	**FRAP (mg TE/g)**	**DPPH (mg TE/g)**
YPL	Un-shaded	930.13 ± 1.76 [b]	14.00 ± 0.26 [b]	9.00 ± 0.17 [b]	209.86 ± 2.44 [b]	2.43 ± 0.06 [b]	1.27 ± 0.05 [b]
YDC	Un-Shaded	966.00 ± 2.19 [a]	20.44 ± 0.06 [a]	11.14 ± 0.02 [a]	249.08 ± 2.59 [a]	3.12 ± 0.01 [a]	1.79 ± 0.02 [a]
	Shaded	661.89 ± 2.31 [c]	6.55 ± 0.01 [c]	3.80 ± 0.01 [c]	207.62 ± 1.26 [c]	1.93 ± 0.01 [c]	0.80 ± 0.08 [c]

TPC: total phenolic content; GAE: gallic acid; FRAP: ferric-reducing antioxidant power; TE: trolox equivalent antioxidant capacity; DPPH: radical-scavenging activity

[a-c] mean values with different superscripts in the same column are significantly different at $p < 0.05$

Table 4. Antioxidant power of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT)

	TPC (mg GAE/g)	**FRAP (mg TE/g)**	**DPPH(mg TE/g)**
BHA	TC	TC	11.73 ± 0.04
BHT	975.98 ± 0.87	TC	11.92 ± 0.01

TPC: total phenolic content; GAE: gallic acid; FRAP: ferric-reducing antioxidant power; TE: trolox equivalent antioxidant capacity; DPPH: radical-scavenging activity

TC: too concentrated

study on ethanol (80%) leaves and stems extract of *C. nutans* (117.00 and 114.42mg GAE/100g dried sample respectively) (Raya *et al.*, 2015). Based on the previous results by Yang *et al.* (2013), methanolic extract of *C. nutans* leaves exhibited 1.77 ± 0.01mg gallic acid equivalent/g, which was much lower than the current results. Furthermore, the water leaves extract of *C. nutans* (extraction time: one hour) showed lower in TPC result than the current result (46.71mg GAE/g of dried sample) (Kosai *et al.*, 2016). Acetone extract of whole aerial part of herbs plant *L. indica* contained 0.35mg GAE/g of dried sample, which is lower than the acetone extract of *C. nutans* (Pranoothi *et al.*, 2014).

In comparison between leaves and stems, leaves have a greater antioxidant potential than the stems. The same result was shown by an antioxidant study on bitter gourd (*Momordica charantia*) plant with the high antioxidative potential in leaves than stems in DPPH, FRAP and TPC (Kubola and Siriamornpun, 2008). The total phenol and total phenolic acid are found exhibiting higher antioxidative potential in leaves than the stems of herbal plant *Moltkia petraea* (Tratt.) Griseb (Končića *et al.*, 2010). Leaf extract has higher total phenolic content (33.67mg GAE/g) than stem (11.11mg GAE/g) extracts determined by Wannes *et al.* (2010) on the methanolic extract of medicinal plant *Myrtus communis* L.

For comparison between un-shaded and shaded samples of YPL, both un-shaded leaves and stems samples exhibited higher ($p < 0.05$) antioxidant activity than shaded samples. High light exposure promoted high phenolic content than under low light condition, hence exhibited high antioxidant activity. Gregoriou *et al.* (2007) have reported that photosynthetic capacity was lowered due to shading. Furthermore, the duration of storage might be one of the factors that were affecting TPC. The previous study showed decreasing trend on TPC result of leaves of *C. nutans* from one day to four days of storage (Raya *et al.*, 2015).

D. Correlation coefficient of antioxidant activities

There was the highest correlation (0.995) between the results of FRAP and DPPH of *C. nutans* leaves and stems, while it showed the lowest value (0.945) between TPC and FRAP (Table 5).

Table 5. Correlation coefficient of antioxidant activities of total phenolic content (TPC), ferric-reducing antioxidant power (FRAP) and DPPH radical scavenging activity

Correlation coefficient (r)	**FRAP**	**DPPH**
TPC	0.945	0.959
FRAP		0.995

Total phenolic content (TPC), ferric-reducing antioxidant power (FRAP) and DPPH radical scavenging activity
Correlation is significant at the 0.01 level (2-tailed)

IV. CONCLUSION

The results showed the presence of various phytochemical compounds in *C. nutans* leaves and stems. These phytochemical compounds contributed to its antioxidant properties. Hence, *C. nutans* has the potential to become an alternative antioxidant source in a food product or for the development of functional food products. In future work, specific types of phytochemical compounds which present in leaves and stems can be investigated.

V. ACKNOWLEDGEMENT

The authors wish to thank Universiti Kebangsaan Malaysia for funding assistance: FRGS/1/2014/STWN03/UKM/02/1 and DIP-2014-007.

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