

Potential antioxidant activities of methanolic extracts of Spider lily (*Hymenocallis littoralis*)Jeevandran Sundarasekar¹, Geethaa Sahgal², Safiah Ahmad Mubbarakh¹, Sreeramanan Subramaniam^{1*}¹School of Biological Sciences, Universiti Sains Malaysia, Georgetown, 11800, Penang, Malaysia²Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Georgetown, 11800, Penang, Malaysia*Corresponding author: sreeramanan@gmail.com / sreeramanan@usm.my**Abstract**

Hymenocallis littoralis has allelochemical importance such as defensive compounds, insect repellents, attractants, and their role in ecological balance. Antioxidant activities of *Hymenocallis littoralis* bulb, anther, flower, stem, leaves and root methanolic extracts were evaluated using Ferric Reducing Antioxidant Power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), total phenolic and flavonoid assays. Dried parts were ground and extracted using sonication method. The extracts were then tested for their antioxidant activity by FRAP, DPPH, total phenolic and flavonoid assays. The flower and anther methanolic extracts shows ferric reducing antioxidant power (FRAP) at 555.12 ± 1.67 and $568.09 \pm 0.42 \mu\text{mol g}^{-1} \text{Fe}^{2+}$. High activity for DPPH free radical molecules was observed in flower (1.29 mg mL^{-1}), stem (1.33 mg mL^{-1}), and anther (0.31 mg mL^{-1}). The phenolic content of *Hymenocallis littoralis* extracts are in ascending order as per root < leaves < stem < bulb < flower < anther. The flavanoid content of *Hymenocallis littoralis* plant extracts in ascending order are bulb < root < leaves < flower < stem < anther. Elucidation and isolation of the active compounds from this plant can help to shed more information of the compound of interest. This finding shows that the *Hymenocallis littoralis* bulb, root and anther extracts possess a good antioxidant activity which can demonstrate a better cytotoxicity activity.

Keywords: *Hymenocallis littoralis*, Spider lily plant, Sonication, Antioxidant activity.**Abbreviation:** Ferric Reducing Antioxidant Power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH).**Introduction**

Reactive oxygen species (ROS) caused oxidative stress to the body system. Highly unstable, unpaired electrons in ROS extract electrons from other molecules to attain stability and cause damage to other molecules which lead to cellular destruction (Ali et al., 2008). The reductions of exogenous antioxidant concentration due to the mutated antioxidant enzymes, toxins, or reduced intake or natural antioxidants are cause for the oxidative stress in human body. Moreover, the oxidative stress is also caused due to the increase number of oxygen/nitrogen/carbon-based reactive species derived from activated phagocytes in the case of chronic inflammation (Somogyi et al., 2004). Oxidative stresses caused by free radicals in our body are eliminated by exogenous and endogenous antioxidants (Tawaha et al., 2007). These antioxidants can be grouped into two based on the mechanism of its action. There is either chain breaking antioxidants or preventive antioxidants (Somogyi et al., 2004). Preventive antioxidants reduce the ROS's chain initiation rate, while the chain breaking antioxidants interfere the propagation of ROS chain (Somogyi et al., 2004). There are several *in vivo* (Somogyi et al., 2004) and *in vitro* (Huang et al., 2005) methods used to determine the antioxidant capacity of plant extracts. Antioxidant activity is used to measure a compound to reduce the pro-oxidants or reactive species of pathologic significance (Somogyi et al., 2004). Ferric reducing antioxidant power (FRAP), total phenolic assay by using the Folin-Ciocalteu reagent, total flavonoid content and DPPH radical scavenging activity are the common methods used to evaluate the antioxidant properties (Karadag et al., 2009). These methods are commonly used to determine the direct interaction of reactive molecules or free

radicals reacting with metal ions. Currently there are abundant search of exogenous antioxidant from natural materials including from plant extracts (Oke et al., 2009). The possible carcinogenic effects of synthetic antioxidants in food prompted many researchers to search for new natural antioxidants (Shahidi, 2008). Moreover, plant has a variety of phytochemicals and thus it contributes for the high antioxidative efficacy (Dorman and Hiltunen, 2004). Leong and Shui (2002) reported that variety of subtropical, tropical and vegetables produced high content of antioxidants. *Maipighia punicifolia* (Ceri), *Garcinia atroviridis* (Asam Gelugor), *Psidium guajava* (Jambu), *Flacouria rukam*, (Rokam Manis), *Ziziphus mauritiana*, (Bidara) are some of Malaysian underutilized fruits which possess abundant antioxidant properties (Ikram et al., 2009). Similarly, various Algerian's plants also were subjected to antioxidant assays such as bark of *Fraxinus angustifolia*, leaves of *Pistacia lentiscus* and *Clematis flammula* and they show incredible antioxidant activity (Atmani et al., 2009). Masoko and Eloff (2007) reported that *Combretum woodii*, *C. colinum spp. taborense*, *C. hereroense*, *Terminalia prunioides*, *T. brachystemma*, *T. sericea*, *T. gazensis*, *T. mollis* and *T. sambesiaca* from South Africa shows a potent antioxidant activity. Since antioxidants from plant source are safe and easily available, potential *Hymenocallis littoralis* with high phytochemical constituents was subjected to determine and quantify various antioxidant activities. This would be the first scientific study to evaluate the antioxidant activity of this plant. Thus methanolic crude extracts from bulb, anther, flower, leaves, stem and roots were used for the antioxidant activities.

Results

Determination of ferric reducing antioxidant power

Hymenocallis littoralis leaves, stem, root, anther, bulb and flower were extracted via methanol solvent and subjected to the FRAP assay. The antioxidant compounds in these extracts could reduce the Ferric ions to ferrous and the reduction was measured spectrophotometrically at 593 nm. The antioxidant capacity of *Hymenocallis littoralis* extracts were determined from regression equation of calibration curve ($y=0.0257x + 0.7434$, $R^2 = 0.9912$) and expressed as μmol ferrous ion equivalent per gram of sample. Only flower and anther displays antioxidant power for ferric ions. The antioxidant capacity of flower and anther are 555.12 ± 1.67 and $568.09 \pm 0.42 \mu\text{mol g}^{-1} \text{Fe}^{2+}$ (Table 1).

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

All the plant parts of *Hymenocallis littoralis* methanol extracts were subjected for DPPH analysis. The analysis reveals the flower, stem and anther of this plant has high scavenging activity for DPPH free radical molecules. The leaves, bulb and root have mild antioxidant activity and L-Ascorbic acid was used as reference antioxidant substance (Table 2). The scavenging activity (%) *Hymenocallis littoralis* are 72.98 ± 0.43 % for anther, 69.61 ± 0.16 % for flower, 66.43 ± 0.60 % for stem, 37.43 ± 0.39 % root, 34.33 ± 0.53 % for bulb, 32.54 ± 1.32 for leaves, and L-Ascorbic acid 82.46 ± 0.21 % (Figure 1). The scavenging activity of *Hymenocallis littoralis* in descending order is L-Ascorbic acid > anther > flower > stem > root > bulb > leaves respectively. The inhibition concentration of extract at 50 % (IC_{50}) value for anther, flower, stem, root, bulb and leaves are 0.31, 1.29, 1.33, 3.68, 5.07 and 5.42 mg mL^{-1} , respectively. In DPPH assay, the lower the IC_{50} the better it is able to scavenge the radicals (Lim et al., 2007). The statistical analysis for methanol extract of anther displayed comparable activity with standard (L-Ascorbic acid). The flower and stem also shows similar antioxidant activity with anther (Table 2). Therefore, the phenolic substance in this plant extracts donate hydrogen atoms to DPPH radicals and stabilize it into hydrazine molecules. The high DPPH scavenging activity shows a significant correlation with phenolic contents (Lim et al., 2007).

Determination of Total Phenolic and Flavonoid contents

In attempt to establish a potential relationship with DPPH and FRAP, phenolic and flavonoid content assays were performed. The phenolic content of *Hymenocallis littoralis* extracts including root (HR), stem (HS), leaves (HL), flower (HF), anther (HA) and bulb (HB) were tested using Folin-Ciocalteu assay. The phenolic content (mg equivalent Gallic acid/ g extract) of *Hymenocallis littoralis* parts are presented in Figure 2 and Table 3. The phenolic content of *Hymenocallis littoralis* parts were determined from regression equation of calibration curve ($y=9.8928x + 0.0924$, $R^2 = 0.997$) and expressed in Gallic acid equivalents (GAE). The outcomes exhibits the phenolic content in ascending as per root < leaves < stem < bulb < flower < anther. The root extract ($5.32 \pm 1.52 \text{ mg GAE g}^{-1}$ extract) produced lowest phenolic content followed by leaves extract ($8.62 \pm 1.52 \text{ mg GAE g}^{-1}$ extract). The stem (21.86 ± 0.83

mg GAE g^{-1} extract) and bulb ($28.97 \pm 3.16 \text{ mg GAE g}^{-1}$ extract) displayed moderate phenolic content compared to the remaining plant extract. The flower ($33.35 \pm 2.51 \text{ mg GAE g}^{-1}$ extract) and anther ($55.35 \pm 6.97 \text{ mg GAE g}^{-1}$ extract) has the highest phenolic content in this evaluation. The flavonoid contents of *Hymenocallis littoralis* different extracts were generated from regression equation of calibration curve ($y=0.0017x + 0.0537$, $R^2 = 0.9861$) and expressed in Catechin equivalents (CE) per gram of dry material. Table 3 and Figure 3 presented the flavonoid content of the extract. The outcomes exhibits the flavonoid content in ascending as per bulb < root < leaves < flower < stem < anther. The methanolic extract of anther and flavonoid has high content of 16.06 ± 3.67 and $16.06 \pm 3.28 \text{ mg CE g}^{-1}$ respectively. Flower ($2.53 \pm 0.59 \text{ mg CE g}^{-1}$), leaves ($1.55 \pm 0.68 \text{ mg CE g}^{-1}$), root ($1.35 \pm 1.18 \text{ mg CE g}^{-1}$), and bulb ($0.76 \pm 0.59 \text{ mg CE g}^{-1}$) has low amount of flavonoid. The high contents of flavonoid in anther and stem in *Hymenocallis littoralis* could enhance the antioxidant activity.

Discussion

Hymenocallis littoralis plant portion such as bulb, flower, anther, stem, leaves and roots were extracted using sonication method via methanol solvent. The methanol extracts were dried in oven at 40°C for 1 week and then subjected antioxidant activities. Ferric ion (Fe^{3+}) is often used as an indicator of electron-donating activity. This activity is mainly involved on an important mechanism of phenolic antioxidant action (Yildirim et al., 2001). This assay has potential redox activity which comparable with ABTS and TEAC assay (Karadag et al., 2009). In FRAP assay, excess Fe^{3+} -TPTZ is reduced to form Fe^{2+} -TPTZ and this formation is due to the reducing ability of the test sample. This assay was chosen since it is simple, precise, has a significant correlation with phenolic content and producing a reproducible results (Singh and Singh, 2012). Moreover, this assay does not require constant stoichiometric factors and pretreatment for the sample (Frankel and Meyer, 2000). *Hymenocallis littoralis* flower and anther methanol extracts displays antioxidant power for ferric ions. Other plants parts do not possess ferric reduction capacity. L-ascorbic acid was used as positive control for this assay and its shows very high reduction power for this assay ($5678 \pm 0.27 \mu\text{mol g}^{-1} \text{Fe}^{2+}$). Even though *Hymenocallis littoralis* shows low ferric reduction power compare to L-ascorbic acid, yet it has high reduction capacity rivaled with other plant species. Li et al. (2008) reported that numbers of Chinese traditional medicinal plants with high phenolic content produced higher level of reduction power for FRAP. Evidence for the correlation between phenolic and FRAP assays was based on their results obtained from *Sargentodoxa cuneata* Rehd. Et Wils (phenolic content: $52.35 \pm 0.25 \text{ mg GAE g}^{-1}$ extract; FRAP: $453.53 \pm 10.3 \mu\text{mol g}^{-1} \text{Fe}^{2+}$), *Scutellaria baicalensis* Ceorgi (phenolic content: $36.30 \pm 0.67 \text{ mg GAE g}^{-1}$ extract; FRAP: $304.86 \pm 14.9 \mu\text{mol g}^{-1} \text{Fe}^{2+}$) and *Trichosanthes Kirilowii* Maxim (phenolic content: $2.13 \pm 0.03 \text{ mg GAE g}^{-1}$ extract; FRAP: $1.85 \pm 0.03 \mu\text{mol g}^{-1} \text{Fe}^{2+}$). In addition, high antioxidant fruits and vegetable apple, banana, and onion also show the similar correlation. Apple (*Malus domestica*) has phenolic content of $48.1 \pm 1.0 \text{ mg GAE g}^{-1}$ extract and FRAP $394 \pm 8.0 \mu\text{mol g}^{-1} \text{Fe}^{2+}$, banana has phenolic content of $38.0 \pm 1.0 \text{ mg GAE g}^{-1}$ extract and FRAP $164.0 \pm 32.0 \mu\text{mol g}^{-1} \text{Fe}^{2+}$ and onion shows phenolic content of $88.0 \pm 1.0 \text{ mg GAE g}^{-1}$ extract and FRAP $369 \pm 13.0 \mu\text{mol g}^{-1} \text{Fe}^{2+}$ (Apak et al., 2007). These reports show a positive correlation

Table 1. The ferric reducing antioxidant power for various plant parts of *Hymenocallis littoralis*.

Extracts	(Mean ± Sd)
Leaves	Nil
Flower	555.12±1.67*
Bulb	Nil
Anther	568.09±0.41*
Stem	Nil
Root	Nil
L-Ascorbic acid	5678±0.27

Nil= no activity; One way ANOVA was used for statistical analysis and all test run in triplicates (n=3) *: Denotes the significance difference when compared to L-Ascorbic acid, p<0.05 using Dunnett's test

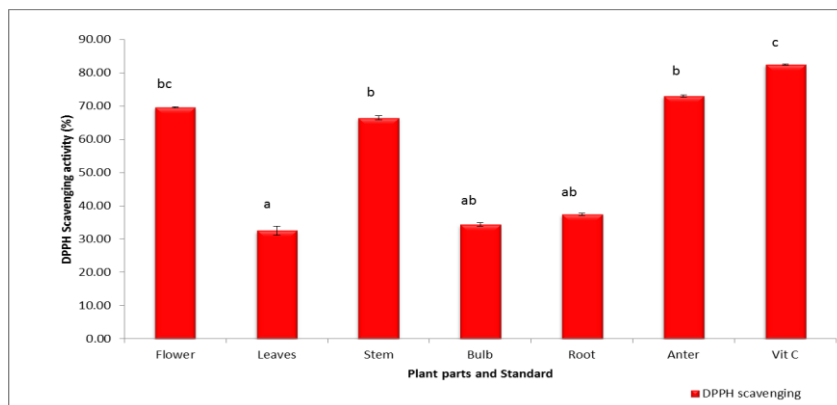


Fig 1. The DPPH scavenging activity (%) of *Hymenocallis littoralis* in different plant parts. Different superscripts indicate statistical significant differences among the concentrations at a p-value < 0.05 (n= 3).

Table 2. The DPPH scavenging activity (%) and IC₅₀ (mg mL⁻¹) for various *Hymenocallis littoralis* parts extracts.

Plant Parts	DPPH Scavenging (%)	
	Mean ± sd	IC ₅₀ (mg mL ⁻¹)
Flower	69.61 ± 0.16*	1.29
Leaves	32.54 ± 1.32	5.07
Stem	66.43 ± 0.60*	1.33
Bulb	34.33 ± 0.53	5.42
Root	37.43 ± 0.39	3.68
Anther	72.98 ± 0.43*	0.31
L-Ascorbic acid	82.46 ± 0.21	0.21

Note: the experiment was conducted in triplicates (n=3) and one way ANOVA test was carried. *: Denotes the significance not difference when compared to L-Ascorbic acid, p<0.05 using Dunnett test

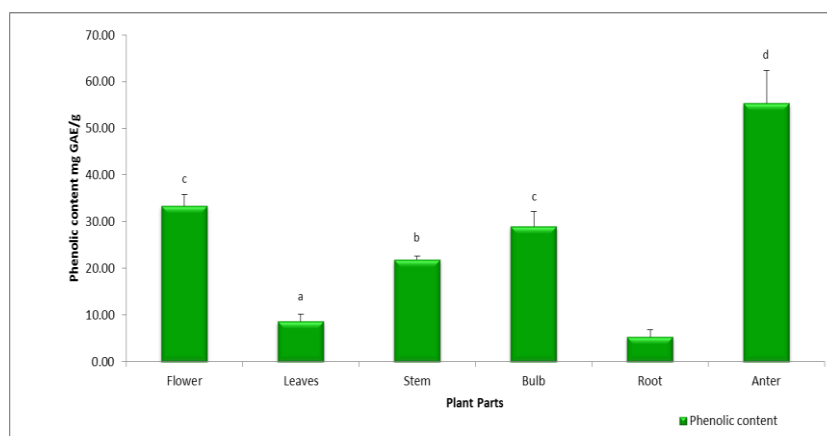


Fig 2. Total phenolic contents of *Hymenocallis littoralis* in various plant parts. Different superscripts indicate statistical significant differences among the concentrations at a p-value < 0.05 (n=3)

among phenolic content and FRAP reducing power as *Hymenocallis littoralis* plant extracts. The flower and anther with highest phenolic content shows high antioxidant power while other plants with low phenolic content fails to provide significant reduction to ferric ions. Even though the anther has low flavonoid content but it is still contributing to a high FRAP capacity. Hence, other phytoconstituents in phenolic groups such as tannins also contributed to the antioxidant activity (Atmani et al., 2009). Non-flavonoid compound such as stilbenes, hydroxycinnamic acid and benzoic from polyphenol group also contributes for their antioxidant activity (Paixao et al., 2007). Consequently, phenolic content contribute for the ferric reducing antioxidant power. DPPH is organic nitrogen radical and give a deep blue colour for UV-visual absorption at 515 nm (Huang et al., 2005). Any antioxidant or reducing compounds reduced the DPPH radical molecules to corresponding pale yellow hydrazine (Karadag et al., 2009). A hydrogen atom from an antioxidant compounds is donated to reactive DPPH molecules to achieve stable condition (Contreras-Guzman and Strong, 1982; Singh and Singh, 2012). Lim et al. (2007) reported that the IC₅₀ value of DPPH has better correlation with phenolic content in guava (phenolic content: 138.0 ± 31 mg GAE g⁻¹ extract; IC₅₀: 1.71 ± 0.61 mg mL⁻¹), orange (phenolic content: 75.0 ± 10 mg GAE g⁻¹ extract; IC₅₀: 5.4 ± 1.3 mg mL⁻¹), kendondong (phenolic content: 33.0 ± 5 mg GAE g⁻¹ extract; IC₅₀: 9.9 ± 1.9 mg mL⁻¹) and star fruits (phenolic content: 131.0 ± 54 mg GAE g⁻¹ extract; IC₅₀: 3.8 ± 2.1 mg mL⁻¹). Therefore, the bleaching action in DPPH molecules is mainly attributed to the presence of polyphenols substances in the crude extracts that were obtained. The DPPH scavenging activity (%) also exhibits a significant correlation with total phenolic content as described by Ikram et al. (2009). *Maipighia puniciroli* with 107.0 ± 0.4 mg GAE/g of phenolic content shows 81.04 ± 0.38 % of scavenging activity. In addition, *Mangifera odorata*, *Carissa carandas* and *Ziziphus mauritiana* with 8.0 ± 0.0, 12.0 ± 0.0, 41.0 ± 0.3 mg GAE g⁻¹ extract of phenolic content express 45.68 ± 11.09, 43.57 ± 1.53 and 74.96 ± 0.44 % of scavenging activity respectively (Ikram et al., 2009). A correlation (r² = 0.02339, p < 0.05) was also obtained between total phenolic and DPPH activities of pulps and peels from eight banana (*Musa* sp.) cultivars (Sulaiman et al., 2011). The phenolic content of the extracts are directly proportion to DPPH scavenging activity. Thus, the high phenolic and flavonoid content in anther, flower and stem extracts donates for the high DPPH scavenging activity. Flavonoid is categorized under phenolic group (Michalak, 2006) and it is a low molecular weight compound which characterized by the flavan nucleus (Heim et al., 2002). The flavonoids are mostly classified according to phenolic and pyrane substitutions. Flavanol, flavone, flavonol, flavanone, isoflavone and antocyanidin are the classes in flavonoids (Heim et al., 2002). Flavonoids mostly attribute to their antioxidant and chelating abilities. Flavonoids could transfer electrons to free radicals, chelate metal catalysts (Ferrali et al., 1997), activate antioxidant enzymes (Elliott et al., 1992), reduce alpha-tocopherol radicals (Hirano et al., 2001) and inhibit Oxidase (Cos et al., 1998; Heim et al., 2002). Thus, flavonoid content of the *Hymenocallis littoralis* plant parts extracts were assessed in this study. The phenolic substances including flavonoids have been shown to be responsible for the antioxidant activity (Hinneburg et al., 2006). Frequent plant species including vegetables, spices, fruits, higher plants were evaluated for the total phenolic contents.

The *Ocimum basilicum* (basil), *Petroselinum crispum* (Parsley), *Electtraria cardamomum* (Cardamom), *Foeniculum vadgare* (Fennel), *Zingiber officinalis* (Ginger) are common spices used in cooking and the total phenolic content of the spices are 141.0 ± 1.6, 29.2 ± 0.44, 24.2 ± 0.29, 30.3 ± 0.76, 23.5 ± 1.26 mg GAE g⁻¹ extract, respectively (Hinneburg et al., 2006). In this study, it was shown that *Hymenocallis littoralis* extracts have comparable phenolic contents with high antioxidant capacity similarly to the other underutilized fruits found in Malaysia. Examples are Rokam Manis (40.0 ± 0.2 mg GAE g⁻¹ extract), Asam Gelugor (29.0 ± 0.2 mg GAE g⁻¹ extract), Jambu (31.1 ± 0.1 mg mg GAE g⁻¹ extract), Bidadar (41.0 ± 0.3 mg GAE g⁻¹ extract), and Jambu Boi (6.0 ± 0.0 mg GAE g⁻¹ extract) (10). A number of Traditional Chinese Medicinal plant such as *Artemisia apiacea* Hance (8.57 ± 0.26 mg GAE g⁻¹ extract), *Baphicacanthus cusia* (Nees) Brem (1.15 ± 0.01 mg GAE g⁻¹ extract), *Lonicera japonica* Thunb (flower) (27.36 ± 0.29 mg GAE g⁻¹ extract), *Lonicera japonica Thunb* (stem) and *Scutellaria baicalensis* Ceorgi (36.30 ± 0.67 mg GAE g⁻¹ extract) (Li et al., 2008). All the reports fortify a significant correlation with phenolic content and antioxidant activity in plant species. Thus, the phenolic content of this *Hymenocallis littoralis* plant parts also shows a corresponding correlation. The flower, anther and stem extracts exhibits highest DPPH scavenging activity while flower and anther extracts reduced the ferric metal in FRAP assay. This outcome reveals higher phenolic content extracts demonstrated better antioxidant activity. The leaves and root shows lower antioxidant activity for both DPPH and FRAP assay and similarly have low amount of phenolic content. However, Sulaiman et al. (2011) reported that non-phenolic compounds also may highly contribute for the antioxidant activities. The non-phenolic compounds such as Vitamin E, Vitamin C (Sulaiman et al., 2011) and triterpenes (ursolic acid, oleanoic acids) (Pilarski et al., 2006) from *Uncaria tomentosa* also highly contributes for the antioxidant activities. Consequently, the non-phenolic and phenolic substances in *Hymenocallis littoralis* extracts might also be accountable in enhancing for the antioxidant activity. *Hymenocallis littoralis*'s anther, flower and stem methanol extracts exhibit good antioxidant property based on both FRAP and DPPH analyses and there is a significant correlation with total phenolic and flavonoid contents. This indicates there is a contribution of phenolic compounds in the extracts to the antioxidant activities. These results can be used as fundamental information for further exposure of responsible antioxidant substance in these extracts. Identification of the dietary minerals and toxicity levels of this plant would aid the incorporation of the plant extract in food industry.

Materials and methods

Samples preparation

The plant materials were washed thoroughly and separated into different parts namely root, bulb, stem, leaves, flower and anther. The different plant parts were dried in oven at 50°C until a constant weight was achieved and then were ground. The powdered plant materials such as bulb, flower, leaves, anther, stem and root were extracted with methanol by means of sonication. The extract was filtered through filter paper (Whatman No.1) and the filtrate was collected and concentrated in a rotary evaporator (RII0, Buchi) at 40°C.

Table 3. The total phenolic and flavonoid contents of *Hymenocallis littoralis* in various plant parts.

Extracts	Phenolic		Flavonoid	
	(mg Gallic acid/g extract)		(mg Cathecin/g extract)	
	Mean	Sd	Mean	Sd
Flower	33.35	2.51	2.53	0.59
Anther	55.35*	6.97	16.06	3.67
Root	5.38	1.52	1.35	1.18
Bulb	28.97	3.16	0.76	0.59
Leaves	8.62	1.52	1.55	0.68
Stem	21.86	0.83	16.06	3.28

Note: All test samples run in triplicates (n=3) and one way ANOVA test was carried. *: Denotes there is significance difference among the groups; p<0.05 using Dunnett test.

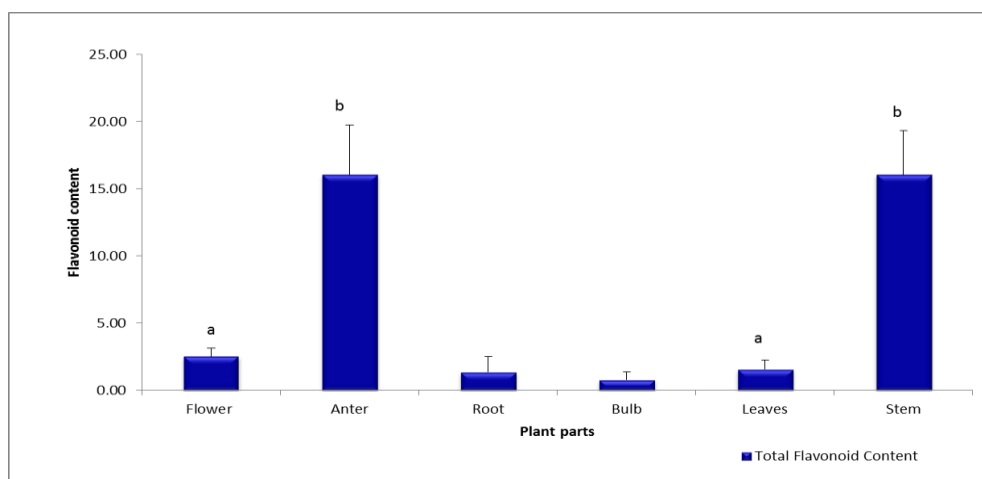


Fig 3. Total flavonoid contents of *Hymenocallis littoralis* in various plant parts. Different superscripts indicate statistical significant differences among the concentrations at a p-value < 0.05 (n=3).

The concentrated extract was dried in an oven at 40°C for three days to obtain a consistent weight prior to storage at -20°C (Masoko and Eloff, 2007).

Evaluation of Ferric-Reducing Antioxidant Power activity (FRAP)

The FRAP reagent was prepared by mixing 25 mL of acetate buffer (300 mmol L⁻¹, pH 3.6), 2.5 mL of 10 mmol L⁻¹TPTZ solution in 40 mmol L⁻¹ HCl and 2.5 mL of 20 mmol L⁻¹ FeCl₃ solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh and warmed to 37°C in a water bath prior to use. One hundred and fifty microlitres of the sample (1 mg mL⁻¹) was added to 4.5 mL of the FRAP reagent. The absorbance of the reaction mixture was then recorded at 593 nm after 4 min and the assay was carried out in triplicates. The standard curve was constructed using FeSO₄ solution (0.5-10 mol.L⁻¹). The results were expressed as μmol g⁻¹Fe (2⁺) dry weight of plant material. L-ascorbic acid was also used as a comparative model for this assay (Li et al., 2008; Sahgal et al., 2009).

Determination of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay

The free radical scavenging activity was measured by using DPPH assay. The quantitative estimation of radical scavenging activity was determined according to the methods described by Wu and Ng (2008). Five milliliters of 0.04 % (w/v) DPPH radical solution was added to test sample solutions ranging from 0.03125, 0.0625, 0.0125, 0.25, 0.5, 1 and 2 mg mL⁻¹. The mixture was vortex-mixed and kept in dark room condition for 30 min. The optical density (OD) was measured at 517 nm. Methanol was used as baseline

control. L-Ascorbic acid (Vitamin C) and BHT were used as positive control. The DPPH radical concentration was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [(A_0 - A_1) \times 100\%] / A_0$$

A₀ is the absorbance of the control reaction and A₁ is the absorbance in the presence of the sample of the tested extracts (Wu and Ng, 2008; Sahgal et al., 2009).

Determination of Total Phenolic Content

The total phenolic content in the methanol extracts was measured using Folin-Ciocalteu reagent method (Djeridane et al., 2006). A linear dose-response regression curve was generated using absorbance reading of Gallic acid at the wavelength of 765 nm using UV-spectrophotometer (UV-160A, Shimadzu). The total phenolic compounds concentration in the extract was expressed as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE g⁻¹) of extract. The content of phenolic compounds in the plant extracts was calculated using the following formula: C= A/B; where C is expressed as mg GAE g⁻¹dry weight of the extract; A is the equivalent concentration of Gallic acid established from calibration curve (mg); and B is the dry weight of the extract (g) (Djeridane et al., 2006; Sahgal et al., 2009).

Determination of Total Flavonoid Content

The total flavonoid content of the extracts was determined according to colorimetric method as described by Zou et al. (2004). In brief, 0.5 mL of the sample solution was mixed with 2 mL of distilled water and subsequently with 0.15 mL of 5 % (w/v) NaNO₂ solution. After 6 min of incubation, 0.15 mL of 10 % (w/v) AlCl₃ solution was added and then allowed to stand for 6 min, followed by adding 2 mL of 4 % (w/v)

NaOH solution to the mixture. Consequently, water was added to the sample to bring the final volume to 5 mL and the mixture was thoroughly mixed and allowed to stand for another 15 min. The mixture's absorbance was determined at 510 nm. The total flavonoid content was expressed in mg of Catechin per gram of extract (Zou et al., 2004; Sahgal et al., 2009).

Statistical Analysis

The data was presented as mean \pm standard deviation (SD) for the three determinations and the statistical significance between groups was analyzed by ANOVA followed by Dunnett's test. P-values less than 0.05 were considered significant.

Conclusion

Hymenocallis littoralis's anther, flower and stem methanol extracts exhibit good antioxidant property based on both FRAP and DPPH analyses and there is a significant correlation with total phenolic and flavonoid contents. This indicates there is a contribution of phenolic compounds in the extracts to the antioxidant activities. These results can be used as fundamental information for further exposure of responsible antioxidant substance in these extracts. Identification of the dietary minerals and toxicity levels of this plant would aid the incorporation of the plant extract in food industry.

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