TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF *CLITORIA TERNATEA* L. (AUNG-ME-NYO) FLOWERS

Khin Myo Myint Tun[1], Zaw Zaw Myint[2]

Abstract

In the present research, the flower of *Clitoria ternatea* L. (Aung-me-nyo) that is one of the most popular Myanmar medicinal plants was selected to study total phenolic content, total flavonoid content and antioxidant activity. Preliminary phytochemical analyses indicated the presence of alkaloids, glycosides, carbohydrates, phenolic compounds, saponins, tannins, α-amino acids, reducing sugars, steroids, flavonoids, anthocyanins, starch and organic acids. Total phenolic contents of various extracts were investigated by Folin-Ciocalteu reagent and total flavonoid contents were determined by aluminium chloride colourimetric method. According to the results, total phenolic contents of petroleum ether, ethyl acetate, methanol and aqueous extracts were 16.36, 33.16, 25.12 and 11.22 µg GAE/mg of extract, respectively. Total flavonoid contents of petroleum ether, ethyl acetate, methanol and aqueous extracts are 132.17, 86.52, 73.48 and 23.48 µg QE/mg of extract, respectively. Antioxidant activity of the extracts of *Clitoria ternatea* flowers was screened by DPPH assay method and powerful antioxidant activity of the flower extracts was observed.

Keywords: *Clitoria ternatea* L., total phenolic content, total flavonoid content, antioxidant activity

Introduction

The present work is directed towards the extensive study of widespread applications of *Clitoria ternatea* L. (Aung-me-nyo) flowers in the treatment of various life threatening diseases and disorders. In Myanmar, Aung-me-nyo plant has been of keen interest due to its wide spectrum of medicinal uses and biological activities. In the present work, phytochemical analyses of the flower of *C. ternatea* (Aung-me-nyo) were carried out. Moreover, *C. ternatea* was selected for the study on its antioxidant activity of various extracts.

Scientific Classification

Family	:	Fabaceae
Genus	:	*Clitoria*
Species	:	*C. ternatea*
Botanical name	:	*Clitoria ternatea* L.
Common name	:	Butterfly pea
Myanmar name	:	Aung-me-nyo (Kress *et al*., 2003)

The photographs of *C. ternatea* (Aung-me-nyo) plants and flowers are shown in Figure 1.

[1] Dr, Associate Professor, Department of Chemistry, West Yangon University
[2] MSc (Chemistry), West Yangon University

Figure 1 *Clitoria ternatea* L. (Aung-me-nyo) plants, leaves and flowers

Medicinal Values of *C. ternatea*

The plant *C. ternatea* is traditionally used for food colouring, stress, infertility and gonorrhea. The plant has been widely used in Ayurveda. The juice of flowers is reported to be used in insect bites and skin diseases (Agrawal *et al*., 2007). The roots are useful in asthma, burning sensation, ascites, inflammation, leucoderma, leprosy, hemicranias, amentia, pulmonary tuberculosis, ophthalmology and reported as bitter refrigerant, ophthalmic, laxative, diuretic, cathartic, aphrodisiac, tonic (Nadkarni, 1976). The root, steam and flower are recommended for the treatment of snakebite and scorpion sting (Kazuma *et al*., 2003). Fresh flowers of *C. ternatea* showed hypoglycemic and hypolipidemic effect (Abhishek *et al*., 2013).

Chemical Constituents of *C. ternatea*

The flowers of *C ternatea* contain flavonol glycosides, 3-O-(2"-O-α-rhamnosyl-6"-O-malonyl)-β-glucoside, 3-O-(6"-O-α-rhamnosyl-6"-O-malonyl)-β-glucoside and 3-O-(2", 6"-di-O-α-rhamnosyl)-β-glucoside of kaempferol, quercetin and myricetin. Delphinidin glycosides, 3-O-β-glucoside, 3-O-(2"-O-α-rhamnosyl)-β-glucoside, 3-O-(2"-O-α-rhamnosyl-6"-O-malonyl)-β-glucoside of delphinidin, and eight anthocyanins (ternatins C1, C2, C3, C4, C5 and preternatins A3 and C4) are also present in the flowers of *C. ternatea* (Kogawa *et al*., 2006; Terahara *et al*., 1996; Terahara *et al*., 1998). Three flavonol glycosides, kaempferol 3-O-(2"-O-α-rhamnosyl-6" -malonyl)-β-glucoside, quercetin 3-O-(2"-O-α-rhamnosyl-6"-malonyl)-β-glucoside and myricetin 3-O-(2", 6"-di-O-α-rhamnosyl)-β-glucoside are also isolated from the petals of *C ternatea* (Kazuma *et al*., 2003).

The aim of the present research is to study the phytochemical constituents, total phenolic content, total flavonoid content and antioxidant activity of *C. ternatea* (Aung-me-nyo) flowers.

Materials and Methods

Collection of Plant Sample

The selected medicinal plant to study in the present research is *C. ternatea* (Aung-me-nyo) flowers. The plant sample was collected from Dawei Township, Taninthayi Region, Myanmar in December 2017. The plant and flower were identified by the Botanists of the Department of Botany, West Yangon University.

Preliminary Phytochemical Investigation

The flower of *C. ternatea* (Aung-me-nyo) was screened for the presence of various bioactive principles. Dried flower sample and crude extracts (petroleum ether, ethyl acetate, methanol and water) were conducted to study the presence of phytochemicals. The analyses were performed according to standard methods for testing different chemical constituents such as alkaloids, glycosides, carbohydrates, phenolic compounds, saponins, tannins, α-amino acids, reducing sugars, steroids, flavonoids, anthocyanins, starch, cyanogenic glycosides and organic acids. After treating the test solution with specific reagents, the tests were detected by virtual observation of colour change or precipitate formation (Harborne, 1998).

Determination of Total Phenolic Content

The total phenolic contents (TPC) in the petroleum ether, ethyl acetate, methanol and watery extracts of *C. ternatea* flowers were estimated by Folin-Ciocalteu Reagent method according to the procedure described by Sahu and Saxena (2013). First, 0.5 mL of prepared extract solution (500 µg/mL in methanol) was mixed with 0.5 mL of methanol. Then, 5 mL of FCR reagent (1:10 v/v) was added to the mixture and incubated for 30 min at 37 ˚C. 4 mL of 1 M sodium carbonate solution was added to each test tube and the test tubes were kept at room temperature for 15 min and the UV absorbance of reaction mixture was read at 760 nm. Gallic acid was used as a standard and calibration curve is shown in Figure 2. The total phenolic contents of the extracts were expressed as µg/mg gallic acid equivalents (GAE).

Determination of Total Flavonoid Content

The total flavonoid contents of *C. ternatea* flower extracts (petroleum ether, ethyl acetate, methanol and water) were determined according to the aluminium chloride colourimetric method (Lin and Tang, 2007). The assay was determined using 0.5 mL of each extract solution (200 µg/mL in methanol) in test tube. To each test tube 1.5 mL methanol, 0.1 mL aluminium chloride, 0.1 mL of potassium acetate solution and 2.8 mL of distilled water were added and mixed well. Sample blank for extracts were prepared in similar manner by replacing aluminium chloride solution with distilled water. After the 40 min incubation at the room temperature, absorbances of all the prepared solutions were measured at 415 nm with UV-visible spectrophotometer. Quercetin was used as standard compound and the amount of total flavonoids content were estimated from a quercetin standand curve shown in Figure 4.

Antioxidant Activity Assay

Screening of antioxidant activity of the crude extracts (petroleum ether, ethyl acetate, methanol and aqueous) of *C. ternatea* flowers were carried out by DPPH free radical scavenging assay using UV spectroscopic method (Brand-Williams *et al*., 1995). Absorbance measurements were done in triplicate for each sample solution. Absorbance values obtained were used to calculate % inhibition, 50 % inhibitory concentration (IC_{50}) and standard deviation. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

Results and Discussion

Phytochemicals Present in *C. ternatea* Flowers

Chemical investigations were carried out on the petroleum ether, ethyl acetate, methanol and water extracts of *C. ternatea* (Aung-me-nyo) flowers sample using standard procedures to identify the constituents. The phytochemical results are described in Table 1.

Table 1 Phytochemical Constituents of *C. ternatea* Flowers

Sr No.	Phytochemicals	Dried sample	PE extract	EtOAc extract	MeOH extract	H_2O extract
1	Alkaloids	+	-	+	+	+
2	Glycosides	+	+	+	+	+
3	Carbohydrates	+	+	+	+	+
4	Phenolic compounds	+	+	+	+	+
5	Saponins	+	-	+	-	+
6	Tannins	+	-	+	+	+
7	α-amino acids	+	+	+	+	+
8	Reducing sugars	+	-	+	+	-
9	Steroids	-	+	+	-	-
10	Flavonoids	+	+	+	+	+
11	Anthocyanins	+	-	-	+	+
12	Starch	+	-	+	+	+
13	Cyanogenic glycosides	-	-	-	-	-
14	Organic acids	+	+	-	+	+

(+) = presence (-) = not detected

Total Phenolic Contents of *C. ternatea* Flowers

Phenolic compounds make up one of the major families of secondary metabolites in plants, and they represent a diverse group of compounds. They possess especially antioxidant properties. Phenolics have been suggested to play a preventive role in development of cancer and heart disease (Marja *et al*., 1999).

In the present study, the total phenolic content for petroleum ether, ethyl acetate, methanol and water extracts of *C. ternatea* flowers were estimated by Folin-Ciocalteu Reagent method using gallic acid as standard. Calibration curve of standard gallic acid is shown in Figure 2. The total Phenolic contents in the *C. ternatea* flower extracts are shown in Table 2 and histogram is shown in Figure 3. It appears that ethyl acetate extract had the highest content of phenolic compounds (33.16 μg GAE/mg of extract) and watery extract had the lowest content (11.22 μg GAE/mg of extract). Methanol extract showed intermediate phenolic content followed by petroleum ether extract with 25.12 and 16.36 μg GAE/mg of extract, respectively. Phenolic extracts have been reported to retard lipid oxidation in oils and fatty foods (Rumbaoa *et al.*, 2009), decrease the risk of heart diseases by inhibiting the oxidation of low-density lipoproteins. They are also known to possess antibacterial, antiviral, antimutagenic and anticarcinogenic properties (Moure *et al*., 2001; Manach *et al*., 2004).

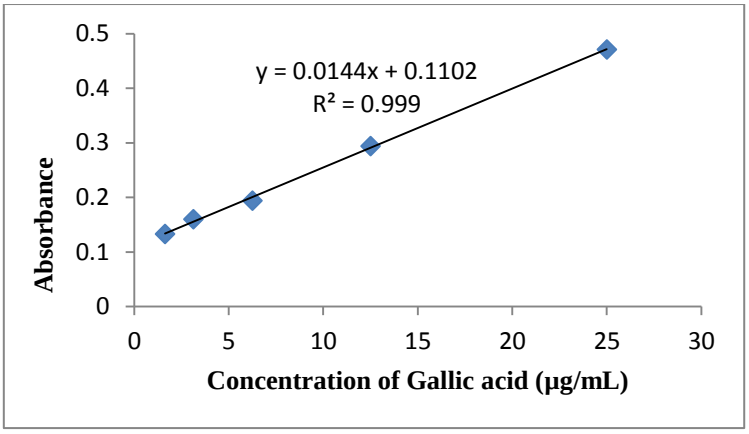

Figure 2 Standard calibration curve of gallic acid

Table 2 Total Phenolic Contents of *C. ternatea* L. Flowers

Sample (500 µg mL^{-1})	Total Phenolic Content (µg GAE/mg of extract)
PE Extract	16.36
EtOAc Extract	33.16
MeOH Extract	25.12
H_2O Extract	11.22

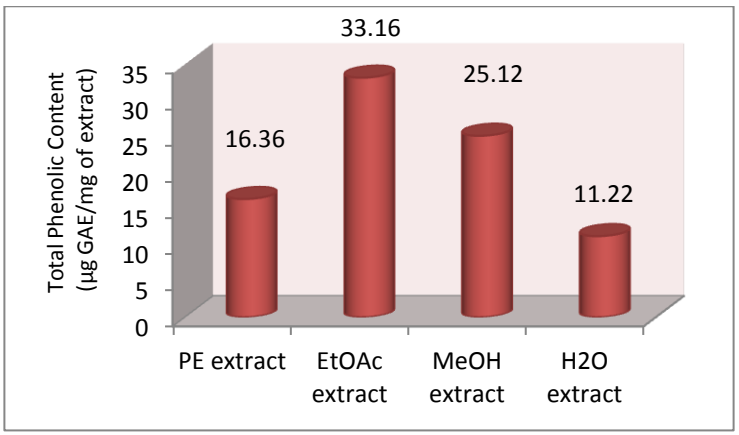

Figure 3 Histogram of total phenolic contents of *C. ternatea* flowers

Total Flavonoid Contents of *C. ternatea* Flowers

Flavonoids are widely distributed in plants, fulfilling many functions. Flavonoids are most important plant pigments for flower colouration, producing yellow, red or blue pigmentation in petals. The total flavonoid contents of *C. ternatea* flower extracts were determined according to the aluminium chloride colourimetric assay method adapted from Lin and Tang (2007). Quercetin was used as a standard and a calibration curve (Figure 4) was constructed from absorbances at 415 nm. Total flavonoid contents of the extracts were expressed as µg QE/mg of extract and given in Table 3 and histogram is shown in Figure 5. Among the four extracts, petroleum ether extract contained highest (132.17 µg QE/mg of extract) amount of total flavonoid content followed by ethyl acetate extract (86.52 µg QE/mg of extract), methanol extract (73.48 µg QE/mg of extract) and then water extract (23.48 µg QE/mg of extract).

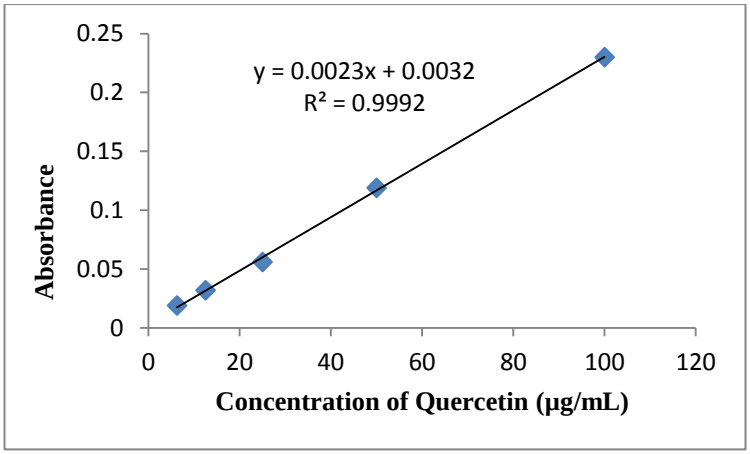

Figure 4 Standatd calibration curve of quercetin

Table 3 Total Flavonoid Content of *C. ternatea* Flowers

Sample (200 µg mL^{-1})	Total Flavonoid Content (µg QE/ mg of extract)
PE Extract	132.17
EtOAc Extract	86.52
MeOH Extract	73.48
H_2O Extract	23.48

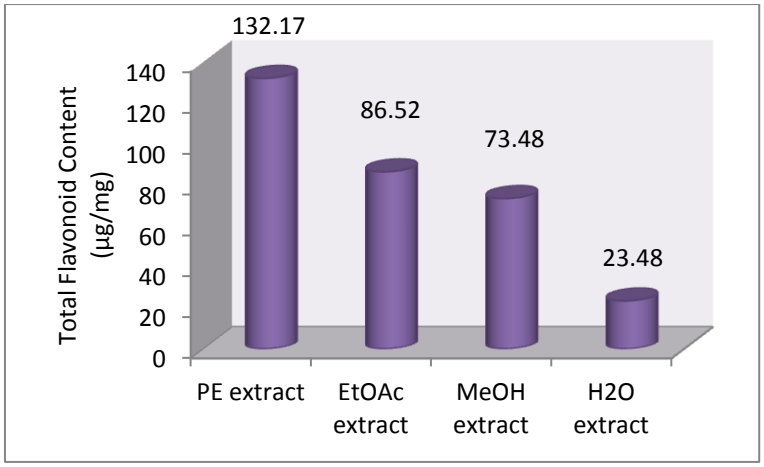

Figure 5 Histogram of total flavonoid contents of *C. ternatea* flowers

Antioxidant Activity of Extracts of *C. ternatea* Flowers

In the present study, the DPPH free radical scavenging activity of petroleum ether, ethyl acetate, methanol and aqueous extracts of *C. ternatea* flower extracts were determined according to the method reported by Brand-Williams *et al*. in 1995. The radical scavenging activity (% RSA) of extracts of *C. ternatea* flower are shown in Table 4 and Figure 6. All four extracts showed a similar increasing trend in antioxidant activity with an increase in their concentration. IC_{50} is used to express the amount or concentration of extracts needed to scavenge 50 % of the

free radicals. The IC_{50} value is inversely proportional to the scavenging activity of the extracts of the *C. ternatea* flower. IC_{50} values of extracts are indicated in Table 5.

IC_{50} value of ascorbic acid was 0.09 µg mL^{-1}. Among the four extracts ethyl acetate extract showed the highest scavenging activity with the IC_{50} value of 9.63 µg mL^{-1} while the petroleum ether extract had the lowest scavenging activity (IC_{50} = 190.77 mL^{-1}). The descending order of radical scavenging activities in the *C. ternatea* flower extracts are as follows: Ethyl acetate extract (IC_{50} = 9.63 µg mL^{-1}) > methanol extract (IC_{50} = 51.72 µg mL^{-1}) > aqueous extract (IC_{50} = 113.89 µg mL^{-1}) > petroleum ether extract (IC_{50} = 190.77 µg mL^{-1}). This means that phytochemicals present in ethyl acetate and methanol extracts possess a stronger potential to scavenge DPPH free radicals.

Table 4 Radical Scavenging Activity (% RSA) of Extracts of *C. ternatea* Flowers

Concentration (µg mL^{-1})	% RSA			
	PE extract	EtOAc extract	MeOH extract	H_2O extract
6.25	13.45	36.36	22.73	27.27
12.5	25.34	61.59	25.45	31.59
25	32.67	77.27	36.59	37.05
50	38.24	90.68	49.32	41.59
100	43.12	96.14	69.09	48.64
200	50.70	96.82	74.23	58.43

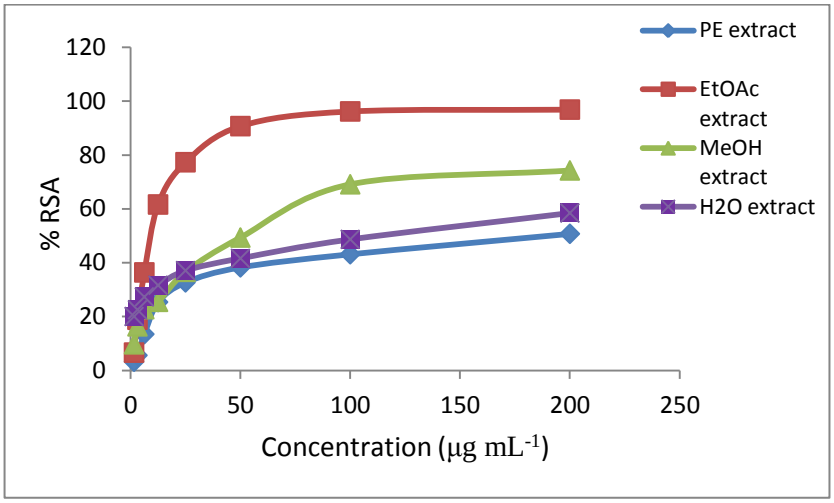

Figure 6 DPPH free radical scavenging activity of *C. ternatea* flowers

Table 5 IC_{50} Values of Ascorbic Acid and Extracts of *C. ternatea* Flowers

Sample	IC_{50} (µg mL^{-1})
PE extract	190.77
EtOAc extract	9.63
MeOH extract	51.72
H_2O extract	113.89
Ascorbic acid	0.09

Conclusion

In this study, the total phenolic contents and antioxidant activity of ethyl acetate extract are likely to show good relationship. Ethyl acetate extract of *C. ternatea* flower showed the highest level of total phenolic content and DPPH radical scavenging activity with the IC_{50} value of 9.63 μg mL^{-1} compared to other extracts and thus potential source for antioxidants. Hence, the *C. ternatea* (Aung-me-nyo) flowers used in this study can be suggested as a suitable source of natural antioxidants.

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