

Chemical characterization of ethanolic extract of Butterfly pea flower (*Clitoria ternatea*)

Tuan Putra, T.N.M., Zainol, M.K., MohdIsa, N.S. and *MohdMaidin, N.

Department of Food Science, Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu

Article history:

Received: 17 December 2020

Received in revised form: 22 January 2021

Accepted: 2 April 2021

Available Online: 25 July 2021

Keywords:

Polyphenols,
Antioxidants capacity,
Clitoria ternatea,
Bunga telang,
Anthocyanins,
Correlation

DOI:

[https://doi.org/10.26656/fr.2017.5\(4\).744](https://doi.org/10.26656/fr.2017.5(4).744)

Abstract

Clitoria ternatea, commonly known as Butterfly pea flower or Bunga telang, is a tropical flower and notable as one of the most vital sources of polyphenols with high antioxidant capacity. *C. ternatea* abundantly produces anthocyanin compounds benefiting natural blue colourants. To date, a blue colourant is the most difficult to obtain since the anthocyanins responsible for this colour is the least stable and easily degraded after extraction. Their stability commonly relies on several operational parameters, such as shifted pH value, high temperature, and light exposure. Apart from colour attributes, anthocyanins have also been identified as a source of functional molecules due to antioxidant activity and beneficial health effects such as anti-cancer, anti-obesity, antimicrobial and more. This study aimed to characterize the chemical constituents of the extractable polyphenols from ethanolic extract of *C. ternatea* quantitatively. The numerical data were evaluated using the Minitab version 18 statistical method. The chemical profiling, including total phenolic content (TPC), total flavonoid content (TFC), total monomeric anthocyanins (TMA) were determined using standard methods. The antioxidant activity was evaluated using DPPH and ABTS methods. The results showed that the ethanolic extract of *C. ternatea* had 35.7 mg QE/g of TFC, 102.4 mg GAE/g_{280nm} and 28.8 mg GAE/g_{750nm} of TPC and 2.7 CE/g and 2.9 ME/g of TMA. The total phenolic content of *C. ternatea* showed a good correlation with the antioxidant activity by the DPPH method when analysed using Pearson correlation and showed an increasing trend of antioxidant power with regards to TPC in both assays. In a nutshell, this study contributes to the knowledge of ethanolic extract of *C. ternatea*. Future research could consider exploring other extraction methods that could enhance the stability of polyphenols particularly the anthocyanins from degradation such as using surfactants.

1. Introduction

Clitoria ternatea, generally known as Butterfly pea flower is a perennial twiner (Taranalli and Cheeramkuzhy, 2000, Lakshan *et al.*, 2019) and is classified in the family of Fabaceae (Lim, 2012; Al-snafi, 2016; Jamil *et al.*, 2018; Lakshan *et al.*, 2019). Owing to its ability to grow easily, this flower is widely found blooming in gardens and wilds (Jamil *et al.*, 2018). *C. ternatea* is broadly planted in many countries, with numerous common names. For instance, 'bunga telang' (Malaysia), Ang Chan (Thailand), Pukingan (Philippines) (Lim, 2012; Ravindran, 2017), Aparajita (India), and Die Dou (China) (Lim, 2012; Al-snafi, 2016; Ravindran, 2017).

Clitoria ternatea flower is also known as a beautiful

ornamental climber as it has several lines or cultivars with varieties of flower colours, thus it is considered as an essential ornamental plant for garden lovers (Michael Gomez and Kalamani, 2003). It possessed two main varieties of petals colours (blue or white) (Taranalli and Cheeramkuzhy, 2000; Kazuma *et al.*, 2003; Lakshan *et al.*, 2019). The assortment of petal colours is primarily resulting from the chemical structure of different anthocyanins presence in the flower, specifically 'Double blue' line flower acknowledged assembling numerous polyacylated anthocyanins and ternatins (Kazuma *et al.*, 2003; Al-snafi, 2016), while white petal line seemed not to consist of anthocyanins (Al-snafi, 2016). The flower of *C. ternatea* is able to produce a vivid blue colour due to the anthocyanins accumulated in its petals thus it is widely used as a food colourant (Nur Faedah Syahirah *et al.*, 2018; Havananda and

*Corresponding author.

Email: nurmahani@umt.edu.my

Luengwilai, 2019).

Phytochemical evaluation of the flower parts significantly showed the presence of secondary metabolites such as flavonols glycosides, myricetin, quercetin (Al-snafi, 2016) phenolic acids, kaempferol (Jamil et al., 2018) and anthocyanins (Al-snafi, 2016; Jamil et al., 2018; Havananda and Luengwilai, 2019). The presence of anthocyanins pigment in *C. ternatea* flower leads to the high antioxidant properties compared to other medicinal plants (Jamil et al., 2018) and reported to have several pharmacological effects including antidiabetic, antimicrobial, anticancer, anti-inflammatory (Al-snafi, 2016; Havananda and Luengwilai, 2019), gastro-protective and aphrodisiac effects (Taranalli and Cheeramkuzhy, 2000).

The main step in order to recover and isolated phytochemicals from the plant was extraction. The sample particle size, types of solvent, operational conditions, extraction methods as well as chemical structure of phytochemicals affect the extraction efficiency (Do et al., 2014). Ethanol was widely used as an extraction solvent possibly it is recognized as safe (GRAS) for applications of extracts in the food or pharmaceutical industry (Jiménez-Moreno et al., 2019). Other than that, several studies report that an aqueous mixture of organic solvent is the best extracting solvent mixture in order to isolate or recover the phenolic compounds from plant origin. For this study, it was of interest to acquire the quantification of phytochemical properties in petals of *C. ternatea* including total phenolic content, total flavonoid content, total anthocyanin content and antioxidant activities in order to have a better understanding of the potential use of *C. ternatea*.

2. Materials and methods

2.1 Preparation of sample

Dried butterfly pea flower (*C. ternatea*) was purchased from Superbee Enterprise located at Melaka. The samples were grounded by using a grinder-mixer and sifted using a vibrating sieve shaker at a particle size of $\leq 2\text{mm}$ (MohdMaidin et al., 2018).

2.2 Extraction of sample

The sifted samples at a particle size of $\leq 2\text{mm}$ were extracted by using the method proposed by Mohd Maidin et al. (2018) with modification. Ethanol was purchased from Eugene Chem. Sdn. Bhd., Penang, Malaysia. Phytochemical constituents were extracted by ethanol-aqueous extraction using 60% (v/v) in a shaking water bath with a circular motion. The shaking water bath was set at 60°C with a speed of 100 rpm for 2 hrs. The ratio

of sample to solvents was 1:8. After 2 hrs, the extract was filtered (vacuum-assisted) and the filtrate was kept in a biomedical freezer (Panasonic, Japan) with temperature (-30°C) prior to analysis.

2.3 Total phenolic content Folin-ciocalteau method

Folin-ciocalteau (FC) colourimetry method was first employed by Singleton and Rossi (1965) to determine the total phenolic content of samples. Briefly, 1.5 mL of Folin-ciocalteau reagent (previously diluted to 10 folds with distilled water) was added into a test tube containing 0.2 mL of sample or standard. The mixture was allowed to react in darkness for 5 mins. Then, 1.5 mL of sodium carbonate (6%) was added to each test tubes and shaken. The sample and standards were allowed to stand in darkness for 90 mins until the final reading at 750 nm wavelength. Gallic acid was used as a calibration curve. Results were expressed as mg GAE/g_{760nm}.

2.4 Total phenolic content using direct measurement

This method was referred to Amendola et al. (2010). Briefly, a set of gallic acid standards and also samples were measured at 280 nm. Results were expressed as mg GAE/g_{280nm}.

2.5 Total monomeric anthocyanins

Lee et al. (2005) established a methodology known as the pH differential method. This methodology was prescribing the use of pH 1 and pH 4.5 buffer solution. This approach has been formally adopted as an official method by AOAC (Lee et al., 2005). Two buffers were prepared; i) pH 1 buffer (potassium chloride (Emsure, Germany), 0.0025M); ii) pH 4.5 buffer (sodium acetate (Sigma Aldrich, United States of America), 0.4M). Prior analysis, a suitable dilution factor was determined by diluting the sample and the absorbance of the sample was adjusted to be in the range of 0.2 – 1.4 at 520 nm.

For the analysis, samples were diluted with both buffers by using the same dilution factor. The test portions added should be 1-part test portion and 4-parts buffer. Both of the buffers were left to stand for 40 minutes and measured at 520 nm and 700 nm. Total monomeric anthocyanin content was calculated according to equation (1).

$$\text{Total anthocyanins} \left(\frac{\text{mg}}{\text{L}}, \text{ME} \right) = \frac{A \cdot \text{MW} \cdot \text{DF} \cdot 10^3}{\epsilon \cdot l} \quad (1)$$

Where A = (A_{520nm} – A_{700nm})_{pH 1.0} – (A_{520nm} – A_{700nm})_{pH 4.5}; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); MW = 493.43g/mol for malvidin-3-glucoside, DF = dilution factor established in D; l = pathlength in cm; ε = 26 900 molar extinction coefficients, in L · mol⁻¹ · cm⁻¹, for cyanidin

- 3-glucoside; $\epsilon = 28000$ molar extinction coefficients, in $L \cdot mol^{-1} \cdot cm^{-1}$ for malvidin-3-glucoside; and $10^3 =$ factor for conversion from g to mg.

2.6 Total flavonoid content

Total flavonoid content was determined by using aluminium chloride colourimetric according to Looi *et al.* (2020) with slight modification. Briefly, 2.8 mL of samples or standards were added into a test tube containing 1.2 mL of distilled water and 90 μ L of sodium nitrite (5%). The mixture was left to stand for 5 mins. Aliquots of aluminium chloride (10%) (90 μ L) were added and allowed to stand for 5 mins. Then, 1.2 mL of sodium hydroxide (0.5M) was added and the mixture was allowed to react in darkness for 30 mins. The absorbance value of mixtures was measured at 510 nm. Quercetin was used as a standard and results were expressed in milligrams of quercetin equivalent per litre (mg QE/L).

2.7 Antioxidant activity using DPPH method

The DPPH scavenging capacity of samples and standards were determined according to Looi *et al.* (2020). Briefly, 125 μ M of DPPH was freshly prepared prior to analysis. Then, 2 mL of DPPH radical solution (125 μ M) was added into a test tube containing 500 μ L of samples/standard. Finally, the mixture was allowed to react in darkness for 15 mins at room temperature. After the incubation period, the absorbance was measured at 517 nm. Ascorbic acid was used as a standard. The antioxidant activity (% inhibition) was calculated as demonstrated in Equation (2).

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{samples or standards}}}{A_{\text{control}}} \times 100 \quad (2)$$

2.8 Antioxidant activity using ABTS method

The ABTS assay was conducted by referring method MohdMaidin *et al.* (2019), with slight modification. The radical solution was prepared by mixing 5 mL of 7mM ABTS with 80 μ L of 150 mM potassium peroxydisulfate. The solution was left to stand for 12-16 hrs. Prior to analysis, the radical solution was diluted with methanol in order to obtain an absorbance value of 0.70 ± 0.02 at 734 nm. The radical was then left to equilibrate at room temperature upon achieving stability.

For the analysis, 3 mL of $ABTS^+$ (0.700 ± 0.02 , A734 nm) was mixed with 30 μ L of sample/blank/standard. In this case, standard solutions of ascorbic acid were prepared in serial dilutions (0-800 μ M). The absorbance of the mixture was measured after being incubated in dark for 6 mins at 734 nm. The antioxidant power (% inhibition) was calculated as Equation (3).

$$\text{Antioxidant power (\% inhibition)} = \frac{A_{\text{Blank}_{t=6}} - A_{\text{samples or standards}_{t=6}}}{A_{\text{ABTS}_{t=0}}} \times 100 \quad (3)$$

2.9 Statistical analysis

All of the analysis was conducted in triplicate ($n = 3$). Results obtained were the average value in which mean \pm standard deviation. Data were analysed using a one-way Analysis (ANOVA) of Minitab18. Differences between different analyses were demonstrated by using Tukey tests. The statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1 Chemical analysis

Phenolic compounds possess a wide variety of functions and structures. Phenols basically acquire an aromatic ring bearing either one or more hydroxyl groups (Alzand and Mohamed, 2012). The total phenolic content of ethanolic extract of *C. ternatea* was tabulated in Table 1. The results showed that the total phenolic content measured at 280 nm was significantly higher (102.37mg GAE/g) than those measured at 750 nm (28.8mg GAE/g). This could be due to the absorbance of all compounds with at least one aromatic cyclic ring happened at this wavelength, hence almost all compounds were quantified here (MohdMaidin *et al.*, 2018). Contrarily to those measured at 750 nm which is based on the reduction of colour from yellow to dark blue which was more selective, thus explained the low amount of TPC quantified. Similarly, a study done by Lakshan *et al.* (2019) obtained the same TPC value (26.7mg GAE/g) although the extraction media used to extract *C. ternatea* was solely water. However, Rabeta and An Nabil, (2013) noted a methanol extract of *C. ternatea* was higher (61.7mg GAE/g) as opposed to water extract (20.7mg GAE/g). The reason for this could be the solubility nature of phenolic compounds were enhanced by the organic solvent (methanol), which facilitates solubilisation through penetration in plant cell structure during extraction.

In this study, the TPC value in our sample was lower than those obtained by Jaafar *et al.* (2020) who found values of 41.17 mg GAE/g dry weight. This might be related to the conditions applied when operating the experiment. Jaafar *et al.* (2020) deduced that the optimum condition for the *C. ternatea*'s extraction was 36.92% of ethanol at 44.24°C for 90 mins. When comparing the experimented value between the optimum conditions and their several observations with varied operational conditions, augmented temperature by 83.64 and ethanol extraction (60%) remarkably enhanced the TPC value (44.91 mg/g). However, reducing ethanol concentration (9.55%) with slightly augmented temperature (50°C) significantly reduced the TPC value by 40.86 mg/g. Whilst, approximately 1.62-fold increase

Table 1. Antioxidant content of ethanolic extract of butterfly pea flower.

Analysis/sample	Results (mg/g dry weight)
Total Phenolic Content 280nm	102.37±1.063
Total Flavonoid Content	35.73±0.978
Total Phenolic Content 750nm	28.75±1.215
Antioxidant activity ABTS (µMolTrolox/g)	5.90±0.080
Antioxidant activity ABTS (µMolAscorbic acid/g)	5.84±0.080
Total Monomeric Anthocyanin Malvidin	2.88±0.408
Total Monomeric Anthocyanin Cyanidin	2.72±0.386
Antioxidant activity DPPH (µMolTrolox/g)	0.55±0.009
Antioxidant activity DPPH (µMolAscorbic acid/L)	0.733±0.002

Results were expressed as mean±standard deviation.

in ethanol concentration (60%), slightly increased temperature (50°C) and decreased extraction time by 39.55 mins resulted in rising the TPC value by 43.20 mg/g. Therefore, operational conditions for example temperature affects the efficiency of the extraction by significantly raise the mass transfer, improving the solubility of solute, reduce the solvent viscosity and surface tension (Cheaib *et al.*, 2018). Apart from that, phenolic compounds are thermolabile compounds. Therefore, the augmented temperature may lead to the degradation of phenols thereby resulting in lower phenolic content (Roselló-Soto *et al.*, 2019). In contrast to those extracted by water, the addition of ethanol may enhance the yield of phenolic compounds during extraction. The reason behind the higher TPC obtained from ethanol extraction over water extraction was owing to the high diversity of polyphenols. As a consequence, the addition of ethanol changes the cell permeability hence prompt to the alteration of the phospholipid bilayer of the cell membranes thus enhance the cell permeability (Cheaib *et al.*, 2018). Therefore, both temperature and concentration of ethanol played a vital role in optimizing the extraction.

Meanwhile, flavonoid compounds are one of the phenolic compounds extracted from vascular plants. These compounds are well-known as blue, red and purple pigments of flowering plants, concerning the presence of anthocyanins sub-group (Alzand and Mohamed, 2012). In this study, the total flavonoid content was found at 35.73 mg QE/g. This was considered low as compared to the findings of TFC from *C. ternatea* ethanolic flower extract noted by Jaafar *et al.* (2020) with 187.05 mg QE/g. This could be due to the different ethanol concentrations used, which was 37%. The higher volume of water indicating the higher polarity of a solvent hence could be the reason for extracting out the bioactive compounds. Apparently, the total flavonoid content in the extract reduces as the concentration of water in ethanol or acetone increases (Do *et al.*, 2014). A recent study by Cheaib *et al.* (2018) found that enhancing ethanol concentration and the

temperature had a positive impact on flavonoid content. Furthermore, different lines of *C. ternatea*, for instance, white petals line, blue petals line and mauve petals line may possess different structures of phenolic and flavonoid compounds. Besides, different sampling locations of the sample also may reflect the value of polyphenols (Jaafar *et al.*, 2020). Their antioxidant properties and colour stability may be susceptible to several environmental conditions including soil aeration, temperature, moisture content, pH, and light (Jamil *et al.*, 2018). These could be the reasons why our result does not in line with previous studies.

Interestingly, the TFC value (35.73 mgQE/g) obtained in this study was higher than the TPC value (28.8 mg GAE/g). Theoretically, the total phenolic content should be above total flavonoid content, considering that flavonoids are classified in phenolic compounds. However, according to Kaisoon *et al.* (2011), plant extracts with a higher amount of flavonoids do not usually possess high total phenolic content, possibly due to the amount of overall phenolic content cannot be quantified by the single Folin-Ciocalteu method (Jaafar *et al.*, 2020). A similar result was also obtained by Jaafar *et al.* (2020) noticed that ethanolic extraction of *C. ternatea* exhibited higher TFC values (187.05mgQE/g) as compared to the TPC values (41.17 mg GAE/g). In addition, Lakshan *et al.* (2019) also found that aqueous extraction of *C. ternatea* had a higher TFC (42 mgQE/L) as compared to the TPC (20.7 mg GAE/g).

Furthermore, the purple colour pigment exhibit by the flower could be attributable to the accumulation of anthocyanins compounds (Rabeta and An Nabil, 2013). The colour of anthocyanins varied when the pH of the solution shifted from acids to base (Jamil *et al.*, 2018). In this study, the total anthocyanin content was measured and results showed that the extract contained 2.88 mg Malvidin Equivalent (ME)/g. A similar finding was found by Jaafar *et al.* (2020), where they noted about 2.86 ME/g of total anthocyanin. Moreover, the result

obtained for total anthocyanin of Cyanidin Equivalent (CE/g) was 2.72 mg CE/g. Our total anthocyanin content (CE/g) was slightly lower than reported by Azima (2014) who noticed the total monomeric anthocyanin was 2.98 mg CE/g. Going further, Al-snafi, (2016) notable that the total anthocyanin content of water extracted *C. ternatea* flower was 1.46 mg CE/g. As mentioned before, the addition of ethanol could enhance the extraction of polyphenols.

3.2 Antioxidant activities

The reaction of ABTS radical solution with antioxidant potential in donating hydrogen atoms led to the discolouration of the blue-green colour of radical solution into colourless and reduced the absorbance at 734 nm (Venkatesan *et al.*, 2019). A similar trend was observed in the DPPH assay. When the stable organic free radical (DPPH) reacts with an antioxidant, hence, led to a visually noticeable discolouration from purple to colourless and reduced the absorbance at 517 nm (Do *et al.*, 2014). In general, plant phenolics exhibit high antioxidant activity and are known as effective free radical scavengers (Rabeta and An Nabil, 2013). It is known that phenolic compounds can neutralize the free radicals by their wide variety of functions, including metal chelating, hydrogen donating and singlet oxygen quenching properties (Venkatesan *et al.*, 2019).

In this study, the antioxidant activity of *C. ternatea* extract was measured using DPPH and ABTS assays. The result was tabulated in Table 2 and showed that there was no significant difference noted among both assays, in terms of percentage of inhibition. In the DPPH assay, the *C. ternatea* extract inhibited the radical at 42.40% whilst in the ABTS assay, the percentage of inhibition noted was 29.16%. On the other hand, with

reference to ascorbic acid content and Trolox content by DPPH method, the extract demonstrates 0.7325 μMol Ascorbic acid/g and 0.55 μMol Trolox/g, respectively. While, for ABTS assay, the extract exhibit 5.90 μMol Ascorbic acid/g and 5.84 μMol Trolox/g.

Table 2. Antioxidant activity of ethanolic extract of butterfly pea flower

Analysis/sample	Results (mg/g dry weight)
Antioxidant activity ABTS (%Inhibition)	29.16 \pm 0.425
Antioxidant activity DPPH (%Inhibition)	42.40 \pm 0.370
Antioxidant activity ABTS (IC ₅₀)	10.23 \pm 0.186
Antioxidant activity DPPH (IC ₅₀)	2.77 \pm 0.020

Results were expressed as mean \pm standard deviation.

As mentioned before, both DPPH and ABTS are similar tests for the antioxidant activity where both tests use strongly coloured stable radical compounds. Owing to the same principles, similar trends of antioxidant activity results were obtained in this study hence, further strengthened the results obtained by both tests. Therefore, a Pearson correlation test was done to understand the correlation between each test and the results were tabulated in Table 3. From Table 3, a strong correlation was only noted between TPC at 280 nm and ABTS. This correlation between antioxidant capacity and phenolics was also reported in previous studies. González-Gómez *et al.* (2010) pointed out that the content of flavonoids was correlated with the antioxidant capacity in the sweet cherry extract. This has also been explored in prior study by Alothman *et al.* (2009), where they deduced that there was a significant correlation between total phenolics with antioxidants activity, possibly due to the presence of phenolic compounds as the main phytoconstituent, which devoted to the

Table 3. Pearson correlation table between bioactive compounds and antioxidant activity

		Correlation						
		TPC 750 nm	TPC 280 nm	TFC	TAC Cyanidin	TAC Malvidin	DPPH	ABTS
TPC 750 nm	Pearson correlation	1	0.211	-0.093	0.842	0.849	-0.691	0.204
	p-value	-	0.864	0.940	0.363	0.355	0.514	0.869
TPC 280 nm	Pearson correlation	0.211	1	-0.993	-0.35	-0.337	0.56	1.000*
	p-value	0.864	-	0.076	0.773	0.781	0.622	0.005
TFC	Pearson correlation	0.013	-0.975	1	0.551	0.539	0.816	0.956
	p-value	0.992	0.144	-	0.629	0.637	0.392	0.189
TAC Cyanidin	Pearson correlation	0.842	-0.35	0.459	1	1.000*	-0.972	-0.356
	p-value	0.363	0.773	0.697	-	0.008	0.151	0.768
TAC Malvidin	Pearson correlation	0.849	-0.337	0.447	1.000*	1	-0.969	-0.344
	p-value	0.355	0.781	0.705	0.008	-	0.159	0.777
DPPH	Pearson correlation	-0.691	0.56	-0.655	-0.972	-0.969	1	0.566
	p-value	0.514	0.622	0.546	0.151	0.159	-	0.617
ABTS	Pearson correlation	0.204	1.000*	-0.994	-0.356	-0.344	0.566	1
	p-value	0.869	0.005	0.071	0.768	0.777	0.617	-

*Correlation is significant at the 0.05 level (2-tailed)

antioxidant activities of fruits extracts such as guava, banana and honey pineapple. This was in agreement with a recent report by Venkatesan *et al.* (2019) that the antioxidant properties of plant extracts are partly related to phenolic compounds.

Nevertheless, a slightly different correlation was noted by Looi *et al.* (2020) in their study where a strong correlation was found between TPC, TFC and both antioxidant assays, ABTS and DPPH in their sample of *Ceri Terengganu*. In this study, *C. ternatea* did follow the trend but only on the correlation between TPC and ABTS assay. However, the differences between the results obtained in this study and those of other studies possibly due to the difference in plant matrices and different solvents (types and concentration) used in extraction, different operational conditions (temperature and time) hence led to variations of compositions and antioxidant activities of the extract (Do *et al.*, 2014). Apart from that, a recent study conducted by Jiménez-Moreno *et al.* (2019), concluded that the extractable compound was not solely phenolic composition of the samples and there could be numerous other compounds with antioxidant activities apart from polyphenols and even some polyphenols or other compounds exhibit negligible or null antioxidant activity.

From the results, it is clear that the total phenolic content of ethanolic extract of *C. ternatea* had the ability to inhibit the free radicals for both assays thus indicating a strong antioxidant potential of the plant. The antioxidant power of the extract was further calculated in each assay and was illustrated in Figure 1(A) for DPPH and Figure 1(B) for ABTS. It was noted that the concentration of TPC was proportional to the inhibition noted in both assays. This result was in agreement with a report by Do *et al.* (2014) who found that the extractable phenolic compound with a high number of hydroxyl groups exhibited a significantly high antioxidant activity. The current result ties well with the previous studies

wherein water extracted *C. ternatea* flower significantly convey to have both high TPC and scavenging activity towards DPPH radicals, thus led to an agreement that plant phenolic highly effective antioxidants and possess an adequate free radical scavenging activity (Rabeta and An Nabil, 2013).

4. Conclusion

The main conclusion that can be drawn is that *C. ternatea* has a wide variety of functions including traditional uses, antioxidants and applications in food and pharmaceutical owing to its ability to scavenge free radicals. Nevertheless, we found that phytochemical evaluation of the ethanolic extract of *C. ternatea* showed a considerably high content of phenols (102.37 mg GAE/g_{280nm} and 28.8 mg GAE/g_{750nm}), flavonoids (35.73 mg QE/g), and anthocyanins (2.88 mg ME/g and 2.72 mg CE/g) when measured using standard methods. Moreover, a good correlation between total phenolic content quantified at both wavelengths (750 nm and 280nm) and DPPH was obtained signified the potential of ethanolic *C. ternatea* extract as a natural antioxidant agent to be employed in food or cosmetic industries. Finally, future studies that could be undertaken is in finding alternative extraction methods that could enhance the stability of these bioactive compounds, particularly its anthocyanins during storage.

Acknowledgement

The authors would like to thank the Ministry of Higher Education Malaysia FRGS/1/2019/WAB01/UMT/03/4 for the financial aid and FPSM, Universiti Malaysia Terengganu for the facilities provided.

References

Al-snaifi, A.E. (2016). Pharmacological importance of *Clitoria ternatea* – A review Pharmacological

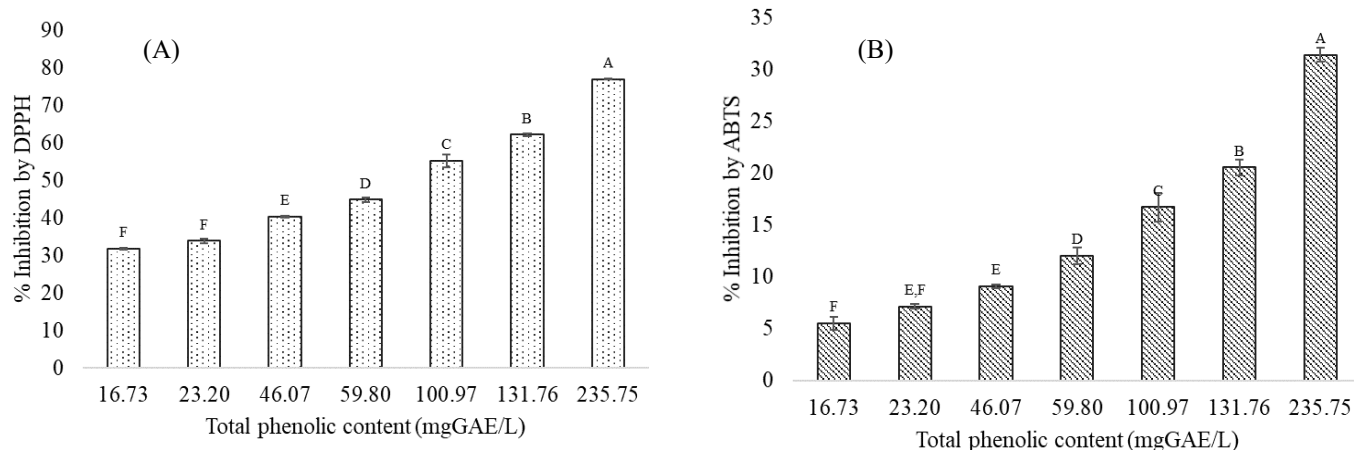


Figure 1. Antioxidant power by DPPH assay (A) and ABTS assay (B). Different alphabet notations above the bars indicate significant difference between each treatment ($p < 0.05$), $n = 3$.

- importance of *Clitoria ternatea* – A review. *IOSR Journal of Pharmacy*, 6(3), 68–83.
- Alothman, M., Bhat, R. and Karim, A.A. (2009). UV radiation-induced changes of antioxidant capacity of fresh-cut tropical fruits. *Innovative Food Science and Emerging Technologies*, 10(4), 512–516. <https://doi.org/10.1016/j.ifset.2009.03.004>
- Alzand, K.I. and Mohamed, M.A. (2012). Flavonoids: Chemistry, Biochemistry and Antioxidant activity. *Journal of Pharmacy Research*, 5(8), 4013–4020.
- Amendola, D., De Faveri, D.M. and Spigno, G. (2010). Grape marc phenolics: Extraction kinetics, quality and stability of extracts. *Journal of Food Engineering*, 97(3), 384–392. <https://doi.org/10.1016/j.jfoodeng.2009.10.033>
- Azima, S. (2014). Anthocyanin content in relation to the antioxidant activity and colour properties of *Garcinia mangostana* peel, *Syzygium cumini* and *Clitoria ternatea* extracts. *International Food Research Journal*, 21(6), 2369-2375.
- Cheab, Di., El Darra, N., Rajha, H.N., Maroun, R.G. and Louka, N. (2018). Systematic and Empirical Study of the Dependence of Polyphenol Recovery from Apricot Pomace on Temperature and Solvent Concentration Levels. *Scientific World Journal*, 2018, 8249184. <https://doi.org/10.1155/2018/8249184>
- Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S. and Ju, Y.H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, 22(3), 296–302. <https://doi.org/10.1016/j.jfda.2013.11.001>
- González-Gómez, D., Lozano, M., Fernández-León, M.F., Bernalte, M.J., Ayuso, M.C. and Rodríguez, A.B. (2010). Sweet cherry phytochemicals: Identification and characterization by HPLC-DAD/ESI-MS in six sweet-cherry cultivars grown in Valle del Jerte (Spain). *Journal of Food Composition and Analysis*, 23(6), 533–539. <https://doi.org/10.1016/j.jfca.2009.02.008>
- Havananda, T. and Luengwilai, K. (2019). Variation in floral antioxidant activities and phytochemical properties among butterfly pea (*Clitoria ternatea* L.) germplasm. *Genetic Resources and Crop Evolution*, 66(3), 645–658. <https://doi.org/10.1007/s10722-018-00738-6>
- Jaafar, N.F., Ramli, M.E. and Salleh, R.M. (2020). Optimum extraction condition of *Clitoria ternatea* flower on antioxidant activities, total phenolic, total flavonoid and total anthocyanin contents. *Tropical Life Sciences Research*, 31(2), 1–17. <https://doi.org/10.21315/tlsr2020.31.2.1>
- Jamil, N., Mohd Zairi, M.N., Mohd Nasim, N.A. and Pa'ee, F. (2018). Influences of Environmental Conditions to Phytoconstituents in *Clitoria ternatea* (Butterfly Pea Flower) – A Review. *Journal of Science and Technology*, 10(2), 208–228. <https://doi.org/10.30880/jst.2018.10.02.029>
- Jiménez-Moreno, N., Volpe, F., Moler, J.A., Esparza, I. and Ancín-Azpilicueta, C. (2019). Impact of extraction conditions on the phenolic composition and antioxidant capacity of grape stem extracts. *Antioxidants*, 8(12), 597. <https://doi.org/10.3390/antiox8120597>
- Kaisoon, O., Siriamornpun, S., Weerapreeyakul, N. and Meeso, N. (2011). Phenolic compounds and antioxidant activities of edible flowers from Thailand. *Journal of Functional Foods*, 3(2), 88–99. <https://doi.org/10.1016/j.jff.2011.03.002>
- Kazuma, K., Noda, N. and Suzuki, M. (2003). Flavonoid composition related to petal color in different lines of *Clitoria ternatea*. *Phytochemistry*, 64(6), 1133–1139. [https://doi.org/10.1016/S0031-9422\(03\)00504-1](https://doi.org/10.1016/S0031-9422(03)00504-1)
- Lakshan, S.A.T., Jayanath, N.Y., Abeysekera, W.P.K.M. and Abeysekera, W.K.S.M. (2019). A commercial potential blue pea (*Clitoria ternatea* L.) flower extract incorporated beverage having functional properties. *Evidence-Based Complementary and Alternative Medicine*, 2019, 2916914. <https://doi.org/10.1155/2019/2916914>
- Lee, J., Durst, R.W. and Wrolstad, R.E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colourants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International*, 88(5), 1269–1278. <https://doi.org/10.1093/jaoac/88.5.1269>
- Lim, T.K. (2012) (Ed.) . Edible medicinal and non-medicinal plants: Volume 2, Fruits. Switzerland, Springer Nature. <https://doi.org/10.1007/978-94-007-1764-0>
- Looi, S.K., Zainol, M.K., Mohd, Z.Z., Hamzah, Y. and Mohdmaidin, N. (2020). Antioxidant and antibacterial activities in the fruit peel, flesh and seed of *Ceri Terengganu* (*Lepisanthes alata* leenh.). *Food Research*, 4(5), 1600–1610. [https://doi.org/10.26656/fr.2017.4\(5\).172](https://doi.org/10.26656/fr.2017.4(5).172)
- Michael Gomez, S. and Kalamani, A. (2003). Butterfly Pea (*Clitoria ternatea*): A Nutritive Multipurpose Forage Legume for the Tropics - An Overview. *Pakistan Journal of Nutrition*, 2(6), 374–379. [https://doi.org/10.26656/fr.2017.4\(5\).172](https://doi.org/10.26656/fr.2017.4(5).172)

doi.org/10.3923/pjn.2003.374.379

MohdMaidin, N., Michael, N., Oruna-Concha, M.J. and Jauregi, P. (2018). Polyphenols extracted from red grape pomace by a surfactant-based method show enhanced collagenase and elastase inhibitory activity. *Journal of Chemical Technology and Biotechnology*, 93(7), 1916-1924. <https://doi.org/10.1002/jctb.5459>

MohdMaidin, N., Oruna-Concha, M.J. and Jauregi, P. (2019). Surfactant TWEEN20 provides stabilisation effect on anthocyanins extracted from red grape pomace. *Food Chemistry*, 271, 224–231. <https://doi.org/10.1016/j.foodchem.2018.07.083>

Nur Faezah Syahirah, L., Muhammad Umar Lutfi, M.Y., Atika, A., Muhammad Hafiz, R., Muhammad Zulhelmi, O.A., Mohd Ariff Adzhan, O. and Khor, P.Y. (2018). A comparative analysis of *Clitoria ternatea* Linn. (Butterfly pea) flower extract as natural liquid pH indicator and natural pH paper. *Dhaka University Journal of Pharmaceutical Sciences*, 17(1), 97–103. <https://doi.org/10.3329/dujps.v17i1.37125>

Rabeta, M.S. and An Nabil, Z.A. (2013). Total phenolic compounds and scavenging activity in *Clitoria ternatea* and *Vitex negundo* linn. *International Food Research Journal*, 20(1), 495–500.

Ravindran, P.N. (2017). The Encyclopedia of Herbs and Spices. Vol. 1 and 2. United Kingdom: CABI. <https://doi.org/10.1079/9781780643151.0000>

Roselló-Soto, E., Martí-Quijal, F.J., Cilla, A., Munekata, P.E.S., Lorenzo, J.M., Remize, F. and Barba, F.J. (2019). Influence of temperature, solvent and pH on the selective extraction of phenolic compounds from tiger nuts by-products: Triple-TOF-LC-MS-MS characterization. *Molecules*, 24(4), 797. <https://doi.org/10.3390/molecules24040797>

Singleton, V.L. and Rossi, J.A.J. (1965). Colorimetry of total phenolics with acid reagents. *American Journal of Enology and Viticulture*, 16, 144–158.

Taranalli, A.D. and Cheeramkuzhy, T.C. (2000). Influence of *Clitoria Ternatea* Extracts on Memory and Central Cholinergic Activity In Rats. *Pharmaceutical Biology*, 38(1), 51–56. <https://doi.org/10.1146/annurev.immunol.22.012703.104702>

Venkatesan, T., Choi, Y.W. and Kim, Y.K. (2019). Impact of Different Extraction Solvents on Phenolic Content and Antioxidant Potential of *Pinus densiflora* Bark Extract. *BioMed Research International*, 2019, 3520675. <https://doi.org/10.1155/2019/3520675>