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RESEARCH ARTICLE

Antioxidant and selected chemical properties of the flowers of three different varieties of Butterfly Pea (*Clitoria ternatea* L.)

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Abstract: Butterfly pea (*Clitoria ternatea* L.) is used for production of herbal teas, herbal juices and having a potential to use in culinary purposes. Even though, it is grown widely in Sri Lanka, comprehensive information and studies conducted on three popular varieties are still lacking. Hence, the aim of this study was to evaluate the elemental compositions, phytochemical properties of three *C. ternatea* L. flower varieties grown in Sri Lanka; white flower with normal keel petals (WSPF), blue flower with normal keel petals (BSPF), and blue flower with enlarged keel petals (BMPF). Total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP) and DPPH radical scavenging activity in aqueous extracts of *C. ternatea* flowers were determined using spectrophotometric methods. Kjeldahl method was used to determine the nitrogen content and crude protein % was calculated by multiplying the nitrogen content by a standard factor. Phosphorus content (UV spectrophotometer) and calcium and potassium contents (flame photometer) of the flower samples were measured by using a general mineralization procedure.The maximum TPC and highest level of TFC were reported in BMPF (31.88 mg GAE eq./g dry weight and 15.96 mg quercetin eq./g dry weight of flower) whereas FRAP of WSPF was significantly lower (10.66 mg trol eq./g dry weight of flower). FRAP of BSPF (14.56 mg trol eq./g dry weight of flower) and BMPF (18.50 mg trol eq./g dry weight of flower) were not significantly statistically different**.** BSPF showed significantly highest level of DPPH radical scavenging activity that was 11.97 mg trol. eq./g dry weight of flower. Meanwhile, the DPPH radical scavenging activity of BMPF and WSPF were not significantly different to each other. Mean P content was highest in WSPF and was lowest in BMPF (4.65 and 4.19 mg/g respectively). The N content was significantly higher ($p<0.05$) in both BSPF and WSPF (43.12 mg/g and 42.35 mg/g respectively). Significantly higher ($p<0.05$) mean protein contents were detected for BSPF and it was 26.95%. This is the first such detailed report on chemical composition and antioxidant properties of three main *C. ternatea* flower varieties grown in Sri Lanka.

Keywords: Antioxidant properties, Chemical composition, *Clitoria ternatea* L.**,** Flower types, Sri Lanka.

INTRODUCTION

Butterfly pea (*Clitoria ternatea* L.) is a perennial vine species native to Malaysia and found enfranchised in all over the world including Sri Lanka. It belongs to the plant family Fabaceae and growing well in neutral, moist soils (Karel *et al*., 2018). It produces solitary, axillary and papilionaceous flowers containing five petals including a standard petal, two wing petals and two keel petals (Bishoyi & Geetha, 2012). This dark blue or white colour flower is the predominant plant part, which is having valuable medicinal properties due to the presence of vital phytochemicals. These phytochemicals are also found in other parts of the creeper such as leaves, roots, bark, and seeds in variable proportions (Karel *et al*., 2018).

'Ela-katarolu'(white flowers) and '*nil-katarolu'*(blue flowers) are two Sinhala common names used for two varieties of butterfly pea found in Sri Lanka based on the petal colour. According to studies done by Jayaweera in 1981, butterfly pea is grown in Sri Lanka as an ornamental plant and a traditional medicinal plant. Blue flowered variety could be found as flowers bearing either a normal keel petal or an enlarged keel petal (Bishoyi & Geetha, 2012).

This plant is used as a hydragogue cathartic in large doses and used to treat conditions such as anasarca, ascites, and other conditions where it is necessary to remove large quantities of fluid from the human organs and systems. *C. ternatea* L. is used in small dosages for cholagogue purgative, acute and chronic congestion of the liver and biliousness. Juice of roots use to treat hemicranias, irritation of bladder and urethra. Seeds are using as a purgative and diuretic for enlargements of abdominal viscera. Leaves are used to treat swollen joints (Jayaweera, 1981). Even though, *C. ternatea* flower has reported with many functional properties like antidiabetic properties, anti-proliferative properties, antioxidant properties, antimicrobial properties and anticomplusive activit y, it is not widely used in Sri

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Lankan traditional medicine (Shende *et al*., 2012; Neda *et al*., 2013; Zingare *et al*., 2013; Havananda and Luengwilai, 2019; Lakshan *et al*., 2019; Pengkumsri *et al*., 2019). However, these properties may differ depending on the growing location, varietal differences, and growing conditions of the plant (Zia-Ul-Haq *et al*., 2008 and Pérez-Lamela *et al*., 2007).

Reactive oxygen species (ROS) are accumulated in the human body due to the biological reactions such as superoxide radicals, singlet oxygen, and hydrogen peroxides (Cerutti, 1991). Excess accumulation of ROS may cause adverse effects to the health by oxidative alteration of biological macromolecules such as lipids, proteins and DNA. This condition may lead to non-communicable diseases; cardiovascular diseases, cancers and neurodegenerative diseases (Halliwell, 2009). Antioxidants act as free radical scavengers, metal chelating agents, oxidative enzyme inhibiting agents, and antioxidant enzyme cofactors minimizing above mentioned ill health (Karadag *et al*., 2009).

Synthetic antioxidants such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) have potential health risks and toxicity. Therefore, scientific research are diverted to extract antioxidants from natural sources (Dudonné *et al*., 2009). Hence, plants with dietary antioxidants would be more beneficial in that context. Those properties may vary in different growing locations, among varieties, and agronomic practices (Zia-Ul-Haq *et al*., 2008 and Pérez-Lamela *et al*., 2007).

The elemental and phytochemical compositions of the *C. ternatea* flower confer for its nutrient and medicinal properties. Even though, the nutrient and the phytochemical profiles of *C. ternatea* flowers have been well studied in countries like Malaysia, it was not studied and reported much about the *C. ternatea* varieties grown in Sri Lanka. Therefore, the main objective of this study is to evaluate the elemental compositions and phytochemical properties of flowers of three *C. ternatea* varieties grown in Sri Lanka; white flower with normal keel petals (WSPF), blue flower with normal keel petals (BSPF), and blue flower with enlarged keel petals (BMPF).

MATERIALS AND METHODS

Plant materials and sample preparation

Fully opened, undamaged fresh flowers of BMPF, BSPF and WSPF were harvested during the peak flowering season in September, 2018 from Athurugiriya, Colombo, Sri Lanka. Flowers were dried at 50 ˚C for 24 hours using a forced air oven (Model SPF-600, SIBATA, Japan) as three separate samples. Dried samples were ground and sieved using a 1 mm sieve. Resulted powder samples were stored at room temperature in 300 gage high density polyethylene bags (Lee & Abdullah, 2011).The *C. ternatea* varieties used for the study, climatic and soil conditions, and Global Positioning System (GPS) coordinates of respective location are given in Table 1.

Aqueous extracts were prepared following the methodology described by Lakshan *et al.,* (2017). Sieved dried powders of flowers were extracted using a hot water bath at a temperature of 59.6 ˚C for 37 minutes with a flower to water ratio of 3 g/1000 mL. Extracts were filtered with 0.45 mm nylon filter and stored at –20 ˚C.

Chemicals and reagents

Folin–Ciocalteu reagent, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), ferric chloride, 2,4,6-tripyridyl-S-triazine (TPTZ), sodium acetate, glacial acetic acid, aluminum chloride, quercetin, sodium carbonate and methanol were purchased from Sigma-Aldrich Inc, USA. All the other chemicals were of analytical grade chemicals.

Total polyphenol content (TPC)

TPC was tested using the Folin-Ciocalteu method described by Singleton *et.al.,* (1999). A sample of 20 µL of extracts (concentrations 3.00, 1.50, 0.75 mg/mL; n=3) were mixed with freshly prepared seven times diluted Folin-Ciocalteu reagent (2N), and then 70 µL of sodium carbonate was added to the mixture in a 96-well micro plate. Then, the mixture was incubated at room temperature for 30 minutes until the absorbance was recorded at 765 nm (Spectr Max Plus, Molecular Device, USA). Gallic acid was used as the standard (assay concentrations: 3, 6, 12, 25, 50 and 100 µg/mL; n=3) and the results were expressed as mg gallic acid equivalents (GAE) per 1 g dry weight of the flower.

Total flavonoid content (TFC)

TFC was assessed using the method described by(Siddhuraju and Becker, 2003). A sample of 100 µL of extracts (concentrations: 3.00, 1.50, 0.75 mg/mL; n=3) were mixed with 100 µL of 2% aluminium chloride solution in methanol in a 96-well micro plate. Thereafter, the mixture was incubated at room temperature for 10 minutes until the absorbance was recorded at 415 nm. Quercetin was used as the standard (assay concentrations: 0.49, 0.98, 1.96, 3.91, 7.82, 15.63, 31.25 and 62.50 µg/mL; n=3) and results were expressed as mg quercetin equivalents per 1 g dry weight of flower.

Ferric reducing antioxidant power (FRAP)

FRAP was tested following the method described by (Benzie and Szeto, 1999), with slight modifications. TPTZ (10 mM) in 40 mM hydrochloric acid, an acetate buffer solution (300 mM, pH 3.6) and a $FeCl_3.6H_2O$ solution (20 mM) were mixed into a ratio of 1: 10: 1 to prepare working FRAP reagent. It was prepared at the time of testing and incubated at 37 ˚C for 10 min. Twenty microliters of aqueous flower extracts (concentrations: 3.00, 1.50, 0.75 mg/mL; n=3), 150 µL of working FRAP reagent and 30 µL of acetate buffer were mixed to make a 200 µL reaction volume in a 96-well micro plate, and incubated at room temperature for 8 minutes. Trolox was used as the standard (assay concentrations: 0.49, 0.98, 1.96, 3.91, 7.82, 15.63, 31.25 and 62.50 µg/mL; n=3) and results were expressed as mg of trolox equivalents per 1 g of flower dry weight.

Table 1: Agro-climatic details and the GPS coordinates of growing locations.

Varieties	Location, Agro-Ecological Region, District	Mean annual rainfall (mm)	Average Annual Temperature (°C)	Soil type	GPS Coordinates
1. White flower with normal keel petals **(WSPF)** 2. Blue flower with normal keel petals **(BSPF)** 3. Blue flower with enlarged keel petals **(BMPF)**	*Athurugiriya*, WL3, Colombo	2726 (Year 2018)	27.1 (Year 2018)	Red-yellow podzolic soils with soft and hard laterite Terrain- Rolling and undulating	6.8723, 80.0004

Source: Climate-Data.Org, (2019); World Weather Online, (2019)

DPPH radical scavenging assay

DPPH scavenging activity was tested using the methods described by (Blois, 1958). Fifty microliters of *C. ternatea* extracts (concentrations: 250, 125, 62.5 and 32.25 µL/mL in methanol; n=3) were used to make a reaction volume of 200 µL with a 125 µM solution of radical DPPH in methanol in a 96-well micro plate. The mixture was incubated at room temperature for 15 minutes and absorbance was measured at 517 nm. trolox was used as the standard (assay concentrations: 0.78, 1.56, 3.13, 6.25 and 12.5 µg/mL; n=3). The results were given in mg of Trolox for 1 g of flower dry weight.

Total nitrogen and crude protein content

The Kjeldahl method was used as the standard method to determine the nitrogen content (Ranst *et al*., 1999). Fresh flower samples were dried at 105 °C until a constant weight was obtained and 0.5 g of dried samples (n=3) were transferred to digestion receptacle of Kjeldahl apparatus. Combined reagent of sulfuric and salicylic acids (7 mL) was added and allowed to react for 30 minutes. Then, 0.5 g of sodium thiosulfate was added and allowed to react for 15 minutes.Concentrated sulfuric acid (3 mL) and 0.2 g catalyst were added. Then, several pumice stones were added and allowed to digest until a clear solution was appeared. After allowing to cool, 150 mL of distilled water was added.

For the distillation; outlet tube of the cooling column was immersed in a 250 mL Erlenmeyer containing 20 mL of the combined boric acid indicator reagent. Digestion tube was connected to the automatic steam distiller, and NaOH was added until formation of copper hydroxide. Distillation was carried out for 125 mL volume mixture until combined boric acid indicator reagent turned from wine red to green passing through blue-grey. Finally, titration was carried out for the distillate on a magnetic stirrer (mixer) with 0.1 M HCl until the wine-red color was reappeared. Crude protein content was determined by multiplying nitrogen content by the standard factor of 6.25.

Determination of P, K and Ca content

Determinations of minerals were carried out by following the dry ash method described by Ranst *et al.,*(1999). First, 0.5 g of previously dried flower samples at 105 °C were transferred into a porcelain crucible. Calcination was done at 450 °C for 2 hours in a muffle furnace. Then, digested with 5 mL of 6M HNO_3 by gentle boiling on a hot plate. 5 mL of 3M HNO_3 was added and reheated for few minutes. The warm solution was filtered into a 50 mL volumetric flask. A glass rod was used to assure a quantitative transfer. Crucibles and the glass rod were rinsed several times with 1% HNO_3 and recovered the residue on the filter. Filtrates were allowed to cool and diluted to 50 mL with water. The diluted filtrates were used to measure absorbance values for P by UV spectrophotometer, Ca and K by flame photometer.

Statistical analysis

The elemental contents measured were subjected to ANOVA procedure in the statistical package SAS 9.4 (SAS Institute, Cary, NC, USA) and DUNCAN mean separation procedure.

RESULTS AND DISCUSSION

TPC, TFC, FRAP, and DPPH radical scavenging activity of three flower types were given in the Table 2.

The maximum TPC was reported in BMPF (31.88 mg GAE/g dry weight of flower) which was significantly higher ($p<0.05$) than other two flower types. Previous studies conducted on aqueous extracts of blue flowers of *C. ternatea* in Malaysia, reported 20.7±0.1 mg GAE/g dry weight of flower (Rabeta and An Nabil, 2013). In another study, Havananda and Luengwilai (2019) has studied 46 accessions of *C. ternatea* from several countries (i.e. Taiwan, Thailand, Kenya, Tanzania, Brazil, United States of America, Cuba, Sudan, Sierra Leone, Mexico, Australia, Former Soviet Union, Dominican Republic, and U.S. Virgin Island) except Sri Lanka and found that the TPC content of those selected accessions have varied from 0.44±0.07 to

Table 2: Antioxidant properties of flower extracts of three varieties

Flower Type	TPC mg GAE/g dry weight of flower	TFC mg quercetin equivalents/g dry weight of flower	FRAP mg trolox equivalents/g dry weight of flower	DPPH mg trolox equivalents/g dry weight of flower
BSPF	26.72±2.17[b]	14.25±0.67[b]	14.56±2.10[a]	11.97±0.38[a]
BMPF	31.88±1.15[a]	15.96±0.58[a]	18.50±2.53[a]	3.95±0.36[b]
WSPF	25.02±0.59[b]	12.50±0.19[c]	10.66±1.57[c]	3.92±0.09[b]

Mean values in a column denoted by different letters are significantly different at $p < 0.05$; n=3

0.79±0.03 mg GAE/g of flower.Comparatively, aqueous extracts of *C. ternatea* varieties grown in Sri Lanka contains higher levels of TPC. Song *et al*., (2010) has investigated the TPC of 56 selected Chinese medicinal plant species and their values have varied from 0.12 to 59.43 mg GAE/g of plant parts which indicates that TPC of *C. ternatea* of the present study is moderate compared to the Chinese medicinal plants.

The highest level of TFC was found in BMPF (15.96 mg quercetin equivalents/g dry weight of flower) which was significantly greater ($p<0.05$) than other two flower types. Second highest TFC was observed in BSPF which was significantly higher than that of WSPF. TFC of 20 selected medicinal plants in India has been investigated by Sulaiman and Balachandran (2012) and reported that, TFC could be varied from 0.08 to 21.58 mg quercetin equivalents per 100 g of plant samples. Results of the present study showed a range from 12.50 to 15.96 mg quercetin equivalents/g dry weight of flower, indicating that the TFC content is much higher in *C. ternatea* varieties grown in Sri Lanka. Study conducted by Attanayake *et.al.,*(2016) showed that three *C. ternatea* varieties have higher TFC out of 11 selected medicinal plants and it has a higher potential for treating oxidative stress related chronic diseases in Sri Lanka.

FRAP of both BSPF and BMPF were not significantly different ($p>0.05$) to each other. However, FRAP of WSPF was significantly lower (10.66 mg trolox equivalents/g dry weight of flower) than that of blue flowered types. Previous studies reported that FRAP of selected 11 Indian traditional medicinal plants has varied from 0.36±0.02 to 18.28±0.41 mg trolox equivalents/g dry weight of the sample (Rajurkar and Hande, 2011). The assays employed were ferric reducing antioxidant power, trolox equivalent antioxidant capacity and scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl free radical. Results obtained indicate that the antioxidant potential varied significantly from plant to plant. The total phenolic contents were determined spectrophotometrically using Folin-Ciocalteu reagent. Significant correlation is observed between ferric reducing antioxidant power and phenolic contents. Our results were in accordance with the results of those studies. However, the FRAP of studied 46 accessions of *C. ternatea* by Havananda and Luengwilai (2019) varied between 4.5±6.0 to 67.7±25.0 mg trolox equivalents/g of flower. The FRAP of tested *C. ternatea* varieties of Sri Lanka was comparatively low as the average FRAP of Havananda and Luengwilai (2019) was 28.30 mg trolox equivalents/g of flowers of *C. ternatea* grown outside Sri Lanka.

BSPF showed the significantly highest level of DPPH radical scavenging activity that was 11.97 mg troloxequivalents/g dry weight of flower. BMPF and WSPF showed a similar level of DPPH radical scavenging activity which was not significantly different ($p>0.05$) to each other. To this regard, results obtained in the present study for BSPF, WSPF and BMPF found to be lesser than the trolox equivalents antioxidant capacities obtained for DPPH radical scavenging activity of tested 46 accessions of *C. ternatea* as it ranged in between 15.4±0.0 to 27.8±3.0 mg trolox equivalents/g of flowers of *C. ternatea* grown outside Sri Lanka (Havananda and Luengwilai, 2019).

Dose response graphs of three types of *C. ternatea* flowers are shown in the Figure 1, for different concentrations (250, 125 and 62.5 µL/mL) and the percentage inhibition values of extracts of three flower types at an assay concentration of 250 µL/mL are shown in the Table 3.

Significantly higher ($p<0.05$) inhibition of DPPH radical was reported in the extracts of BSPF than other two extracts at a given concentration. BMPF and WSPF have shown a similar level of inhibition of DPPH radicals at a given assay concentration.

Nitrogen, phosphorus, potassium, calcium and protein contents of three *C. ternatea* varieties tested are given in the Table 4. The mean phosphorus content is highest in WSPF while it is lowest in BMPF (4.65 and 4.19 mg/g respectively). The N content was significantly higher in both BSPF and WSPF (43.12 mg/g and 42.35 mg/g). The significantly higher mean protein contents were detected for BSPF and it was 26.95%. Mean protein content is lowest in BMPF and it may be due to differences in soil fertility or due to genetic/ physiological characteristics of the variety. Advanced technologies such as ICP-MS elemental analysis could be used for the confirmation of these results and this study could be used as an initial step to continue research on this important medicinal creeper. Based on the results, three varieties can be recommended as a good source of nitrogen and protein for consumption.

Variety WSPF claims the highest amount of mean K (1.15 g/kg), while BMPF has the lowest amount of mean K (8510.95 mg/kg). The highest mean Ca content was

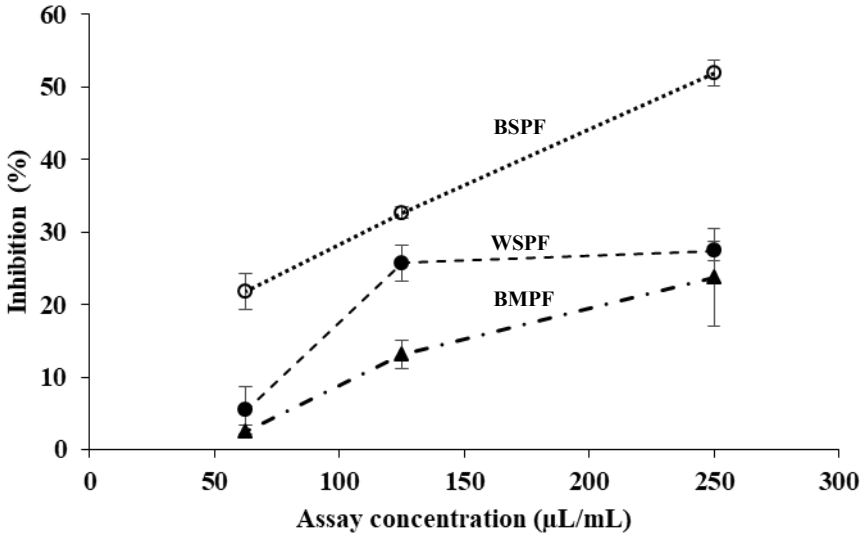

Figure 1: Dose response graph of DPPH radical scavenging assay of three flower extracts (IC_{50} value for trolox; 8.68 µg/mL).

Table 3: % Inhibition of DPPH radicals by three *C. ternatea* flower extracts at 250 µL/mL.

Flower type	% inhibition of DPPH radical
BSPF	51.92±1.77[a]
BMPF	23.75±0.80[b]
WSPF	27.38±3.25[b]

Mean values in a column denoted by different letters are significantly different at $p < 0.05$ and IC_{50} value of trolox: 8.68 µg/mL.

Table 4: N, P, K, Ca and protein contents in the three *C. ternatea* varieties.

Element	BSPF	BMPF	WSPF
N (mg/g)	43.12±0.28	40.74±0.14	42.35±0.35
P (mg/g)	4.22±0.03	4.19±0.05	4.65±0.07
K (g/kg)	10.07±0.05	8.51±0.06	11.49±0.19
Ca (g/kg)	3.63±0.49	2.98±0.72	5.92±0.8
Protein content (%)	26.95±0.18	25.46±0.09	26.47±0.22

reported in variety WSPF (5.91 g/kg). Variety BSPF has the second highest mean Ca content (3.63 g/kg). Lowest mean Ca content was reported in variety BMPF (2.98 g/kg). As summarized in Table 4, mineral content in the *C. ternatea* flower samples is not same among three varieties. Varieties having comparatively high amount of minerals can be considered as good products for consumption. The differences of the mineral content could be due to availability of the particular mineral in the soil.

CONCLUSIONS

The present study is the first detailed study on chemical composition and antioxidant activity of three main varieties of *C. ternatea* flowers in Sri Lanka. Chemical profiles of three flower types were determined and it shows significant amounts of biologically active compounds and presence of nutritionally important elements. It also implies that presence of significantly higher amounts of TPC, TFC and free radical scavenging activity of flower samples are responsible for a given type of biological activities inside the human body such as increasing immunity, reducing inflammation and angiogenesis.

Comparatively higher amount of TPC, TFC and antioxidant power of these three varieties confer their potential as an anticancerous natural herbal drug. It could be trusted as a potential botanical radio protector, which increases the efficiency of chemo radiation drugs while minimizing the acute and chronic damages to target site cells. Herbal juices, teas and curries of *C. ternatea* flowers could be considered as highly nutritious since it contains considerably higher amounts of dietary essential macro nutrients.

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DECLARATION OF CONFLICT OF INTEREST

Authors declare no conflict of interest.

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