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Phytochemical Properties of *Clitoria ternatea* L. (Fabaceae)—A Distinct Flower Morphometric Plants Available in Sri Lanka †

H. D. K. Buddhika [1], R. M. Dharmadasa [2,*], L. D. A. Menuka Arawwawala [2] and Kandiah Pakeerathan [1,*]

[1] Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna, Ariviyal Nagar, Kilinochchi 44000, Sri Lanka; e-mail@e-mail.com
[2] Industrial Technology Institute, Bauddhaloka Mawatha, Colombo-7, Sri Lanka
* Correspondence: dharmadasarm@gmail.com (R.M.D.); pakeerathank@univ.jfn.ac.lk (K.P.); Tel.: +94-112-379-800 (R.M.D.); Tel.: +94-077-696-9511 (K.P.)
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Abstract: Butterfly Pea (*Clitoria ternatea* L.) is a twining herbal plant belonging to family Fabaceae. The *C. ternatea* has potential medicinal values, therefore, traditionally, used in Ayurvedic medicine to cure stress, infertility, gonorrhoea, and as a food colouring agent. The modern science reported a variety of phytochemicals present in *C. ternatea*, and its application in agriculture and medicine. Therefore, the present study was conducted to investigate the variations in agronomic features and phytochemical contents in different morphotypes (blue single petal, blue multi-petal, white single-petal, white multi-petal, and purple single-petal) of *C. ternatea* plants available in Sri Lanka. Morphological characters of flowers, leaves and roots were carried out. Preliminary phytochemical analysis for phenols, flavonoids, tannins, alkaloids, glycosides, steroids and Thin Layer Chromatography (TLC) fingerprint analysis were conducted according to the previously published methods. Total phenol content, total phenol content and total antioxidant capacity were conducted using a spectrophotometer. Data were analyzed using SAS 9.4 statistical packages and DMRT was performed to know the best treatment combination at *p*-value of 0.05. The results showed that all five types of plants were exhibited the presence of all major phytochemicals, but flavonoids were significantly higher in flowers of blue multi-petal and purple single-petal plants. Total phenolic content was significantly higher in flower (53.62 mg GAE/g), leaves (73.32 mg GAE/g), and roots (52.67 mg GAE/g) of purple single-petal and blue colour multi-petal plants, respectively. Total flavonoid content was significantly higher in flower (34.01 mg QE/g), leaves (40.02 mg QE/g), and roots (18.40 mg QE/g) of blue multi-petal plant type. Similarly, radical scavenging capacity tested by DPPH assay exhibited significantly higher scavenging capacity in flower (2.93 mg TE/g), leave (3.77 mg TE/g), and root (3.40 mg TE/g) of white colour multi-petal, blue colour single petals and blue colure multi-petals, respectively at $p < 0.05$. This study revealed that tested phytochemical contents vary according to the different flower colours of *C. ternatea*. Therefore, it is suggested to cultivate varieties/morphotypes with a higher content of phytochemicals and antioxidant capacity for commercial purposes.

Keywords: *Clitoria ternatea*; total phenolic content (TPC), total flavonoid content (TFC), DPPH assay

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1. Introduction

Butterfly Pea (*Clitoria ternatea*) L. belongs to family Fabaceae generally known as Katarolu (Sinhala), Kokkattam (Tamil), Aparajitha (Bengali), and Asian Pigeon wing (English), is a perennial twinning medicinal plant, which has been widely used in traditional and Ayurveda systems of medicine in many parts of the world [6]. Butterfly Pea is well adapted to a variety of soil types (pH 5.5–8.9) including calcareous soil and It exhibit excellent re growth after cutting or grazing within a short period and produce high yield

[3]. Moreover, it survives in both the extended rainfall regions and prolonged periods of drought. Butterfly Pea has pinnate leaves with 5 or 7 leaflets. Flowers auxiliary, single or paired; color range from white, dark blue to purple. Seeds 8–11/pod, oblong, somewhat flattened, 4.5–7 mm long, 3–4 mm wide, olive-brown to, almost black, shiny, often mottled, minutely pitted [6]. Since this is a multipurpose plant, this has been used in traditional systems of medicine, herbal tea, cover crop, green manure, animal feed, nitrogen fixation crop, and as a weed-suppressing purpose [3,6]. At the same time, phytochemical profiles of different plant parts like, root, leaves, flower extracts are widely used in Ayurveda and traditional systems of medicine to cure a number of diseases, including asthma, burning sensation, ascites, inflation, leucoderma, leprosy, hemicranias, amentia, pulmonary tuberculosis, ophthalmology, insect bites snakebite, scorpion sting and skin diseases [6]. Moreover, the plant is used in a number of ailments including body-aches, infections, urinogenital disorders as antihelmintic and antidote to animal stings. They are considered safe for colic, dropsy, and enlargement of abdominal viscera [19].

The extracts from butterfly pea flower lowered the serum glucose levels of diabetic rats and increased their body weight. Further, the anti-diabetic effect was comparable to the diabetes drug glibenclamide [18]. A study found that petals of the butterfly pea flowers contain flavonol glucosides, kaempferol, myricetin, and quercetin. Other chemicals that contribute to the tea's pharmacological properties include phenols, saponins, anthocyanins, flavonoids, and triterpinoids [4]. And since it not made from the camellia sinensisplant, butterfly pea tea is naturally caffeine-free, making it an ideal beverage for people with caffeine sensitivity. At the same time the flower popular in Thailand, Malaysia, and Philippines as an edible dye. The petals are used to prepare ice creams and soups. At the same time, it can use as tea [17]. Even though the plant is used for diverse purposes and widely distrusted in many parts of the country, it has not been yet systematically explored for its actual potential as an industrial crop [17]. Therefore, the present study was undertaken to explore different *C. ternatea* species by means of taxonomic, phytochemical, and antioxidant capacity [18].

2. Methods

The experiment was conducted at the Herbal Technology Section laboratory of the Industrial Technology Institute (ITI) situated in Halbarawa Gardens, Malabe. Planting materials of different plant types were collected from the research plots that prepared in ITI Malabe for this research.

2.1. Sample Collection and Preparation

The fresh *C. ternatea* plants were separated according to different plant morphology and labeled as blue single petal, blue multi-petal (1A), white single-petal (1B), white single-petal (2A), white multi-petal (2B) and purple single-petal (3A). Roots, leaves, and flowers from labelled plants were separated. The plant parts were dried at room temperature without loss of volatile phytochemicals, and subjected to phytochemical analysis.

2.2. Phytochemical Analysis

Phytochemical screening of flavonoids, tannins, alkaloids, glycosides, steroids were carried out using standard protocols. In addition, Thin Layer Chromatography (TLC) fingerprints were developed. Total phenol content (TPC) and total antioxidant capacity (TAC) were measured using spectrophotometric analysis.

2.3. Development of TLC Fingerprint Profiles and Phytochemical Screening

From each sun dried sample 5 g was mixed with 50 mL of 99% methanol and kept in a mechanical shaker for overnight. The liquid part was filtered and filtrate was concen-

trated using a rotary evaporator. As the mobile phase mixture of dichloromethane, cyclohexane and ethyl acetate was used in a ratio of 2:3:0.5 (v/v). Phytochemical screening was carried out as described by Jayasinghe and co-workers [8].

2.4. Antioxidant Activities

Total phenolic content (TPC) [16], total flavonoid content (TFC) [5] and 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay [13] were carried out to determine the antioxidant activities.

2.5. Statistical Analysis

The data were subjected to analysis of variance (ANOVA) using SAS 9.4 statistical package and DMRT was performed to know the best treatment combination at *p*-value of 0.05.

3. Results and Discussion

Flower morphometric and phytochemical analysis was performed and following results were obtained.

3.2. Morphomatric Variation in Flowers of C. ternatea

There were three different colour producing *C. ternatea* plants were observed and visual examination revels that the three different number of petal produced by *C. ternatea* observed and categorized as 1A: Blue colour more petals; 1B: Blue colour single petal; 2A: white colour more petals; 2B: white colour single petal, and 3A: purple colour single petal (Figure 1).

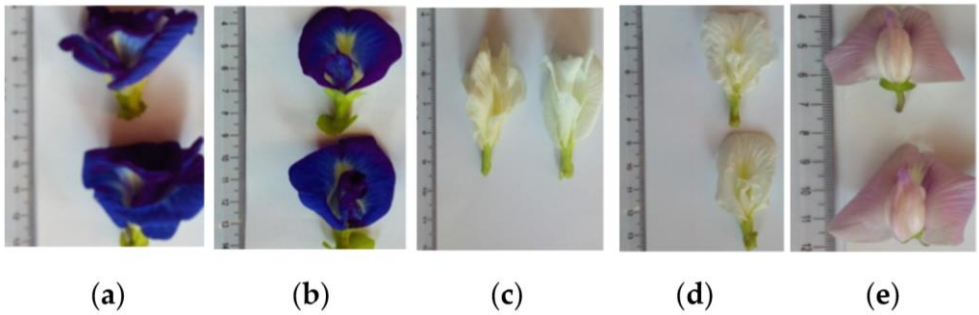

Figure 1. The different flower morphology of ***Clitoria ternatea***; (**a**) 1A: Blue colour more petals; (**b**) 1B: Blue colour single petal; (**c**) 2A: white colour more petals; (**d**) 2B: white colour single petal, and (**e**) 3A: purple colour single petal.

3.3. Variation of Phytochemicals in Different Plant Parts of C. ternatea

The TLC analysis results exhibits that the amount of flavonoids, tannins, alkaloids, glycosides, terpinoids, cardiac and steroids varied greatly in different parts of plant (Table 1). Flavonoids concentration was very high in flowers of 1A: Blue colour more petals, whereas, high concentration was observed in leaves, leaves and flowers and flowers of 1A: Blue colour more petals and 1B: Blue colour single petal and 2B: white colour single petal of respectively. Alokolids concentration was high and more or less equal concentration was observed in root extracts of 1A: Blue colour more petals and 2A: white colour more petals. High concentration of terpinoids was recorded in 3A: purple colour single petal. Other phytochemical concentrations were varied (less or absent) in each part of the plants tested.

Table 1. Phytochemical variation in different Clitoria ternatea Plants parts TLC analysis.

Sample	Plant Part	Phenols	Flavanoids	Tannins	Alkaloids	Terpinoids	Cardiac Glycosides	Steroids
1A: Blue colour more petals	Roots	+	+	-	++	-	-	-
	Leaves	+	++	+	+	+	+	+
	Flowers	+	+++	+	+	+	+	+
1B: Blue colour single petal	Roots	+	+	+	+	-	-	-
	Leaves	+	++	+	+	+	+	-
	Flowers	+	++	+	+	+	+	+
2A: white colour more petals	Roots	+	+	+	++	+	-	+
	Leaves	+	+	+	+	+	-	-
	Flowers	_	+	+	-	-	+	+
2B: white colour single petal	Roots	+	+	-	+	+	+	-
	Leaves	+	+	+	+	+	-	+
	Flowers	-	++	+	-	-	+	+
3A: purple colour single petal	Roots	+	+	+	+	++	-	+
	Leaves	+	+	-	+	+	-	+
	Flowers	-	+++	+	-	-	+	+

"-": Absent, "+": Present (less), "++": High, "+++": Very high concentration of phyto chemicals.

3.3. Antioxidant Activities

Total phenolic content of *C. ternatea* was significantly varied in different parts (Table 2) of the C. ternatea plant producing different colour flower with different number of petals at $p < 0.05$, and considerably higher in leaves than flower and roots. Total flavonoids content of C. ternatea was significantly influenced by plant parts. Table 2 show that TFC was significantly highest at $p < 0.05$ in leaves of 1A: Blue colour more petals (40.02 ± 4.00), and non-significant when compare to leaves of 1B: Blue colour single petal (39.37 ± 2.39) and 2A: white colour more petals (36.69 ± 5.54) mg QE/g of extract. Incontrast, TFC was high in flowers of 2B: white colour single petaland 3A: purple colour single petals with the values of 30.3 4 ± 1.22 and 25.10 ± 4.58 mg QE/g of extract, respectively. In comparison to leaves and flowers, TFC present in roots are significantly less.

The highest DPPH scavenging ability was observed in leaves of 1B: Blue colour single petal with the value of 3.76 ± 1.23 followed by roots (3.40 ± 0.54) and leaves (3.14 ± 0.65) of 1A: Blue colour more petals and roots of 2A: white colour more petals (3.13 ± 0.62) without significant at $p < 0.05$. The lowest DPPH scavenging ability was recorded in 3A: purple colour single petals with the value of 0.61 ± 0.21 mg TE/g of extract.

The current research aims to evaluating the phytochemical composition present in the morphologically different *Clitoria ternatea* plant leaf, flowers and roots to choose the best plant for the commercial production of medically important phytochemicals. It is evident from our findings that authentic part is very crucial for obtaining maximum pharmaceutical quality and avoiding any alternation of medicinal potency of *C. ternatea*. Raya et al. (2015) reported that the different phytochemicals are in different concentration in different plant parts highest initial phenolic content was found in young leaves of *C. nutans* [15]. Moreover, Males et al. (2010) who reported that *I. candida* contains higher phenolic compounds in leaves (1.031–1.423%) compared to stem (0.411–0.516%) [10]. Rafat et al. (2009) also recorded higher phenolic content in leaves than in stems of *A. paniculata*. This finding is in agreement with the current research [14].

The TFC is attributed mainly in leaves and followed by flowers. Lakshan et al. (2020) reported that TFC 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 [9]. 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 [1]. These findings further validating the current research.

Table 2. TFC, TPC and Antioxidant capacity of different Clitoria ternatea Plants parts.

Samples	TPC (mg GAE/g of Extract)	TFC (mg QE/g of Extract)	DPPH (mg TE/g of Extract)
1A: Blue colour more petals			
flower	46.97 (±4.4) ^{d}	34.02 (±3.60) ^{a}	2.61 (±1.2) ^{b}
leaves	73.31 (±7.1) ^{a}	40.02 (±4.00) ^{a}	3.14 (±0.65) ^{a}
roots	52.68 (±5.7) ^{c}	18.40 (±2.86) ^{bc}	3.40 ±0.54) ^{a}
1B: Blue colour single petal			
flower	24.11 (±3.59) ^{ef}	14.06 (±2.49) ^{c}	1.95 (±0.64) ^{c}
leaves	68.61 (±7.42) ^{a}	39.37 (±2.39) ^{a}	3.76 (±1.23) ^{a}
roots	24.41 (±6.14) ^{ef}	16.38 (±3.15) ^{c}	2.64 (±0.43) ^{b}
2A: white colour more petals			
flower	24.07 (±2.29) ^{ef}	23.70 ± (6.04) ^{b}	2.93 (±1.2) ^{b}
leaves	65.89 (±2.43) ^{b}	36.69 (±5.54) ^{a}	2.19 (±1.2) ^{bc}
roots	28.74 (±1.32) ^{e}	15.56 (±2.44) ^{c}	3.13 (±0.62) ^{a}
2B: white colour single petal			
flower	30.77 (±2.17) ^{e}	30.3 4 (±1.22) ^{ab}	2.61 (±0.68) ^{b}
leaves	63.75 (±2.22) ^{b}	26.16 (±5.54) ^{b}	1.90 (±0.76) ^{c}
roots	20.69 (±0.56) ^{f}	13.30 (±2.17) ^{c}	1.23 (±0.38) ^{c}
3A: purple colour single petals			
flower	53.62 (±2.78) ^{c}	25.10 (±4.58) ^{b}	0.61 (±0.21) ^{d}
leaves	67.76 (±5.67) ^{a}	14.08 (±1.52) ^{c}	1.01 (±0.43) ^{cd}
roots	42.08 (±6.34) ^{d}	7.20 (±2.13) ^{d}	1.23 (±0.42) ^{c}

The DPPH activity is higher in roots and followed by leaves and flower. Havananda and Luengwilai (2019) studied 46 accessions of *C. ternatea* and reported that DPPH by varied between 4.5 ± 6.0 to 67.7 ± 25.0 mg trolox equivalents/g of flower [6]. The current findings of DPPH is lower than the Havananda and Luengwilai (2019) results. This variation may be due to the variety differences or maturity differences. Rafat et al. (2009) also reported similar findings from their study with *A. paniculata* [12]. Ghasemzadeh et al. (2014) on the other hand, recorded higher DPPH value in 1-year -old buds than in 6-month-old buds of *C. nutans*. Therefore, selection of plants to be utilized for commercial phytochemical extraction is important, which might result in changes in phytochemical content with course of time.

4. Conclusions

This report that phytochemical content of the *C. ternatea* plants, showing different flower morphology, varied greatly. The Flavanoids, Alkaloids, TPC and TPC is high in *C. ternatea* plants producing Blue colour more petals and Blue colour single petal. The finding concludes that *C. ternatea* plants producing Blue colour more petals and Blue colour single petal are highly suitable for commercial level medically important phytochemical extraction.

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