

A Comparative Elemental Analysis and Pharmacological Evaluation of *Clitoria Ternatea* (Butterfly Pea) Flowers: Blue and White Varieties from Coastal Regions of Kerala, India

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Abstract

Butterfly Pea (*Clitoria ternatea* Linn.), renowned in traditional medicine across diverse cultures, is explored in this study for its pharmacological and phytochemical aspects. Focusing on blue and white varieties from Kerala, India, the research compares elemental phytochemistry and pharmacological activities of aqueous extracts. The plant's medicinal applications, from Ayurvedic treatments to culinary uses, are highlighted. The investigation identifies the White variant as beneficial for bone health, while the blue variant offers antioxidant, antidiabetic, and antifungal properties. The study emphasizes Butterfly Pea flower tea as a caffeine-free alternative with diverse therapeutic potentials for health-conscious individuals.

Keywords: *Clitoria Ternatea* Linn.; Elemental analysis; α -Amylase inhibition; Antifungal Activity.

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1. Introduction

Many traditional remedies still have their foundation in medicinal plants with therapeutic qualities. Because of their long history of usage and a number of advantages, such as fewer side effects, improved patient tolerance, cost-effectiveness, and acceptance, medicinal plants are becoming more and more popular [1]. The increasing demand in the global herbal medicines market warrants more studies on medicinal plants' pharmacological and toxicological evaluations [2]. *Clitoria ternatea* Linn. (C. T.), a prominent twining plant native to the Caribbean, which is now widely popular in Asian countries, produces flowers in shades of blue or white. It is widely recognized by various names such as butterfly pea, conch flower, and shankupushpi. In Indian medicinal texts, it is referred to as Aparajit, Aparajita, or Kakkattan [3]. The Indian Ayurveda system utilizes C.T.'s roots, seeds, and leaves to enhance memory and intelligence in medicines like the Medhya Rasayana that

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encompasses intellect-enhancing properties of not just C.T. but also other medicinal plants like *Celastrus panniculatus*, *Acorus calamus*, *Centella asiatica*, and *Withania somnifera* [4-7]. In the Kancheepuram district of Tamil Nadu, the powdered root of C.T. is combined with water and consumed orally to alleviate indigestion, eye ailments, and headaches [8]. In Assam, the juice extracted from the leaves is mixed with salt and applied around the ears to relieve headaches and reduce swelling in nearby glands.

Additionally, this juice is used as a remedy for snakebites, and its in-silico phytochemical screening has shown the flower to contain inhibitors of snake venom phospholipase A2 (PLA2) [9,10]. According to information gathered from villagers in Dharmapuram Taluk, Tamil Nadu, a mixture of C. T. seed powder and pepper is administered to address constipation [11]. C. T. possesses various therapeutic effects, including its use in managing CNS symptoms during alcohol withdrawal. It exhibits anticancer, antidiabetic, antioxidant, antihistamine, hypolipidemic, and asthmatic properties [12]. Along with its seed extract, the plant is employed in treating conditions such as stomatitis, piles, female sterility, hematemesis, insomnia, epilepsy, psychosis, and leucorrhea [13]. The root of C. T. exhibits anti-inflammatory, analgesic, and antipyretic properties [14], making it beneficial for treating a wide range of conditions such as sore throat, skin diseases, eye ailments, tubercular glands, amentia, hemicrania, burning sensation, stranguary, leprosy, leucoderma, elephantiasis, arthritis, bronchitis, asthma, pulmonary tuberculosis, ascites, ulcers, visceromegaly, and joint-related issues [12-15], while the leaves are recommended for managing neurodegenerative diseases and diabetes mellitus, as well as regulating excessive sweating [16,17]. The plant's medicinal properties in China are utilized to treat various reproductive organ ailments. In Cuba, a decoction of the roots or flowers is employed as an emmenagogue. By soaking and macerating the cleaned roots in water, a mixture is created, which promotes menstruation and stimulates uterine contractions when consumed in the evening. Combining the flowers and roots in a bottle of wine produces a magnificent medicine capable of treating chlorosis, liver problems, and intestinal issues [18]. In Malaysia, the leaves are employed to add a green color to food preparations, while the flowers are used to give rice cakes a blue hue. The flowers, shoots, and leaves are used as vegetables in Kerala (India) and the Philippines, and due to their attractive color, the flowers also serve as ornamental plants [19,20]. This flower's vibrant, deep blue petals have been traditionally used as a popular dye for centuries.

The butterfly-pea (BFP) flower tea is used worldwide for its potent pharmacological effects, such as tea dust, dye material, and edible petals. C.T.'s flower harbors a diverse array of phytochemical compounds, like the major flavonol glycosides, namely 3-O-(2"-O-alpha-rhamnosyl-6"-O-malonyl)-beta-glucoside, 3-O-(6"-O-alpha-rhamnosyl-6"-O-malonyl)-beta-glucoside, and 3-O-(2",6"-di-O-alpha-rhamnosyl)-beta-glucoside of kaempferol (I), quercetin (II), and myricetin (III). These compounds were isolated from the petals. Additionally, eight anthocyanins (ternatins C1, C2, C3, C4, C5, and D3, and preternatins A3 and C4) were identified in the flowers, with six of the ternatins characterized as acylated derivatives of delphinidin. White petal is said to lack anthocyanins [21]. The flowers also contain kaempferol, kaempferol 3-neo hesperidoside, kaempferol 3-2G-

rhamnosylrutinoside, kaempferol 3-rutinoside, kaempferol 3- glucoside, quercetin, quercetin 3-2G-rhamnosyl rutinoside, quercetin 3-neohesperidoside, quercetin 3-rutinoside, quercetin 3-glucoside, myricetin 3-neohesperidoside, myricetin 3- rutinoside, and myricetin 3-glucoside [22,23]. The nutritional composition of *Clitoria ternatea* Linn. flowers revealed that they contain 2.1 % fiber, 2.2 % carbohydrates and 2.5 % fat with a moisture content of 92.4% [50]. The total phenolic content in the leaf and flower of *C. T.* was assessed using the Folin Ciocalteu's reagent (FCR) method [24], while the flavonoid content was determined using the Aluminium chloride colorimetric method [25]. The anthocyanin content was measured using the pH differential method. The findings revealed that the ethanolic *C. T.* extract contained approximately 53 ± 0.34 mg gallic acid (phenolics), 11.2 ± 0.33 mg catechin(flavanoid), and 1.46 ± 0.04 mg cyanidin-3-glucoside(anthocyanin) and the water extract of *C. T.* flowers exhibited the presence of mome-inositol (38.7 %) and pentanal (14.3 %) [26,27]. Extensive pharmacological research conducted on this plant has unveiled its potential, demonstrating a wide range of activities, including antimicrobial, antifungal, antipyretic, diuretic, anti-hyperglycemic, proteolytic, antidiabetic, larvicidal, analgesic, and antioxidant properties [28]. The flower extracts of *C. T.* have been found to exhibit antioxidant activity against in-vitro-generated free radicals [29]. Anthocyanin extract derived from the BFP flower demonstrates both in-vitro and cellular antioxidant activities [28]. These anthocyanins from the flower contribute to the prevention of lipid oxidation, reduction of glutamine concentration in APPH-induced hemolysis, inhibition of protein carbonyl group formation, and suppression of membrane lipid peroxidation [30]. The anthocyanins present in the blue flower are also believed to have the potential to prevent cardiovascular and neurological diseases [31]. The BFP flower anthocyanin extract protects erythrocytes from AAPH-induced hemolysis and oxidative damage [32]. In a study, the aqueous extract was found to exhibit very strong antioxidant activity [33,34]. Methanolic, phenolic, ethanolic, and acetone extracts of *C. T.* have also been investigated in various studies. The flower extracts exhibit effects that are beneficial for managing elevated blood sugar levels and excessive lipid levels [35]. The presence of bioactive compounds, including anthocyanin, in *C.T.* can inhibit the activity of pancreatic alpha-amylase and intestinal alpha-glucosidase, which are enzymes responsible for digesting carbohydrates. This inhibition helps in reducing postprandial hyperglycemia [36]. The ethanolic extract of *C. T.*'s flowers has also demonstrated in vitro antidiabetic activity [37]. The aqueous extract of *C.T.* flower showed antimicrobial activity against bacteria causing dental cavities [38]. The secondary metabolites produced by the plant are suggested to make them potent antimicrobial agents [39]. Recent studies have indicated that treatment with *C.T.* extract can cause a permanent change in the brain, which may explain the improved learning and memory associated with its use [40]. The sample also demonstrated properties that helped with memory loss, increased levels of acetylcholine, and enhanced acetylcholinesterase activity in the brain [41]. BFP Flowers demonstrate significant larvicidal activity against 3 major mosquito vectors. Of all the prepared extracts, seed extract showed larvicidal activity against the larvae of *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* [42]. Out of the blue and white varieties of *C. T.*, the leaf extract of the white variety exhibited a

more hepatoprotective effect than the blue variety's leaf extracts [43,44]. In addition to these properties, other activities such as anti-carcinogenic, insecticidal, and blood platelet aggregation inhibition were also observed in CT [28].

The consumption of butterfly pea flower tea and its incorporation into regular diets has only recently gained popularity in India despite its long-standing use in Ayurvedic preparations. Butterfly-pea flower tea is a good alternative for vegans and those trying to avoid caffeine. In this work, we compare the elemental phytochemistry and some selected pharmacological activities of aqueous extracts of matured Indian variants of BFP blue and white variety flowers available in the coastal regions of Kerala (India) to suggest the better one in terms of availability and benefits, to be used as tea dust.

2. Materials and Methods

2.1. Chemicals and reagents

Nitric acid, iodine, potassium iodide, starch, hydrochloric acid, phosphate buffered α -amylase, potassium ferricyanide, trichloroacetic acid, ferric chloride, sulphuric acid, sodium phosphate, ammonium molybdate, and gallic acid are used.

2.2. Aqueous extract preparation from *Clitoria ternatea* Linn. flowers

In October 2023, fully bloomed and undamaged Blue and White *Clitoria ternatea* Linn. (C. T.) flowers were systematically harvested during their peak flowering season in Thiruvananthapuram, Kerala, India. Stringent selection criteria were applied to ensure the quality and uniformity of the flowers representing both color variants. Both plants were authenticated at the Department of Botany, University of Kerala, by E. A Siril, where the blue variety's taxon was *Clitoria ternate*.var *ternate*. f. *ternate* L and that of the white was *Clitoria ternate*.var *ternate*. f. *albiflora* (Voigt) Fantz. The harvested flowers underwent a carefully controlled drying process and were exposed to natural sunlight for a day. Subsequently, the dried flowers were ground into a fine powder using a grinder and sieved through a 2 mm sieve to achieve consistent particle size. For the preparation of aqueous extracts, 3 g of the resulting powdered samples from both Blue and White CT flowers were precisely measured and mixed with 1000 mL of water. The mixture underwent controlled heating on a water bath set at 80 °C for 20 min, facilitating the extraction of bioactive compounds from the plant material. After the extraction process, the aqueous extracts were filtrated using a 0.45 mm nylon filter to eliminate solid residues and impurities. The clarified extracts were then carefully stored at a constant temperature of 8 °C to preserve their chemical composition and prevent degradation over time. This meticulous extraction methodology [45] guarantees the retention of the valuable phytochemicals present in *Clitoria ternatea* Linn. flowers, forming the basis for subsequent analyses of elemental phytochemistry and pharmacological activities. The study aims to provide comprehensive insights into the potential benefits and versatile applications of Blue and White CT variants, particularly in the context of their suitability as tea dust.

2.3. Determination of N, P, K, and Ca content and crude protein content

The nitrogen content in fresh flower samples was determined using the Kjeldahl method, established as the standard procedure [46]. The flowers were dried at 105°C until a constant weight was achieved, and 0.5 g of the dried samples were placed in the digestion receptacle of the apparatus. A combined reagent of sulphuric acid and salicylic acid (7 mL) was added, allowing it to react for 30 min. Subsequently, 0.5 g of sodium thiosulfate was introduced and allowed to react for 15 min. The addition of concentrated sulphuric acid (3 mL) and 0.2 g of catalyst followed, and the mixture was allowed to digest until a clear solution emerged with the inclusion of several pumice stones. After cooling, 150 mL of distilled water was added. For distillation, the outlet tube of the cooling column was immersed in a 250 mL Erlenmeyer flask containing 20 mL of the combined boric acid indicator reagent. The digestion tube was connected to an automatic steam distiller, and NaOH was added until the formation of copper hydroxide. Distillation was conducted until a 125 mL volume mixture was obtained, causing the combined boric acid indicator reagent to transition from wine red to green and ultimately to blue-grey. Finally, the distillate was titrated on a magnetic stirrer with 0.1 M HCl until the wine-red color reappeared. The crude protein content was determined by multiplying the nitrogen content by the standard factor of 6.25.

Mineral analysis was conducted using the dry ash method, as detailed by Ranst *et al.* [47]. Initially, 0.5 g of flower samples dried at 105°C were carefully transferred into a porcelain crucible. Calcination ensued at 450 °C for a duration of 2 h within a muffle furnace. Subsequently, the calcined samples were digested with 5 mL of 6 M HNO₃ through gentle boiling on a hot plate. An additional 5 mL of 3 M HNO₃ was introduced, and the mixture was reheated briefly. The resulting warm solution was meticulously filtered into a 50 mL volumetric flask, ensuring a quantitative transfer using a glass rod. Crucibles and the glass rod were rinsed multiple times with 1 % HNO₃, and the residue was recovered on the filter. The filtrates were allowed to cool and were then diluted to a final volume of 50 mL with water. These diluted filtrates were employed to measure absorbance values for phosphorus (P) using a U. V. spectrophotometer, while calcium (Ca) and potassium (K) were determined using a flame photometer.

2.4. α -Amylase inhibition assay

The assessment of α -amylase inhibition for the selected extracts and compounds was conducted in accordance with the methodology outlined by Ononamadu *et al.* [48], with minor adjustments. In triplicates, 250 μ L of each extract at varying concentrations (62.5, 125, 250, 500, and 1000 μ g/mL) were dispensed into test tubes. Subsequently, the following components were sequentially added: 250 μ L of phosphate buffer (200 mM, pH 6.9, and containing 6 mM sodium chloride), 250 μ L of phosphate buffered α -amylase (0.05 mg/mL), and 250 μ L of starch (1% w/v). The reaction mixture was then incubated for 15 minutes at 37 °C. To halt the enzymatic reaction, 20 μ L of 1 M HCl was introduced, followed by the addition of 100 μ L of iodine reagent (5 mM I₂ and 5 mM K. I.). The resulting color change was observed, and the absorbance was measured at 625 nm. The control reaction,

representing 100 % enzyme activity, lacked any test extract or compound. Acarbose served as the reference standard.

The inhibition of enzyme activity was determined using the formula:

$$\text{Inhibition (\%)} = (C - S) / C \times 100,$$

Where S is the absorbance of the sample, and C is the absorbance of the control (no extract).

2.5. Reducing power assay

The assessment of reducing power was conducted following the protocol described by Gow- Chin and Pin-Deer [49]. Different extracts (500 μ L) concentrations were combined with 1.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 1.5 mL of 1 % potassium ferricyanide. The resulting mixture was incubated at 50°C for 20 min, and then 5 mL of 10 % trichloroacetic acid was added. Subsequently, the mixture underwent centrifugation at 6000 rpm for 5 minutes at 4 °C. The upper layer of the solution (1.5 mL) was mixed with 1.5 mL of distilled water and 300 μ L of ferric chloride (0.1 %), and the absorbance was measured at 700 nm. Ascorbic acid was utilized as the standard. An increase in absorbance of the reaction mixture indicated an elevation in reducing power. The concentration at 0.5 absorbance was designated as the reference point to assess the scavenging potential of each gelatin sample, as per the method outlined by Uddin *et al.* [50].

2.6. Total antioxidant capacity

Determining Total Antioxidant Capacity (TAC) in the samples was carried out using the phosphomolybdic acid method described by Prieto *et al.* [51]. To 0.1 mL of the extract (1 mg/mL in distilled water), 1 mL of a reagent mixture containing 0.6 M sulfuric acid, 20 mM sodium phosphate, and 4 mM ammonium molybdate was added. The tubes were covered and placed for incubation in a water bath at 95 °C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm against a blank. Gallic acid served as the standard, and the Total Antioxidant Capacity was calculated using the equation:

$$T=C \times (V/M)$$

where *T* is the total antioxidant content (mg/mL) of the extract, *C* is the concentration of Gallic acid (mg/mL) obtained from the calibration graph, *V* is the volume of the extract taken (mL), and *M* is the weight of the extract (g). The total antioxidant capacity (TAC) of the extract is expressed as milligrams per gram (mg/g).

2.7. Antifungal activity

The fungal strain *Candida albicans*, obtained from the collections maintained at Biovent Innovations Pvt Ltd., Department of Biotechnology, University of Kerala, Thiruvananthapuram, was utilized in this antifungal activity study. Sabouraud's Dextrose Agar (SDA, served as the testing medium. The preparation of SDA involved dissolving 65 g of SDA in 1000 mL of double-distilled water, followed by sterilization in an autoclave at

121 °C for 15 lbs pressure for 15-20 min. For the screening of antifungal activity, concentrations of the sample at 25, 50, and 100 µL were used. Imidazole (at a concentration of 100 µg/mL) and 0.05 % DMSO were employed as the positive and negative controls, respectively. The antifungal activity of the extracts was assessed using the test tube method as described by Pandey [52]. The extracts were directly added to the molten SDA media and adjusted to 1 mL in each tube. Thorough mixing of the extracts with the media was ensured, and the tubes were positioned in a slanting manner to facilitate solidification. After confirming the sterility of the tubes, the test fungi were inoculated into the respective test tubes. Tubes inoculated with *Candida albicans* were incubated at room temperature, and the results were recorded after 3 to 4 days. The antifungal activity was evaluated based on the observed inhibition of fungal growth in response to the extracts.

3. Results and Discussion

Table 1. N, P, Ca, K and Protein contents in Blue and White CT variants.

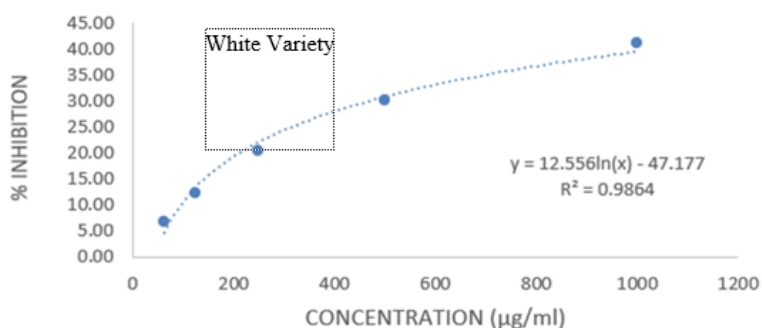
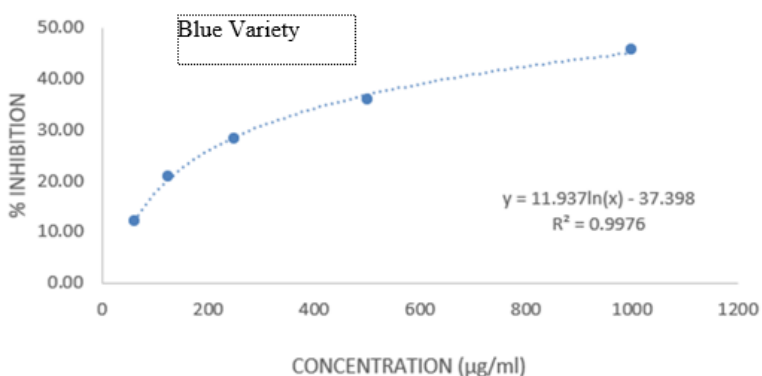
Elements	Blue CT	White CT
P(mg/g)	3.95±0.20	4.45±0.18
N(mg/g)	43.12±0.28	42.35±0.35
K/(g/kg)	10.09±0.04	12.00±0.18
Ca(g/kg)	3.67±0.40	6.12±0.32
Protein Content (%)	26.95±0.18	26.47±0.22

Table 1 presents the Nitrogen, Phosphorus, Potassium, Calcium, and Crude Protein Content of the two C.T. variants tested. The results obtained are in agreement with the earlier investigations where P, N, K, Ca, and protein content were found to be similar [53]. The white variety exhibits the highest mean phosphorus content. In contrast, the blue C.T. variant has a higher mean nitrogen content, and the blue variant also demonstrates a greater mean protein content. The observed disparity in protein content may be attributed to variations in the genetic characteristics of the varieties. Consequently, based on the findings, both variants can be recommended as valuable sources of nitrogen and protein.

The white C. T. variant stands out with the highest potassium and calcium levels. Table 1 clearly indicates that mineral content differs between the two variants. The white variety, characterized by relatively elevated mineral content, emerges as a favorable choice for consumption.

Table 2. Percent α -amylase inhibition by White and Blue CT variants.

Concentration (µg/mL)	% inhibition for White CT	% inhibition for Blue CT
62.5	6.62	12.02
125	12.23	20.70
250	20.54	28.16
500	30.23	35.89
1000	41.13	45.79

Fig. 1. % α -amylase inhibition by White CT variant.Fig. 2. % α -amylase inhibition by Blue CT variant.

Based on the findings presented in Table 2 and Figs. 1 and 2, it is apparent that the blue variant exhibits superior inhibition against α -amylase. The aqueous extract of the blue flower was found to inhibit α -amylase during *in vitro* starch digestion, pointing to the potential of the extract in regulating postprandial blood glucose level [54]. The potential constituent responsible for this inhibitory effect is likely polyphenols, given their known antioxidant properties, which can mitigate damage caused by reactive oxygen species. Additionally, polyphenols have the capacity to bind and inhibit both α -amylase and α -glycosidase, supporting their role in the observed inhibition [55].

Table 3. Average Optical density in to assess scavenging activity of White and Blue CT variants.

Concentration (µg/mL)	Average O.D. for white C.T.	Average O.D. for blue CT
62.5	0.113	0.079
125	0.528	0.448
250	1.112	1.027
500	1.794	1.463
1000	2.345	2.051

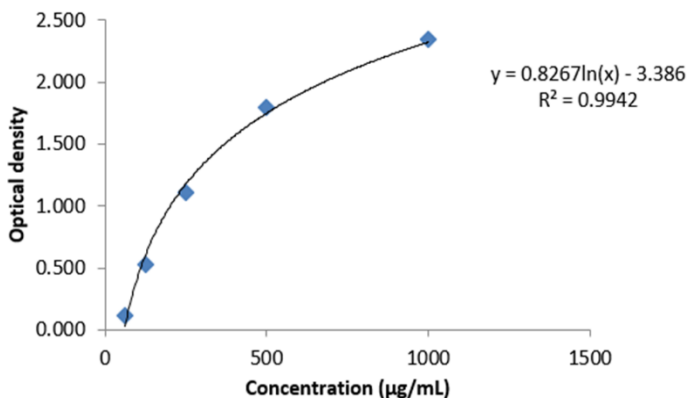


Fig. 3. Scavenging activity of white C.T. variant.

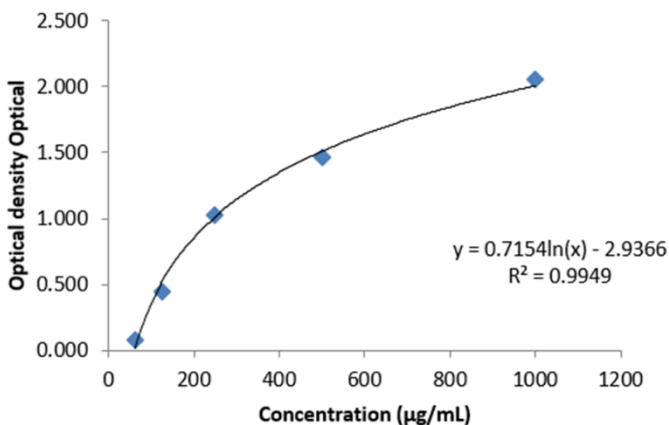


Fig. 4. Scavenging activity of blue C.T. variant.

Based on the data presented in Table 3 and Figs. 3 and 4, the White Variant demonstrates superior reducing power and scavenging activity. Various investigators have reported reduced power activity of C.T. flower extracts [56,57]. The higher reducing power observed in this variant implies a heightened capability to mitigate oxidative stress. This is particularly significant as oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and the body's capacity to neutralize them, is associated with a range of health issues, including aging and chronic diseases.

Table 4. Total antioxidant capacity of white and blue C.T. variants.

Sample	O.D. at 695 nm	mg GAE/g of extract
White	0.322	32.96
Blue	0.367	35.45

As indicated in Table 4, the blue C. T. variant demonstrates elevated total antioxidant capacity. An earlier study also reported increased antioxidant activity for the root extracts of the blue variety in comparison with the white variety [58]. The contrast between the white C.T. extract's superior reducing power and the blue C.T. extract's higher total antioxidant capacity can be explained by the distinct focus on reducing power assays on electron donation and the broader evaluation of total antioxidant capacity assays in neutralizing diverse reactive species. This disparity suggests divergent antioxidant mechanisms between the white and blue C.T. extracts, emphasizing the significance of employing a range of assays to assess antioxidant potential in future studies comprehensively. Recently, Jeyaraj *et al.* have reported more potent antioxidant activity for the anthocyanin-rich fraction of 50% ethanol extract of the blue variant. This further supports the potential of the blue C.T. variant as a functional food ingredient or nutraceutical agent [57].

Table 5. Antifungal activity of blue and white C.T. variants. Symbols have the following meaning: N.G.- No growth, + - Mild growth, ++ - Moderate growth, +++ - Intense growth.

Sample	<i>Candida albicans</i>			+ve control	-ve control
	Tested concentrations				
	25 μ L	50 μ L	100 μ L		
White	++	++	+	NG	+++
Blue	++	+	NG	NG	+++

Findings from Table 5, indicate that the blue C.T. extract exhibited superior antifungal activity compared to the white C.T. extract against *Candida albicans*. The observed differences in antifungal efficacy suggest that the two extracts may contain distinct bioactive compounds or possess varying concentrations of antifungal constituents. Recently, another group of investigators has also reported the activity of C.T. ethanolic extract against *Candida albicans* and suggested its potential as a mouthwash that could be effective against Candidiasis [59]. Angel *et al.* have also reported potent antifungal activities for C.T. extract and suggested its significance as a novel and safe alternative for developing therapeutics against Fungal Keratitis [60]. The enhanced antifungal activity of the Blue CT extract may be attributed to specific compounds, such as polyphenols or secondary metabolites, known for their antifungal properties.

4. Conclusion

A comprehensive analysis of the biochemical composition and functional properties of blue and white *Clitoria ternatea* Linn. variants was conducted, and it was found that both the blue and white C.T. variants offered distinct health benefits. The White variant stands out with the highest mean phosphorus content, indicating its potential role in supporting bone health and energy metabolism. Additionally, the white C. T. variant exhibits the highest potassium and calcium levels, suggesting its suitability for promoting cardiovascular health and bone strength. The blue C. T. variant demonstrates superior inhibition against α -

amylase, which may contribute to better blood sugar management and potential antidiabetic effects. It also exhibits higher total antioxidant capacity, attributed to its polyphenol content, which suggests enhanced protection against oxidative stress. The blue variant shows superior antifungal activity against *Candida albicans*, indicating potential benefits in combating fungal infections. While both variants have notable health benefits, the white C. T. variant may be favored by individuals seeking a tea variant rich in essential minerals like phosphorus, potassium, and calcium. On the other hand, the blue C. T. variant could be preferred for those looking for antioxidant-rich tea with potential antidiabetic and antifungal properties. The choice between the two variants ultimately depends on individual health goals and preferences. Incorporating a variety of teas into one's diet may provide a diverse range of health benefits. Further studies can be conducted on the synergic effect of drinking tea with mixed tea dust of both variants.

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