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Original Research

Environmental, Antimicrobial, and Nutritional Evaluation of *Clitoria ternatea* Flower Extracts: Implications for Food and Health Applications

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Abstract

Clitoria ternatea, also known as butterfly pea or blue pea, has many pharmacological and nutritional values. Stock solutions of flower extracts were prepared in different solvents, i.e., methanol (M), water (W), ethanol (E), glycerol (G), and glycerol/water (GW) to get extracts coded as (ME), (WE), (EE), (GE), and (GWE), respectively. A number of evaluations, such as microbial evaluation, phytochemical properties, mineral contents, and antioxidant activities, have been conducted on the blue flowers. The titration acidity reported 0.018 g citric acid/100 mL (CA) with a pH of 8.1. The total monomeric anthocyanin of blue pea flower was reported to be 77.31 mg/L cyanidin-3-glucoside according

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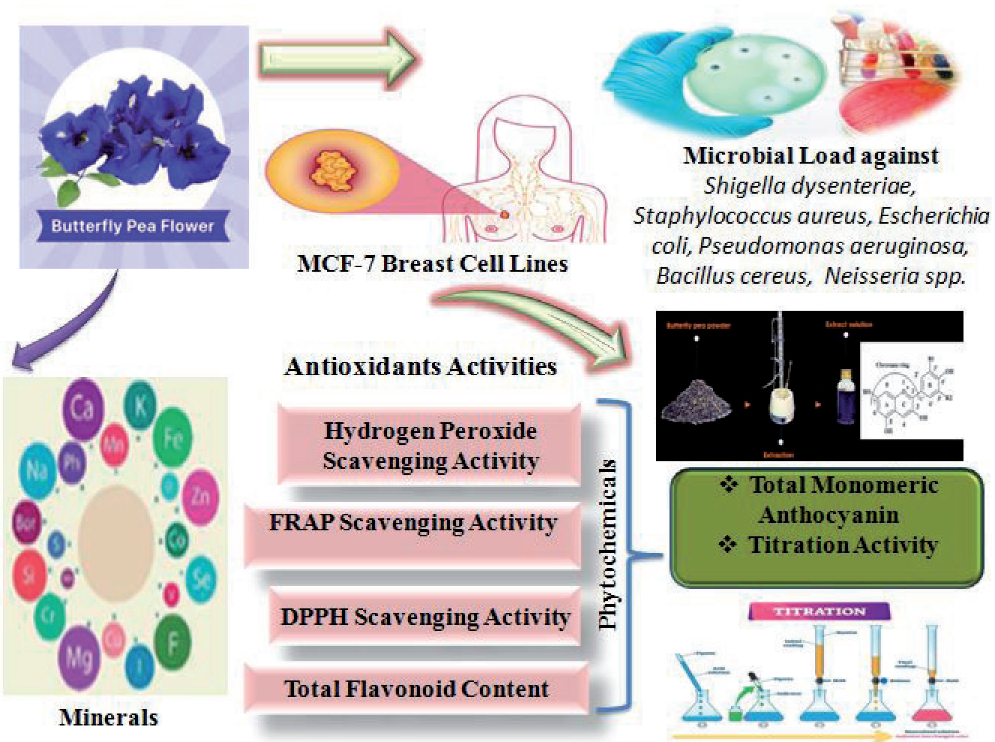
to the spectrophotometric assay. The hydrogen peroxide scavenging activity value of the ethanolic blue pea extract reported IC_{50} = 25.37%. The results of the FRAP scavenging activity were expressed as IC_{50} (%) and mg TE/L to reach IC_{50} = 5.44% and 15.28 mg TE/L. The ethanolic extract was the most effective in the DPPH scavenging activity (EE) (IC_{50} = 14.11 mg/mL), followed by ME (IC_{50} = 12.25 mg/mL), and WE (IC_{50} = 6.77 mg/mL). EE and WE reported the lowest values for total flavonoid contents (3.75 mg QEg^{-1} and 2.49 mg QEg^{-1}, respectively). The antibacterial analysis reported the highest value, 25.14 mm, for *Escherichia coli*, followed by 20.87 mm for *Bacillus cereus*. The results for the cytotoxic impacts on Michigan Cancer Foundation-7 (MCF-7) breast cell lines demonstrated that the highest concentrations (500 µg/mL and 1000 µg/mL) showed significant cytotoxicity activities, lowering viability to <15%. Pea flowers can be considered as a food additive in acidic or even neutral foods. The high nutrient (anthocyanin and flavonoids) levels in blue pea flowers tend to increase their functional properties, such as antioxidant, antibacterial, and anticancer activities.

Keywords: blue pea, butterfly flower, *Clitoria Ternatea*, cytotoxic impacts, microbial load, oxidation

Graphical abstract



Introduction

Herbal extracts have attracted significant attention in cancer treatment and therapeutic research as natural remedies. *Clitoria ternatea* L. belongs to the Fabaceae legume family and is normally well-known as Asian pigeonwings, bluebellvine, butterfly pea, or blue pea flower. In beverages such as herbal teas and drinks, blue pea flowers are often used as a beneficial addition and natural colorant [1]. Blue pea flowers are considered miraculous plants and can generally treat different diseases, such as circulatory and nervous system problems; help in the detoxification processes; maintain the human body's resistance; and inhibit anxiety and constipation [2]. Those flowers are used medicinally for insect bites, skin problems, anasarca, amnesia, asthma, ascites, inflammation, hepatopathy, leprosy, hemicrania, and pulmonary tuberculosis [3]. Another recent study by ALshamrani et al. [4] focused on the antiproliferative effects of *Clitoria ternatea* L. ethanolic extracts on colorectal, breast, and medullary thyroid cancer cells. Also, the plant's flowers have numerous antioxidant and anti-diabetic activities. The major nutrients mentioned that increase the functional properties of the flower are anthocyanins (especially ternatins and cyanidin derivatives), flavonoids, and phenolics. The major phytochemical components found in the blue pea flower are resins, glycosides, flavonoids, saponins, and quercetin.

Quercetin can help inhibit inflammation at early stages by boosting the immune system. *Ciltoria* *ternatea* blue tea can be prepared by boiling 5 petals (approximately 1 g) in 250 ml of water [5]. Regular consumption of blue tea is helpful for thin phlegm due to its non-caffeinated properties [6]. Compared to other plants, blue blooms are very effective at providing the body with essential enzymes [7]. *Clitoria ternatea* L. can also be used for food coloring in desserts, edible ices, candy, ice cream, jelly, and beverages due to the presence of anthocyanin compounds, especially delphinidin 3, 3, 5-diglucoside [8]. Blue pea flower has an incredible coloring function as it can be changed due to the acidity; the lower pH≤3.2 gives a red color; pH≥3.2 to 5.2 gives a violet to the blue color; pH≥5.2 to 8.2 gives a light blue color; pH≥8.2 to 10.2 gives a dark green color. Artificial food colors are derived from coal tar, a source of azo compounds, which are harmful [9].

Based on their safety profiles and polarity, the solvents methanol (M), water (W), ethanol (E), glycerol (G), and glycerol/water (GW) were chosen. The best solvents for extracting polar, water-soluble chemicals are glycerol and water, which are suitable for use in food and medicine. Both polar and relatively non-polar substances are efficiently extracted by ethanol, which is also safe to drink. Despite its toxicity, methanol is frequently utilized in research because of its high extraction efficiency. By combining the extraction capabilities of both solvents, the glycerol/water mixture provides a secure and efficient medium for the recovery of a wide variety of bioactive substances [10].

As the demand for safer, more efficient, and multipurpose treatment options grows, research on breast cancer that focuses on cytotoxic, antioxidant, and antibacterial qualities, particularly utilizing natural plant-based sources like blue pea (*Clitoria ternatea* L.), is becoming increasingly significant. The experimental design was arranged to evaluate the impact of solvent type (methanol, water, ethanol, glycerol, and glycerol/water) on the nutritional and medicinal qualities of butterfly blue pea flower extracts. Five bacterial strains were evaluated for their ability to inhibit microbial growth. Also, some photochemical properties (monomeric anthocyanin content, titration activity), minerals (calcium, phosphorus, potassium, sodium, magnesium), and antioxidant activities, such as hydrogen peroxide, FRAP, DPPH, and flavonoid contents of the extracts will be adequately determined. *Clitoria ternatea* L. flower methanolic extract's cytotoxic activities on MCF-7 breast cancer cells were evaluated to focus on the potential uses against cancer.

Materials and Methods

Flowers Collection and Preparation

Ciltoria ternatea blue flowers (200 g) were collected in the rainy season from a public orchard in Taif City, Alshafa, Saudi Arabia, according to the identification by the Department of Biotechnology at Taif University, with no permission for collecting specimens. The flowers were washed with sterile water and prepared according to the previous study by Rusaczonek. The fresh flowers were kept in sterile plastic bags at a temperature of 4°C; the dried flowers were achieved by air-drying until constant weight according to the previous study carried out by Chemat and Khan [11].

Aqueous Extracts Preparation

A variety of extraction techniques were used, particularly for the bioactive components, to produce a number of aqueous extracts. In order to obtain numerous extracts classified as (ME), (WE), (EE), (GE), and (GWE), respectively, stock solutions of flower extracts were made by dissolving 10 g into 100 mL of various solvents, such as methanol (M), water (W), ethanol (E), glycerol (G), and glycerol/water (GW). The solvents were filtered for additional examination after refluxing for an hour [12].

Parameters of Extraction for Reproducibility

Extractions were performed under controlled conditions to ensure reliable, consistent findings. The ratio of solvent to sample was kept at 10:1 (v/w). To encourage consistent solvent penetration and chemical release, each combination was reflux extracted for 60 min at 70°C while being continuously stirred by a magnetic stirrer (IKA C-MAG HS 7, Shanghai, China) running at 300 rpm. All solutions were left to cool after extraction, filtered using Whatman No. 1 filter paper, and kept at 4°C until additional examination [12].

Antibacterial Activity Determination

Shigella dysenteriae, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, and *Neisseria* spp., as food-borne bacterial indicators in Tryptone Soya Agar, were purchased from the Agricultural Culture Collections, Egypt. The agar-well diffusion method was applied to the ethanolic extract for antibacterial activity, with minor modifications [13]. The 6 bacterial indicators were suspended and diluted to reach 1×10^5 CFU/mL. Approximately 100 µL of the suspension was spread onto Tryptone Soya Agar. The plates were incubated at 37°C for two days, and the results were expressed as the zone of inhibition in (mm).

Total Monomeric Anthocyanin Determination

Approximately 5 g of butterfly blue pea flowers were adjusted with buffers to a pH of 4.5: KCl (0.025 M) and Na·3 H_2O (0.40 M). The mixture was kept in the dark for 30 minutes and then absorbed at 515 and 700 nm. Distilled water is used as the blank. The result of the total monomeric anthocyanin content was expressed as mg/L against cyanidin-3-glucoside as a standard [14].

Titration Activity Determination

The titration acidity of butterfly blue pea flowers was evaluated by the electrometric titration with sodium hydroxide (0.1 N) after the addition of buffers to reach a pH of 8.1. The results of the titration acidity content were expressed as g CA/100 mL in citric acid [15].

Minerals Determination

The butterfly blue pea flowers were dissolved in 100 mL of 2% HNO_3. Some individual minerals, such as calcium (Ca), phosphorus (P), potassium (K), sodium (Na), and magnesium (Mg), were detected by using a spectrophotometer and a flame photometer [16].

Antioxidant Activity

Determination of Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity (H_2O_2) was detected according to the described assay by Mukhopadhyay et al. [17]. Approximately 5 g of butterfly blue pea flowers were treated with ferrous $(NH_4)_2SO_4$ at a concentration of 1 mM and H_2O_2 at a concentration of 5 mM. Distilled water was used as the blank. The mixture was kept in the dark at the ambient temperature for 10 minutes and then absorbed at 510 nm. The H_2O_2 results were expressed as IC_{50} (%).

Determination of FRAP Scavenging Activity

FRAP scavenging activity was detected according to the described assay by Young-Jun et al., according to the reaction of ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+}) reductions [18]. Approximately 5 g of butterfly blue pea flowers were extracted in water, and then 7.5 µL of WE was mixed with 142.5 µL of FRAP reagent and 200 µL DMSO in a 96-well plate. DMSO was used as the blank. The mixture was kept in the incubator at 37°C for 30 min and then absorbed at 760 nm. The results of the FRAP scavenging activity were expressed as IC_{50} (%) and mg TE/L.

Determination of DPPH Scavenging Activity

The antioxidant activities of *C. ternapea* flower extract in various solvents of varying polarity were detected by using the stable radical (DPPH). DPPH activity results for the various extracts, (ME), (WE), (EE), (GE), were evaluated according to Qiao et al. [8]. DPPH at a concentration of (0.0062 mM) was mixed with 20 mL of the stock solutions (M), (W), (E), (G), (GW), and 0.04 mL of butterfly blue pea flower extracts, shaken thoroughly. The absorbance was detected at 715 nm, while the results were expressed as IC_{50} (mg/mL).

Determination of Total Flavonoid Content

Approximately 30 µl of (M), (W), (E), (G), (GW) butterfly blue pea flower extracts were diluted in 6 µl of 10% $AlCl_3$, and 6 µl of 1 mol/L CH_3CO_2K [19]. Quercetin was a standard. The absorbance was detected at 415 nm, while the results were expressed as mg QEg^{-1}.

Anticancer Activity of the Methanolic Extracts

Cell Culture

The Human Michigan Cancer Foundation-7 (MCF-7) breast cancer cell line was established from Egypt's VACSERA. The cells were cultivated in RPMI, BRL, 1640 media, and 10% DMEM supplemented with 10% fetal bovine serum (Fisher, Carlsbad, CA, USA) and 1% penicillin-streptomycin. The cells were cultured at 37ºC with 5% CO_2 in an incubator [4]. A 10:1 (v/w) solvent-to-sample ratio, refluxing each sample for 60 min at 70±2ºC while stirring continuously at 300 rpm.

Cell Viability

Cell viability was determined using a mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma, MO, USA). After that, the MCF-7 cells were exposed to different concentrations (0, 10, 25, 50, 100, 500, and 1000 µg/mL) in comparison to the cells treated with the vehicle (same methanol content), whose viability was determined to be 100% for a 24-hour period. Following a 48-hour incubation period with MTT (5 mg/mL), treated MCF-7 cells containing the methanolic leaf extracts ($1×10^5$ cells/mL) were dissolved in 150 µL/well of dimethyl sulfoxide with the blue dye and agitated for 15 minutes in 96-well plates. A microplate reader (HT, BioTek, USA) was used to detect the absorbance at 595 nm following two washings in order to assess the extent of MTT reduction within the cells following the conversion of the yellow dye to purple in the live MCF-7 cells. The concentrations of the methanolic leaf extracts were used to calculate the percentage of cell viability as defined [4].

Statistical Analysis

The data for *C. ternatea* extracts of all experiments were performed in triplicate and statistically analyzed by ANOVA with post-hoc and Duncan's Multiple Range Tests to evaluate the differences at a significance level of $P \leq 0.05$, while the results were presented in triplicate as mean values with standard deviation (±SD).

Results and Discussion

Antibacterial Activity

The laboratory work reported that the *C. ternatea* extract, whether as a tea or as a food additive, was hygienically safe and suitable for consumption. The antibacterial analysis reported the highest value, 25.14 mm, for *Escherichia coli,* followed by 20.87 mm for *Bacillus cereus*, while *Pseudomonas aeruginosa* and *Shigella dysenteriae* reported the lowest values, 12.05 mm and 13.22 mm, respectively (Table 1). The results of the antibacterial activity were linked with Dacullo and Bitacura [20], who reported its function to inhibit Gram-negative bacteria. The dichloromethane and ethyl acetate components in the blue pea flower are responsible for the antibacterial activities. This could be because ethanol effectively extracts the active antibacterial components by solubilizing both polar and somewhat nonpolar chemicals. Ethanol is considered more appropriate for use in food and medicine. The results further emphasize ethanol-based extracts as prospective options for antimicrobial applications produced from butterfly pea flowers and corroborate the idea that solvent polarity is a critical factor in the extraction of antibacterial chemicals [21].

Total Monomeric Anthocyanin Content

The total monomeric anthocyanin (TMA) of blue pea flower was reported as 77.31 mg/L cyanidin-3-glucoside according to the spectrophotometric assay (Table 2). Acar et al. [22] reported that using a high temperature of 90ºC for more than 20 min can increase TMA content. Another study reported that the increase in TMA contents can be due to the interaction of substances with the solvent, which can allow the migration of phenol components into water. The decrease in TMA contents can happen at a lower temperature of 80ºC due to pigment degradation [23].

Table 1. Antibacterial activity of the ethanolic extract of butterfly blue pea flowers.

Antibacterial Activity Evaluation	Zone of Inhibition (mm)
Shigella dysenteriae	13.22±0.090
Staphylococcus aureus	16.55±0.025
Escherichia coli	25.14±0.012
Pseudomonas aeruginosa	12.05±0.046
Bacillus cereus	20.87±0.023
Neisseria spp.	16.25±0.066

*Each value presents as mean±standard deviation, (n = 3).

Table 2. Phytochemical analysis of the ethanolic extract of butterfly blue pea flowers.

Phytochemical Analysis	Value
Titration Activity	0.018±0.002 g CA/100 mL
Total Monomeric Anthocyanin	77.31±0.070 mg/L
Hydrogen Peroxide Scavenging Activity	25.37±0.090 IC_{50} (%)

*Each value presents as mean±standard deviation, (n = 3).

Titration Activity

The color indexes, such as (L^*, a^*, and b^*), of blue flower extracts can be varied according to several factors, such as temperatures, flower variety, particle sizes, times, acidity (pH), and titration acidity. The current work detected the titration acidity to be 0.018 g CA/100 mL after the addition of buffers to reach a pH of 8.1 (Table 2). The increase in temperature can lead to a decrease in titration acidity values, as the same results were obtained for acacia, squash blossom, lilac, pumpkin flower, clove, and bud teas. The pigments obtained for the blue tea extracts were used as pH indicators in the acid-base titration assay [24].

Mineral Contents

The mineral contents in the blue pea flower varied in descending order which reported that K was the highest (7.25 g/Kg), followed by Ca (5.23 g/Kg), Mg (5.12 g/Kg), P (2.15 g/Kg), and Na (1.01 g/Kg), Fig. 1. Potassium helps in decreasing blood pressure [10]. Calcium helps lymphocytes in mining Ca^{2+} at a low level, adjusting the immune responses (Vig and Kinet, 2009). Phosphorus is a significant element of the metabolic pathways for the thyroid glands and hormones. Sodium is essential for blood clotting, while magnesium can be used in a lot of essential processes such as protein synthesis, cellular energy production, cell growth, and nucleic acid synthesis [25].

Hydrogen Peroxide Scavenging Activity

The H_2O_2 value of the ethanolic blue pea extract reported IC_{50} = 25.37% which is considered an excellent value compared to other types of tea (Table 2). Jayanti et al. [26] reported that the concentrations of the blue tea extracts can depend on H_2O_2 values. H_2O_2 activity is a vital molecule (not toxic), but it can convert to some toxic radicals, such as (OH) by Fenton reactions or (acid) by myeloperoxidase enzyme [26].

FRAP Scavenging Activity

Antioxidant activities were measured using various methods. FRAP can evaluate the reductive ability

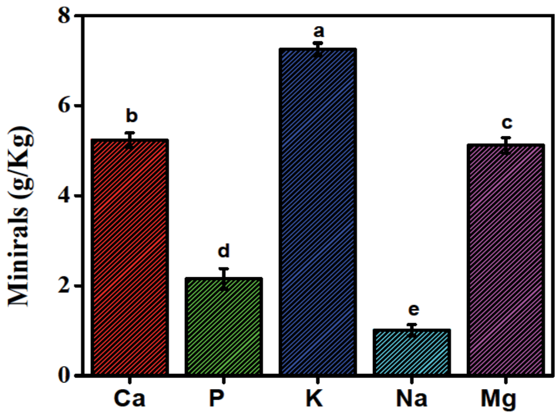


Fig. 1. Mineral contents in blue pea flower.

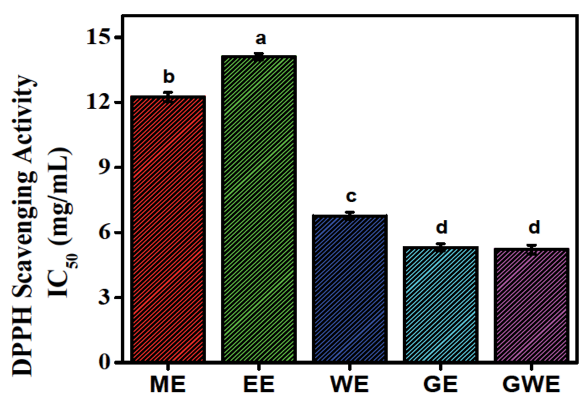


Fig. 3. DPPH scavenging activity in blue pea flower.

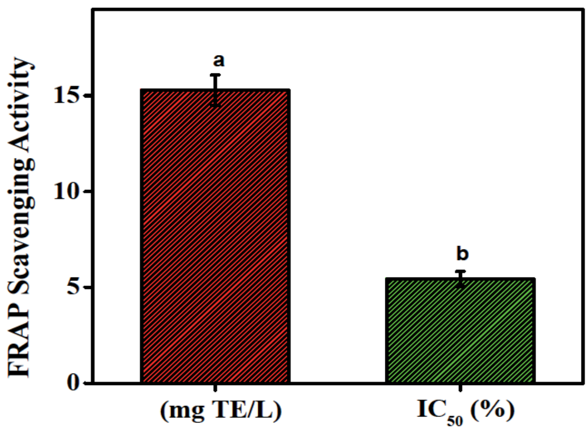


Fig. 2. FRAP scavenging activity in blue pea flower.

of the antioxidants. According to Fig. 2, the results of the FRAP scavenging activity were expressed as IC_{50} (%) and mg TE/L to reach IC_{50} = 5.44% and 15.28 mg TE/L. Chang et al. [27] reported that the increase in FRAP activity values can be due to an increase in temperature and the time of blue tea samples. The bioactive components in the blue tea became more readily available upon heating to 90ºC for more than 20 min [22]. The results for the FRAP activity were in line with the previous study by Safitri et al. [28]. Higher FRAP activity has been detected by using young leaves for preparing herbal blue tea, while lower FRAP values have been detected by using mature leaves and old leaves [29].

DPPH Scavenging Activity

The butterfly pea flowers contain antioxidants in the corolla of their flowers. DPPH activity values of the butterfly pea flowers are presented in Fig. 3. DPPH values were expressed (IC_{50} (mg/mL)). The ethanolic extract was the most effective in the DPPH scavenging activity (EE) (IC_{50} = 14.11 mg/mL), followed by ME (IC_{50} = 12.25 mg/mL), and WE (IC_{50} = 6.77 mg/mL). followed by ME (IC_{50} = 12.25 mg/mL), and WE (IC_{50} = 6.77 mg/mL). GE and GWE reported similar values (IC_{50} = 5.31 mg/mL and IC_{50} = 5.22 mg/mL), respectively. The results were in agreement with Zakaria [30], who detected DPPH assay on blue pea flowers of various solvents. Juswardi et al. [31] reported that the values of the DPPH activity can vary according to the flowering phases of the butterfly pea flowers; the highest value was detected in the blooming flower at 6.58 ppm compared to the bud phase (2.55 ppm) and withered phase (1.74 ppm). Fresh butterfly flowers contain tannin, saponins, triterpenoids, phenol, flavonoids, flavonol glycoside, alkaloids, anthraquinone, anthocyanin, glycoside, and steroids. Antioxidants are helpful as they may act as anthelmintic, antiparasitic, antimicrobial, and insecticidal agents [31]. Chayaratanasin et al. [32] reported that the concentration of DPPH activity values may be influenced by the temperature and the time duration for extraction.

Total Flavonoid Contents

TFC of *C. ternapea* flower extract in various solvents was detected, and the results were expressed in mg QEg^{-1}. GWE value was found to be the highest (5.77 mg QEg^{-1}), followed by GE (5.58 mg QEg^{-1}), and ME (5.25 mg QEg^{-1}), Fig. 4. EE and WE reported lower values (3.75 mg QEg^{-1} and 2.49 mg QEg^{-1}), respectively. Since water provided a lower flavonoid concentration (2.49 mg QEg^{-1}) than ethanol, it was less successful in extracting flavonoids. The results were consistent with those of Escher et al. [33], who detected TFC in blue pea flowers using various solvents. Different results can be due to the polar structure of flavanols [34]. Both GWE and GE are polar, so they have better recovery rates when compared to other solvents [35]. The deep blue color of blue pea flowers reflects the presence of flavonoid antioxidants [31]. Flavonoids may neutralize free radicals by preventing cellular damage [36]. Al-snafi [2] reported the use of *C. ternapea* flowers in medication for high fever, reducing pain, lowering blood sugar, treating Alzheimer's disease, and treating ulcers, in addition to enhancing the immune system and healing wounds.

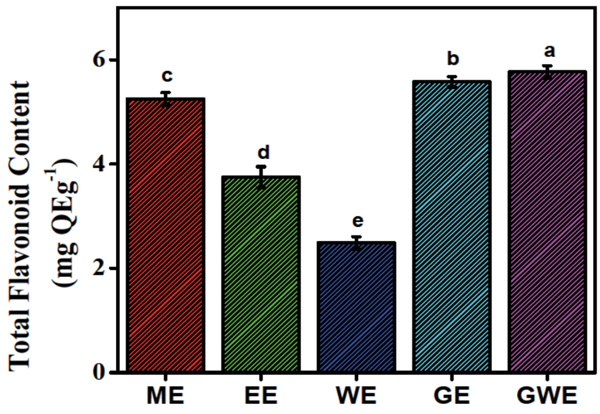


Fig. 4. Total flavonoid content in blue pea flower.

Cytotoxicity on MCF-7 Breast Cancer Cells

The MTT test method was used to evaluate the cytotoxicity of samples by observing the color change of the MTT reagent, which was reduced in response to active cells that had metabolized the reagent through succinate dehydrogenase in the mitochondria. Approximately 10% DMSO must be added to stop the reaction and dissolve the formazan crystals. The formazan product that is produced is a sign of living cells. This study assessed the methanolic extracts for *C. ternapea* flowers' potential for toxicity in the MCF-7 breast cancer cell lines. Different dosages of methanolic extract for flowers (10, 25, 50, 100, 250, 500, and 1000 µg/mL) were applied for 24 hours in a 96-well plate. MCF-7 breast cancer cells were shown to be cytotoxically affected by the methanol extracts of *C. ternatea L.* flower, as indicated by a reduction in cell viability. Furthermore, as shown in Table 3, the different extracts decreased the MCF-7 cell line's viability in a concentration-dependent way, reaching 93.41%, 82.47%, 68.83%, 46.19%, 28.41%, 12.40%, and 4.77%, respectively. The outcomes were compared to control cells. The results demonstrated that the highest concentrations (500 µg/mL and 1000 µg/mL) showed significant cytotoxicity, lowering viability to <15%. At the higher concentration, viability decreased from 12.40% to 4.77%, suggesting a more potent cytotoxic effect. This effect was probably caused by bioactive substances in the fraction that used particular molecular groups to target the cancer cells [37]. It was also discovered that *C. ternatea* flowers exhibited cytotoxic activity, with a particular preference for the breast cancer cell lines MCF-7 and MDA-MB-231 [38]. As a result, *C. ternatea*'s cytotoxic and anticancer qualities demonstrated a significant bias toward cancer cells as opposed to healthy cells. There have been several proposals to use *C. ternatea* as an anticancer medication due to the extracts' possible cytotoxicity. Its complex phytochemical composition, which includes flavonoids, anthocyanins (such as ternatins), and phenolic acids, may be connected to the cytotoxicity of extracts from *Clitoria ternatea* L. These substances have been shown to interfere with cancer cell signaling pathways, cause apoptosis, and impair mitochondrial function. While flavonoids can encourage oxidative stress in cancer cells, which results in cell death, anthocyanins may also stop the cell cycle. Together, these activities point to a potential mechanism by which the extracts cause cytotoxicity, especially in MCF-7 breast cancer cells [38]. It has anti-inflammatory properties that help reduce inflammation and promote general health, as well as antioxidant properties that neutralize free radicals and lessen oxidative stress [39].

Table 3. Cytotoxic activity of the methanolic leaf extracts on breast cancer cells (MCF-7).

Concentration (µg/mL)	Cell Viability (%)
10	93.41±2.02
25	82.47±2.51
50	68.83±1.91
100	46.19±2.85
250	28.41±3.14
500	12.40±2.61
1000	4.77±1.77

*Each value presents as mean±standard deviation, (n = 3).

Oxidative stress is used in cytotoxic breast cancer treatments to kill cancer cells. The blue pea flower, which is high in anthocyanins and flavonoids, is a source of natural antioxidants that may help protect healthy cells and reduce adverse effects [30, 39]. The effects of these antioxidants during chemotherapy or radiation therapy are yet unknown, though, as they may also protect cancer cells from harm brought on by treatment. Blue pea blossom is probably safe to eat as part of a balanced diet under medical supervision.

Conclusions

Food additives are chemical substances that can be added to foods to enhance flavor, taste, and storage period. There are many disadvantages to these chemical substances, including long-term harm to human health. Using a natural food additive, such as *Clitoria ternatea* L. flower, is cheap and safe for health due to its nutritional content. Blue flowers can be used in desserts, edible ice, candies, ice cream, jelly, and beverages. The current data indicate that blue pea flowers may be recommended for medication and pharmacological uses. Anthocyanins and the high antioxidant activity of blue pea flowers have been used in the food industry for intelligent packaging. The antioxidant properties of *C. ternatea* have recently been reported, making the plant a potential treatment for cancer. Even though these in vitro antioxidant results provide important information about the antioxidant potential of blue pea flower extracts, it is crucial to remember that they are preliminary and that additional validation through *in vivo* studies and mechanistic evaluations is required to confirm their biological relevance and application. *Clitoria ternatea* has strong antioxidant, antibacterial, and cytotoxic properties due to the bioactive components such as anthocyanins, flavonoids, and phenolics. The flower is a useful natural food ingredient and a potential therapeutic agent due to its bioactive chemicals, which also have health benefits. Additional evaluations and mostly anthropological research are needed for blue pea flowers.

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Conflict of Interest

The authors declared no conflict of interest.

Author's Contribution

Research concept and design: MA; RS; SAA; AMA; HAA. Collection and assembly of data: RS; RIB; AMA; SMA. Data analysis and interpretation: RHK; AMB; SHQ; AMAB Writing the article: RS; RHK; FA; AMI; AE; SAA; AMA. Critical revision of the article: MA; RAJ; MHA; NHJA, RS; SAA; AMB; SHQ. Final approval of the article: RS; SHQ, RIB; AMA; HAA.

References

1. RAHAYU S., MUHTADI M., INDRAYUDHA P. Apoptosis induction activity and inhibition of MCF-7 cell proliferation in active isolate of butterfly pea flower (*Clitoria ternatea* L.). Trends in Sciences. **20** (9), 5518, **2023**.
2. AL-SNAFI A.E. Pharmacological importance of Clitoria ternatea – A review. IOSR Journal of Pharmacy. **6** (3), 68, **2016**.
3. INDRIANINGSIH A.W., WULANJATI M.P., WINDARSIH A., BHATTACHARJYA D.K., SUZUKI T., KATAYAMA T. In vitro studies of antioxidant, antidiabetic, and antibacterial activities of Theobroma cacao, Anonna muricata and Clitoria ternatea. Biocatalysis and Agricultural Biotechnology. **33**, 101995, **2021**.
4. ALSHAMRANI S.M., SAFHI F.A., MOBASHER M.A., SALEEM R.M., ALHARTHI A., ALSHAYA D.S., AWAD N.S. Antiproliferative Effect of Clitoria ternatea Ethanolic Extract against Colorectal, Breast, and Medullary Thyroid Cancer Cell Lines. Separations. **9** (11), 331, **2022**.
5. RAO A.S., KL S., D'ALMEIDA M.P., RAI K.S. In vitro antimicrobial activity of root extract of Clitoria ternatea. Asian Journal of Pharmaceutical and Clinical Research. **10** (11), 52, **2017**.
6. TRILOKCHANDRAN B., VIJAYAKUMAR G., THIPPAREDDY K.S. Formulation and evaluation of cosmetic cream from cabbage extract. Research Journal of Pharmacy and Technology. **12** (8), 3589, **2019**.
7. SEKAR M., SIVALINGGAM P., MAHMAD A. Formulation and evaluation of novel antiaging cream containing rambutan fruits extract. International Journal of Pharmaceutical Sciences and Research. **8** (3), 1056, **2017**.
8. QIAO G.H., WENXIN D., ZHIGANG X., SAMI R., KHOJAH E., AMANULLAH S. Antioxidant and anti-inflammatory capacities of pepper tissues. Italian Journal of Food Science. **32** (2), **2020**.
9. CHAYARATANASIN P., BARBIERI M.A., SUANPAIRINTR N., ADISAKWATTANA S. Inhibitory effect of Clitoria ternatea flower petal extract on fructose-induced protein glycation and oxidation-dependent damages to albumin in vitro. BMC Complementary and Alternative Medicine. **15**, 1, **2015**.
10. SAMI R., ALSHEHRY G., ELGARNI E., HELAL M. Saudi community care awareness food facts, nutrients, immune system and COVID-19 prevention in taif city among different age categories. African journal of food, agriculture, nutrition and development. **21** (1), 17213, **2021**.
11. CHEMAT F., ZILL E.H., KHAN M.K. Applications of ultrasound in food technology: Processing, preservation and extraction. Ultrasonics Sonochemistry. **18** (4), 813, **2011**.
12. SAMI R., KHOJAH E., ALHARBI M., AM AL-MUSHHIN A., SAEED ALKALTHAM M., MOHAMMAD SALAMATULLAH A., AHMED ISMAIL K., HELAL M. Functional effects of pomegranate peel extracts on milk: Antibacterial measurements, antioxidant activities, and photochemical characterizations. Journal of Biobased Materials and Bioenergy. **15** (5), 571, **2021**.
13. GABER A., HASSAN M.M., DESSOKY E.-D.S., ATTIA A.O. In vitro antimicrobial comparison of Taif and Egyptian pomegranate peels and seeds extracts. Journal of Applied Biology and Biotechnology. **3** (2), 012, **2015**.
14. GOMEZ S., PATHROSE B., KURUVILA B. Comparative evaluation of anthocyanin pigment yield and its attributes from Butterfly pea (*Clitorea ternatea* L.) flowers as prospective food colorant using different extraction methods. Future Foods. **6**, 100199, **2022**.
15. ROKAYYA S., JIA F., LI Y., NIE X., XU J., HAN R., YU H., AMANULLAH S., ALMATRAFI M. M., HELAL M. Application of nano-titanum dioxide coating on fresh Highbush blueberries shelf life stored under ambient temperature. LWT. **137**, 110422, **2021**.
16. SAMI R., ALSHEHRY G., MA Y., ABDELAZEZ A., BENAJIBA N. Evaluation of some specific components existences in okra (*Abelmoschus esculentus* L.(moench)) cultivated from different areas. Journal of Food and Nutrition Research. **7** (2), 155, **2019**.
17. MUKHOPADHYAY D., DASGUPTA P., ROY D.S., PALCHOUDHURI S., CHATTERJEE I., ALI S.,

DASTIDAR S.G. A sensitive in vitro spectrophotometric hydrogen peroxide scavenging assay using 1, 10-phenanthroline. Free Radicals and Antioxidants. **6** (1), 124, **2016**.

18. LEE Y.-J., KIM D.-B., LEE J.S., CHO J.-H., KIM B.K., CHOI H.-S., LEE B.-Y., LEE O.-H. Antioxidant activity and anti-adipogenic effects of wild herbs mainly cultivated in Korea. Molecules. **18** (10), 12937, **2013**.
19. ROKAYYA S., LI C.-J., ZHAO Y., LI Y., SUN C.-H. Cabbage (*Brassica oleracea* L. var. capitata) phytochemicals with antioxidant and anti-inflammatory potential. Asian Pacific Journal of Cancer Prevention. **14** (11), 6657, **2013**.
20. DACULLO R.M.A., BITACURA J.G. In Vitro Antibacterial, Anticoagulant, and Antioxidant Screening of Aqueous Extracts of Blue Ternate (*Clitoria ternatea* L.) Flower. Herbal Medicines Journal. **7** (4), 123, **2022**.
21. MUKHERJEE P.K., KUMAR V., KUMAR N.S., HEINRICH M. The Ayurvedic medicine Clitoria ternatea – From traditional use to scientific assessment. Journal of Ethnopharmacology. **120** (3), 291, **2008**.
22. ACAR A., AYDıN M., ARSLAN D. Development of infusion tea formulations with food wastes: Evaluation of temperature and time effects on quality parameters. Applied Food Research. **2** (1), 100087, **2022**.
23. MEHMOOD A., ISHAQ M., ZHAO L., YAQOOB S., SAFDAR B., NADEEM M., MUNIR M., WANG C. Impact of ultrasound and conventional extraction techniques on bioactive compounds and biological activities of blue butterfly pea flower (*Clitoria ternatea* L.). Ultrasonics Sonochemistry. **51**, 12, **2019**.
24. DINÇER C., TONGUR T., ERKAYMAZ T. Investigation of the effect of different extraction methods on the quality properties of hibiscus extracts. GIDA - Journal of Food. **45** (3), 409, **2020**.
25. ZHANG M., ZHANG Q., ZHAO W., CHEN X., ZHANG Y. The mechanism of blood coagulation induced by sodium dehydroacetate via the regulation of the mTOR/ERK pathway in rats. Toxicology Letters. **392**, 1, **2024**.
26. JAYANTI M., ULFA A.M., YASIR A.S. The Formulation and Physical Evaluation Tests of Ethanol in Telang Flower (*Clitoria ternatea* L.) Extract Losio Form as Antiox idant. Biomedical journal of Indonesia. **7** (3), 488, **2021**.
27. CHANG M.-Y., LIN Y.-Y., CHANG Y.-C., HUANG W.-Y., LIN W.-S., CHEN C.-Y., HUANG S.-L., LIN Y.-S. Effects of infusion and storage on antioxidant activity and total phenolic content of black tea. Applied Sciences. **10** (8), 2685, **2020**.
28. SAFITRI F.W., AHWAN A., QONITAH F. Uji aktivitas antioksidan ekstrak etanol daun adas (*Foeniculum vulgare* Mill) dengan metode DPPH dan FRAP. **2020**.
29. DIAN-NASHIELA F., NORIHAM A., NOORAAIN H., AZIZAH A.H. Antioxidant activity of herbal tea prepared from Cosmos caudatus leaves at different maturity stages. International Food Research Journal. **22** (3), 1189, **2015**.
30. ZAKARIA N.N.A., OKELLO E.J., HOWES M.J., BIRCH-MACHIN M.A., BOWMAN A. In vitro protective effects of an aqueous extract of *Clitoria ternatea* L. flower against hydrogen peroxide-induced cytotoxicity and UV-induced mtDNA damage in human keratinocytes. Phytotherapy Research. **32** (6), 1064, **2018**.
31. JUSWARDI J., YULIANA R., TANZERINA N., HARMIDA H., AMINASIH N. Anthocyanin, Antioxidant and Metabolite Content of Butterfly Pea Flower (*Clitoria ternatea* L.) Based on Flowering Phase. Journal Pembelajaran dan Biologi Nukleus. **9** (2), 349, **2023**.
32. CAHYANINGSIH E., YUDA P.E.S.K., SANTOSO P. Skrining fitokimia dan uji aktivitas antioksidan ekstrak etanol bunga telang (*Clitoria ternatea* L.) dengan metode spektrofotometri uv-Vis. Jurnal Ilmiah Medicamento. **5** (1), 51, **2019**.
33. ESCHER G.B., MARQUES M.B., DO CARMO M.A.V., AZEVEDO L., FURTADO M.M., SANT'ANA A.S., DA SILVA M.C., GENOVESE M.I., WEN M., ZHANG L., OH W.Y., SHAHIDI F., ROSSO N.D., GRANATO D. *Clitoria ternatea* L. petal bioactive compounds display antioxidant, antihemolytic and antihypertensive effects, inhibit α-amylase and α-glucosidase activities and reduce human LDL cholesterol and DNA induced oxidation. Food Research International. **128**, 108763, **2020**.
34. JAAFAR N.F., RAMLI M.E., SALLEH R.M. Optimum extraction condition of Clitorea ternatea flower on antioxidant activities, total phenolic, total flavonoid and total anthocyanin contents. Tropical Life Sciences Research. **31** (2), 1, **2020**.
35. HUAMÁN-CASTILLA N.L., MARIOTTI-CELIS M.S., MARTÍNEZ-CIFUENTES M., PÉREZ-CORREA J.R. Glycerol as alternative co-solvent for water extraction of polyphenols from Carménère pomace: Hot pressurized liquid extraction and computational chemistry calculations. Biomolecules. **10** (3), 474, **2020**.
36. ELHAKEM A.H., BENAJIBA N., KOKO M.Y., KHOJAH E., ROK A. DPPH, FRAP and TAEC Assays with Postharvest Cabbage (*Brassica oleracea*) Parameters During the Packaging Process. Pakistan Journal of Agricultural Sciences. **24** (2), 182, **2021**.
37. ROLLANDO R., AMELIA M.A., AFTHONI M.H., PRILIANTI K.R. Potential cytotoxic activity of methanol extract, ethyl acetate, and n-hexane fraction from *Clitoria ternatea* L. on MCF-7 breast cancer cell line and molecular docking study to P53. Journal of Pure and Applied Chemistry. **12** (1), 7, **2023**.
38. AKTER R., UDDIN S.J., GRICE I.D., TIRALONGO E. Cytotoxic activity screening of Bangladeshi medicinal plant extracts. Journal of Natural Medicines. **68** (1), 246, **2014**.
39. OGUIS G.K., GILDING E.K., JACKSON M.A., CRAIK D.J. Butterfly pea (*Clitoria ternatea*), a cyclotide-bearing plant with applications in agriculture and medicine. Frontiers in Plant Science. **10** (645), 1, **2019**.