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Original Research

Studies of the Biochemistry Behavior of Herbal Butterfly Pea Blue Pea Flower with some Antimicrobial and Antioxidant Potentials

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Abstract

Butterfly pea flowers are commonly used in food manufacturing as coloring agents. The current research aimed to study some phytochemical, antimicrobial, and antioxidant potentials for their extracts. Several assays have been applied to prepare five extracts coded as ME, WE, EE, GE, and GWE for methanol, water, ethanol, glycerol, and glycerol/water, especially for phenolic determination. At the same time, ultrasound processing has been achieved for both fresh and dried flowers, especially for the anthocyanin determination. The antibacterial potential for *Staphylococcus aureus* reported a 1.2 mm zone of inhibition. *C. ternatea* flower extract reported 6.4 and 10.29ºBrix for acidity and total soluble sugar, respectively. The *C. ternatea* flower extract had a transparent, dark, and more opaque blue color due to a higher *L** value (29.52) than lower *a** and *b** values (2.5 and 40.32-), respectively. *C. ternatea* flower extract exhibited a high total antioxidant capacity (59.11%), while ORAC reported (109.22 mg TE/L). ABTS results were IC_{50} = 35.27 µL/mL and 189.68 mg TE/L. The total phenolic contents presented various results on a blue color, GE had the deepest blue color and reported (18.76 mg $GAEg^{-1}$), followed by EE (18.55 mg $GAEg^{-1}$), GWE (18.53 mg $GAEg^{-1}$), and ME

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(15.17 mg GAEg^{-1}). WE had the lightest blue color and reported the lowest value (9.79 mg GAEg^{-1}). Four extractions were prepared for the total anthocyanin contents, which showed the efficacy of the ultrasound process to get the highest anthocyanin content of 4.21 mg Cyanidin-3-glucoside/g on fresh petals. Butterfly pea flowers might be used safely in food, beverage, and cosmeceutical products.

Keywords: *Clitoria ternatea*, antibacterial agent, antioxidants, phenols, anthocyanin

Introduction

The butterfly blue pea flower is considered one of the medical plants with ethnic medicinal uses. *Clitoria ternatea* is widely grown within the tropical belt, while some species are grown in temperate areas as ornamentals, fodders, or even medicinal plants [1]. It contains anthocyanins alkaloid, tannin, resin, steroid, saponin, phenol, ternatins, and flavonol glycoside [2]. These variable components can be used efficiently in the pharmaceutical industries, cosmetics, and drugs due to their huge role in healthcare [3]. The flowers are recommended for the curing of dangerous bites such as snakes and scorpions, as well as for boosting memory, improving the intellect, inhibiting platelet aggregation, and controlling some neurological disorders [4]. It has excellent antioxidant, antidiabetic, anti-obesity, anti-inflammation, anticancer, antihyperlipidemic, and anti-asthma properties. Parimaladevi et al. [5] reported the presence of some anti-fungal proteins that were homologous to the defensin plants. Several techniques have been applied for the bioactive extractions of fresh or dried blue pea flowers, especially brewing and boiling them for daily use [6]. Those bioactive components can be influenced by those techniques, especially anthocyanin, which is affected by temperature, according to several studies [7, 8]. Several anthocyanin types have been detected in the blue butterfly flowers, such as ternatins A3, (C1-C5), (B2-B4), and (D2-D3) [9]. Antioxidants can slow down lipoxygenase, chelate metals, lipid peroxidation, singlet oxygen quenching, and free radical formation to defend the body from a lot of serious diseases [10]. Heydari et al. [11] reported the negative effect of the dried flower intake against the bioactive compounds and the antioxidant activities. Marpaung et al. [12] confirmed that the color indicators of the blue flower extracts, such as intensity, violet, and browning indexes, can be varied according to the extraction techniques. Parimaladevi et al. [5] reported that changes in antioxidant values can be influenced by various extract solvents; DPPH activity was the highest with the ethanolic extract of the flower. Jain et al. [9] noted that the methanolic extracts had higher values compared to acetone extracts for the free radical scavenging activities.

The current research was arranged on the various extracts and preparation assays on butterfly blue pea flower with evaluating the antibacterial potential against Staphylococcus aureus, some phytochemical properties such as (acidity, total soluble sugar, color index), total antioxidant capacity, oxygen radical absorbance capacities (ORAC), ABTS scavenging activity with phenolic and anthocyanin contents.

Experimental

Flower Materials

The pea flowers (*C. ternatea*) were collected from a private farm in Taif, KSA. The petal parts were divided from the sepals. The flowers were separated into three sets. The fresh ones were preserved in a refrigerator at 4ºC. The dried ones were achieved by air drying at an ambient temperature of 25ºC and then were preserved at -80ºC for advanced analysis. In contrast, the ultrasound processing at 40ºC for 60 min has been achieved for both fresh and dried flowers, especially for the anthocyanin determination.

Preparation of *Clitoria Ternatea* Extracts

Several assays have been applied to prepare five extracts coded as ME, WE, EE, GE, and GWE for methanol, water, ethanol, glycerol, and glycerol/water, especially for phenolic determination. Solvents were prepared according to the previous study by Sami et al. [13]. Fig. 1. shows the experimental work processes of *Clitoria ternatea* extracts.

Microbial Determination

Staphylococcus aureus is well-known as a ubiquitous communal bacterium on the skin and anterior nare, while it can cause infection in the case of the bacterial generation increase [14]. The antimicrobial potential determination was detected for *C. ternatea* flower against Gram-positive *Staphylococcus aureus* as a food-borne bacterial on blood agar, then Tryptone Soya, which was purchased from the cell bank, Jeddah, Saudi Arabia. *Staphylococcus aureus* bacteria was suspended and diluted, then incubated at 37ºC for 24 h. The result for the antimicrobial potential determination of *Staphylococcus aureus* bacteria was expressed as the zone of inhibition in (mm).

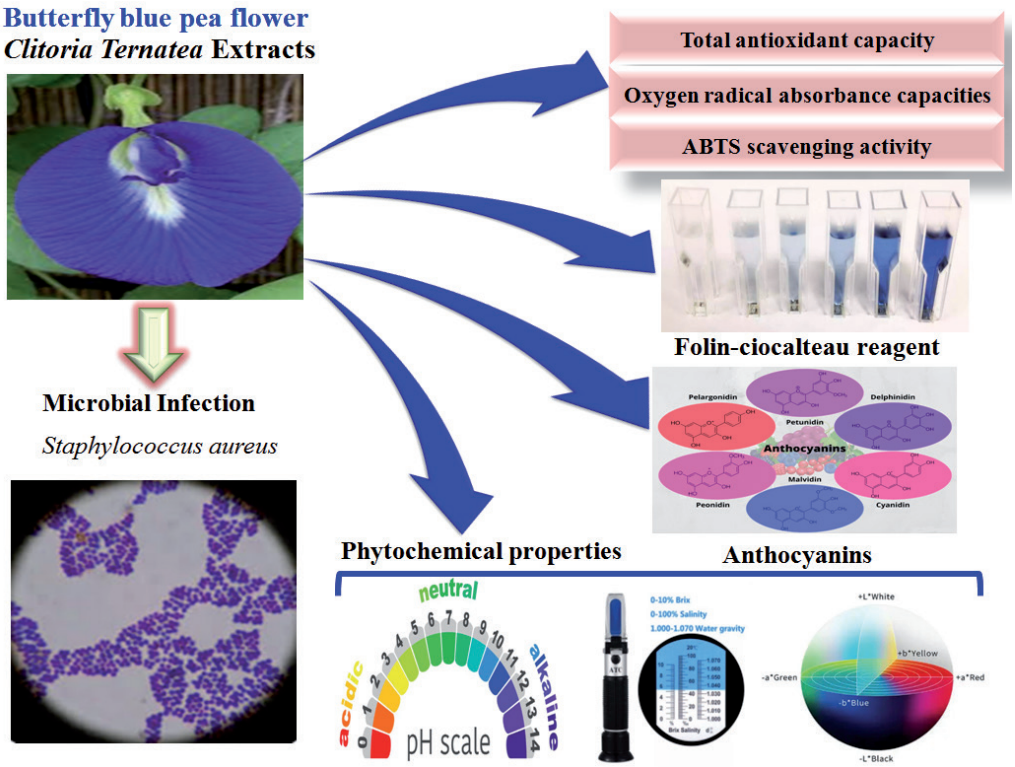

Fig.1. The summary of the experimental work on *Clitoria ternatea* extracts.

Determination of some Phytochemical Properties

Acidity Determination

pH is the quantity of acidity in liquids by using a digital pH meter (LPV2500.97.0002, PH1, Spain) at the ambient temperature, which equals the activity Log (-) of the (H) ions. pH determination may help for prolonging the shelf-life of liquids and it also can control the customer's acceptability [15]. High acidic values may cause some harmful health effects, such as gastroesophageal reflux diseases and dental [16].

Total Soluble Sugar Determination

Total soluble sugar is a percentage of the presence of sucrose in a sugary *C. ternatea* flower solution [17]. Determination of the total soluble sugar content is essential in beverages to evaluate customer acceptability. The total soluble sugar content can be detected via the gravimetric assay using a hand refractometer (Kyowa, HR-1, Japan) [18].

Color Index Determination

The color index is an essential factor for sensorial evaluation and can influence the selection of the beverage itself [19]. Furthermore, *C. ternatea* flower extracts are affected by acidity due to their anthocyanin contents [20]. The color ranged from purple-blue near pH=7, then it turned to red at pH≤7 pH, while it turned to dark red at pH≤3 and turned to green at pH≥10.5 [21]. The color index profile (L^*, a^*, and b^*) was detected by a Miniscan XE Plus spectrophotometer. The final average was calculated when (n = 3) for various values [22].

Determination of some Antioxidant Potentials

Total Antioxidant Capacity Determination

The antioxidant capacity of *C. ternatea* flower was detected by the phosphomolybdate assay by using the ascorbic acid as a blank [23]. It was determined with the help of a reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4, and four mM $(NH_4)6Mo_7O_{24}$). The results were detected using a spectrophotometer (Helios Zeta, UV-VIS, Thermo Fisher, USA) and were expressed in percentage after absorbance at 695 nm wavelength [24].

Determination of Oxygen Radical Absorbance Capacity

The ORAC was detected according to [25] assay. Approximately 75 mM Phosphate-buffered saline with (pH = 7.4) was mixed with 40 mg/mL 2, 2'-azobis (2-amidino-propane) dihydrochloride, 4.8 µM fluorescein, and 1.5 and 0.75 µg/mL Trolox standard solutions. In comparison, *C. ternatea* flower was dissolved in 2% dimethyl sulfoxide. The mixture

was incubated for 10 mins at 37ºC. The result was absorbed at 494 nm and 535 wavelengths. Results were expressed as mg TE/L.

Determination of ABTS Scavenging Activity

The ABTS scavenging activity was detected by using diammonium salt ABTS+ 2, 2-Azinobis-(3 ethylbenzothiazoline-6-sulfonate) [26]. Approximately 2 µL of *C. ternatea* flower was mixed with 198 µL of ABTS reagent against 200 µL of Trolox solution [27]. The mixtures were incubated for 10 mins at 37ºC. The mixture was absorbed at 745 nm wavelength. The lightening of the colors indicates the ABTS scavenging activity effects. The results were detected using a spectrophotometer and expressed as IC_{50} (µL/mL) and mg TE/L [28].

Determination of Total Phenolic Contents

The total phenolic contents (TPC) of *C. ternatea* flower (5 mL) extracts as (ME), (WE), (EE), (GE), and (GWE) were detected according to the Folin-Ciocalteu assay by Mazzucotelli et al. [29]. Approximately 0.5 mL of the Folin-Ciocalteu reagent was mixed by vortex with 1.5 mL 7.5% Na_2CO_3 and 7.9 ml H_2O against 0.1 mL gallic acid as a standard. The mixtures were diluted to reach a concentration of 10 mg/mL and incubated for 1 hour in a dark area. The mixtures were absorbed using a spectrophotometer at a wavelength of 750 nm, while the results were expressed as gallic acid equivalents (mg $GAEg^{-1}$).

Determination of Total Anthocyanin Contents

The total anthocyanin contents (TAC) were evaluated according to the previous pH-differential assay [30]. Approximately 1 mL of *C. ternatea* extracts was mixed in a beaker glass with KCl buffer (pH= 1.0) and CH_3COONa buffer (pH= 4.5) to reach a volume of 10 mL. The absorbance using a spectrophotometer was detected at 520 nm and 700 nm wavelengths against using distilled water a blank. The results were expressed as mg Cyanidin-3-glucoside/g and calculated according to the protocol described by Ahmad et al. [31].

Statistical Analysis

The data from all replications of the current study with standard deviations have been reported as final results. The significant differences between all means were determined by Duncan's Multiple Range Test ($p \leq 0.05$).

Results and Discussions

Antimicrobial Potential

The result of the antimicrobial potential for *C. ternatea* extract is exposed in Table 1. The antimicrobial potential in the agar media for *Staphylococcus aureus* was detected in a zone of inhibition and reported a 1.2 mm zone of inhibition. The antimicrobial potential was linked with the previous study for several micros, such as *Staphylococcus aureus*, *Enterococcus faecalis*, and *Salmonella typhimurium* [32]. The result was also linked with Dacullo and Bitacura [33], who reported that the function inhibited the antimicrobial potential activity.

Acidity and Total Soluble Solids

Clitoria ternatea is a simply accessible flower the acidity may be influenced by several indicators. As shown in Table 1, the acidity value reported was 6.4, which is linked with Rangel et al. [34], who contributed that the changes in color for the butterfly pea beverages can be influenced by the variation in pH values and high temperature. Based on the research work, *C. ternatea* flower extract reported 10.29ºBrix. The results were in agreement with Featherstone [35], who detected the ºBrix and acid ratios. Heating can cause changes in pH values in several herbal teas, such as acacia and squash blossom, and a reduction in the titration value in pumpkin flower tea. On the other hand, heating can reduce the acidity values in other herbal teas, such as honeysuckle, lilac, and clove [36]. The heating period for the butterfly flower can reduce the pH value and increase the titration value [37, 38]. Also, Adams [39] reported a deep purple color can be detected in sports drinks with lower acidity.

Color Index Profile

The color index is one of the essentially important signs of the customer's acceptance [40]. The *C. ternatea* flower extract had a transparent, dark, and more opaque blue color due to a higher *L** value (29.52) than lower *a** and *b** values (2.5 and 40.32-), respectively (Fig. 2.). Amaolo [41] used *C. ternatea* flower as a low-cost pH indicator. The flavylium cations and quinonoid anion can absorb various wavelengths of light, and hence color gradients are formed, especially with lower pH. The best method for using *C. ternatea* flower as a food additive for a long-term storage period is in a powdered form [42].

Total Antioxidant Capacity and Oxygen Radical Absorbance Capacity

The total antioxidant capacity and ORAC of *C. ternatea* extract were detected by phosphomolybdate assay, and the results were expressed in percentage and

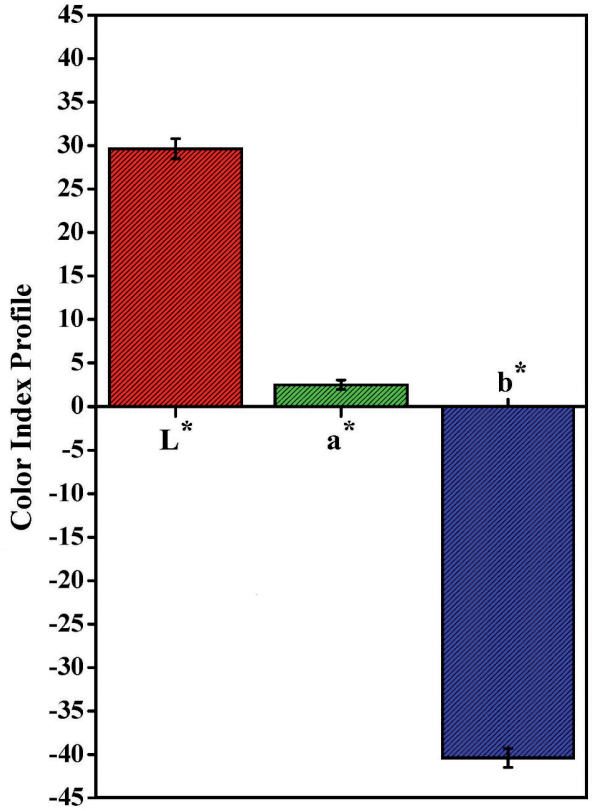

Fig. 2. Color index profile of blue pea flowers

mg TE/L of Trolox, respectively. *C. ternatea* flower extract exhibited a high total antioxidant capacity (59.11%), while ORAC reported (109.22 mg TE/L), Table 1. Rabeta and Nabil [43] reported that the high values of oxidation were due to the contributions of phenolics and oxygen radical absorbance capacities in the *C. ternatea* flower.

ABTS Scavenging Activity

ABTS scavenging activity may determine the free radical diammonium salt of ABTS+ 2, 2-Azinobisn [44]. The results were expressed as IC_{50} (µL/mL) and mg

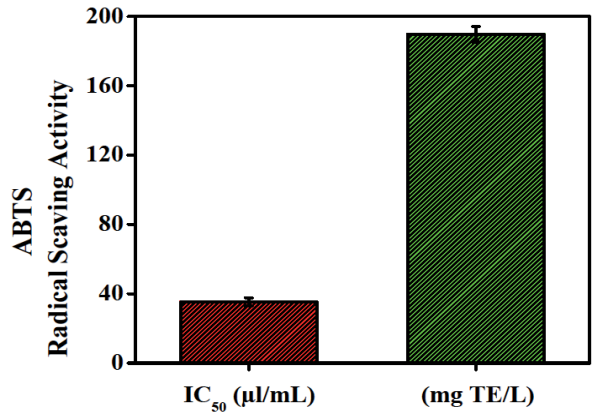

Fig. 3. ABTS radical scavenging activity of blue pea flowers.

TE/L in blue-green solutions with the phosphomolybdate assay. Fig. 3. shows ABTS results as IC_{50} = 35.27 µL/mL and 189.68 mg TE/L. Previous studies reported a relationship between heating temperature and the infusion times of the flower extract with the increase in ABTS scavenging activity [45, 46]. The previous factors, temperature and time, allow the bioactive components to become free due to tissue degradation.

Total Phenolic Contents

The calibration curve of gallic acid represents the oxidation by phosphotungstic and phosphomolybdic acids [47]. TPC different solvents were evaluated, while the data were expressed in mg $GAEg^{-1}$. The experiment presented various results on the blue color. GE had the deepest blue color and reported (18.76 mg $GAEg^{-1}$), followed by EE (18.55 mg $GAEg^{-1}$), GWE (18.53 mg $GAEg^{-1}$), and ME (15.17 mg $GAEg^{-1}$), Fig. 4. WE had the lightest blue color and reported the lowest value (9.79 mg $GAEg^{-1}$). The glycerol extracts had a better ability to extract TPC than the ethanol, methanol, and water due to the polar structure of the phenols [48]. The TPC results were in range with the previous research by Widowati et al. [26], who reported

Table 1. Antioxidant activities, phytochemical analysis, and microbial infection of butterfly pea flowers.

Antioxidant Activities	
Total Antioxidant Capacity	59.11±2.48%
Oxygen Radical Absorbance Capacity	109.22±5.78 mg TE/L
Phytochemical Analysis	
Total Soluble Sugar	10.29±1.24 °Brix
pH	6.40±1.14
Antibacterial Potential	
Staphylococcus aureus	1.20±0.10 Zone of Inhibition (mm)

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

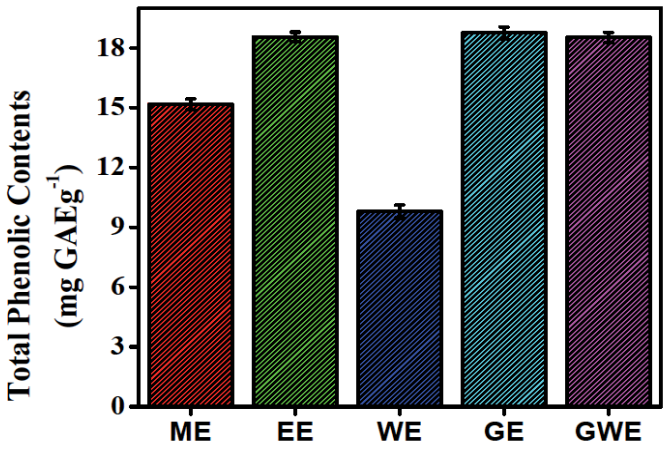

Fig. 4. Total phenolic contents of blue pea flowers.

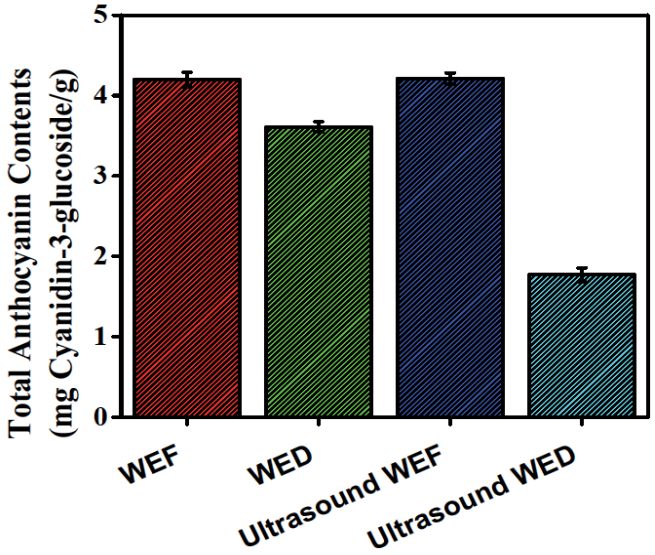

Fig. 5. Total anthocyanin contents of blue pea flowers.

16.20 µg GAE%. Cacace and Mazza [49] reported the migration of phenolic components into water during higher temperatures and infusion time in milled berries tea. The same increase in TPC was reported by (corn tassels, walnut shells, cherry stalks, banana, pomegranate, mandarin, eggplant, and red onion peel) teas [50, 51].

Total Anthocyanin Contents

The anthocyanin content in butterfly peas is extremely high in a delphinidin group with a red-blue color and a high number of hydroxyls, which tend to have high antioxidant activities. Anthocyanins may prevent brain inflammation, blood clogging, and tumor cells, inhibit bacterial growth, control blood sugar, improve vision with analgesic, and exhibit antiasthma, hepatoprotective, anthelmintic, antiparasitic, antiulcer, and anticholesterol activities [52]. Four extractions were prepared for TAC, which showed the efficacy of the ultrasound process to get the highest anthocyanin content as 4.21 mg Cyanidin-3-glucoside/g on fresh petal compared to 1.77 mg Cyanidin-3-glucoside/g for the dry one, Fig. 5. On the other hand, the commercial water extract reported similar value 4.20 mg Cyanidin-3-glucoside/g on fresh petal, while the dry one reported 3.61 mg Cyanidin-3-glucoside/g. Therefore, both procedures were efficient in extracting the TAC in fresh condition, as anthocyanins are well-known as polar substances which are easily soluble in water droplets [53]. The current research was in agreement with Sofyan et al. [54], who applied some treatments for serving the butterfly pea flower drinks. Shen et al. [55] reported that anthocyanin is quite stable during the extraction at high temperatures under both neutral and alkaline conditions. Zor et al. [56] reported a reduction in the TAC at 80ºC due to the degradation of pigment.

Conclusions

C. ternatea is generally used in food and beverage preparations. It gives an acceptable blue tint without artificial food coloring additives. It has antimicrobial activity against *Staphylococcus aureus* bacteria. Glycerol extract had the highest extraction effectiveness for the total phenolic contents. The ultrasound extraction method was the most effective, followed by water extract to get the highest anthocyanin content on fresh petals. It is essential to carry out more research about butterfly pea flowers to use as antioxidants and anti-inflammatory boosters in food and cosmeceutical products.

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Conflict of Interest

The authors declare no conflict of interest.

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