World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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"Silky Blue" antioxidant-rich collagen drink: An innovation from butterfly pea flower extract (*Clitoria ternatea L.*) as anti-aging nutritional supplement

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World Journal of Advanced Research and Reviews, 2025, 27(01), 1811-1815

Publication history: Received on 07 June 2025; revised on 15 July 2025; accepted on 17 July 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.27.1.2678

Abstract

The problem of premature aging often arises among adults. Collagen and antioxidants are compounds believed to be effective in addressing this issue. One of the plants that contains strong antioxidants is the butterfly pea flower. The purpose of this research is to determine the antioxidant capacity of collagen drink from the butterfly pea flower and to know the antibacterial activity of butterfly pea flower extract on *Salmonella typhi* bacteria. To test the antioxidant capacity of this research is to use the DPPH method is used, namely by measuring the antioxidant percentage of the sample against DPPH free radicals. The results of this study were obtained from the equation y = bx + a, and the IC_{50} value was calculated from the obtained results. Based on the results of the antioxidant activity test, a linear regression equation was obtained, namely y = 0.1087x + 43.722, with R^2 = 0.9849, which indicates that the sample extract has a strong antioxidant activity with an IC_{50} value of 57.755 ppm. With categories <50 is very strong, 50-100 is strong, 101-150 is moderate, and >150 is weak. Thus, it can be seen that the value of the sample is included in the category with strong antioxidant activity. Besides antioxidant testing, an antibacterial test was also conducted in this research. Antibacterial activity of *Clitoria ternatea L.* extract against *Salmonella typhi* gives the result of an inhibition zone of 20 mm, which is categorized as a powerful antibacterial agent. The conclusion is that collagen drink from butterfly pea flower extract has a strong antioxidant activity and antibacterial activity, so it can be used as a skin care product to prevent premature aging.

Keywords: Butterfly Pea Flower (*Clitoria ternatea L.*); Collagen; Antioxidant; Anti-aging; Functional drink

1. Introduction

Skin aging is a continuous process associated with the thinning of the skin's physiological functions. Over time, both human and animal organs undergo physiological changes caused by natural and unnatural factors. Skin aging is a multifactorial activity dependent on intrinsic factors (genetic, hormonal, and metabolic) and extrinsic factors (exposure to UV rays, smoking, air pollution, chemicals, and poor nutrition). Aging has adverse effects on the skin's connective tissues, leading to a decrease in elastin and collagen fibers, resulting in fine lines and wrinkles. Additionally, aging reduces the production of proteoglycans and glycosaminoglycans (such as hyaluronic acid) in the skin and cartilage. As a result, the skin weakens, loses its integrity, and becomes dry, unable to retain sufficient moisture. Skin wrinkles also develop due to the reduction in skin thickness over time, due to decreased collagen (1).

There are two types of skin aging: Intrinsic or chronological aging and extrinsic or environmental aging. In intrinsic aging, free radicals are naturally formed through regular metabolism. Extrinsic aging is characterized by skin damage caused by free radicals from exposure to UV rays, cigarette smoke, and air pollution. These environmental stressors not only accelerate skin aging but also contribute to skin damage, causing issues like wrinkles, hyperpigmentation, chronic inflammation, abnormal elastin formation, and cancer (2).

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Efforts to prevent premature skin aging can be made by inhibiting free radicals using substances with antioxidant properties. Apart from slowing down the aging process, antioxidants also help combat degenerative diseases.

The benefits of antioxidants for the skin include protecting the skin from various cell damage caused by UV radiation, slowing down aging, and more. Antioxidants are widely used in cosmetics and skincare products, including collagen beverages. In recent years, collagen supplements have become increasingly popular, advertised as potential remedies against the aging process. It has been found that marine fish collagen is homologous to human collagen and has been widely used as a nutritional supplement along with collagen peptides. They have excellent safety profiles, high bioavailability in the human gastrointestinal barrier, safety, and high bioactivity (1). Research conducted by Ganceviciene R, et al. (3), showed that fish collagen peptides, often used as functional food or dietary supplements, have better antioxidant capacity, photoprotection, immune regulation properties, and better skin aging repair quality. Hence, there is an increasing interest in new and potent anti-aging substances for skincare.

External treatments alone are not sufficient for our skin; internal care is necessary. One of the treatments is collagen beverages. Collagen beverages are beneficial in maintaining skin hydration to prevent dryness. Inside our bodies, collagen is produced by fibroblast cells. However, as we age, the skin loses collagen and becomes thinner and drier. (4). Therefore, external collagen intake is needed. External collagen intake can be found in fish body parts, such as fish scales, because they contain collagen protein.

Considering the above-discussed points, we initiated this research entitled "Innovation of Silky Blue Collagen Drink from Butterfly Pea Flower Extract as a preventive nutrition for premature aging." The selection of butterfly pea flower as an ingredient in collagen beverages is due to its antioxidant content.

Research Problem and Objectives

Since butterfly pea flower (*Clitoria ternatea L.*) extract has the potential to be used as an anti-aging material based on its antioxidant properties, this research will assess the value of its antioxidant capacity using the DPPH method. Furthermore, besides its antioxidant properties, it is also important to determine its antibacterial properties to get important information before using it as a high antioxidant drink supplement.

2. Materials and methods

The equipment used in this research includes a rotary evaporator, incubator, desiccator, mortar and pestle, petri dishes, beakers, digital balance, blender, test tubes, measuring flasks, and pipettes. This study relies on the following materials: butterfly pea flowers, fish scale collagen extract, sucrose, skim milk, blueberry extract, 96% food-grade ethanol solvent, and xanthan gum.

2.1. Butterfly Pea Flower Extraction

A total of 125 grams of butterfly pea flowers were crushed using a blender and then macerated with 96% food-grade ethanol solvent at a ratio of 1:10 for 3x24 hours. The filtrate was then filtered and concentrated using a rotary evaporator (5).

2.2. Qualitative Anthocyanin Confirmation Test

The first technique involves observing the sample's color after heating for 2 minutes at 100°C with 2M HCl. If the red hue of the sample remains unchanged, it is likely due to anthocyanin. The second method involves adding the sample to a small container containing 2M NaOH and pouring it slowly (6). The presence of anthocyanin is indicated by a slow and stable transition from red to blue-green (7).

2.3. Determination of Phytochemical Content in Butterfly Pea Flower Extract

Phytochemical content testing in butterfly pea flower extract includes the following methods:

2.3.1. Steroid/Triterpenoid Examination

Acetic anhydride, concentrated H2SO4, and acetic anhydride are added to the sample. If steroids are present, the solution will turn bluish-green, while triterpenoids will turn reddish-purple.

2.3.2. Flavonoid Examination

One milliliter of the extract is mixed with reagents that include Wilstater, a few drops of strong HCl, and some magnesium powder. A good reaction is indicated if there is a yellow shift. A few drops of strong HCl are added to one milliliter of the extract after it has been heated. An affirmative response is indicated if the color becomes red. Reagent for Sodium Hydroxide (NaOH 10%) When a few drops of 10% NaOH reagent are added to 1 milliliter of the extract, a positive reaction is shown by an orange color shift.

2.3.3. Alkaloid Examination

When a few drops of Wagner's reagent are added to 1 ml of the extract, a positive reaction is indicated by the formation of a brown precipitate. In contrast, a negative reaction is observed if there is merely a change in the color of the extract without any precipitate. Similarly, the addition of two drops of Mayer's reagent to 1 ml of the extract results in a positive reaction when a white or yellow precipitate appears, signifying the presence of certain alkaloids.

2.3.4. Phenolate Examination

Ethanol extract of moringa stem bark positivity is determined by adding 1% $FeCl_3$ until a color change occurs, then comparing the new color with pure extract. Intensity is adjusted as a response to the color shift.

2.3.5. Tannin Examination

To detect the presence of tannins, the sample is first boiled in 20 milliliters of water and then filtered to eliminate impurities. A few drops of 1% ferric chloride ($FeCl_3$) solution are added to the filtrate; the appearance of a greenish-brown or dark blue coloration signifies a positive reaction. In another test using the gelatin reagent, one milliliter of the extract is mixed with five milliliters of gelatin solution and ten milliliters of 10% sodium chloride (NaCl). The formation of a yellow precipitate in this mixture indicates a positive result, confirming the presence of tannins.

2.4. Antioxidant Capacity Test

Absorbance measurements were conducted using a UV-Vis spectrophotometer set at λ=517 nm, and the antioxidant effects were observed using the DPPH free radical scavenging method. Preparation and dilution of antioxidant test solutions involved the following steps:

The extract was prepared at a concentration of 1 mg/mL or 1000 ppm and dissolved in a combination of ethanol and methanol solvents with a 1:1 ratio. The solution was vortexed at 5000 rpm until homogeneous. The extract was further diluted to concentrations of 200 ppm, 150 ppm, 125 ppm, 100 ppm, 75 ppm, 50 ppm, 35 ppm, 25 ppm, and 15 ppm in methanol, reaching a volume of 133.33 µl. Finally, 66.66 µl of DPPH solution (1 mg/ml) was added to each well. The total volume per well was 200 µl.

2.5. Antibacterial Capacity Test

This research utilized an antibacterial assay to determine the activity of butterfly pea flower extract against *Salmonella typhi* bacteria. In this study, inhibition zones similar to disc diffusion were formed around the discs at each concentration, indicating an inhibitory response against the growth of *Salmonella typhi* bacteria. The Sterile Brain Heart Infusion Agar (BHIA) medium was placed in a petri dish, and the test bacteria were added to it. Careful cup transfer ensured thorough mixing of bacteria and agar, and the mixture was allowed to solidify. Over 24-48 hours at 37 degrees Celsius, the solidified mixture was incubated with discs saturated with butterfly pea flower extract. A calliper was used to measure the diameter of the inhibition zones formed.

2.6. Collagen Powder Drink Formulation

The formulation of the collagen powder drink was carried out by the study by Anto et al. (8). The collagen powder, extracted from fish scales and weighing 100 mg, was placed into a reaction glass. Then, 30 mg (0.30%) of xanthan stabilizer was weighed and added to the reaction glass containing collagen. Next, add sucrose (10% (w/v)) and skim milk (0.25% (w/v)) to the reaction glass containing collagen and xanthan powder. Subsequently, incorporate butterfly pea flower extract (0.01%, (w/v)) and blueberry flavouring (0.01%, (w/v)). In the final step, mix thoroughly until homogeneous and neatly package the product (8).

3. Results and discussion

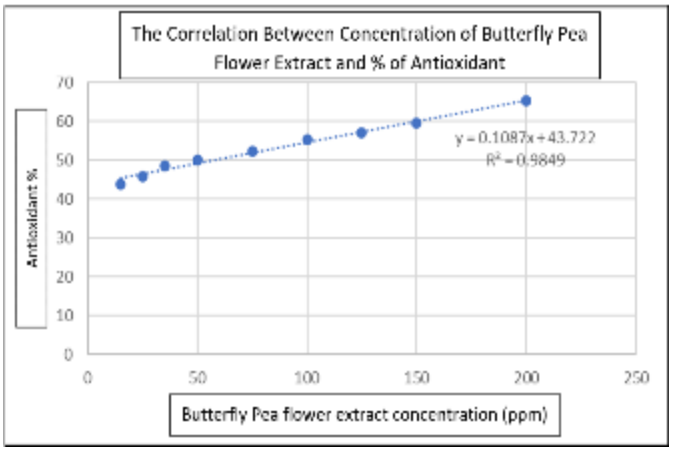

Figure 1 The Correlation Between the Concentration of Butterfly Pea Flower Extract and 5% of Antioxidant

Based on the graph above, the correlation between the concentration of the test solution and the percentage of antioxidants yielded the regression equation y = 0.1087x + 43.722, with an R^2 value of 0.9849. From the R^2 value, it can be concluded that there is a strong and significant correlation between the solvent concentration and the observed percentage of antioxidants, with a coefficient of determination of 0.9849. This indicates that 98% of the inhibition degree is influenced by the concentration of the substance, while less than 2% is affected by other factors such as precision in weighing, solvent addition, pipetting, or impurities in the solution. The obtained R^2 value suggests that the Butterfly Pea Flower Extract has a coefficient of determination approaching +1 (positive value), indicating that the research data obtained is very good. (9). The IC_{50} criteria are as follows: Less than 50 is very strong, 50-100 is strong, 101-150 is moderate, and more than 150 is weak.

Concentration %	Inhibition Zone (mm)		
	Test 1	Test 2	Test 3
25	10.4	10.7	10.2
50	12.65	12.8	12.9
75	15.7	15.4	15.55
100	20.55	20.55	20.5
C+	26	25.55	24.85
C-	8.9	8.75	10.1

Description: 1. Positive control (Chloramphenicol antibiotic)
2. Negative control (Ethanol 96%)

Figure 2 The Effect of Concentration on the Inhibition of Antibacterial Properties

In addition to testing antioxidant properties, antibacterial properties were also tested against *Salmonella typhi* bacteria. This was done to ensure that the product created is safe for digestion. Based on the table, the inhibition produced by the butterfly pea flower extract in this silky blue product is categorized as very strong, as it produces an average of 20.5 mm inhibition zone. The method used was the disc diffusion method and replica testing. The disc diffusion method is commonly used to test the antimicrobial activity of antibiotics against pathogenic microorganisms. The sensitivity of pathogenic microorganisms to antibiotics is indicated by the size of the clear zones formed (10). The parameter used is the clear zone, which is the clear area around the paper disc indicating the absence or inhibition of microorganism growth due to the antimicrobial substance excretion by its competitors (10). The antibacterial activity criteria are as follows: a clear zone diameter of 5 mm or less is categorized as weak, a clear zone of 5-10 mm is categorized as moderate, a clear zone of 10-20 mm is categorized as strong, and a clear zone larger than 20 mm is considered very strong.

4. Conclusion

Based on the results of the conducted research, several conclusions can be drawn from this proposal. Firstly, according to the DPPH method, butterfly pea flower extract is categorized as a strong antioxidant. Secondly, using the disc diffusion method against *Salmonella typhi* bacteria, a clear zone of 20.5 mm was obtained, categorizing it as a very strong antimicrobial agent.

Building upon the findings and conclusions presented, several important recommendations can be made for future research. First, further laboratory tests should be conducted to assess the safety level of the product before consumption. Additionally, organoleptic tests should be performed to determine the appropriateness of the formulated product.

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