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RESEARCH ARTICLE

Phytochemical Screening and Antioxidant Activity of Aqueous and Ethanolic Extracts of *Clitoria ternatea* L. Flowers

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Abstract: Oxidative stress triggers cellular damage via reactive oxygen species, necessitating the search for plant-derived antioxidants to mitigate degenerative pathologies. *Clitoria ternatea* L., commonly known as butterfly pea, is a valuable source of bioactive metabolites with therapeutic potential. This investigation involved the preparation of aqueous (WEC) and ethanolic (EEC) extracts from dried flowers using ultrasonic-assisted maceration techniques. Preliminary phytochemical screening indicated the presence of flavonoids and saponins in both extracts, while terpenoids were uniquely identified in the aqueous fraction. Quantitative analysis of total flavonoid content revealed higher concentrations in WEC (7.804 ± 0.08 mg QE/g) compared to EEC (3.722 ± 0.01 mg QE/g). Antioxidant capacity was evaluated through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The results demonstrated that the aqueous extract possesses superior radical neutralizing capability with an IC_{50} value of 86.67 ± 0.485 ppm, whereas the ethanolic extract yielded an IC_{50} of 113.31 ± 0.142 ppm. Quercetin, used as the reference standard, exhibited an IC_{50} of 22.056 ± 0.196 ppm. The significant activity in the aqueous medium correlates with the higher concentration of polar flavonoids and anthocyanins. Differential solvent polarity dictates the extraction efficiency of these secondary metabolites, positioning the aqueous extract as a more potent candidate for therapeutic applications. The observed radical scavenging properties prove the traditional use of these flowers in health-promoting infusions and natural food colorants.

Keywords: *Clitoria ternatea*; Butterfly pea; Antioxidant activity; DPPH; Total flavonoid content.

1. Introduction

Modernization and shifting lifestyle patterns have led to increased exposure to environmental toxins and unhealthy dietary habits, which significantly contribute to the onset of various chronic diseases. At the molecular level, one of the primary drivers of these conditions is the phenomenon known as oxidative stress [1]. This arises due to an imbalance between the generation of reactive oxygen species (ROS) and the efficacy of the biological antioxidant defense system. Excessive oxidation within the human body results in cellular damage through the loss of electrons and the subsequent formation of highly reactive free radicals. These unstable species target essential biomolecules, including lipids, proteins, and nucleic acids [2]. The resulting structural alterations to these molecules are implicated in the progression of degenerative conditions, including cardiovascular diseases, neurodegeneration, and various forms of malignancy.

Antioxidants function by inhibiting the initiation or propagation of oxidative chain reactions, thereby protecting biological structures from damage. These substances neutralize free radicals by donating electrons or hydrogen atoms, effectively terminating the oxidative cascade [3]. Given the potential side effects of synthetic antioxidants, there is a growing interest in identifying natural, plant-based compounds that offer comparable or superior efficacy with minimal toxicity.

Clitoria ternatea L., a member of the Fabaceae family, is widely recognized for its vibrant blue flowers and diverse pharmacological applications. In various Asian cultures, infusions of these flowers are traditionally consumed to enhance skin health and delay the physiological markers of aging [4]. The intense pigmentation of the butterfly pea flower is attributed to high concentrations of anthocyanins, particularly delphinidin derivatives. Apart from its utility as a natural food colorant, the various parts of the plant, including the roots, leaves, and flowers, exhibit significant biological activities. These include analgesic, antipyretic, anti-inflammatory, antidiabetic, and antimicrobial effects [5, 6]. The presence of specific secondary metabolites, such as ternatins and flavonol glycosides, suggests that the plant serves as a robust source of natural antioxidants [7].

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2. Materials and Methods

2.1. Collection and Authentication of Plant Material

Fresh flowers of *Clitoria ternatea* were sourced from the Plantzone plantation located in Rajahmundry, East Godavari District, Andhra Pradesh, India. The botanical identity of the plant was verified, and the materials were processed to ensure consistency. The chemical reagents utilized in the experimental procedures included DPPH and ABTS from Sigma, quercetin standard, 96% ethanol, and ferric chloride from Smart-Lab. Additional reagents such as potassium persulfate, TPTZ, and various phytochemical testing reagents like Wagner's, Dragendorff's, and Mayer's were obtained from analytical grade suppliers including Loba Chemie and Asahimas Chemical.

2.2. Solvent Extraction

2.2.1. Preparation of Aqueous Extract (WEC)

The fresh flowers were cleaned to eliminate extraneous matter and dried at a controlled temperature of 40°C for a duration of 12 hours. The dried floral material was then processed into a fine powder. For the aqueous extraction, the powdered material was subjected to maceration in distilled water followed by filtration through a double-layer filter paper. To enhance the yield of bioactive compounds, the filtrate underwent ultrasonic extraction for 15 minutes, with intermittent cooling periods to maintain thermal stability. The resulting solution was concentrated using a rotary evaporator and finally dried through lyophilization to produce the water extract [8].

2.2.2. Preparation of Ethanolic Extract (EEC)

In a similar manner, the cleaned and dried floral powder was macerated in 96% ethanol. The mixture was processed using ultrasonic-assisted extraction for 15 minutes, incorporating 5-minute intervals to mitigate overheating. Following extraction, the mixture was filtered, and the ethanol was removed using a rotary evaporator. The concentrated residue was collected as the ethanol extract for subsequent phytochemical and antioxidant analysis [9].

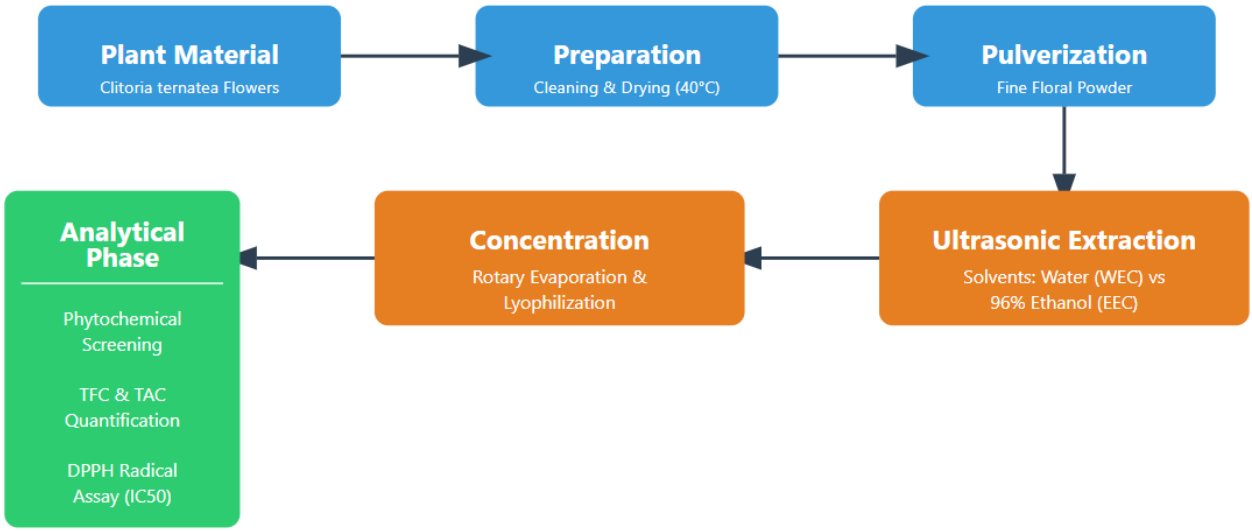

Figure 1. Methodology of the Extraction and Screening

2.3. Qualitative Phytochemical Tests

The detection of flavonoids was performed using the Shinoda test, where approximately 40 mg of each extract was dissolved in boiling water and filtered. The addition of magnesium powder and concentrated hydrochloric acid to the filtrate resulted in a color change to red, yellow, or orange, confirming the presence of flavonoid structures [10]. Terpenoid content was evaluated by reacting the aqueous solution of the extract with concentrated hydrochloric acid and sulfuric acid, where the development of a red or purple hue served as a positive indicator [11]. For the identification of saponins, the extract was heated in water, filtered, and shaken vigorously. The formation of a persistent foam lasting over 10 minutes indicated the presence of these glycosides [12, 13].

2.4. Quantitative Determination of Bioactive Components

2.4.1. Estimation of Total Flavonoid Content (TFC)

The total flavonoid content was quantified using the aluminum chloride colorimetric method. Both the aqueous and ethanolic extracts were treated with a mixture of methanol, 10% aluminum chloride, 1 M potassium acetate, and distilled water. After an incubation period of 30 minutes at room temperature, the absorbance was recorded at the maximum wavelength. Quercetin served as the reference standard, and the final concentrations were expressed as mg of quercetin equivalents (QE) per gram of extract [14].

2.4.2. Determination of Total Anthocyanin Content (TAC)

The pH differential method was employed to calculate the total anthocyanin content based on the structural shifts that occur at pH 1.0 and pH 4.5. Samples were diluted with potassium chloride buffer and sodium acetate buffer respectively. After standing for 60 minutes, absorbance was measured at 520 nm and 700 nm. The calculation accounted for the molar extinction coefficient and molecular weight of cyanidin-3-glucoside or malvidin-3-glucoside to determine the concentration in mg per liter [15, 16].

2.5. Assessment of Free Radical Scavenging Activity

The antioxidant potential was evaluated using the DPPH radical scavenging assay. A stock solution of DPPH was prepared in ethanol and stored under refrigerated conditions. Quercetin was prepared in varying concentrations to establish a standard reference curve. For the sample analysis, 2 mL of the extracts at concentrations between 40 and 150 ppm were mixed with an equal volume of the DPPH working solution. Following a 2-hour incubation in a dark environment, the absorbance was measured at 520 nm. The percentage of inhibition was calculated by comparing the sample absorbance against a control, and the IC_{50} values were determined through linear regression analysis [17, 18].

3. Results

3.1. Phytochemical Screening of Floral Extracts

The qualitative analysis of the secondary metabolites in *Clitoria ternatea* revealed a distinct distribution of bioactive compounds based on the solvent used for extraction. Both the aqueous extract (WEC) and the ethanolic extract (EEC) showed a positive response for the presence of flavonoids and saponins. These results indicate that these specific classes of phytochemicals possess sufficient solubility across the polarity range of water and 96% ethanol.

Table 1. Qualitative Phytochemical Screening of Floral Extracts

S. No.	Phytochemical	Method	Aqueous Extract (WEC)	Ethanolic Extract (EEC)
1	Flavonoids	Shinoda Test	+	+
2	Saponins	Froth Test	+	+
3	Terpenoids	Salkowski/HCl-H_2SO_4 Test	+	-

Note: (+) indicates presence; (-) indicates absence.

Interestingly, terpenoids were detected exclusively in the aqueous extract. This suggests that the terpenoid structures present in the butterfly pea flower may be associated with glycosidic moieties or other polar functional groups that favor aqueous solubility under ultrasonic-assisted conditions. The absence of terpenoids in the 96% ethanolic fraction highlights the selective nature of the extraction process. These findings provide a preliminary basis for the medicinal potential of the extracts, particularly the flavonoid content, which is a primary determinant of radical scavenging efficacy.

3.2. Quantitative Analysis of Total Flavonoid Content (TFC)

The quantification of total flavonoids was performed by referencing a quercetin calibration curve. The absorbance was recorded at 432 nm, and the resulting linear regression equation was determined to be $y = 0.0726x + 0.0758$, demonstrating high statistical reliability with a correlation coefficient of $r = 0.9986$.

The concentration of flavonoids in the aqueous extract was found to be 7.804 ± 0.08 mg QE/g, which is more than double the concentration observed in the ethanolic extract (3.722 ± 0.01 mg QE/g). This significant disparity suggests that the primary flavonoids in *Clitoria ternatea* flowers, such as flavonol glycosides and anthocyanins, possess high affinity for water. The higher TFC in WEC is a critical observation, as it directly correlates with the superior antioxidant performance observed in subsequent assays.

Table 2. Quantitative Estimation of Total Flavonoid Content (TFC)

Extract Type	Absorbance (432 nm)	Regression Equation (y = mx + c)	Correlation Coefficient (r)	Total Flavonoid Content (mg QE/g)
Aqueous (WEC)	Variable	y = 0.0726x + 0.0758	0.9986	7.804 ± 0.08
Ethanolic (EEC)	Variable	y = 0.0726x + 0.0758	0.9986	3.722 ± 0.01

**Values are expressed as Mean ± SD (n=3). The difference between WEC and EEC is statistically significant ($p < 0.05$).*

3.3. Evaluation of DPPH Radical Scavenging Capacity

3.3.1. Antioxidant Performance of Aqueous Extract (WEC)

The radical scavenging activity of WEC exhibited a clear dose-dependent relationship. At the lowest tested concentration of 40 ppm, the inhibition was approximately 29.8%, which escalated to a maximum of 72.15% at 150 ppm. The calculated mean IC50 for the aqueous extract was 86.67 ± 0.485 ppm. This value indicates a potent ability to neutralize the DPPH radical, aligning with the high flavonoid levels identified in the quantitative analysis.

Table 3. DPPH Radical Scavenging Activity of Aqueous Extract (WEC)

Concentration (ppm)	Mean Absorbance (520 nm)*	Percentage Inhibition (%)*	IC50 Value (ppm)
40	0.525 ± 0.002	29.81 ± 0.31	86.67 ± 0.485
50	0.469 ± 0.001	37.26 ± 0.20	
60	0.430 ± 0.001	42.39 ± 0.08	
100	0.331 ± 0.001	55.64 ± 0.08	
150	0.208 ± 0.000	72.15 ± 0.00	

**Blank Absorbance: 0.747. Values are Mean ± SD (n=3).*

3.3.2. Antioxidant Activity of Ethanolic Extract (EEC)

The ethanolic extract demonstrated a comparatively lower capacity for radical inhibition. At 40 ppm, the inhibition was recorded at 21.5%, reaching 64.52% at the 150 ppm concentration level. The calculated IC50 for EEC was 113.31 ± 0.142 ppm. While this indicates significant antioxidant activity, it is markedly lower than that of the aqueous counterpart. In comparison, the reference standard quercetin exhibited an IC50 of 22.056 ± 0.196 ppm, which represents the benchmark for high-potency antioxidant behavior.

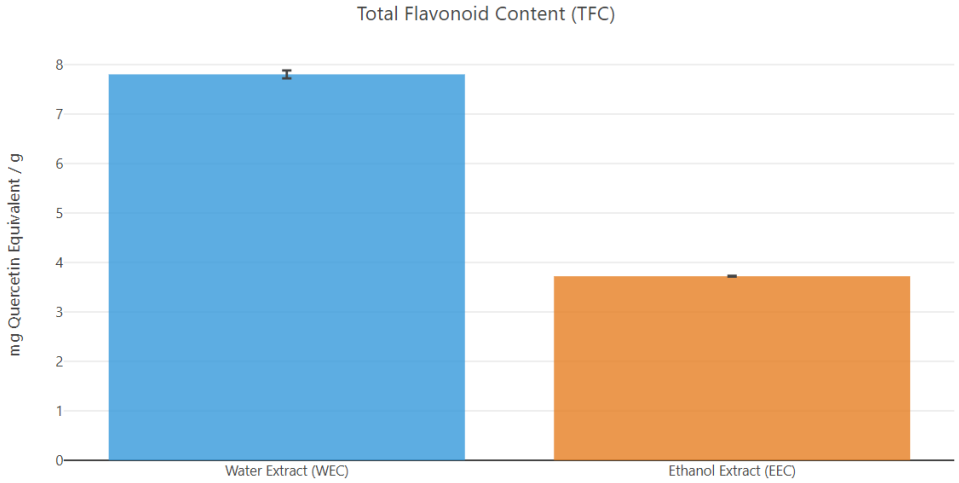

Figure 2. Comparison of Bioactive Content and Antioxidant Potential

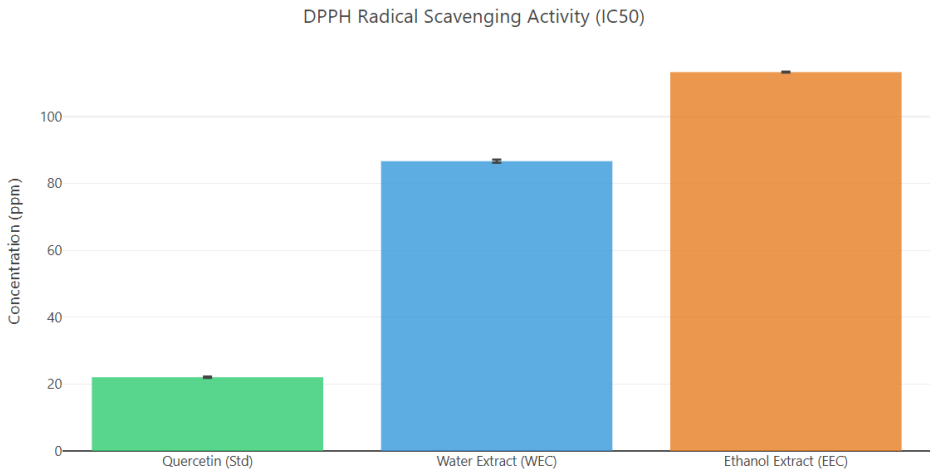

Figure 3. Comparison of (A) Total Flavonoid Content and (B) IC50 values across extracts and standard. Error bars represent Standard Deviation (n=3).

Table 4. DPPH Radical Scavenging Activity of Ethanolic Extract (EEC)

Concentration (ppm)	Mean Absorbance (520 nm)*	Percentage Inhibition (%)*	IC50 Value (ppm)
40	0.586 ± 0.001	21.51 ± 0.08	113.31 ± 0.142
50	0.558 ± 0.001	25.30 ± 0.13	
60	0.511 ± 0.001	31.64 ± 0.08	
100	0.422 ± 0.001	43.51 ± 0.13	
150	0.265 ± 0.000	64.52 ± 0.00	

**Blank Absorbance: 0.747. Values are Mean ± SD (n=3).*

Table 5. Comparative Antioxidant Efficacy of Extracts and Reference Standard

Sample Code	IC50 (ppm)*	Relative Antioxidant Potency	Category
Quercetin (Standard)	22.056 ± 0.196	1.00 (Reference)	Very Strong
Aqueous Extract (WEC)	86.670 ± 0.485	0.25	Strong
Ethanolic Extract (EEC)	113.31 ± 0.142	0.19	Moderate

**Values are Mean ± SD. Potency is calculated relative to the standard; lower* IC50 *indicates higher potency.*

4. Discussion

4.1. Role of Solvents and Extraction Efficiency

The selection of extraction solvents is a pivotal factor in determining the phytochemical yield and biological potency of plant extracts. In this study, the observed superiority of the aqueous extract in terms of both flavonoid content and radical scavenging activity can be explained by the principle of solvent polarity. Water, being highly polar, is more effective at solvating the hydroxyl-rich structures of the flavonoids and anthocyanins predominant in *Clitoria ternatea* [19]. While 96% ethanol is an effective solvent for many organic molecules, it may fail to extract the more polar glycosidic forms of these antioxidants as efficiently as water, especially when assisted by ultrasonic energy. The detection of terpenoids only in the water extract further emphasizes this point. Terpenoids in certain plants can exist as polar saponins or be bound to sugars, making them more amenable to aqueous extraction [20]. The presence of these additional metabolites in WEC may contribute to a synergistic antioxidant effect that is less pronounced in the ethanolic fraction.

4.2. Mechanism of Antioxidant Action

The DPPH assay functions through a mechanism involving the transfer of hydrogen atoms or electrons from the antioxidant molecule to the stable DPPH radical. The reduction of the deep purple radical into a yellow-colored hydrazine derivative is a quantifiable measure of this donation capacity [21]. The higher flavonoid concentration in WEC provides a greater density of hydroxyl groups available for radical neutralization.

Flavonoids such as quercetin and its derivatives, which are known to be present in *Clitoria ternatea*, possess a characteristic structure that stabilizes the resulting phenoxyl radical through resonance. The significantly lower IC50 of the aqueous extract (86.67 ppm) compared to the ethanolic extract (113.31 ppm) confirms that the water-soluble fraction contains the most active hydrogen-donating species. The difference in these values is statistically significant and underscores the importance of solvent choice in preparing therapeutic infusions or functional food ingredients.

4.3. Comparative Efficacy

The results indicate that while both extracts possess antioxidant properties, the aqueous extract is categorized as having strong activity, whereas the ethanolic extract falls into the moderate category [22]. This distinction is vital for the development of natural health products. The efficacy of the aqueous extract at lower concentrations suggests it may be more effective in preventing lipid peroxidation and protecting cellular DNA from oxidative damage *in vivo* [23, 24].

Discrepancies between these findings and other studies might be attributed to the geographic origin of the plant material or the specific extraction techniques employed. Environmental factors such as soil composition, altitude, and UV exposure can alter the metabolic profile of the flowers, leading to variations in the IC50 values reported across different scientific literature [25]. However, the internal consistency of the data presented here where high flavonoid content directly maps to lower IC50 values validates the reliability of the current experimental model.

5. Conclusion

The investigation into the floral extracts of *Clitoria ternatea* L. confirms that the choice of solvent profoundly impacts the recovery of bioactive secondary metabolites and the resulting antioxidant capacity. The aqueous extract demonstrated a superior phytochemical profile, particularly in terms of total flavonoid content and the presence of terpenoids. These chemical advantages translated into a significantly higher radical scavenging efficacy in the DPPH assay compared to the ethanolic extract. The data suggests that the polar constituents of the butterfly pea flower are the primary drivers of its antioxidant potential. Aqueous extracts of these flowers not only preserve their traditional use as healthy infusions but also provide a more potent source of natural antioxidants for pharmaceutical and nutraceutical applications.

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