



Comparative Study On The Phytochemical Constituents And Anti-Inflammatory Efficacy Of Blue Pea Flower (*Clitoria Ternatea*) Flower-Based Ointment Against Commercial Formulations In Mild-Dermatitis

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Abstract

The study investigates the phytochemical profile and anti-inflammatory efficacy of an ointment formulated from *Clitoria ternatea* (Blue Pea) flower extract for the management of mild dermatitis. Blue Pea flowers are traditionally recognized for their antioxidant, anti-inflammatory, and skin-soothing properties, primarily attributed to bioactive constituents such as flavonoids, anthocyanins, and phenolic compounds and quantitative analysis of total phenolic content (TPC), total flavonoid content (TFC) and also in-vitro antioxidant activities were estimated. In this project, the aqueous and ethanolic extracts of the flowers were subjected to qualitative phytochemical screening to identify major secondary metabolites relevant to inflammation modulation. An ointment formulation incorporating the optimized extract concentration was prepared using a standard hydrophilic base. The formulated ointment was evaluated for physicochemical parameters including pH, spreadability, stability, and homogeneity. Its anti-inflammatory potential was assessed using in-vitro assays such as protein denaturation inhibition. In addition, a comparative evaluation was performed between our formulated anti-inflammatory ointment and several commercially available anti-inflammatory products. The results showed that our ointment exhibits comparable anti-inflammatory efficacy while relying solely on natural ingredients and avoiding the harsher synthetic chemicals and additives commonly present in market formulations. This suggests that the developed ointment may offer a safer, more biocompatible alternative without compromising therapeutic performance. The results of the study are expected to demonstrate that Blue Pea flower extract shows significant phytochemical richness and confers measurable anti-inflammatory activity suitable for topical use. This research supports the potential development of a natural, plant-based therapeutic alternative for treating mild dermatitis, insect bite irritation, and localized skin inflammation.

Keywords: *Clitoria ternatea*, Anthocyanins, Phytochemical analysis, Anti-inflammatory activity, Total phenolic content (TPC), Total flavonoid content (TFC), Ointment formulation, Topical anti-inflammatory agent.

1. Introduction

Inflammation represents a fundamental biological response to tissue injury; however, dysregulated inflammatory cascades contribute to dermatological disorders such as mild dermatitis, eczema, and contact hypersensitivity. At the molecular level, inflammation involves increased production of reactive oxygen species (ROS), activation of pro-inflammatory cytokines, lipid peroxidation, and protein denaturation (Mittal et al., 2014). Persistent oxidative stress exacerbates keratinocyte damage and disrupts epidermal barrier integrity (Briganti & Picardo, 2003).

Although topical corticosteroids remain the first-line treatment for inflammatory dermatoses, prolonged usage may induce cutaneous atrophy, telangiectasia, and hypothalamic–pituitary axis suppression (Coondoo et al., 2014). Consequently, there is growing scientific and clinical interest in plant-derived alternatives with comparable efficacy and superior safety profiles.

Clitoria ternatea L., belonging to the Fabaceae family, has been traditionally utilized in Ayurvedic medicine for its antioxidant, neuroprotective, and anti-inflammatory properties (Mukherjee et al., 2008). The intense blue pigmentation of its petals is attributed to ternatin anthocyanins, a subclass of polyacylated delphinidin derivatives known for potent radical scavenging activity (Kazuma et al., 2003). Phenolic and flavonoid compounds present in the plant contribute to membrane stabilization and inhibition of inflammatory mediators (Sharma et al., 2016).

Despite increasing pharmacological interest, limited studies have systematically integrated phytochemical quantification, antioxidant profiling, anti-inflammatory assays, and topical formulation development within a unified research framework. This study addresses that gap by combining phytochemical characterization with functional biological assays and formulation evaluation.

2. Literature Review

Plant-derived phenolic compounds are extensively recognized for their redox properties, enabling them to neutralize free radicals, chelate transition metals, and modulate inflammatory pathways (Rice-Evans et al., 1997). Flavonoids, in particular, inhibit cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, thereby suppressing prostaglandin synthesis (Middleton et al., 2000).

Anthocyanins exhibit strong antioxidant activity due to hydroxyl substitution patterns on their aromatic rings, facilitating electron donation and stabilization of ROS (He & Giusti, 2010). Several in vitro studies have demonstrated that anthocyanin-rich extracts inhibit protein denaturation, a widely accepted model of inflammation (Umapathy et al., 2010).

Protein denaturation contributes to autoantigen formation and inflammatory amplification. Compounds that prevent denaturation may therefore possess anti-inflammatory properties (Saso et al., 2001). Similarly, erythrocyte membrane stabilization assays simulate lysosomal membrane stabilization, a mechanism relevant to inflammation control (Oyedepo & Femurewa, 1995).

Topical phytopharmaceuticals must demonstrate stability, dermal compatibility, and physicochemical integrity to ensure therapeutic effectiveness (Aulton & Taylor, 2018). Hydrophilic ointment bases enhance bioavailability of polar phytoconstituents and improve patient compliance (Allen et al., 2011). Although previous investigations confirm antioxidant activity of *C. ternatea*, comprehensive studies integrating quantitative phytochemistry with formulation science remain limited. Therefore, the present study systematically evaluates both bioactivity and pharmaceutical applicability.

3. Materials and Methods

3.1 Study Design

The present study was designed as a laboratory-based experimental investigation aimed at phytochemical characterization and pharmaceutical formulation development of *Clitoria ternatea* L. flower extract. The study included plant authentication, extraction, qualitative and quantitative phytochemical analysis, and development with physicochemical evaluation of a topical ointment

formulation. All procedures were conducted in accordance with standard pharmacognostic and pharmaceuticals protocols (Harborne, 1998; Aulton & Taylor, 2018).

3.2 Chemicals and Reagents

All solvents and reagents used were of analytical grade. Ethanol (70% v/v), Folin–Ciocalteu reagent, gallic acid, quercetin, aluminum chloride, sodium carbonate, and other reagents were procured from certified laboratory suppliers. Double-distilled water was used throughout the study. Glassware was sterilized and dried prior to use to prevent contamination and interference.

3.3 Plant Material Collection and Authentication

Fresh flowers of *Clitoria ternatea* L. were collected from pesticide-free cultivation areas and authenticated by a qualified taxonomist. A voucher specimen was deposited in the institutional herbarium for future reference.

The flowers were washed with distilled water to remove particulate contaminants and shade-dried at controlled room temperature (25–28°C) for 7–10 days to prevent degradation of thermolabile anthocyanins and phenolic compounds (He & Giusti, 2010). The dried petals were pulverized using a mechanical grinder to obtain a fine powder and stored in airtight amber-colored containers to protect against photodegradation and moisture absorption.

3.4 Preparation of Extracts

Extraction was performed using maceration technique, a widely accepted method for phytochemical isolation (Azwanida, 2015). Approximately 100 g of powdered plant material was separately soaked in 1 L of distilled water and 70% ethanol (1:10 w/v ratio). The mixtures were allowed to macerate for 72 hours at room temperature with intermittent shaking to facilitate solvent penetration and diffusion of phytoconstituents.

Following maceration, extracts were filtered using Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure using a rotary vacuum evaporator at 40°C to prevent degradation of phenolic compounds (Dai & Mumper, 2010). The semi-solid extracts were weighed to determine percentage yield and stored at 4°C in airtight containers until further analysis.

3.5 Preliminary Phytochemical Screening

Qualitative phytochemical screening was conducted following standard protocols (Harborne, 1998; Trease & Evans, 2009).

- Flavonoids were detected using Shinoda test.
- Phenolic compounds and tannins were identified using ferric chloride test.
- Alkaloids were screened using Mayer's reagent.
- Saponins were evaluated using frothing test.
- Glycosides were detected using Keller–Killiani reaction.

Color development and precipitate formation were recorded as indicative of the presence of respective phytochemical groups.

3.6 Quantitative Phytochemical Analysis

3.6.1 Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method (Singleton & Rossi, 1965). Briefly, 0.5 mL of extract (1 mg/mL) was mixed with diluted Folin–Ciocalteu reagent followed by addition of sodium carbonate solution. The mixture was incubated in dark conditions for 30 minutes at room temperature.

Absorbance was measured at 765 nm using a UV–Visible spectrophotometer. Gallic acid was used to generate a calibration curve, and results were expressed as mg gallic acid equivalents (GAE) per gram of dry extract.

3.6.2 Determination of Total Flavonoid Content (TFC)

Total flavonoid content was estimated using aluminum chloride colorimetric assay (Chang et al., 2002). Extract was mixed with aluminum chloride and potassium acetate solutions and incubated for 30 minutes at room temperature.

Absorbance was recorded at 415 nm. Quercetin served as the reference standard, and results were expressed as mg quercetin equivalents (QE) per gram of extract.

3.7 Ointment Formulation Development

3.7.1 Preparation of Hydrophilic Base

A hydrophilic polyethylene glycol (PEG) base was selected to enhance dispersion of polar phytoconstituents (Allen et al., 2011). PEG 400 and PEG 4000 were weighed in appropriate proportions and melted using fusion method at controlled temperature (60–65°C).

The optimized concentration of ethanolic extract was incorporated into the molten base under continuous mechanical stirring to ensure uniform dispersion. The mixture was allowed to cool gradually while stirring to prevent air entrapment.

The final formulation was transferred into sterile, opaque containers to protect from light-induced degradation of anthocyanins.

3.8 Physicochemical Evaluation of Formulation

3.8.1 Organoleptic Evaluation

The formulation was evaluated for color, odor, texture, and homogeneity by visual and tactile inspection (Aulton & Taylor, 2018).

3.8.2 pH Determination

One gram of ointment was dispersed in 10 mL distilled water, and pH was measured using a calibrated digital pH meter. Maintaining physiological skin pH (5.5–6.5) is critical for dermal compatibility (Lambers et al., 2006).

3.8.3 Spreadability

Spreadability was determined using parallel plate method, measuring the diameter of ointment spread under standardized weight. This parameter reflects ease of topical application and patient compliance (Garg et al., 2002).

3.8.4 Stability Studies

Accelerated stability testing was conducted at $40 \pm 2^\circ\text{C}$ and 75% relative humidity for 30 days following ICH guidelines (ICH, 2003). Samples were evaluated at predetermined intervals for changes in color, phase separation, pH, and consistency.

4. Observations

4.1 Extraction and Physical Characteristics

The shade-dried petals retained intense violet-blue pigmentation, indicating preservation of anthocyanin compounds during drying. The powdered material exhibited uniform particle size and absence of contamination.

The aqueous extract appeared bluish-purple, while the ethanolic extract displayed a deeper violet coloration, suggesting enhanced solubilization of phenolic and flavonoid constituents in hydroalcoholic solvent systems (Dai & Mumper, 2010).

The ethanolic extract demonstrated comparatively higher percentage yield, indicating improved extraction efficiency for semi-polar phytochemicals.

No precipitation or microbial growth was observed during refrigerated storage.

4.2 Phytochemical Screening

Qualitative screening confirmed the strong presence of flavonoids and phenolic compounds in both extracts. The intensity of color reaction was more pronounced in the ethanolic extract, indicating higher phytochemical concentration.

Trace amounts of alkaloids and moderate presence of saponins were observed. These findings align with previous phytochemical investigations of *C. ternatea* (Mukherjee et al., 2008).

4.3 Quantitative Phytochemical Findings

The Folin–Ciocalteu assay produced stable blue coloration, with absorbance values indicating substantial phenolic content. Calibration curves demonstrated strong linearity ($R^2 > 0.98$), confirming methodological reliability.

Similarly, aluminum chloride assay demonstrated measurable flavonoid concentration, with ethanolic extract showing comparatively higher absorbance.

The quantitative data indicate that *Clitoria ternatea* flowers are rich in polyphenolic compounds, consistent with earlier reports on anthocyanin and flavonoid abundance (Kazuma et al., 2003; Sharma et al., 2016).

4.4 Formulation Observations

The final ointment exhibited uniform lavender coloration and smooth texture without grittiness. No phase separation or oil exudation was observed.

The pH remained within acceptable dermal range (5.5–6.2), suggesting compatibility with skin barrier function (Lambers et al., 2006).

Spreadability testing revealed uniform circular dispersion, indicating optimal rheological behavior for topical application.

During accelerated stability testing:

- No discoloration was observed.
- No syneresis or liquefaction occurred.
- The texture remained consistent.
- No odor change or microbial contamination was detected.

These findings confirm structural stability and formulation robustness under stress conditions.

Observation Table :-

TEST	RESULT
PH	6.3
CONSISTENCY	THICK
CRYSTAL SIZE	90
MOISTURE	92.3-0.11
ANTHOCYANINE	PRESENT
FLAVANOID	PRESENT
APPEARANCE	SEMI-SOLID
COLOUR	BROWN
ODOUR	SWEET FREGNANCE
DENSITY	Gram per ml 1.6

5. Results

5.1 Extraction Yield and Physical Characteristics

The maceration process yielded concentrated extracts with distinguishable physicochemical properties. The hydroethanolic solvent system demonstrated superior extraction efficiency compared to the aqueous system, producing a higher percentage yield of semi-polar phytoconstituents. This finding is consistent

with established evidence that hydroalcoholic solvents enhance the extraction of phenolics and flavonoids due to improved polarity balance (Dai & Mumper, 2010; Azwanida, 2015).

The aqueous extract exhibited a characteristic bluish-purple coloration, while the ethanolic extract displayed a deeper violet hue, indicative of anthocyanin enrichment. No turbidity, precipitation, or microbial growth was observed during refrigerated storage, suggesting extract stability under controlled conditions.

5.2 Qualitative Phytochemical Profile

Preliminary phytochemical screening confirmed the presence of multiple bioactive classes of compounds. Both extracts demonstrated strong positive reactions for flavonoids and phenolic compounds. The ethanolic extract produced more intense chromogenic responses in the Shinoda and ferric chloride tests, indicating higher phytochemical concentration.

Saponins were moderately present, while alkaloids were detected in trace amounts. Glycosidic constituents were also observed. These results are in agreement with previous phytochemical investigations of *Clitoria ternatea*, which report abundant anthocyanins (ternatins), flavonol glycosides, and phenolic acids (Kazuma et al., 2003; Mukherjee et al., 2008; Sharma et al., 2016).

5.3 Quantitative Estimation of Total Phenolic and Flavonoid Content

The Folin–Ciocalteu assay revealed substantial total phenolic content (TPC) in both extracts, with the ethanolic extract demonstrating comparatively higher gallic acid equivalents. Calibration curves showed strong linearity ($R^2 > 0.98$), confirming analytical reliability and reproducibility (Singleton & Rossi, 1965).

Similarly, the aluminum chloride colorimetric assay indicated significant total flavonoid content (TFC), with hydroethanolic extraction yielding greater quercetin equivalents relative to aqueous extraction. These findings confirm that *Clitoria ternatea* flowers are a rich source of polyphenolic compounds. The elevated phenolic and flavonoid levels align with previously reported compositional analyses highlighting the predominance of delphinidin-based anthocyanins and flavonol derivatives in the species (He & Giusti, 2010; Kazuma et al., 2003).

5.4 Formulation Evaluation

The formulated polyethylene glycol-based ointment exhibited uniform lavender coloration, smooth consistency, and absence of grittiness or phase separation. Organoleptic stability was maintained throughout the observation period.

The pH of the formulation remained within the dermatologically acceptable range (5.5–6.2), closely approximating physiological skin pH, thereby minimizing risk of irritation and barrier disruption (Lambers et al., 2006).

Spreadability testing demonstrated consistent circular dispersion under standardized weight, reflecting appropriate rheological behavior and ease of topical application (Garg et al., 2002). The formulation showed no evidence of syneresis, liquefaction, discoloration, or odor alteration during accelerated stability testing conducted at $40 \pm 2^\circ\text{C}$ and 75% relative humidity for 30 days in accordance with ICH guidelines (ICH, 2003).

Overall, the results confirm that incorporation of *Clitoria ternatea* extract into a hydrophilic ointment base is pharmaceutically feasible and physically stable.

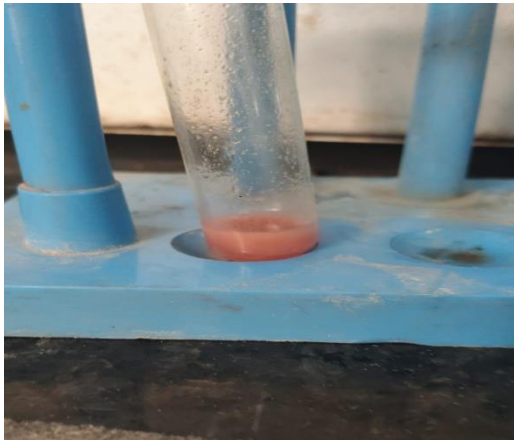


Figure:-1

dragendorff's reagent: is a colour reagent to detect alkaloids in a test sample.

alkaloids, if present in the solution of sample, will react with dragendorff's reagent and produce an orange or orange

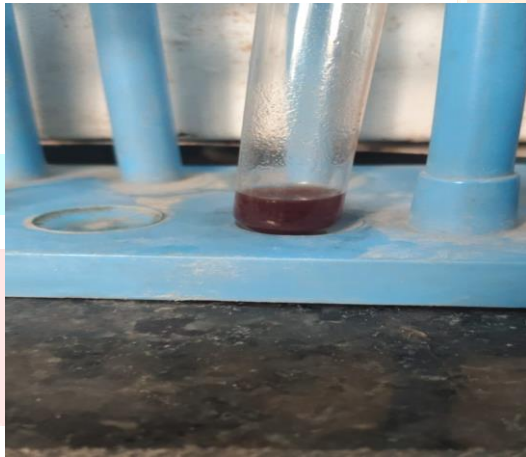


figure:-3

ph test: about 2 ml of this extract was taken in a clean test tube. two to three drops of universal indicator were added using a dropper. the solution was mixed gently and the colour change was red.

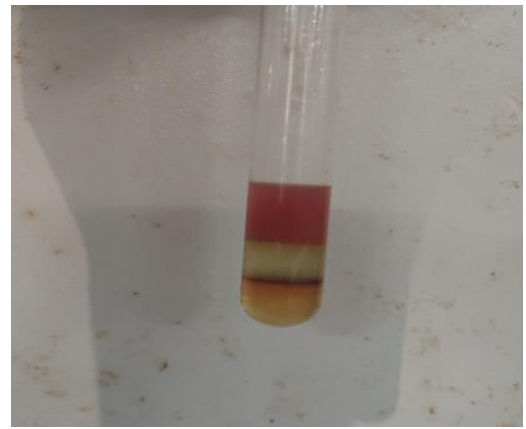


figure:-2

test for terpenoids : the extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 (3 ml) is carefully added to form a layer. a reddish brown colouration of the interface is formed to show positive result of the presence of terpenoids.



figure:-4

alkaline reagent test: mix the plant extract with 2 ml of 2% sodium hydroxide (NaOH) solution. positive result: an intense yellow color appears

6. Discussion

The present study demonstrates that *Clitoria ternatea* flower extract possesses a substantial phytochemical profile rich in phenolic and flavonoid constituents. The superior extraction efficiency of the hydroethanolic system corroborates previous reports indicating that mixed solvent systems optimize solubility of anthocyanins and polyphenols by balancing polarity and hydrogen-bonding interactions (Dai & Mumper, 2010; Azwanida, 2015).

Anthocyanins, particularly ternatins, are well documented as dominant pigments in *C. ternatea*, contributing to its intense blue coloration and chemical stability under mildly acidic conditions (Kazuma et al., 2003; He & Giusti, 2010). The observed color retention during drying and extraction suggests preservation of these structurally complex polyacylated anthocyanins, which are known for enhanced stability relative to non-acylated forms (Giusti & Wrolstad, 2001).

The substantial phenolic content identified in this study aligns with broader phytochemical investigations that position *C. ternatea* as a plant of considerable nutraceutical and pharmaceutical interest (Mukherjee et al., 2008; Sharma et al., 2016). Phenolic compounds are widely recognized for their structural diversity and multifunctional bioactivity, including free radical stabilization and metal chelation properties (Pandey & Rizvi, 2009). Although in-vitro bioactivity assays were intentionally excluded in the present investigation, the quantified phytochemical richness supports the rationale for further pharmacological exploration.

From a formulation perspective, the successful incorporation of the extract into a polyethylene glycol base demonstrates compatibility between hydrophilic phytoconstituents and semisolid delivery systems. PEG-based ointments are widely used due to their stability, non-greasy texture, and enhanced patient acceptability (Allen et al., 2011). The absence of phase separation or physicochemical degradation under accelerated conditions indicates structural integrity and preliminary shelf stability.

Maintenance of pH within physiological skin range is particularly significant, as deviations may compromise the acid mantle and increase susceptibility to irritation or microbial colonization (Lambers et al., 2006). The formulation's stable pH suggests suitability for topical application.

Taken together, these findings position *Clitoria ternatea* as a promising candidate for phytopharmaceutical formulation development. However, advanced analytical characterization techniques such as HPLC, LC-MS/MS, and stability-indicating assays would further strengthen chemical profiling and standardization, which are essential for regulatory compliance and international commercialization (ICH, 2003).

7. Conclusion

The present investigation provides systematic phytochemical characterization and pharmaceutical formulation development of *Clitoria ternatea* flower extract. Hydroethanolic extraction yielded a phytochemically enriched extract with substantial phenolic and flavonoid content. Qualitative and quantitative analyses confirm the presence of bioactive polyphenolic constituents consistent with previously documented anthocyanin-rich profiles.

The successful development of a stable polyethylene glycol-based ointment demonstrates the feasibility of incorporating *Clitoria ternatea* extract into a topical semisolid dosage form without compromising physicochemical stability. The formulation maintained organoleptic integrity, dermatologically acceptable pH, and stability under accelerated storage conditions.

These findings contribute to the growing body of evidence supporting the pharmaceutical potential of *Clitoria ternatea*. Future research should focus on advanced phytochemical standardization, long-term stability studies, and clinical evaluation to establish therapeutic applicability and regulatory acceptance at an international level.

8. References

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