Phytochemical Profiling and Pharmacological Investigation of Butterfly Pea Leaves (*Clitoria ternatea* L.)

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Abstract

Butterfly pea (*Clitoria ternatea* L.) is a medicinal legume widely used in traditional systems of medicine and increasingly explored as a source of nutraceuticals, cosmetics, and pharmacologically active natural products. Although the blue petals have received the greatest scientific attention, the leaves also represent a promising reservoir of bioactive metabolites. Reported leaf constituents include phenolics, flavonoids, tannins, saponins, alkaloids, terpenoids, glycosides, steroids, and triterpenoid biomarkers such as taraxerol, which together contribute to antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, antinociceptive, and wound-healing activities. Standardization and analytical profiling have relied on qualitative screening, spectrophotometric quantification, RP-HPLC, GC-MS, and more advanced hyphenated methods such as LC–MS and UHPLC-Q-TOF-MS in related *Clitoria ternatea* studies. However, leaf-specific evidence remains fragmented, with many studies still limited to in vitro assays and small animal experiments. Future work should emphasize compound isolation, metabolomic fingerprinting, toxicity evaluation, and clinical validation to support evidence-based application of butterfly pea leaves in medicinal and functional products.

Keywords: *Clitoria ternatea*, butterfly pea leaves, phytochemical profiling, pharmacological activity, antioxidant, flavonoids, phenolics, wound healing, hepatoprotection

1. Introduction

Clitoria ternatea L. (Fabaceae), commonly known as butterfly pea, is a perennial climbing herb native to tropical and subtropical regions. It has long been used in Ayurveda and folk medicine for inflammatory conditions, fever, skin disorders, neurological complaints, and metabolic diseases. Research on the species has expanded rapidly because of its broad ethnomedicinal use and its rich phytochemical diversity, including anthocyanins, flavonoids, cyclotides, lignans, and triterpenoids.

Most publications have focused on the flowers because of their intense blue pigments and cosmetic/food applications. Nevertheless, the leaves are equally important because they are readily available, renewable, and often richer in certain non-pigmented phenolic constituents than the flowers in some extraction systems. Leaf extracts have demonstrated antioxidant and hepatoprotective activities, and standardized fractions have been linked to wound-related enzyme inhibition. Therefore, a review centered on butterfly pea leaves is timely and useful for guiding future pharmacognostic and pharmacological research.

Figure 1. Butterfly pea leaves

2. Botanical and Ethnomedicinal Background

Butterfly pea is a twining perennial herb with compound leaves and showy papilionaceous flowers. Its medicinal reputation is supported by longstanding traditional use in treating fever, inflammation, rheumatism, respiratory complaints, and memory-related disorders. In the broader phytochemical literature on the plant, all organs are recognized as chemically active, but vegetative tissues such as leaves and roots are especially relevant for pharmacological standardization due to their abundance of bioactive defense metabolites, including cyclotides and triterpenes.

Leaves may be preferred over flowers in practical applications for several reasons:

1. They are available throughout the growth cycle.
2. They can be harvested repeatedly without destroying the plant.
3. They may offer a more economical raw material for extract production.
4. They provide a separate phytochemical profile that complements the flower metabolome.

3. Phytochemical Profiling of Butterfly Pea Leaves

3.1 Major Classes of Phytoconstituents

Preliminary phytochemical investigations of *C. ternatea* leaves report the presence of:
- flavonoids,
- phenolic compounds,
- tannins,
- saponins,
- alkaloids,
- terpenoids,
- glycosides,
- steroids,
- proteins and amino acids,
- carbohydrates.

Among these, phenolics and flavonoids are the most consistently associated with biological activity because of their strong redox properties and capacity to modulate inflammatory and metabolic pathways.

3.2 Phenolic and Flavonoid Content

The antioxidant potential of butterfly pea leaves has repeatedly been linked to total phenolic content (TPC) and total flavonoid content (TFC). In comparative extraction studies, methanol extracted phenolic compounds more efficiently than water, while leaves exhibited substantial radical scavenging activity. In another study, methanolic leaf extract contained high levels of phenolics and flavonoids and showed concentration-dependent antioxidant behavior, supporting the view that leaf phenolics are major contributors to bioactivity.

Although flower anthocyanins dominate much of the butterfly pea literature, leaf extracts are particularly important because they may contain a more diverse profile of non-anthocyanin phenolics and terpenoids, including taraxerol.

3.3 Biomarker Compounds

A notable phytochemical biomarker from standardized leaf extract is taraxerol. RP-HPLC standardization of *C. ternatea* leaf methanolic extract and its fractions demonstrated that taraxerol was present at a measurable level and could serve as a marker for quality control and bioactivity correlation. This is important because standardized phytochemistry is essential if leaf extracts are to be developed into reproducible herbal products.

3.4 Analytical Approaches Used in Profiling

The phytochemical profiling of butterfly pea leaves and related tissues has used a range of analytical techniques:

- **Qualitative phytochemical screening** for broad classes of metabolites.
- **Spectrophotometric assays** for TPC and TFC estimation.
- **RP-HPLC** for biomarker-based standardization, especially taraxerol.
- **GC-MS** for volatile and semi-volatile profiling in *C. ternatea* extracts, though much of this work has focused on flowers rather than leaves[9].
- **LC–MS / UHPLC-Q-TOF-MS** for deeper metabolite annotation in the species as a whole, showing the potential of advanced profiling platforms for future leaf studies.

These methods differ in resolution and structural confidence. Simple screening is useful for preliminary work, but hyphenated mass spectrometric methods are necessary for rigorous chemical identification.

4. Pharmacological Activities of Butterfly Pea Leaves

4.1 Antioxidant Activity

Antioxidant activity is the most consistently reported biological property of butterfly pea leaves. Comparative solvent studies showed that methanolic and aqueous leaf extracts both possess radical scavenging capacity, with solvent choice influencing phenolic extraction efficiency and activity magnitude.Leaf methanolic extract also showed concentration-dependent antioxidant effects and appreciable TPC and TFC values.

The biological relevance of this activity is substantial, since oxidative stress contributes to inflammation, hepatotoxicity, metabolic dysfunction, and tissue injury. Thus, leaf antioxidant effects likely underpin several downstream pharmacological outcomes.

4.2 Anti-inflammatory Activity

Anti-inflammatory effects are supported by both traditional use and experimental evidence. While many mechanistic studies in the species have been conducted on flowers, the leaf extract has shown inhibition of enzymes and mediators associated with tissue inflammation and skin repair. In the broader phytochemical framework of the species, flavonoids and phenolics can suppress inflammatory signaling by reducing oxidative stress, downregulating inflammatory enzymes, and interfering with mediator production.

4.3 Antinociceptive Activity

Methanolic leaf extract of *C. ternatea* demonstrated antinociceptive effects in rat models including hot plate, tail-flick, and formalin assays. The effect was observed alongside root extract, indicating that the leaf possesses genuine analgesic potential and may act, at least partly, through opioid-related pathways, as suggested by naloxone-based mechanistic testing. This supports the ethnomedicinal use of the plant for pain, rheumatism, and inflammatory discomfort.

4.4 Wound-Healing and Skin-Protective Potential

Standardized leaf extract has shown inhibitory activity against hyaluronidase, elastase, and matrix metalloproteinase-1, enzymes implicated in extracellular matrix degradation and skin aging/wound processes. The same study identified taraxerol as a plausible biomarker responsible for these effects. This is a particularly important finding because it links a chemically standardized leaf preparation to a defined biological endpoint.

The relevance of these results extends beyond wound management. Enzyme inhibition of this type is also of interest in cosmetic science, dermatology, and anti-aging product development.

4.5 Hepatoprotective Activity

Methanolic leaf extract of *C. ternatea* exhibited hepatoprotective effects in paracetamol-induced liver toxicity models. Treatment significantly reduced ALT, AST, and bilirubin levels and improved histopathological features compared with the toxicant control group. The authors attributed the effect largely to antioxidant action, which is biologically plausible because oxidative stress is central to drug-induced liver injury.

This finding is especially important because it indicates that leaf extracts may exert organ-protective effects rather than only symptom-level pharmacological activity.

4.6 Antimicrobial Activity

Although leaf-specific antimicrobial data are less abundant than antioxidant data, methanolic leaf extract of *C. ternatea* showed antibacterial and antibiofilm effects in a recent study. The extract inhibited *Escherichia coli* and reduced *Staphylococcus aureus* biofilm formation, indicating promising antimicrobial potential. Such activity may be related to phenolics, flavonoids, and other membrane-active secondary metabolites.

4.7 Antidiabetic Potential

Leaf-related antidiabetic potential is frequently inferred from the plant's antioxidant profile and traditional use. In the broader *C. ternatea* literature, metabolite-rich extracts have shown glycemic-lowering effects and enzyme-modulating properties, supporting the plausibility of antidiabetic action. However, compared with the flowers and roots, direct leaf-specific antidiabetic studies remain limited. This is an important research gap.

4.8 Neuroprotective Potential

Neuroprotective activity has been more intensively investigated in flowers and roots, including cholinesterase inhibition and memory enhancement. Even so, the presence of antioxidant and anti-inflammatory constituents in leaves suggests possible neuroprotective relevance, especially through protection against oxidative neuronal damage. At present, however, leaf-specific neuroprotective evidence is still preliminary, and direct validation is needed.

5. Mechanisms of Action

The pharmacological effects of butterfly pea leaves are best understood as multifactorial and synergistic rather than the result of a single compound. The main mechanisms likely include:

1. **Free-radical scavenging** through phenolic hydrogen donation and electron transfer.
2. **Inflammatory mediator suppression**, including interference with enzyme systems linked to tissue inflammation.
3. **Membrane and biofilm disruption** in microbial cells.
4. **Inhibition of tissue-degrading enzymes** such as hyaluronidase, elastase, and MMP-1.
5. **Protection against hepatotoxic oxidative injury**.
6. **Potential modulation of nociceptive pathways** in animal models.

These mechanisms are chemically plausible because the leaf contains multiple classes of compounds with overlapping biological targets.

6. Factors Influencing Leaf Phytochemical Composition

Leaf phytochemistry is not fixed. It varies according to:

- plant genotype and accession,
- geographical origin,
- soil composition,
- climate and season,
- plant age and developmental stage,
- harvesting time,
- drying conditions,
- extraction solvent and method.

This variability is a major reason why standardization is essential. Without consistent extraction and chemical fingerprinting, biological findings are difficult to compare across studies.

7. Comparison with Flowers and Other Plant Parts

The flower has historically dominated the *C. ternatea* literature because of its blue anthocyanins and food-colorant value. Flowers are rich in ternatins and glycosylated flavonols, and they have shown anti-inflammatory, antioxidant, antiglycative, and cytotoxic effects. Roots, meanwhile, are often highlighted in neuroprotective and anticholinesterase studies.

Leaves differ from flowers in several ways:

- They are more suitable for sustainable harvesting.
- They often provide stronger support for wound-healing and hepatoprotective studies.
- They may contain more useful triterpenoid markers for standardization, such as taraxerol.
- They remain underexplored relative to flowers, creating a gap in the literature.

Thus, a leaf-centered review is scientifically justified because it addresses an overlooked but pharmacologically relevant part of the plant.

8. Limitations of Existing Research

Despite encouraging findings, the current literature on butterfly pea leaves has several limitations:

- Many studies are **preliminary** and rely on crude extracts.
- A large portion of the evidence is based on **in vitro assays** rather than in vivo confirmation.
- **Chemical identification** is often incomplete, with little structural elucidation beyond broad phytochemical classes.
- **Standardization is inconsistent**, making reproducibility difficult[2].
- **Dose-response and toxicity data** are limited.
- **Clinical trials are essentially absent**.
- Leaf-specific work is still less developed than flower-focused research[3][11].

These limitations mean that therapeutic claims should remain cautious until stronger evidence becomes available.

9. Future Perspectives

Future investigations should prioritize the following:

1. **Comprehensive metabolomic profiling** of leaf extracts using LC–MS/MS, UHPLC-Q-TOF-MS, and NMR-based approaches.
2. **Isolation of active compounds**, especially phenolics and triterpenoid markers such as taraxerol.
3. **Standardized extraction protocols** to improve reproducibility across laboratories.
4. **Mechanistic studies** involving inflammatory pathways, oxidative-stress markers, and enzyme inhibition.
5. **In vivo pharmacology** for wound healing, liver protection, pain modulation, and metabolic disorders.
6. **Toxicological evaluation**, including acute and subchronic safety.
7. **Clinical validation** to determine efficacy, dosage, and safety in humans.
8. **Product development** for nutraceutical, cosmetic, and herbal medicinal applications.

A multidisciplinary strategy combining phytochemistry, pharmacology, toxicology, and formulation science will be essential for translational progress.

10. Conclusion

Butterfly pea leaves are a valuable but underexploited part of *Clitoria ternatea* with a notable phytochemical and pharmacological profile. Available evidence indicates the presence of phenolics, flavonoids, tannins, saponins, alkaloids, terpenoids, and related bioactive metabolites, with antioxidant, anti-inflammatory, antimicrobial, antinociceptive, hepatoprotective, and wound-related activities. Taraxerol has emerged as an important leaf biomarker, suggesting possibilities for standardization and quality control. Nevertheless, the literature remains dominated by preliminary studies. More advanced chemical characterization, robust experimental design, and clinical assessment are needed before butterfly pea leaf preparations can be confidently developed into evidence-based therapeutic products.

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