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Clitoria ternatea flowers: Phytochemical composition, biological activities and emerging applications—A review

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Abstract
Clitoria ternatea L., commonly known as butterfly pea, is a flowering plant recognized for its vivid blue petals and potential applications in food science, phytochemistry and natural-product research. The flowers contain several classes of secondary metabolites, particularly ternatin-type anthocyanins, flavonoids and other phenolic compounds. These constituents have attracted attention because of their contribution to flower pigmentation, antioxidant capacity and potential biological activities. In addition, the pH-responsive colour characteristics of the flower pigments make the plant a promising source of natural colourants for beverages, food products and emerging indicator systems. This review discusses the botanical characteristics, phytochemical composition, extraction approaches and reported biological activities of *C. ternatea* flowers. Particular attention is given to antioxidant, anti-inflammatory, antimicrobial, metabolic, neuroprotective and cytotoxic effects, while distinguishing preliminary experimental findings from established therapeutic evidence. The technological potential of the flowers as natural pigments and functional-food ingredients is also considered. The interpretation of published findings is complicated by differences in plant material, extraction solvents, analytical procedures, experimental concentrations and biological models. Moreover, evidence obtained from other plant parts cannot automatically be attributed to the flowers. Future investigations should prioritize standardized extraction, advanced chemical characterization, bioavailability assessment, appropriate toxicological evaluation and controlled human studies. Overall, *C. ternatea* flowers represent a promising botanical resource, particularly for natural pigmentation and food applications, while their pharmacological potential requires further systematic investigation.

Keywords: *Clitoria ternatea*, butterfly pea flowers, ternatins, anthocyanins, phytochemicals, antioxidant activity, natural colourants, pharmacological potential, functional foods

Introduction

Medicinal and food-associated plants represent important sources of structurally diverse natural compounds that are investigated for their nutritional, technological, and potential biological applications (Castaneda Ovando *et al.*, 2009; Khoo *et al.*, 2017) [2, 9]. Among plant-derived constituents, natural pigments have received particular attention because they contribute to product appearance and may possess chemical properties relevant to food formulation and the development of functional ingredients (Khoo *et al.*, 2017) [9]. *Clitoria ternatea* L., commonly known as butterfly pea, is a flowering plant belonging to the family Fabaceae that has attracted scientific interest because of its traditional uses, characteristic flower pigmentation, and potential applications in food and pharmaceutical research (Mukherjee *et al.*, 2008; Oguis *et al.*, 2019) [11, 12]. Its flowers are particularly recognized for their vivid blue colour, although variations in pigmentation may occur among flower varieties (Oguis *et al.*, 2019) [12]. The characteristic pigmentation of *C. ternatea* flowers is primarily associated with anthocyanins, particularly ternatins, which constitute a distinctive group of polyacylated anthocyanin derivatives (Terahara *et al.*, 1998; Oguis *et al.*, 2019) [12, 15]. The structural characteristics of anthocyanins influence their colour expression and stability, making these compounds relevant to investigations of natural colourants and their technological applications (Castaneda Ovando *et al.*, 2009; Khoo *et al.*, 2017) [2, 9]. Because anthocyanins exhibit changes in colour under different pH conditions, butterfly pea flower extracts have attracted interest as potential ingredients for food products and colour-responsive systems (Castaneda Ovando *et al.*, 2009; Khoo *et al.*, 2017) [2, 9]. Nevertheless, their performance in practical applications depends on several factors, including extraction conditions, pigment concentration, pH, temperature, light exposure, and interactions with other formulation components (Castaneda Ovando *et al.*, 2009) [2]. In addition to their pigmentation properties, *C. ternatea* flowers have been investigated for antioxidant activity and other potential biological effects (Mukherjee *et al.*, 2008; Oguis *et al.*, 2019) [11, 12]. However, the interpretation of such findings requires careful consideration of the experimental system, extract composition, tested concentration, and biological endpoint. Results obtained through chemical antioxidant assays indicate activity under the conditions of the assay but do not demonstrate therapeutic efficacy in humans (Castaneda Ovando *et al.*, 2009; Oguis *et al.*, 2019) [2, 12]. Chemical antioxidant assays, cell-based investigations, animal experiments, and human clinical studies provide different levels of evidence and should not be interpreted interchangeably (Halliwell, 2007) [6]. Another important consideration is the selection of plant material. Research on *C. ternatea* encompasses several plant organs, including flowers, leaves, roots, and seeds, which differ in their chemical composition and may exhibit different biological

properties (Mukherjee *et al.*, 2008; Oguis *et al*., 2019) [11, 12]. Consequently, findings obtained from the leaves, roots, or whole plant cannot automatically be attributed to the flowers. Flower-specific investigations are therefore necessary to establish the identity, concentration, stability, and biological relevance of compounds present in floral extracts. Against this background, the present review focuses specifically on the flowers of *C. ternatea*. It examines their phytochemical composition, extraction and analytical methods, reported biological activities, potential applications as natural food colourants, safety considerations, and prospects for future research. Particular emphasis is placed on distinguishing established chemical findings from preliminary biological observations and on identifying the evidence required to support their potential use in food-related, biotechnological, and pharmaceutical applications.



Image: *Clitoria ternatea* L.

Botanical Characteristics and Taxonomic Position

Clitoria ternatea L., belonging to the family Fabaceae and subfamily Papilionoideae, is a climbing or trailing plant known for its ornamental flowers and traditional uses (Mukherjee *et al*., 2008; Oguis *et al*., 2019) [11, 12]. Its flowers are predominantly deep blue, although white and other colour variations also occur (Oguis *et al*., 2019) [12]. The characteristic blue pigmentation is primarily attributed to ternatins, a group of polyacylated anthocyanins that have attracted interest for their chemical properties and potential applications as natural food colourants (Terahara *et al*., 1998 [15]; Castaneda Ovando *et al*., 2009; Khoo *et al*., 2017) [2, 9]. Their pH-dependent colour changes and sensitivity to environmental conditions make butterfly pea flowers relevant to natural pigmentation and colour-responsive food systems (Castaneda Ovando *et al*., 2009; Khoo *et al*., 2017; Oguis *et al*., 2019) [2, 9, 12].

Table 1: Taxonomic classification of *Clitoria ternatea* L.

Taxonomic rank	Classification
Kingdom	Plantae
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Fabales
Family	Fabaceae
Subfamily	Papilionoideae
Genus	*Clitoria*
Species	*Clitoria ternatea* L.

Distribution, Traditional Use and Contemporary Interest

Clitoria ternatea is a tropical leguminous plant cultivated in tropical and subtropical regions. It is valued for its ornamental flowers and traditional applications, which have contributed to scientific interest in its botanical and phytochemical properties (Mukherjee *et al*., 2008; Oguis *et al*., 2019) [11, 12]. The flowers are particularly appreciated for their intense blue pigmentation, which can be extracted into aqueous preparations. This characteristic has encouraged research into their potential use as naturally derived colourants in foods and beverages (Oguis *et al*., 2019) [12]. Traditional medicinal uses of *C. ternatea* have been documented in ethnopharmacological literature, providing a historical context for investigating its chemical constituents and potential biological properties (Mukherjee *et al*., 2008) [11]. Nevertheless, traditional use should be distinguished from experimentally established efficacy. Historical use

alone does not demonstrate that a preparation is effective for a particular disease or that it is safe at every concentration or under all conditions of use. The composition of a traditional preparation may differ substantially from that of a laboratory extract because of differences in plant material, extraction solvent, temperature, duration, and concentration. Consequently, the biological properties of a concentrated flower extract should not be assumed to be equivalent to those of a traditionally consumed flower infusion. Contemporary interest in butterfly pea flowers is strongly associated with their distinctive pigmentation and the chemical behaviour of their anthocyanins. Changes in pH can alter anthocyanin colour, making these pigments relevant to research on naturally coloured beverages, food formulations, and pH-responsive materials (Castaneda Ovando *et al*., 2009; Khoo *et al.*, 2017) [2, 9]. However, the practical performance of flower extracts depends on pigment stability, processing conditions, interactions with other ingredients, and storage conditions. Research into the potential biological activities of *C. ternatea* has also expanded beyond its traditional applications. Nevertheless, claims concerning disease prevention or treatment require evidence appropriate to the specific biological effect being investigated. Findings from chemical assays or preliminary laboratory experiments should not be presented as established clinical benefits. Similarly, the safety of concentrated extracts requires appropriate evaluation and cannot be inferred solely from the historical consumption of dilute flower preparations. Therefore, the contemporary study of *C. ternatea* flowers should integrate botanical authentication, phytochemical characterization, standardized experimental methods, and appropriate safety assessments. Such an approach can help establish the scientific basis for their potential applications in food science, biotechnology, and pharmaceutical research.

Phytochemical Composition of the Flowers

The phytochemical properties of *Clitoria ternatea* flowers are associated with their secondary metabolites, particularly anthocyanins and other phenolic constituents. The composition of a flower extract may vary according to cultivar, geographical origin, developmental stage, environmental conditions, sample processing, extraction solvent, and analytical methodology (Castaneda Ovando *et al.*, 2009; Dai and Mumper, 2010) [2, 5]. Consequently, the chemical profile reported for one preparation should not automatically be considered representative of all *C. ternatea* flower extracts. Appropriate phytochemical characterization is therefore necessary to establish the identity and relative abundance of constituents in individual preparations.

1. Anthocyanins and Ternatins

Anthocyanins are water-soluble flavonoid pigments responsible for the red, purple, and blue colours of numerous flowers, fruits, and other plant tissues (Castaneda Ovando *et al*., 2009; Khoo *et al*., 2017) [2, 9]. In *C. ternatea* flowers, the characteristic blue pigmentation is primarily associated with ternatins, a group of structurally distinctive, polyacylated anthocyanins derived from delphinidin-based structures (Terahara *et al.*, 1996; Oguis *et al*., 2019) [12, 16]. Their complex glycosylation and acylation patterns contribute to the distinctive pigment chemistry of butterfly pea flowers (Terahara *et al*., 1998) [15]. The structural characterization of ternatins has provided important information about the molecular basis of the unusual blue coloration of *C. ternatea* petals (Terahara *et al*., 1996, 1998) [15, 16]. In addition to their molecular structures, anthocyanins exhibit pH-dependent colour behaviour resulting from changes in their chemical equilibria and molecular forms (Castaneda Ovando *et al*., 2009) [2]. Accordingly, butterfly pea flower extracts may display different colours under different pH conditions, although the resulting colour depends on pigment composition, concentration, solvent environment, and other formulation variables (Khoo *et al*., 2017) [9]. These properties have attracted interest in the use of butterfly pea anthocyanins as natural colourants and in the development of pH-responsive food materials. Nevertheless, their suitability for particular applications requires evaluation of pigment stability under the relevant processing, storage, and formulation conditions (Castaneda Ovando *et al.*, 2009; Khoo *et al*., 2017) [2, 9].

2. Flavonoids

Flavonoids constitute a diverse class of plant secondary metabolites that includes anthocyanins, flavonols, flavones, flavanones, and other structurally related compounds (Panche *et al.*, 2016). Anthocyanins are themselves members of the flavonoid family; therefore, their occurrence in *C. ternatea* flowers should be distinguished from that of other flavonoid subclasses. The presence and abundance of individual flavonoids in a flower extract depend on the plant material and the analytical methods used to investigate it. Flavonoid-rich plant extracts are frequently investigated for their chemical and biological properties. However, the identification of a broad flavonoid group does not establish the presence of every compound belonging to that group in *C. ternatea* flowers. Reliable identification of individual constituents requires appropriate analytical evidence, preferably obtained through chromatographic separation combined with spectrometric techniques, such as liquid chromatography–mass spectrometry (LC–MS), and comparison with suitable reference standards where available (Dai and Mumper, 2010) [5]. Accordingly, phytochemical studies of butterfly pea flowers should distinguish between compounds identified unambiguously, compounds tentatively assigned from analytical data, and constituents inferred only from preliminary screening tests. This distinction is important for producing a chemically reliable account of the flower's composition.

3. Phenolic Compounds

Phenolic compounds comprise a broad group of plant metabolites containing one or more aromatic rings with hydroxyl substituents. Their chemical structures vary considerably, and different phenolic compounds may exhibit different responses in antioxidant assays and other analytical procedures (Dai and Mumper, 2010) [5]. Phenolic constituents in *C. ternatea* flower preparations are therefore relevant to investigations of their chemical composition and potential biological properties. The recovery of phenolic compounds depends on factors such as solvent polarity, extraction temperature, extraction duration, and the condition of the starting material (Dai and Mumper, 2010) [5]. Total phenolic content is commonly estimated using the Folin–Ciocalteu method. However, this assay measures overall reducing capacity under the specified experimental conditions and is not exclusively specific to phenolic molecules; other reducing substances may contribute to the

observed response (Singleton *et al*., 1999) [14]. Consequently, total phenolic content is useful for comparing preparations when the analytical procedure, calibration standard, and reporting units are consistent. Nevertheless, this measurement does not establish the identity or concentration of individual phenolic compounds. More detailed characterization using chromatographic and spectrometric methods is required to determine the specific molecules responsible for the measured response (Dai and Mumper, 2010) [5].

4. Variation in Phytochemical Composition

The phytochemical composition of *C. ternatea* flowers may vary with cultivar, environmental conditions, flower maturity, sample drying, storage, and extraction procedures. These variables can influence pigment stability and the recovery of individual chemical constituents (Castaneda Ovando *et al*., 2009; Dai and Mumper, 2010) [2, 5]. Differences in extraction solvent and processing conditions may also produce preparations with different relative proportions of anthocyanins and other extractable compounds. Such variation is important when comparing results across studies. Differences in measured anthocyanin concentration or total phenolic content may reflect differences in the starting material, extraction efficiency, analytical procedure, or reporting method rather than genuine biological differences between plant samples. Future investigations should therefore report the botanical identity and source of the material, flower colour and maturity where relevant, drying and storage procedures, extraction solvent and conditions, analytical methods, calibration standards, and quantitative results. Where possible, researchers should also include chromatographic profiles and appropriate measures of analytical uncertainty. Standardized reporting would improve reproducibility and facilitate meaningful comparisons among *C. ternatea* flower extracts.

Table 2: Major Phytochemicals of *Clitoria ternatea* Flowers

Phytochemical group	Representative constituents or description	Potential significance	Reference
Anthocyanins	Delphinidin-derived pigments	Contribute to flower pigmentation and pH-dependent colour behaviour	Terahara *et al.* (1996) [16]; Castaneda Ovando *et al*. (2009) [2]
Ternatins	Polyacylated anthocyanins derived from delphinidin-based structures	Responsible for characteristic blue pigmentation; relevant to research on pigment stability	Terahara *et al.* (1998); Oguis *et al.* (2019) [12, 15]
Flavonoids	Flavonol derivatives and glycosides	May contribute to the chemical and biological properties of flower extracts	Kazuma *et al.* (2003) [8]; Panche *et al.* (2016)
Phenolic compounds	Diverse phenolic constituents	May contribute to measured reducing capacity and radical-scavenging activity	Dai and Mumper (2010); Jeyaraj *et al.* (2021) [5, 7]
Other extractable metabolites	Constituents whose recovery depends on plant material and extraction conditions	May contribute to the overall chemical profile; individual compounds require appropriate analytical identification	Dai and Mumper (2010); Jeyaraj *et al.* (2021) [5, 7]

Extraction and Analytical Approaches

Extraction is a fundamental step in investigating the phytochemical composition and potential biological properties of *Clitoria ternatea* flowers. The efficiency and selectivity of extraction depend on several factors, including solvent composition, solvent-to-sample ratio, extraction time, temperature, pH, and the physical characteristics of the starting material (Dai and Mumper, 2010) [5]. Consequently, extracts prepared using different procedures may exhibit differences in pigment concentration, phenolic composition, and measured chemical activity. Water is particularly relevant when preparing butterfly pea flower extracts intended for beverages and food applications because it allows the recovery of water-soluble pigments, including anthocyanins (Castaneda Ovando *et al.,* 2009) [2]. Ethanol and hydroethanolic mixtures may also be used to investigate a broader range of extractable phenolic constituents, depending on the extraction conditions and the polarity of the target compounds (Dai and Mumper, 2010) [5]. Methanol is frequently used in analytical investigations of plant constituents; however, methanolic extracts should not be assumed suitable for direct food consumption without an appropriate, validated solvent-removal and safety process. Anthocyanin stability is another important consideration during extraction and subsequent storage. Temperature, pH, light exposure, oxygen, and the composition of the surrounding medium can influence anthocyanin stability and colour expression (Castaneda Ovando *et al.,* 2009; Patras *et al.,* 2010) [2, 13]. Therefore, extraction and storage conditions should be selected according to the intended application, with appropriate precautions taken to minimize pigment degradation.

1. Analytical Techniques for Phytochemical Characterization

Several complementary analytical techniques can be used to characterize *C. ternatea* flower extracts. UV–visible spectroscopy provides information about pigment absorption and can be used to monitor changes in colour under different experimental conditions (Castaneda Ovando *et al.,* 2009) [2]. High-performance liquid chromatography (HPLC) facilitates the separation and quantification of selected constituents, while liquid chromatography–mass spectrometry (LC–MS) provides additional information about their molecular masses and fragmentation patterns (Dai and Mumper, 2010) [5]. Total phenolic assays are commonly used to estimate the overall reducing response of plant extracts. However, the Folin–Ciocalteu method is not specific exclusively to phenolic compounds because other reducing substances may also contribute to the measured response (Singleton *et al.,* 1999) [14]. Accordingly, total phenolic content should be interpreted as an assay-dependent estimate rather than a direct measurement of the concentration of every phenolic constituent. Chemical antioxidant assays, including DPPH, ABTS, and ferric reducing antioxidant power (FRAP), provide complementary measurements of antioxidant-related chemical activity. These assays operate through different

reaction mechanisms and experimental conditions, so their results are not necessarily interchangeable (Apak *et al.*, 2016) [1]. Moreover, activity demonstrated in a chemical assay does not independently establish biological efficacy in cells, animals, or humans. Combining chromatographic and spectrometric characterization with appropriately selected chemical or biological assays provides a stronger basis for investigating relationships between extract composition and observed activity. Nevertheless, a relationship between the presence of a compound and an observed effect does not establish causation without additional experimental evidence (Dai and Mumper, 2010) [5].

Table 3: Common extraction and analytical approaches for investigating *Clitoria ternatea* flower extracts

Method	Principal application	Important consideration	Reference
Aqueous extraction	Recovery of water-soluble pigments for research and potential food applications	Extraction efficiency depends on pH, temperature, time, and solvent-to-sample ratio	Castaneda-Ovando *et al.* (2009) [2]
Ethanol or hydroethanolic extraction	Recovery of selected phenolic and other extractable constituents	Solvent composition influences extraction selectivity	Dai and Mumper (2010) [5]
UV–visible spectroscopy	Examination of pigment absorption and monitoring of colour changes	Absorption depends on pigment concentration, pH, and measurement conditions	Castaneda Ovando *et al*. (2009) [2]
High-performance liquid chromatography (HPLC)	Separation and quantification of selected constituents	Reliable quantification requires suitable standards and validated analytical conditions	Dai and Mumper (2010) [5]
Liquid chromatography–mass spectrometry (LC–MS)	Characterization of compounds using chromatographic separation and mass-spectrometric data	Compound identification should consider mass accuracy, fragmentation, and reference evidence	Dai and Mumper (2010) [5]
Folin–Ciocalteu assay	Estimation of total reducing capacity commonly reported as total phenolic content	The assay is not specific exclusively to phenolic compounds	Singleton *et al*. (1999) [14]
DPPH assay	Measurement of free-radical-scavenging activity under defined conditions	Results depend on solvent, reaction time, and assay conditions	Apak *et al.* (2016) [1]
ABTS assay	Evaluation of antioxidant-related activity using the ABTS radical cation	Results should be interpreted using the specified assay protocol	Apak *et al.* (2016) [1]
FRAP assay	Measurement of ferric reducing antioxidant power	Measures reducing capacity rather than every possible mechanism of antioxidant activity	Apak *et al.* (2016) [1]

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2. Methodological Considerations and Future Directions

Comparisons between studies require careful attention to sample preparation, extraction procedures, analytical calibration, and reporting units. Researchers should document the botanical identity of the flowers, collection conditions, drying procedure, extraction solvent, solvent-to-sample ratio, extraction duration, temperature, and storage conditions. Where possible, quantitative measurements should be accompanied by replicate data, appropriate reference standards, and estimates of analytical uncertainty. Future research would benefit from standardized protocols for quantifying major ternatins and comparing pigment stability across different extraction conditions. Validated chromatographic methods could improve the comparability of results between laboratories, while LC–MS-based characterization could support more detailed investigation of individual constituents. Linking these chemical data with carefully designed biological experiments would help clarify which components contribute to particular observed effects and whether those effects have relevance beyond the analytical system in which they were measured.

Neuroprotective and Cognitive Potential

Clitoria ternatea has a history of traditional use associated with memory and cognitive function, which has encouraged scientific investigations into its potential neuroactive properties (Mukherjee *et al*., 2008) [11]. Research on the species has explored possible biological mechanisms relevant to neurological function; however, findings must be interpreted according to the plant part, preparation, and experimental model investigated (Oguis *et al.,* 2019) [12]. Anthocyanins and other phenolic compounds present in *C. ternatea* flowers provide a rationale for investigating their potential effects on oxidative processes associated with neuronal function. Nevertheless, the presence of these compounds does not establish that flower extracts possess neuroprotective or cognition-enhancing effects. Evidence obtained using roots, leaves, or whole-plant preparations cannot automatically be attributed to the flowers because different plant organs may contain different chemical constituents. The interpretation of potential neuroprotective effects also requires consideration of absorption, metabolism, distribution, and the ability of relevant compounds or their metabolites to reach the central nervous system. These factors influence whether concentrations associated with experimental effects could plausibly be achieved following consumption. General research on dietary polyphenols emphasizes that biological activity observed *in-vitro* does not necessarily translate into comparable effects in humans (Manach *et al.,* 2004) [10]. Future investigations should therefore prioritize chemically characterized flower extracts, validated neurological models, and clearly defined endpoints. Studies assessing neuronal viability, oxidative stress, inflammatory responses, and relevant molecular pathways may help clarify possible mechanisms. Ultimately, controlled human studies using validated cognitive assessments would be required to determine whether any observed effects translate into meaningful cognitive benefits.

Anticancer and Cytotoxic Potential

Plant-derived compounds have attracted considerable interest in cancer research because some can influence cellular processes associated with proliferation, oxidative

stress, apoptosis, and intracellular signalling. The anthocyanins and other phenolic constituents reported in *Clitoria ternatea* flowers provide a basis for investigating their potential biological effects. However, the presence of these compounds does not establish that a particular flower extract possesses anticancer efficacy. Evidence must be interpreted according to the preparation tested. Findings involving isolated compounds, other plant organs, or whole-plant extracts cannot automatically be attributed to an aqueous flower extract or a chemically different preparation. The composition, concentration, and stability of the tested material may substantially influence the observed response. *In-vitro* cytotoxicity studies can provide preliminary information about the effects of an extract on cancer-cell viability. However, reduced viability does not necessarily demonstrate selective anticancer activity. An extract may damage both malignant and non-malignant cells, and apparent cytotoxicity may also be influenced by experimental conditions or interference with the analytical assay. Consequently, appropriate controls and independent validation are essential when interpreting cell-based results (Riss *et al.*, 2016). A rigorous evaluation should compare cancer cells with suitable non-cancerous cell models and investigate concentration-dependent effects at defined exposure times. Where preliminary findings justify further investigation, additional experiments may examine apoptosis, cell-cycle changes, and relevant molecular pathways. These experiments should distinguish between demonstrated mechanisms and hypotheses requiring further testing. Preclinical investigations may subsequently help establish whether an observed effect is reproducible in more complex biological systems. Nevertheless, activity in cultured cells or animal models does not establish clinical efficacy. Further research is required to determine the selectivity, bioavailability, safety, and potential therapeutic relevance of chemically characterized *C. ternatea* flower preparations.

Hepatoprotective Potential

The liver plays a central role in nutrient metabolism, the biotransformation of foreign compounds, and the regulation of several physiological processes. Oxidative stress, inflammatory responses, and other cellular disturbances can contribute to liver injury, encouraging research into substances that may influence these processes (Cichoz Lach and Michalak, 2014) [4]. The phytochemical composition of *Clitoria ternatea* flowers provides a basis for investigating their potential biological effects in appropriate experimental models. However, the presence of anthocyanins or other compounds with antioxidant-related properties does not establish that a flower extract protects the liver. Direct experimental evidence is necessary to determine whether a particular preparation produces measurable effects in relevant models of hepatic injury. A rigorous investigation should begin with botanical authentication and chemical characterization of the flower extract. Preclinical studies may assess relevant biochemical markers alongside histological observations and other validated indicators of liver injury. Changes in individual biochemical measurements should not be interpreted in isolation because they may reflect different physiological processes and do not independently establish a protective mechanism. The dose, route of administration, duration of exposure, and composition of the preparation must also be reported clearly. Safety assessment is particularly important because a preparation that produces an apparent beneficial effect at one concentration may have different effects at higher concentrations or under different exposure conditions. Furthermore, evidence from animal experiments should be distinguished from evidence obtained in human studies. The therapeutic use of *C. ternatea* flower extracts for liver disease cannot be recommended solely on the basis of their phytochemical composition or antioxidant activity. Additional research is required to establish reproducibility, biological relevance, safety, and potential clinical value.

Food and Nutraceutical Applications

The flowers of *Clitoria ternatea* have attracted interest in food science because their water-extractable pigments can impart distinctive blue coloration to aqueous preparations. Their anthocyanin composition has encouraged investigations into their potential use in beverages and other food products requiring naturally derived colourants (Oguis *et al.*, 2019) [12]. In addition to their colouring properties, the flowers contain phytochemicals that warrant further investigation for potential applications in functional-food and nutraceutical research. However, the presence of phytochemicals does not automatically establish a health benefit. Functional-food claims should be supported by appropriate evidence demonstrating the relevant biological effect under the conditions of intended consumption. Similarly, findings obtained using concentrated extracts or isolated compounds cannot automatically be extrapolated to traditionally prepared flower beverages. The development of butterfly pea flower ingredients requires consideration of sensory properties, pigment stability, processing conditions, formulation compatibility, and microbiological quality. Anthocyanin stability may be influenced by temperature, pH, light exposure, oxygen, and interactions with other ingredients (Castaneda Ovando *et al.*, 2009; Patras *et al.*, 2010) [2, 13]. These factors should be evaluated under the conditions relevant to the intended food product. For nutraceutical applications, additional requirements include chemical standardization, reproducibility between batches, appropriate intake levels, bioavailability, and preparation-specific safety assessment. The identity and concentration of the principal constituents should be established before biological findings are used to support product-development claims. Consequently, further research is needed to determine whether standardized flower preparations offer benefits beyond their established technological properties as natural pigments.

Table 4: Potential food and nutraceutical applications of *Clitoria ternatea* flowers

Application	Relevant flower property	Development consideration	Reference
Herbal beverages	Water-extractable blue pigments	Colour stability, taste, preparation consistency, and microbiological quality	Chusak *et al.* (2018); Oguis *et al.* (2019) [3, 12]
Natural food colourants	Anthocyanin-based pigmentation	Stability during processing and storage	Castaneda Ovando *et al.* (2009); Oguis *et al.* (2019) [2, 12]
pH-responsive beverages	pH-dependent anthocyanin colour behaviour	Reproducibility of colour changes within the formulation	Castaneda Ovando *et al.* (2009) [2]
Functional-food research	Presence of potentially bioactive phytochemicals	Requires evidence of biological relevance at realistic exposure levels	Oguis *et al.* (2019) [12]

Natural Colourant and pH-Responsive Applications

One of the most distinctive technological characteristics of *Clitoria ternatea* flowers is their ability to provide blue pigmentation through anthocyanin-rich extracts. Natural pigments have attracted interest in food science as potential alternatives to selected synthetic colourants, although their suitability depends on stability, performance, safety, and applicable regulatory requirements (Khoo *et al.,* 2017) [9]. Anthocyanins exhibit pH-dependent structural changes that can alter their visible colour. In butterfly pea flower extracts, the observed colour depends on factors including pH, pigment composition, concentration, and the surrounding chemical environment (Castaneda Ovando *et al.,* 2009) [2]. Consequently, the colour produced by a simple aqueous extract may differ from that obtained in a complex food formulation containing sugars, acids, proteins, or other ingredients. These characteristics make butterfly pea flowers relevant to investigations involving beverages, confectionery, desserts, and other products requiring distinctive blue or pH-responsive coloration. Their pigments may also be investigated for indicator applications in which a visible colour change provides information about changes in the surrounding environment. However, such applications require systematic evaluation of response time, colour reproducibility, stability, and performance under the intended conditions. Commercial development requires additional consideration of pigment yield, batch-to-batch variation, processing stability, storage conditions, ingredient compatibility, and microbiological quality. The chemical composition of the pigment preparation should also be characterized to support reproducibility and quality control. Finally, natural origin alone does not establish regulatory approval for a particular food application. The intended use, preparation, purity, exposure level, and applicable jurisdiction must be considered before a flower-derived colourant is introduced into a commercial product.

Table 5: Technological properties relevant to natural colourant development

Property	Potential application	Technical challenge	Reference
Blue pigmentation	Colouring beverages and selected foods	Maintaining the desired colour during processing and storage	Oguis *et al.* (2019) [12]
pH-dependent colour response	Visual indicators and responsive formulations	Establishing reproducible colour responses under different conditions	Castaneda Ovando *et al.* (2009) [2]
Water-based extraction	Aqueous food and beverage preparations	Optimizing pigment recovery and maintaining microbiological quality	Dai and Mumper (2010) [5]
Anthocyanin composition	Development of standardized colourant preparations	Establishing chemical consistency between batches	Terahara *et al.* (1998) [15]
Natural-origin pigments	Potential alternatives to selected synthetic colourants	Demonstrating stability, safety, suitability, and regulatory compliance	Khoo *et al.* (2017) [9]

Safety and Toxicological Considerations

Safety assessment is essential when evaluating botanical ingredients for food, nutraceutical, or pharmaceutical development. Although *Clitoria ternatea* has a history of traditional use, this does not establish the safety of every extract, dose, or formulation (Mukherjee *et al*., 2008) [11]. The safety profile of a preparation may depend on the plant material, extraction solvent, chemical composition, concentration, route of administration, and duration of exposure. Concentrated extracts and isolated compounds may produce exposures substantially different from those associated with traditional flower beverages. Consequently, safety findings obtained using one preparation should not automatically be generalized to another preparation with a different chemical profile or concentration. Appropriate toxicological investigations should be selected according to the intended application and available evidence. Depending on the circumstances, these may include acute and repeated-dose toxicity studies, assessment of organ-specific effects, and genotoxicity testing where scientifically justified. Reproductive and developmental toxicity may also require consideration when the intended population or pattern of use makes these endpoints relevant. Human studies should clearly report the preparation investigated, amount consumed, duration of exposure, participant characteristics, and adverse events. Evidence from a small or short-term human investigation may provide information about tolerability under the conditions studied, but it cannot establish the long-term safety of all flower preparations. For food applications, the extract must also satisfy the applicable requirements for the intended use. Botanical origin alone does not establish regulatory approval, and the regulatory status of a traditional beverage may differ from that of a concentrated extract, isolated pigment, or standardized nutraceutical preparation. Overall, further preparation-specific research is needed to establish the safety, appropriate exposure levels, and potential interactions of *C. ternatea* flower products. A scientifically defensible assessment should integrate botanical authentication, chemical characterization, toxicological evidence, and the regulatory requirements relevant to the proposed application.

Table 6: Safety considerations for *Clitoria ternatea* flower preparations

Safety aspect	Information required	Importance	Reference or evidence requirement
Botanical identity	Verified species and plant part	Prevents confusion between different botanical materials	Mukherjee *et al.* (2008) [11]
Human tolerability	Adverse events and relevant clinical observations	Assesses safety under the conditions investigated	Chusak *et al.* (2018) [3]

Research Gaps and Future Perspectives

Despite increasing scientific interest in *Clitoria ternatea* L. flowers, several methodological and evidential limitations continue to restrict comparisons among studies and the translation of laboratory findings into practical applications. Variations in plant material, extraction procedures, phytochemical composition and experimental design can influence reported outcomes. Addressing these limitations through standardized methods and more rigorous research would improve the reproducibility and practical relevance of future investigations (Dai and Mumper, 2010; Oguis *et al.*, 2019) [5, 12].

1. Standardization of Plant Material

Reliable characterization of *C. ternatea* flowers requires consistent documentation of botanical identity, flower colour, developmental stage, geographical origin, harvesting conditions and post-harvest processing. Differences in flower colour and developmental characteristics may be associated with variations in flavonoid composition and pigment profiles (Kazuma *F*, 2003) [8]. Future studies should provide authenticated botanical identification and clearly describe sample collection, drying, storage and preparation procedures. Such reporting would help researchers determine whether differences in experimental outcomes arise from biological variation or methodological inconsistencies.

2. Improved Phytochemical Characterization

Although ternatin-type anthocyanins are important contributors to the characteristic blue pigmentation of *C. ternatea* flowers, comprehensive chemical characterization remains essential for understanding the composition of individual extracts. Future investigations should combine validated chromatographic separation with suitable spectrometric techniques, such as high-performance liquid chromatography coupled with mass spectrometry (HPLC–MS), where appropriate. Quantification of individual compounds, rather than relying exclusively on measurements of total anthocyanins, flavonoids or phenolic content, would provide a more detailed chemical profile (Castaneda Ovando *et al*., 2009; Dai and Mumper, 2010) [2, 5]. Linking these profiles with biological or technological outcomes may help identify constituents associated with specific effects, although such relationships would require further experimental confirmation.

3. Harmonization of Extraction Methods

Extraction conditions can substantially influence the recovery and stability of plant pigments and other phytochemicals. Solvent composition, pH, temperature, extraction duration, sample-to-solvent ratio and subsequent storage conditions should therefore be reported consistently. Differences in these parameters can complicate comparisons of extraction yields, chemical composition and measured biological activity (Dai and Mumper, 2010) [5]. Future research should establish reproducible extraction protocols appropriate to the intended application and report sufficient methodological detail to permit independent replication. Where different protocols are compared, extraction efficiency and chemical stability should be assessed alongside biological activity.

4. Stronger Biological Evidence

Chemical antioxidant assays and preliminary cell-based experiments are useful for screening plant extracts, but their results should not be interpreted as direct evidence of therapeutic efficacy. Antioxidant assays measure particular chemical reactions under defined experimental conditions, and their outcomes may not reflect biological activity in living systems (Apak *et al.*, 2016) [1]. Similarly, cytotoxicity observed in cultured cells does not, by itself, demonstrate that a preparation is effective or safe in humans. Further investigations should use well-characterized extracts and appropriate controls to establish concentration–response relationships, assess selectivity between target and non-target cells, and investigate plausible mechanisms of action. Studies of absorption, metabolism, bioavailability and toxicity would also be necessary before considering clinical applications.

5. Clear Distinction Between Plant Parts

A recurring challenge in evaluating the literature is the potential for findings obtained from different parts of *C. ternatea* to be discussed collectively. Flowers, leaves, roots and seeds may differ in their chemical composition, and evidence obtained from one plant part cannot automatically be attributed to another. Although the species has been investigated for a range of agricultural and medicinal applications, each preparation must be evaluated according to its specific plant source and composition (Mukherjee *et al.*, 2008; Oguis *et al.*, 2019) [11, 12]. Future publications should clearly identify the plant part examined, the extraction procedure and the composition of the tested preparation. This distinction is particularly important when assessing flower-specific pharmacological claims.

6. Translation into Food Applications

The characteristic pigmentation and pH-responsive colour behaviour of *C. ternatea* flowers provide a clear rationale for continued investigation as a source of natural colourants. However, practical application requires more than demonstrating a colour change in a simple laboratory solution. Anthocyanin stability can be affected by pH, temperature, light, oxygen and interactions with other food constituents (Castaneda Ovando *et al.*, 2009; Patras *et al.*, 2010) [2, 13]. Future studies should evaluate pigment retention, colour consistency and degradation during processing and storage in representative food formulations. Compatibility with other ingredients, packaging conditions and intended storage periods should also be investigated. These assessments would help determine whether the pigments remain suitable under realistic conditions of use.

7. More Robust Human Evidence

Human studies involving *C. ternatea* preparations remain important for determining whether effects observed in laboratory experiments translate into measurable physiological outcomes. For example, a randomized crossover study investigated the acute effects of a *C. ternatea* flower beverage on glycaemic response and antioxidant capacity in healthy participants (Chusak *et al.*, 2018) [3]. However, an acute study involving a specific preparation cannot establish long-term health benefits or be generalized to all flower extracts and formulations. Future clinical investigations should use chemically characterized preparations, appropriate control groups, adequate sample sizes, clearly defined primary outcomes and transparent statistical methods. Dose, duration, adverse events and relevant participant characteristics should also be reported. Long-term health claims should remain provisional until supported by suitable clinical evidence.

Conclusion

Clitoria ternatea L. flowers represent a promising botanical source of naturally occurring pigments, particularly ternatin-type anthocyanins, which contribute to their distinctive blue coloration (Kazuma *et al.*, 2003; Oguis *et al.*, 2019) [8, 12]. Their pigmentation and pH-responsive colour behaviour support continued investigation into their potential use as natural colourants and ingredients in food-related applications. In addition to anthocyanins, the flowers contain other phytochemical groups, including flavonoids and phenolic compounds, that warrant further investigation in relation to their chemical properties and possible biological activities. Antioxidant activity and other potential pharmacological effects have attracted scientific interest; however, the strength of evidence varies according to the activity investigated, the preparation tested and the experimental model used. Chemical antioxidant assays, *in-vitro* experiments and preliminary human studies provide different levels of evidence and should not be treated as interchangeable. In particular, activity demonstrated by an extract in a laboratory model does not establish clinical efficacy, while findings from leaves, roots, seeds or other plant-derived preparations cannot automatically be attributed to the flowers. Among the potential applications discussed, the use of *C. ternatea* flowers as a source of natural pigmentation offers a clear technological rationale for further development. Nevertheless, successful application will depend on reproducible extraction, reliable pigment characterization and adequate evaluation of colour stability, processing compatibility and storage performance. Pharmacological applications remain areas for further investigation and require stronger evidence concerning active constituents, mechanisms of action, selectivity, bioavailability and safety. Future progress will depend on integrating phytochemical analysis, food science and pharmacological research through standardized methods and transparent reporting. Well-designed experimental and clinical studies will be essential to distinguish promising preliminary observations from established benefits. With these limitations addressed, research on *C. ternatea* flowers may help establish reproducible and evidence-based applications while ensuring that scientific conclusions remain proportionate to the available evidence.

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