Clitoria ternatea L.: A Review of Traditional Uses, Phytochemistry and Pharmacological Activities

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Abstract

Clitoria ternatea L. (Fabaceae), known commonly as butterfly pea, Aparajita or blue pea, is a perennial climbing legume that grows across the tropics. Almost every part of the plant, including root, stem, leaf, flower and seed, is used in one or other traditional system of medicine. In Ayurveda the plant is counted among the Medhya Rasayana group, the brain tonics used to sharpen memory and learning. The flowers give a deep blue colour and are used as a natural food colourant in many Asian countries. This review collects and checks the primary literature on the plant. The botany, vernacular names, distribution and morphology come first, followed by the available pharmacognostic work, mainly chromatographic fingerprints of the leaves, stem and seeds. The chemistry is dominated by three groups of compounds: the ternatins, polyacylated delphinidin glycosides responsible for the blue colour; flavonols such as kaempferol, quercetin and myricetin glycosides; and the cyclotides, a family of circular cystine-knot peptides of which C. ternatea is the only known legume source. Extraction work has moved from maceration and decoction towards ultrasound-assisted extraction, deep eutectic solvents and microencapsulation. The pharmacological section covers central nervous system effects, antidiabetic and hepatoprotective activity, and antioxidant, anti-inflammatory, antimicrobial, wound healing, anticancer, cardiovascular and insecticidal effects. Most of this evidence comes from in-vitro assays and rodent models; only three small human studies exist. Acute and subchronic rodent toxicity studies suggest a wide safety margin, but these are preclinical data. The review ends with current formulations, food applications and the remaining gaps.

Keywords: *Clitoria ternatea*; Fabaceae; butterfly pea; Aparajita; ternatins; anthocyanins; cyclotides; nootropic; pharmacognosy; extraction.

List of Abbreviations

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); AChE: acetylcholinesterase; BChE: butyrylcholinesterase; BHT: butylated hydroxytoluene; CAT: catalase; CFA: Freund's complete adjuvant; DCFH-DA: 2',7'-dichlorofluorescein diacetate; DPPH: 2,2-diphenyl-1-picrylhydrazyl; DW: dry weight; EGF: epidermal growth factor; FRAP: ferric reducing antioxidant power; GSH: reduced glutathione; GSK-3β: glycogen synthase kinase 3 beta; HbA1c: glycosylated haemoglobin; HPTLC: high-performance thin-layer chromatography; IC50: half-maximal inhibitory concentration; IL: interleukin; LC50: half-maximal lethal concentration; LC-MS: liquid chromatography-mass spectrometry; MDA: malondialdehyde; MIC: minimum inhibitory concentration; MMP: matrix metalloproteinase; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NADES: natural deep eutectic solvent; NMR: nuclear magnetic resonance; ORAC: oxygen radical absorbance capacity; PCR: polymerase chain reaction; POD: peroxidase; RSM: response surface methodology; SOD: superoxide dismutase; TAC: total anthocyanin content; TPC: total phenolic content; UAE: ultrasound-assisted extraction; VLDL: very low-density lipoprotein; TLC: thin-layer chromatography.

1. Introduction

Legumes are among the oldest crops of humanity and many of them carry a long record of medicinal use. *Clitoria ternatea* L. is one such plant. It is a perennial climbing herb of the family Fabaceae (also placed under the subfamily Papilionoideae, and older books write the family as Papilionaceae) [1, 2, 3]. The plant is known to farmers as a forage crop and nitrogen-fixer, to cooks as a natural blue colourant, and to Ayurvedic physicians as a Medhya Rasayana, a group of drugs used to sharpen the mind [4, 5]. In the last two decades the plant has also become interesting to chemists because its flowers contain a rare class of pigments called ternatins, and to molecular biologists because it is the only legume known to produce cyclotides, circular peptides that are being studied as drug leads and as crop-protection agents [1, 6].

The scientific interest in *C. ternatea* is easy to explain. The blue colour of the flower is stable over a useful pH range, which makes the extract attractive as a replacement for synthetic dyes in foods, cosmetics and intelligent packaging films [7, 8]. The same extract is rich in phenolics and shows measurable antioxidant activity in chemical assays [9]. Root and leaf extracts, on the other hand, have repeatedly shown activity on the central nervous system in rodents, which matches the traditional use of the plant as a brain tonic [10, 11, 12]. A small set of human studies has now also been done, mostly on the flower beverage and its effect on blood sugar and plasma antioxidant status [13].

The literature, however, is scattered. Botanical descriptions sit in agricultural journals, pharmacognostic fingerprints in local pharmacy journals, extraction optimisation in food science journals, and pharmacology in pharmacology journals. Several reviews have covered parts of this material, for example the phytochemistry and pharmacology profile of 2009 [2], the Ayurvedic assessment of 2008 [4], the cyclotide and agriculture review of 2019 [1] and the anthocyanin review of 2021 [7]. What has been missing is a single account that a student of pharmacognosy can read from botany through to clinical work, with the numbers taken directly from the primary papers. This review tries to fill that gap.

The aims of this review are: (i) to describe the botanical identity of the plant, its vernacular names, distribution, morphology, varieties and cultivation; (ii) to collect the pharmacognostic work that exists on the drug; (iii) to link the traditional uses to the plant parts that are actually used; (iv) to summarise the phytochemistry compound class by compound class; (v) to compare the extraction and purification methods that have been reported, with conditions and yields; (vi) to present the pharmacological evidence activity by activity, marking clearly whether a result came from an in-vitro assay, an animal model or a human trial; (vii) to describe what is known about mechanisms, clinical studies, toxicity, formulations and food applications; and (viii) to point out where the evidence is thin or contradictory.

Details of the literature search, including the databases, search strings and selection criteria, are given in the Literature Search Strategy section below.

Literature Search Strategy

A structured search of the published literature on *Clitoria ternatea* was carried out to identify the studies reviewed in this article. The bibliographic sources searched were PubMed/MEDLINE, Scopus, Web of Science, ScienceDirect and Google Scholar. In addition, the reference lists of every retrieved review and monograph chapter were hand-searched for primary studies, and older records that are not indexed in PubMed were located through CrossRef and DOI lookups.

The core search string, applied to titles, abstracts and keywords, was: ("Clitoria ternatea" OR "Clitoria ternatea L." OR "butterfly pea" OR "Aparajita" OR "Shankhpushpi" OR "Asian pigeonwings"). In PubMed this was run as `"Clitoria ternatea"[Title/Abstract]`, which returned 283 records on 13 October

2026; this count is quoted so that the search can be repeated exactly. The same terms were combined with (phytochem *OR extract* OR pharmacolog *OR toxic* OR traditional OR ethnobotan*) to identify pharmacological and ethnobotanical records in the other platforms. No date restriction was applied, from database inception to 13 October 2026. The English-language limit was applied because the primary literature for this species is published almost entirely in English; sources in other languages were not retrieved.

Records were screened in two passes, first on titles and abstracts and then on full texts. Studies were included when they reported original experimental data on C. ternatea itself — pharmacognostic or standardisation work, phytochemical analysis, extraction and formulation studies, pharmacological or toxicological experiments — whatever the model, with in-vitro, animal or human evidence recorded separately for each result. Records were excluded when they concerned other species sold under the same common names (in particular the four Shankhpushpi sources), when C. ternatea appeared only in passing, when they were duplicates across databases, or when they reported no primary data. The final reference list comprises 96 sources: 84 primary experimental studies and 12 reviews, systematic reviews and assessment articles, namely Oguis et al. 2019, Gamage et al. 2021, Jeyaraj et al. 2021, Dighe et al. 2009, Mukherjee et al. 2008, Sethiya et al. 2009, Raza et al. 2024, Nandhini et al. 2026, Indriyati and Dewi 2022, Arshad et al. 2026, Aulakh et al. 1988 and Mehla et al. 2020. The reviews are used for background statements and citation chasing only; every quantitative result and every experimental finding reported in the text is drawn from the primary studies.

The record totals returned by the subscription databases are not reported here because they change daily and cannot be reproduced without an institutional subscription; the PubMed count and the exact strings above are given instead so that the core search can be rerun by anyone. During extraction, numerical results were transcribed exactly as printed, including their units, and any disagreement inside a source (conflicting percentages, inconsistent group sizes, mismatched units) was carried into the text and flagged rather than silently corrected, as described in Sections 6 and 12.

2. Botanical Overview

2.1 Taxonomy and nomenclature

Clitoria ternatea L. belongs to the family Fabaceae, subfamily Papilionoideae [3]. The genus *Clitoria* comprises about 58 valid species placed in three subgenera, and *C. ternatea* is the type species of the subgenus *Clitoria*, which makes it the archetypal member of its genus [1]. The specific epithet is thought to come from the island of Ternate in the Molucca Sea of eastern Indonesia, because Linnaeus based his description on material from that island, although the exact native range of the species is still debated [1]. Most older Indian texts list the family as Papilionaceae, and a few recent papers still use that name [2, 5].

The complete taxonomic position is given in Table 1. The accepted author citation is *Clitoria ternatea* L., and the spelling "ternatea" is used in almost all modern papers, although older literature contains the variants "ternata" and "ternatae" [1].

Table 1. Taxonomic classification and vernacular names of *Clitoria ternatea* L.

Rank	Entry	Source
Kingdom	Plantae	[5]
Order	Fabales	[5, 3]
Family	Fabaceae (Papilionaceae in older works)	[1, 2]

Subfamily	Papilionoideae (Faboideae)	[3, 5]
Genus	*Clitoria* L. (about 58 species, 3 subgenera)	[1]
Species	*C. ternatea* L.	[1]
Vernacular names	Aparajita (Hindi, Bengali); Sangu Pushpam (Tamil); Shankhapuspi (southern India, in some texts); butterfly pea, blue pea, Asian pigeonwings, bluebell vine, cordofan pea, Darwin pea (English); Bunga telang (Malay); Anchun (Thai)	[5, 3, 14, 15, 16]

2.2 Vernacular names

The name most often heard in North India is Aparajita, which is also the name used in the Ayurvedic literature [17, 5]. In Bengali the plant is called Aparajita as well, and in Tamil it is Sangu Pushpam [3]. A recent pharmacological review notes that in parts of southern India the name Shankhapuspi is applied to this plant, which matters because Shankhpushpi is more usually the name of a different set of plants (discussed in Section 3.5) [5]. In English the common name is butterfly pea or blue pea, and older trade names include Asian pigeonwings, bluebell vine, cordofan pea and Darwin pea [1, 5]. In Malaysia the flower is called Bunga telang, and the root has a history of use there as a brain tonic [14]. In Thailand the flower is known as Anchun and is used as a food colouring [15], and in Indonesia and Malaysia the name Bunga telang appears in the food literature [16].

2.3 Origin, distribution and habitat

The native range of *C. ternatea* is disputed, and it is worth being careful here. The older botanical literature places the centre of origin in tropical Asia, and the species is described as native to tropical equatorial Asia, from where it was introduced into Africa, Australia and the Americas [2, 3]. A 2024 review, however, states that the species originated in tropical America [5]. The most detailed treatment says that the exact origin is hard to determine because the type material came from Ternate island, while all other species of the subgenus *Clitoria* are African or Indian, and suggests the plant may be an ancient hybrid that later spread through Southeast Asia [1]. This disagreement is not resolved by the present literature and is flagged as such.

Whatever its origin, the present distribution is pantropical. The plant is naturalised across tropical Asia, Africa, Australia and the Americas, and it also grows in subtropical regions [1]. Its range is reported from roughly 20° N to the Salta district of Argentina at about 24° S, from sea level up to about 1,500 m in India, where it is most common in the Andaman Islands and southern India [3]. In the United States it is found from Florida to Texas and north to New Jersey, Kentucky and Arkansas [3]. In India it has been collected from many states including West Bengal, Odisha, Andhra Pradesh, Kerala, Gujarat, Madhya Pradesh, Maharashtra and Rajasthan [17, 5].

As for habitat, the plant prefers open, sunny places. In Australia it is described in open mesic forest and shrubland, often near places where fresh water collects, such as the edges of wetlands, small gullies or the foot of rocky hillsides; where it establishes well it grows vigorously and can smother other vegetation [1]. It tolerates a wide soil range, including calcareous soils between pH 5.5 and 8.9, and

heavy cracking clay soils [3]. It needs about 400 mm of rainfall or supplementary irrigation, survives brief flooding but not waterlogging, and is sensitive to frost although woody parts often recover [1, 3].

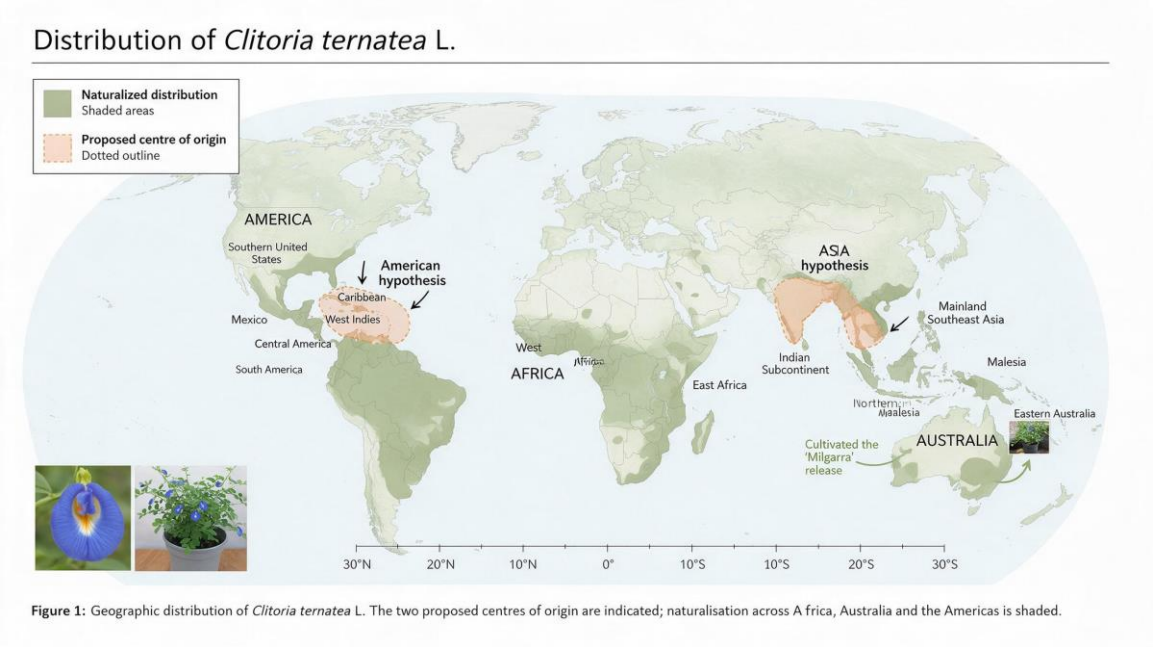


Figure 1: Geographic distribution of *Clitoria ternatea* L. The two proposed centres of origin are indicated; naturalisation across A frica, Australia and the Americas is shaded.

Figure 1. Geographic distribution of Clitoria ternatea L. The two proposed centres of origin are indicated; naturalisation across Africa, Australia and the Americas is shaded.

2.4 Morphology

Clitoria ternatea is a slender perennial twining herb with a deep taproot. The main morphological features are described organ by organ below and are collected in Table 2.

Root. The plant has an extensive deep root system that allows it to survive seven to eight months of drought, and the roots form large nodules that fix atmospheric nitrogen [1, 5]. This nodulation is the reason the crop is used in soil improvement and revegetation work, including the revegetation of coal mine sites in Australia [2]. The root bark is the part richest in the triterpenes taraxerol and taraxerone [17].

Stem. The stems are thin, green to brownish, and climb by twining; the plant behaves as a creeper or vine in moist neutral soil [2].

Leaf. The leaves are pinnately compound with obovate, entire leaflets that have emarginate (notched) tips [1, 5]. Anatomically, the epidermis on both surfaces is a single layer of cells below a thick cuticle with trichome outgrowths; below the upper epidermis lies a palisade layer, and the stomata are paracytic [1, 5].

Flower. The flowers are solitary and zygomorphic, of the typical pea type. The calyx is tubular with five sepals fused for about two thirds of their length. The corolla has five free petals: one large rounded banner (standard) petal, two wrinkled wings that are often about half the length of the banner, and two white keels that enclose the floral organs [1, 5]. Flowers are about 4 cm long and 3 cm wide, deep blue with light yellow markings on the banner [2]. The androecium is diadelphous, with ten filaments of which nine are fused and one free, each carrying a four-lobed white anther; the ovary is monocarpellary with about ten ovules and a long style with a bent tip [1].

Fruit and seed. The fruit is a narrow, flattened pod with a pointed tip, 5–7 cm long, usually containing about ten seeds; the pods are edible when tender [1, 2]. Seeds are reported to contain about 500 calories per 100 g, with a fatty acid profile of palmitic acid 19%, stearic acid 10%, oleic acid 51–52%, linoleic acid 17% and linolenic acid 4% [1, 5].

Table 2. Morphological features of *Clitoria ternatea* by organ.

Organ	Description	Key numbers
Root	Deep taproot with nitrogen-fixing nodules; root bark rich in taraxerol	Survives 7–8 months of drought
Stem	Slender twining herb	Creeper in moist neutral soil
Leaf	Pinnately compound, obovate leaflets, emarginate tips; paracytic stomata; trichomes on both surfaces	Single-layer epidermis under thick cuticle
Flower	Solitary, zygomorphic; calyx fused 2/3; banner + 2 wings + 2 white keels; diadelphous stamens (9+1); monocarpellary ovary	Flower about 4 × 3 cm; 10 ovules
Pod	Narrow, flattened, pointed	5–7 cm long, about 10 seeds
Seed	Oil-rich; oleic acid the main fatty acid	Palmitic 19%, stearic 10%, oleic 51–52%, linoleic 17%, linolenic 4%; about 500 cal/100 g



Figure 2. Habit and organ morphology of *Clitoria ternatea* L. (Fabaceae).

Figure 2. Habit and organ morphology of Clitoria ternatea L. (Fabaceae).

2.5 Varieties and flower colour forms

The flowers of *C. ternatea* are most often dark blue, but white flowers and intermediate shades are common [1]. A chemodiversity study of 20 accessions collected from 11 populations across India recognised four petal types on colour: blue, white, white with a blue tinge, and lilac [17]. Blue and white

forms were found in most populations, the tinged form was seen in Maharashtra and Assam, and the lilac form in Odisha [17]. This is interesting chemically, because white flowers do not produce anthocyanins at all, and mauve flowers accumulate delphinidins that lack the 3′ and 5′ glucosyl substitutions, which shows that glucosylation at those two positions is what produces the blue colour [1, 2]. A double-petal or double-flowered variety is mentioned in some general accounts, but none of the primary sources used for this review documents it, so it is not described further here.

The colour forms are sometimes believed to differ in medicinal strength, and older Indian practice prefers the white-flowered plant for some preparations. The chemodiversity data do not support this belief: comparing white and blue accessions from the same region, taraxerol was higher in white in four populations and higher in blue in three, and β-sitosterol showed the opposite split, leading the authors to conclude that there is no correlation between flower colour and the content of these two markers [17].

2.6 Cultivation, propagation and agricultural value

C. ternatea is cultivated both as an ornamental and as a forage and soil-improvement crop. It grows quickly in warm humid weather, and seeds are the usual means of propagation [3]. Germination and establishment are best between 24 and 32 °C, with seeds sown 2.5–5 cm deep and 20–30 cm apart [1]. The crop does well with ample moisture, ideally 650–1,250 mm of rainfall, though about 400 mm with irrigation is sufficient in drier areas, and it grows best at 27 °C or above [1, 3].

A practical problem is hard seed. About 20% of freshly harvested seed does not imbibe water, and storing seed for a further six months raises germination only to about 15–20%; mechanical scarification of six-month-old seed raised germination from 30% to 71%, and soaking in concentrated sulfuric acid for at least 10 min gave a reported 100% germination in one study [1, 3]. Seeds usually take one to two weeks to germinate and the plants flower about four weeks later [3].

As a forage, the plant is productive: yields of 17–29 tons per hectare of palatable hay have been reported, on par with alfalfa [1]. The Australian cultivar 'Milgarra' was released in 1991 as a composite of 21 introduced and naturalised lines, and harvest at about 45 days gives the best dry matter content and digestibility; pruning every 42 days at 20 cm gave dry matter yields of 1,122 kg/ha in one trial [1]. The hay has consistently lower acid detergent fibre than other legumes and carotenoid content up to 600 mg/kg dry matter at six months [1]. Nodulation is favoured at soil moisture of 25–45% and light durations of 11–14 h, and Rhizobium isolated from the plant is compatible with cowpea and soybean but does not nodulate common bean, alfalfa or pea [1].

Tissue culture work has also been reported. Encapsulated embryogenic callus in 3% sodium alginate survived storage at 4 °C for 90 days with more than 50% survival, and hairy root cultures transformed with *Agrobacterium rhizogenes* reached a transformation frequency of 85.8% while producing about four times the taraxerol content of soil-grown roots [1]. These figures matter for the future supply of the triterpenoid part of the phytochemistry.

3. Pharmacognostic Evaluation

Pharmacognostic evaluation is the first step towards a standardised herbal drug, because it establishes the identity, purity and quality of the crude material. For *Clitoria ternatea* the published work is uneven. The leaf anatomy has been described in general botanical treatments, and two solid chromatographic fingerprinting studies exist, one on the flavonoid profile of the leaf, stem and seed and one on the triterpenoid and sterol markers of the root. Classic pharmacopoeial constants such as total ash, acid-insoluble ash, water-soluble extractive values and loss on drying have not been reported

in the indexed primary literature that was searched for this review, and this gap is stated plainly rather than filled with invented numbers.

3.1 Macroscopy

The crude drug consists of the dried root, stem, leaves, flowers and seeds. Roots are slender to moderately thick, woody, brownish externally, with a sharp bitter taste and a cooling property in the traditional descriptions, and the root bark is the part richest in taraxerol [18, 17]. Leaves are pinnately compound with obovate, entire leaflets with notched tips, dark green when dry [1, 5]. Flowers keep their blue colour reasonably well on drying, which is one reason they have been used as colourants for cloth and food [1]. Seeds are small, hard and brown, about ten per pod [2]. Standardised macroscopic descriptions with dimensions and colour codes, of the kind required by a pharmacopoeia, are still to be written for this species.

3.2 Microscopy

The anatomical information available comes mainly from general treatments of the species. The leaf has a single-layered epidermis on both surfaces beneath a thick cuticle, with trichome outgrowths; a palisade layer lies below the upper epidermis; the stomata are paracytic; and lignified xylem elements are present in the veins [1, 5]. These characters are useful because they distinguish the leaf powder of a papilionaceous legume from powders of the Convolvulaceae plants that are sometimes mixed with it (Section 3.5). Detailed transverse-section reports of the root, stem and seed, and a formal powder microscopy description with diagnostic cellular elements, are not available in the sources used here.

3.3 Physicochemical constants and fluorescence analysis

No primary study in the searched literature reports the standard physicochemical constants for *C. ternatea* drug, that is total ash, sulphated ash, acid-insoluble ash, alcohol-soluble and water-soluble extractive values, foreign matter or loss on drying. The only figure of this type that appears in the reviewed set is a value of 6% ash quoted for seeds, and it comes from a secondary source rather than a primary determination, so it is treated with caution [5]. Fluorescence analysis of the powder under short-wave and long-wave ultraviolet light, and under treatment with various reagents, has also not been reported. These are straightforward studies that a pharmacognosy laboratory could add, and their absence is one of the clear gaps in the literature.

3.4 Chromatographic fingerprints and marker estimation

Two HPTLC studies provide the quantitative core of the pharmacognostic work.

Ponnusamy and colleagues built a flavonoid profile of the leaves, stem and seeds [19]. Shade-dried powders were extracted with 150 mL of methanol by Soxhlet extraction for 8–12 h, and the extracts were run on HPTLC plates with ethyl acetate–butanone–formic acid–water (5:3:1:1) as the mobile phase. After spraying with 1% ethanolic aluminium chloride and heating at 100 °C for 2 min, the plates showed 24 bands with 18 different Rf values in the range 0.01–0.96. Ten of the bands, covering seven Rf values (0.04, 0.10, 0.28, 0.37, 0.49, 0.65 and 0.85), were identified as flavonoids. The distribution was informative: the leaf showed six flavonoid bands, the stem three and the seed only one, at Rf 0.65, which was also common to the stem and leaf. Rutin run as a standard on each plate gave Rf 0.58. The authors proposed the profile as a tool to distinguish the drug from adulterants, although they did not test any adulterant sample themselves [19].

Makasana and colleagues developed and validated an HPTLC method for the simultaneous estimation of β-sitosterol and taraxerol and used it to measure chemical diversity across 20 accessions from 11 Indian populations [17]. Root, stem and leaf powders were extracted with six solvents in turn (petroleum ether, chloroform, ethyl acetate, acetone, methanol and ethanol, 3 × 150 mL under reflux at 85 ± 5 °C).

The plates were developed in petroleum ether:acetone (9.1:0.9, v/v) to 8 cm, derivatised by immersion in 5% sulphuric acid in methanol followed by heating at 110 °C for 10 min, and scanned at 601 nm. Retention factors were 0.22 ± 0.04 for β-sitosterol and 0.38 ± 0.05 for taraxerol. Linearity was 100–600 ng per spot, the detection limits were 30 ng per spot for β-sitosterol and 35 ng per spot for taraxerol, the quantitation limit was 90 ng per spot for both, and the recovery on spiking was 98.23% for β-sitosterol and 98.41% for taraxerol. The paper describes the procedure as validated per the ICH Q2(R1) guidelines (2005); that text has since been superseded by ICH Q2(R2), adopted in 2023 together with Q14. Recovery at three spiking levels is only one element of a validation, and there is no pharmacopoeial monograph for this drug against which the method could be checked independently. Petroleum ether gave by far the best extraction of both markers, 126 ± 4.368 µg taraxerol and 13.2 ± 1.706 µg β-sitosterol per mg of root extract, followed by chloroform and then by the more polar solvents. Across the 20 accessions, taraxerol ranged from 0.358 ± 0.006 to 1.04 ± 0.024 mg/g dry weight and β-sitosterol from 0.183 ± 0.004 to 0.334 ± 0.009 mg/g, with the Kerala white accession highest in taraxerol. Taraxerol was consistently richer in the root and β-sitosterol richer in the leaf.

Table 3. Pharmacognostic parameters available for *Clitoria ternatea*.

Parameter	Reported value / finding	Source
Leaf epidermis	Single layer under thick cuticle, trichomes present, paracytic stomata	[1, 5]
HPTLC flavonoid profile (leaf, stem, seed)	24 bands, 18 Rf values (0.01–0.96); 10 flavonoid bands at Rf 0.04, 0.10, 0.28, 0.37, 0.49, 0.65, 0.85; rutin standard Rf 0.58	[19]
Flavonoid distribution	Leaf 6 bands, stem 3 bands, seed 1 band (Rf 0.65)	[19]
HPTLC markers (root)	β-Sitosterol Rf 0.22 ± 0.04; taraxerol Rf 0.38 ± 0.05; scanned at 601 nm	[17]
LOD / LOQ	30 / 90 ng per spot (β-sitosterol); 35 / 90 ng per spot (taraxerol)	[17]
Recovery	98.23% (β-sitosterol); 98.41% (taraxerol)	[17]
Best extraction solvent for markers	Petroleum ether (126 µg/mg taraxerol, 13.2 µg/mg β-sitosterol in root extract)	[17]
Taraxerol across 20 accessions	0.358 ± 0.006 to 1.04 ± 0.024 mg/g dry weight	[17]
β-Sitosterol across 20 accessions	0.183 ± 0.004 to 0.334 ± 0.009 mg/g dry weight	[17]
Total ash, extractive values, foreign matter, loss on drying	Not reported in the indexed primary literature searched	gap

3.5 Adulterants and substitutes: the Shankhpushpi problem

The common name Shankhpushpi is applied in Ayurvedic practice to four different botanicals, and *Clitoria ternatea* is only one of them. A review of the Ayurvedic and Sanskrit literature identified the four sources as *Convolvulus pluricaulis* Choisy (Convolvulaceae), *Evolvulus alsinoides* L. (Convolvulaceae), *Clitoria ternatea* L. (Papilionaceae) and *Canscora decussata* Schult. (Gentianaceae), and noted that the drug supplied under this name varies from region to region [20]. An earlier review had already compared the pharmacognosy, chemistry and pharmacology of the same four varieties [21]. Because the four plants belong to three different families, the mix-up is not merely academic: powders and extracts sold as Shankhpushpi may or may not contain *C. ternatea* at all, and the memory-enhancing activity measured in one preparation cannot be transferred to the others without evidence.

Some work has been done to separate them. A TLC-DPPH bioautography method differentiated the four claimants by their antioxidant markers: *C. pluricaulis* showed scopoletin; *E. alsinoides* showed β-carotene, scopoletin and chlorogenic acid; *C. ternatea* showed β-carotene, scopoletin and rutin; and *C. decussata* showed β-carotene, scopoletin and mangiferin [22]. Rutin and the blue anthocyanin pigment of the petals are the most practical diagnostic features for *C. ternatea* itself. A later study compared the neuropharmacological actions of all four herbs, testing protection against β-amyloid-induced neurotoxicity in Neuro 2A cells, acetylcholinesterase and 5-lipoxygenase inhibition, and scopolamine-induced memory retrieval in rats [23]. Another comparison of the three memory-enhancing sources found that aqueous methanol extracts of *C. pluricaulis* at 100 mg/kg gave the strongest nootropic and anxiolytic effect in the elevated plus-maze and step-down models, while *E. alsinoides* and *C. ternatea* were also active, at 200 and 100 mg/kg respectively, and all three showed CNS-depressant action at higher doses [24]. *Evolvulus alsinoides* on its own has been reported to improve learning and memory in rodents and to reverse scopolamine-induced amnesia [25].

For the working pharmacognosist the practical message is that authentication of *C. ternatea* material should rest on a combination of features: the papilionaceous leaf anatomy with paracytic stomata, the HPTLC flavonoid fingerprint of Ponnusamy and colleagues, the blue anthocyanins that give a strong reaction in the petal, and the taraxerol and β-sitosterol markers of the root [19, 17].

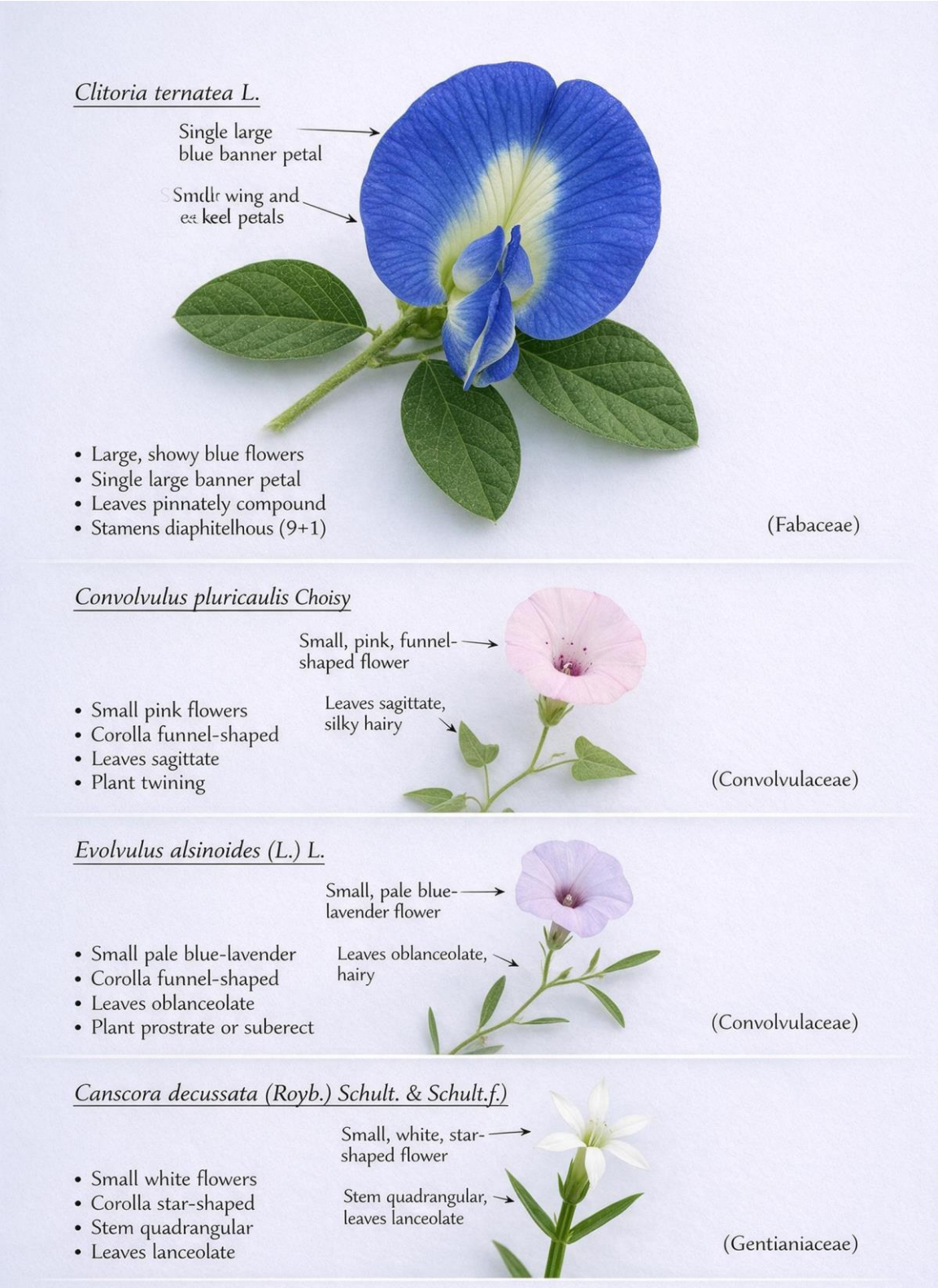


Figure 3. Diagnostic features of the four botanical species associated with the name Shankhpushpi: Clitoria ternatea L. distinguished from Convolvulus pluricaulis, Evolvulus alsinoides and Canscora decussata.

4. Traditional and Ethnomedicinal Uses

4.1 Ayurveda

In Ayurveda *C. ternatea* is placed in the Medhya Rasayana group, the class of rejuvenative drugs used to sharpen intellect and memory [4, 5]. The four faculties that the Medhya Rasayana group is said to

support are Dhi (intellect), Dhrti (attention or steadiness), Smruti (memory) and Mana (the mind) [5]. The root is the part most often named in this context. It is described as being administered with honey and ghee as a general tonic to children, to improve mental faculties, muscular strength and complexion, and in epilepsy and insanity [2].

The list of classical indications is long. The roots are used for tubercular glands, amentia, hemicrania, burning sensation, strangury, helminthiasis, leprosy, leucoderma, elephantiasis, inflammation, vitiated pitta conditions, bronchitis, asthma, pulmonary tuberculosis, ascites, ulcers, ear diseases, visceromegaly and fevers [2]. Leaves are said to be useful in otalgia, liver complaints and skin eruptions, and the seeds in visceral pain [2]. A recent review adds skin, eye and throat disorders, migraine, jaundice, urinary tract infection, constipation, snake bite, headache and indigestion to the folk list [3]. Root extracts are also said to be useful in whooping cough when taken orally, and the white-flowered form is traditionally credited with a goitre remedy [2]. A modern assessment of the Ayurvedic sources confirmed that the plant is used as a nootropic, memory booster, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizer and sedative, and concluded that scientific studies have partly reconfirmed these uses [4].

4.2 Other systems and regional folk practice

Unani and Siddha descriptions of *C. ternatea* were not found in the primary literature searched for this review, and no claim is made for them here. What can be documented is the regional folk practice. In Malaysia the roots are traditionally used as a brain tonic to improve memory, which is the starting point of the recent anticholinesterase studies on the root [14]. In Thailand the flower is used as an everyday food colourant and the plant is used in anti-aging health drinks [15]. In the Philippines and other parts of Southeast Asia the flowers are eaten as a vegetable, and in Malaysia and Thailand the extract goes into desserts and drinks [7]. Thai ethnobotanical work on legumes records the use of butterfly pea among edible and medicinal legumes of the region [26]. Indian field collections show the plant in use across West Bengal, Odisha, Andhra Pradesh, Kerala, Gujarat, Madhya Pradesh, Maharashtra and Rajasthan [17, 5].

4.3 Uses by plant part

The traditional uses can be organised by the part used, which is also how the modern pharmacological studies organise their extracts.

Root. The root is the brain tonic of the tradition, and it is also used as a diuretic, in fevers, in bronchitis and asthma, and as an anthelmintic [2, 14, 27]. The diuretic use has an experimental counterpart in a 1962 study in dogs (Section 7.11).

Leaf. Leaves are used for liver complaints, ear pain, skin eruptions and body aches, and as an anthelmintic [2, 19].

Flower. The flower is the food part. It colours rice, desserts and drinks, and it is also used in fever and inflammation, following the general antipyretic reputation of the plant [7, 28, 15].

Seed. Seeds are described as purgative and cathartic and are used in visceral pain; they are also used against stomatitis, piles, sterility in women, haematemesis, insomnia, epilepsy, psychosis, leucorrhoea and polyuria in the folk literature [19, 2].

Table 4. Traditional uses by plant part with the modern evidence covered in this review.

Part	Traditional uses reported	Modern work supporting or testing the use
Root	Brain tonic, memory, epilepsy, insanity, fevers, bronchitis, asthma, diuretic, anthelmintic	Nootropic and anticholinesterase studies in rodents [10, 11, 12]; antihistaminic and antiasthmatic models [29, 30]; diuretic study in dogs [27]
Leaf	Liver complaints, ear pain, skin eruptions, body ache, anthelmintic	Wound-healing enzyme inhibition [31]; antidiabetic work in alloxan rats [2]
Flower	Food colourant, fever, inflammation, cooling drinks	Antioxidant and glycaemic human trial [13]; antimicrobial and cytotoxic studies [32]; anti-inflammatory hamster model [33]
Seed	Purgative, cathartic, visceral pain, insomnia, epilepsy	Antifungal and insecticidal proteins [34, 35]; larvicidal activity [2]

4.4 Food and colourant uses

The use of the flower as a food colour is the oldest industrial application of the plant and the one with the largest modern literature. The petals give teas, desserts, rice, yoghurt and drinks their blue colour, and the extract has been used to colour cloth [1, 7]. Because the pigment changes colour with pH, turning violet and green as the medium becomes less acidic, it has also been used as a pH indicator in intelligent packaging films that signal food spoilage [7, 36]. A study of blue colourants compared extraction yields on a dry basis and found 8.27–8.66% for phycocyanin, 10.7–11.8% for genipin-derived pigments and 21–29% for butterfly pea anthocyanins, and noted that butterfly pea anthocyanins form no aggregates in acidic beverages around pH 3, unlike the other two blue colourants [7]. Recent work has used the petals as a natural blue colorant for traditional rice cooking, and reports the colour and stability outcomes of that application [28].



Figure 4. The pH-dependent colour of Clitoria ternatea flower extract, blue at neutral to mildly acidic pH and violet under acidic conditions.

5. Phytochemical Constituents

The chemistry of *Clitoria ternatea* has been studied for more than sixty years, and the picture that has emerged is a fairly simple one. Three groups of compounds dominate: the polyacylated anthocyanins called ternatins, which are responsible for the blue colour; a set of ordinary flavonols and phenolic acids that occur through most of the plant; and a family of small circular peptides, the cyclotides, which are of interest far beyond this species. Triterpenoids, phytosterols, fatty acids and a few small proteins fill out the rest. The major classes are collected in Table 5.

Table 5. Major compound classes of *Clitoria ternatea* by plant part.

Compound class	Plant part	Representative members	Source
Anthocyanins (ternatins)	Flower	Ternatin A1–A3, B1–B4, C1–C5, D1–D3; preternatins A3 and C4; all derivatives of delphinidin 3,3′,5′-triglucoside	[7, 1, 2, 32]
Flavonols	Flower, leaf, seed	Kaempferol, quercetin and myricetin glucosides, rhamnosides and malonyl glucosides; rutin	[2, 1, 15, 19]
Phenolic acids	Flower, leaf	Gallic acid, protocatechuic acid, chlorogenic acid, p-coumaric acid, epicatechin	[1, 15]

Triterpenoids	Root bark, leaf	Taraxerol, taraxerone	[17, 31, 2]
Phytosterols	Root, seed, flower	β-Sitosterol, stigmasterol, campesterol	[17, 14, 5]
Norneolignans	Root	Clitorienolactones A–C (free and glycosylated)	[37, 1]
Cyclotides	All tissues	Clotides T1–T12, Cter A–R, CT2–CT20	[38, 6, 39, 40]
Proteins	Seed	Finotin (about 20 kDa, antifungal/insecticidal); Ct protein (14.3 kDa, antifungal); butelase-1 (ligase)	[34, 35, 1]
Fatty acids	Seed, root	Oleic 51–52%, palmitic 19%, linoleic 17%, stearic 10%, linolenic 4% (seed); palmitic and linoleic acids dominant in root GC-MS	[1, 5, 14]

5.1 Anthocyanins: the ternatins

The ternatins are the reason the flower is blue. They were first isolated in 1985 as six acylated anthocyanins, and all of them turned out to be derivatives of delphinidin 3,3′,5′-triglucoside [1, 7]. The structure of the largest member, ternatin A1, was determined in 1989 and it proved to be one of the most stable of the group in neutral solution [1]. Since then the family has grown to fifteen named compounds, A1–A3, B1–B4, C1–C5 and D1–D3, and the pattern of assembly is known: ternatin C5 is the simplest member and the other fourteen are built from it by adding glucosyl and acyl groups, four glucosylation steps and four p-coumaric acid acylation steps being needed to reach ternatin A1 [7, 1]. Two of the enzymes on this pathway have been characterised, and the key point for colour is that glucosylation at the 3′ and 5′ positions of the delphinidin nucleus is what produces the blue shade [7, 41, 42].

The colour forms of the flower fit this chemistry exactly. White flowers make no anthocyanin at all, while mauve flowers accumulate delphinidins that lack the 3′ and 5′ glucosyl groups [1, 2]. The acyl groups matter as much as the sugars: they shield the flavylium ion from hydration, which is why the extract keeps its colour in mildly acidic and neutral media where an ordinary anthocyanin would fade to colourless [7].

Modern chromatographic work has put numbers on the mixture. Fractionating a 50% ethanol petal extract on an Amberlite XAD-16 resin and running the enriched fraction by LC-Q-TOF-MS, eleven ternatins were detected and sized by peak area: ternatin B2 was the largest at 23.93%, followed by ternatin D1 at 20.44% and D2 at 20.03%, then B1 6.62%, B3 5.94%, B4 5.62%, C1 5.24%, D3 4.38%, C2 4.08% and two D3 isomers at 1.83% and 1.88% [32]. The same group measured the total anthocyanin content of the crude extract at 5.5 ± 0.9 mg cyanidin-3-glucoside equivalents per gram and raised it about five-fold, to 25.0 ± 3.5 mg/g on C18-OPN and 25.9 ± 2.4 mg/g on Amberlite XAD-16, while total phenolics rose from 50.4 ± 3.2 mg gallic acid equivalents per gram in the crude extract to 176.0 ± 11.9 mg/g and 122.7 ± 8.0 mg/g in the two fractions [32]. An aqueous flower extract characterised by

high-resolution LC-MS in human keratinocytes was reported to contain polyacylated ternatins derived from delphinidin together with quercetin and kaempferol glycosides as the two main groups [43]. Water extracts made at different pH values give different profiles: at the natural pH of the flower, 6.8–7.0, compounds such as delphinidin-3-(cis-p-coumaroyl)glucoside, cyanidin-3-(p-coumaroyl)glucose and several kaempferol and quercetin glycosides were identified that were not seen in extracts made at pH 3 [44].

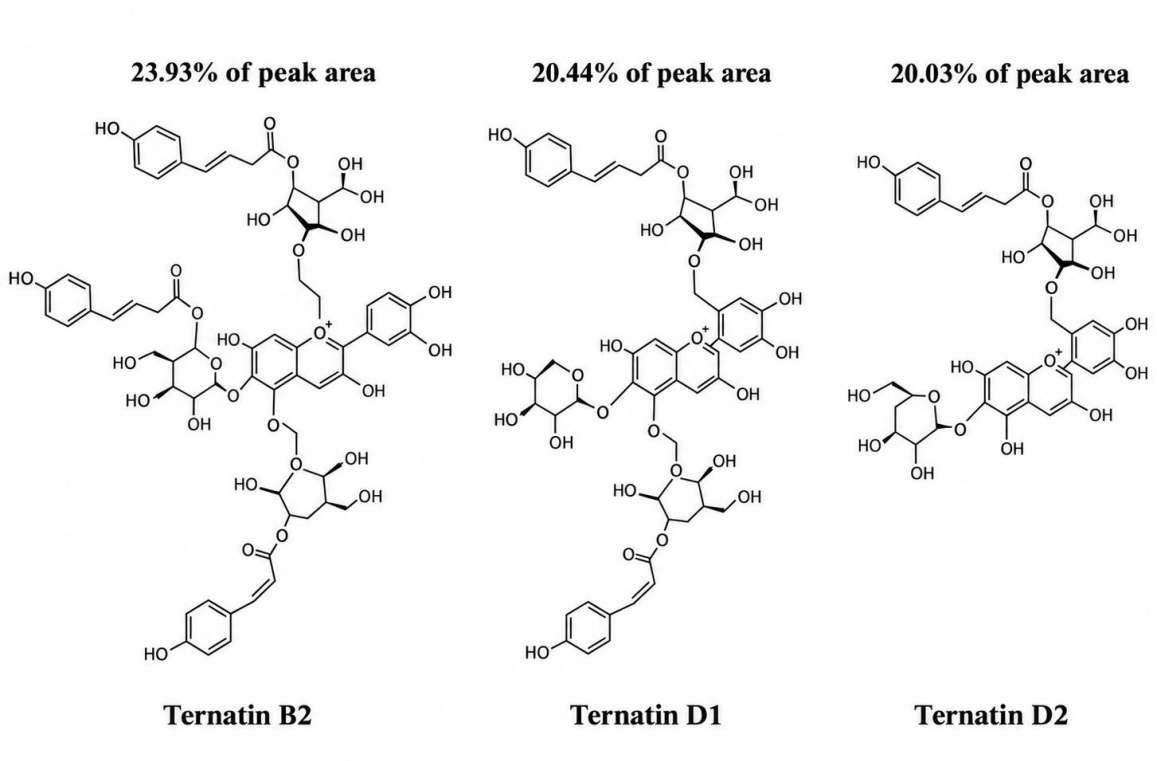


Figure 5. The three most abundant ternatins reported in the anthocyanin-rich petal fraction by LC-Q-TOF-MS: ternatin B2 (23.93% of peak area; C75H81O41, [M+H]+ m/z 1637.39), ternatin D1 (20.44%; C84H87O43, m/z 1783.42) and ternatin D2 (20.03%; C69H71O36, m/z 1475.35).

5.2 Flavonols, phenolic acids and other phenolics

Alongside the pigments sits an ordinary flavonoid chemistry. Five flavonols were isolated from the plant as early as 1985 — kaempferol, kaempferol 3-glucoside, kaempferol 3-robinobioside-7-rhamnoside, quercetin and quercetin 3-glucoside — and the petal glycosides were later characterised as the 3-O-(2″-O-α-rhamnosyl-6″-O-malonyl)-β-glucosides and related sugar combinations of kaempferol, quercetin and myricetin [1, 2]. Leaf flavonols and delphinidin glycosides have been catalogued in the same way, and the malonylated forms dominate the fresh tissue because malonylation is lost on storage and hydrolysis [1, 45].

Free phenolics are also present. Floral extracts contain rutin, epicatechin, gallic acid, protocatechuic acid and chlorogenic acid [1], and an HPLC assay on an anti-aging drink prepared from the flowers measured gallic acid at 10.23 ± 0.23 µg per mg of extract and rutin at 14.05 ± 0.54 µg per mg [15]. Class-level quantification shows how rich the flower is: an 80% ethanol extract contained 55.24 ± 0.68 mg gallic acid equivalents of total phenolics per gram, 33.48 ± 0.44 mg rutin equivalents of total flavonoids, 52.96 ± 0.40 mg of total flavonols, 0.43 ± 0.02 mg of total flavanols and 14.83 ± 0.23 mg of total phenolic acids; after AB-8 macroporous resin purification these rose to 236.78 ± 0.35, 171.22 ± 0.91, 197.83 ± 1.69, 1.48 ± 0.05 and 60.04 ± 1.17 mg/g respectively, that is 2.4- to 4.1-fold enrichment [46]. Leaf and flower extracts made by ultrasonication for an anti-aging drink gave total phenolics of 0.68 ± 0.05 µg gallic acid equivalents per gram in the best case and total flavonoids of

1.53 ± 1.01 µg quercetin equivalents per milligram [15]. A hydroalcoholic root extract has been profiled by UHPLC-ESI-MS/MS, and methanolic leaf and flower extracts were shown to contain phenolics and flavonoids in screening work [47, 48].

5.3 Triterpenoids, phytosterols and norneolignans

The root is the triterpenoid part of the plant. Taraxerol and taraxerone, two pentacyclic triterpenes, were isolated from the root bark in the early 1960s and have been used as chemical markers ever since [1, 2]. The validated HPTLC method described in Section 3.4 measured taraxerol across 20 Indian accessions at 0.358 ± 0.006 to 1.04 ± 0.024 mg/g dry weight and β-sitosterol at 0.183 ± 0.004 to 0.334 ± 0.009 mg/g, with taraxerol consistently higher in the root and β-sitosterol higher in the leaf [17]. The same markers appear in the leaf: a standardised methanolic leaf extract contained 5.32% w/w taraxerol, and the ethyl acetate fraction from which the compound was crystallised gave taraxerol at 4.55% w/w and a purified yield of 5.27% w/w [31]. Root material is also the source of the clitorienolactones, a group of norneolignans that appear to be unique to this species; a UHPLC-DAD-MS/MS study put 32 compounds in the root extract and found the clitorienolactones, both free and glycosylated, together with flavone, flavonol, isoflavone and isoflavanone derivatives as the main constituents of the ethyl acetate and 50% aqueous methanol fractions [37, 1].

The phytosterols are widely distributed. GC-MS of the ethanol root extract resolved 27 compounds, of which n-hexadecanoic (palmitic) acid was the largest peak at 12.95% of the chromatogram, followed by β-sitosterol at 3.92%, linoleic acid at 2.92%, trans-oleic acid at 2.46%, stigmasterol at 1.20%, campesterol at 0.62% and lupenone at 0.45% [14]. The seed oil profile is different again, with oleic acid the dominant fatty acid at 51–52%, then palmitic 19%, linoleic 17%, stearic 10% and linolenic 4% [1, 5]. Work on seeds and petals together has confirmed four phytosterols plus α- and γ-tocopherols, with linoleic acid the most abundant fatty acid in both parts [5], and the older leaf literature records delphinidin and malonyl glucosides alongside the flavonols [41, 42, 45].

5.4 Cyclotides and cliotides

C. ternatea is the only member of the Fabaceae known to make cyclotides, and this single fact has made the plant important to peptide chemists [1, 39]. Cyclotides are small proteins of about 28 to 37 amino acids whose backbone is closed head-to-tail and whose three disulfide bonds form a knotted core; the arrangement, called the cyclic cystine knot, makes them unusually resistant to heat, chemical treatment and proteases [40, 6].

The first survey of the species found twelve novel cyclotides in seed extracts, all in the bracelet subfamily, and extended the family to the legumes [39]. A year later, two groups working on the flowers and seeds described a set of peptides that they named cliotides: fifteen heat-stable cyclotides were isolated from a boiling-water extract of the whole plant, twelve of them new and designated cliotide T1 to T12, with the remainder being the previously reported Cter A, Cter B and Cter D [38]. A parallel study added a Möbius-subfamily member called Cter M, the first Möbius cyclotide found in a Fabaceae plant, along with six further sequences Cter N to Cter R from leaf and flower [6]. At least 74 cyclotide sequences have since been catalogued from transcriptomes of the species [1].

What makes the legume case distinctive is the way the peptides are made. In every other cyclotide-producing family the peptides come from dedicated genes carrying one to three cyclotide domains with flanking pro-domains. In *C. ternatea* the cyclotide domain sits inside an albumin-1 gene: it takes the place of the albumin chain-b subunit, so the precursor is a chimeric albumin with an endoplasmic reticulum signal sequence immediately followed by the mature cyclotide, a short linker and an albumin a-domain [6, 38]. Processing is carried out by signal peptidase I at the N-terminus and by an asparaginyl endopeptidase at a conserved asparagine at the C-terminus, the same enzyme that later became famous

as butelase-1, described from this plant and now the fastest peptide ligase known [38, 1]. The distribution across the plant is broad: each of eight tissues examined contained at least ten different cliotides, cliotides T2 and T3 made up more than 70% of the cyclotides in flowers and pods, T8, T9 and T10 dominated the seeds, and T1 and T4 were present in every tissue examined [38].

The stability of these peptides has direct practical consequences. About 80% of the roughly 3 kDa peptides in a boiling-water extract of fresh flowers survived heating at 100 °C for 30 min [38], purified Cter M gave an unchanged proton NMR spectrum after heating to 95 °C for 5 min and was not hydrolysed by trypsin or chymotrypsin after 8 h at 37 °C [6]. This is the reason the cyclotides, unlike the anthocyanins, can be expected to survive the traditional decoction of the roots and flowers described in Section 4.

Selected cliotides and their properties are given in Table 6.

Table 6. Selected cliotides from *Clitoria ternatea*: mass, charge and measured activity.

Peptide	Subfamily	Monoisotopic mass (Da)	Net charge	Reported activity	Source
Cter M	Möbius	3057.56 (mature; [M+H]+ at m/z 3058.57)	not stated	Heat stable; insecticidal against *Helicoverpa armigera* at 1 µmol/g diet; weakly haemolytic (HD50 > 100 µM)	[6]
Clotide T1	bracelet	not stated	not stated	MIC 1.1 µM (*E. coli*), 2.7 µM (*K. pneumoniae*), 4.7 µM (*P. aeruginosa*); HeLa IC50 0.6 µM; HD50 7.1 µM	[38]
Clotide T2	Möbius	not stated	not stated	Inactive against all five test bacteria up to 100 µM; HeLa IC50 8.0 µM; only 18% haemolysis at 40 µM	[38]
Clotide T4	bracelet	not stated	not stated	MIC 1.0 µM (*E. coli*), 5.5 µM (*K. pneumoniae*), 7.5 µM (*P. aeruginosa*); HeLa IC50 0.6 µM; HD50 8.4 µM	[38]
CT4	bracelet	3098	+2	Most cytotoxic of the seven cliotides tested: A549 IC50	[40]

				0.21 µM; 0.12 µM with paclitaxel	
CT2	Möbius	3260	−1	A549 IC50 7.59 µM; 1.62 µM with paclitaxel	[40]
CT10	bracelet	3251	0	A549 IC50 0.70 µM; 1.01 µM with paclitaxel	[40]
CT19, CT20	bracelet	3125, 3152	+4, +3 (as printed in Table I)	Not significantly cytotoxic up to 10 µM	[40]

Beyond the chemistry, three biological points are worth recording here. First, cliotides T1 and T4, both bracelets, killed Gram-negative bacteria in the low micromolar range while the Möbius peptides T2 and T3 were inactive at up to 100 µM, which links subfamily and charge to antimicrobial action [38]. Second, in the cancer assay the cytotoxicity of the seven cliotides rose with net positive charge up to +2 and the positively charged cliotides at +3 and +4 were not active, so charge rather than size was the determinant [40]. Third, cyclotides from this plant delayed β-amyloid-induced paralysis and improved chemotaxis in transgenic *Caenorhabditis elegans* models of amyloid toxicity, and reduced amyloid deposits, which places the peptides in the neurodegeneration field as well [49, 50].

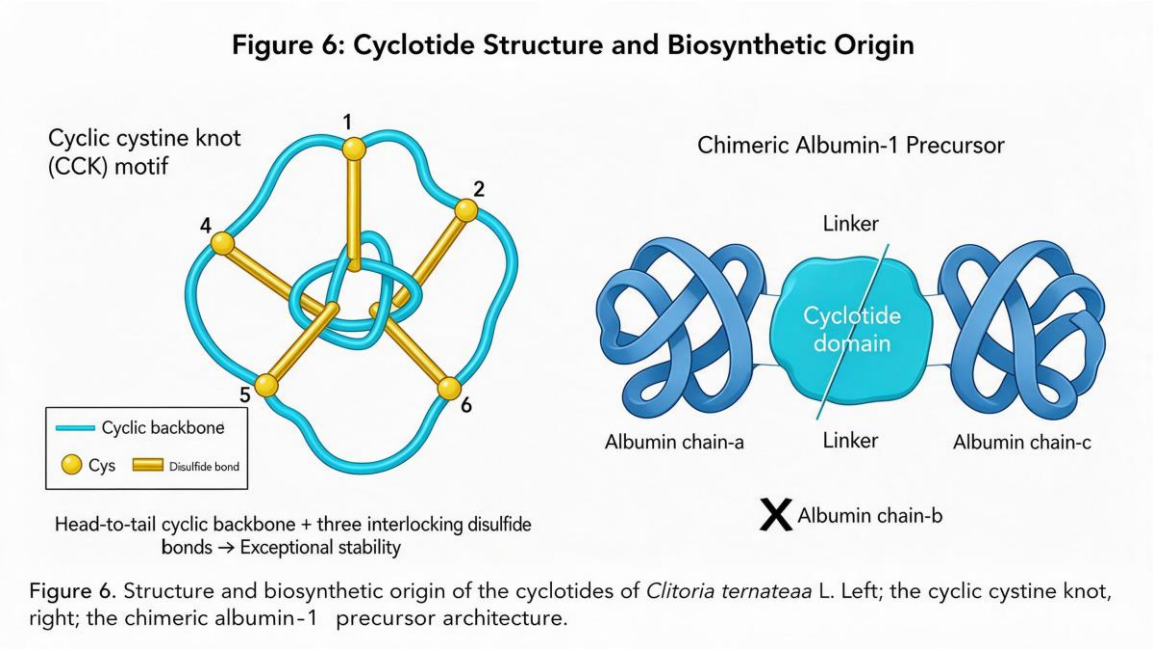


Figure 6. Structure and biosynthetic origin of the cyclotides of Clitoria ternatea L. Left: the cyclic cystine knot; right: the chimeric albumin-1 precursor architecture.

5.5 Proteins, lectins and other constituents

Two seed proteins deserve mention because they were purified to homogeneity and characterised rather than screened. Finotin, a highly basic protein of about 20 kDa isolated by ultrafiltration and isoelectric focusing, inhibited the growth of seven important fungal plant pathogens including *Rhizoctonia solani*, *Fusarium solani*, *Pyricularia grisea* and *Colletotrichum gloeosporioides*, blocked the bacterial blight pathogen *Xanthomonas axonopodis* pv. *phaseoli*, and killed the bean bruchids *Zabrotes subfasciatus* and *Acanthoscelides obtectus* [34]. A second seed protein, of 14.3 kDa and named

Ct protein, was purified by ammonium sulphate precipitation, DEAE-cellulose chromatography and gel filtration; it lysed *Micrococcus luteus*, killed clinically relevant yeasts including *Cryptococcus neoformans* and *Candida albicans*, and inhibited mycelial growth of *Aspergillus*, *Alternaria*, *Cladosporium*, *Rhizopus* and other moulds [35]. Both papers describe proteins that would be destroyed by boiling, which raises an interesting contrast with the cyclotides in the same seeds.

Other seed constituents recorded in the older literature include cinnamic acid, an anthoxanthin glucoside, fixed oil, tannic acid and adenosine, and condensed tannins in raw mature seeds at 0–2.48 mg catechin equivalents per gram with protein-precipitable polyphenols at 0.16–0.77 mg tannic acid equivalents per gram [2, 5]. Ash content of the seed is quoted as 6% in a secondary source, and as noted in Section 3.3 no primary determination of this value was found [5].

5.6 How the chemistry has been measured

It is worth noting briefly how uneven the analytical standardisation is, because it affects how the numbers in this review should be read. Ternatins have been identified properly by LC-MS in only a handful of studies [32, 43, 44, 1]. Most of the extraction papers in Section 6 report only total anthocyanin by the pH-differential method, expressed as cyanidin-3-glucoside or delphinidin-3-glucoside equivalents, and total phenolics by the Folin–Ciocalteu assay, with no separation of individual compounds at all [51, 52, 53, 46]. That is adequate for comparing extraction conditions within a study, but totals from different studies are not strictly comparable because the equivalence standards differ. Only two marker compounds, taraxerol and β-sitosterol, have validated quantitative HPTLC or HPLC methods with reported limits of detection, recovery and linearity [17, 31].

6. Extraction and Purification Methods

Extraction is where most of the modern work on *Clitoria ternatea* is concentrated, because the value of the flower lies in pigments and phenolics that are easily destroyed by heat, light and long contact with water. The literature runs from simple maceration, still used in pharmacognosy teaching laboratories, to ultrasound and microwave assistance, natural deep eutectic solvents, high-pressure homogenisation, resin purification and spray drying. This section describes what has actually been done, with the conditions and the numbers each study reported.

6.1 Conventional extraction

The classical methods are still common. Cold maceration of 1 kg of fresh leaf in 95.5% methanol for 72 h, repeated three times, gave a crude yield of 1.6% w/w and was the starting material for the taraxerol work described earlier [31]. Soxhlet extraction is used for the root and leaf: 95% ethanol for 12 h gave a root extract yield of 15.89% from 27.8886 g of powder [14], and methanol Soxhlet was used for the muscle-relaxant flower extract [54]. Successive extraction with eleven solvents of decreasing polarity has also been applied to the petals to survey the distribution of constituents across the polarity range [55].

Decoction is the traditional preparation and it is the method used whenever a study wants to mirror use. Flowers boiled at 100 °C for 30 min and lyophilised gave a water extract with a yield of 3.06% and total phenolics of 71.916 ± 1.816 mg gallic acid equivalents per litre of extract [56]. Root powder boiled with distilled water at 1:10 for 30 min, twice, centrifuged and dried gave the aqueous root extract used in the hippocampal dendrite study [57]. For the human trial the flowers were boiled twice in water at 1:20 w/v, 90–95 °C for 4 h, then spray dried at an inlet temperature of 178 °C and an outlet of 85–95 °C; the resulting powder contained 53.08 ± 0.08 mg gallic acid equivalents of total phenolics and 1.08 ± 0.12 mg delphinidin-3-glucoside equivalents of total anthocyanins per gram [13].

6.2 Ultrasound-assisted extraction

Four studies have optimised ultrasound-assisted extraction (UAE) with formal experimental designs, and their results are directly comparable in structure if not in units.

Salacheep and colleagues ran a Taguchi L9 design on dried petals ground to 0.17–0.25 mm, using water at 160 W with time (30, 45, 60 min), temperature (40, 60, 80 °C) and liquid–solid ratio as factors [51]. Grey relational analysis across three responses gave an optimum of 45 min at 40 °C with a liquid–solid ratio of 10 mL water per gram of petal (the paper prints mL/mg in its methods and mL/g in its table, an inconsistency carried over here), which in a verification run delivered 1.77 ± 0.22 mg cyanidin-3-glucoside equivalents of anthocyanin per gram dry weight, 7.35 ± 0.04 mg gallic acid equivalents of total phenolics and 63.80% DPPH scavenging. The liquid–solid ratio was the dominant factor for anthocyanin (77.72% contribution, $P = 0.016$) and for total phenolics (84.00%, $P = 0.046$), while temperature dominated the antioxidant response (77.78%, $P = 0.046$); time mattered least in all three cases [51].

Shu and colleagues screened water against glycerol–water mixtures and selected 60:40 glycerol–water, then optimised a Taguchi L27 design at 40–60 °C and 10:1 to 30:1 solvent-to-solid ratio [53]. Again the solvent-to-solid ratio was decisive, contributing 94.90% of the variation in total anthocyanin ($F = 753.19$, $P < 0.001$) against 2.19% for temperature and 1.65% for time. The optimum of 30 min at 50 °C and 10:1 gave an experimental 115.22 ± 4.20 mg/L against a predicted 112.25 mg/L, a bias of 2.58% [53].

Maia and colleagues replaced water entirely with a natural deep eutectic solvent, choline chloride:glycerol 1:2 with 30% water, and optimised a Box–Behnken design of time (15–50 min), temperature (40–80 °C) and petal-to-solvent ratio (2–7%) [58]. Experimental total anthocyanin ranged from 12.95 to 85.48 mg delphinidin-3-glucoside equivalents per litre with $R^2 = 0.8336$, and the best conditions were 80 °C, 50 min and 7% petal ratio. Averaged over all conditions the eutectic solvent gave 374.65 ± 47.21 mg DGE/L against 211.63 ± 56.67 mg DGE/L for water, and antioxidant capacity of 156.43 ± 85.52 against 93.01 ± 37.53 µmol Trolox per mL of extract, both $P < 0.0001$ [58].

Shiau and colleagues compared intact flowers with powder and found the interaction that matters: raising the temperature from 30 to 90 °C at a fixed 30 min raised total anthocyanin in intact flowers by 531.60% (147 ± 16 to 931 ± 55 mg/kg dry weight) but in the powder by only 9.03% (1,051 ± 28 to 1,146 ± 28 mg/kg), because grinding had already released most of the pigment [52]. Ultrasound at 60 °C for 30 min raised anthocyanin yield from intact flowers by 66.70% and total phenolics by 45.30%, but slightly reduced anthocyanin and DPPH values from powder, again because the powder needed no assistance [52].

A response-surface study on Indonesian flowers optimised ethanol–water mixtures and found that the highest flavonoid content, 53.347 ± 0.201 mg quercetin equivalents per gram, occurred in batches extracted with 100% ethanol, although the same batches gave the lowest yields, 4.279–6.303%, below the 10% minimum of the Indonesian Herbal Pharmacopoeia [59]. The authors note the tension between yield and flavonoid concentration rather than resolving it.

6.3 Other assisted and green methods

Microwave-assisted extraction of dried petals at 1:20 g/mL, 770 W for 1 min gave 30.9 mg cyanidin-3-glucoside per litre, but the anthocyanin content was not better than conventional hot-water extraction [7]. High-speed shearing extraction, 30 g of flower powder in 750 mL of 80% ethanol run for ten cycles of 200 s, followed by AB-8 macroporous resin column chromatography, gave the purified phenolic fraction whose class composition is reported in Section 5.2 [46]. High-pressure processing at 300 MPa produced liposomes from petal extract with a mean particle size of 240.7 nm, a polydispersity index

of 0.37 and an encapsulation efficiency of 77.8%, and was better than magnetic stirring or ultrasonication for size and reproducibility [60]. A green extraction designed for Brazilian-grown flowers used a multivariate design over temperature, acidified solvent and time and identified the optimum as 45 °C for 16 min with 22.5 mL of extraction solution; thirteen anthocyanins and nine non-anthocyanin compounds were quantified in the product, and the extract was safe up to 2.2 g per kg body weight in a *Galleria mellonella* toxicity model [61].

pH has been examined as a variable in its own right. Extracting across the range pH 1 to 13, total anthocyanin peaked at pH 3.0 while total phenolics and DPPH activity peaked at pH 7.0, and the two conditions yielded different compound profiles by LC-HRMS [44, 62]. Response surface optimisation of ethanol concentration, time and temperature gave 37% ethanol, 90 min and 45 °C as a practical optimum, delivering 41.17 ± 0.5 mg gallic acid equivalents per gram of total phenolics, 63.53 ± 0.95% DPPH inhibition, 187.05 ± 3.18 mg quercetin equivalents per gram of total flavonoids and 28.60 ± 0.04 mg/L of anthocyanin [63].

Solvent choice is not a minor detail for the pigments. Aqueous extraction of fresh petals at 60 °C for 60 min gave 56.1% yield and 4.0 mg cyanidin-3-glucoside per gram, while the same material extracted with 50% ethanol gave a DPPH IC50 of 1.24 mg/mL and the water extract 1.18 mg/mL [7, 9]. In a direct comparison, ultrasound-assisted water extraction gave a 246.48% better anthocyanin yield than conventional ethanolic extraction, 1.126 against 0.325 mg delphinidin-3-O-glucoside equivalents per gram, with 47.21% more DPPH scavenging [7].

Table 7. Extraction methods reported for *Clitoria ternatea* with conditions and key outcomes.

Method	Material and solvent	Optimised conditions	Key outcome	Source
Decoction	Flower, water	90–95 °C, 4 h, 1:20 w/v, spray dried at 178 °C inlet	TPC 53.08 ± 0.08 mg GAE/g; TAC 1.08 ± 0.12 mg D3G eq/g	[13]
Decoction	Flower, water	100 °C, 30 min, lyophilised	Yield 3.06%; TPC 71.916 ± 1.816 mg GAE/L	[56]
Maceration	Leaf, 95.5% methanol	72 h, three cycles, ≤45 °C	Yield 1.6% w/w; taraxerol 5.32% w/w	[31]
Soxhlet	Root, 95% ethanol	12 h	Yield 15.89%	[14]
Maceration	Petal, ethanol 96% + citric acid 2.5%	24 h shaker, rotary evaporation 55 °C	Yield 17.09 ± 0.25%; anthocyanin 939.31 mg/L	[64]
UAE (Taguchi L9)	Dried petal, water, 160 W	45 min, 40 °C, 10 mL/g liquid–solid ratio	1.77 ± 0.22 mg C3G eq/g; TPC 7.35 ± 0.04 mg GAE/g; DPPH 63.80%	[51]

UAE (Taguchi L27)	Dried petal, glycerol–water 60:40	30 min, 50 °C, 10:1 ratio	TAC 115.22 ± 4.20 mg/L (predicted 112.25)	[53]
UAE (Box–Behnken)	Petal powder, choline chloride:glycerol NADES	80 °C, 50 min, 7% petal ratio	NADES 374.65 ± 47.21 vs water 211.63 ± 56.67 mg DGE/L	[58]
UAE	Intact flower vs powder, water	60 °C, 30 min, 162.5 W	+66.70% TAC from intact flowers, −6.9% from powder	[52]
RSM (Box–Behnken)	Flower, ethanol–water	14.5 material-to-solvent ratio, 100% ethanol, 60 min	TFC up to 53.347 ± 0.201 mg QE/g; yield as low as 4.279%	[59]
High-speed shearing + AB-8 resin	Flower, 80% ethanol	10 cycles × 200 s; elution with 80% ethanol	TPC 55.24 → 236.78 mg GAE/g	[46]
RSM	Flower, ethanol–water	37% ethanol, 90 min, 45 °C	TPC 41.17 ± 0.5 mg GAE/g; TFC 187.05 ± 3.18 mg QE/g; TAC 28.60 ± 0.04 mg/L	[63]
Green multivariate	Flower, acidified solvent	45 °C, 16 min, 22.5 mL	13 anthocyanins quantified; safe to 2.2 g/kg in *Galleria mellonella*	[61]
pH-controlled	Flower, aqueous buffers	pH 3 for anthocyanin; pH 7 for phenolics	Different LC-HRMS profiles at pH 3 and pH 7	[44]
High-pressure processing	Petal extract liposomes	300 MPa, room temperature	240.7 nm, PDI 0.37, encapsulation 77.8%	[60]

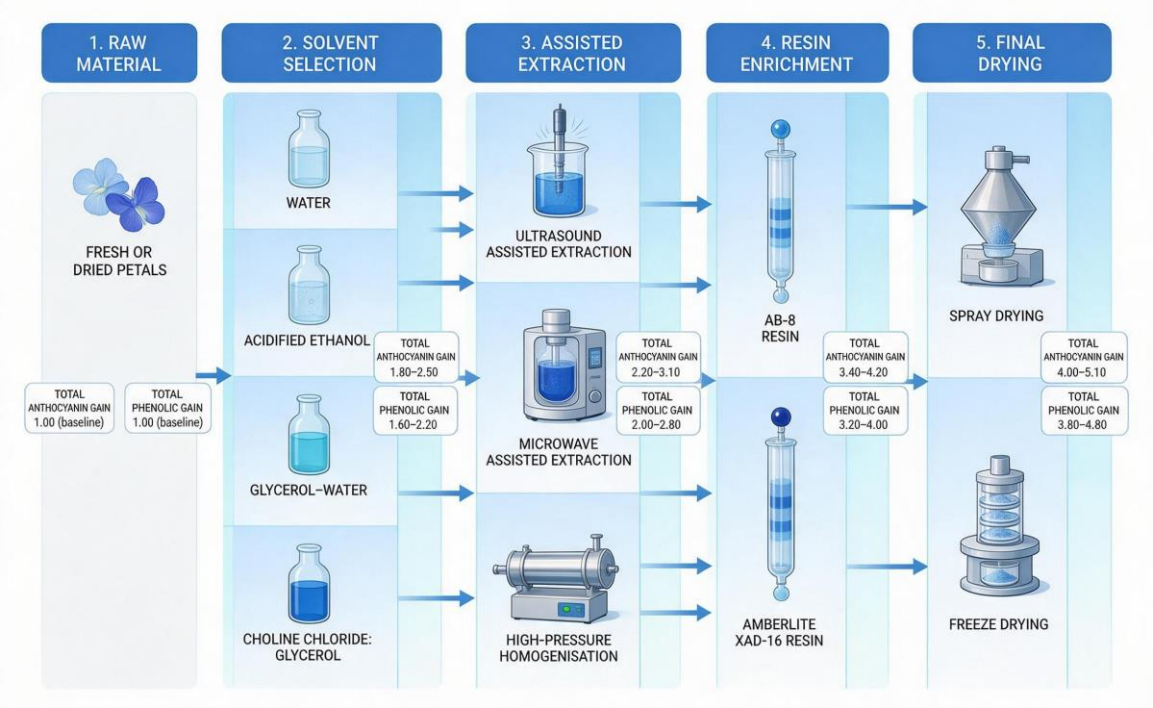


Figure 7. Extraction and purification train reported for Clitoria ternatea flowers: solvent selection, assisted extraction, resin enrichment and drying. The annotated gains are indicative fold-ranges summarising the enrichment of anthocyanins and phenolics at each stage; the measured yields and fold changes are given in Table 7 and Section 6.4.

6.4 Purification, standardisation and drying

Purification is where the largest gains are seen. AB-8 macroporous resin chromatography raised total phenolics 3.29-fold and total flavonoids 4.11-fold [46], and open column chromatography on Amberlite XAD-16 raised total anthocyanin about five-fold while removing flavonols into the ethyl acetate wash [32]. Resin choice was not neutral: C18-OPN gave a higher total phenolic content (176.0 ± 11.9 mg GAE/g) than Amberlite XAD-16 (122.7 ± 8.0 mg/g), but the Amberlite fraction was chosen for further work [32].

Drying and encapsulation are the last step before any practical use. Spray drying with whey protein isolate as wall material at 150 °C inlet temperature gave the best microcapsules: moisture 4.65%, powder recovery 50.75%, encapsulation efficiency 97.30%, anthocyanin 50.10 mg/L, solubility 83.67% and particle size 1.22 µm [64]. Raising the inlet temperature to 170 °C degraded anthocyanin and lowered encapsulation efficiency to 89.66%, so the intermediate temperature was best. Elsewhere, microwave encapsulation with 40% maltodextrin reached 73.24% efficiency and spray drying with 20% maltodextrin reached 87.3% [7], and biopolymeric wall materials have been shown to improve the stability of the pigments further [65].

6.5 Stability of the extracts

Because the whole point of the pigment is its colour, several groups have measured how long it lasts. The numbers in Table 8 are the ones a formulator would need.

Table 8. Stability of *Clitoria ternatea* anthocyanin extracts under heat, light and storage.

Condition	Result	Source
Heating, 60–90 °C, pH 2.5–5.5, 0–5 h	First-order degradation, k = 0.0131–0.0541 h^{-1}; t½ 52.94 h at 60 °C/pH 2.5 and 12.87–15.07 h at 90 °C; activation energy 19.52–45.23 kJ/mol (pH 5.5 to 2.5)	[52]
Heating at pH 3.6 and 5.4	Stable at 60 and 70 °C for 360 min; rate constant rose from 5.57×10^{-4} to 3.41×10^{-3} min^{-1} between 80 and 100 °C at pH 3.6	[7]
Storage, 7 days	Degradation 15.61 ± 1.76% under fluorescent light, 11.42 ± 1.78% in dark, 10.56 ± 0.79% at 4 °C, 13.64 ± 2.33% at 25 °C	[53]
Storage, 21 days	Water extract lost about 38% of anthocyanin; choline chloride:glycerol extract stored refrigerated lost only 16.3%; colour change imperceptible ($\Delta E < 2$) up to 14 days	[58]
Photostability, white fluorescent lamp, 32 °C	Retention 34.4% exposed versus 90.1% covered at pH 3.6; 48.3% versus 94.6% at pH 5.4	[7]
Encapsulation	Spray drying 87.3% efficiency with 20% maltodextrin; microencapsulated colour stability index 0.83 against 0.57 for the free pigment after 21 days	[7]
pH indicator films	Gelatin film with 15% extract: pinkish purple at pH 2, light blue at pH 5–7, bluish green at pH 9–11; turned bluish green after 24 h in contact with spoiling mackerel	[51]
Nanoparticle protection	Extract-loaded silk fibroin nanoparticles retained about 85% DPPH scavenging after 4 h at 70 °C against about 17% for the free extract	[66]

The consistent message is that the ternatins are far more robust than a typical anthocyanin, that light is a bigger enemy than moderate heat, and that encapsulation or a deep eutectic solvent extends shelf life noticeably [7, 52, 58, 65]. Colour stability also depends on pH, which is discussed in Section 11 where the intelligent-film applications are described, and the pigment outlasts its commercial rivals in a direct comparison: at pH 3 and 100 °C the flower extract had a half-life of about 14 h against 7 h for red cabbage and 2 h for hibiscus [8].

7. Pharmacological Activities

The pharmacology of *Clitoria ternatea* has been studied at three levels, and the difference between them matters. Most of the numbers in this section come from in-vitro assays, where a crude extract is tested against an enzyme, a microorganism or a cancer cell line. A second and substantial body of work is in animals, mainly mice and rats, using extracts whose composition is rarely standardised beyond a solvent name. Human evidence is thin and is reserved for Section 9. Throughout this section the level of evidence is marked explicitly, and doses are given as they were reported.

7.1 Central nervous system effects

This is the area where traditional use and modern testing overlap most clearly, because the plant is a Medhya Rasayana in Ayurveda (Section 4.1).

Memory and learning in rodents. Alcoholic extracts of the root and aerial parts at 300 and 500 mg/kg orally attenuated electroshock-induced amnesia in rats, with the root the more effective part at 300 mg/kg, and the effect was accompanied by a rise in brain acetylcholine content and a change in acetylcholinesterase activity in cortex, midbrain, medulla and cerebellum in a pattern the authors compared with the cerebroprotective drug pyritinol [10, 67]. Giving aqueous root extract to rat pups from day seven of life at 50 or 100 mg/kg for 30 days improved retention in passive avoidance and performance in a T-maze both immediately after treatment and 30 days later, while open-field behaviour was unchanged, which the authors read as a lasting change in the brain rather than a stimulant effect [11]. The same group later showed that 50 and 100 mg/kg for 30 days increased dendritic intersections, branching points and somal processes in amygdaloid neurons of young adult rats at 100 mg/kg, correlating with better passive avoidance learning [68].

The clearest modern dataset comes from a chronic cerebral hypoperfusion model. Rats subjected to permanent bilateral occlusion of the common carotid arteries and treated orally for 28 days showed restored memory at 200 and 300 mg/kg, reduced neuronal damage in the CA1 region of the hippocampus, and inhibition of the raised acetylcholinesterase activity in frontal cortex and hippocampus at 300 mg/kg; blood and organ chemistry over the same period showed no toxicity [12]. In normal mice a water extract of the flowers at 200, 400 and 800 mg/kg improved the discrimination index in the object recognition test after 14 days at 200 and 400 mg/kg (0.498 ± 0.039 and 0.521 ± 0.136 against a vehicle value of 0.156 ± 0.078, $P < 0.05$), and the effect appeared at the 30 min test but not the 6 h test; in the same animals prefrontal cortex malondialdehyde fell from 0.081 ± 0.015 to 0.020–0.023 nmol/mg protein and acetylcholinesterase from 9.907 ± 2.153 to 2.035–3.511 µmol/min g protein, both at $P < 0.001$ [56].

Diabetes-linked cognitive decline has also been addressed. An ethanolic leaf extract at 200 and 400 mg/kg given for two weeks to streptozotocin-diabetic rats raised spontaneous alternation in the Y-maze from 34.27 ± 2.52% in diabetic controls to 52.5 ± 5.83% and 58.98 ± 6.77%, shortened Morris water maze transfer latency from 77.8 ± 1.42 s to 53.2 ± 6.00 s and 56.4 ± 1.28 s, lowered whole-brain acetylcholinesterase from 6.36 ± 0.30 to 4.07 ± 0.42 µM/l/min/g at the higher dose, and reduced lipid peroxide from 52.70 ± 2.36 to 25.14 ± 0.27 nmol MDA/mg protein while restoring superoxide dismutase, catalase and glutathione [69] (animal study).

Anxiety, sedation and motor effects. Methanolic extracts of the stems and leaves of the blue variety tested in the elevated plus maze showed mild anxiolytic activity: stems at 200 mg/kg raised open-arm entries from 1.60 ± 0.54 in controls to 5.80 ± 1.48 and open-arm time from 3.66 ± 0.73 s to 10.08 ± 2.51 s, while diazepam 2 mg/kg gave 10.60 ± 1.14 entries; the leaf extract peaked at 100 mg/kg and then fell at 200 mg/kg, which the authors attributed to reduced locomotion or a CNS-depressant effect at the higher dose [70]. In vitro the same extracts were weak antioxidants, with DPPH IC50 of 264.32

µg/mL (stem) and 251.13 µg/mL (leaf) against 20.01 µg/mL for ascorbic acid [70]. The methanolic flower extract reduced rotarod fall-off time from 49.33 ± 0.028 s in controls to 5 ± 0.032 s at 200 mg/kg, close to diazepam 10 mg/kg at 6 ± 0.019 s, indicating a muscle-relaxant effect [54].

Cholinesterase inhibition. Three studies approach the enzyme from different directions. In vitro, an ethanolic root extract inhibited acetylcholinesterase with an IC50 of 118.5 ± 8.6 µg/mL and butyrylcholinesterase with an IC50 of 83.59 ± 6.23 µg/mL (86.57 ± 1.92% and 91.74 ± 0.84% inhibition at 800 µg/mL) against physostigmine at 0.03 and 0.09 µg/mL, so the extract is selective for butyrylcholinesterase but far weaker than the standard [14]. In vivo, whole-brain acetylcholinesterase in diabetic rats fell from 6.36 ± 0.30 to 4.07 ± 0.42 µM/l/min/g with the leaf extract [69], and in the hypoperfusion model frontal cortex and hippocampal acetylcholinesterase was reduced by the root extract at 300 mg/kg [12]. In prefrontal cortex of normal mice the flower water extract lowered the enzyme from 9.907 ± 2.153 to 2.035 µmol/min g protein at 800 mg/kg [56].

Stress, dendrites and newer models. Restraint stress for 6 h a day over six weeks reduced apical and basal dendritic branching and intersections of hippocampal CA3 neurons in mice; aqueous root extract at 100 mg/kg/day given throughout the stress period raised branching in the 40–120 µm apical zones and in all basal zones compared with stressed controls ($P < 0.05$ to $P < 0.001$) [57]. Root fractions have been tested in reserpine-treated zebrafish, where the ethyl acetate and 50% aqueous methanol fractions significantly shortened freezing time and increased distance travelled 72 h after treatment, an effect the authors linked to the clitorienolactones and isoflavonoids they contain [37]. An ethyl acetate fraction of the flower suppressed nitric oxide production in lipopolysaccharide-activated BV-2 microglia and SK-N-SH neuroblastoma cells, with docking pointing at inducible nitric oxide synthase as the target and no toxicity in a zebrafish embryo model [71]. Cyclotide-rich fractions from the plant delayed β-amyloid-induced paralysis and improved chemotaxis in transgenic Caenorhabditis elegans and reduced amyloid deposits, apparently by inhibiting oligomerisation [49], and molecular dynamics simulations show the peptides binding amyloid fibrils and weakening the inter-strand hydrogen bonds that hold the β-sheet together [50].

Table 9. Central nervous system studies on *Clitoria ternatea* (all animal or in-vitro studies).

Study	Part and extract	Model	Dose	Key result	Evidence
[10]	Root and aerial, alcoholic	Electroshock amnesia in rats	300, 500 mg/kg p.o.	Memory retention improved at 300 mg/kg; brain acetylcholine and regional AChE changed like pyritinol	Animal
[11]	Root, aqueous	Passive avoidance and T-maze, rat pups from day 7	50, 100 mg/kg for 30 days	Better retention and spatial learning immediately and 30 days	Animal

				later; open field unchanged	
[68]	Root, aqueous	Amygdala Golgi morphometry, rats	50, 100 mg/kg for 30 days	Increased dendritic intersections and branching at 100 mg/kg	Animal
[12]	Root, extract	Chronic cerebral hypoperfusion (PBOCCA) rats	100–300 mg/kg for 28 days	200 and 300 mg/kg restored memory; 300 mg/kg lowered cortical and hippocampal AChE; no toxicity	Animal
[56]	Flower, water (decoction)	Object recognition in mice	200, 400, 800 mg/kg for 14 days	Discrimination index 0.498 ± 0.039 and 0.521 ± 0.136 vs vehicle 0.156 ± 0.078 ($P < 0.05$); prefrontal MDA and AChE reduced ($P < 0.001$)	Animal
[69]	Leaf, ethanol 95%	STZ-diabetic rats, Y-maze, water maze, radial maze	200, 400 mg/kg for 14 days	Alternation 34.27 ± 2.52 → 58.98 ± 6.77%; AChE 6.36 ± 0.30 → 4.07 ± 0.42 µM/l/min/g	Animal
[70]	Stem and leaf, methanol, blue variety	Elevated plus maze in mice; DPPH	50, 100, 200 mg/kg p.o.	Open-arm time 3.66 ± 0.73 → 10.08 ± 2.51 s (stem 200 mg/kg); mild activity only	Animal + in vitro
[18]	Seed, ethanol and benzene	Clonidine catalepsy in mice	75, 100 mg/kg i.p.	Benzene extract at 100 mg/kg cut catalepsy at 90 min from	Animal

				175.5 ± 14.18 to 14.7 ± 3.121 s	
[54]	Flower, methanol	Rotarod in mice	50, 100, 200 mg/kg p.o.	Fall-off time 49.33 ± 0.028 → 5 ± 0.032 s; comparable to diazepam 10 mg/kg	Animal
[14]	Root, ethanol	AChE and BChE (Ellman assay)	20–800 µg/mL in vitro	IC50 118.5 ± 8.6 µg/mL (AChE) and 83.59 ± 6.23 µg/mL (BChE)	In vitro
[57]	Root, aqueous	Restraint stress, hippocampal CA3 dendrites in mice	100 mg/kg for 6 weeks	Restored branching and intersections in multiple Sholl zones ($P < 0.05–0.001$)	Animal
[37]	Root, ethyl acetate and 50% methanol fractions	Reserpine-induced zebrafish stress	Not stated in abstract	Reduced freezing, increased distance and velocity at 72 h	Animal (zebrafish)
[71]	Flower, ethyl acetate fraction	LPS-activated BV-2 and SK-N-SH cells	Not stated in abstract	Reduced nitric oxide; docking suggests iNOS; no zebrafish embryo toxicity	In vitro
[49, 50]	Cyclotide fractions	Transgenic *C. elegans* amyloid models; molecular dynamics	Not stated in abstract	Delayed Aβ paralysis, improved chemotaxis, reduced deposits	In vitro / model organism

7.2 Anti-inflammatory, analgesic and antipyretic activity

Methanolic root extract given orally to rats inhibited carrageenin paw oedema and acetic acid-induced vascular permeability, reduced yeast-induced pyrexia, and cut the number of writhings in mice at 200 and 400 mg/kg [72]. The same review literature reports the antipyretic effect as comparable to paracetamol 150 mg/kg and the paw oedema inhibition as comparable to diclofenac sodium [2].

Ethanolic root extract at 200 and 400 mg/kg reduced paw oedema in carrageenin- and histamine-induced models in female Wistar rats and, in Freund's complete adjuvant arthritis, significantly reduced paw diameter from day 7, with joint damage reduced at 200 and 400 mg/kg alongside improvements in haematological and antioxidant parameters [5].

In vitro, ethanolic leaf and flower extracts inhibited non-enzymatic glycosylation of haemoglobin, glucose uptake in yeast cells and salivary α-amylase, and showed anti-inflammatory action by albumin denaturation and red-cell membrane stabilisation: at 50 µg/mL the leaf extract inhibited albumin denaturation by 89.3 ± 6.25% and the flower extract by 80 ± 5.60% against aspirin at 73.3 ± 5.13%, and membrane stabilisation reached 80 ± 5.60% and 72.7 ± 5.08% against 62 ± 4.34% for aspirin [73]. A standardised leaf extract also inhibited hyaluronidase with an IC50 of 18.08 ± 0.46 µg/mL against oleanolic acid at 41.51 ± 0.50 µg/mL, inhibited elastase at 42.68 ± 0.46 µg/mL (a value the authors call insignificant against the standard at 9.61 ± 0.36 µg/mL), and inhibited matrix metalloproteinase-1 more strongly than oleanolic acid, which is the experimental basis for the traditional use of the leaf on wounds [31].

7.3 Respiratory and antihistaminic effects

Two studies from the same group test the root in models that match the traditional use in bronchitis and asthma. Ethanol root extract at 100–150 mg/kg intraperitoneally inhibited milk-induced leucocytosis and eosinophilia, protected against egg-albumin-induced mast cell degranisation and inhibited blue dye leakage in passive cutaneous anaphylaxis in rats; the acute lethal dose was above 1,300 mg/kg and the extract contained steroids, saponins, flavonoids and glycosides [30]. The same extract at 100, 125 and 150 mg/kg significantly inhibited clonidine-induced catalepsy ($P < 0.001$) but not haloperidol-induced catalepsy, which points to an antihistaminic rather than a dopaminergic mechanism, since clonidine releases histamine from mast cells [29]. In the flower, an ethanolic extract at 400 mg/kg reduced histamine-induced dyspnoea in guinea pigs and reduced cough, lung inflammation and inflammatory markers in mice [1].

7.4 Antidiabetic activity

The evidence here falls into three tiers: enzyme and glycation assays in vitro, a small number of animal studies, and one systematic review that pulled the two together.

In vitro. Ethanolic leaf and flower extracts at 10–50 µg/mL inhibited non-enzymatic glycosylation of haemoglobin (86.51 ± 6.05% and 89.24 ± 6.24% at 50 µg/mL against vitamin E at 84.94 ± 5.94%), increased glucose uptake by yeast cells (87.48 ± 6.12% and 93.56 ± 6.54% against metformin at 90.28 ± 6.31%) and inhibited salivary α-amylase (81.40 ± 5.69% and 86.16 ± 6.03% at the top concentration) [73]. A purified phenolic fraction from the flower inhibited pancreatic lipase with an IC50 of 0.28 mg/mL against orlistat at 0.16 mg/mL, α-amylase at 1.70 mg/mL against acarbose at 2.76 mg/mL and α-glucosidase at 1.04 mg/mL against acarbose at 0.94 mg/mL, so it was more potent than acarbose on amylase and roughly equipotent on glucosidase [46]. Water extracts of the flower at 0.25–1.00 mg/mL inhibited fructose-induced and methylglyoxal-induced advanced glycation end-product formation, though less than aminoguanidine [16].

Animal studies. A systematic review following PRISMA, searching PubMed and Scopus for 2012–2021, retained five studies: three with in-vitro endpoints and two in animals [16]. In alloxan-diabetic rats a flower protein extract at 500 mg/kg for 30 days lowered blood glucose to a degree comparable with metformin 100 mg/kg, raised PPARγ, Glut2, Tcf7l2 and Capn10 expression and lowered MCP1; a chloroform flower extract at 300 mg/kg for 12 days cut fasting blood glucose from 378.33 ± 3.51 to 136.33 ± 5.51 mg/dL ($P < 0.001$) with a significant gain in body weight [16]. Older work in the same tradition gave aqueous leaf and flower extracts at 400 mg/kg for 84 days to alloxan-diabetic rats and

reported lower serum glucose, glycosylated haemoglobin, cholesterol, triglycerides, urea, creatinine and glucose-6-phosphatase with higher insulin, HDL, liver and muscle glycogen and glucokinase activity [2].

Two recent studies used the same design: high-fat diet plus 0.01% propylthiouracil for 28 days followed by nicotinamide 120 mg/kg and streptozotocin 60 mg/kg, then 200, 400 and 800 mg/kg of a 70% ethanol flower extract for 28 days [74, 75]. The first reported that the extract lowered blood glucose, raised insulin, raised pancreatic catalase and superoxide dismutase and lowered pancreatic malondialdehyde, IL-18 and IL-6, glycogen gene expression and hepatic GSK-3β, with 400 mg/kg usually the most effective dose and 800 mg/kg the best for liver histology [74]. The second study printed actual values: in the positive control group liver malondialdehyde was 7.45 nmol/mL and 29.69 nmol/mg protein, superoxide dismutase 5.08 U/mL and 12.59 U/mg protein, catalase 35.19 U/mL and 144.23 U/mg protein, lactate dehydrogenase 77.78 U/L, acid phosphatase 41.10 U/L and alanine aminotransferase 31.61 IU/L; treatment with 800 mg/kg brought these to 4.46 nmol/mL and 12.71 nmol/mg protein, 11.39 U/mL and 31.64 U/mg protein, 72.16 U/mL and 200.45 U/mg protein, 49.62 U/L, 24.83 U/L and 15.16 IU/L respectively, in several cases better than glibenclamide 0.45 mg/kg or simvastatin 0.9 mg/kg alone [75]. Both papers present most of their data as figures with letter-coded group comparisons rather than printed numbers, and neither states the group size in the text, which limits how far the results can be taken.

7.5 Hepatoprotective effects

Methanolic leaf extract standardised at 358.99 ± 6.21 mg gallic acid equivalents and 123.75 ± 2.84 mg catechin equivalents per gram, with a DPPH IC50 of 420.00 µg/mL, protected mice against paracetamol: at 200 mg/kg it significantly lowered the raised alanine and aspartate aminotransferases and bilirubin ($P < 0.01$) and improved liver histology [76]. The diabetes model described above also produced liver endpoints, with the extract reducing inflammation, necrotic cells and lipid degeneration, the 800 mg/kg dose being most effective [74]. Anthocyanin-rich butterfly pea extract at 175 and 700 mg/kg for a month reduced inflammatory cells and periductal fibrosis in *Opisthorchis viverrini*-infected hamsters, lowered plasma nitrite, alanine aminotransferase and 8-oxodG, and shifted the expression of NF-κB and inducible nitric oxide synthase down and catalase, superoxide dismutase and glutathione peroxidase up [33].

7.6 Cardiovascular, lipid and metabolic effects

Hypertension and vascular function were tested in rats given L-NAME at 40 mg/kg in drinking water for five weeks and treated with flower extract at 300 mg/kg/day or lisinopril at 2.5 mg/kg/day [77]. The extract reduced the rise in blood pressure, improved acetylcholine-induced vasorelaxation and the enhanced contractile response to sympathetic stimulation, partially prevented left ventricular hypertrophy and dysfunction, lowered plasma nitrate and nitrite, reduced oxidative stress, and suppressed serum angiotensin-converting enzyme activity, plasma angiotensin II, cardiac angiotensin II type 1 receptor, NADPH oxidase 2, NF-κB and tumour necrosis factor-alpha expression; the extract was standardised on flavonoids [77].

For lipids, an aqueous petal extract tested in high-fat diet rats gave total anthocyanin of 78.0943 mg/100 g and tannin of 1424.90 mg/100 g in the material used, and at 750 mg/kg significantly improved cholesterol (mean difference 111.12 mg/dL, 95% CI 99.57–122.67, $P < 0.001$), LDL (76.38 mg/dL, 95% CI 56.77–96.00, $P < 0.001$), VLDL, body weight (56.20 g, 95% CI 13.89–98.51, $P = 0.012$) and the thickness of the tunica of the thoracic aorta (22.76 µm, 95% CI 20.11–24.40, $P < 0.001$) [78]. Hydroalcohol extracts of seed and root at 500 mg/kg reduced total cholesterol, triglycerides and VLDL in Poloxamer 407-induced hyperlipidaemia [1]. In a mouse model of low-grade inflammation,

an anthocyanin-rich flower extract lowered plasma glucose, lipopolysaccharide and tumour necrosis factor-alpha, restored lipid metabolism and the balance of regulatory and Th17 cells, increased tight-junction protein expression in the colon, and reshaped the gut microbiota towards short-chain-fatty-acid producers such as *Akkermansia* and Butyricicoccaceae [79].

Table 10. Metabolic, hepatic and cardiovascular studies.

Study	Model	Treatment	Key numbers	Evidence
[73]	In-vitro glycation, yeast glucose uptake, α-amylase, albumin denaturation, membrane stabilisation	Ethanolic leaf and flower extracts	Hb glycation 86.51% and 89.24% at 50 µg/mL; membrane stabilisation 80% and 72.7% vs aspirin 62%	In vitro
[46]	Lipase, α-amylase, α-glucosidase	Purified flower phenolics	IC50 0.28, 1.70, 1.04 mg/mL vs orlistat 0.16 and acarbose 2.76/0.94 mg/mL	In vitro
[16]	PRISMA review of flower antidiabetic studies (2012–2021)	5 studies retained of 13 records	Chloroform extract 300 mg/kg: glucose 378.33 ± 3.51 → 136.33 ± 5.51 mg/dL in 12 days	Review
[74]	STZ + nicotinamide + HFD/PTU rats, 28 days	70% ethanol flower extract 200–800 mg/kg	Glucose and insulin improved (best 400 mg/kg); pancreatic CAT, SOD up; MDA, IL-18, IL-6, GSK-3β down	Animal
[75]	Same model, liver endpoints	200–800 mg/kg	MDA 7.45 → 4.46 nmol/mL; CAT 35.19 → 72.16 U/mL; ALT 31.61 → 15.16 IU/L at 800 mg/kg	Animal
[76]	Paracetamol liver injury in mice	Methanolic leaf extract 200 mg/kg	ALT, AST and bilirubin lowered ($P < 0.01$); DPPH IC50 420.00 µg/mL	Animal + in vitro

[33]	*O. viverrini*-infected hamsters	Anthocyanin complex 175 and 700 mg/kg for 1 month	Reduced inflammation, periductal fibrosis, nitrite, ALT and 8-oxodG	Animal
[77]	L-NAME hypertensive rats, 5 weeks	Flower extract 300 mg/kg/day vs lisinopril 2.5 mg/kg/day	Blood pressure, vasorelaxation, ACE, Ang II, NF-κB and TNF-α all improved	Animal
[78]	High-fat diet rats	Aqueous petal extract 750 mg/kg	Cholesterol −111.12 mg/dL, LDL −76.38 mg/dL, body weight −56.20 g ($P < 0.05$)	Animal
[79]	Low-grade inflammation mouse model	Anthocyanin-rich flower extract	Lower LPS and TNF-α; restored Treg/Th17 balance; raised *Akkermansia* and Butyricicoccaceae	Animal

7.7 Antimicrobial activity

The antimicrobial tests on this plant fall into three groups: disc-diffusion screens with crude extracts, minimum inhibitory concentration determinations, and work on purified proteins. The three give quite different pictures of how active the plant really is.

The most complete study is on the flower as a food biopreservative. An acidified ethanolic petal extract was tested against thirteen food-borne organisms by disc diffusion at 50 mg/mL and by broth microdilution [80]. Zones of inhibition were 15.8 ± 1.7 mm for *Bacillus subtilis*, 15.5 ± 1.3 mm for *Penicillium expansum*, 14.5 ± 2.1 mm for *Bacillus cereus*, 14.0 ± 1.1 mm for *Proteus mirabilis*, 13.4 ± 1.4 mm for *Staphylococcus aureus* and 12.0 ± 0.4 mm for *Klebsiella pneumoniae*, while *Escherichia coli*, *Yersinia enterocolitica*, *Bacillus spizizenii*, two yeasts and two other moulds showed no zone at all. Notably the *S. aureus* zone was larger than the chloramphenicol control zone of 10.0 ± 1.0 mm at the single concentration tested. Minimum inhibitory concentrations ranged from 1.6 mg/mL (*K. pneumoniae*) to 50 mg/mL (*S. aureus*), and minimum bactericidal concentrations were consistently higher than the MICs, from 25 mg/mL (*K. pneumoniae*, *P. expansum*) to 100 mg/mL (*S. aureus*), which the authors read as concentration-dependent killing [80]. Against *P. expansum* on cooked rice the extract produced roughly a 5-log suppression of growth over five days, and scanning electron microscopy showed flattened, empty hyphae with disrupted cell walls and complete suppression of conidial development. The paper attributes the activity to the penta-acylated anthocyanins, ternatins A1, A2, B1, B2, D1 and D2.

The root has also been tested. Aqueous and ethanolic root extracts applied by agar well diffusion at 100 µg/mL gave zones of 35 mm (alcoholic) and 18 mm (aqueous) against *E. coli*, 22 and 14 mm against *Pseudomonas aeruginosa*, and 28 and 15 mm against a clinical multidrug-resistant *Klebsiella*

pneumoniae isolate; *Candida albicans* was resistant to both extracts at every concentration, and the alcoholic extract was significantly more active than the aqueous one at each dose ($P < 0.001$) [81]. Minimum inhibitory and minimum bactericidal concentrations were not determined in that study.

A combined screen of methanolic leaf and flower extracts reported disc zones of 7.00–13.00 mm against *S. aureus*, *E. coli*, *Salmonella typhi* and *Pseudomonas* sp. at 50–200 µg/mL, with the largest zone, 13.00 ± 1.00 mm, for both leaf and flower against *E. coli* at 200 µg/mL, against gentamicin control zones of 15–20 mm; biofilm inhibition ranged from 21% to 58% [48]. The same paper docked GC-MS-derived leaf and flower compounds against the *E. coli* acrB efflux pump (PDB 5ENR), with the best binding energies at −8.2 kcal/mol for a leaf compound and −5.4 kcal/mol for a flower compound [48].

The cleanest minimum inhibitory concentration data come from fractionating the petal extract. The anthocyanin-rich fraction from Amberlite XAD-16 was active only against *B. cereus* and *B. subtilis*, at 0.63 mg/mL, and *E. coli*, at 10 mg/mL; neither crude extract was active below 40 mg/mL against any of nineteen strains including three MRSA, two VRE and five *P. aeruginosa* isolates, and the fraction was about sixteen times more potent than the crude extracts against the two *Bacillus* species [32]. The authors note that the deep blue colour interfered with broth microdilution, which is why agar dilution was used.

Purified seed proteins work at entirely different concentrations. Finotin inhibited seven fungal plant pathogens and the bean blight bacterium, and the 14.3 kDa Ct protein lysed *Micrococcus luteus* and killed *Cryptococcus neoformans* and *Candida albicans* [34, 35]. The cliotides T1 and T4 inhibited *E. coli* at about 1 µM and *Klebsiella* and *Pseudomonas* in the low micromolar range, whereas the Möbius cliotides were inactive up to 100 µM [38]. Taken together the picture is that the crude extracts are weak by clinical standards, with inhibitory concentrations in the mg/mL range, but that the purified peptides and proteins are genuinely potent.

Table 11. In-vitro antimicrobial activity of *Clitoria ternatea* preparations.

Study	Preparation	Method	Key results
[80]	Flower, acidified ethanol	Disc diffusion (50 mg/mL) and broth microdilution	Zones: *B. subtilis* 15.8 ± 1.7 mm, *P. expansum* 15.5 ± 1.3 mm; MIC 1.6 (*K. pneumoniae*) to 50 mg/mL (*S. aureus*); rice model 5-log suppression
[81]	Root, aqueous and ethanolic	Agar well diffusion, 12.5–100 µg/mL	At 100 µg/mL: alcoholic 35 mm (*E. coli*), 28 mm (MDR *K. pneumoniae*); *C. albicans* resistant; no MIC
[48]	Leaf and flower, methanol	Disc diffusion 50–200 µg/mL; biofilm; docking	Largest zone 13.00 ± 1.00 mm; biofilm inhibition 21–58%; acrB docking to −8.2 kcal/mol
[32]	Petal, crude 50% ethanol and water; anthocyanin-rich fraction	Agar dilution, 19 strains, 0.16–40 mg/mL	Fraction MIC 0.63 mg/mL (*B. cereus*, *B. subtilis*) and 10 mg/mL (*E. coli*); crudes > 40 mg/mL against all strains

[34]	Seed protein finotin, purified	Spore germination and mycelial growth	Inhibited *R. solani*, *F. solani*, *P. grisea*, *C. gloeosporioides* and *X. axonopodis* pv. *phaseoli*
[35]	Seed Ct protein, 14.3 kDa	Broth dilution, haemolysis	Lysed *M. luteus*; fungicidal against *Cryptococcus* and *Candida*; inhibited several moulds
[38]	Cliotides T1–T4	MIC in Mueller-Hinton broth	T1 and T4 MIC 1.0–1.1 µM (*E. coli*), 2.7–5.5 µM (*K. pneumoniae*), 4.7–7.5 µM (*P. aeruginosa*); T2 and T3 inactive at 100 µM

7.8 Wound and skin healing

The wound work runs from enzyme assays to a rat cream study to a human oral-wound trial, which is described in Section 9.

At the enzyme level, a methanolic leaf extract standardised on taraxerol inhibited hyaluronidase with an IC50 of 18.08 ± 0.46 µg/mL, about two-fold better than the oleanolic acid standard at 41.51 ± 0.50 µg/mL, inhibited human leukocyte elastase at 42.68 ± 0.46 µg/mL (described by the authors as insignificant against the standard at 9.61 ± 0.36 µg/mL), and inhibited matrix metalloproteinase-1 more strongly than oleanolic acid at the concentrations tested; the ethyl acetate fraction was the most active fraction on matrix metalloproteinase-1 [31]. Since hyaluronidase, elastase and matrix metalloproteinase-1 all degrade connective tissue matrix, this is a coherent mechanism for a traditional wound application.

In vivo, a flower extract cream at 5%, 10% and 15% was applied to incision wounds on the backs of 75 Sprague Dawley rats, five per group at three termination points [82]. Interleukin-6 staining was 100% strong in all treated groups on day 3, then fell in the 5% and 10% groups; epidermal growth factor was 100% strong in the 5% and 10% groups on day 3 and in the 10% group on day 7; and epithelial thickness on day 15 reached 96.04 ± 7.97 µm in the 5% group against 66.31 ± 4.46 µm in the povidone-iodine control. The differences against povidone-iodine were not statistically significant at any time point, but the authors concluded that re-epithelialization was faster than in the controls and that the 10% cream was comparable to povidone-iodine [82]. An earlier study cited in that paper reported complete healing at day 7 with a 15% cream in Wistar rats against day 12 in untreated controls.

A niosome formulation of an anthocyanin complex made from this plant and *Zea mays* has been tested in the same setting. The complex promoted collagen production in human gingival fibroblasts, where neither source alone did, and in incisional wounds of the rat buccal cavity a 10% anthocyanin-complex niosome gel reduced wound size after three days more than fluocinolone acetonide gel ($P < 0.05$) and gave complete wound healing by day 5, with histology showing the adverse effect of the steroid gel and the healing effect of the niosome gel [83].

Aqueous flower extract has also been tested on human cells. At the optimal concentration of 0.08 mg/mL, chosen from an MTT range of 0.01–0.1 mg/mL, viability of human corneal epithelial cells reached 183.34 ± 3.70% of control at 72 h, and in a scratch assay the extract closed 67.50 ± 1.58% of the wound at 6 h and 100% at 12 h against 33.67 ± 0.71% and 51.83 ± 1.11% in untreated controls (P

< 0.05); connexin 43 expression rose significantly at 12 h within the treated group [84]. The extract contained 33.06 mg cyanidin-3-glucoside equivalents of anthocyanin and 24.14 mg gallic acid equivalents of total polyphenol per gram, and fifty-one constituents were tentatively identified by LC-MS.

Table 12. Wound-healing studies with *Clitoria ternatea*.

Study	Model	Preparation	Key results	Evidence
[31]	Hyaluronidase, elastase, MMP-1 in vitro	Standardised methanolic leaf extract and fractions	Hyaluronidase IC50 18.08 ± 0.46 µg/mL vs oleanolic acid 41.51 ± 0.50 µg/mL; MMP-1 inhibition significant	In vitro
[82]	Rat incision wound, 75 animals	5%, 10%, 15% flower extract cream vs povidone-iodine	Day 15 epithelial thickness 96.04 ± 7.97 µm (5% cream) vs 66.31 ± 4.46 µm (povidone-iodine); IL-6 and EGF immunohistochemistry scored	Animal
[84]	Human corneal epithelial cells, scratch assay	Aqueous flower extract, 0.08 mg/mL	67.50 ± 1.58% closure at 6 h, complete at 12 h vs 33.67 ± 0.71% and 51.83 ± 1.11% control (P < 0.05)	In vitro (human cells)
[85]	Animal and human oral wounds	Anthocyanin complex gel	Faster closure in animals; clinical improvement in orthodontic patients	Animal + human
[86]	In vitro and 60-volunteer oral wound trial	Anthocyanin complex niosome gel	Accelerated healing in animals; significant clinical healing in volunteers	In vitro + human

7.9 Anticancer and cytotoxic activity

Cancer activity has been reported at two levels: purified cyclotides with sub-micromolar potency, and crude extracts with conventional phytochemical potencies.

The cliotides are the striking result. Of seven peptides isolated from flowers and seeds, cliotide CT4, a bracelet with a net charge of +2, killed paclitaxel-resistant human non-small-cell lung cancer A549/paclitaxel cells with an IC50 of 0.12 µM and sensitive A549 cells with an IC50 of 0.21 µM, and it sensitised the resistant cells to paclitaxel at 10 nM; CT10 was next at 0.70 µM in A549, CT10 and CT12 at 0.76 µM in the resistant line, CT2 at 7.59 and 1.62 µM, and CT19 and CT20 were not significantly cytotoxic at all [40]. The ranking followed net positive charge up to +2 and then reversed, with the +3 and +4 peptides inactive. In a separate screen of twelve new cliotides, T1 and T4 killed HeLa cells with an IC50 of 0.6 µM, T3 gave 2.0 µM and T2 gave 8.0 µM, values in the range of the

benchmark cyclotides varv A and varv F (2.6–7.4 µM) though above cycloviolacin O2 (0.1–0.3 µM) [38].

The plant's anthocyanin complex has been tested against bile-duct cancer. In rats with chemically induced cholangiocarcinoma the complex suppressed tumour markers and improved the efficacy of gemcitabine [87]. A hydroalcoholic seed extract gave IC50 values of 45.4 µg/mL against MDA-MB-231 breast cancer cells and 96.5 µg/mL against PANC-1 pancreatic cancer cells, with raised reactive oxygen species, upregulated caspase-8, caspase-9 and caspase-3 and apoptotic morphology [47].

The crude-extract cytotoxicity results are more ambiguous. Methanolic leaf and flower extracts were toxic to brine shrimp with LC50 values of 62.54 and 38.88 µg/mL and up to 83.33% and 93.33% mortality at 200 µg/mL, so the flower extract was the more toxic of the two [48]. In HEK-293 human embryonic kidney cells both crude petal extracts were non-toxic from 19.5 to 156.3 µg/mL and strongly cytotoxic at 312.5 µg/mL, while the anthocyanin-rich fraction was already strongly cytotoxic at 156.3 µg/mL, that is, concentration enriched but not selectively so [32]. In the same cellular system the fraction at 78.1 µg/mL inhibited only about 20% of the reactive oxygen species generated by AAPH, against quercetin 20 µg/mL, although the crudes had previously given 75–80% inhibition at 156.3 µg/mL [32]. Earlier work quoted in that paper reported IC50 values of 27.2–68.2 µg/mL on MCF-7, K562 and SKBR cells and of 36 and 57 µg/mL on Dalton's lymphoma ascites cells, with no toxicity to Hs27 fibroblasts up to 100 µg/mL.

Table 13. Anticancer and cytotoxicity data.

Preparation	Model	Key results	Source
Cliotide CT4	A549 and A549/paclitaxel lung cancer cells	IC50 0.21 and 0.12 µM; sensitised resistant cells to paclitaxel 10 nM	[40]
Cliotides CT2, CT7, CT10, CT12	Same lines	IC50 0.70–7.59 µM (A549); cytotoxicity tracked net charge up to +2	[40]
Cliotides T1–T12	HeLa cells, 72 h MTT	IC50 0.6 µM (T1, T4), 2.0 µM (T3), 8.0 µM (T2)	[38]
Anthocyanin complex	Rat cholangiocarcinoma model, with gemcitabine	Tumour markers suppressed; drug efficacy improved	[87]
Hydroalcoholic seed extract	MDA-MB-231 and PANC-1 cells	IC50 45.4 and 96.5 µg/mL; ROS raised; caspase-8/-9/-3 up; apoptosis	[47]
Methanolic leaf and flower	Brine shrimp lethality	LC50 62.54 and 38.88 µg/mL	[48]
Crude petal extracts and anthocyanin-rich fraction	HEK-293 cells	Non-toxic to 156.3 µg/mL (crudes); strongly cytotoxic at 312.5 and 156.3 µg/mL respectively	[32]

7.10 Insecticidal, larvicidal and anthelmintic activity

The plant has a history as a forage legume and its defensive chemistry shows up in every screen. Methanolic seed extract was the most active of four parts against three mosquito vectors, with LC50 values of 54.4 ppm against *Culex quinquefasciatus*, 65.2 ppm against *Anopheles stephensi* and 154.5 ppm against *Aedes aegypti*, and it was the most promising of the three plants compared, alongside *Saraca indica* and *Nyctanthes arbor-tristis* [88]. Ethanol root extract at 50 mg/mL paralysed and killed *Pheretima posthuma* earthworms more effectively than piperazine citrate, aqueous and ethanolic extracts at 100 mg/mL acted on *Eisenia foetida* more slowly than levamisole, and methanolic leaf extract prevented 93% of *Meloidogyne incognita* eggs from hatching [5]. Purified finotin at 1% w/w killed 100% of *Acanthoscelides obtectus* larvae and at 5% w/w killed *Zabrotes subfasciatus*, and cliotide Cter M at 1 µmol/g of diet caused larval mortality in *Helicoverpa armigera* [34, 6].

7.11 Diuretic activity

The root's diuretic reputation is old and the evidence is thin. In anaesthetised dogs the ethanol root extract given orally at 16 or 19 mg/kg produced no diuretic or natriuretic effect at all, while intravenous doses of 4 or 6 mg/kg increased urinary sodium and potassium excretion and reduced chloride, without changing urine volume [27]. Reviews continue to list the plant as a diuretic on the strength of this and similar old experiments [5, 1].

7.12 Other reported activities

A few further lines of work are worth recording briefly. Supplementing rabbits with a crude butterfly pea extract at 0.5 g/kg body weight from 35 to 90 days of age had no effect on the phagocytic function of blood polymorphonuclear leukocytes or alveolar macrophages, but produced lower blood urea nitrogen, higher liver weight, brighter and less yellow meat colour, delayed lipid peroxidation in meat stored at 4 °C and improved digestibility of organic matter and ether extract ($P < 0.05$ in each case) [89], which is more a story about the extract as a feed additive than as a medicine. A review of Indian herbs for Alzheimer's disease placed the plant among the traditional materia medica with a memory-enhancing reputation, and mapped the Ayurvedic claims to the cholinesterase and antioxidant data described in Section 7.1 [90]. On the computational side, four compounds isolated from the flower, trilinolein, β-sitosterol, palmitic acid and isoquercetin, were screened against inflammatory proteins, and isoquercetin bound the IκB kinase-β complex with a docking score of −10.1 kcal/mol, the highest affinity in the set; commercial isoquercetin also scavenged 83% of DPPH radicals at 10 µg/mL and retained antibacterial, haemocompatible and cytocompatible behaviour in a dose-dependent manner [91]. Anti-ulcer, local anaesthetic, platelet aggregation-inhibiting, smooth-muscle-relaxing, antidepressant and anticonvulsant activities are all listed in reviews of the plant [1, 5, 3], but for several of these no primary study was located in the present corpus, and they are best treated as claims awaiting primary confirmation.

Table 14. Other reported biological activities.

Activity	Model	Result	Evidence level	Source
Larvicidal	Mosquito larvae	LC50 54.4 ppm (*C. quinquefasciatus*), 65.2 ppm (*A. stephensi*), 154.5 ppm (*A. aegypti*)	In vitro	[88]
Anthelmintic	*Pheretima posthuma*, *Eisenia foetida*	Root extract 50 mg/mL more active than piperazine citrate	In vitro	[5]
Nematicidal	*Meloidogyne incognita* egg hatch	93% inhibition by leaf methanolic extract	In vitro	[5]
Insecticidal protein	*Acanthoscelides*, *Zabrotes*, *Helicoverpa*	Finotin 1–5% w/w larval mortality; Cter M 1 µmol/g diet	In vitro / diet assay	[34, 6]
Diuretic	Anaesthetised dogs	No effect orally at 16–19 mg/kg; IV 4–6 mg/kg raised Na^+ and K^+ excretion	Animal	[27]
Feed additive / immune	Rabbits, 0.5 g/kg from weaning	No change in phagocytosis; lower blood urea nitrogen; delayed meat lipid peroxidation	Animal	[89]
Anti-cholangiocarcinoma	Rat model with gemcitabine	Tumour markers suppressed; drug efficacy improved	Animal	[87]
Computational anti-inflammatory	Docking of flower isolates	Isoquercetin bound IKK-β at −10.1 kcal/mol	In silico	[91]

8. Proposed Mechanisms of Action

The pharmacological results described in Section 7 can be organised into a smaller number of mechanisms. Some are supported by direct measurement in the same study that reported the effect, some rest on docking calculations or on inference from a related model, and some are still only plausible. This section separates the three.

8.1 Cholinergic and neuronal protection

Three lines of evidence point at the cholinergic system. Brain and regional acetylcholinesterase was measured directly in three animal studies and fell in each: in the prefrontal cortex of mice given a flower decoction from 9.907 ± 2.153 to 2.035 µmol/min g protein [56], in whole brain of diabetic rats given a leaf extract from 6.36 ± 0.30 to 4.07 ± 0.42 µM/l/min/g [69], and in cortex and hippocampus of hypoperfused rats given a root extract at 300 mg/kg [12]. In vitro, a root ethanolic extract inhibited both acetylcholinesterase and butyrylcholinesterase, with a preference for the latter [14]. The older work is consistent: alcoholic root extract raised rat brain acetylcholine content while changing regional enzyme activity in the pattern seen with pyritinol [10].

Around the cholinergic effect sits a consistent antioxidant story in the brain. Malondialdehyde fell in every study that measured it, superoxide dismutase, catalase and glutathione rose in the diabetic and stress models, and the same extracts that moved these markers also improved behaviour [56, 69, 57]. The structural correlate is dendritic remodelling: more intersections and branching points in amygdaloid neurons and in hippocampal CA3 after treatment with the root extract [68, 57]. Whether the cholinergic, antioxidant or structural effect comes first is not resolved by any of these studies.

8.2 Scavenging and cytoprotection

The ternatins and flavonols are chemically good reducing agents, and the assay numbers are correspondingly strong, but they are also method-dependent. Reported DPPH IC50 values for flower preparations span a wide range, from 0.86 ± 0.07 mg/mL for an anthocyanin-rich fraction to 195.5 µg/mL for a water extract of dried petals, with ABTS IC50 of 42.9 µg/mL for the latter [32, 1], and cellular assays tell a more modest story: the same fraction gave only about 20% inhibition of AAPH-generated radicals in RAW264.7 macrophages at 78.1 µg/mL, where the crude extracts had given 75–80% at 156.3 µg/mL [32].

Direct cytoprotection has been shown twice. In human keratinocytes, pre-incubation with 100, 250 and 500 µg/mL of a flower water extract reduced hydrogen peroxide-induced cytotoxicity and significantly reduced mitochondrial DNA damage in ultraviolet-exposed cells ($P < 0.05$), with polyacylated delphinidin anthocyanins and quercetin and kaempferol glycosides identified as the main constituents by high-resolution LC-MS [43]. And in the human corneal epithelial model, the extract doubled the rate of scratch closure and raised connexin 43 at the wound margin [84].

8.3 Anti-inflammatory signalling

The most detailed measurement is in the hypertensive rat. There the flower extract lowered plasma nitrate and nitrite, reduced the rise in serum angiotensin-converting enzyme activity and plasma angiotensin II, and suppressed cardiac expression of the angiotensin II type 1 receptor, NADPH oxidase 2, NF-κB and tumour necrosis factor-alpha, with the accompanying functional improvements in blood pressure, vasorelaxation and cardiac hypertrophy [77]. In the low-grade inflammation mouse model the same class of effect appears as a restored balance of regulatory and Th17 cells, higher tight-junction protein expression in the colon, lower lipopolysaccharide and tumour necrosis factor-alpha, and a microbiota shift towards short-chain-fatty-acid producers [79].

Three studies approach the same pathway computationally or in cell culture. An ethyl acetate flower fraction reduced nitric oxide in lipopolysaccharide-activated BV-2 microglia, and docking pointed at inducible nitric oxide synthase [71]. Isoquercetin isolated from the flower docked the IκB kinase-β complex at −10.1 kcal/mol, the strongest binding among the inflammatory proteins examined, and molecular dynamics simulations were run on that complex [91]. And downstream of signalling, the extract blocks the enzymes that execute tissue damage: hyaluronidase, elastase and matrix metalloproteinase-1 [31], albumin denaturation and red-cell membrane destabilisation [73], and the

histamine responses in tracheal tissue and in the mast cell models [30, 1]. Docking is hypothesis-generating evidence only, and none of the three studies confirmed its predictions experimentally.

8.4 Metabolic enzyme inhibition

The antidiabetic and anti-obesity data reduce to three enzymes. A purified flower phenolic fraction inhibited pancreatic lipase at an IC50 of 0.28 mg/mL, α-amylase at 1.70 mg/mL and α-glucosidase at 1.04 mg/mL, values that are more potent than acarbose on amylase and similar on glucosidase [46], and crude leaf and flower extracts inhibited salivary amylase, yeast glucose uptake and haemoglobin glycation at 50 µg/mL in the 80–93% range [73]. Advanced glycation end-product formation was also reduced by flower water extracts, though less than by aminoguanidine [16]. In animals these enzyme effects translate into the glucose, insulin, glycogen and glucokinase changes of Section 7.4.

8.5 Membrane-directed mechanisms of the peptides

The cyclotides and the seed proteins act differently from the small molecules, and the structure-activity relationships are unusually clean. In the cancer assay, cliotide cytotoxicity rose with net positive charge up to +2 and the +3 and +4 peptides were inactive, which points at an interaction with negatively charged membrane surfaces rather than at a receptor [40]. The same subfamily split appears in the antimicrobial assay: the positively charged bracelet cliotides T1 and T4 were active against Gram-negative bacteria at about 1 µM while the neutral or negative Möbius peptides were inactive up to 100 µM [38]. Haemolysis tracks the same property, with HD50 values of 7.1 and 8.4 µM for T1 and T4 against more than 100 µM for Cter M [38, 6]. Scanning electron microscopy of *Penicillium* hyphae treated with the flower extract showed cell wall disruption and empty hyphae [80], which is what a membrane-directed mechanism looks like, though the purified cliotides were not tested in that system.

8.6 Absorption, metabolism and the gut

A question that runs through the whole literature is how much of the administered material survives digestion. The best data come from a study of the extract in a food matrix: after simulated gastrointestinal digestion 42.03 ± 2.74% of the total anthocyanins remained, four prototype compounds and twenty-seven metabolites were detected in rat plasma and urine, and the plasma Cmax of delphinidin-3-glucoside and cyanidin-3-glucoside were 69.034 ± 8.05 nM and 51.65 ± 3.205 nM, with quercetin, cyanidin-3-glucoside and delphinidin-3-glucoside identified as the metabolites carrying the antioxidant effect in a D-galactose-induced ageing model [92]. Those nanomolar circulating concentrations are far below the concentrations used in most of the in-vitro work in Section 7, and they are the main reason the in-vitro results should be read as demonstrations of capability rather than as evidence of clinical effect.

The cyclotides sit at the other extreme. Their cyclic cystine knot survives boiling [38], digestion [6] and, in the case of the seed proteins, requires separation from the matrix to be destroyed [34, 35]. Whether intact cliotides reach the systemic circulation after oral dosing in this plant has not been demonstrated.

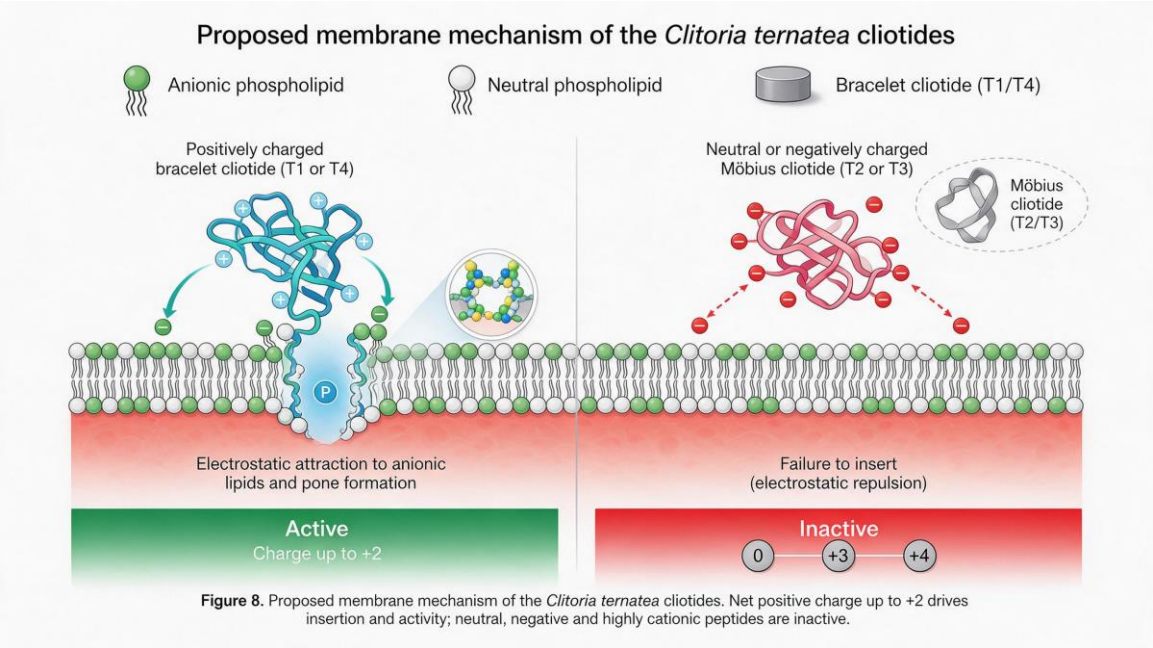


Figure 8. Proposed membrane mechanism of the Clitoria ternatea cliotides. Net positive charge up to +2 drives insertion and activity; neutral, negative and highly cationic peptides are inactive.

9. Human Studies

Only three human studies were located, all small, all Thai or Southeast Asian, and all acute.

The best-designed is a randomised crossover trial in fifteen healthy men aged 22.53 ± 0.30 years with a body mass index of 21.57 ± 0.54 kg/m² [13]. Each participant received, on five separate days with a one-week washout, 50 g of sucrose in water, 1 g or 2 g of a spray-dried flower extract in water, and the same doses of extract combined with the sucrose, all as 400 mL beverages consumed within five min after a 10–12 h fast. The extract was the decoction described in Section 6.1, standardised at 53.08 ± 0.08 mg gallic acid equivalents and 1.08 ± 0.12 mg delphinidin-3-glucoside equivalents per gram. Alone, the extract changed nothing in fasting glucose or insulin. With sucrose it lowered plasma glucose at 30 and 60 min ($P < 0.05$), cut the incremental area under the glucose curve to 0.67-fold and 0.60-fold of sucrose alone, suppressed insulin at 60 min at the 2 g dose, and left uric acid unchanged. The antioxidant effects were larger: ferric reducing ability of plasma rose 3.71-fold and 5.48-fold, oxygen radical absorbance capacity 2.09-fold and 2.49-fold, Trolox equivalent antioxidant capacity about 1.4-fold and plasma protein thiol 2.63-fold relative to sucrose, while the rise in malondialdehyde caused by sucrose at 30, 60, 150 and 180 min was prevented at every time point. No adverse events were recorded. The authors note that the results cannot be extended to people with glucose intolerance, to women or to older ages.

The other two studies concern oral wounds. In 68 orthodontic patients with acute wounds randomly assigned to a 10% anthocyanin-complex mucoadhesive gel or placebo, assessed double-blind, wound size reduction and closure were greater in the treated group on day 3 ($P < 0.05$) [85]. In a larger randomised, placebo-controlled, double-blind trial in 60 volunteers aged 18–60 with oral wounds, a niosome gel of the same complex accelerated wound closure, reduced pain and improved quality of life more than the plain gel, triamcinolone gel and placebo [86]. Both used an anthocyanin complex of *C. ternatea* and purple waxy corn rather than the plant alone, so neither isolates the contribution of this species.

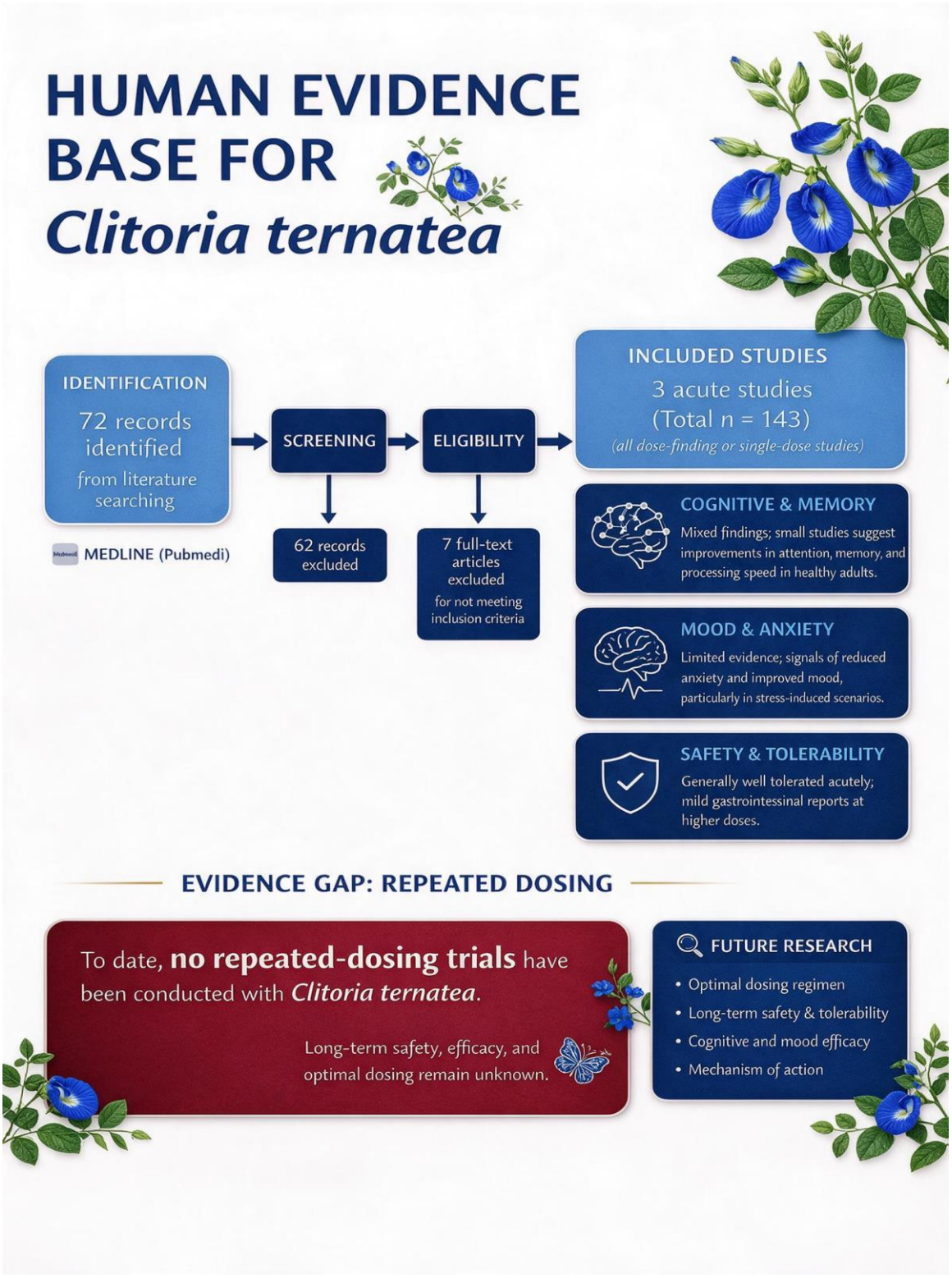


Figure 9. The complete human evidence base for Clitoria ternatea to date: three acute studies, 143 participants in total, no repeated-dosing trial.

Table 15. Human intervention studies with *Clitoria ternatea*.

Study	Design and participants	Intervention	Main outcomes
[13]	Randomised five-way crossover, n = 15 healthy men, one-week washout	1 or 2 g spray-dried flower decoction, with or without 50 g sucrose, as 400 mL beverages	Glucose iAUC 0.60–0.67-fold of sucrose; FRAP 3.71–5.48-fold, ORAC 2.09–2.49-fold, protein thiol 2.63-fold higher; sucrose-induced MDA rise prevented at all time points; no adverse events
[85]	Randomised double-blind, 68 orthodontic patients with acute oral wounds	10% anthocyanin-complex mucoadhesive gel vs placebo	Greater wound size reduction and closure on day 3 ($P < 0.05$)
[86]	Randomised block placebo-controlled double-blind, 60 volunteers aged 18–60 with oral wounds	Anthocyanin complex niosome gel vs plain gel, triamcinolone gel and placebo	Faster wound closure, less pain and better quality of life than all comparators

No published randomised trial on cognition, diabetes, blood pressure or any chronic condition was found in the material reviewed here, and no dose-ranging or repeated-dosing human study exists. The gap between the animal literature of Section 7 and these three acute trials is the single largest feature of the evidence base.

10. Toxicity and Safety

The safety profile of this plant is better documented than most of its pharmacology, and the general finding across every test is a wide margin of safety in mammals, with one important exception in fish.

Acute oral toxicity in rodents has been examined several times. An ethanolic flower extract given to rats at 2,000 mg/kg under OECD guideline 423 produced no mortality and no changes in breathing, ingestion, excretion, motility or behaviour at 24 h or after 14 days, and haematology at the end of the observation period was unremarkable [15]. A methanolic flower extract at 200 and 400 mg/kg and an ethanolic leaf extract at the same doses were likewise without mortality in the acute setting, and no organ toxicity was reported at these doses [54, 69, 32]. The root extract was placed in OECD category 5 with a reported median lethal dose above 1,300 mg/kg in mice after intraperitoneal dosing [30]. Sub-chronic work is thinner: the same flower extract at 200, 400 and 800 mg/kg for 28 days in diabetic rats left renal and hepatic chemistry within the reference range and improved liver histology at the highest dose [74], and a leaf extract at 200 and 400 mg/kg for two weeks in diabetic rats did not worsen the hepatic and haematological profile [69].

A chronic feeding study in a food animal gives a different perspective. Rabbits given a crude butterfly pea extract at 0.5 g/kg body weight from 35 to 90 days of age showed no change in phagocytic function

or gut histology, but had lower blood urea nitrogen, higher liver weight and meat with delayed lipid peroxidation [89], which suggests the extract is well tolerated when eaten daily.

Two non-mammalian tests are worth flagging because they do not agree with the mammalian picture. In a *Galleria mellonella* waxworm model a green flower extract was reported safe up to 2.2 g/kg [61], and methanolic leaf and flower extracts killed brine shrimp with LC50 values of 62.54 and 38.88 µg/mL [48]. Brine shrimp lethality is a crude and non-specific screen, and the concentrations that kill shrimp are far above anything achieved in mammalian tissue in the studies reviewed here.

The exception is aquatic. In yellow rasbora (*Rasbora lateristriata*) exposed for four days to the flower extract in the aquarium water at 50, 75 and 100 ppm, liver histopathology showed concentration-dependent vacuolisation, pyknosis, necrosis and haemorrhage, with the highest damage scores at 75 and 100 ppm, the most severe damage at 75 ppm, and haemorrhage peaking at 100 ppm [93]. The authors attribute the effect to oxidative damage of hepatopancreatic membranes and note that the non-linear response may reflect precipitation of phytochemicals at the highest concentration. Only histology was done, no serum enzymes, and the group size is stated inconsistently in the source (four or six fish per group). The authors draw the practical conclusion that disposal of concentrated extracts into fresh water is a real ecological concern.

Cyclotide safety deserves a separate word. Clotides are haemolytic in the low micromolar range (HD50 7.1–8.4 µM for T1 and T4) and cytotoxic to cancer cells at sub-micromolar concentrations [38, 40], so in principle the peptides are toxic. Their behaviour in the whole-plant context is different: the traditional food and beverage uses involve boiling, which does not destroy them [38], yet no adverse event was reported in the acute human trial at 2 g of dried decoction [13], and no haemolysis has been reported in any animal study of the whole extract. Whether this reflects low peptide content in the extracts used, poor absorption, or rapid clearance has not been examined.

Table 16. Toxicity studies on *Clitoria ternatea*.

Test	Material and dose	Outcome	Source
Acute oral toxicity, OECD 423	Flower, ethanolic, 2,000 mg/kg in rats	No mortality; no behavioural or haematological abnormality at 14 days	[15]
Acute oral toxicity	Flower methanolic and root ethanolic, up to 2,000 mg/kg	No mortality	[69, 54]
Acute intraperitoneal toxicity	Root extract, mice	Median lethal dose above 1,300 mg/kg	[30]
Sub-chronic, 28 days	Flower 70% ethanol, 200–800 mg/kg in diabetic rats	Renal and hepatic chemistry within reference range; best liver histology at 800 mg/kg	[74]
Chronic feeding, 55 days	Crude extract 0.5 g/kg in rabbits	No change in phagocytosis or gut histology; lower blood urea nitrogen;	[89]

		delayed meat lipid peroxidation	
Galleria mellonella larvae	Green flower extract to 2.2 g/kg	Reported safe at the highest dose	[61]
Brine shrimp lethality	Methanolic leaf and flower	LC50 62.54 and 38.88 µg/mL	[48]
Fish liver histopathology	*Rasbora lateristriata*, 50–100 ppm, 4 days	Dose-dependent necrosis, vacuolisation, pyknosis, haemorrhage; worst at 75 ppm	[93]
Human, acute	1–2 g dried decoction, single dose	No adverse events in 15 men	[13]

11. Formulations, Nano-delivery, Food Uses and Commercial Development

Because the ternatins are coloured, water-soluble and fragile, most of the formulation work on this plant has been driven by colour stability rather than by the pharmacology. The result is a body of delivery research that is ahead of the therapeutic one.

11.1 Vesicular and nanoparticulate delivery

Niosomes have produced the two strongest results. An anthocyanin complex of *C. ternatea* and purple waxy corn encapsulated in niosomes improved permeation through mucosa in vitro, promoted collagen production in human gingival fibroblasts where neither source alone did, and in rat buccal wounds a 10% niosome gel gave complete healing by day 5, better than fluocinolone acetonide gel after three days [83]; the same formulation then performed best in the 60-volunteer trial described in Section 9 [86]. In fibroblast scratch wounds, niosomes at 0.002 mg/mL restored the monolayer within 24 h and upregulated type I, III and IV collagen, fibronectin and laminins [86].

Liposomes have been made by high-pressure processing. Petal extract liposomes produced at 300 MPa had a mean particle size of 240.7 nm, a polydispersity index of 0.37 and an encapsulation efficiency of 77.8%, better in size and reproducibility than magnetic stirring or ultrasonication, and the resulting formulation was tested as a pH indicator film [60]. Silk fibroin nanoparticles carrying the extract retained about 85% of their DPPH scavenging activity after four h at 70 °C against about 17% for the free extract, which is a direct demonstration of the protection a nanocarrier can give [66]. Phycocyanin–anthocyanin nanoparticles from the plant have been used as emulsifiers to build high-internal-phase Pickering emulsions, a food-grade structuring route rather than a drug delivery one [94].

Green nanoparticles have attracted a parallel literature. Silver nanoparticles made from a flower and purple rose mixture have been synthesised and characterised [95], and silver nanoparticles fabricated from the flower extract showed angiosuppressive activity in vitro and in vivo, with an IC50 of 19.37 µg/mL against human umbilical vein endothelial cells, about 1.2-fold better than the reference standard, and angiogenesis inhibition in the chorioallantoic membrane model with vessel density reduced by 67.3% at 125 µg/mL ($P < 0.05$) [96]. The toxicity of such particles to normal tissue at therapeutic concentrations has not been worked out.

11.2 Drying, encapsulation and food matrices

Spray drying is the most developed route. With whey protein isolate as wall material at an inlet temperature of 150 °C the microcapsules reached 97.30% encapsulation efficiency, 50.10 mg/L anthocyanin, 83.67% solubility, 4.65% moisture and 1.22 µm particle size [64]. The human trial beverage used a spray-dried decoction powder made at 178 °C inlet temperature [13], and microwave encapsulation with 40% maltodextrin and spray drying with 20% maltodextrin gave 73.24% and 87.3% encapsulation efficiency respectively [7].

In food matrices the question is bioaccessibility. After simulated gastrointestinal digestion of a corn-snack formulation, 42.03 ± 2.74% of the total anthocyanins remained, four prototypes and twenty-seven metabolites appeared in rat plasma and urine, and the plasma peak concentrations of delphinidin-3-glucoside and cyanidin-3-glucoside were 69.034 ± 8.05 nM and 51.65 ± 3.205 nM [92]. Those numbers, which are the only human-relevant pharmacokinetic data for this plant, are the yardstick against which the in-vitro concentrations in Section 7 should be judged.

11.3 Intelligent packaging and sensors

The ternatins change colour with pH, and several groups have turned that into packaging. Starch films loaded with the anthocyanins and titanium dioxide shifted colour with pH and gave a freshness-sensing function [36], gelatin films with 15% extract changed from pinkish purple at pH 2 through light blue at pH 5–7 to bluish green at pH 9–11 and turned bluish green after 24 h in contact with spoiling mackerel [51], and sodium alginate films with 10–40% extract were antibacterial against *E. coli* at the highest loading, changed colour with pH and with ammonia gas, and biodegraded in soil and beach sand within 15 days [3]. These are food-contact applications, not drug ones, but they are the closest thing to a commercial product line in the current literature.

11.4 Commercial and intellectual-property status

One commercial product is documented. Sero-X®, a cyclotide-containing ethanolic extract of *C. ternatea* vegetative tissue, was registered for commercial use in Australia in 2017 as an eco-friendly pesticide for cotton and macadamia, with the prototype cyclotide Cter M enriched in the extract; it showed no toxicity to tested rodents or bee pollinators and is classified as non-hazardous under the Globally Harmonized System [1]. On the agricultural side the cultivar 'Milgarra', released in Queensland in 1991 as a grazing composite of 21 introduced and naturalised lines, remains the reference cultivar [1].

No patent documents for *C. ternatea* pharmaceutical or nutraceutical formulations were located in the material reviewed here, and no commercial-scale production of the plant for anthocyanins exists; large-scale harvest is not currently thought economically feasible, and plant cell suspension cultures have been proposed as an alternative [1]. Food, cosmetic and pigment applications are therefore the only ones with a real market at present.



Figure 10. Nano-delivery and encapsulation systems for Clitoria ternatea extracts, with reported particle sizes and encapsulation efficiencies (see Table 17).

Table 17. Formulations, delivery systems and commercial products based on *Clitoria ternatea*.

Product or system	Type	Key figures	Source
Anthocyanin complex niosome gel	Mucoadhesive topical gel	Complete rat buccal wound healing by day 5; best of four arms in 60-volunteer trial	[83, 86]
High-pressure liposomes	Liposomal dispersion, 300 MPa	240.7 nm, PDI 0.37, 77.8% encapsulation	[60]
Spray-dried microcapsules	Whey protein isolate wall material	97.30% encapsulation at 150 °C inlet; 1.22 µm particles	[64]
Maltodextrin microcapsules	Microwave or spray dried	73.24% and 87.3% encapsulation	[7]
Silk fibroin nanoparticles	Polymeric nanocarrier	~85% DPPH retained after 4 h at 70 °C vs ~17% free	[66]
Silver nanoparticles	Green metallic nanoparticles	HUVEC IC50 19.37 µg/mL; 67.3% vessel reduction at 125 µg/mL	[96]
Phycocyanin–anthocyanin nanoparticles	Pickering emulsifier	Food-grade high-internal-phase emulsions	[94]
Intelligent films	Starch, gelatin and alginate matrices	Colour change at pH 2–11; ammonia sensing; antibacterial at 40% loading; soil	[36, 51, 3]

		biodegradation within 15 days	
Sero-X®	Registered pesticide, Australia 2017	Ethanol extract of vegetative tissue; Cter M enriched; no rodent or pollinator toxicity	[1]
'Milgarra'	Forage cultivar, Queensland 1991	Composite of 21 naturalised lines	[1]

12. Limitations and Gaps in the Evidence

Any honest review of this plant has to start with what is missing, because the gaps are as informative as the data.

The first problem is standardisation. Most studies report only a solvent name and a dose in mg/kg, with no marker compound and often no extract yield [70, 81, 48, 54]. Where standardisation does exist it varies between a total phenolic content, a total anthocyanin content expressed against different standards, and a single marker such as taraxerol [31, 17, 76]. Total anthocyanin values from different papers are therefore not comparable, and a dose of "500 mg/kg of flower extract" in one study may contain ten times less pigment than the same dose in another.

The second problem is the level of evidence. Of the studies reviewed here, the large majority are in vitro or in animal models. Only three human studies exist, all acute, all small, all from one region, and two of them used an anthocyanin complex shared with another plant [13, 85, 86]. There is no repeated-dose human study, no dose-ranging study, no study in any patient group with a chronic condition, and no pharmacokinetic data in humans beyond the plasma metabolite work done in rats [92]. The gap between an in-vitro IC50 in the low µg/mL range and the nanomolar plasma concentrations actually achieved after oral dosing is the single most important unresolved issue in this literature.

The pharmacognostic profile is incomplete. As set out in Section 3, no primary determination of pharmacopoeial ash values, extractive values or foreign matter was found for any part of the plant, powder microscopy has not been described, and no fluorescence analysis under ultraviolet light exists [2, 1]. The HPTLC work on taraxerol and β-sitosterol is the only validated quantitative marker method available [17], and even that was applied to one population in one country.

The botanical and phytochemical picture has its own soft spots. The origin of the species is genuinely disputed between tropical Asian and tropical American sources, and the two hypotheses cannot be separated with the molecular data in hand [1, 5]. Flower colour is a single-locus trait whose genetics remain uncharacterised [2, 19]. The distribution and expression of the cyclotides, the plant's most distinctive chemistry, has been mapped in only one transcriptome study [1], and the naming of the peptides is inconsistent between research groups, with "cliotides" and "Cter" series used for overlapping sets [38, 6, 40].

There are also reporting problems inside individual papers that limit their use. Several display most of their results as figures without printed numbers and do not state group sizes [74, 75]. One antimicrobial paper prints conflicting strain identifiers in its tables and abstract [81], another has an abstract and table that disagree about which organism a biofilm result refers to [48], one extraction paper gives inconsistent units for its liquid-to-solid ratio [51], one toxicity study reports group sizes of four and six in different sections [93], and one reports different figures for the same percentage in its abstract and results [59]. These are not fatal, but they are reasons to treat individual numbers as approximate.

Finally, several traditional uses remain untested in any primary study. Unani and Siddha documentation for this plant was not found in the sources reviewed here, the double-petal flower form has no separate chemical or pharmacological literature, and claims such as anti-ulcer, local anaesthetic, antidepressant and platelet-inhibiting activity survive in reviews without an identifiable primary source [1, 5, 3].

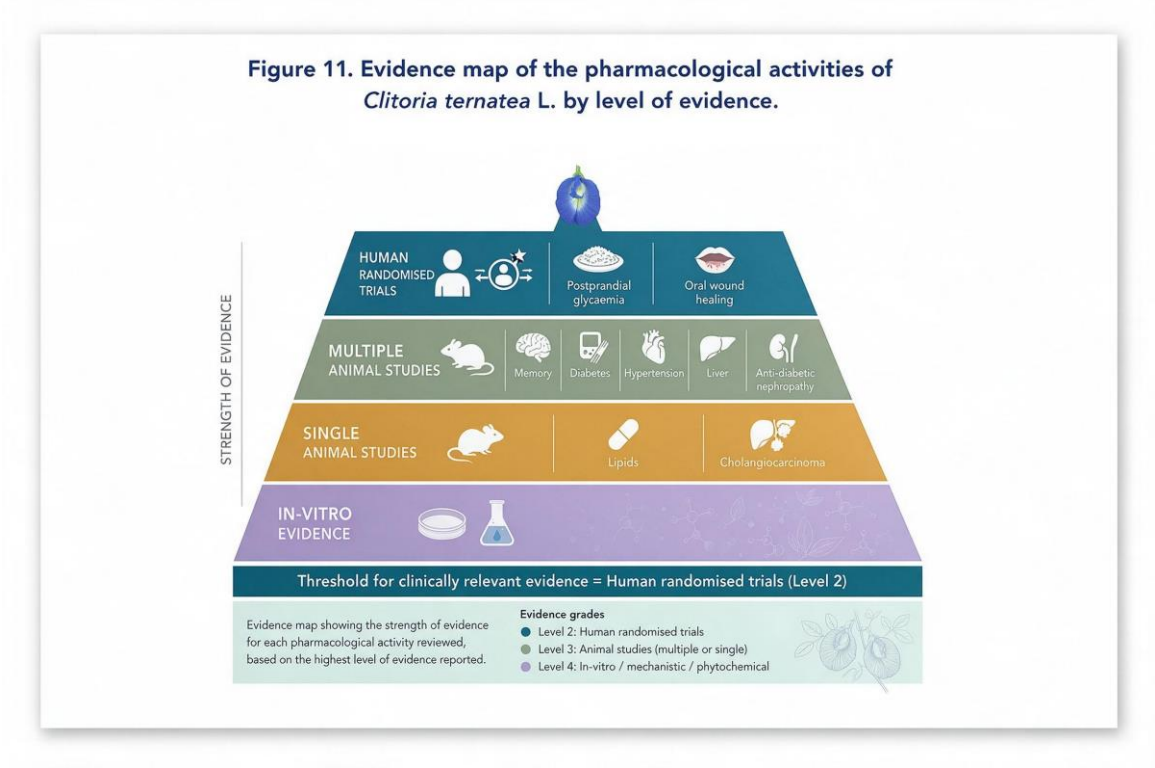


Figure 11. Evidence map of the pharmacological activities of Clitoria ternatea L. by level of evidence.

13. Conclusion

Clitoria ternatea L. is a plant whose reputation rests on two chemistries that pull in different directions. The ternatins are unusually stable anthocyanins, resistant to heat, light and modest pH change, cheaply extracted from a fast-growing tropical legume; on the preclinical evidence and the small short-term human trials they are well tolerated, and on that basis the plant already has a real, if modest, commercial life as a food colour, an intelligent-packaging ingredient and a topical oral-wound gel. The cliotides are a different proposition: sub-micromolar toxins with clean structure-activity relationships and a biosynthetic route found nowhere else in the legumes, but with no delivery system, no pharmacokinetics and no path from the petal to the clinic.

Between the two sits a middle layer of ordinary pharmacology. The plant lowers blood glucose in diabetic rodents, improves memory in hypoperfused, diabetic and stressed animals, reduces blood pressure and cardiac damage in L-NAME hypertensive rats, protects the liver against paracetamol and against parasite-associated inflammation, and closes wounds faster than controls in rats and in small human trials. The mechanisms that have been measured converge on acetylcholinesterase inhibition, antioxidant defence, NF-κB and angiotensin pathway suppression, metabolic enzyme inhibition and membrane-directed peptide action, though in most cases the causal chain from compound to effect has not been closed.

The preclinical safety record is encouraging: acute lethal doses above 1,300–2,000 mg/kg in rodents, no organ damage in sub-chronic rodent studies, and no adverse events in the small short-term human trials at 2 g of dried decoction. None of this establishes long-term safety in humans, which has never

been tested. The exception on the animal side is aquatic toxicity, which matters for the environment rather than for the consumer.

What the field needs next is not more screening. It needs marker-standardised extracts, repeated-dose human trials on the two indications with the best animal support, namely postprandial glycaemia and oral wound healing, a proper pharmacokinetic study of ternatins and cliotides together, and a pharmacognostic standard with ash values, powder microscopy and validated marker assays. Until those exist, *Clitoria ternatea* remains what it has been for sixty years: a promising and chemically unusual plant whose best-known uses are still ahead of its evidence.

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