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BUTTERFLY PEA (*CLITORIA TERNATEA*), A CYCLOTIDE-BEARING PLANT WITH APPLICATIONS IN AGRICULTURE AND MEDICINERA

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Abstract:

The perennial leguminous herb Clitoria ternatea (butterfly pea) has attracted significant interest based on its agricultural and medical applications, which range from use as a fodder and nitrogen fixing crop, to applications in food colouring and cosmetics, traditional medicine and as a source of an eco-friendly insecticide. In this article we provide a broad multidisciplinary review that includes descriptions of the physical appearance, distribution, taxonomy, habitat, growth and propagation, phytochemical composition, and applications of this plant. Notable amongst its repertoire of chemical components are anthocyanins which give C. ternatea flowers their characteristic blue colour, and cyclotides, ultra-stable macrocyclic peptides that are present in all tissues of this plant. The latter are potent insecticidal molecules and are implicated as the bioactive agents in a plant extract used commercially as an insecticide. We include a description of the genetic origin of these peptides, which interestingly involve the co-option of an ancestral albumin gene to produce the cyclotide precursor protein. The biosynthesis step in which the cyclic peptide backbone is formed involves an asparaginyl endopeptidase, of which in C. ternatea is known as butelase-1. This enzyme is highly efficient in peptide ligation and has been the focus of many recent studies on peptide ligation and cyclization for biotechnological applications. The article concludes with some suggestions for future studies on this plant, including the need to explore possible synergies between the various peptidic and non-peptidic phytochemicals.

The study examines the affordability and health effectiveness of blue tea in India with a special focus on pharmacological characteristics and sensory evaluation in comparison to black tea. A mono-method quantitative technique has been utilized to gather information on the health effects of Clitoria ternatea (CT) using random sampling techniques and SPSS analytical tool for data analysis. Further, thirty-five untrained panelists have been employed to conduct the sensory analysis. Blue tea made of butterfly pea flower has been identified as a potential source of phytochemical characteristics that can be crucial in addressing human health hazards. Blue tea has several health benefits including antioxidative, anti-inflammatory, anti-stressor, anti-diabetic, and anti-carcinogenic effects. The research has reflected the acceptance of blue tea in the Indian market considering market awareness and its affordability among the population. The average spending of 66 surveyed Indian consumers on tea consumption is less than 300 INR each month and they are most likely to spend the same amount of money on purchasing blue tea. However, its acceptability has been limited in India in comparison to conventional black tea whose acceptability is higher among Indian consumers with better taste and aroma

Keywords: *Anthocyanin, Antibacterial, Anticancer, Anti-inflammatory, Antioxidant, Flavanol blue tea, blank tea; heath benefited*

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INTRODUCTION:

Clitoria ternatea, commonly known as butterfly pea, is a perennial herbaceous plant from the Fabaceae family. It has recently attracted a lot of interest as it has potential applications both in modern medicine and agriculture, and as a source of natural food colorants and antioxidants. *C. ternatea* has long been cultivated as a forage and fodder crop, and early studies assessed the plant for these purposes (Reid and Sinclair, 1980; Barro and Ribeiro, 1983; Hall, 1985). Numerous field trials in Queensland, Australia, eventually led to the registry of *C. ternatea* cv. 'Milgarra' (Oram, 1992), the only cultivar in Australia that was released for grazing purposes (Conway and Doughton, 2005). Additionally, *C. ternatea* has been widely used in traditional medicine, particularly as a supplement to enhance cognitive functions and alleviate symptoms of numerous ailments including fever, inflammation, pain, and diabetes (Mukherjee et al., 2008).

In as early as the 1950s, studies on *C. ternatea* sought to elucidate its pharmacological activities, phytochemical composition and active constituents (Grindley et al., 1954; Piala et al., 1962; Kulshreshtha and Khare, 1967; Morita et al., 1976). The novel *C. ternatea* anthocyanins termed "ternatins" which render *C. ternatea* flowers with their vivid blue colour, were first isolated in 1985 (Saito et al., 1985). Following further isolation and structural characterization of numerous other ternatins, the ternatin biosynthetic pathway was postulated a decade later (Terahara et al., 1998). In 2003, comparison of *C. ternatea* lines bearing different floral colors provided insights into the role of acylation on *C. ternatea* floral colour determination (Kazuma et al., 2003a). The abundance of these unique anthocyanins alongside other secondary metabolites in *C. ternatea* makes the plant an ideal source of natural additives that can enhance the appearance and nutritive values of consumer products (Pasukamonset et al., 2016, 2017, 2018; Siti Azima et al., 2017). Although a number of recent studies has endeavored to elucidate the pharmacological activities of *C. ternatea* (Adhikary et al., 2017; Kavitha, 2018; Singh et al., 2018), the contribution of individual extract components on any bioactivity measured remains unknown.

summarizes some of the key agricultural and biochemical studies conducted on *C. ternatea* from the 1950s to the present, providing a convenient timeline of discoveries. The corresponding references to the key studies and milestones are listed in Table 1. In recent years, the small circular defense molecules called cyclotides, in *C. ternatea* (Nguyen et al., 2011; Poth et al., 2011a,b; Nguyen et al., 2014) have fueled scientific innovations that may have impact in modern agriculture, biotechnology and medicine. In 2017, Sero-X®, a cyclotide-containing eco-friendly pesticide made from extracts of *C. ternatea*, was approved for commercial use in Australia[1]. In addition, the *C. ternatea* cyclotide processing enzyme, butelase-1, which is the fastest ligase known to date and is capable of ligating peptides across a vast range of sizes (26 to >200 residues), can potentially be used in the large scale synthesis of macrocycle libraries and peptide-based pharmaceuticals (Nguyen et al., 2014, 2015)

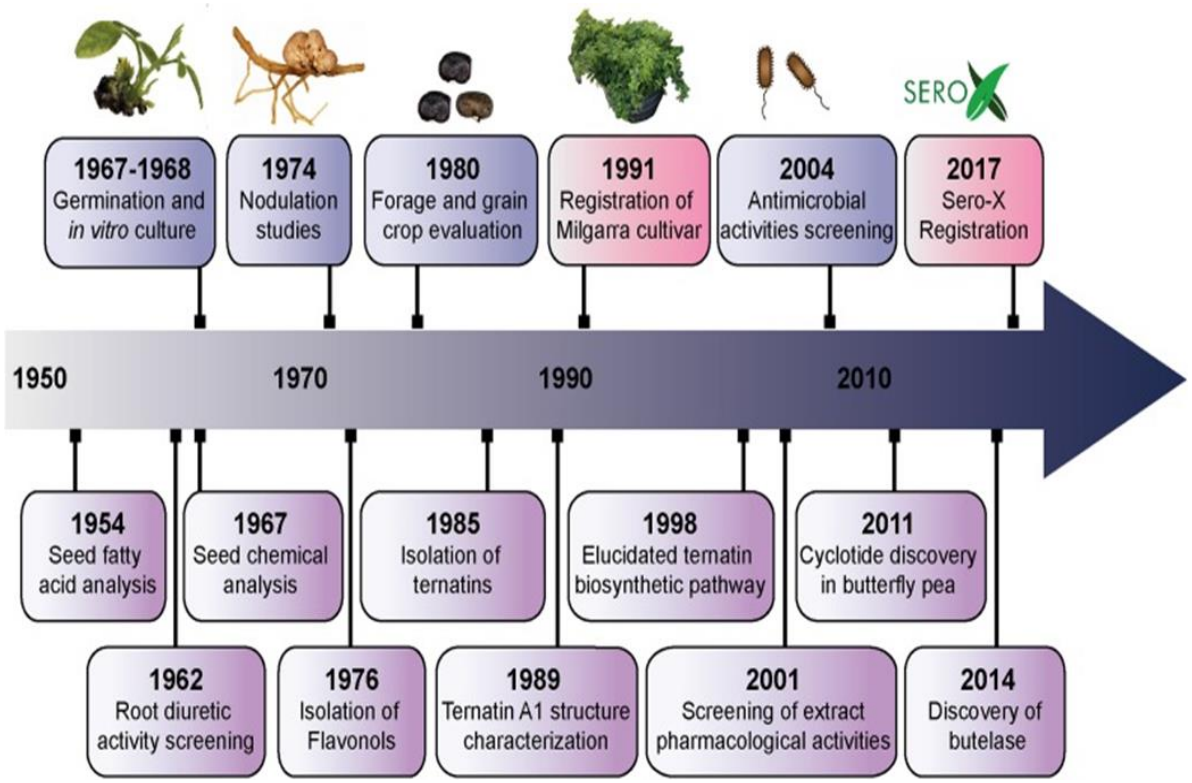

Table 1
Milestones in *Clitoria ternatea* studies.

Years	Milestones	References
1954	Seed fatty acid composition analyzed	Grindley et al., 1954
1962	Root diuretic properties screened	Piala et al., 1962
1967	Phytochemical composition of seeds analysis	Kulshreshtha and Khare, 1967
1967–1968	Germination studies and *in vitro* propagation	Mullick and Chatterji, 1967
1974	Nodulation pattern characterized	Oblisami, 1974
1976	Kaempferol-glycosides in leaves isolated	Morita et al., 1976
1980–1990	Forage and grain crop properties evaluated	Reid and Sinclair, 1980; Barro and Ribeiro, 1983; Hall, 1985
1985	Ternatins isolated from flowers	Saito et al., 1985
1989	Structure of Ternatin A1 determined	Terahara et al., 1989a
1991	Milgarra cultivar registered in Australia	Oram, 1992
1998	Ternatin biosynthetic pathway determined	Terahara et al., 1998
2000	Pharmacological activities of the extracts determined	Rai et al., 2001
2004	Antimicrobial properties characterized	Kelemu et al., 2004
2011	Cyclotides in *C. ternatea* discovered	Poth et al., 2011a,b
2014	Butelase discovered	Nguyen et al., 2014
2017	Sero-X® registered	Innovate Ag, 2018[1]

Plant Description

Clitoria ternatea produces pentamerous zygomorphic pea-shaped flowers with a tubular calyx consisting of five sepals which are fused about two thirds of their length. The showy corollae consists of five free petals, with one large and rounded banner, two wrinkled wings which are often half the length of the banner and two white keels which aid in protecting the floral organs (Cobley, 1956; Biyoshi and Geetha, 2012) (Figure 2A). The corollae are most often dark blue in color but may also occur in white and various blue and white shades in between (Morris, 2009; Biyoshi and Geetha, 2012). The diadelphous *C. ternatea* stamens consist of 10 filaments where nine are fused and one is free lying (Cobley, 1956; Biyoshi and Geetha, 2012). Attached to each filament is a pollen-bearing white anther, which consists of four lobes (Cobley, 1956; Pullaiah, 2000). *C. ternatea* produces a monocarpellary ovary bearing ten ovules (Pullaiah, 2000; Biyoshi and Geetha, 2012). Surmounting this is a long and thick style with a bent tip (Cobley, 1956; Biyoshi and Geetha, 2012). *C. ternatea* pods are narrow and flattened with pointy tips, and they typically contain around 10 seeds (Cobley, 1956) (Figure 2B). The seeds contain palmitic acid (19%), stearic acid (10%), oleic acid (51-52%), linoleic acid (17%) and linolenic acid (4%) (Grindley et al., 1954; Joshi et al., 1981). The caloric content of the seed is reported to be around 500 cal/100 g (Joshi et al., 1981). *C. ternatea* produces pinnate compound leaves that are obovate and entire with emarginate tips (Taur et al., 2010) (Figure 2C). The epidermis on both leaf surfaces consists of a single layer of cells protected by a thick cuticle and with trichome outgrowths (Taur et al., 2010). A layer of palisade cells, lignified xylem and paracytic stomata lie underneath the upper epidermis (Taur et al., 2010). *C. ternatea* produces an extensive deep-root system, which enables the plant to survive up to 7–8 months of drought (Cobley, 1956). The roots also produce large nodules for nitrogen fixation (Cobley, 1956) (Figure 2D).

Taxonomy, Geographic Distribution and Habitat

The genus *Clitoria* occurs in tropical and subtropical environments across the globe. The number of subfamilial taxa remains unclear, and as in the case of *Clitoria*, the descriptions of species and citations of type specimens are noted as being incomplete or incorrect according to Fantz (1977). Thus, it is difficult to estimate species richness of the genus. Within *Clitoria*, three subgenera have been described and held as valid according to the monograph of *Clitoria*. Across all three subgenera, Fantz retains 58 species as valid, with numerous lower classifications of varieties and subspecies (Fantz, 1977).

Clitoria ternatea is the holotype of *Clitoria* subgenus Clitoria, and represents the archetypical *Clitoria*. The etymology of the specific name is postulated to be from the island of Ternate in the Indonesian archipelago because it is from specimens from that location that Linnaeus produced the specific description. Ternate is not in the Indian Ocean but is instead in the Molucca Sea and in eastern Indonesia, lending ambiguity to the native range of the species. The distribution of all other taxa in subgenus Clitoria is restricted to Southern and Eastern Africa, India, Madagascar, and other islands of the Western Indian Ocean (Figure 3). The exact geographic origin of *C. ternatea* is thus difficult to determine, but we may infer from the center of diversity for subgenus Clitoria, that *C. ternatea* arose in or around the Indian Ocean and not the Pacific Ocean or South China Sea where it has been in use as a food coloring historically (Fantz, 1977; Staples, 1992). It is also entirely possible that the taxon we know as *C. ternatea* is an ancient hybrid of one or more members of the subgenus Clitoria that had subsequently been introduced to Southeast Asia. Testing of this synthetic origin hypothesis would require large scale genetics work on *C. ternatea* and related taxa like *Clitoria biflora*, *C. kaessneri*, *C. lasciva*, and *C. heterophylla*. Regardless of the specific geographical origin and evolutionary history of *C. ternatea*, the present day distribution of naturalized populations of *C. ternatea* is pantropical, as facilitated by key characteristics of the species: tolerance to drought conditions, non-reliance on specific pollinators because of self-pollination, and nitrogen fixation capability (Cobley, 1956; Staples, 1992; Conway et al., 2001). It is also possible to cultivate and maintain populations in subtropical regions (ex. Wee Waa NSW, located at -30.2, 149.433333).

Figure 3

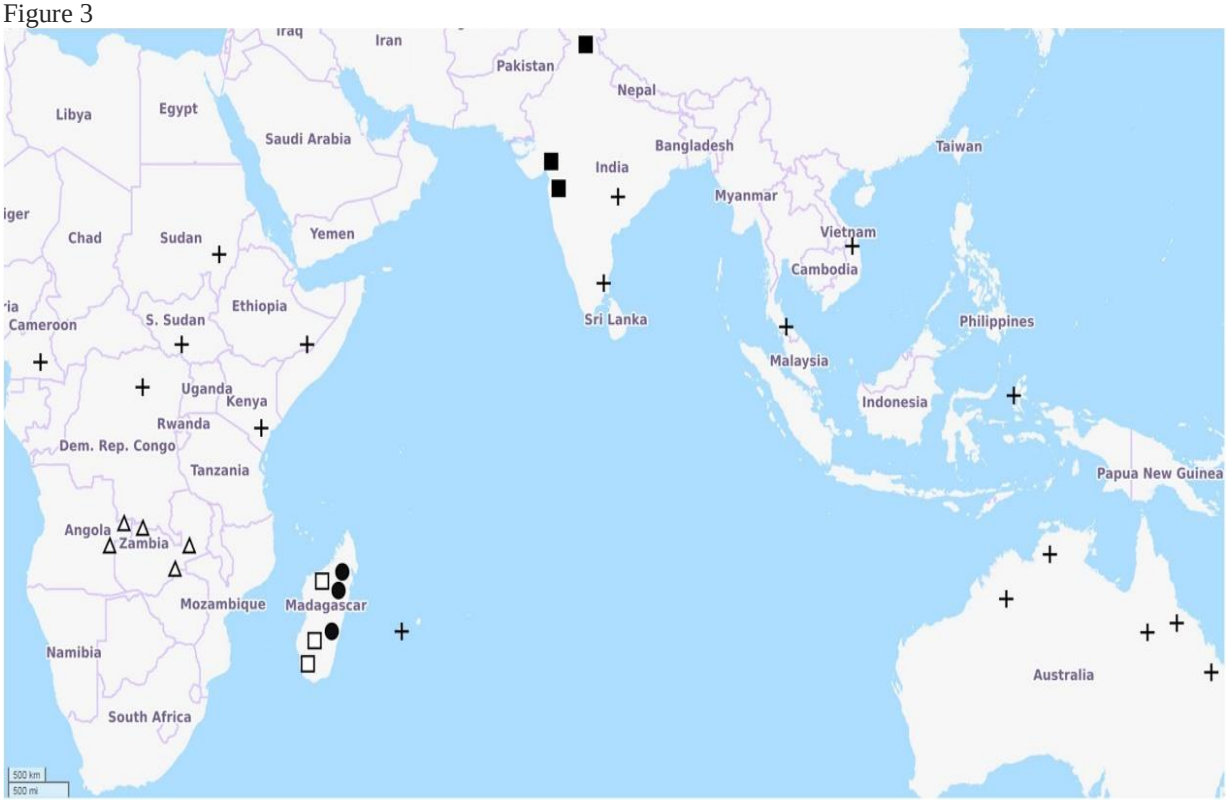

FIGURE 3. Distribution of *Clitoria* subgenus Clitoria species adapted from Fantz, 1977. Points of occurrence are approximate. Map data from Openstreetmap.org. Symbols represent: ▯ *C. biflora*, □ *C. heterophylla*, Δ *C. kaessneri*, • *C. lasciva*, + *C. ternatea*.

The habitat of *C. ternatea* is open mesic forest or shrub land (personal observations of authors and records in the Australasian Virtual Herbrarium[2]). In Australia, the authors note that populations of *C. ternatea* occur in tropical regions in open areas where sunlight is plentiful due to a sparse canopy and in areas near where fresh water would collect such as the border of wetlands, small gullies, or at the base of rocky hillsides. When present, the plants are often vigorous and smother other vegetation.

Chemical Constituents

Butterfly pea (Clitoria ternatea) contains a wide range of chemical compounds, each contributing to its therapeutic benefits. The plant's chemical composition includes tannins, phlobatannins, carbohydrates, saponins, triterpenoids, phenols, flavonoids, flavanol glycosides, proteins, alkaloids, anthraquinones, anthocyanins, cardiac glycosides, Stigmast-4-ene-3,6-dione, volatile oils, and steroids. These diverse chemical constituents play a significant role in the medicinal properties of Butterfly pea. Here is a list of the principal chemical components found in this plant [29,30].

S.No.	Phytoconstituents	CTE
1.	Protein	+
2.	Carbohydrates	+
3.	Resins	+
4.	Tannins	+
5.	Saponins	+
6.	Flavonoids	+
7.	Alkaloids	+
8.	Steroids	+
9.	Phenols	+
10.	Glycosides	+

Medical uses

Antimicrobial Effect Clitoria ternatea extracts have demonstrated inhibitory effects against a variety of pathogens, including bacteria, fungi, and viruses. The methanol extracts from both the leaf and root exhibited the most potent antimicrobial activity, with MIC (Minimum Inhibitory Concentration) values ranging from 0.3 mg/ml to 100.00 mg/ml against various bacterial, yeast, and fungal species. Among the tested organic solvent extracts (petroleum ether, ethyl acetate, and methanol), the methanol extract proved to be the most effective [33].

Anti-inflammatory Antipyretic and Analgesic Effects The ethanol extract of Clitoria ternatea root (ECTR) showed antihistaminic activity by inhibiting clonidine-induced catalepsy in mice, but it did not exhibit the same effect against haloperidol-induced catalepsy. In contrast, the methanol extract of Clitoria ternatea root with blue flowers (MECTR) demonstrated anti-pyretic potential. It effectively reduced normal body temperature and yeast-induced pyrexia in rats, comparable to the effects of paracetamol [34,35].

Antiparasitic and Insecticidal Effects Clitoria ternatea's ethanolic extract (100mg/ml) induced paralysis in Indian earthworms (Pheretima posthuma) within 15-20 minutes and led to their death within 28-30 minutes. Among the various extracts, the methanol extract of Clitoria ternatea roots exhibited the most potent anthelmintic activity. Both the aqueous and ethanolic extracts of Clitoria ternatea leaves demonstrated significant anthelmintic activity against Eisenia foetida, with the ethanolic extract showing higher efficacy. Furthermore, the seed extract of Clitoria ternatea displayed promising mosquito larvicidal activity against Aedes aegypti, Culex quinquefasciatus, and Anopheles stephensi [36,37,38]

Antioxidant Activity Clitoria ternatea flower petal extract (CTE) demonstrated potent antioxidant activity and protected erythrocytes against oxidative damage. Clitoria ternatea leaf extracts showed significant antioxidant effects and reduced DNA damage. Aqueous extracts exhibited stronger antioxidant activity compared to ethanol extracts. Methanolic extract of Clitoria ternatea leaf showed antioxidant properties and a hepatoprotective effect in mice against paracetamol-induced liver toxicity [39,40].

Neuroprotective Activity Clitoria ternatea has been found to have neuroprotective qualities, which may be linked to its antioxidant and anti-inflammatory capabilities. It has showed promise in preventing neurodegenerative disorders and increasing cognitive function

Anthelmintic Activity The ethanolic extract of Clitoria ternatea (CT) leaves exhibited anthelmintic activity at 100 mg/ml. However, in another study, anthelmintic activity was observed with the methanolic extract of CT leaves at 10 mg/ml and 25 mg/ml, while no such activity was found with the ethanolic extract at the same concentrations

Medicinal Properties Butterfly pea has a wide range of medicinal applications due to its diverse pharmacological properties. Here are some of the notable medicinal applications of Butterfly pea.

Diuretic and Anti-urolithiasis Effect The alcoholic extract derived from Clitoria ternatea leaves exhibited

robust inhibitory potency against the formation of calcium oxalate crystals, which is comparable to the effect of the proprietary drug Cystone used for dissolving kidney stones. Notably, the extract showed a higher percentage of inhibition of calcium oxalate crystallization in vitro compared to Cystone.

Central Nervous Effect Clitoria ternatea extracts have shown promising memory-enhancing and cognitive effects, including significant anxiolytic, antidepressant, and CNS-depressant activities. The extracts increased acetylcholine content in the hippocampus, leading to improved learning and memory. Additionally, they enhanced passive avoidance learning and retention by increasing dendritic intersections and branching in Sahu et al.; Int. J. Plant Soil Sci., vol. 35, no. 18, pp. 942-951, 2023; Article no. IJPSS.104094 948 amygdaloid neurons. The extracts also exhibited anti-amnesic effects, boosted acetylcholine content, and increased acetylcholinesterase activity in the brain. Overall, Clitoria ternatea extracts demonstrated nootropic, anxiolytic, antidepressant, anticonvulsant, and antistress activities, making them promising candidates for cognitive enhancement and neuroprotective interventions.

Diabetes Control Clitoria ternatea extracts exhibited noteworthy antidiabetic effects in diabetic rats. Methanol, water, petroleum ether, and chloroform extracts were able to reduce blood glucose levels. The aqueous extracts of leaves and flowers showed improvements in glucose and insulin levels while reducing enzyme activity. The alcoholic root extract demonstrated preventive effects against diabetic complications in the brain and pancreas. Moreover, Clitoria ternatea extract showed inhibition of advanced glycation end product (AGE) formation and displayed strong antioxidant properties, indicating its potential as a therapeutic intervention for diabetic complications. 2.17 Wound Healing Effect Clitoria ternatea seed and root extracts have demonstrated remarkable wound healing activity in various models, including excision, incision, and dead-space models, whether administered orally or applied topically. These extracts exerted their effects on all phases of wound healing: the inflammatory, proliferative, and remodelling phases. Moreover, the standardized leaf extract exhibited inhibitory activity against enzymes involved in skin wound healing, such as hyaluronidase and matrix metalloproteinase-1 (MMP-1). The presence of the bioactive compound taraxerol in the extract and ethyl acetate fraction is believed to contribute to its wound healing potential. Overall, Clitoria ternatea extracts hold promise as natural agents for promoting effective wound healing [41-45]

CONCLUSION:

In conclusion, Clitoria ternatea, commonly known as Butterfly pea, holds immense medicinal potential and has been utilized since ancient times for various therapeutic purposes. Its traditional use in Ayurvedic medicine for treating health concerns such as indigestion, arthritis, and skin diseases has been well-documented. Furthermore, the flowers of C. ternatea have gained global popularity as ornamental flowers and natural food colorants. The plant has demonstrated significant effects in wound healing, memory enhancement, antidiabetic activity, and antimicrobial properties. Additionally, its antioxidant, neuroprotective, and anthelmintic activities have been well-documented. The various bioactive compounds identified in C. ternatea, such as anthocyanins, flavanols, and lipophilic compounds, contribute to its therapeutic benefits and add to its value as a natural remedy. Moreover, its adaptability to different climates and wide distribution across various regions make it a promising candidate for functional food applications. The micropropagation techniques discussed in this review offer valuable tools for mass-producing homogeneous and disease-free plantlets, contributing to the conservation and commercial production of Clitoria ternatea.

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