**Biological activities and phytochemicals of clitoria *ternatea***

**(Butterfly pea)**

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**Abstract**

Clitoria *ternatea* or commonly known as ‘Butterfly pea’ has been used traditionally in Ayurvedic medicine in which various parts of the plants are used to treat health issues such as indigestion, constipation, arthritis, skin diseases, liver and intestinal problems. The flowers, leaves, bark, root, stem and other parts of C. *ternatea* are used treatments of many diseases. C. *ternatea* flowers worldwide as ornamental flowers and traditionally used as a food colorant. This book chapter has recent advances biological activities and phytochemicals from C. *ternatea* plants.

Various phytochemicals such as kaempferol, quercetin and myricetin glycosides as well as anthocyanins have been isolated from C. *ternatea* flowers. Clitoria *ternatea* flower extracts were found to possess antimicrobial, antioxidant, anti-inflammatory, cytotoxic and antidiabetic activities which are beneficial to human health. Clitoria *ternatea* flower is a promising candidate for functional food applications owing to its wide range of pharmaco therapeutic properties as well as its safety and effectiveness.

**Introduction**

Since ancient times, people of all civilizations and cultures have used aromatic and medicinal herbs for therapeutic, religious, cosmetic, nutritional, and beautifying purposes [1-2]. The plant species Clitoria *ternatea* belongs to the kingdom Plantae, the phylum Tracheophyta, the class Magnoliopsida, and the family Fabaceae [3]. Clitoria *ternatea*, a perennial climber that grows to a height of 2 to 3 metres, is sometimes referred to as the butterfly pea or blue pea flower[4]. The blue flower pigment is traditionally used as a food colouring in Southeast Asia, but it is also frequently grown as a decorative plant and utilised as a species for revegetation [5-6]. It is well recognised that the plant is suitable as a cover crop and green manure, able to not only stifle perennial weeds but also able to enrich the soil by fixing nitrogen [7-8].

The C. *ternatea* plant is extensively distributed in Madagascar, South and Central America, the Caribbean, India, the Phillipines, and other tropical Asian nations [9-10]. In Ayurvedic medicine, Clitoria ternatea is regarded as a nootropic herb [11]. It thrives in areas with both full sunlight and some shade. Seed germination takes place in approximately 1-2 weeks, while flowering takes place in 4 weeks [12-13]. There are numerous C. *ternatea* lines with 4-5 cm long flowers in a variety of colours, including light blue, dark blue, white, and mauve (Fig. 1). Ternatin anthocyanins and different flavanol glycosides of kaempferol, quercetin, and myricetin are among the substances said to be present in the flowers [14-15]. The leaves are eliptic-oblong, pinnate, and have 5-7 leaflets. Their length and width range from 2.5 to 5.0 and 2.0 to 3.2 cm, respectively. When tendered, their flat, linear, beaked seed pods, which range in length from 5-7 cm, are delicious. The seed is oval-shaped, blackish or yellowish brown in colour, and is 3–4 mm in width and 4.5–7.0 mm in length. It has a taproot system with several lateral roots that are thin [16-17].



Fig. 1 Flower of Clitoria ternatea

Nutritional examination of C. *ternatea* flowers showed that their moisture content was 92.4% and that their percentages of protein, fibre, carbohydrate, and fat were 0.32, 2.1, 2.2, and 2.5%, respectively. A high concentration of calcium (3.09 mg/g), magnesium (2.23 mg/g), potassium (1.25 mg/g), zinc (0.59 mg/g), sodium (0.14 mg/g), and iron (0.14 mg/g) were also discovered in the flower[18]. The bioactive components from C. *ternatea* flower were examined, recognised, and isolated in a number of researches. The blue ternatin anthocyanins are acylated on the basis of delphinidin and are known as anthocyanins (Fig. 2).



Fig. 2 Delphinidin 3-malonyl glucoside

Ayurvedic medicine has traditionally employed Clitoria ternatea for a number of medical conditions. Its seeds are used as a laxative, to treat colic, and to treat swollen joints. Its roots are used to treat indigestion, constipation, fever, arthritis, sore throat, skin illnesses, and eye ailments. Traditional Cuban medicine utilises a decoction of the roots alone or in combination with flowers to produce uterine contractions, encourage menstruation, and heal liver and intestinal issues[20-21]. On the roots, seeds, flowers, and leaves of C. *ternatea*, numerous research studies have been conducted. Numerous research have demonstrated that the crude extract from the Clitoria *ternatea* flower has antidiabetic [22], antioxidant [23], antibacterial [24], and anti proliferative/anticancer [25] properties. As a result, C. *ternatea* flowers can be used as a supplement or as a natural source of antioxidants in the food and pharmaceutical industries. This study summarises the most recent information on C. *ternatea* flower extraction techniques, their impact on the phytochemicals, and the biological actions of these phytochemicals. This book chapter includes the current update on the C. *ternatea* plants and its effect on the phytochemicals as well as the biological activities of these phytochemicals.

**Blue Pea Flower Anthocyanins**

The blue pea blossom, Clitoria *ternatea* L., is a rich source of polyacylated anthocyanins, and because they are more stable than non-acylated anthocyanins, they can be used as a natural food colour [26-27]. Like other anthocyanins, the pH affects how the colour of the blue pea flower anthocyanin extracts looks. Red colour exists at pH levels below 3.2, violet colour changes to blue between pH levels 3.2 and 5.2, light blue colour exists between pH levels 5.2 and 8.2, and light blue colour changes to dark green colour between pH levels 8.2 and 10.2 [28]. The structural changes in anthocyanin molecules and the altered H and OH concentration in the medium could both be responsible for this colour change. The presence of the flavylium ion is responsible for the red hue, the neutral quinoidal base for the blue colour, and the ionic chalcone for the green colour. When pH rises, the flavylium ion in non-acylated anthocyanins changes into the colourless carbinol pseudo base. However, the acyl groups in blue pea flower anthocyanins inhibit the hydrolysis of the flavylium ion to the less stable carbinol pseudo basic form and instead produce the blue colour quinoidal that has reduced sensitivity to pH fluctuations in mildly acidic or neutral medium. As a result, in both acidic and neutral food systems, blue pea flower anthocyanins could be employed as a blue colouring agent. The blue pea blossom, Clitoria ternatea L., is a rich source of polyacylated anthocyanins, which have the advantage of being utilised as a natural food colouring agent due to their better stability when compared to non-acylated anthocyanins[29]. Like other anthocyanins, the pH affects how the colour of the blue pea flower anthocyanin extract looks. Red colour exists at pH levels below 3.2, violet colour turns to blue between 3.2 and 5.2, light blue colour exists between 5.2 and 8.2, and light blue colour changes to dark green colour between 8.2 and 10.2. The structural change in anthocyanin molecules and the altered H+ and OH- concentration in the medium could both be responsible for this colour change. The presence of the flavylium ion is responsible for the red hue, the neutral quinoidal base for the blue colour, and the ionic chalcone for the green colour [30]. When pH rises, the flavylium ion in non-acylated anthocyanins changes into the colourless carbinol pseudo base. However, the acyl groups found in blue pea flower anthocyanins stop the hydrolysis of the flavylium ion into the less stable carbinol pseudo basic form, resulting in the formation of the blue hue quinoidal, which is less sensitive to pH fluctuations in moderately acidic or neutral medium [31-32]. As a result, in both acidic and neutral food systems, blue pea flower anthocyanins could be employed as a blue colouring agent.

**Others Phytochemicals**

* **Flavonoids:** Clitoria *ternatea* is rich in flavonoids, including anthocyanins (e.g., delphinidin, cyanidin, and petunidin) and flavonols (e.g., quercetin, kaempferol, and myricetin). These compounds contribute to the blue and purple pigmentation of the flowers and have shown antioxidant and anti-inflammatory properties. Anthocyanins like delphinidin and cyanidin, as well as flavonols like quercetin and kaempferol, are among the most prominent flavonoids found in this plant.
* **Alkaloids:** Clitoria *ternatea* contains alkaloids, which are organic compounds that often have pharmacological activities. The major alkaloid identified in the plant is called "clitorine." Alkaloids can exhibit a range of biological effects, but their specific activities in Clitoria *ternatea* are still being studied.
* **Triterpenoids and Sterols:** Clitoria *ternatea* has been found to contain various triterpenoids and sterols. These compounds are known for their diverse biological activities, including anti-inflammatory and antioxidant properties.
* **Saponins:** Saponins are glycosides with soap-like properties. Clitoria *ternatea* contains certain saponins, which have exhibited various biological activities, including antifungal and antimicrobial effects.

**Biological activities of Clitoria *ternatea***

Clitoria *ternatea* flower contains a significant amount of phytochemicals which exhibits great antioxidant, antimicrobial, antidiabetic, anti-inflammatory and antiproliferative/ anticancer properties [38-42]. Acute toxicity study using albino Wistar rats treated orally with aqueous ethanol extract (2000 mg/kg bodyweight) of the flower showed no signs of mortality or abnormality and there was no significant difference in the haematological values The extract did not display acute toxicity effects and are safe for consumption [43]. Clitoria *ternatea* flowers can potentially be utilised as a functional food incorporated into various food products or even as a pharmaceutical supplement/drug combined with commercial drugs to improve treatment efficacy of patients.

**Antioxidant properties**

Clitoria ternatea is rich in flavonoids, anthocyanins, and other polyphenolic compounds that act as potent antioxidants. Antioxidants help neutralize free radicals, which are unstable molecules that can cause oxidative damage to cells and contribute to aging and various diseases. The antioxidant activity of Clitoria *ternatea* may protect cells and tissues from oxidative stress and support overall health [44-45]. The antioxidant activity of C. *ternatea* flowers was examined in several studies using antioxidant assays such as 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP), hydroxyl radical scavenging activity (HRSA), hydrogen peroxide scavenging, oxygen radical absorbance capacity (ORAC), superoxide radical scavenging activity (SRSA), ferrous ion While the water extract of the C. ternatea flower was found to be less strong than ascorbic acid (vitamin C), the 100% methanol extract was shown to be more potent than vitamin E [46-49] in the DPPH experiment. certain research, the antioxidant activity (DPPH assay) of the extracts made using various solvents was investigated and compared; at 15 minutes of extraction time, it was discovered that the water extract was more effective than the 100% ethanol extract[50].

The water extract and 50% methanol were found to be similarly potent and to have higher activity than the 100% methanol extract in another investigation, which revealed the ideal extraction time (6 h) for the three extracts [51].

**Nootropic effects**

Nootropics, also known as "smart drugs" or cognitive enhancers, are substances that may improve cognitive functions such as memory, learning, and focus. Clitoria *ternatea* has been traditionally used as a brain tonic and is believed to enhance cognitive abilities. Some studies have suggested potential memory-enhancing effects of Clitoria *ternatea* extracts, likely attributed to the presence of certain compounds that may support brain health.

**Anxiolytic and antidepressant effects**

Clitoria *ternatea* has been traditionally used for its calming and mood-enhancing properties. Preclinical studies in animals have shown that Clitoria *ternatea* extracts may have anxiolytic (anxiety-reducing) and antidepressant effects, possibly due to interactions with neurotransmitter systems in the brain.

**Antimicrobial activity**

Several studies have investigated the antimicrobial activity of Clitoria *ternatea* extracts against various microorganisms, including bacteria, fungi, and even some viruses. The plant's bioactive compounds, such as flavonoids, alkaloids, and tannins, are believed to be responsible for its antimicrobial effects.

For instance, a study published in the Journal of Traditional and Complementary Medicine in 2015 found that Clitoria *ternatea* leaf extract exhibited potent antibacterial activity against both Gram-positive and Gram-negative bacteria. Another study in the Journal of Pure and Applied Microbiology in 2017 demonstrated its antifungal properties against various pathogenic fungi.

Moreover, researchers have also explored the antiviral potential of Clitoria ternatea. A 2018 study in the Journal of Ethnopharmacology revealed that the plant extract displayed inhibitory effects against the herpes simplex virus.

The antimicrobial activity of Clitoria ternatea offers promising possibilities for the development of natural antimicrobial agents and could potentially aid in the treatment of various infectious diseases. However, further research is required to fully understand its mechanisms of action and to validate its potential in clinical applications.

**Anti-inflammatory properties**:

Inflammation is a natural response by the body to protect against harmful stimuli. However, chronic inflammation can lead to various health problems. Some studies have indicated that Clitoria *ternatea* extracts exhibit anti-inflammatory effects, which could be beneficial in managing inflammatory conditions. Since they are known to influence both COX-1 and COX-2, the currently available non-steroidal anti-inflammatory medicines (NSAIDs), such as acetaminophen and aspirin, are linked to adverse effects, mainly gastrointestinal and cardiovascular problems [55]. To lessen the hazards associated with NSAIDs while providing adequate pain management, new or alternative approaches must be found. With healthy albino rats of either sexe, the petroleum ether extract of C. *ternatea* flowers was tested for anti-inflammatory efficacy using the carrageenan paw edoema technique. In Eddy's hot plate method, the treatment group (400 mg/kg) significantly increased reaction time (time recorded when animals licked their fore or hind paws or jumped response, whichever appeared first) compared to control untreated group. The extract (200 and 400 mg/kg) significantly inhibited paw edoema compared to control untreated group. In carrageenan induced edoema, the study hypothesises that the extracts may have a protective impact against the release of prostaglandins, kinnins, and other chemicals [56].

**Anti-diabetic potential**

Research has shown that extracts from Clitoria *ternatea* have the ability to lower blood glucose levels by enhancing insulin sensitivity and stimulating glucose uptake in cells. Additionally, it may help preserve pancreatic beta-cell function, which is responsible for insulin production, thereby improving overall glucose control [57]. Furthermore, Clitoria *ternatea* has been found to mitigate diabetic complications such as diabetic nephropathy and retinopathy due to its protective effects on kidney and retinal tissues [58].

Although promising, it is essential to note that the majority of studies have been conducted on animals or in vitro, and human clinical trials are limited. As such, further research is required to validate and better understand the anti-diabetic potential of Clitoria *ternatea* fully [59]. Recently Studies have explored the potential anti-diabetic effects of Clitoria *ternatea* extracts. Compounds in the plant may help reduce blood glucose levels and enhance insulin sensitivity, making it potentially useful in managing diabetes [60].

**Wound healing**

Clitoria *ternatea* has been traditionally used for wound healing. The plant's antioxidant and anti-inflammatory properties may play a role in promoting wound healing and tissue repair. current research on Clitoria *ternatea's* wound healing potential is promising, it is essential to note that most studies have been conducted in animal models or in vitro. Further clinical research and human trials are necessary to fully validate the efficacy and safety of Clitoria ternatea for wound healing applications.

**Anti bacterial Activity**

The advent of bacteria resistant to antibiotics severely reduces the efficacy of modern medications, leading to infection treatment failure [62]. In order to overcome this difficulty, it is necessary to create different strategies in addition to looking for novel antibacterial substances. There are several ways to test an antimicrobial agent's (antibacterial or antifungal) in vitro activity, including the disc diffusion method and broth or agar dilution [63]. Several studies investigated on the antibacterial potential of C. ternatea flowers. The methanol extract of C. ternatea flower was tested against 12 bacterial species (Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Staphylococcus aureus, Streptococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Enterobacter aerogens, Proteus mirabilis and Herbaspirillum spp.) and was found to have the most potent activity against Bacillus thuringiensis with a minimum inhibitory concentration (MIC) of 12.5 mg/mL and minimum bactericidal concentration (MBC) of 25 mg/mL with an inhibition zone of 15.7 mm using agar disc diffusion technique [64]. In a different investigation, the antibacterial activity of a flower extract from C. ternatea (4 mg) against E. coli, S. enteritidis, K. pneumoniae, S. typhimurium, and P. aeruginosa was examined. The extract was tested in hexane, water, methanol, petroleum ether, and chloroform. With an inhibitory zone range of 16–26 mm for E. coli, , and P. aeruginosa, K. pneumonia, the methanol extract was found to have the maximum activity when evaluated using the agar disc diffusion technique, while it had no effect on S. typhi and S. enteritidis. K. pneumonia and P. aeruginosa showed the maximum zone of inhibition, measuring 26 mm[65]. The ethanol extract paste of the C. ternatea flower contains anthocyanins that have antibacterial action against Yersinia enterocolitica, E. coli, B. cereus, B. subtilis, S. aureus, Proteus mirabilis, B. subtilis subsp. spizizenii, and B. subtilis subsp. spizizenii. The anthocyanin fraction obtained from the ethanol extract of C. ternatea flower had the best effect against B. subtilis with a disc diffusion inhibition zone of 10 mm in one study, while the anthocyanin fraction obtained from the ethanol extract of C. ternatea flower had the best effect against B. subtilis with a MIC of 1.6 mg/mL and minimum lethal concentration (MLC) of 25 mg/mL in another study [66].

**Cytotoxic and anti-proliferative/anticancer activities**

Among the methods used to treat and manage cancer include chemotherapy, radiation therapy, and targeted therapy, however these treatments do not offer a permanent cure and have a number of toxicities and side effects. [67]. Therefore, there is an urgent need for new agents that are secure, accessible, and efficient. The anticancer potential of C. *ternatea* flowers extracted using various solvents was examined in several research. In the in vitro cytotoxic experiment against Dalton's lymphoma ascites (DLA) cells at 3 h, the 100% petroleum ether extract (IC50=36 g/mL) was discovered to be more powerful than the 100% ethanol extract (IC50 value of 57 g/mL), which may be related to the differing phytochemical content of the two extracts. While the ethanol extract only contains flavonols, the petroleum ether extract was discovered to contain saponins, tannins, steroids, and triterpernoids. [68]. On the human epithelial laryngeal carcinoma (Hep-2) cell line, the hydrophilic (100% methanol) extract had a stronger anticancer impact than the lipophilic (hexane:ethyl acetate, 1:1) extract [69]. In contrast to the lipophilic extract, which is made up of fatty acids, phytosterols, and tocopherols, the powerful active substances found in the hydrophilic extract were primarily ternatins, kaempferol, and quercetin, which are responsible for the antiproliferative impact. floral extract with diabetes-preventing properties. The water extract significantly decreased the level of fructosamine in glycated bovine serum albumin (14.47-36.66%) as well as the generation of fluorescent advanced glycation end products, with the peak action at day 28 (49.4% at 1 mg/mL). According to the study, the extract's capability to inhibit the formation of advanced glycation end products may be mediated by its capacity to scavenge free radicals, which is primarily attributable to the presence of the active components ternatin anthocyanins, delphinidin derivatives, and kaempferol [70]. These studies collectively suggested that the flavonoid principles (flavonol glycosides and anthocyanins) and alkaloids present in the extract may exert the hypoglycemic activity, which may involve enhancing the transport of blood glucose from plasma to peripheral tissues or potentiating insulin secretion from the -cell.

**Diuretic and anti urolithiasis effect**

When given orally in a non-toxic dose, Clitoria *ternatea* roots or their extract in 95% alcohol had no discernible diuretic or natriuretic impact on dogs. The extract exhibited symptoms of kidney injury, although intravenous doses also moderately increased the excretion of salt and potassium in the urine. [71]. By using a titrimetric method, it was determined whether different Clitoria *ternatea* extracts might suppress the in vitro development of calcium oxalate crystals, which is a common primary component of most urinary stones. It was discovered that the inhibitory power of the alcohol extract of Clitoria *ternatea* was equivalent to that of Cystone, a patented medication for the removal of kidney stones. In vitro studies using an alcohol extract of Clitoria ternatea leaves revealed greater calcium oxalate crystallization inhibition (72.991.2%) compared to cystone (90.551.27%) in terms of calcium oxalate precipitation [72].

**Conclusion**

The edible blue pea flower, also called Clitoria ternatea, is significant both aesthetically and therapeutically. The blue pea flower contains a lot of polyacylated anthocyanins, which are more stable than non-acylated anthocyanins. It is simpler to employ anthocyanins from blue pea flowers as a blue food colouring agent in acidic food systems because they exhibit a very bright and uniform blue colour in acidic medium. Many different pharmacological effects of Clitoria ternatea have been reported, including memory improvement, increased acetylcholine levels, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquillizing, sedative, antimicrobial, antipyretic, anti-inflammatory, analgesic, diuretic, local anaesthetic, antidiabetic, insecticidal, and blood platelet aggregation inhibition. There have been reports of numerous secondary metabolites from this plant, including flavanoids, anthocyanin glycosides, pentacyclic triterpenoids, and phytosterols. It may be used successfully as a memory booster and as a lead for the development of novel phytoceuticals for the treatment of CNS illnesses. There are currently no known very effective curative therapies for this indication. The mode of action of these bioactive elements has been uncovered in some investigations, and this information can be used to comprehend the biological effect that is responsible for its occurrence. Further research is therefore required to better understand how the extract/active chemicals work to elicit the biological response and how they might influence/modulate certain pathways/molecular targets in the human body. Numerous studies have demonstrated the potential antioxidant activity of C. ternatea flowers in cell-based assays as well as in vivo studies and chemical-based tests. Future research comparing the impact on people with specific medical issues to subjects in good health is advised to better understand the impact and assess the potential. Because this flower offers so many advantages, it would be important to do the suggested additional research in order to better understand the biological impacts that have already been noted and to look into potential bioactivities. The bioactive components of C. *ternatea* flowers make excellent prospects for research and development as cutting-edge pharmacological agents and applications as functional foods to advance human health and wellness.

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