South Asian Journal of Research in Microbiology

11(2): 18-26, 2021; Article no.SAJRM.77676
ISSN: 2582-1989

Clitoria ternatea - Shifting Paradigms: From Laboratory to Industry

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2021/v11i230247
Editor(s):
(1) Dr. Bagiu Iulia Cristina, University of Medicine and Pharmacy "Victor Babeş" Timişoara, Romania.
Reviewers:
(1) Henrilyn E. Loñez, Kalinga State University, Philippines.
(2) Ola Saleh Mahdi, University of Babylon, Iraq.
Complete Peer review History, details of the editor(s), Reviewers and additional Reviewers are available here: https://www.sdiarticle5.com/review-history/77676

Mini-review Article

Received 20 September 2021
Accepted 30 November 2021
Published 03 December 2021

ABSTRACT

Clitoria ternatea commonly known as Butterfly pea is a standard Ayurvedic medicinal plant used in many parts of south Asian countries. Traditional medicinal plants are a great alternative to find new treatments and for the development of novel antimicrobials to combat many diseases. In Ayurveda and traditional and folk medicine in several countries, decoction and extracts made from *C. ternatea* are recommended to be used for various medical treatments. *C. ternatea* extracts claimed to possess antibacterial, antiviral, and antifungal properties, which had been supported and validated by many *in vitro* and *in vivo* experiments. However, biologically active compound/s isolation and development novel compounds still remain in its infancy. Despite its enormous potential health benefits, only a single commercial product managed to reach industrial level production. *C. ternatea* cyclotide studies are also limited despite the fact that it the fastest known natural ligase discovered to date. These cyclotides are rapid peptide ligators and has been the focus of many recent studies on peptide ligation and cyclization for biotechnological applications. In this mini summary we have tried to point out innate unique biological properties of *C. ternatea* and suggested few future studies, more specifically on *C. ternatea* cyclotides development against bacterial heat shock proteins (Hsp 100) for novel antimicrobial discovery and development.

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Keywords: Clitoria ternatea; plant cyclotides, bioactive compounds; antimicrobial activity; discovery of novel antibiotics; bacterial clpb; eskape pathogens.

1. INTRODUCTION

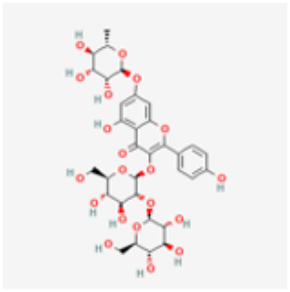
Clitoria ternatea (CT), commonly known as 'blue pea' in English is an evergreen perennial plant which grows naturally in several tropical countries. There are two varieties of the plant: Blue flowered and white flowered plant (Fig. 1). These flowers resemble to a conch shell and bear five petals. The leaves are compound, alternate, stipulate, and imparipinnate and the root system consists of a stout tap root with few branches and many slender lateral roots. The main root is thick which grows to more than two meters and has one to several glaucous, purplish, nodules. The roots fix nitrogen and have an acrid and bitter taste. In Ayurvedic and traditional medicine, decoctions and extracts made from *Clitoria ternatea* and many other plants have been used extensively to treat many ailments, infections/disorders [1,2,3,4,5,6,7]. *Clitoria ternatea* seeds contain mainly palmitic acid (19%), steric acid (10%), oleic acid (52%), linoleic acid (17%) as fatty major fatty acid compounds [8].

Traditional medicinal plants are a great alternative to find new treatments and for the development of novel antimicrobials to combat many diseases. In Ayurveda and traditional and folk medicine in several countries, decoction and extracts made from *C.ternatea* are recommended to be used in treatments of several ailments / disorders. These include ingestion, constipation, fever, eye and ear ailments, mucus disorders, sore throat, skin disorders, irritation of bladder and urethra, vaginal disorders, liver disorders, enlargement of abdominal viscera, anxiety, depression, impaired learning and memory, inflammatory conditions [1,9]. According to Ayurveda and traditional and folk medicine, it is also used in the treatment of rabies and rheumatoid arthritic conditions [9]. Some of these claimed therapeutic effects of CT have been experimentally tested by using *In vitro* and *in vivo* studies and have been scientifically validated: such as anxiolytic, antidepressant, learning and memory enhancement, antipyretic, diuretic, and anti-inflammatory effect. However, CT is one of the most underutilized (both medicinally and commercially) medicinal plant. In this mini summary we have attempted to direct readers attention towards innate specific properties of *C. ternatea* and suggested few possible future studies, for novel antimicrobial discovery and development.

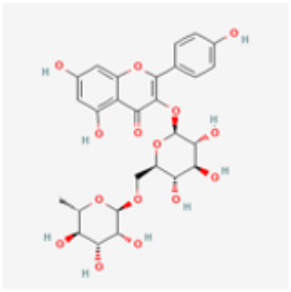
Fig. 1. *C. ternatea* Blue, White colour flowers, Pods and plant are shown in clockwise arrangement.
Figures were adapted from Royal Botanic Gardens, Kew, Plants of the world Online. http://www.plantsoftheworldonline.org/taxon/urn:lsid:ipni.org:names:486606-1 Accessed on 05th November 2021.

2. MAJOR PHYTOCHEMICAL COMPOUNDS

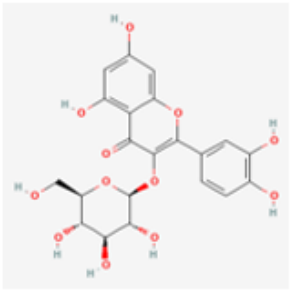
Clitoria ternatea contains a significant number of phytochemicals (Fig. 2) which are believed to be the major contributors for its unique antioxidant, antimicrobial, antidiabetic, anticancer and anti-inflammatory properties [8,10, 40]. Flavanols and Anthocyanins are the major natural products found in *Clitoria ternatea* [8]. It has been reports that blue flowers contain Kaempferol 3-robonoside-7-rhamnoside, Kaempferol 3-rutinoside, Quercetin 3-glucoside and Myricetin 3-neophesperodosie while white flowers contain Kaempferol 3-glucoside, Kaempferol 3-rutinoside and Quercetin. *Clitoria ternatea* leaves mainly contain Kaempferol 3-glucoside and Kaempferol 3-rutinoside. Anthocyanins such as Ternatin A1 to A3, B1 and B2, C1 to C5 and D1 to D3 have been reported in blue flowers while Delphinidin has been reported in mauve flowers [8]. We urge the readers to refer to [8] for a detailed description of major phytochemical compounds.



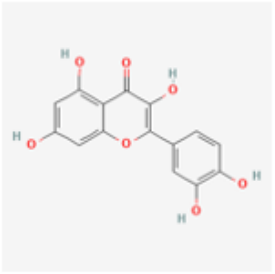
Kaempferol 3-sophoroside-7-rhamnoside



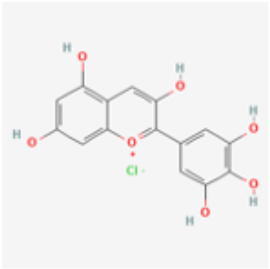
Kaempferol-3-O-rutinoside



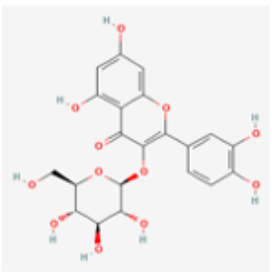
Quercetin 3-glucoside



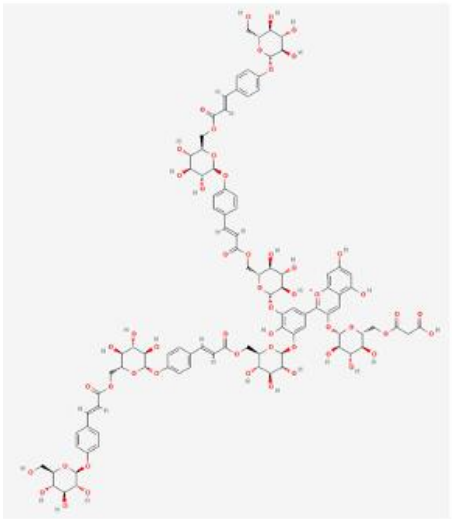
Quercetin



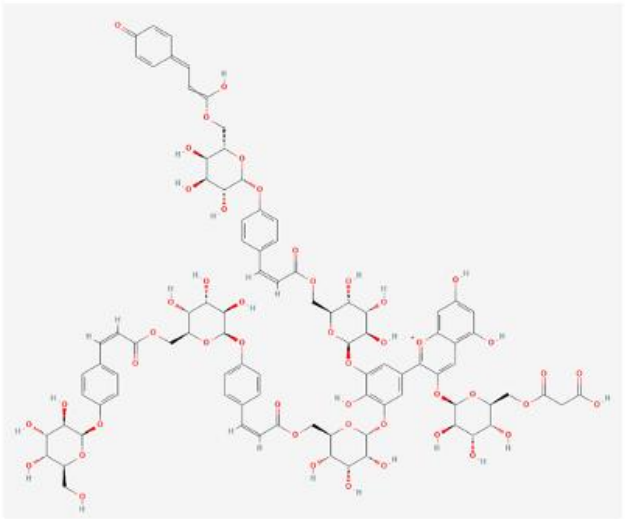
Delphinidin



Quercetin 3-glucoside



Ternatin A1



Ternatin B1

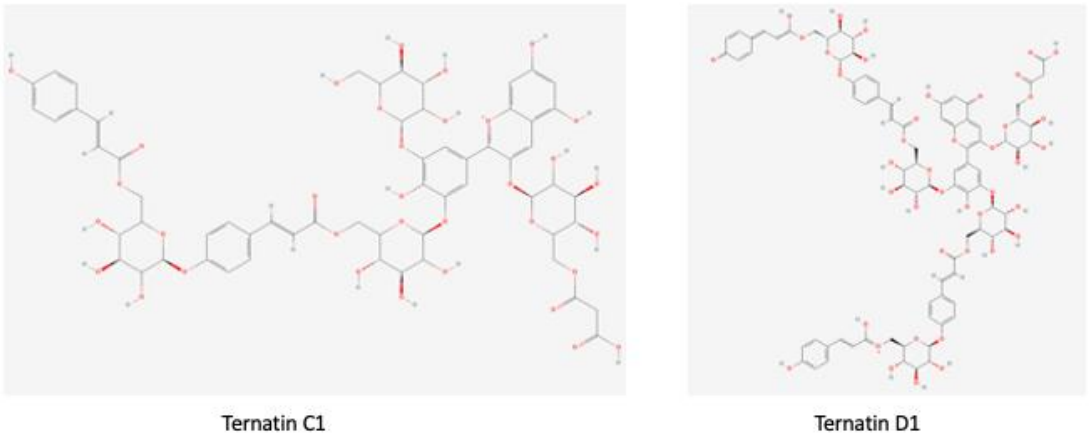


Fig. 2. Few reported Phytochemical compounds found in *C. ternatea*. Compound structures were taken from PubChem, https://pubchem.ncbi.nlm.niwetwetwetdryh.gov/ Accessed on 05th November 2021

3. MEDICINAL PROPERTIES AS REPORTED IN LITERATURE

As mentioned earlier CT has been used in traditional medicine for over centuries. This has stimulated investigators to unravel pharmacological effects of CT, using various plant extracts from CT. It has been well documented that CT extracts from leaves and roots had some significant anti-inflammatory, analgesic, and anti-pyretic activities. Recently in 2014, Ranaweera and colleagues [1] reported that aqueous root extracts of CT possessed a significant *in vitro* inhibitory activity against heat induced albumin denaturation process. This was a significant observation as this demonstrated that aqueous root extracts may content compounds for antirheumatic arthritic activity. Subhash C Mandal and colleagues and Debapriya Garabadu and colleagues had reported that methanolic root extracts showed a significant inhibition in Carrageenin induced paw oedema [11,12]. Interestingly, Subhash C Mandal and colleagues observed that methanolic extracts of CT had a noteworthy anti-pyretic activity in albino rats [11]. This effect was dose dependent, and they stated that ant-pyretic activity observed was comparable to the control drug (paracetamol) as well.

C. Kulkani in 1988 reported about local anaesthetic effect of CT [13]. According. To Manisha Bhatia, Jagbir Chahal and Sumeet Gupta, both petroleum and ether extracts of CT possessed a long-lasting analgesic effect up to 2 hours in albino male wistar rats [14]. S. B Kasture and his group conducted an interesting study to investigate the spectrum of activity of the methanolic extract of *Clitoria ternatea* (CT) on the central nervous system [15]. They used methanolic extracts of CT and studied for its effect on cognitive behaviours, anxiety, depression, stress, and convulsions induced by pentylenetetrazol (PTZ) and maximum electroshock. Their study indicated that methanolic extracts of CT were found to possess nootropic, anxiolytic, antidepressant, anticonvulsant and antistress activity. In 2018, Sirichai Adisakwattana and colleagues conducted a series of elegant experiments to assess the effect of the *Clitoria ternatea* flower extract, on the inhibition of pancreatic α-amylase, in vitro starch hydrolysis, and predicted the glycemic index of different type of flours including potato, cassava, rice, corn, wheat, and glutinous rice flour [16]. According to Sirichai Adisakwattana and colleagues, *Clitoria ternatea* flower extract could reduce the starch digestibility, predicted glycemic index and the hydrolysis index, of flour through the inhibition of carbohydrate digestive enzymes.

N Kamkaen and J M Wilkinson studied potential antioxidant activity of CT extracts and an extract containing eye gel formulation in 2009 [17]. They reported that aqueous extracts of CT had a stronger antioxidant activity (as measured by DPPH scavenging activity) than ethanol extracts. At the same time, they reported that when aqueous CT extracts were incorporated into the formulation eye gels, these gels were also shown to retain antioxidant properties. In a separate study where protective effect of CT flower extracts with antioxidant activity on male

reproductive parameters were conducted by Sitthichai Iamsaard and colleagues in 2014 [18]. They had performed few well planned *in vitro* and *in vivo* experiments and they reported that CT flower extracts possessed antioxidant activity and extracts were not harmful to the male reproductive system. Their findings indicated that CT flower extracts can be used for protection against testicular damage in Ketoconazole induced rats. According to a study on chemical composition and anti-proliferative properties of flowers of *Clitoria ternatea* [19], showed that the water extracted of CT had significant effects against hormone dependent breast cancer cell line MCF-7 with an IC_{50} value of 175.35 µg/ml. Methanol extracts of CT had been used to study *in vivo* to evaluate its anticancer activity in Dalton's lymphoma (DLA) bearing mice [20]. The results from DLA bearing mouse study indicated suggest that methanol extract had a significant antitumour effect in DLA bearing mice.

4. ANTIMICROBIAL PROPERTIES

The antimicrobial properties of *C. ternatea* extracts have been well documented. Sreenivasan Sasidharan and colleagues used methanolic extracts of CT of the leaf, stems, flower, seed, and roots to study its antimicrobial properties against 12 bacterial species, 2 yeast species, and 3 filamentous fungal by agar diffusion and broth dilution methods [21]. Their results showed that the leaf and root extracts were the most effective against all the tested organisms. In 2019, Kathirvel Brindhadevi and the group studied antimicrobial activities of crude extracts from *Clitoria ternatea* tested against the urinary tract infection causing pathogen *Proteus mirabilis* [22]. Kathirvel Brindhadevi and colleagues had used clinical samples for the respective study. According to their findings, the highest antibacterial activity was observed for acetone and the lowest antibacterial activities were observed for isopropyl alcohol, and petroleum ether extracts against *Proteus mirabilis*. Interestingly, in a recent review in 2021 stated that *Clitoria ternatea* plant extracts contain antiviral potential and can be used for COVID-19 prophylaxis efforts based on natural antiviral Plant extracts and their compounds [23]. At the same time, it is worth mentioning here that in Srilankan traditional medicine treatment regime *Clitoria ternatea* has been mentioned as a suitable plant to treat rabies as well [9].

Clitoria ternatea extract showed a favourable antifungal activity against *Aspergillus niger* [24]. S. Sasidharan and colleagues reported that the extract had an antifungal activity against *A. niger* with a minimum inhibition concentration 0.8 mg/mL and minimum fungicidal concentration 1.6 mg/mL, respectively. In 2014, Ajesh & Sreejith have reported a discovery of a novel antifungal protein with lysozyme-like activity from seeds of *Clitoria ternatea* [25]. They reported that this novel protein had lytic activity against *Micrococcus luteus* and broad-spectrum, fungicidal activity against clinically relevant yeasts, such as *Cryptococcus neoformans*, *Cryptococcus albidus*, *Cryptococcus laurentii*, *Candida albicans* and *Candida parapsilosis*. Sitthichai Iamsaard and colleagues [18] showed that *Clitoria ternatea* flower extracts can be used for protection against testicular damage in Ketoconazole induced rats. David J. Craik and colleagues conducted an extensive RNA-sequencing (RNA-seq) and gene expression profiling on *Clitoria ternatea* plant defence protein known as Cyclotides [26]. Cyclotides are plant proteins which has six conserved cysteines (Fig. 3) that form a cystine-knot motif and protect plants from various attacks including pathogens [27, 28]. They had designed few elegant experiments using *C. elegans* to study *in vivo* functions of *C. ternatea* cyclotides. They discovered that cyclotide contained fractions from soil contacting organs were effective at killing nematodes, whereas similar enriched fractions from aerial parts contained cyclotides with stronger interactions with insect like membrane lipids. Based on reported data, generally *C. ternatea* root extracts seem to contain more potent anthelmintic components [29, 30, 31]. It is important to mention here that these cyclic peptides have been used recently to develop eco-friendly pesticide known as Sero-X® [32].

5. CURRENT AND POTENTIAL COMMERCIAL PRODUCTS

C. ternatea plant is used extensively in traditional medicine and reported to have several health benefits with recent findings at the international level. However, to date, scientifically validated value-added products from *C. ternatea* are very limited worldwide. Currently, *C. ternatea* plant is being used by an Australian company known as 'Innovate Ag' to commercially produce a bio-insecticide with a trade name Sero-X (https://innovate-ag.com.au/ Accessed on 24/10/2021). According to 'Innovate Ag', Sero-X is a non-toxic, bee-friendly, world first plant extract bio-pesticide, which can be used as an

alternative to traditional synthetic pesticides. Sero-X is a natural product that has a high efficacy against a wide range of pests, and no impact on non-target insects (https://innovate-ag.com.au/sero-x/ Accessed on 24/10/2021).

A research group from Sri Lanka attempted to develop a beverage using blue pea flower extract having additional health benefits [33]. Interestingly, they developed a range of blue pea flower incorporated beverages comprising a natural sweetener (*Stevia* extract) and a flavour (lime). This experimental beverage had a significant antioxidant activity and was shelf stable for a period of 28 days without preservatives [33]. A lemon based mocktail drink with butterfly pea flower extract was used as a market trial [34] and found out that it could be introduced to the consumers as a non-alcoholic drink. Initial test results revealed that the product provides consumers with an appealing and uniquely coloured non-alcoholic drink with a pleasing taste.

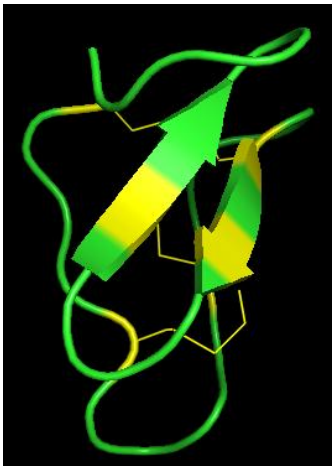


Fig. 3. The structure of cyclotide kB1 from *O. affinis* is shown here, PDB code 1NB1. Location of Cysteine residues are shown in yellow and disulfide bonds are shown as yellow sticks. Figure prepared using Pymol molecular visualization system, https://pymol.org/2/

A natural enzyme known as Butelase 1 is found in pods of the common medicinal plant *Clitoria ternatea* and it is the fastest known ligase to date [35]. Butelase 1 is a cyclase involved in the biosynthesis of cyclotides, the largest family of plant cyclic peptides [36]. Butelase 1 has the ability to ligase and catalyse peptide cyclization at an extraordinary rate. Butelase 1 cyclization reactions are 20,000 times faster than those of sortase A, a commonly used enzyme for backbone cyclization [35]. A detailed protocol for Butelase 1 purification has been established by James T Pam and his group [35]. According to this protocol Butelase 1 can be purified from pods of *C. ternatea* by a four-step chromatographic procedure to give ~5 mg of enzyme per kg of fresh plant material. Hence, Butelase 1 can be used as a versatile tool in protein engineering and general biological research.

6. CONCLUDING REMARKS

Here we have attempted to outline enormous potentials and applications of *C. ternatea* as a medicinal plant. *C. ternatea*, has long been used as a traditional Ayurvedic medicinal plant and various pharmacological activities of *C. ternatea* has been reported in traditional medicine. However, it is worth mentioning here, that only a few biologically active compounds have been isolated from *C. ternatea.* Recently it has been shown that bacterial chaperone ClpB (Caseinolytic Peptidase B) can be used as novel antimicrobial target and development of novel antimicrobials [37,38,39]. Hence it would be interesting to investigate the effects of *C. ternatea* plant cyclotides against bacterial ClpB and ESKAPE pathogens as ClpB is essential for survival of pathogens under stress. At the same time, it is important to mention that most of these *in vitro* experiments for *C. ternatea* were done by different research groups under different conditions. Hence, these experiments need to be performed under comparable conditions using valid control experiments. Claims such as *C. ternatea* being used to treat rabies needs to be investigated thoroughly using suitable animal models. Despite there are many exciting research about potential benefits of *C. ternatea*, only a single product which truly managed to find its ways to industrial applications. Therefore, it is important explore ways to translate these initial laboratory findings to next level with the idea of product commercialization.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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