

Pharmacological and Phytochemical studies on *Clitoria ternatea* L. –A review

Arya M R^{1*}, Suhara Beevy S², Mathew Dan³

¹ Research Scholar, Department of Botany, University of Kerala, Thiruvananthapuram, Kerala, India

² Professor, Department of Botany, University of Kerala, Thiruvananthapuram, Kerala, India

³ Senior Scientist and Head, Plant Genetic Resource Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Kerala, India

Abstract

Medicinal plants are a rare gift to humans since they are used to cure innumerable diseases; these plants have been used immensely in Ancient Ayurveda and Folk medicine. The plant *Clitoria ternatea* L. commonly known as Aparajitha in Sanskrit has been mentioned as one of the most prominent herb in Ancient Ayurveda. The species belongs to the subgenera Clitoria, is a common garden plant in most parts of the world. The extracts of the plant plants like leaf, flower and roots have been scientifically evaluated for anti-inflammatory, antidepressant, antimicrobial, antipyretic, antioxidative and sedative properties. The flower extracts have been used as a dyeing agent and also is an active ingredient in the cosmetic industry. This review mainly unveils the potential bioactivities of the medicinal plant *Clitoria ternatea* L.

Keywords: *Clitoria ternatea* L., ternatin, cyclotides, asparaginy endopeptidases, butelase-1

Introduction

The plant – *Clitoria ternatea* L.

Clitoria ternatea L., commonly known as Butterfly pea, is a climber with a fine stem. The plant is called ‘Shankupushpi’ in Ancient literature, denoting the shape of its flowers. “*Shankupushpi*” is an important drug in Ancient Ayurveda and reported as a brain tonic. In Ayurvedic literature, it is considered a major component of “Medhya-Rasayana” which are a group of medicinal plants described in Ayurveda with various benefits, precisely to improve memory and intellect¹. Seeds and leaves are thought to act as a memory booster and a brain tonic. Seeds are specifically used to cure swollen joints, and crushed seeds can be consumed with cold or hot water for urinary difficulties. The plant has two types of flower form- zygomorphic (single form), consisting of a typical papilionaceous corolla and diadelphous stamen and the double petal form, with five petals of similar sizes and free stamens². The shape of flowers is a reflection of its genus name since it resembles the shape of the female genital organ “Clitoris”, hence the Latin name of the genus; and the name of the species originated from “Ternate”, an Eastern Indonesian island³. The colour of flowers varies from white, light blue, dark blue, violet and mauve. Leaves are obovate and pinnately compound. *C. ternatea* pods are flattened with pointed tips, and they typically consist of about eight to ten seeds. The plant has an extensive deep-root system that enables the plant to survive extensive periods of drought. The roots also possess root nodules for nitrogen fixation typical to most leguminous crops.



Fig 1: *Clitoria ternatea* L. The two Morphoforms: A- Single form, B- Double form Picture courtesy – Anju Prithviraj

Geographic distribution

The genus is an indigenous climber distributed in the tropical and subtropical regions of the world. The plant commonly called Butterfly pea likely originated in Tropical Asia, though its true origin is masked by extensive cultivation around the globe^[4]. The number of subfamilial taxa remains imprecise, and as in the case of *Clitoria*, the descriptions of species and citations of type specimens are noted as being incomplete or incorrect⁵. The genus *Clitoria* is classified into three subgenera- Subgenus *Bractearia*, *Clitoria* and Subgenus *Neurocarpum* and a total of eight sections. Across all three subgenera mentioned, Fantz maintains 58 species as valid. *Clitoria ternatea* L. belongs to the Subgenus *Clitoria*.

Pharmacological Studies

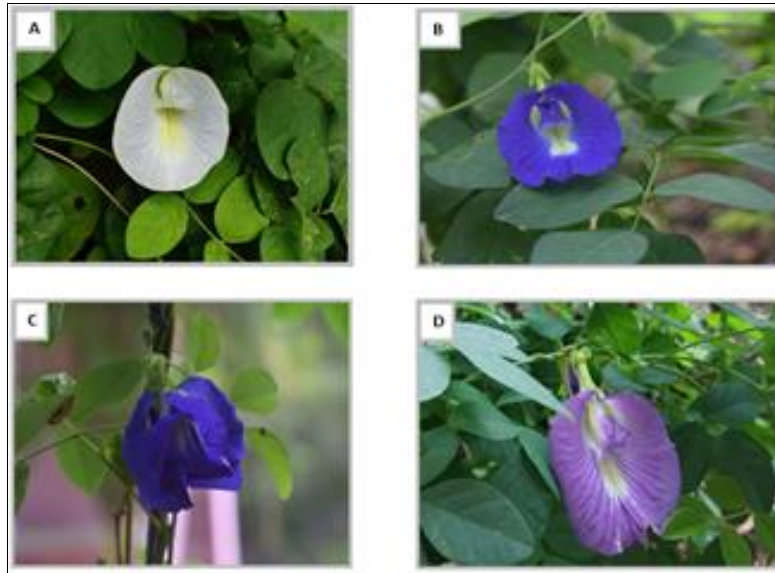


Fig 2: The different varieties of *Clitoria ternatea* L. screened for Pharmacological and Phytochemical studies
Picture courtesy – Anju Prithviraj

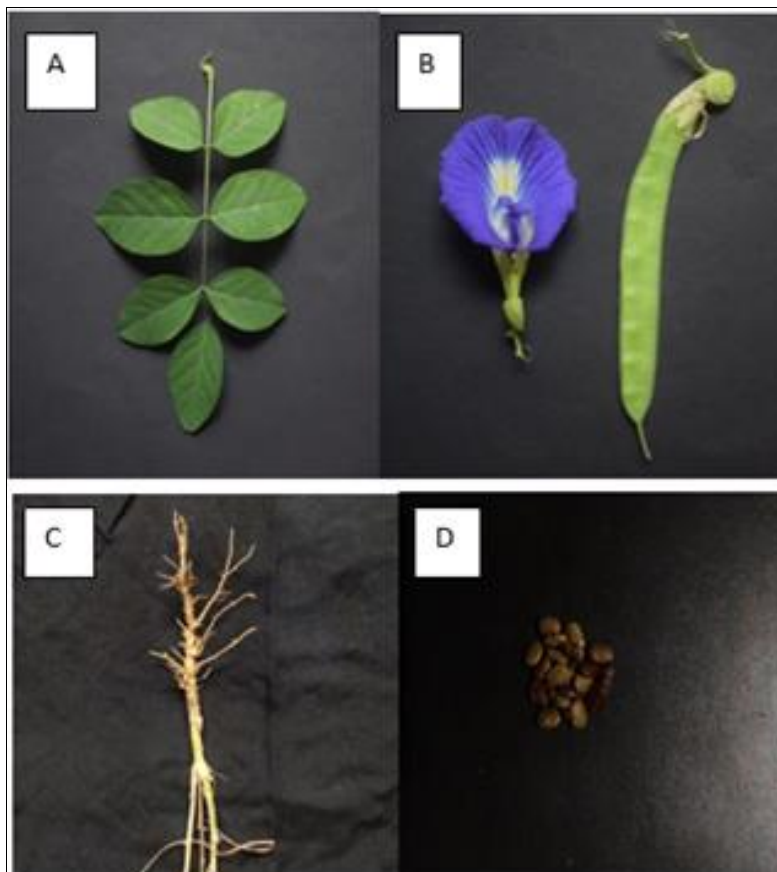


Fig 3: The different plant parts used for pharmacological and phytochemical analysis (A- Leaves, B- Flower and Pod, C- Roots, D- Seeds)

Anticancer studies

The cytotoxicity of methanolic leaf and flower extract and aqueous extract of *C. ternatea* flower has also been demonstrated in various cancer cell lines [6]. The anticancer and chemo sensitising activities of Cyclotides (peptides) from the plant in paclitaxel (Taxol)-resistant lung cancer cells was demonstrated [7].

Hypolipidemic and Antidiabetic activity

The oral administration of the hydroalcoholic extract of the roots and seeds of the plant resulted in a significant ($p < 0.005$) reduction of Total serum cholesterol, triglycerides, very-low-density lipoprotein (VLDL) cholesterol and low-density lipoprotein cholesterol levels (LDL). In diet-induced hyperlipidemic rats, the atherogenic index and the HDL/LDL ratio were also found to be in a standard range after the oral treatment, and the effects were compared with atorvastatin (50 mg/kg) and gemfibrozil (50 mg/kg). Studies on the plant have shown that oral administration of aqueous leaf extract of the plant (400mg/kg body weight) and aqueous flower extract (400mg/kg body weight) for 84 days showed significant reduction in the activity of the gluconeogenic enzymes like glucose-6-phosphatase, serum glucose, glycosylated haemoglobin, total cholesterol, triglycerides, urea, creatine but increased HDL-cholesterol, serum insulin, protein, liver and skeletal muscle glycogen content [8].

Antioxidant studies

The antioxidant studies on the aqueous extracts of the plant showed stronger antioxidant potential than ethanolic extracts with IC₅₀ (Half maximal Inhibitory concentration) values of 2mg/ml and 5 mg/ml, respectively), as confirmed by performing 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) scavenging assay. The total phenolic content (TPC) was found to be 2.0 mg/g extract as gallic acid equivalents. The data obtained accounts for the use of *C. ternatea* flower extracts as antioxidant inclusions in the cosmetic industry. The different solvent extracts of the leaves were assessed for their *in-vitro* free radical scavenging potential by DPPH free radical scavenging assay and all extracts exhibited potential *in vitro* free radical scavenging activity with respect to an increase in concentrations, the most potent being the methanolic extract followed by chloroform and petroleum ether extracts [9]. Under another study, the DPPH free radical scavenging assay, hydroxyl radical scavenging assay and reducing power assay on the petroleum ether, chloroform and methanolic extracts of the roots of white and blue varieties were carried out. The results showed that all three root extracts significantly inhibited the DPPH free radical scavenging activity at concentrations ranging from 50-600 μ g/ml (Microunits). The extracts from the white coloured varieties showed the highest inhibition when compared to the blue coloured ones [10].

Antiasthmatic studies

The ethanolic and aqueous extract of the plant roots were screened for antiasthmatic activity in histamine aerosol induced bronchospasm in Wister rats¹¹. The results showed that aqueous extract of the plant decreases bronchial hyperreactivity and also possess broncho-dilating activity.

Antipyretic studies

A study was carried out for the evaluation of the antipyretic potential of the root methanolic extract of the blue-flowered variety in albino rats. The rats were screened at two conditions for the study - one was the rats at normal body temperature and the second, in rats induced with pyrexia using yeast. The results showed an increase in rectal temperature after 19 hours of yeast suspension (10 ml/kg body wt.) subcutaneous injection. The extract induced a significant reduction in normal body temperature at doses of 200, 300 and 400 mg/kg body wt., and the yeast elicited elevated temperature in a dose-dependent manner. The effect lasted up to 5 hours after the drug administration. The antipyretic effect shown by the methanolic extract of the plant can be compared to that of paracetamol (150 mg/kg body wt.) which is a well-known standard antipyretic agent [12].

Cytotoxic activity

A study investigated the stem-bark, leaves and seeds for the examination of cytotoxic activity and demonstrated a significant value of cytotoxicity under the brine shrimp lethality bioassay test.^[13] The Lethal concentration at 50% (LC₅₀) values of the crude methanolic extract of the stem-bark, leaves and seeds were found out to be 179.89, 25.82, 110.92 μ g/ml, respectively. A very satisfactory result was obtained from the values of the activity in the methanol fraction of leaves (22.28 μ g/ml) and from the crude methanol extract of leaves (25.82 μ g/ml).

Memory booster

In a study, the oral treatment of *C. ternatea* root extract in rats at varied doses significantly increased memory. The study analysed the alcoholic extracts of aerial parts and roots of the plant and it was reported to impair electroshock induced amnesia.^[14] The same study evaluated the acetylcholine (ACH) content of the whole brain and acetylcholinesterase activity at different regions of the rat brain (cerebral cortex, midbrain, medulla oblongata and cerebellum) and it was advocated that an increase in ACH in rat hippocampus can be the neurochemical foundation of memory improvement in rats. The maximum manifestation of *C. ternatea* extracts for the memory-enhancing and anxiolytic activity ($p < 0.001$) was at 200 and 100 mg/kg, respectively [15].

Blood platelet aggregation inhibition studies

C. ternatea was evaluated for Blood platelet aggregation activity. A study carried out in a rabbit model, showed significant inhibition of collagen and adenosine diphosphate (ADP) induced aggregation of platelets. This maybe the result of a one the major anthocyanin isolated from the petals – Ternatin D1. The study put forward the *in vitro* inhibitory effect of the same on rabbit platelets. [16].

CNS depressant activity

A tail suspension test on mice was carried out for CNS depressant activity. The extract of *C. ternatea* significantly lowered the duration of tail immobility at the doses of 100 and 400 mg/kg. This was more at a dose of 400 mg/kg of the plant extract as compared to fluoxetine (10 mg/kg), which is a well-known antidepressant. The antidepressant effect was also analysed in the ethanolic extract of the plant root and leaves and the results were found to be at the dose of 150 mg/kg and 300 mg/kg respectively [17]. The final results from the study gave a satisfactory indication that two compounds, (Z)-9, 17-octadecadienal and n-hexadecanoic acid isolated from the plant roots could be comparable with those of the Monoamine Oxidase Inhibitors (MAO-A), which can put into light a herbal remedy for the treatment and cure of psychiatric disorders [18].

Immunoregulatory effects

The studies on the oral administration of aqueous extract of *C. ternatea* on alloxan-induced diabetic rats for a period of 60 days were carried out. The aqueous extract noticeably reduced the serum glucose and cholesterol levels on diabetic rats. The total WBCs, RBCs, T-lymphocytes and B-lymphocytes were significantly up regulated in the diabetic treated rats, while monocytes and eosinophils showed a contrasting shift. Cationic cyclotides (peptides) from the plant reduced the secretion of various cytokines and chemokines (cell signalling molecules) in human monocytes at both resting and lipopolysaccharide-stimulated states. The study states the plant as a likely candidate for novel immunoregulatory curatives [19]. The antioxidant and anti-inflammatory properties of the plant may also be responsible for this immunomodulatory effect.

Neuropharmacological activity

Innumerable studies have been reported on improving cognitive behaviour from *C. ternatea* root extract [14]. In one such study, it was put forward that neonatal rats administered with 100 mg/kg of aqueous root extract have remarkably improved acetylcholine content in the hippocampus from 52.79 ± 12.36 to 68.83 ± 9.87 nmol/g tissue while for young adult's rats, the increase was from 33.9 ± 6.92 to 52.79 ± 12.36 nmol/g tissue. Also, intubation of neonatal rat pups with 50 and 100 mg/kg of the *C. ternatea* plant root extract for 30 days also showed increased percentage of response [14]. In addition, it was believed that the root extract of *Clitoria* causes perpetual changes in the brain, which can be correlated to the enhancement of learning abilities [14]. Furthermore, the aqueous root extract was also spotted to elevate the synthesis of neurotransmitters such as acetylcholine which is a good memory booster and has learning capabilities comparable to synthetic drugs such as Nefiracetam [20] and Dehydroepiandrosterone sulphate (DHEA-S) [21].

Anti-ulcer activity

Anti-ulcer activity in rats was studied on the ethanolic and chloroform leaf extract and alcoholic and aqueous extract of the whole plant. The result is justified and maybe due to the antioxidant and anti-secretory potential of the whole plant. [22-23]

Phytochemical composition

The roots, seeds, leaves and flowers of the plant are the reported parts used from ancient times. The major phytochemical constituents in *C. ternatea* fall under the major groups like flavonoids, alkaloids anthocyanins, ternatins, tannins saponins, taraxerol, and taraxerone [24]. Non- proteinaceous and proteinaceous components had also been isolated from the plant. Spectroscopic studies revealed a total of 14 different types of flavonol glycosides. [25] The compounds found in the flower are quercetin 3-(2^G-rhamnosylrutinoside), kaempferol 3-(2^G-rhamnosylrutinoside), kaempferol 3- neohesperidoside, quercetin 3- neohesperidoside, myricetin 3- neohesperidoside, kaempferol 3-rutinoside, quercetin 3-rutinoside, myricetin 3-rutinoside, kaempferol 3- glucoside, quercetin 3- glucoside, myricetin 3- glucoside, kaempferol 3-O-(2^{''}-O- α -rhamnosyl-6^{''}-0-malonyl)- β -glucoside, quercetin 3-O-(2^{''}-O- α -rhamnosyl-6^{''}-0-malonyl)- β -glucoside and myricetin 3-O-(2^{''}-O- α -rhamnosyl-6^{''}-0-malonyl)- β -glucoside, Taraxerol and taraxerone, pentacyclic triterpenoids and flavonol glycoside. The roots showed the presence of 3, 5, 4[']- trihydroxy-7-methoxyflavonol-3-O- β -d-glucopyranoside. *C. ternatea* seeds contain contain p-hydroxycinnamic acid, β -sitosterol, γ -sitosterol adenosine, flavonol-3-glycoside, ethyl- α -d-galactopyranoside, 3,5,7,4[']-tetrahydroxyflavone, 3-rhamnoglucoside, hexacosanol and an anthoxanthin glucoside in addition to protein and fatty acid. Another study reported the presence of finotin in the seeds of the plant. Finotin is a major insecticidal protein in plants [26].

The major breakthrough research on the blue *C. ternatea* flowers were carried out for the isolation of the six major acylated anthocyanins. All the acylated anthocyanins were derivatives of delphinium 3, 3', 5'-triglucoside, a prime anthocyanin cation with three beta-D-glucosyl residues attached to the 3-, 3'- and 5-hydroxy groups [27]. The structural elucidation of the largest anthocyanin isolated from the blue variety, ternatin A1, was studied²⁸, which is also one of the most stable in neutral solutions. The structure of ternatin A2²⁹, B1³⁰,

B2³¹ and D2³² were elucidated afterwards. The structural framework and function of several other ternatins like A3, B3-B4, C1-C5, D3 and preternatins such as A3 and C4 were also determined^[31, 33]. The latter study also revealed that the ternatins with lower molecular weight are more prevailing in young flowers, while the ones with higher molecular weight are more frequent in mature flowers^[33]. Since anthocyanins are groups that impart colour to a tissue, it was put forward from the study that the white variety did not possess anthocyanins in the petals. The study deduced the fact that glucosylation (the addition of glucose units) of delphinidins at 3' and 5' positions are the key to the production of *C. ternatea* flowers in varied colours^[25].

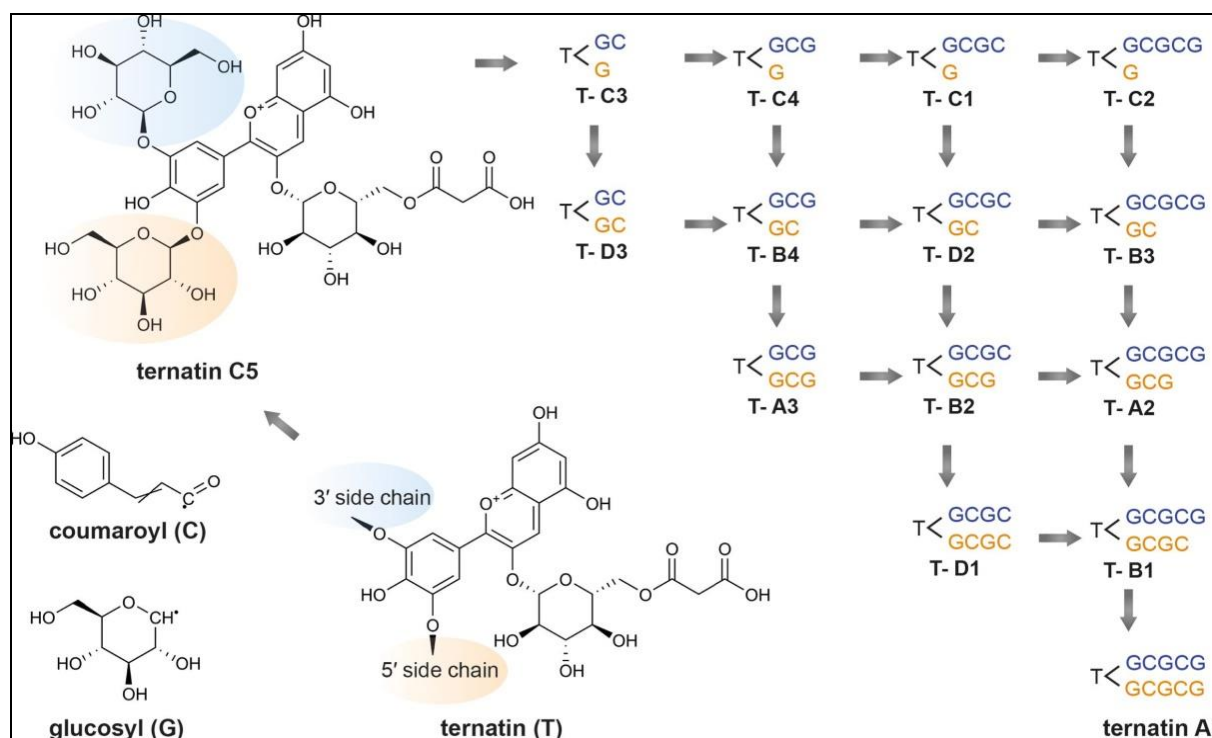


Fig 4: Suggested ternatin biosynthetic pathway. Adapted from the study of Terahara *et al.* (1998). The pathway commenced with ternatin C5 (PubChem CID 10843319). Ternatin A1 (PubChem CID 16173494) can be made through the addition of four p-coumaroyl (C) and four glucosyl moieties (G) at the 3' sidechain (given in blue) and 5' sidechain (given in orange). All the other ternatins mentioned are products from the intermediate stages.

Traditionally, phytochemical studies on the plant focused on non-proteinaceous compounds. Today as we have come a far way in sequencing of nucleotides, spectroscopy and characterisation of protein peptides as well as structural elucidation of the same, noticeably more number of proteinaceous components have been determined and classified. Cyclotides are one of the major class of plant defence peptides identified^[34]. The linear precursors of the peptide backbone undergo enzymatic transpeptidation reaction in order to form into cyclic molecules of nearly 30 amino acid residues. The most astonishing and at the same time relevant fact is that these cyclic peptides are only found in *C. ternatea*^[35], and not in any other members of the family Fabaceae. Cyclotides recently discovered in the plant are produced from genes embedded within an albumin precursor protein. The novel RNA sequencing methods are useful in identifying the sequences that code for Cyclotides in the plant and it was noticed that the albumin – 1- gene family members of *C. ternatea* are nearly 74 in number^[35]. The presence of the abundant albumin-1-gene sequences backs up the study that the presence of Cyclotide peptides increases the fitness of a plant. The knowledge of partitioning and distribution of cyclotide precursors aided by transcriptomic expression analysis of the various plant parts in certain organs specifically help in cyclotide expression on those organs, while other precursors are expressed constitutively throughout the plant.^[35] Glycine is the most abundant amino acid present in *C. ternatea*. The biophysical properties of peptides play a major role in determining the activity of the cyclotides and it is found that the properties in *C. ternatea* is different from most of the other plant members in which the cyclotides are present. For example, Centre 13 cyclotide contains eight Arginine residues that confer a predicted charge of +7 and the isoelectric point value of 10. These values are above those projected for the cyclotide peptides from *Momordica cochinchinensis* (MCoTI-I)^[36]. Since cyclotides are major insecticidal as well as nematicidal proteins, numerous works on the effect of the same on model species were carried out. In one such study, it was put forward that the cyclotide extracts from roots, in comparison to the leaves, exhibit elevated toxicity against the juvenile LI stage of the nematode *C. elegans*, whereas adults and late-stage juveniles were unaffected^[35]. The charges of the cyclotides from *C. ternatea* are comparable to other nematicidal peptides earlier discovered.^[37] Another study put forward the fact that in *C. ternatea*, Cyclotide sequences observed in aerial tissue generally have reduced charges and pi (Isoelectric point) values than cyclotides in those tissues which are in direct contact with the soil³⁸. These cyclotides isolated and studied have been shown to have a high capacity to bind to insect-mimetic plasma

membranes and thereby act as an insecticidal protein. Post translational modifications in the cyclotide precursors can also be a reason of variant properties of the peptides in the different families.

The present model of cyclotide biosynthesis in the plant commences with the signal peptide inducing the docking of the RTC (ribosome-transcript complex) with the rough endoplasmic reticulum (RER) ³⁸. The signal peptide cleavage releases the N-terminus of the cyclotide domain in the plant. One of the protein folding enzymes called PDI (protein disulphide isomerases) aids in the folding of the cyclotide domain as the propeptide is stacked within the Endoplasmic reticulum. There are only few studies on the structure and transcriptomic sequence of PDIs of the plant. A study on the activity-guided protein-fractionation identified a single Asparaginyl endopeptidases (AEP) isoform from *C. ternatea* called butelase-1 that has high capability in the intermolecular peptide cyclisation and thereby formation of plant cyclotides ^[39].

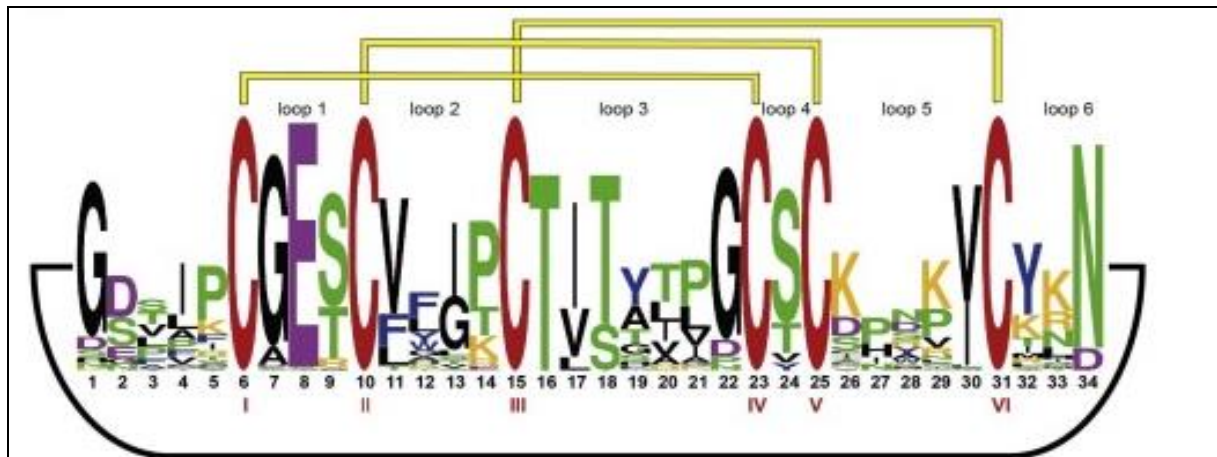


Fig 5: Amino acid sequence logo representation (Crooks *et al.*, 2004) of the 74 previously reported cyclotide sequences with the six conserved cysteine residues (shown in red, numbered I - VI) forming three disulfide bridges (shown in yellow). The cyclic backbone is represented by the black line.

Pesticidal activity

Anthelmintic studies

The anthelmintic activity of the leaf extract of the plant was determined by taking *Eisenia foetida* as the experimental model organism. The study revealed that both the aqueous and ethanolic leaf extracts showed promising anthelmintic activity at three different concentrations (25, 50 and 100 mg/ml). ^[40] The former study was carried out for the deduction of time of paralysis (P) and time of death (D) of the worms. The result showed that the anthelmintic activity of ethanolic extract of the leaf extract was more potent than the aqueous extract of the plant. In another such study, *C. elegans* was utilised as the model organism where the plant extracts were found to successfully kill nematode larvae, where the root extracts showed higher lethality than the leaf extracts ^[35]. Another similar study used *Pheritima posthuman* as the model organism; and the study showed that the ethanolic extract (50 mg/ml) caused a significantly higher mortality rate and incidence of worm paralysis when compared to a commonly used drug for controlling parasitic worms - piperazine citrate ^[40].

Insecticidal studies

Proteins and peptides isolated from the plant reportedly aid to the insecticidal properties ^[26]. A study showed that the plant extracts were able to penetrate and permeabilise the lipids on the membranes of the insects, and the greatest potency was exhibited by the shoot extracts (0.31 µg/ml LC₅₀) ^[35]

Larvicidal activity

The methanolic seed extracts of the plant was effective against larvae of the three model species with lethal concentration values 65.2, 154.5 and 54.4 ppm, respectively, for *A. stephensi*, *A. aegypti* and *C. quiquefascitus* whereas the values of chloroform extract of the leaf extract was 302.2, 517.2 and 422.2 ppm for *A. stephensi*, *A. aegypti* and *C. quiquefascitus*, respectively. The methanol and chloroform flower extracts also showed satisfactory activity against larvae of *A. stephensi* with LC₅₀ values of 254.4 and 7.48.7 ppm respectively. The studies proved that *C. ternatea* shows promising larvicidal activity ^[41].

Antimicrobial activity

The plant's potential for antimicrobial activity was analysed in the study against a group of Extended-Spectrum Beta-Lactamase (ESBL) producing model organisms viz, *Salmonella Typhimurium*, *Salmonella enteritidis*, *Klebsiella pneumonia*, Enteropathogenic *E. coli*, Uro-pathogenic *E. coli*, and *Pseudomonas aureginosa*. All of these organisms were isolated from patients suffering from urinary tract infection and acute gastroenteritis ^[42]. The activity of these microbes were analysed using the Disc diffusion method. The flower extracts of *C. ternatea* also exhibited activity against the microbes under study. Methanolic extracts of the plant exhibit high activity in comparison to chloroform and aqueous extracts. The identification of the potent protein from the plant –

“Finotin” was sufficient to put forward the vigorous inhibitory effect on the growth of fungal pathogens in a variety of plants. ^[26]

Other major areas of work

Physicochemical applications

The evaluation of different physicochemical parameters like total Ash content, Acid insoluble ash, Alcohol insoluble ash, Water-soluble extractives, soluble mineral content, crude protein content, total lipid content, crude fibre content and soluble carbohydrates in *C. ternatea* was carried out. A study estimated the total ash, insoluble ash, soluble minerals, crude protein, total lipid, crude fibre and soluble carbohydrate in various parts of *C. ternatea* (mg /100g dry weight) and the results showed that the maximum amount of total ash was found in the leaves, and the minimum was recorded in the seed. Insoluble Ash content was found to be highest in the leaves, followed by root, flowers; however, no insoluble ash content was reported in stem and seeds. Soluble mineral content was recorded highest in the stem, followed by flowers and leaves, and lowest in the seeds. Crude protein content was found to be high in seeds, whereas the roots showed the lowest amount. The total lipid content was high in the stem, followed by seeds and leaves, while the lowest was in roots. The roots showed high amounts of Crude fibre, followed by stem and seeds, whereas it was lowest in the leaves. Soluble carbohydrate varied from higher quantities in leaves, followed by roots, flowers, seeds and stem ^[43].

Also, Nickel and Lead in the flowers of *C.ternatea* was within the admissible range of less than 0.2 mg/kg as calculated by The National standard of China on Maximum Levels of Contaminants in Foods, 2005. The Proximate analysis showed that the flower contained high levels of moisture (92.40 %). The percentage of fat composition was 2.50 %, carbohydrate and crude fibre was 2.23% and 2.10 %, respectively. The percentage composition of Ash and Protein were in fewer amounts.

Genetic Improvement studies

Variability results due to the differences in either the genetic constitution of the individuals of a population or in their adaptable environments. The requisite of variability is that it is a very potent factor in determining the resistance to biotic and abiotic factors and for wider adaptability any plant species. The high phenotypic (PCV) and genotypic (GCV) coefficient of variability in the population of the cross *C.ternatea* x *C.purpurea* was analysed for the following traits like leaf length and plant height, seed weight, crude protein, crude fibre, leaf breadth, number of leaf per plant, the total number of pods per plant and the study pointed out their scope for selection and its significance in sound breeding programmes for forage improvement ^[44].

In vitro propagation studies

Studies on outshoot regeneration accompanied by callus formation in *C. ternatea* leaf explants were carried out ^[45]. A study found that abundant multiple shoot buds were induced from the young shoot tip of *C. ternatea* by the incorporation of MS medium + auxins (NAA- Naphthalene Acetic acid or IAA- Indole Acetic acid) + 6-Benzylaminopurine (BAP) (0.5mg-1).

Other Applications

The different parts of the plant like roots, seeds, leaves and flowers are reported to be used from ancient times.

Food source

From time immemorial, the plant has been used as a forage legume. Every year, the plant produces dry matter nearing around 30 tons per acre. The seeds are a high source of protein; hence the plant has high potential in increasing the nitrogen levels in run-down cultivated paddocks. The levels of crude fibre and crude protein in the leaves were estimated and are within the consumable range ^[44].

Ability to fix atmospheric nitrogen

The roots of the plant produce large round root nodules, which house nitrogen-fixing bacteria capable of fixing atmospheric nitrogen onto the tissues thereby making the plant suited for use in a crop rotation system ^[46]. Studies provide insights as to the list of legumes, when grown along with *C. ternatea*, are more efficient in inducing root nodules and thereby increasing the soil yield. In a very recent study from Thailand, researchers were able to isolate 11 rhizobial strains from the plant substantiating the fact that the plant aids in improvement of nutrients in the soil ^[47].

Ornamental value

C.ternatea is typically grown in warm climates, usually as an ornamental plant. Attractive flowers always provide an aesthetic value to any species. Though the genus *Clitoria* is widespread and produces many species, *C.ternatea* is the only species in the genus that adds to the aesthetic value by producing flowers. The colours range from creamy-white, light blue, dark blue, violet, pink and mauve. The single petal form is the typical papilionaceous flower, while the double form is non-papilionaceous with free stamens. Mauve, deep violet, dark blue coloured flowers are widely exploited for ornamental purposes ^[48].

Conclusion and Future perspectives

The butterfly pea is considered to be one of the most important medical plants studied so far. In Ayurvedic formulations, the different parts of the plant have been widely used as a brain and nerve tonic to promote memory and intelligence. The plant has been widely screened for its wide range of phytochemical and pharmacological properties. A recent study has put forward the effectiveness of cyclotides for the treatment of Alzheimer's disease as it was found that these can help in neuroprotection and prevent progressions that cause the ailment. The roots have been screened for their diuretics and laxative activities. The leaves are important in hepatopathy, and seeds are cathartic. The plant shows a very promising mosquito larvicidal activity. In general, the plant extracts have been studied for their vitalizing nootropic, anxiolytic, antidepressant, anticonvulsant, sedative, antipyretic, anti-inflammatory, analgesic, memory-enhancing activity. There is a growing need to cultivate legumes like *C. ternatea* taking into account its wide adaptability. Antioxidant properties of the plant have been widely researched on and there is adequate evidence for the potentiality of the plant for usage as natural antioxidants. Innumerable studies have been conducted to analyse the memory enhancement activity of the plant. Even so, the true prospects of the plant in clinical trials for cognitive impairment should be further studied. At present, there are numerous industries including the cosmetic and food industry that takes the aid of this plant for various purposes. Furthermore, the first *C. ternatea* based insecticide (Sero-X[®]) is also in the market and is widely used for insect control on cotton and macadamia nut crops. Biotechnological applications of the plant have also been researched on and the prominent one from the lot is the discovery of the butelase-1 enzyme from the plant since it is an important tool for peptide ligation and cyclisation. A large number of cyclotides have been tested for pesticide activity. Further work is required for the knowledge on the different factors that specifically express the Cyclotides in the plant. Likewise, the various other medical applications of cyclotides from the plant need to be investigated for aiding future studies.

Acknowledgements

The authors are grateful to Kerala State Council of Science, Technology and Environment (KSCSTE) for providing fellowship to carry out the research and to the Head, Department of Botany, University of Kerala for providing necessary facilities throughout the work.

References

1. Kulkarni R, Girish KJ, Kumar A. Nootropic Herbs (Medhya Rasayana) In Ayurveda: An Update. *Pharmacognosy Reviews*,2012;6(12):147. Doi: 10.4103/0973-7847.99949
2. Shammad J, Mathew D. Assessment Of Intraspecific Variability In *Clitoria Ternatea* L. (Fabaceae) – Morphological Traits. *Phytomorphology*,2017;67(1&2):1-7.
3. Linnaeus, C. *Critica Botanica* (In English), 1737, 45.
4. Fantz, Paul R. "Ethnobotany of *Clitoria* (Leguminosae)". *Economic Botany*. New York Botanical Garden Press,1991;45(4):511-20.
5. Fantz PR. Taxonomic Notes And New Sections Of *Clitoria* Subgenus *Bractearia* (Leguminosae). *SIDA, Contributions To Botany*,1979;8(1):90-94.
6. Akter R, Uddin SJ, Grice ID, Tiralongo E. Cytotoxic Activity Screening Of Bangladeshi Medicinal Plant Extracts. *Journal Of Natural Medicines*,2014;68(1):246-252. DOI 10.1007/S11418-013-0789-5
7. Sen Z, Zhan XK, Jing J, Yi Z, Wanqi Z. Chemosensitising Activities Of Cyclotides From *Clitoria Ternatea* In Paclitaxel-Resistant Lung Cancer Cells. *Oncology Letters*,2013;5(2):641-644. <https://doi.org/10.3892/OL.2012.1042>
8. Daisy P, Rajathi M. Hypoglycemic Effects of *Clitoria Ternatea* Linn. (Fabaceae) In Alloxan-Induced Diabetes In Rats. *Tropical Journal of Pharmaceutical Research*,2009;8(5):393-398. DOI: 10.4314/Tjpr.V8i5.48082
9. Rabeta MS, An Nabil Z. Total Phenolic Compounds And Scavenging Activity In *Clitoria Ternatea* And *Vitex Negundo* Linn. *International Food Research Journal*,2013;20(1):495-500
10. Patil AP, Patil VR. Comparative Evaluation Of In Vitro Antioxidant Activity of Root of Blue And White Flowered Varieties of *Clitoria Ternatea* Linn. *International Journal of Pharmacology*,2011;7(4):485-491. 10.3923/Ijp.2011.485.491
11. Chauhan N, Rajvaidhya S, Dubey BK. Antiasthmatic Effect Of Roots Of *Clitoria Ternatea* Linn. *International Journal Of Pharmaceutical Sciences And Research*,2012;3(4):1076.
12. Devi BP, Boominathan R, Mandal SC. Anti-Inflammatory, Analgesic And Antipyretic Properties Of *Clitoria Ternatea* Root. *Fitoterapia*,2003;74(4):345-349. [https://doi.org/10.1016/S0367-326X\(03\)00057-1](https://doi.org/10.1016/S0367-326X(03)00057-1)
13. Rahman AS, Saha R, Talukder N, Khaleque SMA, Ali HA. Bioactivity-Guided Cytotoxic Activity Of *Clitoria Ternatea* Utilising Brine Shrimp Lethality Bioassay. *Bangladesh Journal Of Physiology And Pharmacology*,2006;22(1-2):18-21. DOI: <https://doi.org/10.3329/Bjpp.V22i1.3564>
14. Rai KS, Murthy KD, Karanth KS, Nalini K, Rao MS, Srinivasan KK. *Clitoria Ternatea* Root Extract Enhances Acetylcholine Content In Rat Hippocampus. *Fitoterapia*,2002;73(7-8):685-689.
15. Malik J, Karan M, Vasisht K. Nootropic, Anxiolytic And CNS-Depressant Studies On Different Plant Sources of Shankpushpi. *Pharmaceutical Biology*,2011;49(12):1234-1242. <https://doi.org/10.3109/13880209.2011.584539>

16. Honda T, Saito N, Kusano T, Ishisone H, Funayama N, Kubota T *et al.* Isolation of Anthocyanins (Ternatin A1, A2, B1, B2, D1, And D2) From *Clitoria Ternatea* Cv.(Double Blue) Having Blood Platelet Aggregation-Inhibiting And Vascular Smooth Muscle Relaxing Activities. *Japan Kokai Tokyo Koho*, 1991, 7.
17. Parvathi M, Ravishankar K. Evaluation of Antidepressant, Motor Coordination And Locomotor Activities of Ethanolic Root Extract Of *Clitoria Ternatea*. *Journal Of Natural Remedies*,2013;13(1):19-24. <https://doi.org/10.18311/Jnr/2013/113>
18. Margret AA, Begum TN, Parthasarathy S, Suvaitenamudhan S. A Strategy To Employ *Clitoria Ternatea* As A Prospective Brain Drug Confronting Monoamine Oxidase (Mao) Against Neurodegenerative Diseases And Depression. *Natural Products And Bioprospecting*,2015;5(6):293-306. DOI 10.1007/S13659-015-0079-X
19. Nguyen GK, Qiu Y, Cao Y, Hemu X, Liu CF, Tam JP. Butelase-Mediated Cyclisation And Ligation of Peptides And Proteins. *Nature Protocols*,2016;11(10):1977-1988. DOI: 10.1038/Nprot.2016.118
20. Van Der Schyf CJ, Geldenhuys WJ, Youdim MB. Multifunctional Drugs With Different CNS Targets For Neuropsychiatric Disorders. *Journal of Neurochemistry*,2006;99(4):1033-1048. <https://doi.org/10.1111/J.1471-4159.2006.04141.X>
21. Moore H, Stuckman S, Sarter M, Bruno JP. Stimulation Of Cortical Acetylcholine Efflux By FG 7142 Measured With Repeated Microdialysis Sampling. *Synapse*,1995;21(4):324-331. <https://doi.org/10.1002/Syn.890210407>
22. Vivek D, Semwal CB, Yadav NH. Evaluation of Anti-Ulcer Activity of *Clitoria Ternatea* Leaves (Linn) Extract In Wistar Rats. *Indian Journal Of Research In Pharmacy And Biotechnology*,2014;2(3):1225.
23. Rai SS, Banik A, Singh A, Singh M. Evaluation of Anti-Ulcer Activity of Aqueous and Ethanolic Extract of Whole Plant Of *Clitoria Ternatea* In Albino Wistar Rats. *International Journal Of Pharmaceutical Sciences And Drug Research*,2015;7(1):33-39.
24. Banerjee SK, Chakravarti RN. Taraxerol From *Clitoria Ternatea* Linn. *Bulletin Of The Calcutta School Of Tropical Medicine*,1963;11:106-107.
25. Kazuma K, Noda N, Suzuki M. Malonylated Flavonol Glycosides From The Petals Of *Clitoria Ternatea*. *Phytochemistry*,2003;62(2):229-237. [https://doi.org/10.1016/S0031-9422\(02\)00486-7](https://doi.org/10.1016/S0031-9422(02)00486-7)
26. Kelemu S, Cardona C, Segura G. Antimicrobial And Insecticidal Protein Isolated From Seeds Of *Clitoria Ternatea*, A Tropical Forage Legume. *Plant Physiology And Biochemistry*,2004;42(11):867-873. <https://doi.org/10.1016/J.Plaphy.2004.10.013>
27. Saito N, Abe K, Honda T, Timberlake CF, Bridle P. Acylated Delphinidin Glucosides And Flavonols From *Clitoria Ternatea*. *Phytochemistry*,1985;24(7):1583-1586.
28. Terahara N, Saito N, Honda T, Toki K, Osajima Y. Structure Of Ternatin A1, The Largest Ternatin In The Major Blue Anthocyanins From *Clitoria Ternatea* Flowers. *Tetrahedron Letters*,1990;31(20):2921-2924. [https://doi.org/10.1016/0040-4039\(90\)80185-O](https://doi.org/10.1016/0040-4039(90)80185-O)
29. Terahara N, Saito N, Honda T, Toki K, Osajima Y. Structure Of Ternatin A2, One Of *Clitoria Ternatea* Flower Anthocyanins Having The Unsymmetrical Side Chains. *Heterocycles (Sendai)*,1990;31(10):1773-1776.
30. Kondo T, Ueda M, Goto T. Structure Of Ternatin B1, A Pentaacylated Anthocyanin Substituted On The B-Ring Asymmetrically With Two Long Chains. *Tetrahedron*,1990;46(13-14):4749-4756. [https://doi.org/10.1016/S0040-4020\(01\)85593-9](https://doi.org/10.1016/S0040-4020(01)85593-9)
31. Terahara N, Oda M, Matsui T, Osajima Y, Saito N, Toki K *et al.* Five New Anthocyanins, Ternatins A3, B4, B3, B2, And D2, From *Clitoria Ternatea* Flowers. *Journal Of Natural Products*,1996;59(2):139-144. <https://doi.org/10.1021/Np960050a>
32. Terahara N, Saito N, Honda T, Toki K, Osajima Y. Acylated Anthocyanins Of *Clitoria Ternatea* Flowers And Their Acyl Moieties. *Phytochemistry*,1990;29(3):949-953. [https://doi.org/10.1016/0031-9422\(90\)80053-J](https://doi.org/10.1016/0031-9422(90)80053-J)
33. Terahara N, Toki K, Saito N, Honda T, Matsui T, Osajima Y. Eight New Anthocyanins, Ternatins C1– C5 And D3 And Preternatins A3 And C4 From Young *Clitoria Ternatea* Flowers. *Journal Of Natural Products*,1998;61(11):1361-1367. <https://doi.org/10.1021/Np980160c>
34. Nguyen GKT, Zhang S, Nguyen NTK, Nguyen PQT, Chiu MS, Hardjojo A *et al.* Discovery And Characterisation Of Novel Cyclotides Originated From Chimeric Precursors Consisting Of Albumin-1 Chain A And Cyclotide Domains In The Fabaceae Family. *Journal Of Biological Chemistry*,2011;286(27):24275-24287. DOI:<https://doi.org/10.1074/Jbc.M111.229922>
35. Gilding EK, Jackson MA, Poth AG, Henriques ST, Prentis PJ, Mahatmanto T *et al.* Gene Coevolution And Regulation Lock Cyclic Plant Defence Peptides To Their Targets. *New Phytologist*,2016;210(2):717-730. <https://doi.org/10.1111/Nph.13789>
36. Felizmenio-Quimio ME, Daly NL, Craik DJ. Circular Proteins In Plants: SOLUTION STRUCTURE OF A NOVEL MACROCYCLIC TRYPSIN INHIBITOR FROM *MOMORDICA COCHINCHINENSIS** 210. *Journal of Biological Chemistry*,2001;276(25):22875-22882. DOI: <https://doi.org/10.1074/Jbc.M101666200>
37. Liu Rui. "Two Antimicrobial And Nematicidal Peptides Derived From Sequences Encoded *Picea Sitchensis*." *Journal Of Peptide Science*,2011;17(9):627-631. <https://doi.org/10.1105/Tpc.112.099085>

38. F Conlan B, A Anderson M. Circular Micro-Proteins And Mechanisms Of Cyclization. *Current Pharmaceutical Design*,2011;17(38):4318-4328. DOI: <https://doi.org/10.2174/138161211798999410>
39. Nguyen GK, Wang S, Qiu Y, Hemu X, Lian Y, Tam JP. Butelase 1 Is An ASX-Specific Ligase Enabling Peptide Macrocyclisation And Synthesis. *Nature Chemical Biology*,2014;10(9):732-738. Doi: 10.1038/Nchembio.1586
40. Salhan M, Kumar B, Tiwari P, Sharma P, Sandhar HK, Gautam M. Comparative Anthelmintic Activity Of Aqueous And Ethanolic Leaf Extracts of *Clitoria Ternatea*. *Int J Drug Dev Res*,2011;3(1):62-9.
41. Mathew N, Anitha MG, Bala TSL, Sivakumar SM, Narmadha R, Kalyanasundaram M. Larvicidal Activity of *Saraca Indica*, *Nyctanthes Arbor-Tristis*, And *Clitoria Ternatea* Extracts Against Three Mosquito Vector Species. *Parasitology Research*,2009;104(5):1017-1025. DOI 10.1007/S00436-008-1284-X
42. Uma B, Prabhakar K, Rajendran S, Lakshmi SY. Antimicrobial Activities Of Some Medicinal Plants Against Extended Spectrum Beta Lactamase Producing Gram Negative Enteric Bacterial Pathogens. *Pharmacology Online*,2009;1:389-392.
43. Deka M, Medhi AK, Kalita JC, Sarma KK, Deka L. Proximate Analysis Of Primary Metabolites In Different Parts Of *Clitoria Ternatea* L. A Comparative Study. *International Archive of Applied Sciences And Technology*,2013;4(3):62-67.
44. Kalamani A, Gomez SM. Genetic Variability In *Clitoria* Spp. *Annals Of Agricultural Research*, 2001;22(2):243-245.
45. Malabadi RB, Nataraja K. Shoot Regeneration In Leaf Explants Of *Clitoria Ternatea* L. Cultured In Vitro. *Phytomorphology*,2001;51:169-171.
46. Cobley LS. An Introduction To The Botany Of Tropical Crops, Longman,1976:2:387.
47. Duangkhet M, Chikoti Y, Thepsukhon A, Thapanapongworakul P, Chungopast S, Tajima S, Nomura M. Isolation And Characterisation Of Rhizobia From Nodules Of *Clitoria Ternatea* In Thailand. *Plant Biotechnology*,2018;35(2):123-129. <https://doi.org/10.5511/Plantbiotechnology.18.0402a>
48. Singh T, Ramakrishnan S, Mahanta SK, Tyagi VC, Roy AK. Tropical Forage Legumes In India: Status And Scope For Sustaining Livestock Production. *Forage Groups*,2018;8:123-136. <https://dx.doi.org/10.5772/Intechopen.81186>.