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Review

The Ayurvedic medicine *Clitoria ternatea*—From traditional use to scientific assessment

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ABSTRACT

Clitoria ternatea L. (CT) (Family: Fabaceae) commonly known as 'Butterfly pea', a traditional Ayurvedic medicine, has been used for centuries as a memory enhancer, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative agent. A wide range of secondary metabolites including triterpenoids, flavonol glycosides, anthocyanins and steroids has been isolated from *Clitoria ternatea* Linn. Its extracts possess a wide range of pharmacological activities including antimicrobial, antipyretic, anti-inflammatory, analgesic, diuretic, local anesthetic, antidiabetic, insecticidal, blood platelet aggregation-inhibiting and for use as a vascular smooth muscle relaxing properties. This plant has a long use in traditional Ayurvedic medicine for several diseases and the scientific studies has reconfirmed those with modern relevance. This review is an effort to explore the chemical constituents, pharmacological and toxicity studies of CT, which have long been in clinical use in Ayurvedic system of medicine along with a critical appraisal of its future ethnopharmacological potential in view of many recent findings of importance on this well known plant species.

Contents

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1. Introduction

Clitoria ternatea L. (CT), Fabaceae is a very well known Ayurvedic medicine used for different ailments, which has been investigated scientifically in considerable detail. CT is commonly called butterfly pea or conch flower or shankapushpi and in Indian traditional medicine is known as Aparajit (Hindi), Aparajita (Bengali), and Kakkattan (Tamil). It seems to be a native of the Caribbean, Central America and México and early after the *conquista* was distributed to the Indian subcontinent (Dan Austin, pers. comm., January 2008) .In the traditional (Asian) Indian systems of medicine particularly in Ayurveda, the roots, seeds and leaves of CT have long been widely used as a brain tonic and is believed to promote memory and intelligence (Mukherjee et al., 2007a).

The disease preventive and health promoting approach of 'Ayurveda', takes into consideration of the whole body, mind and spirit while dealing with the maintenance of health, promotion of health and treating ailments is holistic way and finds increasing acceptability in many regions of the world (Mukherjee et al., 2007b). 'Medhya rasayana' is one of the major aspects of Ayurvedic pharmacology which ascribed intellect promoting activities of medicinal plants (Mukherjee, 2002; Govindarajan et al., 2005). Several aspects on integrated approaches of drug development from Ayurveda have explored many potential lead components from herbs (Mukherjee and Wahile, 2006). There are several reported Ayurvedic 'medha' drugs which include *Clitoria ternatea*, *Celastrus panniculatus*, *Acorus calamus*, *Centella asiatica*, and *Withania somnifera* (Sivaranjan and Balachandran, 1994).

With the advancement of Ayurvedic tradition and its scientific exploration, several classes of plant species have been studied in order to evaluate their therapeutic potentials and to isolate the lead compounds. *Clitoria ternatea* has witnessed a pharmacological and toxicological evaluation of these claims pointing to some important therapeutic benefits of this traditional drug which are highlighted in this review. CT has been used as an ingredient in 'Medhya Rasayana' a rejuvenating recipe used for treatment of neurological disorders and considered to strengthen a person's intellect (Sharma and Bhagwan, 1988). CT is a potential candidate for enhancing learning and memory (Taranalli and Cheeramkuczhi, 2000; Rai et al., 2001, 2002, 2005). In traditional systems of medicine transmitted orally or in writing (esp. Ayurveda) various therapeutic effects have been attributed to roots, leaves and seeds of CT. A number of bioactive secondary metabolites and pharmacological activities of the plant have been reported. Hence, this review is a critical assessment of the currently available information on ethnobotanical and ethnomedical uses, pharmacognosy, and medicinal uses as recorded in traditional systems of medicine transmitted orally or in writing. It also reviews secondary metabolites, pharmacological and toxicological studies of this useful plant.

2. Botany and pharmacognosy of *Clitoria ternatea*

It is an ornamental perennial climber, up to 2–3 m in height, growing wild and also in gardens, bearing conspicuous blue or white flowers (Fig. 1) resembling a conch-shell. While presumably of American origin, today it is cultivated and naturalized throughout the humid tropics of the old and new world below 1600 m elevation (Morton, 1981). It is distributed in India, The Philippines, other tropical Asian countries, South and Central America, the Caribbean and Madagascar (Anonymous, 1988; Sivaranjan and Balachandran, 1994). Within the Americas, this species ranges from Florida to Texas, and from New Jersey to Kentucky and Arkansas, it is widely distributed in Mexico (Sonora and Tamaulipas south), the Bahamas, Cuba, Dominican Republic, Haiti, Jamaica, Puerto Rico, Turks and Caicos Islands, several Virgin and Leeward Islands, and in South America to Paraguay and Argentina (Austin, 2004).

The scientific name of the genus is derived from Greek kentron, a spur, prickle, sharp point, the center, and sema, a signal, referring to the spurred standard petal (Austin, 2004). Later, there was a debate as to whether or not it should be included in *Centrosema* or *Bradburya*. Ranges of several species of Clitoria are similar to some in Centrosema. One of the distinctive traits of both *Centrosema* and *Clitoria* are flowers rotated 180° and with the banner points downward. It species name is named for the island of Ternata in Moluccaa archipelago.

The root system of CT consists of a fairly stout taproot with few branches and many slender lateral roots. The thick horizontal root, which may grow to more than 2 m long, bears one to several purplish, glaucous, wiry stems. The plant possesses imparipinnate leaves consisting of five to seven leaflets, 6–13 cm long. The leaflets

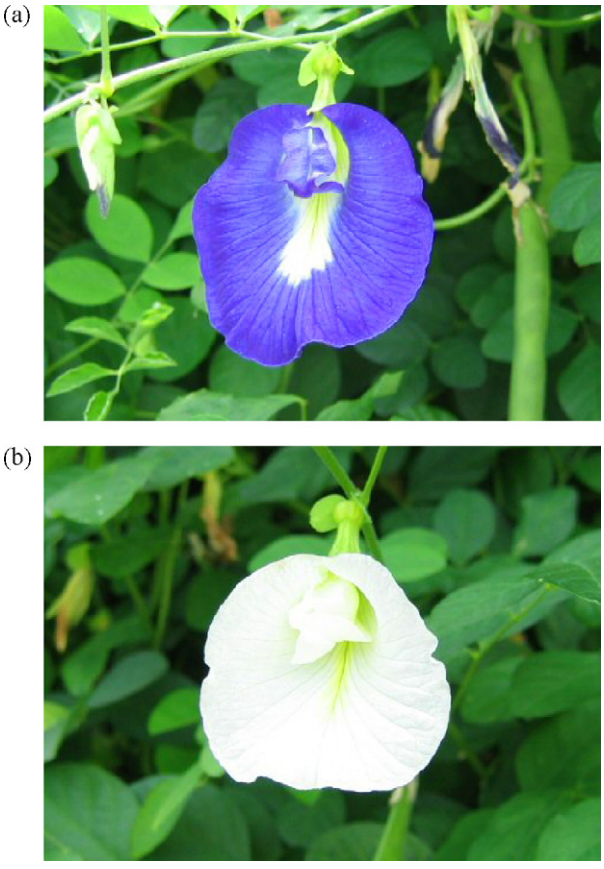


Fig. 1. *Clitoria ternatea* L.

are ovate or oblong, 2–5 cm long and subcoriaceous, rubiaceous stomata with wavy cell walls are present on both upper and lower epidermis of the leaflets. Multicellular trichomes, with two basal cells smaller than the terminal cells, are present. In transverse section leaf shows a dorsiventral structure. The lower epidermal cells of midrib region are papillose whereas those of lamina are not so noticeably papillose. All along the veins prismatic crystals of calcium oxalate are present. The vein islet number is 7.5 and palisade ratio is 6.0. The plant bears solitary, axillary, papilionaceous flowers, white or bright blue in colour with yellow or orange center. The pods are 5–10 cm long, flat, nearly straight, sharply beaked and 6–11 seeded (Karandikar and Satakopan, 1959; Pillai, 1976).

The seeds are yellowish-brown or blackish in color and subglobose or oval in shape. Root system consists of a fairly stout taproot with few branches and many slender lateral roots. The root is woody, cream white with a few lenticels united to form transverse cracks. The fresh root is slightly bitter and acrid in taste. The transverse section shows outermost phloem composed of 12–25 rows of thin walled longitudinally elongated cells some of which are compressed and a few exfoliating. Phellogen is single layered and phelloderm is two to three layered, some cells contain rhomboidal crystals of calcium oxalate. Cortex is composed of 10–12 layers of thin-walled almost polygonal or tangentially elongated cells, packed with mostly compound starch grains (Shah and Bole, 1961). Some of the cortical cells contain rhomboidal crystals of calcium oxalate. Central core consists of vascular elements. Phloem appears as conical strands separated by narrow medullary rays. Phloem fibers in groups of two to eight or a few solitary fibers are present. Some of the phloem parenchyma cells contain starch grains and a few others contain calcium oxalate crystals. Woody elements form the major and central part of the root consisting of vessels, wood parenchyma, wood fibers and mostly uniseriate to triseriate medullary rays. Three rays which start from the center is wider and four to five seriate. All the ray cells are fully packed with starch grains and few contain calcium oxalate crystals (Anonymous, 2001; Kalamani and Michael, 2001, 2003). Information on possible adulterants or species which may substitute CT are not available.

3. Medicinal uses in Asian Indian medicine

The drug 'Sankhapushpi' of Ayurveda consists of the roots and seeds of CT and is used as a 'tonic of the nerves', alterative and laxative. The leaves and roots are used in the treatment of a number of ailments including body aches, especially infections, urinogenital disorders, and as an anthelmentic and antidote to animal stings. Among the two varieties, the white-flowered one is found to be therapeutically more active, and hence preferred. The blue-flowered variety is generally used as a substitute for the white-flowered one. The roots have an acrid and bitter taste and are credited with purgative, laxative and diuretic properties. The root is used in the treatment of various diseases, like indigestion, constipation, fever, arthritis and eye ailments. It is also employed in cases of ascetics, enlargement of the abdominal viscera, sore throat and skin diseases (Anonymous, 1995). They are also demulcent and given in chronic bronchitis. Though they are purgative, they cause griping and tenderness, and hence are not recommended (Nadkarni, 1976). They are, however, administered with honey and ghee as a general tonic to children for improving mental faculties, muscular strength and complexion tonics and in epilepsy and insanity (Anonymous, 1976). The root-juice of the white-flowered variety is blown up the nostrils as a remedy for hemicrania. The decoction or powder of root is given in rheumatism, and ear-diseases. Powdered seeds are mixed with ginger and given as laxative, the action, however, is accompanied by griping in lower abdomen. The seeds are considered for colic, dropsy and enlargement of abdominal viscera; they are also used in swollen joints (Morris, 1999; Anonymous, 2001). The root, stem and flower are recommended for the treatment of snakebite and scorpion sting in India (Kirtikar and Basu, 1935).

4. Medical uses in the Americas

There are more than 50 species of *Clitoria*. *Clitoria* spp. potentially could be an economically important genus, but many species are known only locally. The mostly frequently reported species is *Clitoria terneata* (Fantz, 1991). In Cuba decoction of roots alone or roots and flowers are considered emmenagogue. This mixture is made by placing a handful of cleaned and macerated roots in a bottle of water. A glass taken in the evening is said to promote menstruation and induce uterine contractions and to aid in *el flujo loquial*. A stronger dose of the same liquid is used as a vaginal douche. An infusion of the flowers is used against the same problems. Combining a handful each of flowers and roots in a bottle of good wine one makes a *medicamento magnífico* (magnificent medicine) that is taken in one cup a day to treat clorosis (a malady of adolescents involving "impoverishment of the blood," probably anemia) and against liver and intestinal problems. Seeds are said to be laxative, vermifugal, and slightly emetic. Fantz (1991) reported economic uses for 23 species of *Clitoria* (incl. a systematic assessment of 8000 voucher specimens), e.g. the seeds as antihelminthic (Crevost and Petelot, 1929); diuretic and antidote in poison, refrigerant (Duke, 1986) as well as numerous uses in reproductive medicine (based on the principle of simile). The root and the root bark have a variety of uses (cf. section 7 on pharmacological activities). In general terms, surprisingly little information is available on local and traditional uses of this species in the Americas (e.g. very limited information is available in Morton, 1981, it is not included, for example, in Martinez, 1969; García Barriga, 1992; Argueta, 1994; Aguilar et al., 1994), and there are doubts about the differentiation between CT and *Centrosema virginianum* (L.) Benth., in the local ethnopharmacopeias (Fantz and Predeep, 1992; Fantz, 1996, other species in the Caribbean and Mexico are reported to have the same medical uses, Austin, 2004). However, these authors did not mention any usage in the Amazon (Schultes and Raffaut, 1990).

Overall and especially based on the uses in *Asian Indian* medicine, an ethnopharmacological evaluation of CT needs to focus on potential CNS-related, anti-inflammatory and antimicrobial activities.

5. Other uses

Clitorea terneta is a highly palatable forage legume generally preferred by livestock over other legumes. It exhibits excellent regrowth after cutting or grazing within short period and produce high yields. It can be grown with all tall grasses for rotational grazing, hay or silage. Butterfly pea is also used as a cover crop and green manure. Due to its attractive flower colours it is also grown as an ornamental plant (Michael and Kalamani, 2003). The young shoots, leaves, flowers and tender pods are eaten as vegetable in Kerala (India) and in the Philippines. In Malaysia, the leaves are employed to impart a green color to food and the flowers to impart a bright blue color to rice cakes. The climber yields a useful green fodder throughout the year, particularly during dry period and also dry feed (Nadkarni, 1976; Anonymous, 1976, 2001). It is grown either alone or with other perennial grasses in Punjab, Rajasthan, Uttar Pradesh, Gujarat, Maharashtra, Madhya Pradesh, Andhra Pradesh, Tamilnadu and Karnataka in India.

Besides suppressing many perennial weeds, it enriches the soil by fixing Nitrogen. It is also used as drought-resistant pasture in arid and semi-arid regions (Kirtikar and Basu, 1935; Anonymous, 1950; Manandhar, 2002).

6. Primary and secondary metabolites

6.1. Roots

The roots form nodules, which contain higher amount of plant growth substance such as indole acetic acid, kinetin and gibberelic acid. The level of tryptophan, precursor of indole acetic acid was also higher in the nodules. *Rhizobium* spp. isolated from root nodules produced higher amount of indole acetic acid in culture when supplemented with tryptophan (Ahmad et al., 1984). Rajagopalan (1964) investigated the presence of free amino acids and amides in the root nodules of CT; the root nodules contains glycine, alanine, valine, leucine, α-aminobutyric acid, γ-aminobutyric acid, aspartic acid, glutamic acid, γ-methyleneglutamic acid, arginine, ornithine, and histidine and γ-aminobutyric acid.

Banerjee and Chakravarti (1963, 1964) reported the isolation and identification of pentacyclic triterpenoids, taraxerol and taraxerone from the roots (Banerjee and Chakravarti, 1963, 1964). Content of Taraxerol (1) in root of CT was determined through high performance thin layer chromatography (HPTLC) by Kumar et al. (2008). Yadava and Verma (2003) isolated antimicrobial flavonol glycoside 3,5,4′-trihydroxy-7-methoxyflavonol-3-O-β-D-xylopyranosyl-(1,3)-O-β-D-galactopyranosyl(1,6)-O-β-D-glucopyranoside from the ethyl acetate soluble fraction of the defatted seeds of CT.

6.2. Seeds

These contain a protein with amino acid sequences similar to that of insulin but with the absence of histidine, threonine, proline and cystine. The seeds yield a greenish-yellow fixed oil (Tiwari and Gupta, 1957). The fatty acid content of CT includes palmitic, stearic, oleic, linoleic, and linolenic acids (Vianni and Souto, 1971; Debnath et al., 1975; Joshi et al., 1981; Husain and Devi, 1998). The seeds also contain a water-soluble mucilage, delphinidin 3,3′,5′-triglucoside useful as a food dye, besides, three unidentified trypsin inhibitors (Macedo and Xavier-Filho, 1992). Other substances present in the seeds are *p*-hydroxycinnamic acid (4), flavonol-3-glycoside, ethyl-α-D-galactopyranoside, adenosine, 3,5,7,4′-tetrahydroxyflavone, 3-rhamnoglucoside, a polypeptide, hexacosanol, β-sitosterol (5), γ-sitosterol and an anthoxanthin glucoside (Sinha, 1960a,b; Kulshrestha and Khare, 1967, 1968; Gupta and Lal, 1968). The seeds also contain oligosaccharides or flutulene (Revilleza et al., 1990). Kelemu et al. (2004) isolated antimicrobial and insecticidal protein finotin from seeds of CT.

6.3. Flowers

They have a vivid blue or white color and are of relatively large size, so it is used as an ornamental around the world. In Southeast Asia, the blue flower pigment is traditionally utilized as food colorant because of the high stability. Ternatins are blue anthocyanins found in the petals of CT (Srivastava and Pande, 1977). They are acylated anthocyanins based on delphinidin (6). The six major anthocyanins ternatins A1, A2, B1, B2, D1, and D2, were isolated, and these structures were characterized as malonylated delphinidin 3,3′,5′-triglucosides (9) having 3′,5′-side chains with alternative D-glucose and *p*-coumaric acid units (Terahara et al., 1989a,b, 1990a,b,c,d; Kondo et al., 1990). Terahara et al. (1996) reported the isolation and characterization of five ternatins A3, B4, B3, B2, and D2 from CT flowers, and the structures were determined by spectroscopic methods using a combination of chemical analysis such as FABMS, ^{1}H and ^{13}C NMR spectroscopies, as delphinidin 3-malonylG having 3′-GCG-5′-GCG, 3′-GCG-5′-GC, 3′-GCGCG-5′-GC, 3′-GCGC-5′-GCG, and 3′-GCGC-5′-GC side chains, respectively, in which G is D-glucose and C is *p*-coumaric acid. Terahara et al. (1998) isolated eight anthocyanins ternatins C1, C2, C3, C4, C5, D3, preternatins A3 and C4. The structures of ternatins C1, C2, C3, C4, C5, and D3 1–6 were postulated as delphinidin 3-malonylglucoside having 3′-GCGC-5′-G, 3′-GCGCG-5′-G, 3′-GC-5′-G, 3′-GCG-5′-G, 3′-G-5′-G, and 3′-GC-5′-GC, and compounds preternatins A3 and C4 as delphinidin 3-glucoside having 3′-GCG-5′-GCG and 3′-GCG-5′-G as side chains, respectively. Ranaganayaki and Singh (1979) reported kaempferol isolation and identification from the flowers of CT and Saito et al. (1985) detected kaempferol, kaempferol 3-2^{G}-rhamnosylrutinoside, kaempferol 3-neohesperidoside, kaempferol 3-rutinoside, kaempferol 3-glucoside, quercetin, quercetin 3-2^{G}-rhamnosylrutinoside, quercetin 3-neohesperidoside, Quercetin 3-rutinoside, quercetin 3-glucoside, Myricetin 3-neohesperidoside, Myricetin 3-rutinoside and Myricetin 3-glucoside. Three flavanol glycosides, kaempferol 3-O-(2″-O-α-rhamnosyl-6″-O-malonyl)-β-glucoside, quercetin 3-O-(2″-O-α-rhamnosyl-6″-O-malonyl)-β-glucoside, and myricetin 3-O-(2″,6″-di-O-α-rhamnosyl)-β-glucoside were isolated from the petals of CT together with eleven known flavanol glycosides (Kazuma et al., 2003a, 2004). Kazuma et al. (2003b) investigated the flavonoids in the petals of several CT, with different petal colors using LCMS/MS. Delphinidin 3-O-(2″-O-α-rhamnosyl-6″-O-malonyl)-β-glucoside, delphinidin 3-O-(6″-O-malonyl)-α-glucoside, delphinidin 3-neohesperidoside and delphinidin 3-O-β-glucoside were isolated from the petals together with three known anthocyanins. All through ternatins, a group of 15 (poly) acylated delphinidin glucosides were identified in all the blue petal lines.

6.4. Leaves

These contain β-sitosterol, kaempferol-3-monoglucoside, kaempferol-3-rutinoside, kaempferol-3-neohesperiodoside, kaempferol-3-O-rhamnosyl-(1,6)-glucoside, kaempferol-3-O-rhamnosyl-(1,6)-galactoside and kaempferol-3-O-rhamlnosyl-(1,2)-O-chalmnosyl-(1,2)-O-[rhamnosyl-(1,6)]-glucoside. Lactones aparajitin and clitorin from leaves were also reported (Tiwari and Gupta, 1959; Morita et al., 1977; Ripperger, 1978). The leaves also contain an essential oil, colouring-matter and mucilage. The mucilage contains anhydrogalacatan, anhydropentosan and methylpentosan and an alkaloid (Sinha, 1960c). The structures of the major constituents of CT (Fig. 2).

7. Pharmacological activities

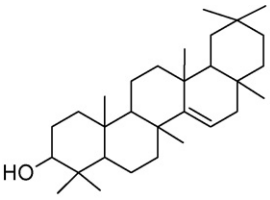
CT has been widely screened for its various pharmacological activities. It has relatively well documented neuropharmacological actions such as enhancing acetylcholine content, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative activities which justify its use in CNS diseases in Ayurvedic system of medicine (Sections 6.1–6.5). It has antimicrobial, antipyretic, anti-inflammatory, analgesic, diuretic, local anaesthetic, antidiabetic, insecticidal, blood platelet aggregation-inhibiting and vascular smooth muscle relaxant properties (Sections 6.6–6.10). The various reported pharmacological activities of CT (Table 1) highlight the therapeutic potential of CT and limitations in our knowledge as claimed in traditional Indian medicine.

7.1. Effect of CT on learning and memory

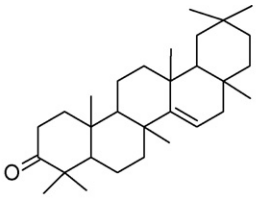
Rai et al. (2000a) evaluated the effect of CT aqueous root extract on learning and memory in rat pups (7 days old) using open field behaviour test, spontaneous alternation test, rewarded alternation test and passive avoidance test. The results of this study showed that the oral treatment of CT roots extract at different doses significantly enhanced memory in rats. In another study, Rai et al. (2001) screened CT aqueous root extract for learning and memory improvement using open field behaviour test, passive avoidance test and, spatial learning test (T-maze test) in neonatal rat pups (7 days old). Neonatal rat pups were intubated during growth spurt period at the dose of 50 and 100 mg/kg of aqueous root extract for 30 days. CT root extract had memory enhancing properties which had little or no effect on the general motor activity but showed improved retention and spatial learning performance at both time points of behavioral tests. This memory enhancing property was marked in neonatal rats (which were in their growth spurt period) treated with CT 100 mg/kg body weight for 30 days. Thus it appears that treatment with CT extract results in permanent change in the brain which were responsible for the improved learning and memory.

The alcoholic extracts of aerial parts and roots of CT at 300 and 500 mg/kg p.o. doses in rats attenuated electroshock-induced amnesia using conditional avoidance response paradigm (Taranalli and Cheeramkuczhi, 2000). At 300 mg/kg the extract produced significant memory retention, and the root parts were found to be more effective, but this doses seems to be very high. The authors also studied the possible mechanism through which CT elicits the anti-amnesic effects on central cholinergic activity by evaluating the acetylcholine content of the whole brain and acetylcholinesterase activity at different regions of the rat brain, viz., cerebral cortex, midbrain, medulla oblongata and cerebellum. The results of this study suggest that roots of CT are more effective in attenuating memory deficits as compared to aerial parts, and the mechanism by which CT produced memory retention appears to be similar to the standard drug pyritinol, since aerial parts, root parts and pyritinol have similar influence on cholinergic activity of the brain.

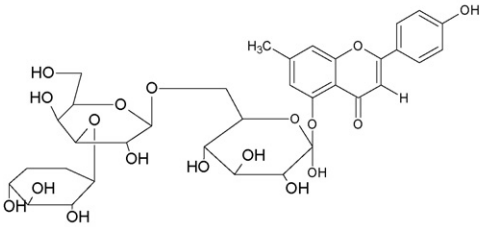
The effects of aqueous root extract of CT on the acetylcholine content of the rat hippocampus were reported (Rai et al., 2002; Mukherjee et al., 2007c). Treatment with 100 mg/kg of CT aqueous root extracts, for 30 days in neonatal period (7 days old) and young adult (60 days old) in rats, significantly increased acetylcholine (ACh) content in their hippocampi as compared to age-matched controls. Hippocampal ACh content was found to be significantly less in 90-day-old control rats as compared to 37-day-old control rats. On the contrary, hippocampal ACh content was found to be higher in 90-day-old CT treated rats than in 37-day-old CT-treated rats. *Acetylcholine* (ACh) is one of the main neurotransmitters of the central nervous system and serves to increase attention and facilitate learning. Therefore, an increase in acetylcholine content in rat hippocampus may be the neurochemical basis for improved learning and memory. CT aqueous root extract treatment may be of value for reinforcing depressed cholinergic transmission in certain age-related memory disorders and to improve learning and



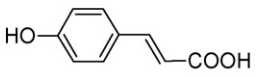
Taraxerol (1)



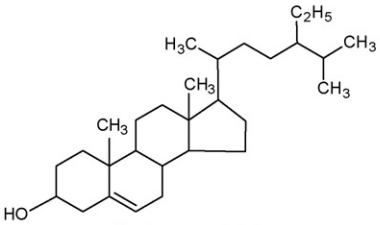
Taraxerone (2)



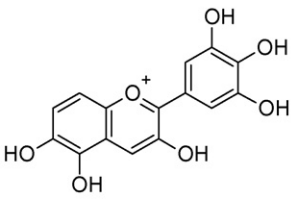
Flavonol glycoside 3,5,4'-trihydroxy-7-methoxyflavonol-3-O-β-D-xylopyranosyl-(1,3)-O-β-D-galactopyranosyl (1,6)-O-β-D-glucopyranoside (3)



p-Hydroxycinnamic acid (4)

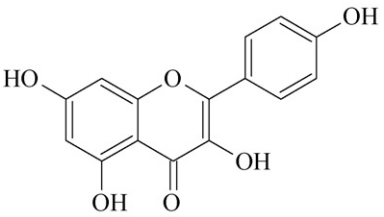


β-sitosterol (5)

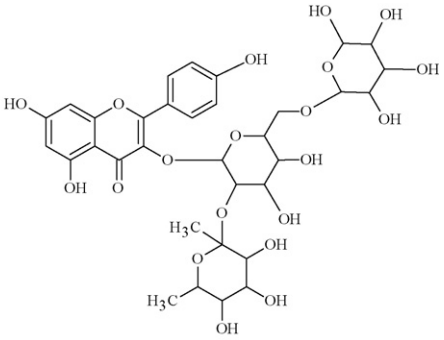


Delphinidin (6)

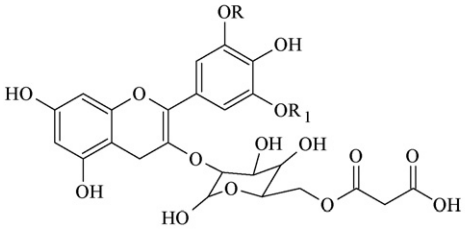
Fig. 2. Secondary metabolites of *Clitoria ternatea* L.



Kaempferol (7)



Clitorin (8)



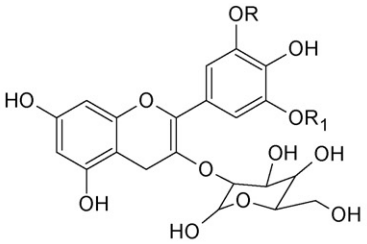
Delphinidin 3 - malonyl glucoside (9) (G is D-glucose and C is p-coumaric acid)

Ternatin	R	R^1
Ternatin A1 (10)	GCGCG	GCGCG
Ternatin A2 (11)	GCGCG	GCG
Ternatin A3 (12)	GCG	GCG
Ternatin B1 (13)	GCGCG	GCGC
Ternatin B2 (14)	GCGC	GCG
Ternatin B3 (15)	GCGCG	GC
Ternatin B4 (16)	GCG	GC
Ternatin C1 (17)	GCGC	G
Ternatin C2 (18)	GCGCG	G
Ternatin C3 (19)	GC	G
Ternatin C4 (20)	GCG	G
Ternatin C5 (21)	G	G
Ternatin D1 (22)	GCGC	GCGC
Ternatin D2 (23)	GCGC	GC
Ternatin D3 (24)	GC	GC

Fig. 2. (*Continued*)

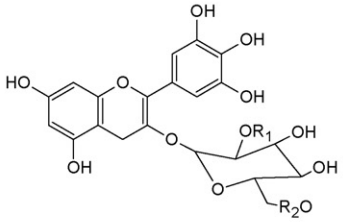
memory in normal individuals. It remains to be seen whether it has a similar action in senile animals (Ng et al., 2006). Rai et al. (2000b) reported that CT significantly improved passive avoidance memory retention. Rats orally intubated with 100 mg/kg/day of CT for 30 days during their young adult age showed increased retention observed 48 h after the learning process in the same group of young adult CT treated rats compared with age-matched saline controls, which indicates an increase in the ability to retain avoidance memories, implying possible effects of CT on amygdaloid neurons. This indicates that CT aqueous root extract treatment has a definite role in increasing dendritic arborization of amygdaloid neurons and also hippocampal CA3 neurons. In another study, Rai et al. (2005) further evaluated the effect of CT aqueous root extract on the dendritic cytoarchitecture of neurons of the amygdala. Wistar rats of either sex were orally intubated with aqueous root extract (50 and 100 mg/kg) for 30 days, along with age-matched saline controls. These rats when subjected to passive avoidance tests showed a significant increase in passive avoidance learning and retention. Subsequent to the passive avoidance tests, these rats were killed by decapitation. The amygdala was processed for Golgi staining and the stained neurons were traced using a camera lucida and analysed. The study showed a significant increase in dendritic intersections, branching points and dendritic processes arising from the soma of amygdaloid neurons in aqueous root extract treated rats compared with age-matched saline controls. The improved dendritic arborization of amygdaloid neurons correlates with the increased passive avoidance learning and memory in the aqueous root extract of CT treated rats. The results suggested that CT aqueous root extract enhances memory by increasing the functional growth of neurons of the amygdala.

CT aqueous root extract affects the brain structures concerned with learning and memory namely hippocampus and amygdala. These behavioral changes are relatively permanent indicating that CT brings about permanent changes in the brain. It may also affects the biosynthesis of neurotransmitter viz. acetylcholine, which has been implicated in the process of learning and memory. On the other hand, CT may also induce long-term potentiation (Rai et al., 2000b). CT aqueous root extract not only promotes neurogenesis in



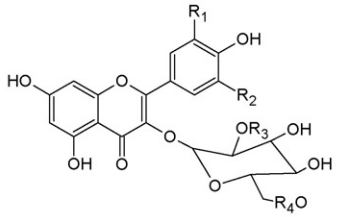
Anthocyanins (10)

Ternatin	R	R^1
Preternatin A3 (25)	GCG	GCG
Preternatin C4 (26)	GCG	G



The structures of anthocyanins

Anthocyanin	R^1	R^2
Delphinidin 3-O- (2″-O-α-rhamnosyl- 6″-O-malonyl)-β -glucoside (41)	Rhamnosyl	Malonyl
Delphinidin 3-O-(6″-O-malonyl)- α -glucoside (42)	H	Malonyl
Delphinidin 3-neohesperidoside (43)	Rhamnosyl	H
Delphinidin 3- O- β -glucoside. (44)	H	H



Flavonol glycosides

Flavonol glycoside	R_1	R_2	R_3	R_4
Kaempferol 3-O-(2″-O-α -rhamnosyl-6″–O-malonyl)-β -glucoside (27)	H	H	rhamnosyl	malonyl
Quercetin 3-O-(2″-O- α -rhamnosyl-6-O-malonyl)- β -glucoside (28)	OH	H	rhamnosyl	malonyl
Myricetin 3-2^G-rhamnosylrutinoside (29)	OH	OH	rhamnosyl	rhamnosyl
Quercetin 3-2^G-rhamnosylrutinoside (30)	OH	H	rhamnosyl	rhamnosyl
Kaempferol 3-2^G-rhamnosylrutinoside (31)	H	H	rhamnosyl	rhamnosyl
Kaempferol 3-neohesperidoside (32)	H	H	rhamnosyl	H
Quercetin 3-neohesperidoside (33)	OH	H	rhamnosyl	H
Myricetin 3- neohesperidoside(34)	OH	OH	rhamnosyl	H
Kaempferol 3-rutinoside (35)	H	H	H	rhamnosyl
Quercetin 3-rutinoside (36)	OH	H	H	rhamnosyl
Myricetin 3-rutinoside (37)	OH	OH	H	rhamnosyl
Kaempferol 3-glucoside (38)	H	H	H	H
Quercetin 3- glucoside(39)	OH	H	H	H
Myricetin 3-glucoside (40)	OH	OH	H	H

Fig. 2. (*Continued*).

Table 1
Pharmacological potentials of *Clitoria ternatea* L.

Sl. No.	Plant part	Extract/compound	Activity	References
1	Roots and aerial parts	Aqueous extract, alcoholic extract	Learning and memory enhancing, enhancement of acetylcholine content.	Rai et al. (2000a,b, 2001, 2002, 2005), Taranalli and Cheeramkuczhi (2000) and Ng et al. (2006)
2	Roots and aerial parts	Alcoholic extract, methanol extract	Nootropic activity, anxiolytic, anxiolytic activity, antidepressant activity, tranquilizing property, sedative activity, anticonvulsant activity, and antistress activity	Kulkarni et al. (1988), Boominathan et al. (2003) and Jain et al. (2003)
3	Flowers	Anthocyanins (Ternatin A1, A2, B1, B2, D1, and D2)	Blood platelet aggregation-inhibiting and vascular smooth muscle relaxing activities.	Honda et al. (1991)
4	Roots, aerial part	Methanol extract, alcoholic extract	Antipyretic activity	Kulkarni et al. (1988) and Parimaladevi et al. (2003, 2004)
5	Roots	Methanol extract	Anti-inflammatory and analgesic activity	Kulkarni et al. (1988) and Parimaladevi et al. (2003)
6	Seeds	Finotin Flavonol glycoside from ethyl acetate soluble fraction	Antimicrobial activity	Kelemu et al. (2004) Osborn et al. (1995) and Yadava and Verma (2003)
7	Flowers	Ethanol extract	Antidiabetic activity	Sharma and Majumdar (1990)
8	Seeds	Finotin	Insecticidal activity	Kelemu et al. (2004)
9	Roots	Alcoholic extract	Diuretic activity in dogs	Piala et al. (1962)
10	Aerial part	Alcoholic extract	Local anesthetic effect	Kulkarni et al. (1988)

the amygdala, but also stimulates the release of hormones or neuromodulators that modulate the activity of neurotransmitters and neuromodulators involved in learning and memory, thereby contributing to enhanced learning and memory. After detailed clinical trials in adults, CT aqueous root extract treatment may be envisaged as a memory enhancer and also might be useful in the treatment of neuronal degenerative disorders involving the amygdala (Rai et al., 2000b, 2005).

7.2. Effect of CT on general behaviour

Ethanol extract of the root of CT was evaluated for different neuropharmacological actions in rats and mice, such as general behaviour, exploratory behavior, muscle relaxant activity and phenobarbitone induced sleeping time. The extract at dose of 100 and 150 mg/kg caused reduction in spontaneous activity, decrease in exploratory behavioural pattern by the head dip and Y-maze test, reduction in the muscle relaxant activity by rotarod, 30° inclined screen and traction tests. In addition CT extract significantly potentiated the phenobarbitone-induced sleeping time (Boominathan et al., 2003). Kulkarni et al. (1988) studied the effect of alcoholic extract of aerial part of CT on special discrimination in rats. Oral treatment with alcoholic extract at a dose of 460 mg/kg significantly prolonged the time taken to traverse the maze, which was equivalent to that produced, by chlorpromazine. The lower dose 230 mg/kg was ineffective.

7.3. Effect of CT on nootropic and anxiolytic activity

The nootropic activity of extract of aerial parts of CT was evaluated by using elevated plus maze (EPM) and object recognition test (Jain et al., 2003). The animals treated with CT (100 mg/kg) showed a significantly increased inflexion ratio on the ninth day. In the object recognition test CT treated mice required significantly less time to explore the familiar object as compared with the new object and significantly reduced the discrimination index. The increase in the inflexion ratio and discrimination index by CT per se provides evidence for the species' nootropic activity. CT met a major criterion for nootropic activity, namely, improvement of memory in absence of cognitive deficit. The decrease in transfer latency by CT in the EPM is in accordance with the hypothesis of nootropic drugs. The improvement in inflexion ratio by CT on the ninth day indicated its weak effect on long-term memory. Jain et al. (2003) assessed the anxiolytic activity of a methanolic extract from aerial part of CT by using EPM and the light/dark exploration test (Jain et al., 2003). The oral administration of CT (100–400 mg/kg) dose dependently increased the time spent in the open arm; the time spent in the lit box and decreased the duration of time spent in the dark box. The oral administration of CT (30 mg/kg) failed to show any significant effect in both animal models of anxiety, a weak effect was observed in both animal models.

7.4. Antidepressant, tranquilizing and sedative activity of CT

Jain et al. (2003) evaluated the antidepressant activity of methanolic extract of aerial part of CT at the doses of 100 and 400 mg/kg using tail suspension test (Jain et al., 2003). Oral administration of CT significantly reduced the duration of immobility. CT decreased total duration of immobility and did not produce sedation and behavioral toxicity but improved cognitive abilities.

Tranquilizing property of alcoholic extract of aerial part of CT was evaluated in rats using conditioned avoidance response test. Oral treatment of alcoholic extract at the dose of 230 mg/kg did not influence conditioned avoidance response in rats whereas a very high dose of 460 mg/kg inhibited conditioned avoidance response in 66% rats with out affecting the unconditioned response. The pharmacological relevance of such a high doses remains doubtful. Similarly the standard drug chlorpromazine produced 83% inhibition of conditioned avoidance response without any effect on the unconditioned response (Kulkarni et al., 1988). They also studied the gross behavioral effect following the administration of an alcoholic extract of CT aerial part in a dose range of 1–2 g/kg. Post drug observations were made at intervals of 30 min, 3 and 6 h. The results indicate that like chlorpromazine, it possesses prominent CNS effects, characterized by tranquilizing properties such as dose-dependent inhibition of alertness, diminution of spontaneous motor activity and increased sedation. Loss of righting reflex was observed in some mice with this extracts and the animals responded well to the acoustic, tactile and nociceptive stumuli were reduced. The same extract showed inhibition of the conditioned avoidance response. No catalepsy was observed even at the highest dose of extract used. Chlorpromazine 10 mg/kg, exhibited marked

reflex and moderate catalepsy. It was reported that this extract of aerial part of CT potentiate the barbiturate induced sleeping time in rats in a dose dependent manner which was comparable to the standard drug chlorpromazine (Kulkarni et al., 1988). Due to the extremely high doses used, the relevance for these results remains very doubtful.

7.5. Anticonvulsant and antistress activity of CT

The anticonvulsant activity of a methanol extract from the aerial parts of CT was screened using PTZ- and maximum electroshock (MES)-induced seizures in mice at the dose of 100 mg/kg po (Jain et al., 2003). CT significantly delayed the onset of convulsions in PTZ-induced convulsions and also delayed the duration of tonic hind limb extension in MES-induced convulsions. These results suggest that CT may be useful in the treatment of seizures (Jain et al., 2003). Kulkarni et al. (1988) studied the anticonvulsant activity of an ethanolic extract of aerial parts of CT using maximum electroshock seizure (MES) test and, pentylenetetrazol (PTZ) test in rats. At the dose of 230 and 460 mg/kg no significant effects were observed in both tests.

The antistress activity of a methanolic extract of aerial part of CT was evaluated using cold-restraint stress-induced ulcers, lithium-induced head twitches, clonidine-induced hypothermia, sodium-nitrite-induced respiratory arrest and, haloperidol-induced catalepsy in rat and mice. The treatment with CT (100, 200, and 400 mg/kg) significantly reduced the ulcer index. CT decreased ulcer index dose-dependently and showed antistress activity. CT (100 mg/kg) significantly reduced the number of head twitches. CT reduced the head twitches significantly, and at the same dose exhibited increased IR in EPM, suggesting a link between cognitive improvements and decreased serotonergic transmission. CT (100 mg/kg po) per se was without any effect on the rectal temperature and CT did not significantly alter clonidine-induced hypothermia. CT failed to reverse clonidine-induced hypothermia, indicating that noradrenergic mechanism was not involved in the central effects of CT. The effect of CT (100 mg/kg po) was not significant in the mice treated with sodium nitrite. CT failed to decrease the effect of sodium nitrite. Oral administration of CT (100 mg/kg po) potentiates haloperidol-induced catalepsy only up to 45 min which is not significant.

7.6. Anti-inflammatory, analgesic and antipyretic activity activities of CT

A methanolic extract of CT roots was reported to have significant anti-inflammatory activity using carrageenin-induced rat paw oedema and acetic acid-induced vascular permeability models in rats (Parimaladevi et al., 2003). In the same study the extract was evaluated for analgesic activity in mice with the acetic acid-induced writhing response, where it markedly reduced the number of writhing. The analgesic activity of ethanolic extract of aerial part of CT was evaluated in mice using mechanical stimulus (tail clip method), acetic acid-induced writhing and, radiant heat method, where the oral treatment of this alcoholic extract showed significant analgesic activity from 30 min to 3 h in the radiant heat method, which was comparable to the reference drug morphine sulphate. The alcoholic extract of CT showed marginal analgesic effect in the tail clip test and no effect in acetic acid-induced writhing in mice (Kulkarni et al., 1988). They studied the effect of an ethanolic extract of aerial parts of CT on rectal temperature and yeast-induced pyrexia in rats. No dose range was provided. Oral treatment of alcoholic extract of CT aerial part produced a marked reduction in normal body temperature and also showed a significant dose-dependent antipyretic effect on yeast-induced pyrexia in rats. The effect produced by higher dose was comparable to the reference drug paracetamol. Yeast suspension (10 ml/kg) increased rectal temperature after 19 h of subcutaneous injection. The extract produced significant reduction in normal body temperature and yeast-provoked elevated temperature in a dose-dependent manner (Parimaladevi et al., 2004).

7.7. Antidiabetic activity

Sharma and Majumdar (1990) evaluated the antidiabetic activity of ethanolic extract in rats. Rats fed with ethanol extracts of flowers for 3 weeks significantly lowered serum sugar level in experimentally induced diabetes due to inhibition of the β-galactosidase and α-glucosidase activities but no inhibition of β-D-fructosidase activity was observed (Sharma and Majumdar, 1990).

7.8. Local anaesthetic effect

Kulkarni et al. (1988) studied the local anaesthetic effect of an alcoholic extract of CT aerial part using corneal anaesthesia in rabbits and, plexus anaesthesia in frogs. 10% solution of alcoholic extract of CT aerial part produced abolition of the foot withdrawal reflex in frogs but failed to produce any surface anaesthetic effect on rabbit cornea. Alcoholic extract of CT aerial part was almost as effective as xylocaine in inducing local anaesthesia.

7.9. Platelet aggregation inhibitory activity

In a study anthocyanin ternatin D1 isolated from petals of CT (double blue) was evaluated for *in vitro* platelet aggregation inhibitory activity in rabbits. Ternatin D1 showed significant inhibition of collagen- and ADP-induced aggregation of platelets (Honda et al., 1991).

7.10. Antimicrobial activity

CT has important agronomic characteristics such as adaptation to a wide range of soil conditions and resistance to drought. It is resistant to a number of pathogens and pests. A highly basic small protein was purified from seeds of CT to homogeneity by using ultrafiltration and preparative granulated bed isoelectric focusing. The protein, designated finotin, has broad and potent inhibitory effect on the growth of various important fungal pathogens of higher plants, namely *Rhizoctonia solani*, *Fusarium solani*, *Colletotrichum lindemuthianum*, *Lasiodiplodia theobromae*, *Pyricularia grisea*, *Bipolaris oryzae* and *Colletotrichum gloeosporioides*. It also inhibits the common bean bacterial blight pathogen *Xanthomonas axonopodis pv. phaseoli* (Kelemu et al., 2004). While not of immediate relevance for humans, the data point to potential antifungal effects. CT contains antifungal proteins homologous to plant defense which is another part of the ethnopharmacological relevance of this plant of being used for its antifungal properties (Osborn et al., 1995).

A flavonol glycoside isolated from the ethyl acetate soluble fraction of the roots of CT showed antimicrobial activity against various bacteria and fungi (Yadava and Verma, 2003).

8. Toxicological assessment

An ethanolic extracts of aerial parts and root of CT when administered orally to mice, in doses 1500 mg/kg and above they were found to be lethargic (Taranalli and Cheeramkuczhi, 2000). Though CT root extracts, up to 3000 mg/kg administered orally failed to

produce any lethality in mice, animals showed signs of central nervous system depression indicated by ptosis (dropping of upper eyelids), and decreased locomotor activity at doses 1500 mg/kg and above. A characteristic observation was the cathartic effect indicated by profuse watery stools in all the root extract treated mice. All mice which received the extracts in the dose 2900 mg/kg and above through the intraperitoneal route died in 6 h, due to severe CNS depression. Kulkarni et al. (1988) studied the gross behavioral and acute toxicity after administration of graded doses of alcoholic extract of CT aerial parts. Post drug observations were made at intervals 30 min, 3 and 6 h. The LD_{50} was determined approximately 30 min to 1 h after drug administration. Dose-dependent inhibition of alertness, diminution of spontaneous motor activity and increased sedation were produced by the extract in a dose range of 1–2 g/kg. At 2 g/kg, responses to acoustic, tactile and nociceptive stumuli were reduced and loss of righting reflex was observed in some mice. No catalepsy was observed even at the highest dose of extract used, while chlorpromazine 10 mg/kg, exhibited marked reflex and moderate catalepsy. LD_{50} of the extract in mice was 2290 mg/kg, ip. Acute toxicity relating to the determination of LD_{50} value was performed with different doses of the methanolic extract of CT root and observed that the extract is safe to use in mice even at the dose of 3.2 g/kg orally (Parimaladevi et al., 2004). There are various doses on which this plant has been studied, which prove this plant should be studied more systematically for its toxicity in long-term effect.

9. Conclusion

Clitoria ternatea, a traditional Ayurvedic medicinal plant has a long tradition of use as a memory enhancing and anxiolytic agent. Extracts of the roots, seeds and leaves of CT have long been in clinical use in the Ayurvedic system of medicine (Mukherjee et al., 2007a) and in other systems of medicine. Various pharmacological activities of *Clitoria ternatea* such as, memory enhancing, enhancement of acetylcholine content, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing, sedative, antimicrobial, antipyretic, anti-inflammatory, analgesic, diuretic, local anaesthetic, antidiabetic, insecticidal, blood platelet aggregation-inhibiting and vascular smooth muscle relaxing properties have been reported. Various secondary metabolites like flavanoids, anthocyanin glycosides, pentacyclic triterpenoids, and phytosterols have been reported from this plant (Mukherjee et al., 2007c). It can be used as a lead for developing new phytoceuticals for the treatment of CNS disorders and may be used effectively as a memory enhancer. So far, no highly active curative treatments have been identified for this indication (Heinrich and Teoh, 2004). Consequently, the development of a novel phytomedicines (Heinrich, 2008) seems to be a more attractive long-term strategic goal. Though the reported evidences supports the safety and efficacy of CT, but the quality of the evidence is limited in respect to its bioactive secondary metabolites, bioavailability, pharmacokinetics, and therapeutic importance including clinical trials, which are not known with sufficient detail. Thus *Clitoria ternatea* merits further phytochemical, pharmacological and clinical investigations for development of an effective natural remedy from Ayurvedic rasayana to provide therapeutically effective lead compounds or extracts.

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