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Clitoria ternatea (APARAJITA): A REVIEW OF THE ANTIOXIDANT, ANTIDIABETIC AND HEPATOPROTECTIVE POTENTIALS

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ABSTRACT

Clitoria ternatea commonly known as Butterfly pea is a medicinal plant belonging to the family Fabaceae. The plant is reported to be used in insect bites, skin diseases, asthma, burning sensation, ascites, inflammation, leucoderma, leprosy, hemicrania, amentia and pulmonary tuberculosis. It is commonly called "Shankpushpi" in the Sanskrit language where it is reported to be a good "Medhya" (brain tonic). The major phytoconstituents found in Clitoria ternatea are the pentacyclic triterpenoids such as taraxerol and taraxerone, ternatins, alkaloids, flavonoids, saponins, tannins, carbohydrates, proteins, resins and starch. Clitoria ternatea has been evaluated for its medicinal properties and shows promising effects as having antioxidant, antidiabetic and hepatoprotective activities.

KEY WORDS

Antidiabetic, Antioxidant, Hepatoprotective, Clitoria ternatea, Ethanol extract, Flavonoids, Methanol extract, Phenolic compounds.

INTRODUCTION

Clitoria ternatea commonly known as Butterfly pea belonging to the family Fabaceae and sub-family Papilionaceae is a perennial leguminous twiner. *Clitoria* Linn. comprises 60 species distributed mostly within the tropical belt with a few species found in temperate areas. The mostly frequently reported species is *Clitoria ternatea*. The plant is mainly used as a forage as it is highly palatable for live-stock and it is well adapted to various climates [12]. Native to the island of Ternate in the Molluca archipelago, this species is now widely grown as ornamental, fodder or medicinal plant [17]. The plant originated from tropical Asia and later was distributed widely in South and Central America, East and West Indies, China and India, where it has become naturalized [4].

Clitoria ternatea is commonly also called Clitoria, blue-pea, kordofan pea (Sudan), cunha (Brazil or pokindong (Philippines). This plant is known as Aparajit (Hindi), Aparajita (Bengali), and Kokkattan (Tamil) in Indian traditional medicine [43]. It has several synonyms in Ayurvedic scriptures like: Sanskrit names: Aparajita, Girikarnu, Asphota and Vishnukranta. English names: Butter-fly pea, Mazerion and Winged leaved Clitoria. Local names: Aparajita (Hin), Aparajita (Beng), Gorani (Guj), Gokarna (Mar) and Buzrula (Arabic).

The juice of flowers is reported to be used in insect bites and skin diseases [1]. The roots are useful in asthma, burning sensation, ascites, inflammation, leucoderma, leprosy, hemicrania, amentia, pulmonary tuberculosis, ophthalmology and reported as bitter, refrigerant, ophthalmic, laxative, diuretic, cathartic, aphrodisiac, tonic [36]. Consequently they are used in the treatment of a number of ailments including body-aches, infections, urinogenital disorders and as antihelmintic and antidote to animal stings. Seeds are cathartic and useful in visceralgia. They are considered safe for colic, dropsy and enlargement of abdominal viscera [32]. The root, stem and flower are recommended for the treatment of snakebite and scorpion sting in India [26].

PLANT DESCRIPTION

Clitoria ternatea has twining fine stems, 0.5-3 m long. The leaves are pinnate, with 5-7 elliptic to lanceolate leaflets, 3-5 cm long and shortly pubescent underneath **(Fig. 1)**. Flowers are solitary, deep blue to blue mauve; very short pedicellate and 4-5 cm long (**Fig. 2**). Pods are flat, linear, beaked, 6-12 cm long, 0.7-1.2 mm wide and slightly pubescent with upto 10 seeds. The seeds are olive, brown or black in colour, often mottled, 4.5-7 mm long and 3-4 mm wide [14].

Fig 1: A *Clitoria ternatea* twiner

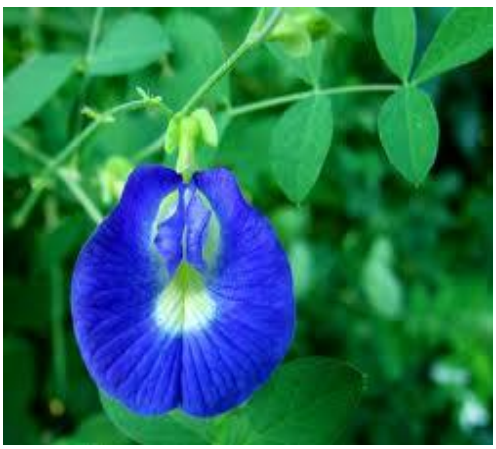

Fig 2: A *Clitoria ternatea* flower

HISTORY

From ancient times “Shankhpushpi” is known as reputed drug of Ayurveda and reported as a brain tonic, nervine tonic and laxative. It is considered as a “Medhya- Rasayana” in Ayurvedic texts. It comprises of entire herb with following botanicals viz. *Convolvulus pluricaulis* (Convolvulaceae), *Evolvulus alsinoides* (Convolvulaceae), *Clitoria ternatea* (Papilionaceae) and *Conscora decusata* (Gentianaceae). It is an Ayurvedic drug used for its action on the CNS (Central Nervous System), especially for boosting memory and improving intellect [52]. The flowers of the plant *Clitoria ternatea* resemble a conch shell; therefore it is commonly called “Shankpushpi” in the Sanskrit language where it is reported to be a good “Medhya” (brain tonic) drug and, therefore, used in the treatment of “Masasika Roga” (mental illness) [10]. Extracts of this plant have been used as an ingredient in Medhya- Rasayana, are juvenating recipe used for treatment of neurological disorders [28].

PHYTOCONSTITUENTS

Roots, seeds and leaves are the reported plant part used from ancient times. The major phytoconstituents found in *Clitoria ternatea* are

the pentacyclic triterpenoids such as taraxerol and taraxerone [5, 6]. Phytochemical screening of the roots shows the presence of ternatins, alkaloids, flavonoids, saponins, tannins, carbohydrates, proteins, resins, starch, taraxerol and taraxerone [61]. A new simple, sensitive, selective and precise High Performance Thin Layer Chromatography method has been developed for the determination of taraxerol in *Clitoria ternatea* Linn. which was being performed on Thin Layer Chromatography aluminium plates [28]. A wide range of secondary metabolites including triterpenoids, flavonol glycosides, anthocyanins and steroids has been isolated from *Clitoria ternatea* Linn. [35]. Four kaempferol glycosides I II, III and IV were isolated from the leaves of *Clitoria ternatea* Linn. Kaempferol-3- glucoside (I), kaempferol-3 - rutinoside (II) and kaempferol-3-neohesperidoside (III) were identified by Ultra Violet, Protein Magnetic Resonance and Mass Spectrometry. (IV), C33H40O19, mp: 198, was characterized as Kaempferol-3-orhamnosyl glucoside from spectral data and was named Clitorin [33].

The seeds contain nucleoprotein with its amino-acid sequence similar to insulin, delphinidin-3,3,5-triglucoside, essential amino-acids, pentosan, watersoluble mucilage, adenosine, an anthoxanthin glucoside, greenish yellow fixed oil,[21] a phenol glycoside, 3,5,7,4-tetrahydroxy-flavone-3-rhamoglycoside, an alkaloid , ethyl D-galactopyranoside, p-hydroxycinnamic acid polypeptide, a highly basic protein-finotin, a bitter acid resin, tannic acid, 6% ash and a toxic alkaloid [45]. According to Yoganarasimhan seeds contain g-sitosterol, ß-sitosterol, and hexacosanol and anthocyanin glucoside [57]. It also contains anti-fungal proteins and has been shown to be homologous to plant defensins [42]. Another study demonstrated that minor delphinidin glycosides, eight anthocyanins (ternatins C1, C2, C3, C4, C5 and D3 and preternatins A3 and C4 were isolated from the young *Clitoria ternatea* flowers [60]. Recent study showed that malonylated flavonol glycosides were isolated from the petals of *Clitoria ternatea* with different petal colors [26]. It was also reported that five new anthocyanins, ternatins A3, B3, B4, B2 and D2 were isolated from *Clitoria ternatea* flowers [60].

ANTIOXIDANT POTENTIAL OF *Clitoria ternatea*

Antioxidants act as radical scavengers, inhibit lipid peroxidation and other free radical-mediated processes, and therefore they protect the human body from several diseases attributed to the reactions of radicals. Various phenolic antioxidants such as flavonoids, tannins, coumarins, xanthenes and, more recently, procyanidins have been shown to scavenge radicals in a dose-dependent manner and therefore are viewed as promising therapeutic drugs for free radical pathologies [49]. Phenolic compounds are a large and diverse group of phytochemicals, which includes many different families of aromatic secondary metabolites in plants [15]. They are known to exert various physiological effects in humans, such as inhibiting platelet aggregation [11], reducing the risk of coronary heart disease and cancer and preventing oxidative damage of lipid and low-density lipoprotein [34, 53, 56]. Phenolic compounds have strong in vitro and in vivo antioxidant activities associated with their ability to scavenge free radicals, break radical chain reactions and chelate metals [54].

Flavonoids and other classes of phenolic Compounds are important phytochemicals [20]. Flavonoids are very effective antioxidants [65] that constitute a large group of naturally occurring plant phenolic compounds including flavones,

flavonols, isoflavones, flavonones and chalcones. Flavonoids contain a characteristic C6–C3–C6 structure, with free hydroxyl groups attached to aromatic rings, and they inhibit lipid oxidation by scavenging free radicals or by other mechanisms such as singlet oxygen quenching, metal chelation, and lipoxygenase inhibition [65]. Many plant phenolics exhibiting antioxidant properties have been studied and proposed for protection against oxidation [41, 62]. Natural antioxidants occur in all parts of the plant (wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen, and seeds) [46]. Flower is an important part of plant which contains a great variety of natural antioxidants, such as phenolic acids, flavonoids, anthocyanin and many other phenolic compounds [24, 68].

Medicinal plants are considered as potential sources of antioxidant compounds. There is an increasing interest in the investigation of naturally occurring antioxidants from plants [39]. One of the plants that deserve attention is *Clitoria ternatea.* The ethanolic extract of *Clitoria ternatea Linn.* was evaluated for its in vitro antioxidant activities [43] by DPPH free radical method. DPPH (Diphenyl picryl hydrazine) is a free radical at room temperature which produces violet colour in ethanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured solution. Ascorbic acid was used as the standard drug for the determination of the antioxidant activity and the EC50 value of ascorbic acid was found to be 6.1 µg/ml. An increased EC50 value was observed (36.5µg/ml) for the plant extract when compared with standard drug ascorbic acid (6.1µg/ml). The extract exhibited potent antioxidant activity with an EC50 of 36.5µg/ml.

The antioxidant properties of *Clitoria ternatea* has also being assayed by using the free radical scavengers Feric reducing power assay (FRAP), super oxide dismutase (SOD), Di phenyl picryl hydrazyl (DPPH) and total poly phenols [29]. The study showed that methanolic extract showed good antioxidant activity than hexane and chloroform extracts.

The antioxidant activities of the ethanol extract of *Clitoria ternatea* on acetaminophen (APAP) induced toxicity in rats suggest that the ethanol extract of *Clitoria ternatea* can prevent renal damage from APAP (Acetaminophen) induced nephrotoxicity in rats and it is likely to be mediated through active phytoconstituents and its antioxidant activities [50]. Acetaminophen (APAP) is a widely used analgesic and antipyretic drug that is safely employed for a wide range of treatments [66]. Phytoconstituents like 1 Cycloprop[e]azulene,1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-,[1aR-(1aa,7a,7aa,7ba)] [Synonyms: Varidiflorene], Pterocarpin, 6H-Benzofuro[3,2-c][1]benzopyran, 6a,11a-dihydro-3,9-dimethoxy-, (6aR-cis)- [Synonyms: Homopterocarpin], Isoparvifuran, Hexadecanoic acid, ethyl ester, Myo-Inositol, Propane, 1,1-diethoxy- were identified from ethanol extract of *Clitoria ternatea* by using a gas chromatograph-mass spectrograph (GC MS). The antioxidant studies revealed that the levels of renal SOD (superoxide dismutase), CAT (catalase), GSH (reduced glutathione) and GPx (glutathione peroxidase) in the APAP treated animals increased significantly along with a reduced MDA (malondialdehyde) content in ethanol extract of *Clitoria ternatea* treated groups [50].

The white flowered leaves had higher content of all the enzymic antioxidants analyzed than the blue flower [22]. The enzymatic antioxidant activity of *Clitoria ternatea* was analyzed by using goat liver slices, in both blue flowered leaf

and white flowered leaf of *Clitoria ternatea* and H_2O_2 was used as oxidant.

The total phenolic compounds (TPC) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity in the flowers and leaves of *Clitoria ternatea* has been analysed [47] and the presence of antioxidant activity in both leaves and flowers showed that *Clitoria ternatea* have the potential to be an alternative source of natural antioxidants. It is concluded that scavenging activity expressed by *Clitoria ternatea* flower is affected by the amount of total phenolic compound.

Phytochemical analysis has revealed that the stem contains phytosterols, phenolic compound, flavonoids and carbohydrates [18]. Various *in vitro* models were applied to evaluate anti oxidant property of these extracts. *In vitro* studies included Free Radical Scavenging Capacity (RSC) on DPPH Radicals, Scavenging capacity for hydroxyl radicals, (by measuring the degradation of 2 - deoxyribose with OH radicals generated in Fenton reaction), scavenging capacity for super oxide radicals (NBT reduction assay, Nitro blue Tetrazolium assay) and Antioxidant using β - Carotene linoleate model system (β-CLAMS). The phytoconstituents responsible for antioxidant activity were isolated by preparative TLC method. The methanolic extract showed the maximum free radical scavenging capacity as compared to acetone extract.

Comparative evaluation of *in vitro* antioxidant activity of root of blue and white flowered varieties of *Clitoria ternatea* [44] showed that methanol extracts of blue and white flowered varieties of *Clitoria ternatea* showed a very powerful antioxidant activity in DPPH radical-scavenging assay. Methanol extracts of *Clitoria ternatea* also showed significant reductive ability as well as hydroxyl radical scavenging activity. Methanol extract of white flowered variety of *Clitoria ternatea* showed more significant antioxidant activity as compared to blue flowered variety of *Clitoria ternatea*.

The phenolic compounds and antioxidant capacities of free and bound phenolics from 12 available Thai edible flowers which have long been consumed as vegetable and used as ingredients in cooking, has been investigated [23], *Clitoria ternatea* was one of them. Major phenolic acids identified in these analyses were gallic acid, ferulic acid and sinapic acid, while predominant flavonoids were quercetin and rutin. The soluble as well as bound fractions of edible flowers are rich sources of phenolic compounds with antioxidant, DPPH radical-scavenging activity and reducing power. DPPH radical scavenging capacity of bound phenolic fraction was found to be 17.6% in *Clitoria ternatea, this suggests that screening* edible flowers as potential sources of bioactive components with high antioxidant properties may be of interest to consumers and public health workers.

A polyherbal formulation (Rheumatone) made using five medicinal plants namely *Clitoria ternatea, Sida cordifolia, Cleodendron serratum, Bacopa monnieri, Cardiospermum Halicacabum*, does not exhibit any side effects and it has the enzymatic antioxidant activity [55]. There was a significant reduction in the levels of Super oxide dismutase (SOD), Catalase, Peroxidase and Glutathione peroxidase (GPx) in the liver and kidney of adjuvant induced arthritic rats and there was an elevated level of Super oxide dismutase, Catalase, Peroxidase noted in the Liver and Kidney of rats that were treated with the polyherbal formulation Rheumatone compared with the toxic rats. There was a

signiciant increase of GPx in Liver and Kidney of rats treated with Rheumatone compared with Group II rats treated with Freund's Complete Adjuvant (FCA).

ANTIDIABETIC POTENTIAL OF *Clitoria ternatea*

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action [19]. Diabetes mellitus is also associated with an increased risk for developing premature atherosclerosis due to independent risk factors such as hypertriglyceridemia and hypertension [51]. Insulin therapy and oral hypoglycemic agents offer effective glycemic control; yet, their shortcomings limit their usage [2]. The world health organization has also recommended the evaluation of the effectiveness of plants in conditions where we lack safe modern drugs [64]. Phytochemicals isolated from plant sources are used for the prevention and treatment of cancer, heart disease, diabetes mellitus and high blood pressure [63]. Plants are reputed in the indigenous systems of medicine for the treatment of various diseases [3], the available literature shows that there are more than 800 plant species showing hypoglycemic activity [30] and *Clitoria ternatea* is one of them.

Chronic administration of plant extracts (100mg/kg) for 14 days reduces the blood glucose level of the diabetes induced animals (Wistar Albino rats) as compared to diabetic control group [13]. There was significant decrease in the blood glucose level in the 7th and 14th days of the diabetes induction, showing antidiabetic effect. The effect was comparable to that of standard antidiabetic drug Glibenclamide. Hyperglycemia was induced by intra peritoneal injection of freshly prepared aqueous solution of alloxan monohydrate. Extensive damage to the islets of langerhans and reduced dimensions of islets were found in control animals. Restoration of normal cellular populatation and size of islets with hyperplasia were seen in extract treated groups. The partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia were indicative of the antidiabetic potential of the plant. Aqueous extracts of *Clitoria ternatea* plant showed anti-hyperglycemic activity in streptozotocin treated rats and this effect is because of increase in glucose uptake and glycogen deposition in isolated rat hemi diaphragm.

Clitoria ternatea leaf and flower extracts exhibit antihyperglycaemic effect in rats with alloxan-induced diabetes mellitus [10]. The effect of orally administered aqueous extracts (400 mg/kg body weight) of *Clitoria ternatea* leaves and flowers on serum glucose, glycosylated hemoglobin, and insulin were examined in control and extract-treated diabetic rats. The aqueous extracts of *Clitoria ternatea* leaves and flowers significantly reduced serum glucose, glycosylated hemoglobin and the activities of gluconeogenic enzyme, glucose-6- phosphatase, but increased serum insulin, liver and skeletal muscle glycogen and the activity of the glycolytic enzyme, glucokinase. For all the biochemical tests performed, the leaf extract-treated rat showed essentially the same profile as those treated with the flower extract.

The alcoholic root extract of *Clitoria ternatea* has shown significant gross impact in preventing the possible complications related to brain hippocampal area CA3 and pancreatic tissue in juvenile diabetic rat experimental models [31]. These benefits could be due to interference of number of chemical compounds present in this extract. Encephalopathy is a major complication in juvenile diabetes mellitus which cripples the

potential physiomorphological growth and development in early childhood. It is very essential to diagnose and initiate the treatment at the earliest to prevent the possible complications. The ancient medical science Ayurveda mentions number of remedies to treat cognitive dysfunctions, the herbal root of *Clitoria ternatea* plant is one among them.

Clitoria ternatea leaf extract shows the synergetic effect along with *Trichosanthes dioica leaf extract* on the Streptozotocin-induced diabetic rats [25]. The ethanolic extracts of *Trichosanthes dioica* leaf and *Clitoria ternatea* leaf exhibited higher degree of antihyperglycaemic activity. With regard to the mechanisms, it cannot be excluded that *Trichosanthes dioica* leaf extract and *Clitoria ternatea* leaf extract may contain some biomolecules that may synthesize the insulin receptor to insulin or stimulate the beta cells of islets of Langerhans to release insulin which may finally lead to improvement of carbohydrate metabolizing enzymes towards the establishment of normal glucose levels. Significant and higher degree of antihyperglycaemic efficacy was achieved with combination (200 mg/kg of *Trichosanthes dioica* leaf + 200 mg/kg of *Clitoria ternatea* leaf) when compared to the extent of efficacy that was obtained with 400 mg/kg dose of individual plant extracts of *Trichosanthes dioica* leaf and *Clitoria ternatea* leaf.

HEPATOPROTECTIVE POTENTIAL OF *Clitoria ternatea*

Despite remarkable advances in modern medicine, hepatic disease remains a worldwide health problem, thus the search for new medicines is still ongoing. Hepatic cells participate in a variety of metabolic activities; therefore the development of liver protective agents is of paramount importance in the protection from liver damage. The literature has constantly shown that hepatoprotective effects are associated with plant extracts rich in antioxidants [16, 38, 7]. Many compounds and extracts from plants have thus been evaluated for hepatoprotective and antioxidant effects against chemically-induced liver damage [8, 67, 9]. Many studies have been done on the hepatoprotective activity of *Clitoria ternatea.*

Ethanolic extract of leaves of *Clitoria ternatea* (EECT, 200 and 400 mg/kg) was evaluated for prophylactic and therapeutic hepatoprotective activity against carbon tetrachloride induced hepatic damage [58]. Silymarin (100 mg/kg) was used as standard drug. Hepatoprotective effect of EECT was evident in prophylactic and therapeutic groups at doses of 200 and 400 mg/kg. Histopathology of liver ascertained the effect of EECT and carbon tetrachloride on cytoarchitecture of the liver. The liver section of normal control animals indicated the presence of normal hepatic parenchyma, whereas administration of carbon tetrachloride in animals showed severe centrilobular necrosis, fatty changes, vacuolization and ballooning degeneration indicating severe damage of liver cytoarchitecture. The EECT 200 mg/kg in both prophylactic and therapeutic studies showed recovery and protection from hepatocyte degeneration, centrilobular necrosis, fatty infiltration, whereas EECT 400 mg/kg showed mild to normal cytoarchitecture that indicated the dose dependent hepatoprotection of EECT. The silymarin treated animals showed slightly altered hepatic parenchyma and uniform spread sheets of hepatocytes which indicated functional liver, on account of regenerative activity. The possible prophylactic and therapeutic hepatoprotective effect of *Clitoria ternatea* leaves was attributed due to the presence of

flavonoids which contributed to its antioxidant property.

Methanolic extracts of blue and white flowered varieties of *Clitoria ternatea* have potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats [44]. Methanol extract of white flowered variety (MEWFV) effectively control SGOT (serum glutamate oxaloacetate transaminase), SGPT (serum glutamate pyruvate transaminase) and ALP (serum alkaline phosphatase) as compared to methanol extract of blue flowered variety (MEBFV). MEWFV of Clitoria *ternatea* showed more significant hepatoprotective activity as compared to MEBFV of *Clitoria ternatea* . The possible mechanism of this activity was due to free radical-scavenging and antioxidant activity, which may be due to the presence of phenolic compounds in the extracts.

The hepatoprotective effect against paracetamol-induced liver toxicity in mice of ME (Methanol Extract) of *Clitoria ternatea* leaf was studied [4] by monitoring the levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and billirubin along with histopathological analysis. The mice treated with the ME of *Clitoria ternatea* leaf (200 mg/kg) showed a significant decrease in ALT, AST, and bilirubin levels, which were all elevated in the paracetamol group; this confirmed the hepatoprotective effect of *Clitoria ternatea* leaf extract against the model hepatotoxicant paracetamol. The hepatoprotective action was likely related to its potent antioxidative activity.

A polyherbal formulation named **"**Ayush-Liv.04" consisting of *Clitoria ternatea* leaves 20% as one of its constituents was evaluated for its hepatoprotective activity against ethanol and CCl4 induced liver damage in rats [37]. The activities of liver marker enzymes in serum namely AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), ALP (Serum alkaline Phosphatase) , ACP(Serum acid Phosphatase) and serum bilirubin level (total) were increased in toxic group animals. But the activities of these enzymes were significantly lowered in post-treated group of rats. This suggests antihepatotoxicity of "Ayush-Liv.04".

CONCLUSION

The scientific research on *Clitoria ternatea* suggests a huge antioxidant, antidiabetic and hepatoprotective potential of this plant. The plant is a rich source of phytochemicals, with high levels of phenolic compounds and antioxidant activities. The study also indicates that the leaf and flower extracts of *Clitoria ternatea* have a hypoglycaemic effect. The extracts were effective in regulating the biochemical indices associated with diabetes mellitus. *Clitoria ternatea* possesses strong hepatoprotective potential.

The hepatoprotective activity of *Clitoria ternatea* leaf may be due to its free radical-scavenging and antioxidant activity, resulting from the presence of some phenolic compounds in the extracts. Further studies are in progress to better understand the mechanism of action of *Clitoria ternatea* responsible for the observed hepatoprotective and antioxidant activity. The organic and aqueous extracts of *Clitoria ternatea* could be further exploited in the future as a source of useful phytochemicals compounds for the pharmaceutical industry and the antioxidant mechanisms and the anti- Proliferative properties of the extracts should be further studied to gain more application for use as natural antioxidants.

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