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Butterfly pea: An emerging plant with applications in food and medicine

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Abstract
Dyes and Food Colorings play an important role in the Food Industry. A trend was seen at that time for using natural dyes instead of artificial dyes. A natural dye can be extracted from *Clitoria ternatea* (CT/Blue pea) flower because of its vivid blue color. The reason for this deep blue color is because of the Anthocyanin compounds contained in the flower. *Clitoria ternatea* extraction was obtained through different methods and they vary with their manufacturing process. This plant was widely used in traditional medicine because it is rich in bioactive compounds. In treating diabetics, blood pressure, retinal damage, edema, and indigestion both the aerial and underground parts of this plant are being used. Researchers proved this plant's medicinal activities such as nootropic activity, antioxidant activity, analgesic activity, anti-inflammatory and antibacterial activity. Currently, this plant's uses are widely spread in the nanotechnology field as well.

Keywords: *Clitoria ternatea*, butterfly pea, ayurveda, antioxidant, nanoparticles

Introduction
Clitoria ternatea is a plant that belongs to the family fabacean and is widely distributed in many different countries. There are 58 species of *Clitoria ternatea* spread all over the world like India, Sri Lanka, Malaysia, Philippine Islands, Australia, Indonesia, South Africa, Australia, and around countries in the Indian ocean.

Soil and Climate Parameters
It has a fibrous root system, fertile loamy soil, and well- drained soil is preferable to its growth. This plant can be grown easily, but it has a short life period and it is a tropical plant that grows under 19°-28° C temperature in a moderate water range (700-1500mm). Furthermore, this plant has the potential to survive up to 7-8 months even in drought conditions. (Oguis *et al.*, 2019) [1]. There are different names used for *Clitoria ternatea* (CT) like Asian pigeon wing which is the English name for blue pea flower and 'blue pea', 'butterfly pea', 'bluebell', 'Cordofan- Pea', and 'Chandra kanta' are also known. (Vidana Gamage *et al.*, 2021) [2].
Flowers, seeds, roots, and leaves are the edible parts of this plant that are being used in food industries, culinary uses, folk medicine & also in religious activities. In Asian countries, mostly *Clitoria ternatea* flower is blue/dark blue/purple color but white and light-yellow color flowers are also seen.

Morphological Characteristics
The blue pea flower has a length, width of nearly 4cm, 3cm respectively and there are five petals attached to the sepals and corolla of the blue pea flower which consists of two wings, two keels, and one banner that has light yellow color markings in the middle. The blue pea plant is a climbing legume with very thin leaves which have a length of around 2.5-5 cm and 1.5-3.5 cm width respectively (Mukherjee *et al.*, 2008) [12]. It is an evergreen plant with a fibrous root system and its large nodules can fix nitrogen into a usable form of plants, with the association of Rhizobium bacteria. *Clitoria ternatea* is a perennial plant that propagates by seeds that are black in color and are located in pods that are about 7-11cm long. (Suarna *et al.*, 2021). [4]
CT flower is used for culinary purposes and its extracted dye is used as a natural colorant in the food industry and its roots and leaves are used for medicinal purposes and herbal drink preparations. (Chusak *et al.*, 2019) [6] Butterfly pea powder is the most famous product in the market which is made from the blue pea flower.

Roots and seeds are mainly used in traditional medicine and young leaves can be used as side dishes.

It has health benefits like helping in digestion, improving eyesight, lowering blood pressure, and making the skin glowing. (Barik *et al.*, 2007) [7], (Oguis *et al.*, 2019) [1].

There are different types of bioactive compounds found in *Clitoria ternatea* which are Anthocyanin compounds, flavonoids, glycosides, steroids, resins, and phenols are some of them. These bioactive compounds of the CT plant help with Anti-diabetic activity, Antioxidant activity, Anti-Bacterial activity, Anti- Inflammatory activity, and Analgesic activity (Chusak *et al.*, 2019) [6], (Gupta *et al.*, 2010) [8].

Clitoria ternatea is rich in anthocyanin compounds and it is the pigment that caused the deep blue color of the CT flower. Therefore, it is used as a food colorant in the food industry. Anthocyanin is one of the most unstable food colorants found in nature and its stability depends upon temperature, pH, and other enzymatic activities. (Vidana Gamage *et al.*, 2021) [2]. Most importantly, in *Clitoria ternatea* the anthocyanin is present in the form of polyacylated anthocyanin also known as Ternatins which is one of the stable forms of anthocyanin (Thuy *et al.*, 2021) [10]. Anthocyanin can play the role of antioxidant activity and antimicrobial activity which help to protect against several health issues like cancers, diabetics, and cardiovascular diseases. (Jeyaraj *et al.*, 2021) [9].

This report is focused on the overall description of *Clitoria ternatea* plants' nutritional & bioactive components, utilization, and applications.

Nutritional Composition

This legume plant is high in minerals like Calcium (1.5-25.9 g/kg), Phosphorous (0.3-3.9 g/kg), Potassium (7.7-23.0 g/kg), Sodium (0.3-1.1g/kg), Magnesium (3.2- 6g/kg), Manganese (28-91 mg/kg), Zinc (25- 44mg/kg), Copper (6-9 mg/kg) etc. Various types of fatty acids are also present in blue pea seeds & petals like Linoleic acid, Arachidic acid, Palmitic acid & Stearic acid which help to boost up brain health. (Mukherjee *et al.*, 2008) [12]. Crude protein and fiber contents are high in *CT* and because of its fiber content, it can be used as forage (Heuzé *et al.*, 2016) [17]. The blue pea plant is loaded with Vitamin A, Vitamin C, and E. B and is also rich in polyphenols like flavonoids, phenolic acids, tannins, lignans, alkaloids, terpenoids, and coumarins. It plays an important role in free radical scavenging hence it can provide antioxidant properties mainly by leaves and flowers and anti-cancer properties. There are different types of extraction methods used to separate polyphenolic compounds of this plant and its quality depends upon the method and types of equipment used. Polyphenols found in roots are pentacyclic triterpenoids, taraxerol, and taraxerone. (Sing J *et al.*, 2012) [14]. Nowadays scientists are *researching* BP (Blue Pea) for making *different medicines*, due to the presence of many functional *compounds* in this plant. Currently, blue pea tea is available in the market, and it has been a trending beverage in Asian countries. Specially, "Starbucks" Asia launched a special edition of cold beverages using blue pea flowers. But only a few value-added products are available in the market. (Pasukamonset *et al.*, 2017) [13].

Applications

Clitoria ternatea has gained significant interest in a number of fields: traditional medicine, food and agricultural applications (Oguis *et al.*, 2019) [1].

In Ayurveda, parts of CT have been using in treating health issues. *Clitoria ternatea* flowers are used all over the world as a food colourant and as ornamental flowers. In food applications, CT flowers are used as a promising candidate because of a wide range of pharmacotherapeutic properties, safety, and effectiveness. CT is used as a nitrogen-fixing and fodder crop (Jeyaraj *et al.*, 2021) [9].

Medicine

In Ayurveda, *Clitoria ternatea* possesses various pharmacological activities, including antidiabetic, nootropic, anesthetic, antimicrobial, antipyretic, analgesic, anti-inflammatory, antidepressant, antistress, diuretic, anticonvulsant, anxiolytic, insecticidal properties (Saptarini *et al.*, 2015) [16].

Researchers have shown that CT can be used in cancer and diabetes which are considered to be some newly emerging diseases. Scientists have found in various experiments that CT is showing the hypoglycemic activity as it lowers the level of blood glucose. In a study, the blood glucose levels were significantly decreased when CT leaves extract and CT flowers extract in aqueous form (@ 400mg/kg) were given orally to alloxan-induced diabetic like conditions in rats (Daisy *et al.*, 2009) [19]. Same could be seen when CT leaves methanolic extract was treated for Streptozotocin- induced diabetic rats at single dose treatment for 15 days (Mishra *et al.*, 2015) [20]. The aqueous extract of CT leaves (@ 100 mg/kg) for alloxan-induced diabetic like conditions in rats caused a reduction in the level of blood glucose when rats were treated for 14 days (Gunjan *et al.*, 2010) [21]. The CT seeds also possess anti-diabetic properties, in a study the antidiabetic effect was shown when the CT seeds ethanolic extract was used in the rats' treatment for Streptozotocin-induced diabetes like conditions (Kalyan *et al.*, 2011) [22]. In a study conducted by Sharma and Majumdar, CT flowers ethanolic extract also decreased the blood glucose level when treated for alloxan-induced diabetic like conditions in rats (Sharma *et al.*, 1990) [23].

All over the world, the lives of many people are affected by cancer, another emerging disease like diabetes. As CT is affecting good on some cancer cell lines it can be used in the treatment of some cancers. In one study, the crude methanolic extract of seeds, stem bark, and leaves of CT was tested for cytotoxic activity. As result, anti- cancerous activity was shown in all the extracts, but compared to other CT extracts, a significant higher cytotoxic activity was shown in the leaves extract (Rahman *et al.*, 2006) [42]. In one study, CT cyclotides were found to possess the chemosensitizing and anticancer activities in paclitaxel-resistant lung cancer cells (Sen *et al.*, 2013) [40]. In a study, CT flower aqueous extract and the methanolic extract of CT leaves and CT flowers has been demonstrated for the cytotoxicity in various cancer cell lines (Akter *et al.*, 2014 & Neda *et al.*, 2013) [44, 45]. In a study conducted by Kumar *et al.*, the cytotoxic effect was also shown by ethanolic and petroleum ether extracts of CT flower in the trypan blue dye exclusion method (Kumar *et al.*, 2011) [48]. CT seed also showed the anticancer activity as it was showing a decrease in packed cell volume, tumor volume, viable count, and an increase in tumor-bearing mice's life span (Latha *et al.*, 2013) [24]. Therefore, researchers have demonstrated CT as a good anticancer drug.

CT possesses antimicrobial properties, as it works against microorganisms like bacteria, and fungi. In a study, the methanolic extract of CT flowers was found to work against *E. coli*, *P. aeruginosa*, and *K. pneumonia* (Uma *et al.*, 2009) [25]. In another study, the methanolic extract of CT flowers was

found to work against *Bacillus thuringiensis* with MIC (Minimum inhibitory concentration) values between 12.5-25 mg/ml (Kamilla *et al.,* 2014) [26]. In a study, CT leaves methanolic extract is also reported bactericidal activity in *Klebsiella pneumonia, Bacillus cereus, Salmonella typhi, Staphylococcus aureus* and *Proteus vulgaris* (Anand *et al.,* 2011) [27]. In other studies, anthocyanins of CT flower ethanol extract paste showed antibacterial activity against *K. pneumonia* and the MIC (value was between 1.6-25 mg/ml (Leong *et al.,* 2017) [28]. In another study, an isolated protein called finotin from CT seeds showed antimicrobial properties (Kelemu *et al.,* 2004) [29]. The antimicrobial activity against *A. formicans, P. aeruginosa, E. coli, B. subtilis, K. pneumonia, S. agalactiae, and A. hydrophila* was shown by the various extracts of CT leaves (Ponnusamy *et al.,* 2010) [30]. Antibacterial activity was shown by the ethanol extract of CT leaves against antibacterial *Bacillus* spp. and *Streptococcus* spp. (Shahid *et al.,* 2009) [31]. The methanolic extract of CT leaves (@0.8 mg/mL) (a minimum inhibitory concentration) showed the antifungal activity against *Aspergillus niger* (Kamilla *et al.,* 2014) [26].

In Ayurveda, CT has been used in the treatment of serious liver problems as it is showing promising hepatoprotective effects. In a study, CT leaves methanolic extract (@200 mg/kg) was used against paracetamol induced liver toxicity in mice. It showed a protective effect as it decreases the alanine aminotransferase level, bilirubin along level, aspartate aminotransferase level and improves histopathological levels (Nithianantham *et al.,* 2011) [32]. In another study, hepatoprotective activity was found to have in the extract of CT leaves in carbon tetrachloride-induced hepatotoxicity in rats. More hepatoprotective effect was shown in the extracts of white-flowered CT leaves than in the extracts of blue-flowered CT leaves (Jayachitra *et al.,* 2012) [33].

Some Antioxidant activities are also possessed by CT. In a study, antioxidant activity was shown when rats were treated with the aqueous extract of CT flowers against ketoconazole-induced testicular damage (Iamsaard *et al.,* 2014) [46]. In one study CT extracts (@400 mg/mL) were found to protect canine erythrocytes against hemolysis and oxidative damage as CT showed antioxidant activity (Phrueksanan *et al.,* 2014) [47]. The aqueous extract of CT flowers (Kamkaem *et al.,* 2009) [34], methanolic extract of CT leaves (Nithianantham *et al.,* 2011) [32], methanolic and acetone extract of CT flowers (Jain *et al.,* 2010) [35], aqueous extract of CT flower petals (Phrueksanan *et al.,* 2014) [47] also showed the antioxidant activity.

CT does possess anti-inflammatory activities. In a study, anti-inflammatory effects were found to have in the methanolic extraction of CT roots (@200 mg/kg and 400 mg/ kg) in rats as CT inhibits acetic acid induced rat paw oedema (Parimala *et al.,* 2003) [36]. Anti-inflammatory activity was also shown in the ethanolic extract of leaves and flowers of CT (Suganya *et al.,* 2014) [37]. In another study, the extract of CT roots (@200 mg/kg and 400 mg/kg) has shown inhibition of oedema at percentages of 21.6% and 31.8%, respectively.

Besides all the above-mentioned uses, in treating various diseases like infections, body aches, anthelmintic and urinogenital problems, the extracts of CT leaves and roots are used. CT leaves are also beneficial for hepatopathy and otalgia, while the seeds are cathartic. Furthermore, in India, flowers, roots, and stems are used to heal snake bites and scorpion stings. CT roots and seeds are used as a tonic and laxative for the nerves (Nithianantham *et al.,* 2011) [32].

Food

The presence of delphinidin anthocyanin contributes to the bright, deep-blue blossoms of *Clitoria ternatea*. Due to its strong anthocyanin pigment content, CT flower is used as a natural blue colorant in food and beverages all over the world. This colorant is commonly used in various quantities with a mocktail, cake, ice cream, candy, and other traditional meals and desserts. It's also recognized for the purplish-blue color of anthocyanins in its blossoms, which can be used as a natural culinary coloring. CT extract is often used as a food colorant. Because the color parameter of CT may be utilized to correlate and anticipate the extract's pH value, there is a lot of room for CT flower extract to be developed further into more inventive uses: to monitor the pH intelligent packaging from CT. The bloom of CT has been utilized as a colorant in many meals, beverages, and sweets throughout Asia, in addition to the biological pigment. This colorant flower is commonly used in various quantities with rice, cookies, bread, flours, and desserts. CT colorant is particularly used in the local culinary scene. (Sutakwa *et al.,* 2021) [43].

Considering positive health effects and attractive colorants in the food processing system, the demand for anthocyanin has been continuously increasing. CT flower rice is loaded with antioxidants. It has anti- inflammatory and anti-carcinogenic properties. It’s linked to reducing cases of obesity, diabetes, and heart disease. And also, fresh pods, young shoots and leaves are used as a vegetable or as a side dish. The leaves' natural green color is used in a variety of meals as a colorant (Chusak *et al.,* 2019) [6].

Extraction methods

For agricultural uses and other industrial uses, extraction should be done to separate components of butterfly pea. Traditional extraction methods (conventional method) and modern methods (Non-conventional method) are the types of extractions. (Jeyaraj *et al.,* 2021) [9] Both these are successful extraction methods, but the efficiency of the methods depends on the compound extracted. The yield of this extraction method depends on various factors like temperature, pH, types of solvent used, & extraction time. Since the 1970s, conventional extraction methods were maneuvering for blue pea plants. (Lakshan *et al.,* 2019) [3] Traditional extraction methods are maceration extraction, Soxhlet extraction, & cold/hot water extraction. Ultrasound-assisted extraction and microwave extraction are examples of non-conventional extraction methods.

Maceration extraction is used for dye extraction from blue pea flower. A powder form sample of a blue pea plant is used for maceration extraction and this is a process done at 25-95 °C, for 40-80 min. Water is added to the power form sample and gradually it is made for heating. The extracted liquid is strained through the process & residue solid material is pressed to increase the liquid yield. This liquid is dried at 60 °C to remove water & strain dye. By maintaining temperature and time 45.52% of yield can be obtained at 54 °C, for 74min. This is a simple method and does not require special equipment or technology. (Baskaran *et al.,* 2019) [11].

Hydroalcoholic extraction is a conventional extraction method, used to extract anthocyanin compounds (Jaafar *et al.,* 2020) [49]. In this process, dried powder form is used as the sample. Ethanol, methanol, or distilled water is the solvents used in hydroalcoholic extraction. According to the FDA (2018), distilled water is class one solvent & best solvent for extraction of anthocyanin compounds. The yield of anthocyanin

extraction of blue pea flower is 56.1% when the water is used as solvent at 60 °C for 60min (Jeyaraj *et al.*, 2021) [9].

Ultrasound-assisted extraction is an effective non-conventional method to extract anthocyanin compounds from blue pea flower. Because high yield can obtain within a short extraction period (Anthika *et al.*, 2015) [50]. The powder form of the sample is taken for the extraction. Distilled water is used as the solvent, at 30 °C for 15-45min. Extraction is taken at an ultrasonic bath and constant temperature should be maintained. In this process, anthocyanin compound will be soluble in distilled water and the extract can strain through filter paper.

However, maceration is more effective than ultrasound extraction in the case of flavonoid extraction under aqueous ethanol. Besides using ultrasound extraction flavonoid content of aqueous ethanol was high after 7 days. So longer time is required to complete this process. In one study RMS (Response Surface Methodology) was used to find the best extraction for anthocyanin. According to a report (Chong *et al.*, 2015) [51], anthocyanin extraction yield was 246.6% higher by RMS compared to ultrasound-assisted extraction. (Baskaran *et al.*, 2019) [11]

Coldwater & hot water extraction methods are most commonly used in conventional extraction methods.

These techniques are used to separate various bioactive components of blue pea that can be taken without leaving any residues behind the extraction chamber (Baskaran *et al.*, 2019) [11]. Phenolic extraction yield is 185.3mg/g in cold water extraction. Total phenolic extraction yield is high in hot water extraction, that of 239.5mg/g. Flavonoid extraction value is high in hot water extraction, that of 128.3mg/g. Ascorbic acid value also can be detected using cold water extraction. The value is 10.36mg/g. Ascorbic acid value could not detect by the hot water method as the increasing temperature is caused due to degrading ascorbic acid. (Minh *et al.*, 2020). [52].

Various solvent mixtures are used to extract bioactive components of the CT plant. Ethanol and Methanol are the major solvents used in most extractions. Anthocyanins extract efficiency in a mixture of ethanol and methanol is one of them. According to the scientists, ethanol extraction is competent because of its high extraction percentage at polar molecules (Pham *et al.*, 2019 & Satria *et al.*, 2022) [53, 15].

Microwave-assisted extraction can be processed in a domestic microwave oven to extract medicinal plants. It is a non-conventional & environmentally friendly technique, used to extract dye from blue pea flowers. Distilled water is the solvent using between 300MHz to 300GHz frequency and wavelength between 1cm to 1m Sample and the solvent ratio is 1:20g/ml. It takes only a few minutes to complete the process. The extracted dye can stain through filter paper. The extraction yield of microwave-assisted extraction is 48.61% (Marsin *et al.*, 2020) [54].

Although traditional extraction methods have been widely employed to extract these flowers, non- conventional extraction methods (ultrasound aid) have proven to be superior and advantageous for phytochemical extraction. Exploration of alternative nontraditional extraction methods that are deemed as "green techniques" might thus be advantageous in determining the efficiency of extraction.

Functional compound and its bioactivities

Anthocyanin is the major functional compound present in *Clitoria ternatea*. They are water-soluble pigments belonging to the Flavonoids. Flavonoids are a subclass of the polyphenol family. Fruits, vegetables and plants look in various colors like violet, blue, orange and red which are produced by the chemicals mentioned above (Vidana Gamage *et al.*, 2021) [2]. Due to this reason, Butterfly pea flowers are looking much more attractive. There were more than 700 types of Anthocyanins identified by the Scientists. Anthocyanins are responsible for protecting the plant cells from UV radiation as well they are contributing to pollination. 6 most common anthocyanidins have been found, they are Pelargonidin, Peonidin, Delphunidin, Cyanidin, Petunidin, and Malvidin (Vidana Gamage *et al.*, 2021) [2]. The pH of the solution is the main factor affected to obtain the various colors of Anthocyanins. That means the chemical structure of the Anthocyanins can be changed by the slight variations in pH of the surrounding medium.

In the Butterfly pea flower, polyacrylate anthocyanins are the most abundant source of Anthocyanins because their stability is higher than the Non-Acylated Anthocyanins. The big advantage of polyacrylate anthocyanins is, that they are known to be used as natural food coloring agents. (Marpaung *et al.*, 2020) [55]. As mentioned earlier, the color of the anthocyanins of Butterfly pea flower extract can change with pH like this, Red color obtains at the pH lower than 3.2, color changes from violet to blue when the pH changes from 3.2 to 5.2, the light blue color occurs at the range of pH 5.2 to 8.2 and the color changes from light blue to dark green color when the pH changes from 8.2 to 10.2 (Sutakwa *et al.*, 2021) [43]. The reason behind this color change is the structural alternation that takes place in molecules of Anthocyanins with the change in hydrogen ions and the concentration of the hydrogen ions present in the medium. Red color occurs due to the presence of ion flavylium, green color occurs due to the presence of ionic chalcone and due to the neutral quinoidal base, the blue color occurs (Salacheep *et al.*, 2020). [56].

The major bioactivity of Anthocyanin is the Antioxidant activity. Antioxidants are substances that inhibit oxidation and protect the cells against free radicals. The antioxidant activity has the ability to donate the hydrogen atoms or electrons to free radicals and then relocate free radicals and prevent the damage occurring from the free radicals (Tan *et al.*, 2015) [58]. Anthocyanins exhibit two types of antioxidant activities that are *in vivo* and vitro (Escher *et al.*, 2020 & Vidana Gamage *et al.*, 2021) [57, 2]. Due to its antioxidant capabilities, Butterfly pea anthocyanins could prevent the types of diseases such as neurological diseases, cardiovascular diseases, cancers, and diabetes (Cazarolli *et al.*, 2009) [59], (López *et al.*, 2019) [60]. Some of the studies have proven the toxicological safety of using the petal extract of the butterfly pea.

The petal aqueous extract of the butterfly pea showed no of cytotoxicity effects in fibroblast cells of human and protective effects have been shown in humans' red blood cells. Moreover, the oxidation of pBR322 plasmid DNA was inhibited by this extract (Mehmood *et al.*, 2019 & Escher *et al.*, 2020) [61, 57]. The *vitro* antioxidant properties of anthocyanin were displayed by extracts of the water of butterfly pea and up to 625µg/mL there were no toxic effects occurred on RAW264.7 cells. In one of the studies, they have found the Anthocyanin extract of Butterfly pea demonstrated considerable antioxidant activity against peroxyl radicals and DPPH. But IC50 of the Anthocyanin extracted from Butterfly pea (0.47mg/L) was considerably high amount when compared to the 0.002mg/L amount of Ascorbic acid. The term IC50 is, how much concentration of Anthocyanin required to reduce the free radical concentration by 50% (Phrueksanan *et al.*, 2014) [47]. One of the scientists studied, how does the antioxidant activity

contribute to the prevention of lipid oxidation (López *et al.*, 2019) [60]. Lipid oxidation takes place, when the free radicals are present in surrounding medium.

After the 48 hours of treatment, the formation of 7- keto cholesterol in an emulsion of cholesterol was prevented by Anthocyanins (6mg/mL) that was extracted from CT by distilled water and then inhibited the generation of free radicals by 79.8% (López *et al.*, 2019) [60]. Due to the dehydration of 7-hydroperoxycholestrol, the cholesterol free radical chain reaction produces 7-keto cholesterol (Xu *et al.*, 2005) [62].

Therefore, the quantity of lipid oxidation can be measured by the amount of 7-keto cholesterol generated. The antioxidant property is helpful to reduce the diseases like hyperglycemia. Hyperglycemia is the disease of increasing the serum glucose levels in the body after a meal. Due to this, some complications occur in the body. In mitochondria, the reactive oxygen species (ROS) are produced and they deplete the serum antioxidant enzymes. Some of the studies proved anthocyanins of butterfly pea are able to inhibit the actions of the digestive enzymes of Carbohydrates like pancreatic α- amylase and intestinal α-glucosidase which caused to the reduction of the postprandial hyperglycemia (McDougall *et al.*, 2005) [63].

Nanosciences for future improvements

Several studies conducted various methodologies to synthesize a variety of nanoparticles from blue pea. Silver nanoparticles, which have antifungal, anti- inflammatory, antiangiogenic, and anti-permeability properties, are employed in the medical industry like Antibacterial agents in catheters that have been surgically placed to prevent infection effects generated in surgery (Gurunathan *et al.*, 2009) [64]. Nanocrystalline silver particles offer a wide range of uses in biomolecular detection and diagnostics, antimicrobials and treatments, catalysis, and microelectronics (Sahayaraj *et al.*, 2011) [65].

For silver nanoparticles synthesis, fresh flowers of CT were randomly picked and washed thoroughly in tap water for about 5 min. Then CT petals were removed from the flower and kept for drying in the tray at room temperature. Next preparation of curd extract, in here finely crushed dried 10g of flower petals mixed with 100ml of methanol and filter it using watchman 1 filter paper. And the filter obtained dried in a vacuum drier and the powder was stored at 40C. Add 100ml distilled water to the powder to get the aqueous extract. Then do the UV-VIS Spectral analysis; 1ml of aqueous flower extract was added into the 10ml of 5mM Sliver Nitrate, then the drop of Ag+ to AgO (silver oxide) remained shown by determining the UV. VIS is spectrum at time range from 5- 120min within the range of 400-480nm wavelengths in a spectrophotometer. Finally, the sample was deducted by Scanning Electrons Microscope for the analysis of the size and the presence of silver nanoparticles. The opinion of that practical was plants have been considered a leading candidate for nanoparticle synthesis. Those biogenic nanoparticles are less expensive, easier to make, and more environmentally friendly. *Clitoria ternatea* flower extract showed a lot of promise. Lowering effect on Ag+ silver nanoparticles reduction at the temperature in the room UV Vis Spectroscopy was used to characterize the samples. The production of silver nanoparticles was confirmed by SEM (scanning electron microscope). The particles produced ranged in size from 5 to 50 nm, with a diameter of 5 to 50 nm. The shape that is consistent (cubic or spherical). The particles also tend to stick together. Aggregate, implying that they could be beneficial in applications that require the process of coating materials Nanoparticles with a wide range of sizes and characteristics can be obtained by tapping the many plants in the wild environment. (Anitha *et al.*, 2013) [66]

Moreover, nanoparticles made by biological techniques have been used in human healthcare systems like nanomedicine, diagnosis, and commercial product manufacturing (Bar *et al.*, 2009; Cruz *et al.*, 2010) [67, 68]. Nanoparticles have been found to have a variety of biological functions due to their unique physical and chemical properties (Haverkamp *et al.*, 2009) [69]. Plant extracts have recently been utilized to make a variety of environmentally benign metal nanoparticles, including silver (Ag), zinc oxide (ZnO), gold (Au), magnesium oxide (MgO), titanium oxide (TiO2), platinum (Pt), and nanoparticles of iron oxide (Fe3O4) (Zhan *et al.*, 2011 & Masarovicova *et al.*, 2013) [71, 72]. Metabolites, both primary and secondary found in crude extracts from plants were used in a redox process to convert the ionic form to metallic nanoparticles that are good for the environment (Kim *et al.*, 2007 & Aromal *et al.*, 2012) [73, 74]. Zinc oxide (ZnO) is a naturally occurring inorganic substance with physical, optical, and antimicrobial properties that are widely used in a variety of products like ceramics, glass, concretes, car tires, lubricants, paints, etc. (Sabir *et al.*, 2014) [70]. Zinc oxide nanoparticles are harmless and can be utilized as an alternative to molecule UV-absorbers to protect against a wider range of UV rays. In this process, whole plants were harvested. All of the pieces were rinsed in distilled water twice and then precisely honed to the small parts. plant extracts preparation was done by 5 gm of plant material and15 minutes in a 250 ml Beaker with 50 mL Milli-Q water, then using standard filtration method herbal extract were collected to a separate conical flask and stored in 4 C in a refrigerator. Then preparation of precursor; Zinc Nitrate Hexahydrate is used to make a 1 mM zinc nitrate solution. [Zn(NO3)2·6H2O] and Milli- Q water and it is stored in the refrigerator. Finally, the synthesis of ZnO nanoparticles is the process of three boiling tubes were used a).10 ml of 1mM Zinc nitrate solution b) 10 ml of aqueous plant extract (plant extract used to reduce metal ions to nanoparticles of metallic oxide) 9 mL Zinc Nitrate Solution (1 mM) c) 9 ml of 1 mM Zinc nitrate solution. After 2 to 3 hours, the mixture (third tube) was centrifuged for 15 minutes at 5000 rpm to extract the tablet. The particle is dissolved in Milli-Q water after the supernatant is discarded. Characterization of ZnO nanoparticles (UV-VIS spectra analysis) the UV-VIS spectrophotometer was used to measure ZnO nanoparticles' absorption spectrum produced by lowering metal ion concentrations in solutions of various extracts. The current study looks at how to make nanoparticles of ZnO from diverse sources of the healing properties of CT. It ensures that the technique is environmentally friendly and that the approach can be obtained easily for future needs. Because of the significance of this plant, the nano compositions could be useful in the prevention and treatment of patients, according to Ayurveda a variety of illnesses. (Jogam *et al.*, 2020) [75].

In another experiment Biosynthesis of silver and gold nanoparticles with the help of ultrasound and the *Clitoria ternatea* flower. UV-Vis spectrophotometric analysis and the scanning electron microscopy (SEM), transmission electron microscope (TEM) and x-ray diffraction, are the methods used to detect nanoparticles. The impact of the ultrasound approach on the nanoparticles' physicochemical properties as compared to that of the reflux method. The antibacterial activity of the nanoparticles was tested by different bacteria to see how the character affected their biological activity. Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, and Streptococcus pyogenes are some of them. The antioxidant

activity of the nanoparticles was assessed using a radical inhibition experiment utilizing 2,2-diphenyl-1-picrylhydrazyl activists (DPPH). *Clitoria ternatea* flower showed decreased ability to create nanoparticles with particle sizes ranging from 18 to 50 nm, according to the findings. In general, when compared to the reflux approach, ultrasound produces lower particle sizes for both silver and gold nanoparticles. When compared to the Reflux approach, the ultrasound-assisted method produced smaller particles with better antibacterial activity against tested pathogens. The antioxidative activity of the nanoparticles was demonstrated by a DPPH inhibition and it should be exceeded 50%. The findings suggest that making silver and gold nanoparticles with *Clitoria ternatea* flower extracts as just a reducing agent with ultrasound-assisted synthesis is a green technique. (Fatimah *et al.*, 2020) [76].

Ternatin, Anthocyanins and Quercetin Glycosides from Butterfly Pea Petals Protect Macrophage Cells from Lipopolysaccharide (LPS)-Induced Inflammation. It is using high-performance liquid chromatography, diode array detection, and electrospray ionization/mass spectrometry, *Clitoria ternatea*'s blue blooms yielded 12 phenolic metabolites such as nine ternatin anthocyanins and three glycosylated quercetins. Three anthocyanins were previously unknown in this species show ternatin fragmentation patterns. Flavonols and ternatin anthocyanins were separated from the extracts. Polyphenols from *Clitoria ternatea* were found to have anti-inflammatory activities in RAW 264.7, macrophage cells when they were exposed to lipopolysaccharide (LPS). COX-2(Cyclooxygenase-2) enzyme activity was powerfully inhibited by flavonols, with a small reduction in ROS The ternatin anthocyanins inhibited nuclear NF-B (nuclear factor that enhance the activity of B cells) translocation, iNOS(Nitric Oxide Synthase) protein expression, and NO production via a non-ROS suppression technique. As a way, quercetin glycosides and ternatin anthocyanins found in the blue flower petals of *Clitoria ternatea* could be used to make pharmaceuticals or nutraceuticals that protect against chronic inflammatory diseases by lowering macrophage cells' excessive production of pro-inflammatory mediators (Nair *et al.*, 2015) [77].

Table 1: Nutritional composition of *Clitoria ternatea*

Component	Amount (%)
Moisture	92.4-0.11
Ash	0.15-1.40
Fat	2.5-0.11
Protein	0.02-0.32
Crude Fiber	0.2-2.0
Carbohydrate	2.23-0.3
Potassium	1.2506-0.235
Manganese	0.0249-0.003
Sodium	0.1413-0.003
Zinc	0.5980-0.006
Arsenic	<0.0001
Nickel	0.001267-0.0001
Boron	0.0150-0.002
calcium	3.0953-0.09
Cobalt	<0.0001
Chromium	0.0007-0.0
Cupper	0.0103-0.007
Iron	0.1441-0.007
Magnesium	2.2306-0.134
Molybdenum	0.0001-0.0001X5.7
Selenium	<0.0001
Cadmium	<0.0001
Lead	0.002333-0.0002

Table 2: An exploration of various parts of *Clitoria ternatea* and their functional importance in the diet

Part used		Utilization in foods	Effect on Health	Supportive Evidence
Above ground parts	Flowers	Tea powder Soft cheeses Cheesecake Jelly Ice cream Yogurt Butter	• Anti-diabetic activity • Anti- hyperglycemic activity • Antioxidant activity • Anti-cancer potential	• Ethanolic extraction of CT flowers induced anti diabetic activity at 200 to 400mg/kg dosage in rats (Sharma *et al.*, 1990) [23]. • Folin-Ciocalteu test, AlCl3 colorimetric method, and pH differential method were used to define the concentration of total quantity of phenolic components, flavonoid, and anthocyanin in an aqueous extract of CTE at 0.25-1.00 mg/ml, etc. (Chayaratanasin *et al.*, 2015) [39] • The antioxidant activity of flower petal and eye gel of CT was 2 mg/mL. (Kamkaem *et al.*, 2009) [34] • Using *Clitoria ternatea* flower extract shows antioxidant activity at 50 and 100 mg/kg and protects rats from testicular type damage caused by ketoconazole (Iamsaard *et al.*, 2014) [46]. • CT flower petal extract at 400 µg/mL protects canine erythrocytes beside free radical- induced hemolysis and oxidative damage. (Phrueksanan *et al.*, 2014) [47] • CT flower has chemical composition for antiproliferative activities (Neda *et al.*, • 2013) [45]. • Ethanolic and petroleum ether extraction of CT flower showed cytotoxicity effect by using trypan blue exclusion method (Sen *et al.*, 2013) [40]. • *Clitoria ternatea* Linn. floral extracts *in vitro* cytotoxic activity research. (Bhat *et al.*, 2011) [41]

	Leaves	Beverages- Herbal tea (caffeine free, cocktail) Natural food dye in- • Ice cream • Yogurt • Bakery industry	• Anti-diabetic activity • Antioxidant activity Anti-Bacterial activity • Anti- Inflammatory activity	• 400mg dosage were lower the blood glucose level, insulin, and glycosylated hemoglobin urea and creatinine. (Chusak *et al.*, 2019) [6], (Oguis *et al.*, 2019) [1] • Methanol extraction on the glycemic response, 1-2g of blue pea extraction was ingested with 50g sucrose resulting plasma glucose level suppressed. (Chusak *et al.*, 2019) [6] • Methanol extraction of *Clitoria ternatea* leaves were showed DPPH scavenging activity & reduced the DNA damage (Jiji *et al.*, 2020) [79]. • Ethanolic and aqueous extraction of *Clitoria ternatea* leaves are showed antibacterial activity against gram negative bacteria in the presence of Bacillus cereus and Bacillus subtilis. (Shahid *et al.*, 2009) [31]. • Powder form of *Clitoria ternatea* shows (400mg/kg) acid induced inflammation in rats-anti-inflammatory activity by methanol extraction. (Singh *et al.*, 2018) [5]. • Anti-inflammatory activity measured by ethanol extraction of *Clitoria ternatea* 100- 400mh/kg reduction in carrageenan induced paw oedmea (Shen *et al.*, 2014) [80].
				• Anti-inflammatory activity evaluated against acetic acid and carrageenan, change the cell count in bronchoalveolar fluid level of immunoglobulin. (Singh *et al.*, 2018) [5].
Below ground parts	Roots	Powder and extraction used as medicine	• Nootropic activity • Anti-inflammatory activity • Anti-pyretic activity • Immunomodulatory activity • Anti-asthmatic activity • Analgesic activity	• Ethanolic extraction of *Clitoria ternatea* root showed same level of memory retentivity by increasing Acetylcholine content at a dose of 300mg/kg for 7 days in electroshocked rats. (Taranalli *et al.*, 2000) [81], (Rai *et al.*, 2002) [82]. • Aqueous extract of *Clitoria ternatea* root showed memory improvement in neonatal rats at the doses of 50 and 100 mg/kg for 30 days. (Rai *et al.*, 2002) [82]. • Aqueous extract of *Clitoria ternatea* root increased the amount of acetylcholine notably in their hippocampus at the dose of 100 mg/kg for 30 days in rats. (Rai *et al.*, 2002 & Mukherjee *et al.*, 2008) [82, 12]. • Methanol extraction of C.T root exhibited anti-inflammatory effect and inhibited rat paw oedema at doses of 200 and 400 mg/ kg, induced by acetic acid in rats (Lijon *et al.*, 2017) [83]. (Singh *et al.*, 2018) [5]. • Methanolic extract of C.T root lowered the yeast-induced elevated body temperature of Wistar rats notably at doses of 200, 300, 400mg/kg that had Barik *et al.*, 2007) [7]. • Aqueous extract of *Clitoria ternatea* roots increased dendritic intersection, branching points and changed dendritic arborization of amygdala neurons at 50 mg/kg and 100 mg/kg for 30 days in rats (Rai *et al.*, 2005) [84]. • Ethanolic extracts of *Clitoria ternatea* roots exhibited anti-asthmatic effect in rats induced by mast cell degranulations and passive cutaneous anaphylaxis at a dose of 100-150mg/kg. (Taur *et al.*, 2010) [85]

Table 3: Health Benefits of *Clitoria ternatea*

Sr. No.	Property		Test model	Dose/ concentration/ method	Mechanism of action and Potential findings	References
1.	Effects on central nervous system	a) Neurological disorders	Electro shocked rats	*Clitoria ternatea* was administrated at 300 mg/kg rate of body weight for 7 days. Passive avoidance test	The extraction obtained from the aerial parts increased the acetylcholine content in the brain and its activity increased in cortex and midbrain except in the medulla oblongata and cerebellum and also exhibited 66.66% of memory retentivity. *Clitoria ternatea* root extraction showed a notable increment in ACh content. However, there was a decrease in its activity in the areas of midbrain and medulla oblongata. but its effect is not so important.	Taranalli *et al.*, 2000 [81] Gollen *et al.*, 2018 [38]
			Electro shocked rats	*Clitoria ternatea* was administrated at 500 mg/kg rate of body weight for 7 days. Passive avoidance test	The aerial extract of CT exhibited memory retentivity of 50%. This effect was not affected to the cholinergic markers when making a comparison with normal rats. When the root extract of C.T is administered to rats, it increased the AChE content in the brain and AChE activity in the cerebral cortex. However, AChE activity in the areas of the medulla oblongata and cerebral cortex notably decreased. Also, slight decrease of ACHE activity is observed in mid brain. Moreover, AChE activity in the cerebellum had not shown any change.	
		b) Learning and	Neonatal rat	*Clitoria ternatea*	Aqueous extraction of CT roots showed	Rai *et al.*, 2001

		memory enhancing	about 7 days old.	administrated at 50 & 100 mg/kg rate of body weight for 30 days. Open field behaviour test, Passive avoidance test, T-maze test	active retention in avoidance. In this test rats increased the responses for alterations and responses. These values were decreased in T-maze test. Rat pups showed constant behavior changes with root extract of CT.	[86]
			Neonatal rats about 7 days old and 60 days old rats.	*Clitoria ternatea* administrated at 100mg/kg rate of body weight for 30 days.	Aqueous extraction of CT roots showed nootropic activity in 48h-30days. CT extract increased the Ach amount in hippocampus of rats.	Rai *et al.*, 2002 [82]
			Young adult rats (60 days old)	*Clitoria ternatea* administrated at 100mg/kg rate. Elevated plus maze test and Recognition test	On 9th day of CT aqueous extract administration, rats showed high amount in inflexion ratio and lowered the transfer latency. Rats showed high discrimination index when new object introduced to the environment in object recognition test.	Rai *et al.*, 2002 [82]
			Young adult rats (60 days old)	*Clitoria ternatea* administrated at 50-100 mg/kg rate of body weight for 30 days.	Aqueous extraction of *Clitoria ternatea* roots showed memory enhancing effect. This extract contained nerve growth factor. It has neuron survival ability, cholinergic effect and dopaminergic plasticity in central nervous system.	Rai *et al.*, 2005 [84]
			Young diabetic rats	*Clitoria ternatea* administrated at 100mg/kg rate of body weight.	Ethanolic extraction of *Clitoria ternatea* roots were showed protective effect on dentate gyrus and frontal cortex neurons.	Ravishankar *et al.*, 2013 [87]
		c) Seizures	Mice	*Clitoria ternatea* administrated at 100mg/kg rate of body weight.	Methanolic extraction of leaves and flowers of *Clitoria ternatea* showed anti-convulsant Activity. It has an effect of reducing limb extension on maximal electroshock.	Jain *et al.*, 2003 [88]
		d) Depression	Mice	*Clitoria ternatea* administrated at 100-400 mg/kg rate of body weight. Tail suspension test	Methanolic extraction of *Clitoria ternatea* showed effect on depression. CT extract decreased the period of immobility. At 400mg/kg dose showed highest effective compared to 10mg/kg fluoxetine.	Jain *et al.*, 2003 [88]
			Mice	*Clitoria ternatea* administrated at 150-300 mg/kg rate of body weight.	Ethanolic extraction of CT roots showed anti- depressant activity. Z)-9,17-octadecadienal and n- hexadecanoic acid are two compounds which has ability to developing novel selective MAO-A(Monoamine oxidase inhibitors) inhibitors. This herbal remedy was used to treat psychiatric disorders. It showed result in anxiety and depression in mice.	Parvathi *et al.*, (2013) [89] Margret *et al.*, 2015 [78]
		e) Anxiety	Mice	*Clitoria ternatea* administrated at 100-400 mg/kg rate of body weight. Light/dark exploration test	Methanol extraction of *Clitoria ternatea* showed anti- anxiety activity. The oral administration of CT for 60 min, increased the time period in the dark/light exploration test. When, the CT dose was increased the time period mice spent also increased. Relationship between, amount of time spent in the box and dose is dependent manner.	Jain *et al.*, 2003 [88]
		f) Lithium induced head twitches	Rats	*Clitoria ternatea* administrated at 100mg/kg rate of body weight.	The CT extract was administered 60 minutes before the injection of lithium sulphate (Li2SO4) of 3 mEq/kg. CT extract was reduced the head twitches above 60 min.	Jain *et al.*, 2003 [88]
		g) Analgesics Nephropathy	Rats and mice	*Clitoria ternatea* administrated at 10mg/kg rate of body weight.	Ethanolic and petroleum extraction of *Clitoria ternatea* leaves showed analgesic activity. It showed higher effect than diclofenac sodium for 1hour treatment.	Shen *et al.*, 2014 [80]
				Clitoria ternatea was administrated at 200-400 mg/kg rate of body weight.	Methanolic extraction of *Clitoria ternatea* roots demonstrated analgesic activity by acetic acid induced withing test. CT showed 50.1%-63.8% withing at 200-400mg/kg dose. In experiments CT leaves mwthanolic extraction showed antinociceptive activity.	Kamilla *et al.*, 2014) [26]
2.	Ulcer		Rats	*Clitoria ternatea* was administrated at	Anti-ulcer activity was detected by measuring ulcer index in rats after	Jain *et al.*, 2003 [88]

			100-400 mg/kg rate of body weight.	administration of CT extract. Rats were held at 4 °C for two hours on wooden plank. Methanolic extraction of CT revealed anti-stress activity when it injected 60min before the test.	
3.	Diabetes	Juvenile diabetic rat experimental model	*Clitoria ternatea* was administrated at 100mg/kg rate of body weight for 30 days.	Hippocampal area of rats was protected by defatted alcoholic extraction of *Clitoria ternatea* roots for 30 days.	Parvathi *et al.*, 2013 [89]
		Rats	*Clitoria ternatea* was administrated at 100mg/kg rate of body weight.	*Clitoria ternatea* leaves ethanol extract showed the antidiabetic activity. Continuous oral administrated for 28 days significantly lowered the blood glucose level in rats.	Gunjan *et al.*, 2010 [21]
4.	Hepatoprotective	Mice	*Clitoria ternatea* was administrated at 200mg/kg rate of body weight.	*Clitoria ternatea* leaves methanolic extraction showed effect opposed to paracetamol. It has effect with liver toxicity by reducing aspartate aminotransferase, bilirubin & alanine aminotransferase with the improvement of histopathology.	Nithianantham *et al.*, 2011 [32]
5.	Oedema and Peritoneal inflammation	Rats	*Clitoria ternatea* was administrated at 200-400 mg/*kg rate of body weight.*	Methanolic extraction of CT roots reduced paw oedema, that vascular permeability and carrageenan caused by lactic acid in rats. Oedema was inhibited 21.6%-31.8% at the doses of 200-400mg/kg. Diclofenac inhibition was high at 400mg/kg dose than 20mg/kg. Peritoneal inflammation also reduced by 35.9%- 55.1% at the dose of 200-400mg/kg. *Clitoria ternatea* ethanolic extraction showed anti- inflammatory activity.	Parimala *et al.*, 2003 [36] Suganya *et al.*, 2014 [37]
6.	Pyrexia	Rats with pyrexia Rats	*Clitoria ternatea* was administrated at 230-460 mg/kg rate of body weight. *Clitoria ternatea*, 400mg/kg sample was administrated.	The ethanolic extract of aerial parts of CT had the effect for antipyretic activity. That had been depended according to the dose. Ethanol and acetone extracted CT leaves showed antipyretic effect in yeast. Significantly it decreased the fever.	Murugalakshmi *et al.*, 2014[18]

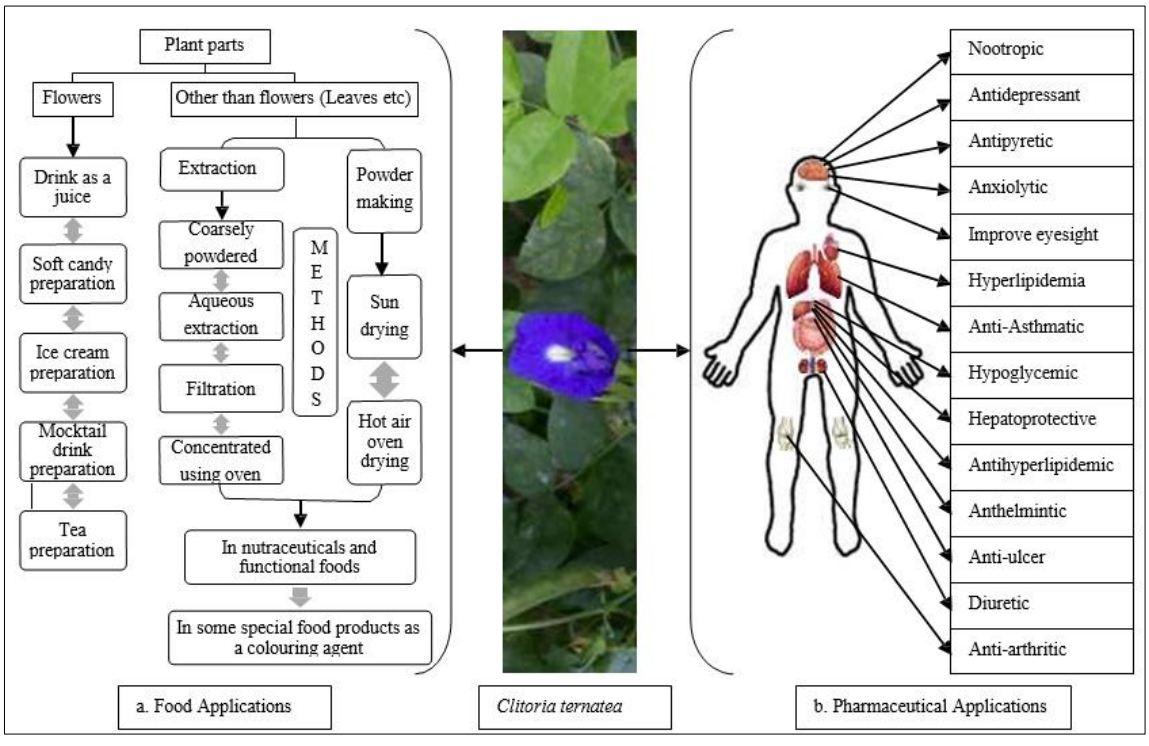

Fig 1: Food and Pharmaceutical applications of *Clitoria ternatea*

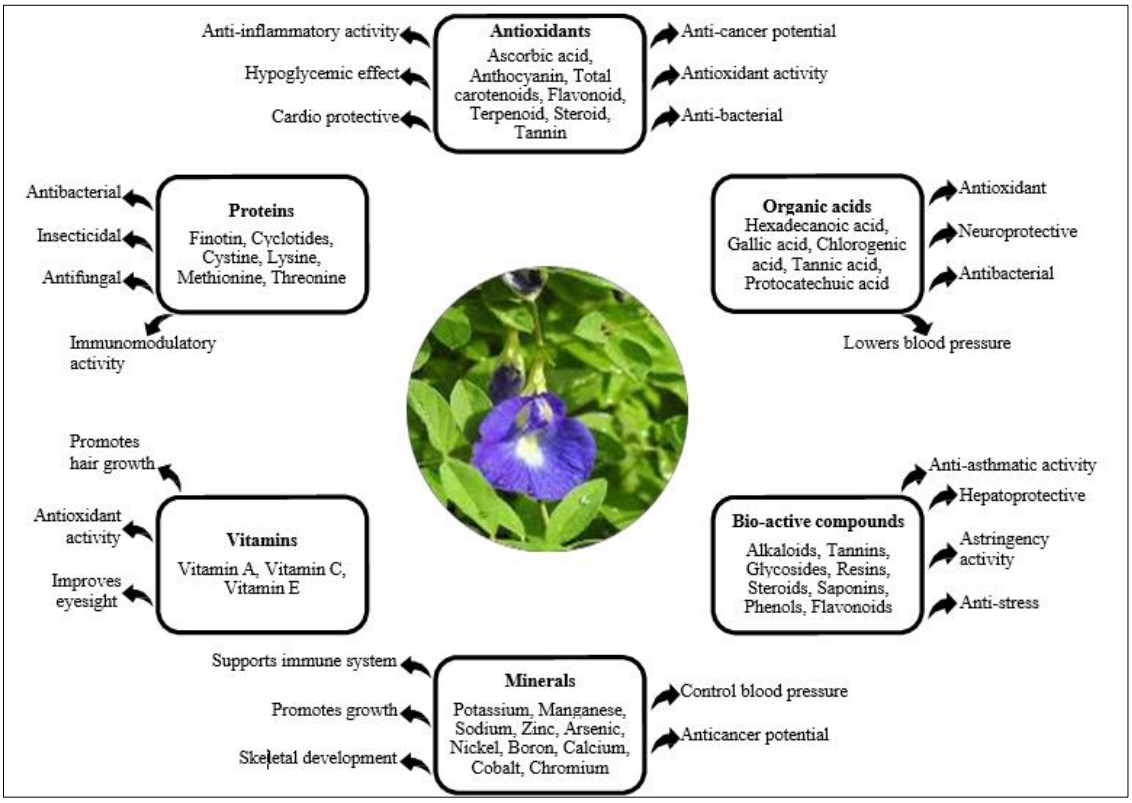

Fig 2: Functional compounds of *Clitoria ternatea*

Conclusions

Clitoria ternatea has a long tradition and is found in several countries. It is not just an ornamental flower but also a good medicinal plant which has numerous benefits. It has been experimented several years back by various scientists and have found a number of pharmacological uses as well. With the advancement of technology and Ayurvedic traditional medicine and the improvement of scientific research, different classes of plant species and their leading compounds have been studied. Extractions obtained through different methods of the roots, seeds, flowers, and leaves of CT have been experimented in Ayurvedic studies. Earlier different parts of *Clitoria ternatea* have been used for the treatment of Asthma, skin diseases, constipation, fever, Inflammation, Indigestion, snakebite and scorpion sting, etc. Later on, this plant is used for many pharmacological activities such as, memory enhancer (by increasing of acetylcholine content), act as a good stress, anxiety, and depression reliever, to gain calmness in mind and helps to have a good sleep. Moreover, it helps to lower the body temperature and also acts as a good pain reliever, the seeds are used to treat when joints in different parts of the body got swollen. It can be used to treat when difficulties in urination occur and improves the flow of urination, treat boils, blisters, and ulcers, and act as a neutralizer for poisons that enter the body. One of the most important benefits of this plant is its antidiabetic activity. Furthermore, it shows properties like antiseizure, tranquilizing, sedative, antimicrobial, insecticidal, and inhibition of blood platelet aggregation, etc.

Extractions of this plant are also useful to treat many diseases still where the proper medications have not been discovered such as cancers, neuro problems, kidney-related disorders, hyperglycemia, urinary disorder, goiter, disorders in the respiratory system, etc. This plant has a good source of evidence to be used as a memory enhancer. and anxiolytic agent. Nowadays different parts of Butterfly pea are incorporated into foods. When considering nanosciences and technology new nanomaterials and concepts have developed. Those can be used to produce energy by using sources like glucose, movement, light, etc. It gives a great conversion efficiency. These nanoparticles are helpful to minimize its side effects and also improving the bioavailability of *Clitoria ternatea*.

The plant has various important phytochemicals. The main phytochemicals enriched in *Clitoria ternatea* are flavonoids, anthocyanins, alkaloids, ternatins, saponins, tannins, taraxerol, and taraxerone. Due to the presence of Anthocyanins, it gives a blue purplish color so that it can also use as a natural food dye. Processing and extraction of *Clitoria ternatea* are done by conventional and non-conventional methods. It can be utilized as a starting point for the development of new phytoceuticals for the treatment of CNS illnesses and to improve memory function. Still, proper treatments and medications are not available for these conditions. Also, depression, stress, and anxiety are some of the major problems which are persisting nowadays. So, this plant can be used to make drugs for medicine in the future. All these data and scientific studies prove that *Clitoria ternatea* is fully safe and effective to be used as phytoceuticals.

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