In Vitro Analysis of Antioxidant, Anti-Inflammatory, and Antimicrobial Properties of Clitoria ternatea (Bluebellvine) Plant

Sai Venkat Tarigoppala[1], Kiranmai Tatini[2], Bhaskara raju V[3], Dhabal kumar satapathy [4], Srinivasu M[5]
[1,2] *Student, B. Pharmacy, Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, Andhra Pradesh, India*
[3]*Principal, Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, Andhra Pradesh, India*
[4,5] *Assoc. Professor, Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, Andhra Pradesh, India*

Abstract: **The plant Clitoris ternatea, also referred to as "butterfly pea", Asian pigeonwings, Bluebellvine ,Blue pea, Cordofanpea, Darwin pea, Nilakantha and Aparajita .It has long been utilisedin Ayurvedic medicine to treat a variety of diseases including constipation, indigestion, arthritis, skin conditions, liver, and gastrointestinal issues. The constituents found in Clitoria ternatea included tannins, phlobatannin, glucose, flavanol glycosides, and amino acids, alkaline substances, antharaquinone, a special pigment called as an anthocyanins, cardiovascular glycosides, volatile oils, and steroids., dietary fibre, saponins, triterpenoids, phenols, flavonoids were all present in Clitoriaternatea. By using agar disc and well diffusion procedures, andhave been examined against Salmonella typhi, B. cereus, Staphylococcus aureus, Klebsiella pneumonia, E.coli, pseudomonas and Proteus vulgaris. the organic solvents (petroleum ether, ethyl acetate, and methanol, ethanol) extracts from leaves of Clitoria ternatea (Papilionoideae)But the intended usage of the solvent should determine what solvent is selected. Clitoria ternatea flower has a broad spectrum of pharmacotherapeutic qualities that make it an ideal choice for use in functional foods. It is additionally safe and effective. The DPPH(1,1-diphenyl-2-picrylhydrazyl) scavenging assay was used to investigate C. ternatea activity and determine antioxidant activity. We used the well diffused test for assessing each extract's antibacterial activity against seven unique bacterial strains. The chemical components and pharmacological properties like anti-microbial, anti-inflammatory, anti-oxidant activity of Clitoria ternatea will be highlighted in this review.**

Index Terms: **Anti-bacterial, Anti-diabetic activity, Anti-inflammatory, Extraction, Phytoconstituents, Therapeutic uses.**

I. INTRODUCTION

Clitoris ternatea belonging to Fabaceae family and the Papilionaceous subfamily. This perennial leguminous plant grows as vines or creeper in tropical climates, bearing deep roots and elliptic to obtuse leaves. Some of its flowers are white to blue. It is propagated by seed and produces significant yields in a short amount of time following grazing, with great regrowth. Many naturally occuring antioxidants and bioactive components, including phenolics, flavonoids, cyan pigments, flavonol glycosides and kaempferol glycosides, flavonol glycosides, terpenoids, myricetin with glycosides, tannins, and steroids, are present in the flower. The potential of these substances to combat oxidative stress there has been well investigated. The root part of the CT has been used as an anthelmintic, laxative, purgative, a diuretic inflammation, constipation, urinary tract infections, fever, arthritis, and eye conditions.

Antioxidants prevent or slow down the free radical induced cellular oxidation associated with cancer, atherosclerosis, diabetes, asthma, and autoimmune disorder(JR Kanwar, 2009).Since there are no adverse reactions and scavenge radicals that are unstable as soon as they are consumed through metabolic processes, natural antioxidants are superior to be manufactured ones(C Proestos, 2013). The process triggered by free radicals will be halted by an antioxidant chemical, protecting bodily cells from harm(Gilgun-Sherki, 2002).In addition, an element high in antioxidants may shield against UV radiation. In order to improve memory and intelligence, seeds and leaves were commonly utilized as a brain tonic. A snakebite remedy that was employed included juice and flowers. We employed seeds for managing inflamed joints, and for urinary issues, we take crushed seeds with cold or boiling water(Nawaz AH, 2009)(SG, 2009)(Mukherjee PK, 2007).Using rat models, the study intended to determine the anti-

inflammatory effect of a methanolic extract obtained from the lower leaves of the plant Clitoria ternatea Linn.The analgesic effect of the ethanolic extract in mice was also assessed in the same investigation(parmila devi B, 2003).In order to find C. ternatea organic solvent extracts that might be helpful in the creation of new instruments as antimicrobial substances for the control of inflammatory disorders, this study was conducted.

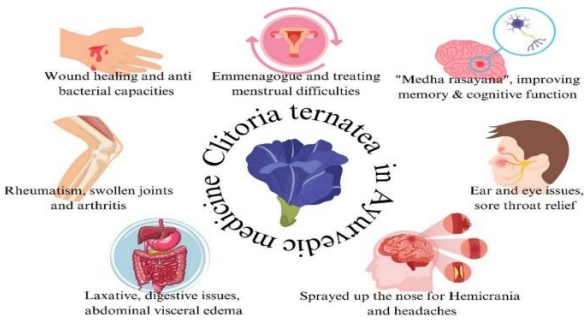

Figure 1: Pharmacological uses of clitoria ternatea in ayurvedic system (shwetali mahesh shirodkar, 2023).

II. EXTRACTION OF PHYTOCHEMICALS

The process of phytochemicals being extracted from plants is a crucial step.A varietyof extraction techniques exist, and choosing the best parameters is crucial to ensuring an increase in phytochemical output(Azmir J, 2013).There are both conventional and nontraditional extraction methods, and each has advantages over the other. Therefore, the compatibility of the samples and the desired result must be taken in mind when carefully selecting the extraction method. Plant samples are eitherfresh, dried, ground, or powdered, and they are often sized out before extraction to maximize the surface area for mixing that is accessible with solvent. Most of the research on C. ternatea flowers used fresh flowers or leaves that had been air- or oven-dried(B)(Kamkaen N, 2009).Or crushed or finely powered (Lakshan SAT, 2019) (Mehmood A, 2019) (Pham TN, 2019) (Adhikary R, 2018).Young flowers orfoliage that was separated into smaller pieces, cleaned, and kept in a freezer at-25 C were used in certain research. The flowers were taken within a month(Chong FC, 2015).Or dried by lyophilization then followed by grounding.

Types of extraction process:

Conventional Extraction process:

Traditional extraction techniques, like soxhlet extraction, maceration, and hydrodistillation, typically use a variety of solvents with either heat or mixing. These techniques can be expensive and take a long time to complete, however in addition, they are very efficient. To ensure explore the possible bioactivities & phytochemical content of the material, the majority of studies used the extraction using aqueous solvent containing either methanol or ethanol instead of water alone and heating, while researched examined the best solvent and/or extraction conditions. Thephytochemical components were extracted using ethanol aqueous extraction at a ratio of 60% (v/v) in an agitated water bath in a circular motion.For two hours, the rotating water bath was set at 60 degrees Celsius and 100 revolutions per minute.1:8 ratio existed between the sample and solvents.A biological freezer (Panasonic, Japan) was used to store the filtrate at a temperature of 30°C until analysis was performed after the extract had been filtered (with vacuum assistance) for two hours.

Non -conventional extraction process:

Alternative extraction techniques, such as ultrasound aided extraction, microwave help the extraction process, extraction with enzyme assistance, supercritical fluid extraction and pressurized liquid extraction, are more recent, extremely effective, safer for the environment, and offer a number of benefits over traditional methods of extraction(Wen C, 2018).

Ultrasound aided extraction process:

Ultrasound assisted extraction works on the concept of acoustic waves production leading to molecular movement of solvent and sample which facilitates the leaching of organic and inorganic compounds (Herrera MC, 2005).Small samples can be extracted using ultrasonic aided extraction, which minimizes solvent usage, maximizes yield, and minimizes the extraction time. The best conditions for UAE of C. ternatea petals using the Taguchi method and gray relational analysis. After 30 minutes of extraction at 40 ◦C and 10 mL of distilled water/mg of material, a high phytochemical yield was detected, particularly for TPC (Salacheep S, 2020). comparing a water extracts with ultrasound aid to the water extract without, the ultrasonically assisted extract revealed more phenolic and flavonoid components. Likewise, the ultrasound-assisted extract of water demonstrated increased antioxidant activity in the DPPH, and FRAP(ferric reducing anti-oxidant power)test.

Microwave aided extraction process:

Ionic conduction and dipole rotation processes in the microwave-assisted convert electromagnetic energy to

heat, which facilitates the release of solvent-bound the solute from this instance matrix (Alupului A, 2012).The process of microwave-aided extraction (MAE) uses the energy from microwaves to help a solvent penetrate a sample and to facilitate the solutes' entry into the solvent by hydrogen bond interference. Because the generated electromagnetic waves encourage the molecules' dipole rotation, heating the sample, this occurrence may happen(Kaufmann B, 2002).

For E.g.:
utilizing microwaves to extract complete C. ternatea species flowers in 95% ethanol. This study's liquid-to solid ratio (v/w) was 20:1, the microwave power was adjusted at 400 W, and the extraction process took three minutes. From since the ethanolic extract used in this investigation, the total alcohol content (TAC) was determined to be 0.2546 mg/g (Saejung T, 2021).

Prior research on C. ternatea flowers has effectively separated flavonoid and phenolic components, as well as exhibited potent antioxidant activity, using Methanol or dilute ethanol (Kaisoon O, 2011)(Mehla J, 2013).While Traditional techniques for extracting have been extensively employed to extract these flowers, nonconventional methods, such as ultrasound assistance, have proven to be more effective and advantageous when it comes to phytochemical extraction. Determining the extraction efficiency of different phytochemicals might therefore benefit from research into the use of alternative non-conventional extraction methods that are regarded as "green techniques".

Consequently, next studies may focus on unconventional extraction techniques on C. ternatea flowers in addition to investigating other safe and environmentally acceptable techniques such enzyme assisted, pressured liquid, pulsed electric field, and pressurized liquid extraction.

III. PLANT CONSTITUENTS

Nutritional examination of C. ternatea petals revealed that the moisture content was 92.4% and the percentages of protein, fiber, carbohydrate, and fat were 0.32, 2.1, 2.2, and 2.5 percent, respectively. In addition, a high concentration of calcium (3.09 mg per gram), magnesium (2.23 mg per gram), potassium (1.25 mg per gram), zinc (0.59 mg/g), sodium (0.14 mg/g), and iron (0.14 mg/g) was discovered in the flower(Neda GD, 2013). Cinnamic acid, phenolic aglycones, and flavanol glycosides were first identified as cod five favanols: quercetin, kaempferol 3-glucoside, kaempferol 3-robinobioside-7 rhamnoside, and kaempferol. Furthermore, six acylated anthocyanins derived from blue flowers were discovered by him. He dubbed them ternatins and they were investigated in later research.

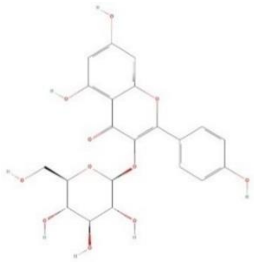

Figure 2 Kaempferol3-glucoside

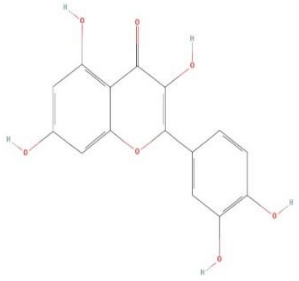

Figure 3 Quercetin

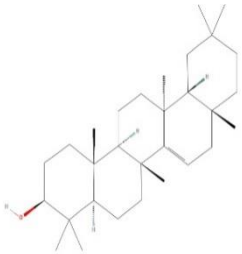

Figure 4 Taraxerol

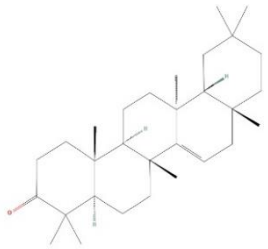

Figure 5 Taraxerone

Catechins, alkaloids, glycosides, carbohydrates, phytosterols, and saponin are among the phytochemical elements of C. ternatea petals (Lijon B, 2017).Combining phenolic chemicals with sugar results in glycosides. The color pigments called anthocyanins, which give plants their characteristic red, blue, and hues of purple that draw pollinators, are the main types of phenolics founds in plants(Lee DW, 2002).

Taraxerol and taraxerone, two pentacyclic triterpenoids, are the main phytoconstituents in Clitoria ternatea (Banerjee SK, 1964).

Ternatins, flavonoids, alkaloids, saponins, the tannins, carbohydrate, protein, resins, starch, as well taraxerol, and taraxeroneare detected by phytochemical screening of the roots (Uma B, 2009). Leaf material from Clitoria ternatea L. was used to isolate four kaempferol glycosides: I, II, III, and IV.Protein Magnetic Resonance, Mass Spectrometry, and Ultra Violet were used to identify kaempferol-3-glucoside (I), kaempferol-3-rutinoside (II), and kaempferol-3-neohesperidoside (III).(IV), C33H40O19, mp: 198. This is obtained the name clitorinafter being identified from spectral data as Kaempferol-3-orhamnosyl glucoside (Morita N, 1977). Protein, phenol, starch,

carbohydrate, fat, and total soluble sugars were all quantitatively assessed after Clitoria ternatea was ground into powder(Shekhawat N, 2010).Polyphenols are essential to the food business because they give food its color, astringency, and bitterness. Moreover, polyphenols' potent antioxidant activity provides significant health advantages such defense against the onset of illnesses and cancer (Maqsood S, 2013).

IV. PHARMACOLOGICAL ACTIONS

A number of substances found in Clitoria ternatea flowers have strong anti-inflammatory, antiproliferative/anticancer, antidiabetic, antioxidant, and microbiological effects (Lo´pez Prado AS, 2019)(Mahmad N, 2018)(Rajamanickam M, 2015).

Anti bacterial activity:

The development of bacteria resistant to antibiotics severely reduces the efficiency of existing medications, leading to the failure of infection treatments (Scheffler RJ, 2013).In addition to looking for novel antibacterial substances, different strategies must be developed to address this challenge. Several ways to assess in vitro behaviour of a particular antimicrobial (antibacterial or antifungal) agent, including disc diffusion methods, broth , agar dilution, and others (Balouiri M, 2016).The antibacterial properties of C. ternatea petals are the subject of several investigations.12 different kinds of bacteria (Bacillus cereus, B. subtilis, Bacillus thuringiensis Staphylococcus aureus, Streptococci faecalis, P. aeruginosa, K. pneumoniae, and Escherichia coli, the Enterobacter aerogens, Vibrio mirabilis, Salmonella typhi, and Herbaspirillum spp.) were evaluated in comparison to the extract of methanol of C. ternatea flower. It was discovered that Bacillus thuringiensis exhibited the strongest activity. By making use of the diffusion of agar wells method, the different extracts of CT leaves shown antibacterial action against fish pathogens, including P. aeruginosa, E. coli, or Escherichia coli K. pneumonia, Bacillus subtilis, A. formicans, A. hydrophila, and S. agalactiae (Ponnusamy S, 2010).Additionally, cyclotides made from CT in a prior work revealed potentially gram-negative-specific antibacterial action (Nguyen KN, 2016).CT leaf hydroalcoholic extract demonstrated antifungal efficacy (Das N, 2014).The antifungal activity of the plant Clitoria leaf extract against A. niger was good; the lowest concentrations of inhibition and fungicidal activity were 0.8 and 1.6 mg/ml, respectively. Upontreatment with Clitoria ternatea extract, the primary alterations seen under scanning electron microscopy were the fungal hyphae's loss of cytoplasm and a noticeably thinner, deformed hyphal wall that caused cell wall disruption. Furthermore, when The Clitorialeaf extract was applied to A. Niger, changes in conidiophores were also noted (Kamilla L, 2009)(55).Antibacterial activity against enteric fever-causing Shigella dysenteriae and Salmonella species was demonstrated (Shahid M, 2009).

Anti-oxidant activity:

Chronic and degenerative condition include autoimmune disorders and cancer, cardiovascular diseases, and neurodegenerative diseases are all influenced by oxidative stress.Antioxidants through There are natural sources of to improve human health(Admassu H, 2018)(Pham-Huy LA, 2008).Using antioxidant assays like 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) radical scavenging, ferrous reducing antioxidant power (FRAP), hydroxyl radical scavenging (HRSA), hydrogen peroxide scavenging, oxygen radical absorption capacity (ORAC), and superoxide radical scavenging activity, an array of studies examined the antioxidant activity of C. ternatea flowers. Antioxidant activity in vitro was established by the flower petal aqueous extract, which also protected erythrocytes from free radical-induced protein oxidation and lipid peroxidation (Phrueksanan W, 2014).The methanolic extract of CT leaves showed the protection against the aluminium induced oxidative damage in hippocampus of rats via restoring the actions of antioxidants like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in hippocampus (Mahalakshmi CHN Durga, 2015).The hepatoprotective activity of Clitoria ternatea leaves against liver damage caused by paracetamol was also investigated in mice with ME. Activity was assessed using histological examination in conjunction with the measurement of aspartate aminotransferase, alanine aminotransferase, and bilirubin levels. However, utilizing a number of antinociception models, the potential mechanism behind the antinociceptive impact of methanolic extracts of the species Clitorialeaves and roots was investigated. Clitoria ternatea Water-based leaf samples were analysed to determine their antioxidant potential by measuring the quantities of enzymatic and non-enzymatic antioxidants. Plant phenolics are effective at consuming free radicals as well asgenerally show strong antioxidant activity with an increase in extract concentration, the percentage among C. ternatea extracts that exhibited radical

scavenging activity increased. This indicated that a C. ternatea extracts eliminated the free radicals of DPPH in a manner that was dependent on concentration. Higher C. ternatea extract concentrations demonstrated greater antioxidant capacity than lower extract concentrations. GWE demonstrated the strongest scavenging activity among the four C. ternatea extracts. When an electron (hydrogen) is accepted by DPPH, which are stable free radicals, they can change into the non-radical form DPPH-H. This reduction is supported and reduced to strong violet colour of DPPH or a change to a yellow to colourless solution.

The following method was used to assess C. ternatea extracts' capacity to reduce DPP

$$\text{Free radical scavenging activity}(\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100\%$$

Anti-inflammatory activity:

The non-steroidal anti-inflammatory medicines (NSAIDs) that are now on the market, such as aspirin and acetaminophen, have been linked to adverse effects, especially cardiovascular and gastrointestinal ones, because they are known to impact both COX-1 and COX-2. Finding new or different approaches is necessary to lessen the hazards of NSAIDs while yet providing enough pain treatment (Brune K, 2015).According to the study, The excerpts could be able to prevent the manufacturing of kinins, prostaglandins, and other chemicals in carrageenan-induced edema (Shyamkumar IB, 2012)(.Additionally, it was discovered that the anthocyanins inhibited the generation of nitric oxide more than the flavanols produced. In western blot analyses, the flavanols markedly reduced COX-2 enzymes expression compared to the anthocyanins, while only the anthocyanins decreased nuclear factor-B translocation &iNOS expression of proteins (Nair V, 2015).Additionally, the ethanolic extract of CT's leaves and flowers shown anti-inflammatory properties in vitro (Suganya G, 2014).Methanol extract from Clitoria ternatea roots, administered orally to rats at 200–400 mg/kg, was observed to prevent acetic acid-induced vascular permeability in rats as well as carrageenin)-induced rat paw oedema. Additionally, the extract significantly reduced rats' pyrexia caused by yeast .The extract of petroleum ether (60–80°C) has strong analgesic and anti-inflammatory consequences (Parimaladevi B, 2004).Another study tested the antipyretic capacity of CT methanol extract in albino rats, and the extract's antipyretic impact was comparable to the instance of the common antipyretic drug paracetamol (PCM) (150 mg/kg).These results suggest that CT could help with the development of medications or dietary supplements that protect against chronic inflammatory disorders.

Anti diabetic activity:

Numerous investigations looked into the antidiabetic potential of C. The ternatea flower extract both in vitro as well as in vivo. In healthy men, acute consumption of C. ternatea flowers extract or beverage with sugar was observed to reduce postprandial plasma insulin and glucose levels in a randomised crossover study (Chusak C, 2018).The extract includes alkaloid substances, which may increase the β-cell's production of insulin or improve the movement of blood glucose from plasma to adjacent tissues .The 400 mg/kg b/w water-based extracts of Clitoria ternatea flowers and leaves significantly ($P<0.05$) decreased serum glucose, glycosylated haemoglobin, and the activities of the gluconeogenic enzyme glucose-6-phosphatase, while increasing serum insulin, the liver and skeletal muscle glycogen, and the action of the glycolytic enzyme glucokinase. Using leaf extract on rats displayed nearly identical profiles to those treated with floral extract in every biochemical test conducted (Daisy P, 2009) (M, 2009).

V. THERAPEUTICAL USES

The pharmacological actions of CT have been extensively screened. Its comparatively well-established neuropharmacological properties, including increased acetylcholine content, nootropic, anti-stress, anxiolytic, antidepressants, anticonvulsant, tranquilizing, and sedative effects, support its application in CNS disorders within the Ayurvedic medical system. In addition to its antimicrobial, antipyretic, anti-inflammatory, a diuretic, local anaesthetics, antidiabetic, and insecticidal qualities, it also inhibits blood platelet aggregation and relaxes vascular smooth muscle. Clonidine-induced hypothermia, lithium-induced head twitches, cold restraint stress (CRS)-induced ulcers, sodium nitrite-induced respiratory arrest, and haloperidol-induced cataleptic seizures in rats and mice were employed to assess the anti-stress properties of aerial components. A stem, root, & flowers have been utilized to cure scorpion stings and snakebite (Singh NK, 2018).The

in vitro inhibitory action of ternatins D1 derived from petals examined the aggregation of rabbit platelets in 2008. According to the findings, there was a significant suppression of collagen & adenosine diphosphate (ADP)-induced platelet aggregation. According to Daisy's 2004 study, giving alloxan-induced diabetic rats aqueous extracts of CT leaf & flower for 60 days markedly raised their white blood cell, red blood cell, T lymphocyte, and B lymphocyte counts. Additionally, these extracts were discovered to have immunomodulatory properties that fortify the immune system (Pasukamonset P, 2016)(80). As a biotechnological method of peptide ligation and cyclization, the butelase-1 enzyme that was isolated from C. ternatea pod is becoming more and more significant.

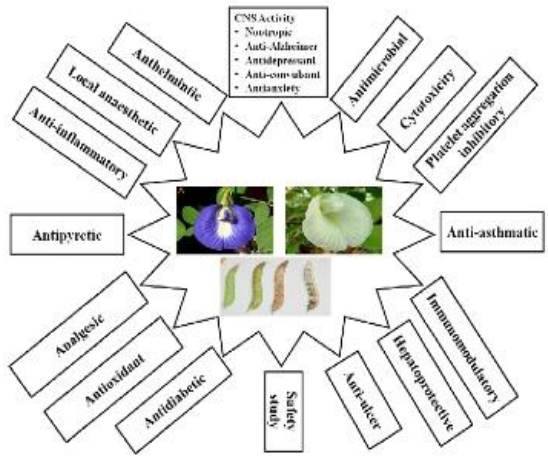

Figure 6: Various pharmacological activities of both white and blue clitoria ternatea

VI. CONCLUSION

The versatile plant Clitoria ternatea is well-known for its traditional uses as a cover crop, culinary coloring, and in ayurvedic medicine. Over the years, numerous useful studies on this plant have been conducted, providing more insight into its prospective applications by demonstrating its numerous health advantages. However, carefully thought-out research is required to assess CT's actual potential in cognitive impairment. Therefore, the effectiveness and safety for CT in treating different kinds of dementia may be clinically assessed .The study examined Clitoria ternatea as a potentially useful medicinal plant with a broad spectrum of pharmacological activity that, due to its efficacy and safety, might be applied in a number of medical applications.

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