International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

https://ijpsat.org/
Vol. 48 No. 2 January 2025, pp. 293-299

Clitorea ternatea L. (Botany, Bioactivity and Secondary Metabolites)

Marina Silalahi

Biology Education Department, Faculty of Teacher Training and Education,
Universitas Kristen Indonesia, Cawang, Jakarta
Email: marina_biouki@yahoo.com

Abstract – Clitorea ternatea is a species belonging to Fabaceae, has long been used by local Indonesian people as a food coloring, traditional medicine and ornamental plant. This study aims to explain the botany, bioactivity and secondary metabolites of C. ternatea. The study was conducted through an online literature study with the keywords C. ternatea and uses of C. ternatea. Data were analyzed qualitatively. C. ternatea is a perennial plant, climbing, odd compound leaves, flower crowns similar to clitoris which are generally blue, and pods. In traditional medicine C. ternatea is used to treat various diseases such as eye infections, boils, digestive system disorders, constipation, arthritis, skin disorders, and liver disorders. The bioactivities of C. ternatea such as antimicrobial, antioxidant, anti-inflammatory, and anti-diabetes mellitus. The anthocyanin found in C. ternatea flowers is ternatin, a polyacylated derivative of delphinidin 3,3',5'-triglucoside. C. ternatea flowers are widely used as food coloring and have antioxidant activity so that they have great potential as functional food ingredients or nutraceutical agents.

Keywords – C. Ternatea, Anti-Microbial, Ternatin.

Introduction

Interest in exploring natural materials for use as initial chemicals or intermediate compounds in the development of new drugs continues to increase [1], because it is considered more effective [2]. *Clitoria ternatea*, which is known by local Indonesian people as *kembang telang* is a multifunctional plant that is used for food, medicine and decoration [3]. The structure, beautiful flowers, and flowering throughout the year make *C. ternatea* easy to find in various yards. *C. ternatea* is able to tolerate various environmental parameters so that it is easy to cultivate in various conditions [4].

The flower extract of *C. ternatea* has great potential to be developed for human health as a food additive/coloring agent [5,6], antioxidant, and cosmetics [5]. In Indonesia, *C. ternatea* has been commercialized as "tea". Foods and drinks that are given *C. ternatea* flower extract have a blue to purplish color [6,7]. Anthocyanin compounds are responsible for the pigment which is a water-soluble compound but is unstable and easily degraded and fades whenever exposed to light [8].

The flowers of *C. ternatea* have been used as traditional medicine by various ethnic groups in Indonesia and other countries. *C. ternatea* flowers are believed to clear the eyes of babies by the Betawi ethnic group [9], to treat eye infections by local people in Lombok [10] and in Bali [11], and to treat boils by the Togian ethnic group [12] so that it has the potential to be used and developed as a nutraceutical ingredient [6]. The Togian tribe in Central Sulawesi uses *C. ternatea* leaves to treat fever [12]. In Indian Ayurvedic medicine, *C. ternatea* is used to treat digestive disorders, constipation, arthritis, skin diseases, liver problems, intestines [13], and eye diseases [14]. *C. ternatea* has been widely used as a brain tonic, improving memory and intelligence [2].

International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

https://ijpsat.org/
Vol. 48 No. 2 January 2025, pp. 293-299

The use of plants as traditional medicine is related to their bioactivity. Various researchers have reported the bioactivity of *C. ternatea* including anti-inflammatory [4,15], anti-diabetic [13,15,16,17], anti-microbial [13,15] anti-hyperlipidemic [15,16,17], antineoplastic [16], anti-cancer [15,17], antioxidant, analgesic, antipyretic, central nervous system, antiparasitic, gastrointestinal [15], anti-allergic, preventing cardiovascular damage [4] (, and cytotoxic [13].

Although there have been many studies on *C. ternatea*, those that link bioactivity to its secondary metabolite content are still limited. This study aims to explain the botany, benefits, bioactivity and secondary metabolites of *C. ternatea*.

METHODS

The research method is based on literature studies published online on Google Scholar using the keywords *C. ternatea* and uses of *C. ternatea*. The information obtained is synthesized to explain the botany, benefits, bioactivity, and secondary metabolites of *C. ternatea* as the basis for its development as a nutraceutical.

BOTANY of *Clitoria ternatea* L.

Fabaceae is morphologically easily recognized from its pods. Fabaceae has about 19,400 species and about 730 genera [18]. One genus that is easily found in the surrounding environment is the Genus *Clitoria* L. which is estimated to have about 90 species [19].

Description: Woody, twining vine, up to 1–3 m long. Stems slender, cylindrical, with lines of small trichomes. Leaves alternate, 5–7 leaflets; leaflets opposite, 1.5–4.5 1–3.5 cm, elliptical or less often ovate or oblong, glabrous, apex rounded, obtuse, sometimes reticulate or mucronate, basally obtuse, margin entire; upper surface dark green, dull, puberulent, with concave midvein; lower surface pale green, dull, puberulent, with prominent venation; ribs 2–7 cm long; petioles minute, pubescent; petioles 2–4 cm long, pubescent, with thickened base; stipules filiform, 1.5 mm long; stipules lanceolate, pubescent, ca. 4 mm long. Flowers solitary, on short pedicels; pedicels 1 cm long, pubescent, with a pair of bracteoles in the middle. Calyx campanulate, 1.5–2.2 cm long, green, puberulent, lobes lanceolate-ovate, 8–10 mm long, with conspicuous central vein; corolla blue-violet, standard broadly ovate, 3.5–5 cm long, resected, with pale yellow base and pale yellow center. Nuts 9–11 1 cm long, oblong, ribbed along both edges, apex acuminate. Seeds numerous, 5–6 mm long, oblong, flat, dark brown [3,20].

International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

https://ijpsat.org/
Vol. 48 No. 2 January 2025, pp. 293-299

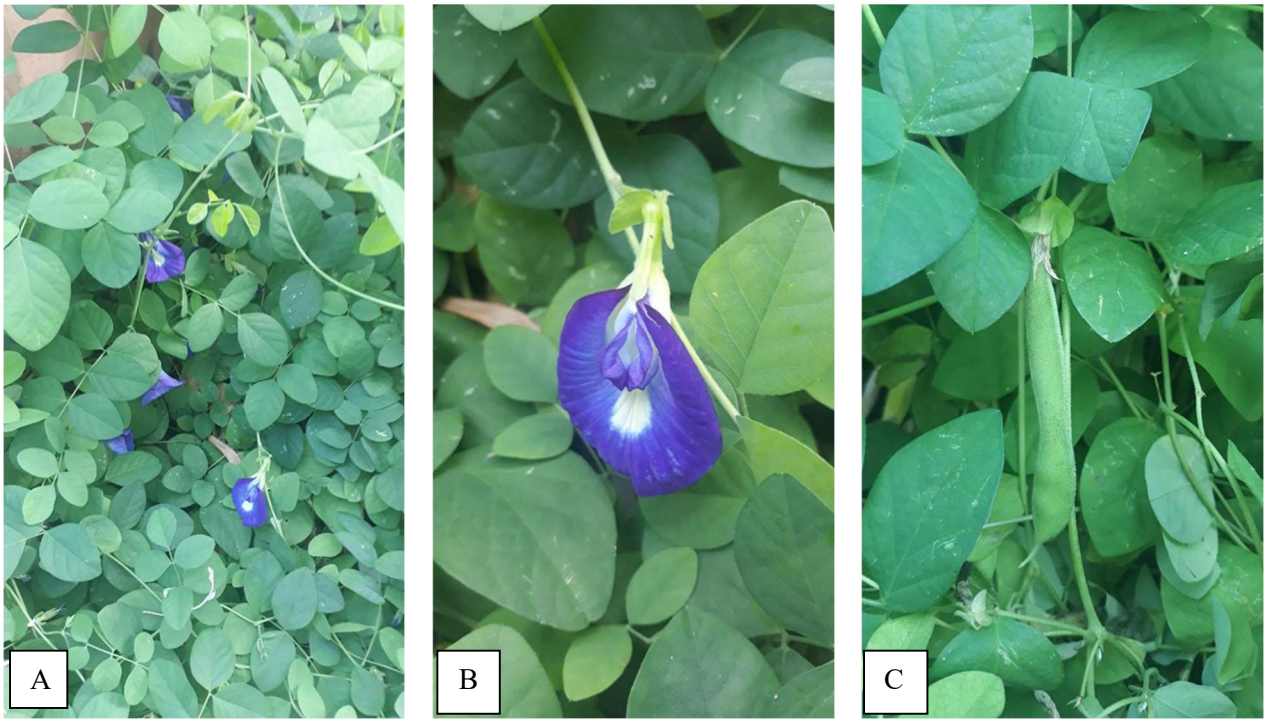

Figure 1. *Kembang telang* or *C. ternatea* A. Habitus; B. The flowers are blooming with purple crowns; C. The young pods.

UTILIZATION AND BIOACTIVITY

ANTI-MICROBIAL

Exploration of natural antimicrobial compounds continues to be carried out as one of the anticipations of the emergence of microbes resistant to commercial antibiotics. Various bacteria, viruses, and pathogenic fungi that contribute to infectious diseases in humans that cause death [21]. Antimicrobials are compounds that can inhibit growth or cause death of microbes. Proteus mirabilis is a microbe that causes urinary tract infections [22]. The anthocyanins of *C. ternatea* have antimicrobial activity in vivo and in vitro (both antibacterial and antifungal) [8].

In laboratory research, compounds used as positive controls as antimicrobials include ciprofloxacin, gentamicin and tetracycline [22]. *C. ternatea* has potential antimicrobial activity against Escherichia coli [6,8,21,23,24,25,26], Bacillus subtlis [6,8,23,26], Bacillus cereus [6,21], Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus agalactiae and Aeromonas hydrophila [21], and Proteus mirabilis [22]. In addition to inhibiting bacterial growth, methanol extract of *C. ternatea* seeds has antifungal activity against A. niger, P. chrysogenum [23], Fusarium sp., Aspergillus niger and Trichoderma sp. [8], Aspergillus parasiticus [25], Candida albicans, Aeromonas hydrophila [26], and Coniochaeta hoffmannii [14].

The bioactivity of *C. ternatea* as an antimicrobial is greatly influenced by various factors, such as the polarity of the compound used in the extraction, the type of microbe, the concentration and the organ used. The highest antibacterial activity of *C. ternatea* against Proteus mirabilis was observed in the acetone extract and the lowest in the petroleum ether extract [22]. The anthocyanin-rich *C. ternatea* flower fraction had stronger antibacterial activity than the crude extract tested on Bacillus cereus, Bacillus subtilis, and Escherichia coli [6]. *C. ternatea* extract inhibited the growth of Proteus mirabilis but its activity was still lower than standard antibiotics (ciprofloxacin, gentamicin and tetracycline) [22]. Gram-positive bacteria were more sensitive to *C. ternatea* extract than Gram-negative bacteria [27].

International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

https://ijpsat.org/
Vol. 48 No. 2 January 2025, pp. 293-299

ANTIOXIDANT

The study of *C. ternatea* bioactivity as an antioxidant is more prominent than other bioactivities. Antioxidant tests in the laboratory were carried out through 1,1-diphenyl-2-picrylhydrazyl (DPPH) tests [25,28], hydroxyl radicals and hydrogen peroxide [28]. The bioactivity of plants as antioxidants is associated with the content of phenolic, phenolic and flavonoid compounds, therefore their activity is proportional to their levels [25,28,29].

Factors that affect the effectiveness of antioxidants include solvent polarity, so that different solvents show different phytochemical results and antioxidant capacities [30]. The all organs of *C. ternatea* including leaves, flowers [28] and seeds [26,29] have antioxidant activity, but the levels vary. The antioxidant activity of immature *C. ternatea* seeds is higher than that of mature seeds [31]. *C. ternatea* leaves show higher amounts of total phenolic content than flowers [32]. The crude methanol extract of *C. ternatea* seeds showed maximum antioxidant activity with DPPH test (86.26%) while the crude methanol extract showed maximum reducing power of 370.19% ± 20.50 [25].

The antioxidant activity of anthocyanin-rich fractions was stronger using the DPPH test compared to the cellular antioxidant test using RAW 264.7 macrophages [6]. This activity is thought to be related to the content of phenolic compounds. The n-hexane extract of CT seeds had the highest phenolic content (272.16 ± 17.96 GAE µg/g), the crude methanol extract had the highest flavonoid content (275.5 ± 8.24 GAE µg/g) [25].

Total phenolic content, flavonoids are proportional to antioxidant activity (DPPH, ABTS/ 2,2-azino-bis-3-Ethylbenzothiazoline-6-Sulfonic Acid, FRAP/ferric reducing antioxidant power, reducing activity, Cu^{2}+ and H_2O_2) [33]. The water extract of *C. ternatea* has the lowest antioxidant activity, while methanol extract has quite high antioxidant activity when compared to control (quercetin), according to DPPH scavenging test [27]. Temperature and time are physicochemical factors of *C. ternatea* flowers that affect the levels of extracted phenolic compounds and their antioxidant activity [30]. The maximum scavenging activity of total phenolics and ABTS for aqueous methanol extract is 80%, while DPPH reducing power and flavonoid content are maximum in methanol extract [29].

ANTI-INFLAMMATORY

The Ayurvedic system of medicine, *C. ternatea* is well known for treating several inflammatory diseases like rheumatism [34]. Ethanol extract of *C. ternatea* root (EECT) has anti-inflammatory and anti-rheumatic activities in animal models. The EECT showed significant reduction in mean paw edema volume in inflammation induced by carrageenan and histamine. A significant reduction in paw diameter was observed in EECT (200 and 400 mg/kg) and diclofenac (10 mg/kg) treated groups after 7th day [34].

ANTI DIABETES MELLITUS

Diabetes mellitus is a metabolic disorder that causes blood glucose levels above normal, also known as hyperglycemia. Natural ingredients that have the potential as anti-diabetes mellitus are compounds that inhibit the breakdown of polysaccharides into glucose such as α-amylase. The *C. ternatea* phenolics significantly inhibit α-amylase [16,30,35], α-glucosidase, and lipase [16,30], angiotensin-I-converting (ACE-I) [30]. Crude methanol extract (α-amylase), n-hexane extract and water-soluble residue showed 50% inhibition of the pepsin enzyme [25].

SECONDARY METBOLITES

Secondary metabolites of plants are produced as an adaptation to the environment, which are utilized by humans for various purposes including dyes, food ingredients and medicines. Based on their formation pathways, secondary metabolites of plants are grouped into alkaloids, terpenoids and phenolic compounds. Anthocyanins are one of the derivatives of phenolic compounds found in *C. ternatea* [30].

The structure, content, and type of secondary metabolites produced by plants vary, which is related to their bioactivity. In general, *C. ternatea* contains tannins, phlobatannins, carbohydrates, saponins, triterpenoids, phenols, flavonoids, flavanol glycosides, proteins, alkaloids, anthraquinones, anthocyanins, cardiac glycosides, Stigmast4-ene-3,6-dione, essential oils, and

steroids [15]. These contents vary between organs. *C. ternatea* can cure various diseases and has high antioxidant activity due to its bioactive compound content such as anthocyanins, alkaloids, steroids, tannins, reducing sugars, and flavonoids [4].

The uniqueness of anthocyanins in *C. ternatea* flowers is the high amount of polyacylated anthocyanins known as ternatin [7]. Ternatin is a polyacylated derivative of delphinidin 3,3′,5′-triglucoside [7]. Anthocyanins of *C. ternatea* flowers have been applied to food, which show good thermal and storage stability, but lower photostability. The anthocyanins of *C. ternatea* show a deep blue color at acidic pH between pH 3.2 to pH 5.2 [7].

C. ternatea leaves contain bioactive compounds such as -(2-ethylhexyl) phthalate, (2S,5S)-2,5-dimethyl-2-phenyl-1,3-dioxolane-4-one, 5,10-dihexyl-5,10-dihydroindolo (3,2-b) indole-2,7 dicarbaldehyde, 9,12-octadecadienoic acid (Z,Z), 2-hydroxy-1-(hydroxyl methyl) ethyl ester (CAS), neophytadiene and nonacosane (CAS) [36]. The stem contains clitorternalactone, clitorienolactone A, myricetin 3-glucoside, quercetin 3-glucoside, kaempferol 3 -glucoside, taraxerol, and taraxerone [37]. Water and methanol extracts of *C. ternatea* seeds contain alkaloids, carbohydrates, glycosides, flavonoids, tannins, saponins, amino acids, proteins, terpenoids. Quantitatively, methanol extracts of seeds and leaves have good phenol, carbohydrate, tannin, flavonoid and terpenoid content compared to other organs [23]. *C. ternatea* flowers contain kaempferol, quercetin and myricetin glycosides and anthocyanins [13]. *C. ternatea* content is related to biological activities such as β-sitosterol, taraxerol, clitorienolactone B and β-sitosterol glycosides [29]

Conclusions

C. ternatea as traditional medicine is used to treat various diseases such as eye infections, boils, digestive system disorders, constipation, arthritis, skin disorders, and liver disorders. The bioactivities of *C. ternatea* such as antimicrobial, antioxidant, anti-inflammatory, and anti-diabetes mellitus. The anthocyanin found in *C. ternatea* flowers is ternatin, a polyacylated derivative of delphinidin 3,3',5'-triglucoside.

References

[1] Makasana, J., Dholakiya, B.Z., Gajbhiye, N.A., & Raju, S. (2017). Extractive determination of bioactive flavonoids from butterfly pea (*Clitoria ternatea* Linn.). Research on Chemical Intermediates, 43, 783-799.

[2] Shahnas, N., & Akhila, S. (2014). Phytochemical, in vitro and in silico evaluation on *Clitoria ternatea* for alzheimer's disease. PharmaTutor, 2(9), 135-149.

[3] Silalahi, M. (2021). *Clitoria ternatea* L. Fabaceae. In Ethnobotany of the Mountain Regions of Southeast Asia (pp. 317-322). Cham: Springer International Publishing.

[4] Jamil, N., Zairi, M.N.M., Nasim, N., & Pa'ee, F. (2018). Influences of environmental conditions to phytoconstituents in *Clitoria ternatea* (butterfly pea flower)–A review. Journal of Science and Technology, 10(2).

[5] Ravindran, D.R., Bharathithasan, M., Ramaiah, P., Rasat, M.S.M., Rajendran, D., Srikumar, S., ... & Mohd Amin, M.F. (2020). Chemical composition and larvicidal activity of flower extracts from Clitoria ternatea against Aedes (Diptera: Culicidae). Journal of Chemistry, 2020(1), 3837207.

[6] Jeyaraj, E.J., Lim, Y.Y., & Choo, W.S. (2022). Antioxidant, cytotoxic, and antibacterial activities of *Clitoria ternatea* flower extracts and anthocyanin-rich fraction. Scientific reports, 12(1), 14890.

[7] Vidana Gamage, G.C., Lim, Y.Y., & Choo, W.S. (2021). Anthocyanins from *Clitoria ternatea* flower: Biosynthesis, extraction, stability, antioxidant activity, and applications. Frontiers in Plant Science, 12, 792303.

[8] Mahmad, N., Taha, R.M., Othman, R., Abdullah, S., Anuar, N., Elias, H., & Rawi, N. (2018). Anthocyanin as potential source for antimicrobial activity in *Clitoria ternatea* L. and Dioscorea alata L. Pigment & Resin Technology, 47(6), 490-495.

[9] Marpaung, A.M. (2020). Tinjauan manfaat bunga telang (*Clitoria ternatea* L.) bagi kesehatan manusia. Journal of Functional Food and Nutraceutical, 63-85.

[10] Eni, N.N.S., Sukenti, K., Aida, M., & Rohyani, I.S. (2019). Studi etnobotani tumbuhan obat masyarakat komunitas Hindu Desa Jagaraga, Kabupaten Lombok Barat, Nusa Tenggara Barat. Biotropika: Journal of Tropical Biology, 7(3), 121-128.

[11] Oktavia, G.A.E., Darma, I.D.P., & Sujarwo, W. (2017). Studi etnobotani tumbuhan obat di kawasan sekitar Danau Buyan-Tamblingan, Bali. Buletin Kebun Raya, 20(1), 1-16.

[12] Tabeo, D. F., Ibrahim, N., & Nugrahani, A. W. (2019). Etnobotani Suku Togian di Pulau Malenge Kecamatan Talatako, Kabupaten Tojo Una-Una, Sulawesi Tengah. Biocelebes, 13(1).

[13] Jeyaraj, E.J., Lim, Y.Y., & Choo, W.S. (2021). Extraction methods of butterfly pea (*Clitoria ternatea*) flower and biological activities of its phytochemicals. Journal of food science and technology, 58(6), 2054-2067.

[14] Yolin Angel, P.A.S.R., Jeyakumar, P., Jasmin Suriya, A.R., Sheena, A., Karuppiah, P., Periyasami, G., ... & Murugan, K. (2024). Topical antifungal keratitis therapeutic potential of *Clitoria ternatea* Linn. flower extract: phytochemical profiling, in silico modelling, and in vitro biological activity assessment. Frontiers in Microbiology, 15, 1343988.

[15] Al-Snafi, A.E. (2016). Pharmacological importance of Clitoria ternatea–A review. IOSR Journal of Pharmacy, 6(3), 68-83.

[16] Li, C., Tang, W., Chen, S., He, J., Li, X., Zhu, X., ... & Peng, Y. (2022). Phytochemical properties and in vitro biological activities of phenolic compounds from flower of *Clitoria ternatea* L. Molecules, 27(19), 6336.

[17] Kumar, M., & More, D. (2019). Phytochemical analysis and bioactivity of selected medicinal plant of butterfly-pea (*Clitoria ternatea* L.) used by Kolam tribe Addjoing region of Telangana and Maharashtra states. Pharma Innovation, 8(1), 417-421.

[18] Lewis, G.P., Schrire, B.D., Mackinder, B.A. & Lock, M. (2005). Legumes of the world. Royal Botanic Gardens, Kew.

[19] Fantz, P.R. (1978). A monograph of the genus *Clitoria* (Leguminosae: Glycineae). Dissertation Abstracts International B 38(11): 5162.

[20] Acevedo-Rodriguez P. (2005). Vines and climbing plants of Puerto Rico and Virgin Islands. Contributons from the United States National Herbarium 51: 1-483.

[21] Jamil, N., & Pa'ee, F. (2018). Antimicrobial activity from leaf, flower, stem, and root of *Clitoria ternatea*–A review. In AIP Conference proceedings. AIP Publishing.

[22] Dhanasekaran, S., Rajesh, A., Mathimani, T., Samuel, S.M., Shanmuganathan, R., & Brindhadevi, K. (2019). Efficacy of crude extracts of *Clitoria ternatea* for antibacterial activity against gram negative bacterium (Proteus mirabilis). Biocatalysis and agricultural biotechnology, 21, 101328.

[23] Chakraborty, S., Sahoo, S., Bhagat, A., & Dixit, S. (2017). Studies on antimicrobial activity, phytochemical screening tests, biochemical evaluation of *Clitorea ternatea* linn. plant extracts. International Journal of Research–Granthaalayah, 5(10), 197-208.

[24] Islam, M.A., Mondal, S.K., Islam, S., Shorna, A., Nourin, M., Biswas, S., ... & Saleh, M.A. (2023). Antioxidant, cytotoxicity, antimicrobial activity, and in silico analysis of the methanolic leaf and flower extracts of *Clitoria ternatea*. Biochemistry Research International, 2023.

[25] Mushtaq, Z., Khan, U., Seher, N., Shahid, M., Shahzad, M.T., Bhatti, A.A., & Sikander, T. (2021). Evaluation of antimicrobial, antioxidant and enzyme inhibition roles of polar and non-polar extracts of *Clitoria ternatea* seeds. JAPS: Journal of Animal & Plant Sciences, 31(5).

[26] Sreekala, S., & Muraleedharan, U.D. (2021). Cationic *Clitoria ternatea* seed peptide as a potential novel bioactive molecule. Protein and Peptide Letters, 28(11), 1259-1271.

[27] Zulkamal, L.M., Zolhalim, N.A.A., Aris, F., Zakaria, N.A., Yusof, F.Z.M., Ibrahim, D., & Jalil, M.T.M. (2023). Bioactivity of *Clitoria ternatea* crude extracts against pathogenic bacteria. Malaysian Applied Biology, 52(2), 41-49.

[28] Lakshmi, C.H.N.D., Raju, B.P., Madhavi, T., & Sushma, N.J. (2014). Identification of bioactive compounds by FTIR analysis and in vitro antioxidant activity of *Clitoria ternatea* leaf and flower extracts. Indo American Journal of Pharmacy and Research, 4(9), 3894-3903.

[29] Makasana, J., Gajbhiye, N., Kumar Bishoyi, A., Savaliya, M., Raju, S., Bansod, S., ... & Dholakiya, B. (2023). Exploration of HPTLC technology for rapid chemical fingerprinting and simultaneous determination of bioactive constituents from *Clitoria ternatea* Linn. Chemistry Select, 8(13), e202203217.

[30] Escher, G.B., Marques, M.B., do Carmo, M.A.V., Azevedo, L., Furtado, M.M., Sant'Ana, A.S., ... & Granato, D. (2020). *Clitoria ternatea* L. petal bioactive compounds display antioxidant, antihemolytic and antihypertensive effects, inhibit α-amylase and α-glucosidase activities and reduce human LDL cholesterol and DNA induced oxidation. Food research international, 128, 108763.

[31] Padmanabhan, V., Kumar, S.S., & Giridhar, P. (2024). Phytochemicals and UHPLC-QTOF-HRMS characterisation of bioactives of butterfly pea (*Clitoria ternatea* L.) seeds and their antioxidant potentials. Food Chemistry, 433, 137373.

[32] Rabeta, M.S., & Nabil, Z. (2013). Total phenolic compounds and scavenging activity in *Clitoria ternatea* and *Vitex negundo* linn. International Food Research Journal, 20(1).

[33] Mehmood, A., Ishaq, M., Zhao, L., Yaqoob, S., Safdar, B., Nadeem, M., ... & Wang, C. (2019). Impact of ultrasound and conventional extraction techniques on bioactive compounds and biological activities of blue butterfly pea flower (*Clitoria ternatea* L.). Ultrasonics sonochemistry, 51, 12-19.

[34] Swathi, K.P., Jayaram, S., Sugumar, D., & Rymbai, E. (2021). Evaluation of anti-inflammatory and anti-arthritic property of ethanolic extract of *Clitoria ternatea*. Chinese Herbal Medicines, 13(2), 243-249.

[35] Pasukamonset, P., Kwon, O., & Adisakwattana, S. (2016). Alginate-based encapsulation of polyphenols from *Clitoria ternatea* petal flower extract enhances stability and biological activity under simulated gastrointestinal conditions. Food hydrocolloids, 61, 772-779.

[36] Sarumathy, K., Rajan, M.D., Vijay, T., & Jayakanthi, J. (2011). Evaluation of phytoconstituents, nephro-protective and antioxidant activities of *Clitoria ternatea*. Journal of Applied Pharmaceutical Science, (Issue), 164-172.

[37] Wu, F.S., Hung, C.J., Lin, C.L., Chang, T.H., Chen, C.L., Sung, P.J., ... & Chen, J.J. (2020). New norneolignan and bioactive constituents of *Clitoria ternatea*. Chemistry of Natural Compounds, 56, 1000-1004.