

Antioxidant activity of Different Extracts of Clitoria *ternatea* (Blue Butterfly pea Flower)

Sakshi Singh, Siddharth Agrawal and Babita Agrawal

Department of Chemistry, C.M.P. Degree College, Prayagraj (University of Allahabad) Email: babitaprashantagrawal@gmail.com

Abstract

A tropical flower with the common names Butterfly pea flower and Bunga telang, Clitoria ternatea is renowned as one of the primary suppliers of polyphenols with potent anti-oxidant characteristics. Natural blue colourants benefit from the abundance of anthocyanin chemicals produced by Clitoria ternatea. As the least stable and most quickly damaged anthocyanins after extraction are those that give this colour, bluish colouring agents are now the most challenging to produce. Their stability frequently depends on a amount of operational factors, including sun exposure, high temperatures, and pH shifts. In addition to its colour properties, anthocyanins They were recognised as a source of beneficial atoms as a result of its antioxidant abilities and advantageous health impacts, which include antibacterial, anti-cancer, and anti-obesity properties. This experiment was done with the intention of quantitatively characterising the molecular components of the polyphenols that can be extracted with ethanol from the C. ternatea extract. Strong antioxidant capabilities are well-known for phenolic chemicals and flavonoids. The Clitoria ternatea floral ethyl acetate extract, standard to gallic acid, showed the highest level of antioxidant properties. As a result, the ethyl acetate extract (90.93%) of the flower demonstrated higher antioxidant activity than the ethanol extract (75.19%). So we can firmly say that novel finding that Flower ethanol extract (Clitoria ternatea) exhibited excellent Antioxidant Activity.

Key Words: Clitoria ternatea, Anthocyanin, Phytochemical, antioxidant activity, Biological activity

Introduction

Clitoria *ternatea*, also referred to as a permanent twiner is the butterfly pea blossom that relates to the Fabaceae family (N, J., M.n.m, Z., & N.A.I.M, N. 2018). This flower can develop quickly; therefore gardens and the wild frequently have it blooming (Mukherjee P.K. et al., 2008) With several common names, Clitoria *ternatea* is widely cultivated in numerous nations for examples, Aparajita (India), Pukingan (Philippines), Ang Chan (Thailand), 'bunga telang' (Malaysia), Aparajita (India), as well Die Dou (China) (Kosai P. et al., 2015, Subramanian M.S.et al., 2011,

Singh, Agrawal & Agrawal (2023), Research Communications

Mukherjee P.K., 2008) Since Clitoria ternatea has numerous cultivars with different bloom hues, it is renowned for being a beautiful decorative climber and regarded as an must-have ornamental plant for garden enthusiasts (Luengwilai, T. H. & K. 2019, Oguis G.K et al., 2019). Its petals came in two main hue varieties: blue and white (Landim Neves et al., 2021, Taranalli A.D et al., 2000, Kazuma K et al., 2003) Different anthocyanins' presence in the flower's chemical makeup is primarily what causes the variety of petal colours. The "Double blue" line flower specifically was noted for assembling a large number of polyacylated anthocyanins with ternatins. (Lakshan S.A.T et al., 2019, Kazuma K et al., 2003) while the line of white petals appeared to lack anthocyanins (Al-Snafi A.E 2016). Considering the anthocyanins that have gathered in the petals of the Clitoria ternatea flower, which can provide a brilliant blue colour (Figure. 1), It's commonly used as a food colouring (Nur F et al., 2018, Luengwilai, T. H. & K. (2019). secondary metabolites include kaempferol, glycosides, myricetin, flavonols, and the phenolic acids are present. (N, J., M.n.m, Z., & N.A.I.M, N. (2018), and anthocyanidins (Luengwilai, T. H. & K. (2019)) was considerably demonstrated by phytochemical analysis to the flower sections. In contrast to other therapeutic plants, the anthocyanin pigment found in Butterfly flowers gives them more antioxidant qualities. These effects include antidiabetic, antibacterial, anticancer, and anti-inflammatory properties (Taranalli A.D et al., 2000)



Figure 1: Clitoria *ternatea Flower*

Clitoria *ternatea* is rich in these bioactive compounds. Extraction was the first step in removing antioxidants from the plant. These extraction efficiency is affected by a number of factors, including sample size of particles, solvent types, operating circumstances, extraction techniques, and phytochemical chemical makeup. (Do Q.D et al., 2014). Perhaps because Ethanol is commonly used as an extraction solvent because it has been determined to be safe (GRAS) for use in food and medicinal applications. Additionally, a number of research show that the optimal organic solvent mixture for isolating or recovering the plant-based phenolic compounds is an aqueous mixture. Quantitating the phytochemical properties from the flower petals, such as their overall amount of flavonoids and phenols, total anthocyanin content, and antioxidant activities, was necessary to complete this task in order to gain a deeper understanding of the potential uses of Clitoria ternatea. (Zhang L et., 2019) Flavonoids and phenolic compounds are recognised for

Singh, Agrawal & Agrawal (2023), Research Communications

their strong antioxidant properties. Recent study investigated the phenolic composition of Clitoria *ternatea* extracts and found a wide range of phenolic substances, likeas quercetin, kaempferol, and catechin. These substances have the ability to scavenge free radicals, shielding cells from oxidative harm. (Jimenez- Moreno et al., 2019) In addition to its direct antioxidant activity, it has been demonstrated that Clitoria ternatea increases the activity of natural antioxidant enzymes. Recent study by Tang et al. (2020) demonstrated that administration of Mice's levels of catalase (CAT) and superoxide dismutase (SOD) were elevated by Clitoria ternatea extract. These enzymes break down dangerous Reactive Oxygen Species (oxygen species that are reactive) (ROS), which are essential to the body's defence against oxidative stress.

Antioxidant activity is among the principal characteristics that has drawn scientific attention. Antioxidants are substances that assist in counteracting harmful the body's free radicals, shielding cells from cellular oxidative damage as well as possible harm. In this article, we shall look into the Clitoria ternatea flowers' capacity as antioxidants. To do this, we will compare studies on the Clitoria ternatea flowers' antioxidant activity in ethanol and ethyl acetate extracts using conventional techniques. In this research paper, Using approved methods; we will look into the antioxidant capacity of Clitoria ternatea flowers in different extracts.

Blooms of Blue Pea Anthocyanins

The class of pigments that are water-soluble known as anthocyanins gives many vegetables, flowers, and fruits their vivid colours. The existence of different anthocyanins contributes to the wide spectrum of colors, ranging blue to the purple and red, reliant on the environment's pH. The concentration and composition of Blue pea blooms contain anthocyanins can differ based on elements such as the plant's age and its growth environment, and genetic factors. In a recent study, researchers discovered that the anthocyanin content of butterfly pea flowers increased during the flower's early developmental stages and reached a peak just before full bloom (Chutichuda c et .al. 2017). These bioactivity and health benefits anthocyanin content of blue pea blossom have attracted significant scientific interest. Strong antioxidant qualities found in anthocyanins aid in the body's defence against dangerous free radicals, lowering the effects of oxidative stress and the risk of tissue and cell damage. Numerous health advantages, including as enhanced cardiovascular wellness, anti-inflammatory effects, and even anticancer qualities, have been connected to these antioxidants.

In conclusion, the blooms of blue pea plants are abundant in anthocyanins. Which contribute to their vibrant blue color. These anthocyanins have a range of possible health advantages due to their antioxidant properties. Traditional medicine has utilized blue pea flowers for their calming and diuretic effects, and they have found a place in culinary applications and natural food colorants. Nonetheless, To fully investigate their bioactivity and therapeutic potential, more research is necessary. The allure of these enchanting blue flowers continues to captivate people, making them a unique and a useful supplement to diverse cultural practices worldwide.

Polyacylated anthocyanins, which are abundant in the blue pea bloom, Clitoria ternatea L are more stable than non-acylated anthocyanins, therefore they can be used as a natural food colouring. (Sowndhararajan K.et. al., 2017). Like other anthocyanins, the pH affects the hue of

Singh, Agrawal & Agrawal (2023), Research Communications

the blue pea blossom anthocyanin extracts. Between pH values of 3.2 and 5.2, red colour occurs; between pH levels of 5.2 and 8.2, pale blue hue exists; and between 8.2 and 10.2, pale blue hue hanges to emerald green colour. (Buchweitz M. et al., 2012, Marpaung A. M et al., 2019 t's possible that structural modifications to anthocyanin molecules and a change in the medium's OH- and H+ concentrations led to this colour shift. (Escher G.B et al., 2020, Liu S et al., 2014)

Preparation of butterfly pea flower (Clitoria ternatea) Extract

In July, the dehydrated Butterfly pea flowers were harvested from Prayagraj, Uttar Pradesh, India's Company Garden. The flowers were manually cleaned, then ground into a powder using an electric blender and a 20-gauge mesh filter (Figure 2). For additional analysis, the BPF powdered was kept at -20 °C after being enclosed in bags of plastic.

Materials with Methods

Methanol AR was purchased from Pvt. Ltd. Loba Chemie. in Mumbai, India, and 1, 1-diphenyl-2-picrylhydrazyl (DPPH*) was purchased from HiMedia Pvt. Ltd. in Mumbai, India. Using a UV-visible spectrophotometer (Systronic, Model No. 119), the absorbance of every sample was measured. In July 2023, the tests were conducted in the Chemistry Department of CMP Degree College that is a constituent college of the University of Allahabad.



Figure. 2: Pictorial view of Clitoria *ternatea* flower A) Clitoria *ternatea* flowers, B) Clitoria *ternatea* flower Powder. DPPH^{*} (1, 1- Diphenyl-2-picrylhydrazyl) radical scavenging activity

The antioxidant capacities were evaluated by means of the DPPH* radical scavenging method. A stable radical known as DPPH* was employed to measure the ability of the different extracts to donate hydrogen or scavenge radicals. Gallic acid was used as standard. As a reagent for our spectrophotometric test, we are using the stable radical DPPH*. Measuring the drop in DPPH* absorbance at its highest absorption of 517 nm is the technique involved. Concentration of DPPH* was 0.002 percent in methanol.

The extraction Stock solutions were created using 1.0 mg/10 ml of methanol. Various volumes of extracts (2.0, 1.5, 1.0, 0.5, 0.25, and 0.125 ml) were taken in separate test tubes and Methanol was added to the volume to get it up to 2 ml. Two millilitres of each concentration of CT flower extracts (2 ml) were mixed with two millilitres of DPPH in 0.004% (v/v) methanol and incubated at room temperature for thirty minutes when it's dark.. Gallic acid was also treated in the same way. The optical absorbance was then determined using a UV-visible spectrophotometer at 517 nm.

The formula that follows was utilised for calculating the results: DPPH* and Methanol was utilised as a control. Each sample was examined in triplicate.

% Inhibition of DPPH* activity = (Ac - As / Ac) X 100

Where Ac = Absorbance of control, As = Absorbance of sample.

Final Results

The Antioxidant Potantial value of ethanol with ethyl acetate extracts of Flowers of Clitoria *ternatea*, were compared and evaluated by DPPH* assay (Table 1).

S.No.	Solvent/ Extract	Flower
1.	Ethanol	75.19%
2.	Ethyl acetate	90.93%

Gallic Acid (Standard): 95.66%

<u>% Antioxidant activity (Through DPPH* analysis)</u>

 Table 1: Antioxidant Capability of ethanol and ethyl acetate extracts of Flowers of the Clitoria ternatea L.

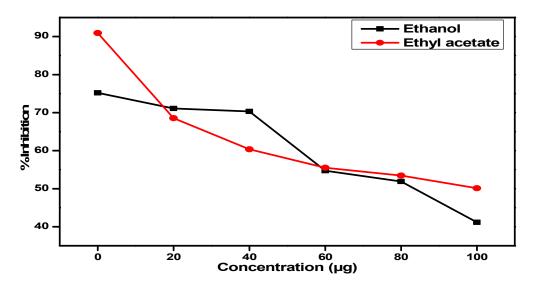


Figure.3: Ethanol and Ethyl acetate extraction of Flower of Clitoria ternatea L.

Discussion

The capacity to give electrons or hydrogen atoms to free radicals in order to displace them and stop harm from free radicals is the antioxidant capability.

Anthocyanins show antioxidant activity both in vivo and in vitro. The antioxidant properties of phenol and extract of ethyl acetate from Clitoria ternatea flowers are thought to be accountable for the blue pea flower's ability to prevent cardiovascular and neurological conditions, cancer, and diabetes. The lowering levels of the ethanol and ethyl acetate extracts from Clitoria ternatea flowers were 75.19% and 90.93%, respectively. The ethyl acetate extracts of flower shows Antioxident activity which are similar to Gallic acid (standard) 95.66%.

Conclusion

Phenolic compounds consist of one or more aromatic ring structures with an abundance of hydroxyl groups attached to them. which have the ability to squelch free radicals, and that their structure is directly in connection with their antioxidant effect a single or many aromatic rings ts, we can confidently state that the phenolic, anthocynin and flavonoid components present in the extracts have antioxidative potential. We observed that medicinal potential of the Clitoria *ternatea* L. plant extracts chosen for Antioxidant activity has not been carefully examined. The present study aimed to ascertain screen for phytochemicals present in a number of excerpts from different part of plant Clitoria *ternatea* L. Flowers extract and examine its Antioxidant potential. The newly investigated that Ethyl acetate extracts of Flowers shows outstanding Antioxidant activity. This research would be very helpful in treating various kinds of oxidative stress and disease.

Acknowledgment

The CMP Degree College's (A Constituents PG College of the University of Allahabad) Chemistry Department, Prayagraj, is acknowledged by the writer for providing the essential resources.

References

- Al-snafi, A.E. (2016). Pharmacological importance of *Clitoria ternatea* A review Pharmacological importance of Clitoria ternatea A review. *IOSR Journal of Pharmacy*, 6(3), 68–83.
- Buchweitz, M., Nagel, A., Carle, R., and Kammerer, D. R. (2012). Characterisationof sugar beet pectin fractions providing enhanced stability of anthocyaninbased natural blue food colourants. Food Chem, 132, 1971–1979. doi: https://doi.org/10.1016/j. foodchem.2011.12.034.
- Chutichude, C., Pattamayutanon, P. (2017). Composition of Anthocyanin Pigments and Total Phenolics in Clitoria ternatea Flowers. HortScience, 52(9), 1311-1315.
- Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S. and Ju, Y.H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content,

and antioxidant activity of Limnophila aromatica. *Journal of Food and Drug Analysis*, 22(3), 296–302. https://doi.org/10.1016/j.jfda.2013.11.001

- Escher, G. B., Wen, M., Zhang, L., Rosso, N. D., and Granato, D. (2020). Phenolic composition by UHPLC-Q-TOF-MS/MS and stability of anthocyanins from Clitoria ternatea L. (butterfly pea) blue petals. Food Chem, 331:127341. doi: https://doi.org/10.1016/j.foodchem.2020.127341.
- Jiménez-Moreno, N., Volpe, F., Moler, J.A., Esparza, I. and Ancín-Azpilicueta, C. (2019). Impact of extraction conditions on the phenolic composition and antioxidant capacity of grape stem extracts. *Antioxidants*, 8(12), 597. https://doi.org/10.3390/antiox8120597
- Kazuma, K., Noda, N. and Suzuki, M. (2003). Flavonoid composition related to petal color in different lines of *Clitoria ternatea*. *Phytochemistry*, 64(6), 1133–1139. https://doi.org/10.1016/S0031-9422(03)00504-1
- Kazuma, K., Noda, N. and Suzuki, M. (2003). Flavonoid composition related to petal color in different lines of *Clitoria ternatea*. *Phytochemistry*, 64(6), 1133–1139. https://doi.org/10.1016/S0031-9422(03)00504-1
- Kosai, P., Sirisidthi, K., Jiraungkoorskul, K. (2015). Review on ethnomedicinal uses of memory boosting herb, butterfly pea, Clitoria ternatea. J Nat Remedies, 15,71–76. https://doi.org/10.18311/jnr/2015/480
- Lakshan, S.A.T., Jayanath, N.Y., Abeysekera, W.P.K.M. and Abeysekera, W.K.S.M. (2019). A commercial potential blue pea (*Clitoria ternatea* L.) flower extract incorporated beverage having functional properties. *Evidence-Based Complementary and Alternative Medicine*, 2019, 2916914. https://doi.org/10.1155/2019/2916914
- Landim, Neves., M. I., Silva, E. K., and Meireles, M. A. A. (2021). Natural blue food colorants: consumer acceptance, current alternatives, trends, challenges, and future strategies. Trends Food Sci. Technol, 112, 163–173. doi: https://doi.org/10.1016/j.tifs. 2021.03.023
- Liu, S., Fu, Y., and Nian, S. (2014). Buffering colour fluctuation of purple sweet potato anthocyanins to acidity variation by surfactants. Food Chem, 162, 16–21.doi: https://doi.org/10.1016/j.foodchem.2014.04.029.
- Luengwilai, T. H. & K. (2019). Variation in floral antioxidant activities and phytochemical properties among butterfly pea (Clitoria ternatea L.) germplasm *Genetic Resources and Crop Evolution An International Journal, 66*, 645–658. https://doi.org/10.1007/s10722-018-00738-6
- Luengwilai, T. H. & K. (2019). Variation in floral antioxidant activities and phytochemical properties among butterfly pea (Clitoria ternatea L.) germplasm *Genetic Resources and Crop Evolution An International Journal, 66*, 645–658. https://doi.org/10.1007/s10722-018-00738-6
- Marpaung, A. M., Andarwulan, N., Hariyadi, P., and Faridah, D. N. (2019). The difference in colour shifting of Clitoria ternatea L. Flower extract at pH 1,4, and 7 during storage. Curr. Nutr. Food Sci, 15, 694–699. doi: https://doi.org/10.2174/1573401314666180503152636.
- Mukherjee, P.K., Kumar, V., Kumar, N.S. (2008). The Ayurvedic medicine Clitoria ternatea-From traditional use to scientific assessment. J Ethnopharmacol, 120,291–301. https://doi.org/10.1016/j.jep.2008.09.009
- Mukherjee, P.K., Kumar, V., Kumar, N.S.(2008). The Ayurvedic medicine Clitoria ternatea-From traditional use to scientific assessment. J Ethnopharmacol, 120, 291–301. https://doi.org/10.1016/j.jep.2008.09.009

- N, J., M.n.m, Z., & N.A.I.M, N. (2018) . Influences of environmental conditions to phytoconstituents in Clitoria ternatea (butterfly pea flower): a review. J Sci Technol, 10, 208-228.
- N, J., M.n.m, Z., & N.A.I.M, N. (2018). INFLUENNCE OF ENVIROMENTAL CONDITIONS TO PHYTOCONSTITUENTS IN CLITORIA TERNATEA, 208–228. J Sci Technol, 10, 208–228.
- Nur F., S, L., Muhammad Umar Lutfi, M.Y., Atika, A., Muhammad Hafiz, R., Muhammad Zulhelmi, O.A., Mohd Ariff Adzhan, O. and Khor, P.Y. (2018). A comparative analysis of *Clitoria ternatea* Linn. (Butterfly pea) flower extract as natural liquid pH indicator and natural pH paper. *Dhaka University Journal of Pharmaceutical Sciences*, 17(1), 97–103. https://doi.org/10.3329/dujps.v17i1.37125
- Oguis, G.K., Gilding, E.K., Jackson, M.A. (2019). Butterfly pea (Clitoria ternatea), a cyclotidebearing plant with applications in agriculture and medicine. Front Plant Sci, 10,645. https://doi.org/10.3389/fpls.2019.00645
- Sowndhararajan, K., Deepa, P., Kim, M., (2017). Parkinson's disease and the therapeutic potential of natural products. Neurochemistry International, 104, 16-31.
- Subramanian, M.S., Prathyusha, P. (2011) . Pharmaco-phytochemical characterization of Clitoria ternatea Linn. Int J Pharmtech, Res 3, 606–612
- Tang, Y., Liu, Y., Zhang, J., Li Z, Chen Z, Zhuang Y.(2020). Protective effect of Clitoria ternatea flower extract against radiation-induced oxidative stress in mice. PLoS One, 15(7), 0236307.
- Taranalli, A.D. and Cheeramkuzhy, T.C. (2000). Influence of Clitoria Ternatea Extracts on Memory and Central Cholinergic Activity In Rats. *Pharmaceutical Biology*, 38(1), 51–56. https://doi.org/10.1146/ annurev.immunol.22.012703.104702
- Taranalli, A.D. and Cheeramkuzhy, T.C. (2000). Influence of Clitoria Ternatea Extracts on Memory and Central Cholinergic Activity In Rats. *Pharmaceutical Biology*, 38(1), 51–56. https://doi.org/10.1146/annurev.immunol.22.012703.104702
- Zhang, L., Lin, G., Kovács, B., Janzen, D., Taylor, C., Zhang, D.(2019). Antioxidant phenolic compounds from the flowers of Clitoria ternatea. J Funct Foods, 62,103542.