Journal of Health Sciences and Innovative Research 2024;5(2):28-34
DOI: https://doi.org/10.31357/jhsir.v5i02.7851

Research Article

Comparison of *in-vitro* antioxidant activity of two varieties of *Clitoria ternatea* flowers in Sri Lanka

Wickramage, C.S.[1], Pathiraja, P.A.R.M.[1], Silva, N.M.G.[1], Thilakarathne, W.K.A.W.[1], Thathsarani, N.B.K.[1], Kumari, K.D.K.P.[2], Ratnayake, W.M.K.M [3*]

[1]*Department of Pharmacy and Pharmaceutical Sciences, Faculty of Health Sciences, CINEC Campus, Malabe, Sri Lanka.*

[2]*Faculty of Health and Social Sciences, Bournemouth University, United Kingdom (UK).*

[3]*Department of Cosmetic Science, Faculty of Health Sciences, CINEC Campus, Malabe, Sri Lanka.*

Abstract

Introduction: *Clitoria ternatea*, commonly known as “butterfly pea” in English and “Nil Katarolu" in Sinhala, is widely distributed in tropical regions. Due to its distinctive deep blue colour, it is now widely used in herbal teas, herbal juices, and cosmetic items. Additionally, flowers, seeds, roots, and leaves of this plant have been used for ages in traditional medicine to treat various diseases. As the antioxidant mechanism is crucial in many diseases, this study aimed to evaluate the antioxidant potential of boiled aqueous extracts of fresh flowers of *C. ternatea* i.e. normal keel blue petals ($AEFC_N$) and enlarged keel blue petals ($AEFC_L$). Methods: The *in-vitro* antioxidant activity of both crude extracts was evaluated by following a 2-2-diphenyl-1-picrylhydrazyl (DPPH) assay. Further, the total phenolic and flavonoid contents were determined using the Folin-Ciocalteu method and $AlCl_3$ method respectively. The data was analyzed using SPSS software and $p<0.05$ was considered as statistically significant. Results: The results showed that the $AEFC_N$ and $AEFC_L$ exhibited dose-dependent DPPH scavenging activity with an IC_{50} value of 20.09 mg/mL and 26.8 mg/mL respectively whereas, 26.61 µg /mL for ascorbic acid. Further, total phenolic present ($p>0.05$) in $AEFC_N$ and $AEFC_L$ were 3.651 ± 0.05 mg GAE/g fresh flower of *C. ternatea* and 3.47 ± 0 mg GAE/g fresh flower weight of *C. ternatea* respectively, while total flavanoids ($p<0.05$) present were 63.58 ± 0.58 mg QE/g fresh flower of *C. ternatea* and 50.42 ± 0.29 mg QE/g fresh flower weight of *C. ternatea* respectively. Conclusion: The presence of a significantly ($p<0.05$) low IC_{50} value of $AEFC_N$ in DPPH assay provided scientific evidence for the high antioxidant activity of normal keel petals compared to the enlarged petals. Although there was no significant difference between phenol amounts, the AEFCN showed higher flavonoid amounts than $AEFC_L$ ($p<0.05$). In addition, AEFCN showed higher total phenols and flavonoid amounts than $AEFC_L$. As phenolic compounds and flavonoids are well-known bioactive compounds with antioxidant properties, a comparatively higher amount of total phenols and flavonoids supports the relatively high antioxidant activity of $AEFC_N$.

Keywords: *Clitoria ternatea,* butterfly pea, “Nil Katarolu", antioxidant, DPPH

Introduction

Clitoria ternatea is an herbal plant that is widely distributed throughout the world. The plant is native to Southeast Asia and mainly in tropical countries [1]. It is an appealing perennial climber with conspicuous blue or white flowers. It is commonly known as Aparajita (Bengali), Chinese:

*Corresponding author: kalpani.ratnayake@cinec.edu
Received: 11 March 2024; Accepted: 17 November 2024

How to cite this article:
Wickramage, C.S., Pathiraja, P.A.R.M., Silva, N.M.G., Thilakarathne, W.K.A.W., Thathsarani, N.B.K., Kumari, K.D.K.P., Ratnayake, W.M.K.M. Comparison of in-vitro antioxidant activity of two varieties of *Clitoria ternatea* flowers in Sri Lanka, Journal of Health Sciences and Innovative Research, 2024;5(2):28-34

die dou; English: blue-pea, Butterfly-pea, Hindi: “Aparajita”, Sanskrit: “Girikarnika”, “Vishnukranta”; Tamil: “Kakkanam”, Telugu: “Dintena” and “Nil karatarolu” in Sinhala [2]. *Clitoria ternatea* belongs to the family Fabaceae [3]. The *C. ternatea* is a long-lived perennial herb with an erect habit. Flowers are blue scabbards, linear and flat. Five sepals present which are fused about two-thirds of their length. The most striking feature of this plant is its vivid deep blue flowers. Germination and establishment of *C. ternatea* are most favourable when the temperature is between 24–32 ˚C [4].

The different parts of *C. ternatea* such as roots, leaves, seeds and flowers have significant interest based on their traditional medicinal applications [3]. The roots have been found to have anti-inflammatory, anthelmintic, laxative, diuretic and cooling effects. They are commonly used in pathological conditions such as asthma, severe bronchitis and hectic fever. The root paste is used in curing abdominal swellings, sore throat, mucous disorders, and fever [5]. The seeds and leaves of this plant are commonly used as a brain tonic and it is believed that they can enhance memory and intelligence [6]. In addition, the seeds are crushed and taken with cold or boiled water as a treatment for urinary problems. Also, seeds are used to treat swollen joints [6]. Further, flowers and juice of *C. ternatea* were used as an antidote for snake bites [6]. The whole plant extract of *C. ternatea* has potential medicinal values and pharmacological effects, such as anti-inflammatory, antipyretic, analgesic and antioxidant effects [6]. According to the Lakshan *et al*. [7], dried flowers of different varieties of *C. ternatea,* showed significant antioxidant activity.

Antioxidants are substances that may protect human cells against free radicals, which may play a role in heart disease, cancer, and other diseases. Also, antioxidants either inhibit or delay the oxidation process. Natural and synthetic antioxidants are routinely used in foods and medicines to protect the final product against oxidation [8,9]. Antioxidants have broad applications. It is well known that spices and herbs are good sources of antioxidants [8]. It has been demonstrated that the administration of specific antioxidants, such as superoxide dismutase (SOD) and/or catalase, are effective at attenuating the tissue inflammation and injury observed in experimental models of ischemia and reperfusion, arthritis, chronic gut inflammation, and immune complex-induced pulmonary injury [10,11,12].

(A) Blue flower with normal keel petal

(B) Blue flower with enlarged keel petal

Plate 1: Different morphologies of *Clitoria ternatea* blue colour flowers

The phytochemicals present in the plants, especially flavonoids and phenols act as organic sources of antioxidants, which act against free radicals and prevent cancers [13]. Also, they have some other important health properties such as anti-inflammatory, and anti-allergic properties. In addition to promoting health, phytochemicals provide beneficial characteristics such as adding to plant colour, shielding plants from pathogens, and safeguarding plants. Further, through the activation of enzymes that lower the risk of certain diseases including cancer and age-related degenerative diseases, flavonoids can also be nutritionally advantageous [13,14,15].

Although there are several pharmacological importance of *C. ternatea*, there are limited studies that have been done to evaluate its pharmacological activities. Specially, to the best of our knowledge, there are no studies conducted on aqueous extract of fresh flowers of *C. ternatea.* As currently there is a trend of using fresh flowers of *C. ternatea* varieties among general public as a beverage also, the present study was focused on investigating the antioxidant potential of fresh

blue flowers of *C. ternatea* with normal keel petals (Plate 1A) and enlarge keel petals (Plate 1B) through 2-2-diphenyl-1-picrylhydrazyl (DPPH) assay. Further as phenol and flavonoids are the major phytochemicals which may contribute antioxidant potential of the plant extract, the present study also aimed to find total phenolic and total flavonoid content in the aqueous extract of *C. ternatea* flowers.

Methods

Chemicals and equipment

Dihydrochloride, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), (Sigma-Aldrich Company) was used for antioxidant assay and Folin-Ciocalteu reagent was used to assess total phenolic content. All other chemicals and reagents were of analytical grade and distilled water was used for the extraction process. UV- Visible spectrophotometer (Jenway 6305) was used to measure absorbances at each assay.

Plant materials

Fresh samples of two varieties of *C. ternatea* i.e. blue flower with normal keel petals and blue flower with enlarged keel petals, were collected from the Colombo District, Sri Lanka. The plant materials of *C. ternatea* were authenticated and voucher specimens were deposited at the National Herbarium, Department of National Botanical Garden, Peradeniya, Sri Lanka (SP-01 and SP-02).

Preparation of the plant extracts

The collected mature fresh flowers of *C. ternatea* were washed with tap water and then with distilled water to eliminate dust particles and other impurities. They were blotted dried by blotting papers and cut into small pieces. The aqueous extract of fresh flowers of *C. ternatea* (AEFC) was prepared by refluxing 50 g of fresh flowers with 150 mL of distilled water for 30 minutes at 40 ^{0}C. The extract was filtered through a filter paper, and the bluish filtrate was collected. The same method was followed for both morphological types of flowers of *C. ternatea* and the resulting filtrates were named $AEFC_N$ (for normal petals) and $AEFC_L$ (for enlarged petals) respectively and they were used for all other assays.

Evaluation of in-vitro antioxidant activity

The *in-vitro* antioxidant activity was assessed by DPPH free radical scavenging assay as described by *Ratnayake et al.* [16]. An aliquot of 1.5 mL of 0.25mM DPPH solution was mixed with 1.5 mL of $AEFC_N$ (50 – 1.562 mg/mL) or $AEFC_L$ (50 – 1.562 mg/mL). Similarly, a series of gallic acid (0.0005 – 0.005 mg/mL) was used as a reference. To avoid the interferences of absorbance by the colour of the test sample, the blank sample was prepared by mixing 1.5 mL of methanol and 1.5 mL of $AEFC_N$ or $AEFC_L$ for each concentration. Methanol was used as the negative control. Then the reaction mixtures were allowed to reach the steady state at room temperature in the dark. After 30 min, the absorbance was measured at 517 nm using a spectrophotometer (Jenway-6305). All the tests were performed in triplicate for each concentration. Antioxidant activity was measured in terms of radical scavenging activity and the percentage scavenging effect was calculated using the following formula.

$$\text{Scavenging activity (\%)} = \frac{[A_o - A_T]}{A_o} \times 100$$

Where, A_0 is the absorbance of the negative control (Methanol) and A_T is the absorbance of the test sample ($AEFC_N$ /$AEFC_L$ or gallic acid). The radical scavenging activity of test samples was expressed as a mean of EC_{50} (µg/mL), which is defined as the mean concentration of the anti-oxidant required to lower the initial DPPH concentration by 50% in each experiment.

Estimation of total phenolic content by Folin-Ciocalteu method

The total phenolic content of the $AEFC_N$ and $AEFC_L$ were determined by the Folin-Ciocalteu (FC) method as described by [17]. The gallic acid

stock solution of 1000 µg/mL was prepared by dissolving 0.1 g of Gallic acid in 100 mL of methanol. A series of test solutions of gallic acid with varying concentrations (50 µg/mL – 0.625 µg/mL) were prepared.

The initial reaction mixtures were prepared by mixing volumes of FC reagent (0.5 mL) and distilled water (in negative control; 1 mL) or gallic acid solution (in positive control; 1 mL) or $AEFC_N$ or $AEFC_L$ (in test sample; 1 mL). The colour blanks were also prepared for the test and standard solutions adding distilled water (0.5 mL) instead of FC reagent.

The reaction mixtures were kept at room temperature for 5 min and 2.5 mL of 5% Na_2CO_3 solution was added to each tube. Then they were incubated in the dark at room temperature for 40 min. After that absorbances at 725 nm were measured. All the tests were performed in triplicate for each concentration.

The mean absorption for each concentration of gallic acid was calculated and a graph was plotted with the concentration of each sample vs. absorbances. The mg/ g of gallic acid equivalents in milligrams per gram (mg GAE/ g) of aqueous extract of *C. ternatea* for two varieties were found by graphs.

Estimation of total flavonoid content by aluminum chloride method

The total flavonoid content of the $AEFC_N$ and $AEFC_L$ was determined by the aluminum chloride method as described by Parimelazhagan [17].

The quercetin stock solution 1000 µg/mL was prepared by dissolving 0.05 g of quercetin in 50 mL of methanol. A series of test solutions of quercetin with varying concentrations were prepared (800 µg/mL – 200 µg / mL).

The initial reaction mixtures were prepared by mixing volumes of 5 % $NaNO_3$ solution (150 µL) and distilled water (in negative control; 1 mL) or quercetin solution (in positive control; 1 mL) or $AEFC_N$ or $AEFC_L$ (in test sample; 1 mL). The colour blanks were also prepared for test and standard solutions distilled water (150 µL) instead of 5% $NaNO_3$ solution.

The reaction mixtures were vortexed and were kept at room temperature for 5 min. After that 150 µL of 10% $AlCl_3$ solution was added to each tube. Then they were vortexed again and incubated at room temperature for 6 min. After that 2 mL of 4% NaOH was added to all tubes. Then all contents were made up to 5 mL using distilled water. The tubes were vortexed and allowed to stand for 15 min at room temperature.

The absorbances at 510 nm were measured. All the tests were performed in triplicate for each concentration. The mean absorption for each concentration of quercetin was calculated and a graph was plotted with the concentration of each sample vs. absorbances. The mg/ g of quercetin equivalents in milligrams per gram of aqueous extract of *C. ternatea* for two varieties were found by graphs.

Statistical analysis

All the results were subjected to descriptive statistics and expressed as mean ± standard deviation (SD). Data were analyzed by using SPSS statistic 21 software. p-values < 0.05 were considered statistically significant.

Results

In-vitro antioxidant activity

The $AEFC_N$ and $AEFC_L$ exhibited significant ($p<0.05$) dose-dependent DPPH scavenging activity compared to the negative control. The percentage inhibition of the DPPH scavenging activity of $AEFC_N$ and $AEFC_L$ (50 – 1.562 mg/mL) are shown in Figure 1(A) and Figure 1(B)

respectively. Ascorbic acid (6.25 – 50 µg/mL) was used as a standard antioxidant and percentage inhibition is shown in Figure 1(C). The IC_{50} value of $AEFC_N$ and $AEFC_L$ were found as 20.09 mg/mL and 26.8 mg/mL respectively whereas, 26.61 µg /mL for ascorbic acid.

Total phenolic content by Folin-Ciocalteu method

The total phenolic content for the gallic acid standard is shown in Figure 2. The total phenolic in $AEFC_N$ and $AEFC_L$ were expressed as milligrams of gallic acid equivalent (GAE) per gram of aqueous extract of fresh flowers of *C. ternatea.* The result obtained showed that the total phenolic content present in $AEFC_N$ and $AEFC_L$ were 3.651 ± 0.05 mg GAE/g for fresh flower of *C. ternatea* and 3.47 ± 0 mg GAE/g for fresh flower of *C. ternatea* respectively.

QE/g fresh flower of *C. ternatea* respectively.

Discussion

Hot water extracts of fresh flowers of *C. ternatea* are commonly used herbal beverages nowadays in Sri Lanka. Also, *C. ternatea* tea is often consumed on its own or mixed with other ingredients for flavour and additional health benefits. In addition to being consumed as a standalone tea, *C. ternatea* flower extract or tea is sometimes used as an ingredient in cocktails and mocktails to add colour and flavour. It's a popular choice for mixologists looking to create visually stunning drinks. Flowers of *C. ternatea* are used not only as drinks but also as a natural food colouring. They are occasionally used to provide a blue colour to savoury meals, rice dishes, and even desserts.

The free radicals and reactive oxygen species are

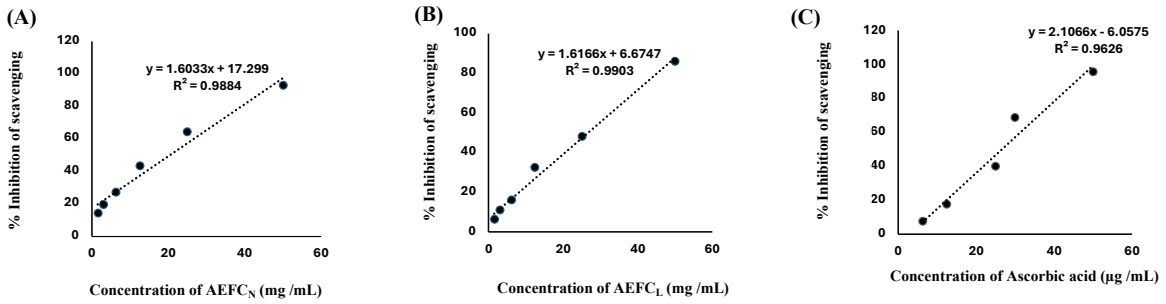

Figure 1: Percentage inhibitions of DPPH radical scavenging effects vs different concentration of solutions; **(A)** Percentage inhibition of DPPH radical scavenging activity for aqueous extract of *C. ternatea* flower with normal keel petals ($AEFC_N$); **(B)** Percentage inhibition of DPPH radical scavenging activity for aqueous extract of *C. ternatea* flower with enlarged keel petals ($AEFC_L$); **(C)** Percentage inhibition of DPPH radical scavenging activity for ascorbic acid.

Total flavonoid content by aluminum chloride method

Total flavonoids content for the quercetin standard is shown in Figure 3. The total flavonoid in $AEFC_N$ and $AEFC_L$ were expressed as milligrams of quercetin equivalent (QE) per gram of aqueous extract of fresh flowers of *C. ternatea*. The result obtained showed that the total flavanoids present in $AEFC_N$ and $AEFC_L$ were 63.58 ± 0.58 mg QE/g fresh flower of *C. ternatea* and 50.42 ± 0.29 mg

involved in creating oxidative stress and act as risk factors for certain chronic diseases. Antioxidants can trap these free radicals [18]. Hence, antioxidants are important in delaying or inhibiting oxidative damage to the human body. In addition to combatting oxidative stress, antioxidants are important for promoting skin health to promote a more youthful appearance and overall skin health and boosting immune function to strengthen the body's natural defenses against

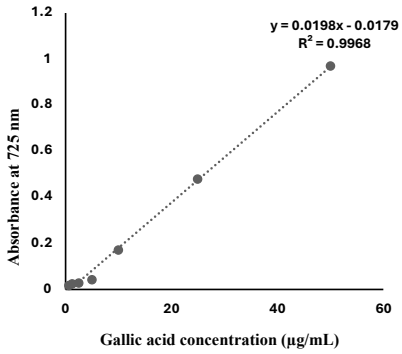

Figure 2: Standard curve for gallic acid in Folin-Ciocalteu method

infections and diseases. Most natural sources such as medicinal plants, are rich in antioxidants. Considering the important health effects of natural antioxidant compounds, it is important to reveal the antioxidant potential of natural sources as it will enhance the utilization of those by providing scientific evidence. Hence, through this study, an attempt has taken to reveal the scientific evidence of the antioxidant potential of hot aqueous extract of fresh leaves of *C. ternatea* which are currently used by the Sri Lankan community.

The DPPH assay was used to measure the antioxidant activity of the test extracts. It measured the ability of an antioxidant to scavenge DPPH radicals, which are stable free radicals. When antioxidants react with DPPH radicals, they donate hydrogen atoms or electrons, leading to a reduction in the purple-coloured DPPH solution which gives maximum absorption at 517 nm from the UV-Visible spectrophotometer. The degree of discolouration is proportional to the antioxidant capacity of the tested sample. Thus, the DPPH assay provides a quantitative measure of the antioxidant activity of a substance. Hence, the dose-dependent decrease of absorbance for $AEFC_N$ and $AEFC_L$ confirms the reducing ability of compounds present in the aqueous solution. According to the results observed from this study $AEFC_N$ and $AEFC_L$ showed DPPH scavenging activity with an IC_{50} value of 20.09 mg/mL and 26.8 mg/mL respectively. This showed comparatively more DPPH scavenging activity in normal keel petals of blue colour C. *ternatea.* As described by Lakshan and his coworkers in 2020 [7], the dried blue flower of *C. ternatea* with normal keel petals and enlarged keel petals showed DPPH scavenging percentage for 250 µL/mL as 51.92 % and 23.75 % respectively. Although, the percentage inhibition values and effective concentration are different in their study compared to the current study, it also showed the same pattern in which normal keel petals are more active than enlarged keel petals. However, these value differences may be due to differences in the fresh and dry nature of the flowers, sample preparation methods as well as geographical differences in the sample collecting locations.

In herbal plants, there are two types of antioxidant compounds i.e., Hydrosoluble antioxidants (phenolics, flavonoids, anthocyanins, stilbene, lignin) and liposoluble antioxidants (alpha-carotene, beta-carotene, lycopene, lutein and zeaxanthine) [19]. As the aqueous extract of fresh flowers of *C. ternatea* showed antioxidant properties, we attempted to screen the hydrosoluble antioxidant compounds such as phenolic and flavonoids quantitatively.

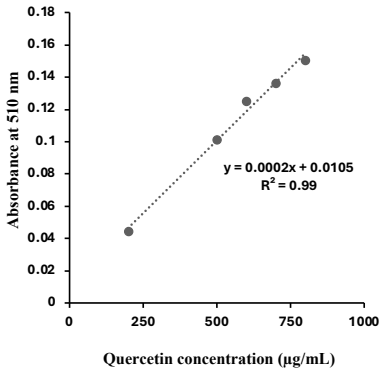

Figure 3: Standard curve for quercetin in aluminum chloride method

To measure the total phenolic content in the $AEFC_N$ and $AEFC_L$ quantitatively, the Folin-Ciocalteu method, which is a widely used colourimetric assay for the quantification of total phenolic content in various samples, particularly plant extracts, was used. The principle behind the

Folin-Ciocalteu method is based on the oxidation-reduction reaction between phenolic compounds and the Folin-Ciocalteu reagent (FCR). The FCR contains phosphomolybdic-phosphotungstic acid complexes, which are reduced by phenolic compounds in an alkaline medium to form blue-coloured complexes. Here, molybdotungsto-phospharic heteropolyanion reagent (yellow colour) converts into molybdotungsto-phosphate (blue colour) [20]. The intensity of the resulting blue colour is proportional to the total phenolic content in the sample. According to the results obtained from the study, the total phenolic present in $AEFC_N$ was comparatively more than it was in $AEFC_L$. Hence, this was made scientific support to show the proportionated relationship between the presence of phenols and antioxidant potential in the flowers of *C. ternatea*.

In addition to the phenol, as the flavonoids are also another phytochemical which can contribute to the antioxidant activity of most plant sources, the total flavonoid content in the $AEFC_N$ and $AEFC_L$ was measured by using an $AlCl_3$ method [17]. The $AlCl_3$ method is a colourimetric method based on the formation of yellow colour acid-stable complexes in between $AlCl_3$ and C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols [21]. In the assay, the intensity of the yellow colour was proportional to the concentration of flavonoids present in the sample. Quercetin which is well-known flavonoid compound, was used as a standard and total flavonoid content in $AEFC_N$ and $AEFC_L$ was expressed as quercetin equivalent. According to the results obtained from the study, the total flavonoid present in $AEFC_N$ was comparatively more than it was in $AEFC_L$. Hence, this was made scientific support to show the proportionated relationship between the presence of flavonoids and antioxidant potential in the flowers of *C. ternatea*.

In overall test results, $AEFC_N$ showed more antioxidant potential compared to the $AEFC_L$. Hence, the antioxidant potential of *C. ternatea* blue colour flowers with normal keel petals, plays a crucial role in promoting health and wellness, both in traditional medicine systems and modern scientific research. Further studies exploring the antioxidant properties of *C. ternatea* flowers may lead to the development of novel therapeutics and functional ingredients for various applications.

Conclusion

The results showed that blue flower with normal keel petals has a significant ($p < 0.05$) antioxidant potential than a blue flower with enlarged keel petals because blue flower with normal keel petals has significantly ($p < 0.05$) higher amount of total phenols and flavonoids than blue flower with enlarged petals. Hence, the present findings provided scientific evidence for the *in-vitro* antioxidant properties of the fresh flowers of *C. ternatea*. Further, the present findings provided scientific evidence for the presence of phenolic and flavonoids in boiled aqueous extract of fresh flowers of *C. ternatea* which may contribute to their antioxidant properties.

Further investigations need to be carried out for the isolation and characterization of the bioactive compounds present in the boiled aqueous extract of fresh flowers of *C. ternatea,* which is responsible for higher antioxidant activity.

References

1. Balaji KS, Priyanka S, Preethi SD, Chandrashekara KT, Lokesh S, Rangappa KS. "Angio-suppressive effect of *Clitoria Ternatea* flower extracts is mediated by HIF-1α and down-regulation of VEGF in Murine carcinoma model". *Medicinal Chemistry*. 2016;6(7):515-20.
2. Silalahi, M. Clitoria ternatea L. Fabaceae. Springer Nature Switzerland AG. 2020 DOI: 10.1007/978-3-030-14116-5_113-1
3. Chakraborty S, Sahoo S, Bhagat A, Dixit S.

"Studies on antimicrobial activity, phytochemical screening tests, biochemical evaluation of *Clitorea ternatea* Linn. plant extracts". *International Journal of Research-Granthaalayah*. 2017;5(10):197-208.

4. McDonald C. K. Germination response to temperature in tropical and subtropical pasture legumes. 1. Constant temperature. *Australian Journal of Experimental Agriculture.* 2002;42:407–419.
5. Devi BP, Boominathan R, Mandal SC. "Anti-inflammatory, analgesic and antipyretic properties of *Clitoria ternatea* root". *Fitoterapia*. 2003;74(4):345-9.
6. Al-Snafi AE, "Pharmacological importance of *Clitoria ternatea*–A review". *IOSR Journal of Pharmacy*. 2016;6(3):68-83.
7. Lakshan SA, Pathirana CK, Jayanath NY, Abeysekara WP, Abeysekara WK. "Antioxidant and selected chemical properties of the flowers of three different varieties of Butterfly Pea (*Clitoria ternate*a L.)". *Ceylon Journal of Science*. 2020;49(2):195-201.
8. Hinneburg I, Dorman HD, Hiltunen R. Antioxidant activities of extracts from selected culinary herbs and spices. *Food chemistry*. 2006;97(1):122-9.
9. Lourenco, SC, Martins, MM, Alves, VD. Antioxidants of Natural Plant Origins: Drom Sources to Food Industry Applications, *Molecules*. 2019;24(22):4132.
10. Skaleric UR, Allen JB, Smith PD, Mergenhagen SE, Wahl SM. "Inhibitors of reactive oxygen intermediates suppress bacterial cell wall-induced arthritis". *Journal of immunology* (Baltimore, Md.: 1950). 1991;147(8):2559-64.
11. Clark DA, Fornabaio DM, McNeill H, Mullane KM, Caravella SJ, Miller MJ. "Contribution of oxygen-derived free radicals to experimental necrotizing enterocolitis". *The American journal of pathology*. 1988;130(3):537.
12. Johnson KJ, Ward PA. "Role of oxygen metabolites in immune complex injury of lung". *Journal of immunology* (Baltimore, Md.: 1950). 1981;126(6):2365-9.
13. Crozier A, Clifford MN, Ashihara H, editors. "Plant secondary metabolites: occurrence, structure and role in the human diet". *John Wiley and Sons*. 2008
14. Jaafar NF, Ramli ME, Salleh RM. Optimum extraction condition of *Clitorea ternatea* flower on antioxidant activities, total phenolic, total flavonoid and total anthocyanin contents. *Tropical life sciences research*. 2020;31(2):1.
15. Prasad KN, Yang B, Yang S, Chen Y, Zhao M, Ashraf M, Jiang Y. Identification of phenolic compounds and appraisal of antioxidant and antityrosinase activities from litchi (Litchi sinensis Sonn.) seeds. *Food Chemistry*. 2009;116(1):1-7.
16. Ratnayake WM, Suresh TS, Abeysekera AM, Salim N, Chandrika UG. Acute anti-inflammatory and anti-nociceptive activities of crude extracts, alkaloid fraction and evolitrine from *Acronychia pedunculata* leaves. *Journal of ethnopharmacology*. 2019; 238:111827.
17. Parimelazhagan T. Pharmacological assays of plant-based natural products. *Springer;* 2015.
18. Cheeseman KH, Slater TF. An introduction to free radicals chemistry. *British Medical Bulletin.* 1993;49:481–93
19. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang JJ, Li HB. "Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources". *International journal of molecular sciences*. 2017;18(1):96.
20. Huang D, Ou B, Prior RL. "The chemistry behind antioxidant capacity assays". *Journal of agricultural and food chemistry*. 2005;53(6):1841-56.
21. Bag GC, Devi PG, Bhaigyabati TH. "Assessment of total flavonoid content and antioxidant activity of methanolic rhizome extract of three Hedychium species of Manipur valley". *International Journal of Pharmaceutical Sciences Review and Research*. 2015;30(1):154-9.