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Proximate analysis of *Clitoria ternatea* flower dye in making jellies and chocolates

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Abstract

From ages *Clitoria ternatea* flower has known for its colour imparting property as a natural food colorant in various parts of the world specifically in Asia. Its abundance and growth in many parts of Asia including India has established it as traditional food colouring agent. It has been explored for its medicinal importance also for its rich antioxidant properties. In the present study taking into consideration of its antioxidant properties, the colour imparting nature was studied under various parameters. The results showed 100% DPPH activity and TPC was also improved in comparison. Proximate analysis of the anthocyanin content revealed promising results in making jellies and chocolates for the food industries. It was shown that the method of preparation has a significant impact in retaining the antioxidant and anti-inflammatory properties of anthocyanin content which is an added advantage for the present study.

Key words: Antioxidant, Proximate, *Clitoria ternatea*

Introduction:

Plant Kingdom always been useful in one way or other for humans from ages. Ayurveda has many decoded descriptions of plants and their useful parts with medicinal properties that are traditionally used. One such plant which is commonly available in Asia known as "Aparajita" of Indian origin has loads of antioxidants and also show anti-inflammatory properties. Knowingly or unknowingly this flower is used as natural colorant in many parts of the world such as Thailand, Malaysia and Indonesia including India both as commercial and domestic ways. The blue colour of the flower has driven the research interest to dive into its antioxidant as well as anti-inflammatory properties which may add more scientific explanation of choosing this plant in the present study. Specially its antioxidants data will attract incorporation of this flower dye into foods and can be better option as nutraceutical and dietary supplement recommendations.

Not only the commercial importance but also its health benefits may help it to be fit in the food industry. *Clitoria ternatea* the scientific name of Aparajita or the Butterfly pea flower is rich in bioactive compounds and show various pharmacological effects such as anti-inflammatory, antioxidant, anticancer, antihistamine, antibacterial, antiparasitic and immunosuppressive activities.

The chemical components like carbohydrates, tannins, saponins, flavonoids, anthocyanins, are found in various parts of plant and flavanol glycosides, kempferol, quercertin are specifically found in petals that have wide range of biological importance.

Taking into consideration of the chemical and biological importance, the extracts of the flower incorporation as food colouring agent in preparation of jellies, chocolates by optimizing its stability and quality, and also evaluating proximate parameters of the formulated compounds is the line of research focussed on present work.

Material and methods:

1.Collection of flowers:

Fresh *Clitoria ternatea* flowers were collected from home garden

2.Raw materials for product preparation:
Agar-agar, coconut water, cocoa butter, milk powder, tender coconut pieces, vanilla essence were purchased from super market.

3.Preparation of flower powder:
3.1.Tray drying:
Fresh *Clitoria ternatea* flowers were dried in drying cabinet at 50^0 C with periodic checking to avoid over drying. Weight lost by the sample was checked at regular intervals of 30 minutes using an electronic digital balance. When there was no weight loss observed after three consecutive weight check then the drying process was stopped. Then the dried sample was transferred to a clean airtight container to avoid moisture absorption and stored in cool and dry place for further use.

3.2.Crude extraction:
3.2.1.Maceration method:
Analytically weighed 5 grams of Clitoria ternatea flower powder was taken. An extraction solvent of 40 mL ethanol and 60 mL distilled water was prepared. Then the powder was transferred into a conical flask containing extraction solvent and extraction process was done by placing the flask in an orbital shaker at 50^0 C for 3 hours at 100 rpm in order to facilitate diffusion of bioactive compounds into the solvent. After extraction time, the extract is filtered using Whatman's No.1 filter paper to separate solid residue. Then on a rotary evaporator under reduced pressure the filtrate was concentrated. The crude extract was collected and stored for further analysis.

3.2.2. Ultra Sonication method:
5 grams of Clitoria ternatea powder was analytically weighed and was thoroughly mixed in prepared extraction solvent of 40 mL ethanol + 60 mL distilled water in a conical flask. The mixture was then subjected to ultra sonication at 50 KHz frequency for 30 minutes at 50^0C. This process enhances the extraction efficiency by disrupting the cell walls to release phytochemicals into solvent. Then the solid residue is separated from liquid using filtration process. Then the filtrate is concentrated using a rotary evaporator and the concentrated crude extract is stored for further analysis and formulation of products.

4. Preliminary phytochemical screening:
By using the standard protocols for preliminary phytochemical screening, the presence of constituents such as carbohydrates, amino acids, alkaloids, flavonoids, proteins, glycosides, tannins, sterols, phenols, quinones, oxalates, terpenoids and saponins were evident by changing in the colour of crude extract precipitate.

5.Phytochemical quantitative determination:
5.1. Total Phenolic content assay:
Using standard protocol of FC reagent and sodium carbonate, the crude extract was quantified spectrophotometrically at 760 nm in GAE/g extract.

5.2. DPPH radical scavenging activity:
Using standard DPPH protocol, the crude extract was quantified spectrophotometrically at 517 nm and the inhibition percentage was calculated.

5.3. Total Anthocyanin determination using pH differentiation method:
Each sample was diluted with pH 1 and pH 4.5 buffers. Using microplate reader scanning at 465 nm the absorbance was recorded, standard was taken as apigeninidin (AP). Total anthocyanin and monomeric anthocyanin were calculated.

6.Products preparation infusing Clitoria ternatea extract:
6.1. Coconut jelly preparation using extract:
For coconut jelly preparation, various concentrations such as 25 mg, 50 mg, 170 mg and 1 gm of *Clitoria ternatea* extract were used in prepared 200 mL coconut water, 2.8 gm agar and 10 gm sugar mixture and after heating for uniform mixing and upon cooling was poured into molds and allowed to settle at room temperature.

6.2. Blue chocolate preparation using extract:
For blue chocolate preparation, various concentrations such as 25 mg, 100 mg, 125 mg, 170 mg of *Clitoria ternatea* extract is infused into the vanilla essence based prepared chocolate mix and mixed thoroughly for even colour distribution. Then poured into molds to set at room temperature followed by refrigeration to get solidified prior to evaluation.
6.3.Sensory evaluation report:
Sensory evaluation of the jellies and chocolates infused with Clitoria ternatea was done using a semi trained panel of 10 members. The panellists were received with a detailed instructions on scoring with a 9 point hedonic scale. The attributes were colour, aroma, consistency, taste and acceptability. The submitted questionnaire was later converted into quantitative terms and mean scores were analysed.

7.Proximate analysis:
All proximate analysis were done using standard protocols.
7.1. Determination of moisture content:
Moisture content was determined by placing weighed samples in hot air oven at 105^0C for 1 hour and then removed and weighed again after cooling at room temperature.

7.2. Determination of ash content:
Pre weighed crucibles were taken and samples were placed in them. Then they were placed in muffle furnace at 550^0 C for 3 to 4 hours. Then the samples were ignited until grey colour was obtained. Upon cooling at room temperature the ash samples were weighed.

7.3. Determination of fat content:
Using Soxhlet extraction method, the fats were extracted. After 4 hours of extraction process, the fat was removed and flasks were kept on rotary evaporator for solvent evaporation. Then further solvent was evaporated at 103^0C for 30 minutes and then kept in desiccator to cool at room temperature and then weighed further.

7.4. Determination of protein content:
Kjeldahl method was used to determine protein content. The digested and distilled samples using Kjeldahl method were titrated further.

Results and Analysis:
Considering various applications of the *Clitoria ternatea* flower extract which is in blue colour, the focus of the work was on its extraction process, proximate analysis and product preparation by infusing it in order to retain the necessary properties of the phytochemicals.

Tray drying:
Through tray drying, significant reduction of the moisture content from 80 to 18% was observed as shown in figure 2, when *C.ternatea* blue flowers were subjected to 50^0C temperature for 4 hours. It is noteworthy that during the process browning was not observed as shown in figure 1(a, b).

Figure 1 a. Before drying

Figure 1 b. After working

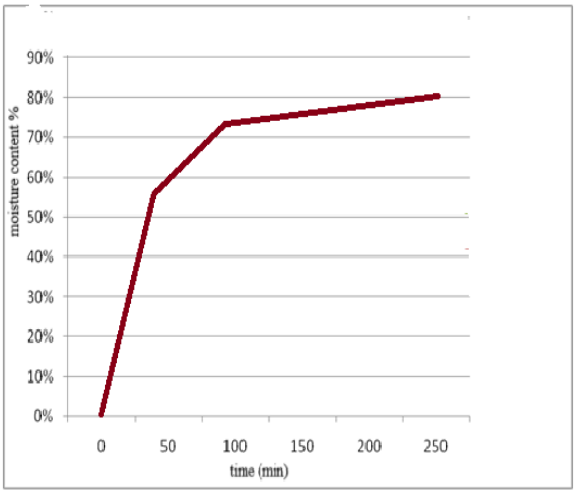


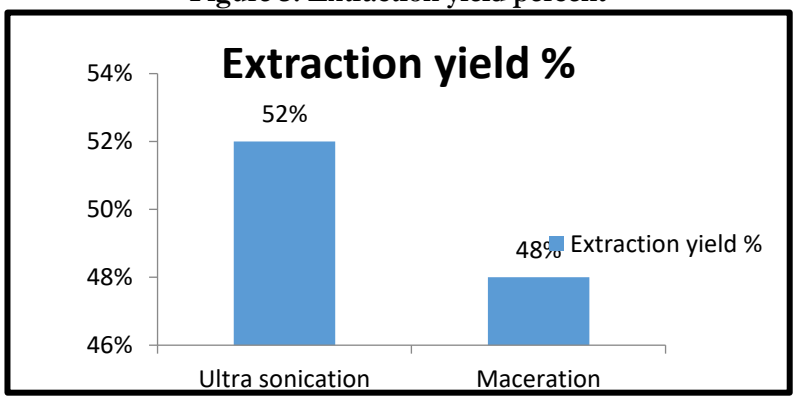
Figure 2.Moisture content

Extraction yield:
The extraction yield of blue pea flower (*Clitoria ternatea*) from tray dried powder through maceration and ultra sonication was shown in table 1 and figure3

Table 1: Extraction yield of *Clitoria ternatea*

Extraction method	Extraction yield %
Ultra sonication	52%
Maceration	48%

Figure 3: Extraction yield percent



In table and figure 3, it was observed that the extraction yield was higher in Ultra sonication process than in maceration. This is because, Ultra sonication enhances the yield by cell wall disruption using high frequency waves which allows better penetration and release of bioactive compounds into the solvent.

Phytochemical screening of *Clitoria ternatea* extract:

Preliminary phytochemical screening of *Clitoria ternatea* (blue pea flower) was done using standard protocols and was reported positive for the presence of tannins, alkaloids, flavonoids, carbohydrates, phenols, terpenoids,glycosides, resins, oxalates and quinones as shown in table 2.

Table2: Preliminary phytochemical analysis of *Clitoria ternatea*

PHYTOCHEMICAL COMPOUND	BLUE PEA FLOWER EXTRACT
Alkaloids	+ + +
Carbohydrates	+ + +
Glycosides	+
Flavonoids	+ + +
Phenols	+ + +
Tannins	+ + +
Amino acids	+
Saponin	+
Sterols	+
Terpenoids	+ + +
Quinones	+ + +
Oxlates	+
Resins	+

(Absent -, Present +, More present ++, Strongly present +++)

Quantitative determination of phytochemicals:

Total Phenolic contents:

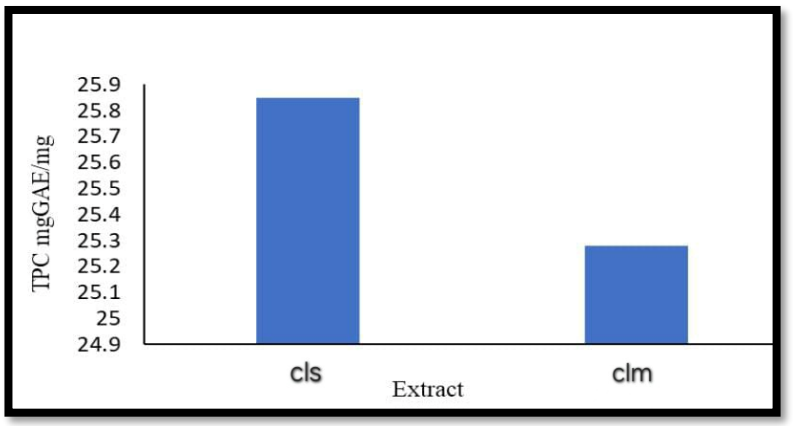


Figure 4: TPC assay of the extract

In the figure 4, it is observed that the total phenolic content of ultra sonication processed extraction (25.8 mg GAE/g) was slightly higher than that of macerated extraction (25.2 mg GAE/g). This indicates the ultra sonication method is more effective and is due to cavitation effect, which breaks plant's cell walls and enhances solvent penetration. This process leads to better phenolic compounds release when compared to passive diffusion and maceration which are slower processes.

DPPH radical scavenging activity:

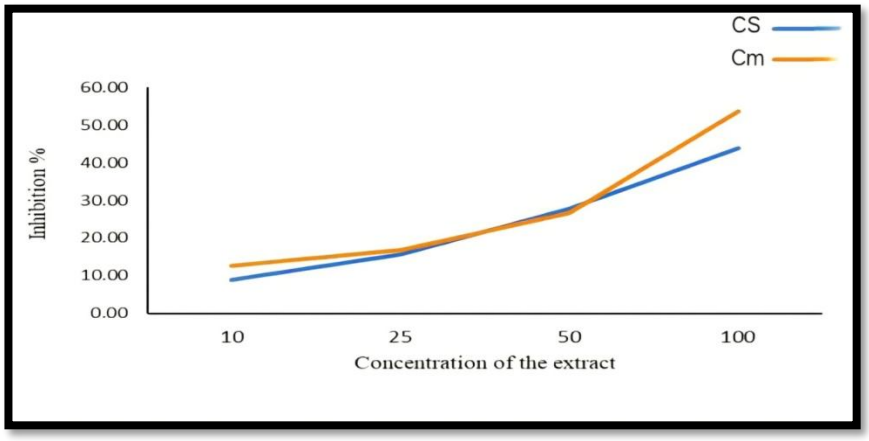


Figure 5: DPPH radical scavenging of the extracts

In figure 5, CS (ultrasonic) and Cm(maceration) exhibited dose dependent DPPH radical inhibition. It is also observed that ultrasonic extracts (CS) showed highest percentage of inhibition at 100 µg/ mL indicating it has superior antioxidant activity.

Total anthocyanin determination by pH differentiation method

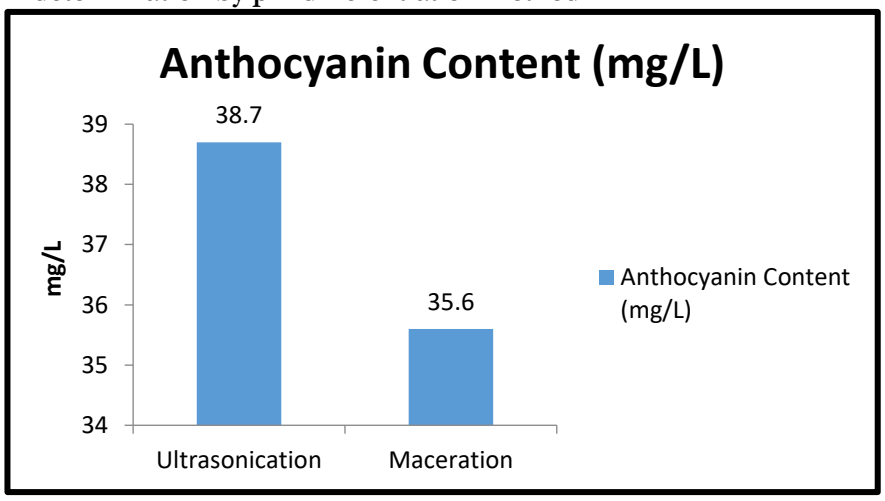


Figure 6: Total anthocyanin content of extracts

Ultrasonication method extract showed 38.70 mg/L anthocyanin presence than maceration method extract which was 35.96 mg/L. The increased efficiency is due to the cavitation effect achieved through ultra sonic waves mediated disruption of cell walls for enhanced intracellular phytochemicals like anthocyanins release into solvent.

Sensory evaluation

For sensory evaluation of *Clitoria ternatea,* various concentrations was blue pea flower extract was infused into coconut jellies and through 10 semi-trained panellists the scores were tabulated in terms of appearance, colour, aroma, texture, taste and overall acceptability of the prepared jelly in table 3.

Table 3: Sensory evaluation of jellies

Sample	Appearance	Colour	Texture	Aroma	Taste	Overall
Control	5.6	6.4	5.8	5.3	6	5.82
S1(25 mg)	7	7.8	7.5	7.3	7.4	7.4
S2(50 mg)	7	7.1	7	7	7.2	7.06
S3(170 mg)	8.6	8.8	8.5	8.3	8.7	8.5
S4(1 gm)	4.3	4.6	4.2	4.7	4.5	4.4

The sample S3 has shown superior sensory attributes among all which was also shown in table 3, figure 7a to 7d and figure 8.

Figure 7a: Control jelly

Figure 7b: 25 mg jelly

Figure 7c: 170 mg jelly

Figure 7d: 1gm jelly

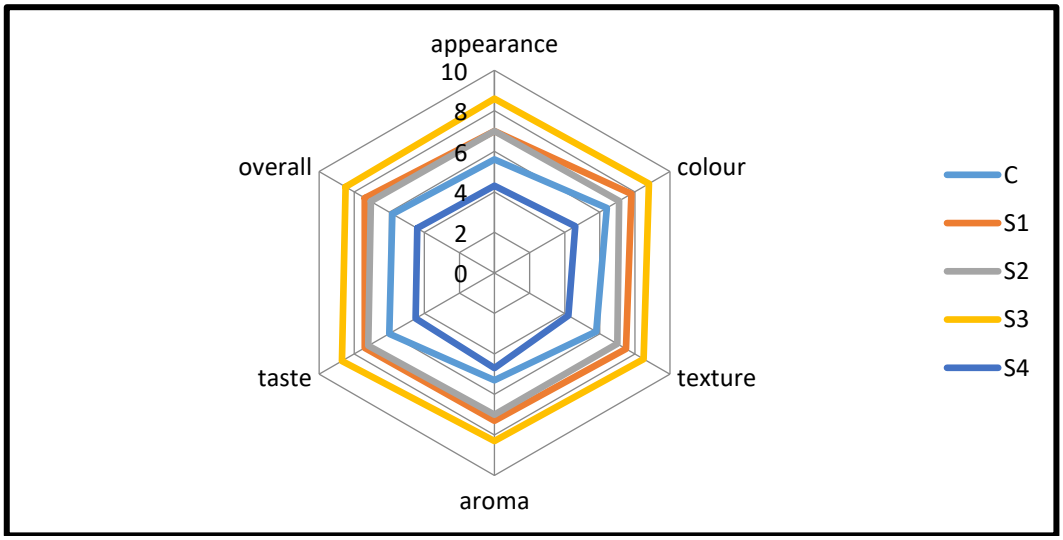


Figure 8: Sensory evaluation chart

Proximate analysis

Proximate analysis of moisture, ash, protein and fat were conducted for selected S3 sample jelly and control from sensory evaluation. The results were shown in table 4

Table 4: Proximate analysis of S3 and control

Parameters	Control	S3
Moisture %	93%	91%
Ash (gm)	0.92	0.95
Fat (gm)	0.09	0.09
Protein (gm)	0.18	0.20

There was no significant difference found in moisture, ash and protein content among the jellies but the fat content was same in both which was also shown in figure 9.

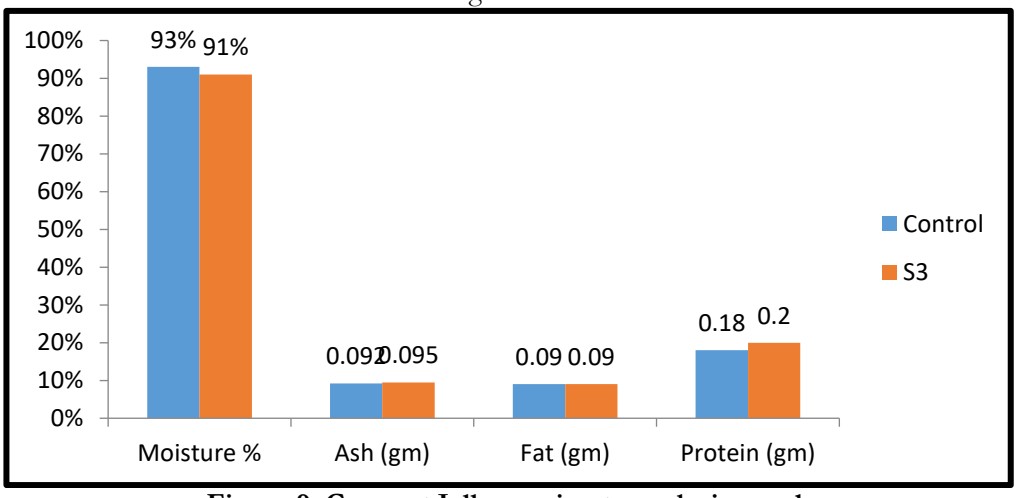


Figure 9: Coconut Jelly proximate analysis graph

Sensory evaluation of *Clitoria ternatea* extract in chocolate

Various concentrations of blue pea flower ultrasonic extract were incorporated into chocolate and sensory evaluation was done through 10 semi trained panellists and the results were tabulated in table 5.

Table 5: Sensory evaluation results of chocolate with extract

Attribute	Control	W1(25 mg)	W2(100 mg)	W3(150 mg)	W4(175 mg)
Appearance	7.2	7.5	8.8	7	5.3
Colour	7.4	7.6	8.9	7.2	6.5
Texture	7.1	7.4	8.7	7	6.2
Taste	7.3	7.2	8.9	7.1	5.4
Aroma	7	7.5	8.8	7	5.3
Overall	7.2	7.44	8.82	7.06	5.74

The W2 chocolate has got highest scores among the other samples in terms of set attributes as shown table 5, figures 10a to 10e and figure 11.

Figure 10a:Control Chocolate

Figure 10b: 50 mg Chocolate

Figure 10c: 100 mg Chocolate

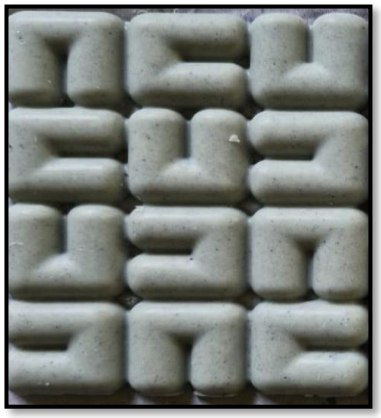


Figure 10d: 150 mg Chocolate

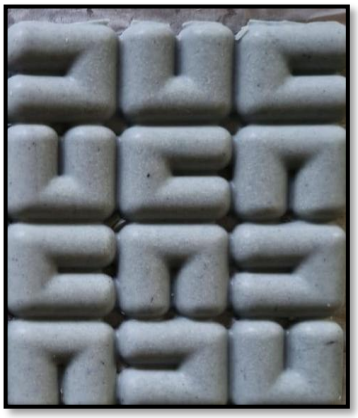


Figure 10e: 175 mg Chocolate

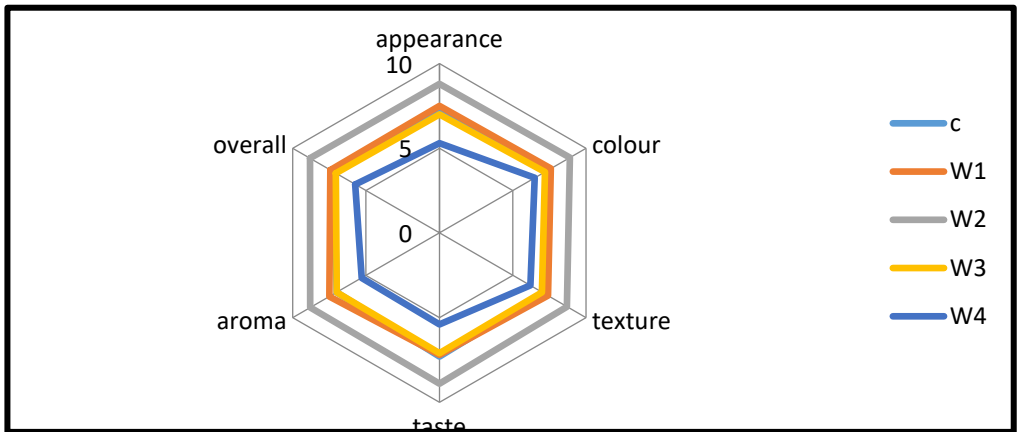


Figure 11: Sensory evaluation of chocolate chart

Proximate analysis of chocolate

Proximate analysis for selected W2 chocolate and control was conducted and found that no significant difference in moisture content an ash content but slight to moderate increase in fat and protein content was seen in W2 chocolate than control as shown in table 6.

Table 6: Proximate analysis of selected W2 chocolate and control

Parameters	Control	W3
Moisture content %	13.3%	13%
Ash (gm)	1.79gm	1.84gm
Fat (gm)	33.7gm	34gm
Protein(gm)	9.45gm	9.49gm

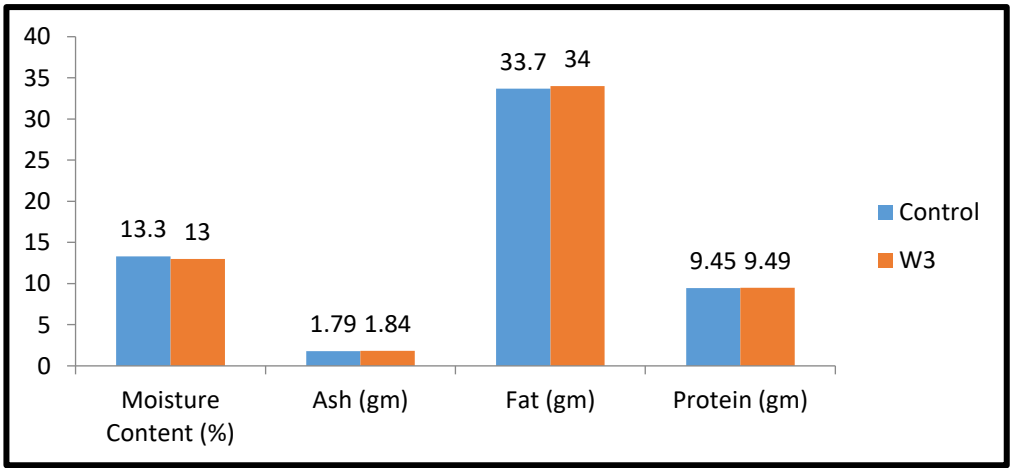


Figure 12: Proximate analysis of Chocolate

Discussion:

In the present market the products related to plant extract infused jellies, chocolates, are more in demand due to their efficient colour, taste, aroma and overall appearance. This type of product can be achieved by optimizing them at various levels such as drying of flower, extraction yield, TPC, DPPH, Total anthocyanin, proximate analysis in terms of moisture, ash, fat and protein content. A comparative discussion in detail has been done in the above mentioned parameters with previously mentioned literature.

Drying: The tray drying method in a drying cabinet at 50^0C done in this study has shown significant reduction of moisture content to 18% from 80% as shown in figure 1 and 2. The results obtained in the present study falls in the range of 5 to 37% as per Sofiah et.al.,2022.

Extraction yield: The Ultra sonication process has shown better yield of 52% than the earlier reported work of 40.3% by Gamage et.al.,2023. In the present study maceration process was also used which showed significantly less yield of 48% than ultra sonication method.

Total Phenolic content: Total Phenolic content of *Clitoria ternatea* flower extract through ultra sonication method has shown good results in the present study as 25.8 mg GAE/g which was more than the reported value of 14 mg GAE/g at 760 nm Samart Sai et.al., 2024.

DPPH scavenging activity: The inhibition of DPPH shows abundance of phenolic compounds. DPPH scavenging activity of *Clitoria ternatea* flower extract through ultra sonication method has shown 100% inhibition in the present study which was better than Nurjamalinaet.al, 2020 where it was reported as 68.12% inhibition.

Total anthocyanin: The presence of Anthocyanin in the *Clitoria ternatea* flower is actually responsible for the blue colour. This extract has shown better results of Anthocyanin content when the method of extraction was done through ultra sonication. The yield was 38.70 mg/L which was higher in comparison with the work of Jaafar et.al., 2020 which was reported as 28.60 mg/L.

Sensory evaluation

Sensory evaluation of white chocolate: Sensory evaluation of white chocolate as per the Hedonic test in the attributes mentioned in table 5, figure 10 and 11 has shown better results with 100 mg extract infused chocolate preparation in comparison to the reports of Pamungkaningtyas et.al., 2024.

Sensory evaluation of coconut jelly: Sensory evaluation of coconut jelly as per the Hedonic test in the attributes mentioned in table 3 and figures 7 and 8, where 170 mg *Clitoria ternatea* extract infusion has shown better results. As per the reports of Lonez and Banwa, 2021 the attributes comparatively showed better in the present study.

Proximate analysis of products:

Proximate analysis of coconut jelly

Moisture: The jelly is an intermediate moisture food which is hard to dry as it is hygroscopic in nature Delgado and Banon, 2015. Determination of moisture content is vital with regards to jelly texture and quality Dewi et.al., 2018. In the present study the moisture content for coconut jelly infused with *Clitoria ternatea* extract was 91% which was slightly less than the control percent which was 93% as shown in table 4 and figure 9. The jelly moisture content for various combinations of fruit pulps range in between 13 to 20% as per Madukokila et al., 2021.

Ash: The food ash content will be reduced when moisture content is high and the range for the ash content of jelly is from 0.67 to 2.29% Delgado ND Banon, 2015. The present infusion of *Clitoria ternatea* extract into the coconut jelly showed 0.95 gm ash content as shown in table 4 and figure 9 which falls in the range as reported earlier, this may be due to the type of components used in jelly preparation.

Fat: The fat content reported for the coconut jellies prepared by infusion of *Clitoria ternatea* flower extract through ultra sonication process was 0.09 gm as shown in table 4 an figure 9.

Protein: The protein content for White chocolate prepared by infusion of *Clitoria ternatea* flower extract through ultra sonication was 0.20 gm as shown in table 4 and figure 9.

Proximate analysis of white chocolate

Moisture: The moisture content in the present study was reported as 13%. As per the table 5Increasing the concentration of *Clitoria ternatea* extract powder decreased the moisture content significantly Pamungkaningtyas et.al., 2024. The moisture content in the present study was compared to control as shown in table 6 and figure 12.

Ash: The ash content has shown slightly higher 1.84 gm than control which is 1.74 gm as shown in table 6 and figure 12.

Fat: The fat content is 34 gm in the infused sample and 33.7 in the control as shown in table 6 and figure 12. The lipid content was generally reported less than 10% for edible flower extracts Carboni et.al., 2025, but the infusion of them into chocolate may enhance the overall fat content of the product.

Protein: The protein content is same for both sample and control that is 9.49 gm as shown in table 5 and figure 12. This falls within the range of 2 to 23% as reported by Carboni et.al., 2025.

Conclusion:

The natural dyes infused product making in the field of food industry has greater importance. In the present study, the antioxidant properties explored for the anthocyanin dye have been an advantage in promoting the *Clitoria ternatea* flower ultra sonication extract as a better and recommended alternative as a natural food colorant. The proximate analysis shown in the study has further added its importance in terms of nutritive value making it a value added product in the food industry.

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Conflict of Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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