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RESEARCH ARTICLE

Formulation and Evaluation of Edible Ink from *Clitoria ternatea* L. (Butterfly Pea) and *Hibiscus rosa-sinensis* L. (Hibiscus)

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Abstract

Background: Colour additives, encompassing pigments and dyes, are applied topically to alimentary products, pharmaceuticals and cosmetics. Synthetic food colourants may pose health risks to consumers and may induce toxicological effects upon ingestion. In contrast, natural food colourants are regarded as innocuous, non-toxic and environmentally biodegradable. Edible ink is a viscous liquid or paste containing dyes or pigments, is used to impart chromatic hues and intricate patterns onto the surface of food products. **Aim:** The objective of this study is to formulate edible ink from *Clitoria ternatea* L. and *H. rosa-sinensis* L. **Methods:** The Pigment extraction was carried out using the solvent extraction method, wherein the factors considered were ethanol concentration, liquid feed ratio, extraction time and temperature. The resultant pigments were used to prepare the edible ink and subsequently subjected to an array of physical, functional and shelf-life analysis. **Result:** The findings indicate that the optimal extraction conditions were achieved with 80% ethanol concentration, 1:4 liquid feed ratio, 60 min extraction time and 50ºC temperature. The physical properties evaluation revealed that the edible ink exhibited desired attributes, while the antioxidant values were 109.72±1.106 for *C. ternatea* L. and 110.87±1.050 for *H. rosa-sinensis* L. edible ink. Furthermore, the cytotoxicity assessment revealed that the formulated inks were non-cytotoxic at lower concentrations, and the microbial study confirmed that both inks remained safe for up to 30 days with excellent colour retention when applied to food substrates. **Conclusion:** This study successfully developed a non-toxic, antioxidant-rich edible ink from *C. ternatea* L. and *H. rosa-sinensis* L. under optimised conditions. The formulated ink has demonstrated effective colour performance, stability and safety, ensuring its potential use in the food industry.

Major Findings: The study concludes by highlighting the optimised formulation of edible inks from *C. ternatea* L. and *H. rosa-sinensis* L., which exhibits antioxidant activity, non-cytotoxic, microbial stability and promising application in food products.

Keywords: Antioxidant, Application, Edible Flowers, Edible Ink, Pigment Extraction

1. Introduction

Food additives are defined as "any substance that is normally ingested as a food by itself and not commonly used as a typical ingredient of food, regardless of its nutritional value, but added to the food to achieve a specific function, known for having safe dose levels and subject to legal permission"[1]. Food additives are molecular entities incorporated into food products to perform specific technological functions, such as imparting color, enhancing sweetness, preserving, modifying texture, facilitating drying, or executing various other technological processes. As the name implies, colour additives serve the purpose of providing chromatic attributes to the food products. Food colourants are substances incorporated into food primarily to impart colour, prevent colour degradation due to environmental factors, or enhance natural

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colours[2]. Additionally, they may be utilised to alter the undesired hues, standardise colour uniformity, enhance colour stability or adjust the natural colour of food for a more or less vibrant or intense[3]. According to the U.S. Food and Drug Administration (FDA), colour additives encompass any pigment, dye or material capable of being applied to the human body or introduced into food, pharmaceuticals, or cosmetics for the purpose of imparting colour, whether used singularly or in combination with other substances[4].

Edible ink is a liquid or paste formulation containing dyes or pigments[5]. It is employed to imprint vibrant colours and patterns onto the surfaces of foodstuffs, pharmaceuticals, confectioneries, edible films, and coatings. Furthermore, it finds application in the printing of packaging materials for food, pharmaceuticals and other products, delivering not only chromatic colours but also biodegradable, environmentally friendly, and non-toxic properties[6]. In the formulation of edible ink, soybean oil functions as a binding agent, sucrose serves as a pigment carrier, and xanthan gum acts as a stabiliser[7].

Natural pigments are pigments directly extracted from plants and microorganisms, have garnered significant attention due to their diverse applications. In comparison to fruits and vegetables, flowers contain greater concentrations of bioactive antioxidants, including vitamin C, carotenoids, anthocyanins and polyphenols[8,9]. Edible flowers are non-toxic and, in addition to their aesthetic appeal, serve as potential sources of valuable phytochemicals. These phytochemicals, including flavonoids, anthocyanins, carotenoids, and phenolics, are primarily responsible for the health-promoting benefits associated with edible flowers[10]. Consequently, the objective of the present investigation is to develop an edible ink formulation by harnessing natural pigments derived from two distinct floral species, *C. ternatea* L. and *H. rosa-sinensis* L. This research aims to mitigate the detrimental effects associated with commercially available inks

2. Materials and Methods

2.1 Procurement of Raw Materials

Clitoria ternatea L. (Butterfly pea) and *H. rosa-sinensis* L. (Hibiscus) flowers were collected from the botanical garden of *Semmozhi poonga*. Soybean oil and sucrose were purchased from a local supermarket. The flowers were authenticated by Siddha Central Research Institute with the following codes: H03012404R for *H. rosa-sinensis* L. and C03012405T for *C. ternatea* L.

2.2 Formulation of Edible Ink from *C. ternatea* L. (*Clitoria*)

2.2.1 Biological Source

Clitoria ternatea L. is a member of the Fabaceae family, class Magnoliopsida, phylum Tracheophyta, and kingdom Plantae[11].

2.2.2 Materials Required

Clitoria ternatea L., Ethanol, Sucrose, Soybean oil and Xanthan gum.

2.2.3 Extraction of C. ternatea L. Pigment by Solvent Extraction Method

The pigment extraction from *C. ternatea* L. petals was carried out using the solvent extraction method. The petals were initially triturated using a laboratory extractor. The extraction procedure was optimised by modelling four key factors: Ethanol concentration, liquid feed ratio, extraction time and extraction temperature. Each parameter was assessed at four distinct levels, culminating in sixteen experimental configurations.

Anhydrous ethanol was employed at concentrations of 50%, 60%, 80% and 90%, with each concentration aliquoted into separate 100 ml beakers. The crushed petal and the ethanol were mixed at the liquid-to-feed ratios of 1:2, 1:4, 1:6 and 1:8. The pH of the solution was meticulously adjusted to 2 for all experimental runs. The extraction temperatures were varied at 40°C, 50°C, 60°C and 70°C for each trial, and the extraction durations were set at 30, 60, 90 and 120 min for optimising the extraction process. The pigment yield was quantified by measuring the absorbance of the extract at 550nm using a spectrophotometer under different experimental condition[7].

2.2.4 Preparation of Edible Ink from C. ternatea

The edible ink is prepared from the optimised pigment of *C. ternatea* L. To prepare the edible ink, 10 ml of optimised pigment and 20 g of sucrose were added to a 100 ml beaker and stirred continuously until it is fully dissolved, and 20 ml of soybean oil was added

as a connecting material. Then, 0.5 g of xanthan gum was added as a stabiliser and dissolved completely. The mixture was set aside for 60 min. The preparation of edible ink was then completed[7].

2.3 Formulation of Edible Ink from *H. rosa-sinensis* L. (Hibiscus)

2.3.1 Biological Source

Hibiscus rosa-sinensis L. is a member of the *Malvaceae* family, class *Magnoliopsida*, phylum *Magnoliophyta* and kingdom *Plantae*[12].

2.3.2 Materials Required

Hibiscus rosa-sinensis L., Ethanol, Sucrose, Soybean oil and Xanthan gum.

2.3.3 Extraction of H. rosa-sinensis L. Pigment by Solvent Extraction Method

The petals of *H. rosa-sinensis* L. were crushed using the laboratory extractor, and the resultant crushed petals were immersed in anhydrous ethanol in varying concentrations of 50%, 60%, 80% and 90% in the liquid-to-feed ratio of 1:2, 1:4, 1:6 and 1:8 (g/ml) each in separate 100 ml beakers. The pH of the extraction solution was uniformly adjusted to 2 across all the experimental trials. The extracting temperature was varied at 40°C, 50°C, 60°C and 70°C, while the extraction time was set at 30, 60, 90 and 120 min for all the experimental trials. The absorbance of the extracts was subsequently measured at 550 nm using a UV Spectrophotometer to optimise the extraction parameters in the pigment yield.

2.3.4 Preparation of Edible Ink from H. rosa-sinensis L

The edible ink is prepared from the optimised *H. rosa-sinensis* L. pigment. For the formulation, 10 ml of optimised pigment and 20 g of sucrose were combined in a 100 ml beaker and stirred continuously until completely dissolved. Then, 20 ml of soybean oil was added as a connecting material and mixed thoroughly. Subsequently, 0.5 g of xanthan gum was incorporated as a stabiliser, ensuring its complete dissolution into the mixture and the resultant mixture was then allowed to rest for 60 min to facilitate the final ink formulation. The preparation of edible ink was completed.

Figure 1 shows the optimisation of pigment using a UV Spectrophotometer, and Figure 2 demonstrates the preparation of edible ink from the standardised pigments of *C. ternatea* L. and *H. rosa-sinensis* L.

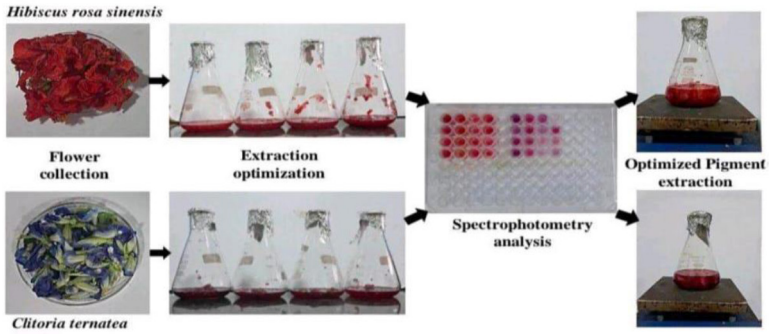


Figure 1. Pigments optimisation under varying conditions using a UV spectrophotometer.



Figure 2. Flowchart: Formulation of edible ink from standardised pigments. **(a).** Pigment, sucrose, soybean oil, **(b).** Xanthan gum, **(c).** Edible ink (Left: Clitoria, Right: Hibiscus).

2.4 Physical Analysis of Edible Ink

The physical properties of edible ink, including colour, odour, viscosity, permanence of colour and drying time, were assessed using various techniques. The Colour Quest XE method with the Hunter Colorimeter Lab system was employed to measure colour values (L, A, B) of edible ink[13]. Odour concentrations and complaints were evaluated using dynamic olfactometry, which relies on sensory-trained assessors[14]. Viscosity was determined by recording the flow rate of 50 ml of ink through a hole in a cup, with three replicative trials performed to compute the mean efflux duration[15]. The permanence of colour was evaluated by submerging the samples in water, and drying time was determined to assess the speed at which the ink set[16].

2.5 Functional Properties

The functional properties of the edible ink were evaluated through DPPH radial scavenging and cytotoxicity activity.

2.5.1 Diphenyl-1-picrylhydrazyl (DPPH)[17]

The Diphenyl-1-picrylhydrazyl (DPPH) technique is used to examine the edible ink's capacity to scavenge free radicals. In methanol solution, DPPH gives off a violet or purple hue, which fades to yellow tones when antioxidants are present. Using 500 L of 1,1-Diphenyl 2-picryl-hydrazyl (0.4 M) solution, 50, 100, 250, 500, and 1000 g/ml of 1ml of Standard and Sample were mixed. The mixture was then allowed to sit at room temperature for 30 min in the dark. A spectrophotometer set up at 517 nm was used to test the mixture's absorbance[17]. The percentage DPPH radical scavenging activity was calculated by the following equation[18].

$$RSA\ \% = \frac{Abs(Control) - Abs(Sample)}{Abs(control)} \times 100$$

2.5.2 Cytotoxicity Activity

The Cytotoxicity experiment measures cell proliferation by evaluating absorbance, which is directly linked to cell activity. Based on the method by Mosmann[19], 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is reduced by mitochondrial enzymes in viable cells to form purple formazan. The level of formazan produced is inversely related to cell viability. In this assay, trypsinised cells were incubated in 96-well plates for 24 h at 37°C, followed by treatment. The samples were added to the cells for a period of 12–24 h at varying doses. The MTT solution was then added, and after 4 h, the formazan crystals were dissolved in 100 µl of DMSO, and the absorbance was measured at 570 nm using a microplate reader[19].

2.6 Shelf-life Assessment of the Edible Ink

The shelf life study was conducted on the 0th, 15th and 30th day following the preparation of edible ink. The prepared inks were stored in air air-tight glass container under ambient conditions for 30 days to evaluate the stability and microbial activity of the edible ink.

2.6.1 Stability Study

The stability study was performed by quantifying the pigment's absorbance using spectrophotometric analysis, which measures light absorption at specific wavelengths, a critical technique for evaluating mixtures like dyes and pigments[20]. To assess pigment stability, 5 mL of the extracted pigment was dissolved in 250 mL of distilled water, and the resulting solution was placed in a cuvette. The absorbance was measured using a dual-beam UV spectrophotometer at 282 nm on the 1st and 15th days after preparation.

2.6.2 Microbial Analysis

Total Bacterial Count (TBC) is the count of the number of bacterial colony-forming units present in the sample, giving a quantitative evaluation of the total number of bacterial colony-forming units per millilitre[21]. The TBC was done by the pour plate method using nutrient agar as a culture medium. Total yeast and mold count is used as an indicator of the general hygienic conditions of the product's life cycle from cultivation to handling, processing, and storage[22]. The total yeast and mold count was done by the pour plate method using potato dextrose sugar as a culture medium.

2.7 Application of Edible Ink on Food Substrates

The prepared ink was applied to selected food material using a plastic stencil. The process began with the preparation of a stencil, followed by pouring the ink over it onto the food surface and the remaining ink was collected. The printed food products were kept at room temperature for drying[23].

3. Results and Discussions

3.1 Standardisation of Pigments using Absorbance Measurement

The pigment yield was evaluated by measuring the absorbance of the extract at 550nm using a UV Spectrophotometer. As shown in Figure 3a, the absorbance of *C. ternatea* L. increased with the ethanol concentration of 80%, reaching a peak of 2.702 at this concentration and fell at the concentration of 60%. Similarly, *H. rosa-sinensis* L. exhibited the highest absorbance of 1.187 at 80% ethanol concentration (Figure 4a). These results are consistent with the findings of Li[24], which demonstrated that an 80% ethanol concentration has increased the extraction efficiency and yielded the highest pigment absorbance response[24].

Figures 3b and 4b show that the highest absorbance values, 1.255 and 2.227, were observed at a liquid-to-feed ratio of 1:4 for both *H. rosa-sinensis* L. and *C. ternatea* L. Conversely, the lowest absorbance values, 1.037 and 1.465, were recorded at a liquid-to-feed ratio of 1:8. According to Wang[25], an increase in the liquid-to-feed ratio can lead to higher oxygen concentrations in the extract, which may cause the oxidation of certain anthocyanins, thereby reducing the total anthocyanin yield. This likely explains the observed decrease in absorbance at the higher liquid-to-feed ratio of 1:8 in the present study[25]. A similar change trend was observed for the effects of extraction time and temperature (Figures 3c,d and 4c,d). The maximum absorbance was achieved at an extraction time of 60 min and an extraction temperature of 50°C for both pigments, while the lowest absorbance occurred at 120 min and 60°C. The prolonged extraction time and higher temperatures may lead to pigment degradation, such as oxidation or pyrolysis, resulting in the formation of other compounds[26]. The highest pigment yield was indicated by maximum absorbance, was achieved under the optimal conditions at 80% ethanol, 1:4 liquid-to-feed ratio, 60 min extraction time, and 50°C temperature. These conditions were most effective for extracting pigments from both *C. ternatea* L. and *H. rosa-sinensis* L. The optimised pigment was then used to formulate the edible ink, which was subsequently subjected to comprehensive physical, functional, and microbial analyses.

3.2 Physical Characteristics

The edible inks were analysed for physical properties like colour, odour, viscosity, permanence of colour and drying time.

3.2.1 Color Measurement

Table 1 shows the colour analysis of *C. ternatea* L. and Hibiscus rosasinensis L. edible inks, using Hunter

Table 1. Color parameters of edible inks

S. No.	Color parameter	*C. ternatea* L. ink	*H. rosa-sinensis* L. ink	pvalue
1	L*	51.78±1.006	37.13±1.035	0.00
2	a*	11.78±1.048	10.07±1.075	
3	b*	4.19±1.002	3.06±1.064	

The values are expressed as mean±standard deviation. The values are ***Significant at 0.1% level (p≤0.001) by using a paired T-test.

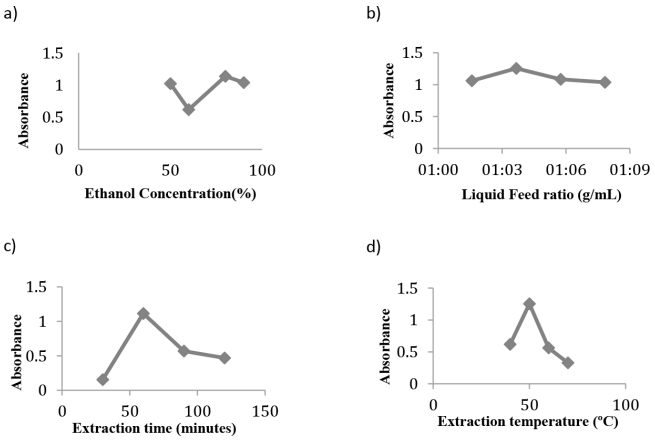


Figure 3. Absorbance of *Hibiscus rosa-sinensis* L. pigment in different conditions.

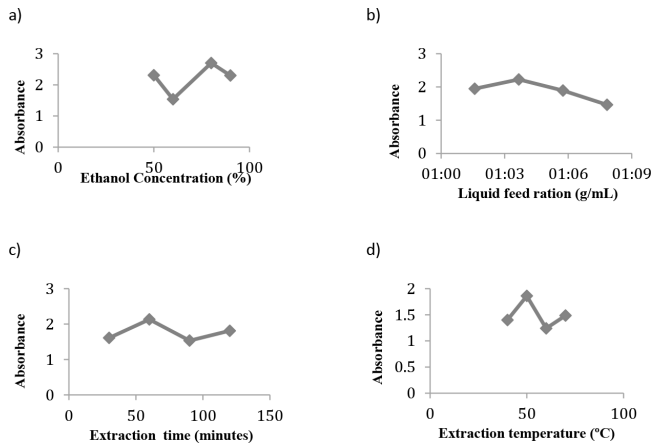


Figure 4. Absorbance of *Clitoria ternatea* L. pigment in different conditions.

Lab parameters, which revealed a highly significant difference of 0.1% ($p \leq 0.001$). The lightness L* of Clitoria ink was 51.78±1.006, whereas the lightness L* of Hibiscus ink was 37.13±1.035. A higher L* value corresponds to a lighter sample[27]. Therefore, the lower L* value of Hibiscus ink indicates a darker color[28]. Both inks showed positive redness values (a* = 10.07±1.075 for Clitoria and a* = 11.78±1.048 for Hibiscus), which are attributed to the presence of anthocyanins and phenolic compounds[29]. Furthermore, the b* values for *C. ternatea* L. and *H. rosa-sinensis* L. and inks were 4.19±1.002 and 3.06±1.064, respectively, with both values being positive, indicating a yellowish hue. This yellowness of the edible ink is due to the change in pH. The anthocyanin exhibits a yellow to green hue in alkaline[30].

3.2.2 Odour Assessment

The odour characteristics of edible inks from *C. ternatea* L. and *H. rosa-sinensis* L. were assessed on the 0th and 7th day after preparation. *C. ternatea* L. ink exhibited a moderate fruity odour, while *H. rosa-sinensis* L. ink had a strong, pleasant fruity odour on both days. These findings are consistent with Delostrico, who reported low to moderate odours in organic ink derived from *Melastoma malabathricum* fruit extract[31]. The floral scents include Volatile Organic Compounds (VOCs) such as terpenoids, fatty acid derivatives, and phenylpropanoids, which vary significantly among plant species, origin, and production methods, contributing to the distinct odours observed in *C. ternatea* L. and *H. rosa-sinensis* L[32].

3.2.3 Determination of Viscosity

Viscosity measures a liquid's resistance to flow. Table 2 demonstrates the flow time for edible inks. For *C. ternatea* L. ink the flow time was 14 min and 16 s, while for *H. rosa-sinensis* L. ink, it was 12 min and 6 s. Solvents serve as the medium for dissolving dyes or suspending pigments in ink, without impacting their colour. However, they significantly affect the ink's viscosity. Solvents with lower viscosity facilitate faster flow, while those with higher viscosity result in slower flow. Since soybean oil is used in the formulation of the edible ink, it results in a higher viscosity and slower flow rate[15].

Table 2. Viscosity estimation of edible inks

S. No.	Edible Ink	Trial 1 (in min)	Trial 2 (in min)	Trail 3 (in min)	Average Time (in min)
1	*C. ternatea* L. ink	14.3	14.7	13.5	14.16
2	*H. rosa-sinensis* L. ink	12.5	12.9	12.4	12.6

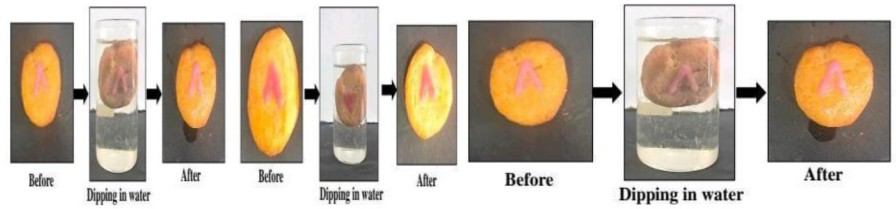


Figure 5. Evaluation of colour permanence using the dipping method. (**a**) *C. ternatea* L., (**b**) *H. rosa-sinensis* L. ink.

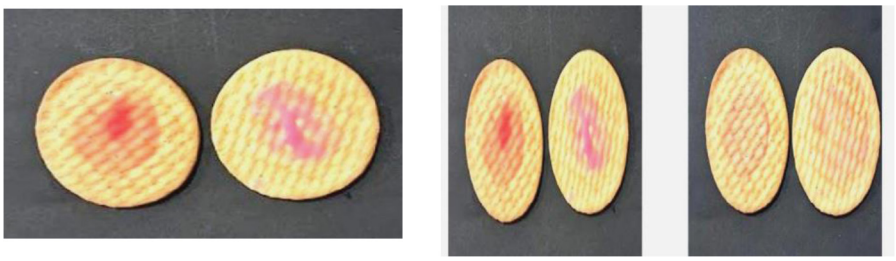


Figure 6. Assessment of drying time for edible ink after application.

3.2.4 Permanence of Colour

Permanence of colour refers to an ink's ability to resist fading and the colour change when exposed to weather or water. In this study, *H. rosa-sinensis* L. ink (Figure 5a) showed slight smearing when submerged in water, while *C. ternatea* L. ink remained intact (Figure 5b). Both inks demonstrated resistance to fading and colour change, indicating good durability against water exposure[31].

3.2.5 Drying Time

Drying time is defined as the interval required for ink to fully evaporate after being applied to a substrate, such as paper or a food material. In the present study, *C. ternatea* L. ink exhibited a drying time of 15 min and 53 s, whereas *H. rosa-sinensis* L. ink showed a drying time of 14 min and 8 s when applied on food material. In comparison, Delostrico *et al*.,[31] reported the drying time of organic inks ranged from 11 min 23 s to 22 min 33 s across different formulations, aligning with the findings of the present study. Silva[33] demonstrated that inks incorporating ethanol dried more expeditiously, suggesting that the ethanol acts as a catalyst in the drying process. Since the ethanol was not used in the preparation of the edible inks in this study, the observed drying times were relatively longer[33] (Figure 6).

3.3 Functional Properties of Edible Ink

3.3.1 DPPH Antioxidant Assay

Dietary antioxidants are the compounds present in food which reduce the harmful effects of reactive species on the human body[34].

From the shown curve, the IC_{50} value has been calculated with the linear equation, y = mx+c. The antioxidant activity was evaluated using the DPPH assay, with the IC_{50} value representing the concentration required to inhibit 50% of DPPH activity[35]. IC_{50} values <50-100 ppm indicate high antioxidant activity, <101-250 ppm indicate moderate activity, and <250-500 ppm suggest weak antioxidant potential[36] (Figure 7).

Table 3 indicates the IC50 values of edible inks. For *C. ternatea* L., the IC50 value was 109.72±1.11 ppm, and for H. rosa-sinensis L. inks, it was 110.87±1.05 ppm, both reflecting moderate antioxidant activity. Statistically significant differences at ($p \leq 0.05$) were observed between the two inks. Similar findings were reported by Widyanto *et al*[37], who reported a strong antioxidant activity in D. rostrata peel (IC_{50} = 59.27 ppm) and moderate activity with 50% ethanol extract of the same (IC_{50} = 121.7 ppm). Therefore, the use of 80% ethanol for pigment extraction may reduce antioxidant activity. These results are consistent with the study undergone by Karim, which reported moderate antioxidant activity (IC_{50} = 171.2±7.3 ppm) found in melanin-free squid ink[38].

3.3.2 Cytotoxicity Assay

Cytotoxicity testing is crucial in assessing the potential toxicity of plant-based products[39]. In this study, the *in vitro* cytotoxic activity of edible inks was determined using the MTT cell viability assay against the vero cell line, with Optical Density (OD) measurements taken at 570 nm. The vero cell line was exposed to various concentrations (1, 4, 8, 16, 32, 64, 128, 256 and 512

Table 3. IC_{50} value of the prepared edible inks

S. No.	Samples	IC_{50}
1	Standard	99.053±1.005
2	*C. ternatea* L. ink	109.72±1.106
3	*H. rosa-sinensis* L. ink	110.87±1.050

The values are represented in mean±standard deviation.
*Significant at 5% level ($p \leq 0.05$) using paired T-test.

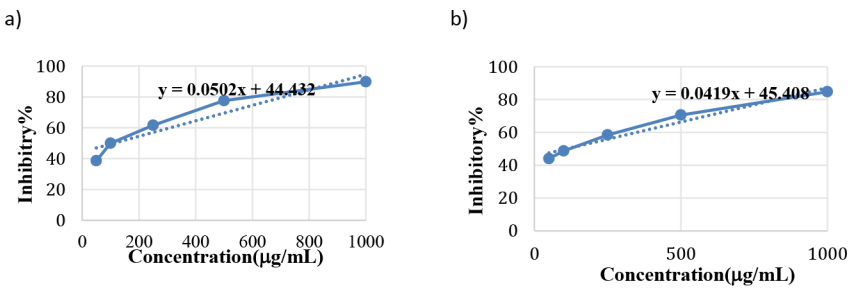


Figure 7. Antioxidant activity curve of edible inks.

µg/mL) of inks. Both edible inks demonstrated no significant inhibition of cell viability.

As illustrated in Figure 8, cell viability decreased with increasing concentrations of inks. Specifically, Hibiscus ink reduced cell viability from 97% to 46% within the concentration range of 1 µg/mL to 512 µg/mL, while Clitoria ink decreased viability from 95% to 39%. This decline in cell viability with increasing sample concentration is consistent with the findings of Olotu[40]. According to ISO 10993-5 guidelines, cell viability above 80% is considered non-cytotoxic, 60%-80% as weakly cytotoxic, 40%-60% as moderately cytotoxic, and below 40% as strongly cytotoxic. Figure 8 shows that at concentrations ranging from 1-4 µg/mL, both edible inks exhibited cell viability between 97% and 80%, indicating a non-cytotoxic activity at lower concentrations.

3.4 Shelf Life Evaluation of Edible Ink

The shelf life of the natural food colorants extracted from plant materials is influenced by factors such as the solvents used, their molecular structure, interaction with ingredients and environmental conditions during processing and storage[41]. The prepared edible inks were stored at a refrigerator temperature of 4°C for 30 days and were analysed for pigment and ink stability, total

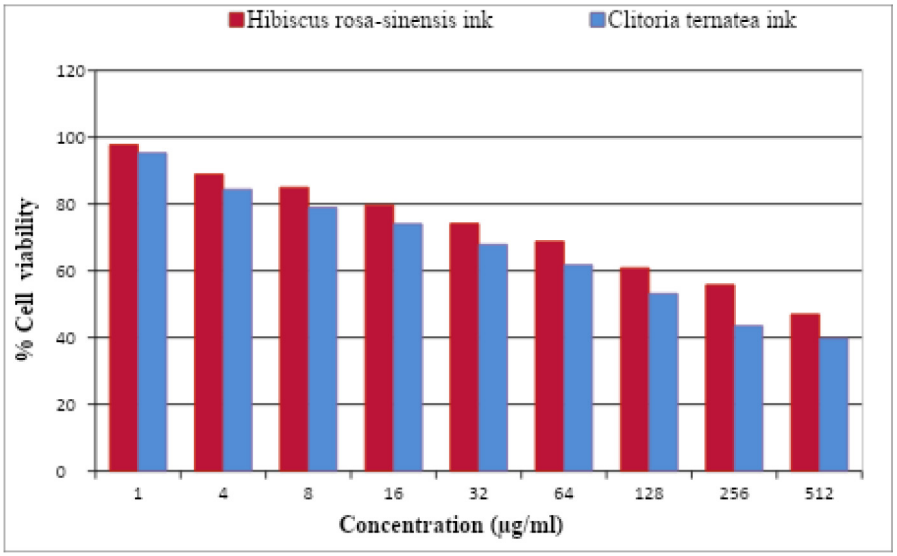


Figure 8. Percentage cell viability of vero cells treated with edible inks.

Table 4. Stability profile of pigment and edible ink

Edible ink	Wave length (nm)	Pigment absorbance			Edible ink absorbance		
		0th day	1st day	15th day	0th day	1st day	15th day
C. ternatea L.	400	1.67±0.49^{b}	1.52±0.26^{b}	0.72±30^{b}	0.45±0.16^{c}	0.50±0.1^{c}	0.24±0.01^{c}
	500	1.73±0.1^{a}	1.73±0.1^{a}	0.83±0.14^{a}	1.34±0.11^{a}	1.35±0.1^{a}	0.73±0.09^{a}
	600	0.83±0.14^{d}	0.83±0.14^{c}	0.35±0.14^{c}	0.15±0.1^{d}	0.15±0.1^{d}	0.07±0.02^{d}
	700	0.32±0.1^{e}	0.31±0.10^{d}	0.08±0.01^{d}	0.04±0.02^{e}	0.04±0.02^{e}	0±0^{e}
	800	0.33±0.10^{e}	0.29±0.1^{e}	0.07±0.01^{e}	0.03±0.01^{e}	0.03±0.01^{e}	0±0^{e}
H. rosa sinensis L.	400	1.67±0.49^{b}	1.52±0.26^{b}	0.72±0.30^{b}	0.72±0.30^{b}	0.83±0.11^{b}	0.52±0.17^{b}
	500	1.72±0.10^{a}	1.73±0.1^{a}	0.83±0.14^{a}	0.83±0.17^{b}	0.82±0.17^{b}	0.48±0.10^{b}
	600	0.90±0.10^{c}	0.83±0.14^{c}	0.35±0.14^{c}	0.37±0.11^{c}	0.36±0.14^{c}	0.21±0.1^{c}
	700	0.34±0.10^{e}	0.31±0.1^{d}	0.08±0.01^{d}	0.07±0.01^{e}	0.08±0.01^{e}	0.05±0.01^{d}
	800	0.33±0.10^{e}	0.27±0.09^{e}	0.07±0.01^{e}	0.07±0.01^{e}	0.07±0.01^{e}	0.05±0.01^{d}

The values are expressed as mean±standard deviation. The values are represented with different superscripts from a-e in a row differ significantly at 5% level ($p \leq 0.05$) using a paired T-test.



Figure 9. Stencil-based application of edible ink.

bacterial growth and yeast and mold proliferation in both the *C. ternatea* L. and *H. rosa-sinensis* L. edible inks.

3.4.1 Stability Analysis of Pigment and Edible Ink

The stability test is done to measure the significant changes that have taken place in the product during the storage[42]. An increase in UV spectrophotometer absorbance signifies greater colour intensity, attributed to a higher solute concentration, which enhances light absorption at specific wavelengths characteristic of the pigment[43]. Table 4 highlights the stability of pigments and edible inks prepared from *H. rosa-sinensis* L. and *C. ternatea* L., demonstrating a statistically significant difference at the 5% level ($p \leq 0.05$) based on a paired t-test. This difference is due to the low storage stability of anthocyanins[44].

The results indicate that the pigments and edible inks showed high absorbance on the 0th and 1st days after preparation, primarily due to the anthocyanin content. The anthocyanins, responsible for red-blue hues in plants, exhibit strong absorption in the UV-visible spectrum[45]. However, by the 15th day, the absorbance significantly declined, reflecting anthocyanin degradation. This instability is influenced by factors such as oxygen, co-pigments, enzymes, light, pH, temperature, sugars, vitamin C, and sulfur dioxide or sulfites[44]. The findings align with prior research. Azwanida *et al*[42] observed that *C. ternatea* L. pigment faded and became dull after five days of storage, primarily due to betalain degradation caused by light exposure. Similarly, Kathiresh[46] reported that *Sesbania sesban* pigments showed reduced colour intensity after 15 days of storage due to the changes in pH and storage temperature[46]. In conclusion, the study confirms that the absorbance and colour intensity of anthocyanin-based pigments and edible inks reduced over a storage period of 15 days, emphasising the limited stability of anthocyanins under typical storage conditions.

Table 5. Microbial analysis of edible ink

Storage day	*C. ternatea* L. edible ink		*H. rosa-sinensis* L. edible ink	
	TBC	TYMC	TBC	TYMC
0th Day	6.5×10^4	Nil	7.4×10^4	Nil
1st Day	6.5×10^4	Nil	7.4×10^4	Nil
15th Day	1.7×10^5	Nil	2.5×10^5	Nil
30th Day	2.8×10^5	Nil	4.8×10^5	Nil

3.4.2 Microbial Evaluation

Microbial analysis, including TBC and Total Yeast and Mold Count (TYMC), was conducted on days 0, 1, 15, and 30 to evaluate the safety and quality of the edible inks. TBC serves as an indicator of food product safety, while TYMC reflects the hygienic conditions during the product's lifecycle[47].

Table 5 indicates that the *C. ternatea* L. ink exhibited lower bacterial counts (6.5×10^4 to 2.8×10^5) when compared to *H. rosa-sinensis* L. ink (7.4×10^4 to 4.8×10^5), which can be attributed to the presence of strong antioxidant and phenolic compounds, like tannins present in the *C. ternatea* L., which are known for their antimicrobial properties[48]. This result is in contrast to research on squid ink, which demonstrated a higher bacterial count of 1.17×10^6 on the first day post-preparation[49]. Additionally, no yeast or mold growth was observed in either ink throughout the study, indicating that proper hygiene and sanitation practices were maintained during production.

3.5 Application of Edible Ink

Figure 9 illustrates the application of the prepared edible ink onto wheat flour biscuits using a plastic stencil, which significantly enhanced the visual appeal with vibrant red and purple hues. This finding aligns with Liu *et al*[7], who showed that purple potato-based edible ink, applied via stencil printing on bread and biscuits, resulted in strong colour effects[21]. These results suggest

that pigment-based edible inks could serve as an effective alternative to commercially available synthetic inks, further supporting the potential of natural edible inks in food applications.

4. Conclusion

This study optimised the extraction of pigments from *C. ternatea* L. and *H. rosa-sinensis* L. under controlled conditions (80% ethanol, 1:4 liquid-to-feed ratio, 60 min, and 50°C), resulting in high yield and stability. The extracted pigments were developed into edible inks with desirable properties, including vibrant colours, optimal viscosity, water resistance, and efficient drying times. Functional analyses demonstrated that the inks exhibited moderate antioxidant activity, non-cytotoxic at low concentrations and desirable shelf life stability with no yeast or mold growth and low TBC. These inks have effectively enhanced the visual appeal of food products, demonstrating their potential in sustainability, functionality, and serving as an eco-friendly alternative to synthetic food colourants. Natural food colourants are gaining popularity among consumers due to their health benefits. These findings highlight the growing demand for plant-based food colourants. The successful application of these pigment-based inks in food substrates has confirmed their potential as a viable substitute for synthetic inks, offering an innovative and environmentally sustainable solution for the food industry.

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6. Author Contribution

Ideation and Conceptualisation, D.S.P. and V.S.; Methodology Formulation, D.S.P. and V.S.; Supervision and Validation, V.S.; Resource provision, V.S.; Investigation and Data collection, D.S.P.; Data analysis, D.S.P. and V.S.; Original draft preparation, D.S.P.; Visualisation and presenting, D.S.P.; Reviewing, V.S. Both the authors have read and agreed to the published version of the manuscript.

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