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Assess the antioxidant and antimicrobial activity of herbal popsicles prepared by *Hibiscus sabdariffa L.* and *Clitorea ternatea* floral waste

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KEYWORDS

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ABSTRACT

In this study, we extracted bio-colour from two commonly available flowers, Rosella (*Hibiscus sabdariffa* L.) and Butterfly pea flower (*Clitoria ternatea*), and evaluated their potential therapeutic benefits by examining their antioxidant and antibacterial activity. To assess the suitability and quality of the extracted bio-colour as a food additive, we formulated ice popsicles using bio-colour derived from *H. sabdariffa* and *C. ternatea*. The crude floral waste extract of *H. sabdariffa* showed the highest reducing capacity (FRAP assay), antioxidant activity (DPPH, ABTS assay), and antibacterial potential. This may be attributed to polyphenols, flavonoids, anthocyanins, ascorbic acids, organic acids, hibiscus acid, and other compounds in *H. sabdariffa* flower parts. The ice popsicles formulated with these two bio-colours contained significant polyphenol and flavonoid content, contributing to their antioxidant potential comparable to ice popsicles available in the local market. The formulated ice popsicles also retained better physical properties (texture, melting, smoothness/hardness) and sensory qualities (as per hedonic scale rating) than market-derived ice popsicles. Therefore, these two crude floral wastes can be utilized as functional food bio-colourants in the food industry.

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1 Introduction

Food colour is a critical factor directly linked to consumers' acceptance of food items (Solymosi et al. 2015; Dey and Nagababu 2022). However, a significant amount of colour is lost while processing various food items. As a result, various synthetic or natural food colouring agents are used to restore the colour intensity, texture, and taste of food items (Xing et al. 2012; Dey and Nagababu 2022). In recent decades, synthetic food colourants have been predominantly used in food processing. It has been reported that these synthetic food colourants, when present in food industrial discharges, can cause water pollution by increasing the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of water bodies (Ardila-Leal et al. 2021; Al-Tohamy et al. 2022; Patil et al. 2022). They can interfere with the process of photosynthesis, inhibit the growth of aquatic plants (Yang et al. 2011; Hussain et al. 2020; Slama et al. 2021), and also cause biomagnification after entering the food chain (Shivani et al. 2020; Alsukaibi 2022). Additionally, numerous reports have highlighted the hazardous effects of these synthetic food colourants, such as hyperactivity, depression, hives, asthma, and brain tumours (Bora et al. 2019; Dey and Nagababu 2022; John et al. 2022). Therefore, scientists face the challenge of developing new, cost-effective, nutritious food colourants that enhance food flavours and are safe to use.

Bio-colourants refer to natural colouring agents from various living organisms such as plants, insects, and animals. The main food bio-colourants include carotenoids, flavonoids, anthocyanidins, and chlorophyll, which are extracted from different parts of plants (Rymbai et al. 2011; Singh et al. 2023; Vega et al. 2023). In India, permitted bio-colours include beta-carotene, beetroot concentrates, grape extract, annatto, lutein, cochineal extract, paprika oleoresin, turmeric oleoresin, phycocyanin, and saffron (Bora et al. 2019). The orange-yellow pigment beta-carotene, isolated from carrot (*Daucus carota*), algae (*Dunaliella salina*), oranges, pumpkins, apricots, mangoes, papayas, and red bell peppers, can act as an antioxidant and antiproliferative agent (Mortensen 2006; Rymbai et al. 2011; Sowmya Shree et al. 2017; Young and Lowe 2018; Renita et al. 2023). Betanin found in beetroot (Beta *vulgaris*) possesses potent radical scavenging, anti-inflammatory, hepato-protective, cardioprotective, antiproliferative, and antimicrobial activity and is widely used as a food colourant in yoghurt, candy, and ice cream (Bora et al. 2019; Silva et al. 2021; Luzardo-Ocampo et al. 2021; Novais et al. 2022).

Annatto is a yellow-orange coloured bio-colourant used in dairy products extracted from *Bixa orellana* tree seeds (Novais et al. 2022). Lutein, a yellow-coloured carotenoid with prominent antioxidant potential, is extracted from the petals of the marigold (*Tagetes erecta*) flower and is widely used in beverages, chewing gums, and oils, as a food colourant (Rymbai et al. 2011; Manzoor et al. 2022; Saini et al. 2023). The yellow pigment Crocin is isolated from the dried stigma of the saffron plant (*Crocus sativa*) and exerts efficient radical scavenging activity, thereby preventing metabolic syndromes (Farrell 1998; Naidu and Sowbhagya 2012; Bora et al. 2019). Similarly, Cochineal Extract, also known as Carmine (Carminic acid), is extracted from *Dactylopius coccus* (AlAshkar and Hassabo 2021; Li et al. 2021). Paprika red colour oleoresin is obtained from *Capsicum annuum* (Pérez-Gálvez et al. 2003; Rymbai et al. 2011; Kostrzewa et al. 2023). Curcumin, a bright yellow-coloured pigment, is isolated from the rhizome of *Curcuma longa* (Rajendran et al. 2022) and is used as a food colouring agent in various food items, having significant antioxidant and antimicrobial activities (Bora et al. 2019). Most non-food plant parts (flowers, leaves, fruit peels, etc.) are discarded as agro waste from the food processing or agricultural industry (Helkar et al. 2016; Torres et al. 2018).

The accumulation of agro-waste poses disposal issues and can lead to environmental pollution. However, these problems can be mitigated by using agro-waste to extract bio-colourants. These bio-colourants not only add colour to food but also contain various bioactive compounds, resulting in greater antioxidant and antimicrobial activity, as well as enhanced therapeutic potential (Rymbai et al. 2011; Bora et al. 2019; Singh et al. 2023; Pasdaran et al. 2023). As a result, natural bio-colourants are becoming preferred over conventional synthetic colourants due to their easy availability, cost-effectiveness, and lack of side effects (Rymbai et al. 2011; Ghosh et al. 2022; Nabi et al. 2023). In this study, we have endeavoured to extract bio-colourants from the natural crude floral waste of Rosella flowers (*Hibiscus sabdariffa*) and Butterfly pea flowers (*Clitoria ternatea*) and evaluate their antioxidant and antibacterial activity. These flowers are known for their beneficial health effects, including anti-inflammatory, radical scavenging, and antiproliferative and anti-carcinogenic potential (Goh et al. 2021; Jeyaraj et al. 2021). We investigated food products' antioxidant properties using these bio-colourants and compared their antioxidant potential with crude floral waste extract. Additionally, this study assessed the radical scavenging and antimicrobial activity of herbal popsicles prepared from the floral waste of *H. sabdariffa* and *C. ternatea*.

2 Materials and Methods

2.1 Preparation of floral waste extracts

The fallen floral parts of the Rosella flower (*H. sabdariffa*) were obtained from Adamas University Campus located in Barasat, West Bengal, India, and the flower parts of the Butterfly pea (*Clitoria ternatea*) were collected from a nearby temple as floral waste. Ten grams of Rosella and Butterfly pea flower petals were dried and blended with a mortar and pestle until the petals became a paste while being extracted with 100 ml of 1% citric acid

solution at a ratio of 1:10. The prepared extracts were filtered through Whatman filter paper no. 1 and transferred into a centrifuge tube, and centrifugation was carried out at 12000 RPM. As a result of the centrifugation, only the supernatant was collected in a new centrifuge tube, dried at 50°C on a hot plate, and stored at 4˚C for further study.

2.2 Estimation of the phenol content

The phenol content of crude floral waste extracts and their formulated products was evaluated using Folin-Ciocalteu's reagent, as Mathur and Vijayvergia (2017) described. In this method, 100 µL of the sample solution was mixed with 1 mL of Folin-Ciocalteu reagent (diluted in a 1:20 ratio, W/V) and 1 mL of 7% Sodium Carbonate was added, followed by 90 minutes of dark incubation. Finally, the absorbance reading was taken at 760 nm using a spectrophotometer (Hitachi, U-2910) (Shirazi et al. 2014), and the values were represented in terms of standard gallic acid.

2.3 Total flavonoid content Estimation

The flavonoid content of crude floral waste samples and their formulated products was determined using a spectrophotometric method (Shirazi et al. 2014). 1 ml of the sample solution and 4 ml of dH_2O were combined in a 10 ml volumetric flask. After incubating for 5 minutes, 0.3 mL of 5% (w/v) Sodium nitrite ($NaNO_2$) and 0.3 mL of 10% (w/v) Aluminium chloride ($AlCl_3$) were added. Then, 1 ml of 1M Sodium hydroxide was added to each tube, and the final volume was adjusted to 10 mL. The absorbance was measured at 510 nm using a spectrophotometer. Quercetin was used as a control, and the data was expressed as equivalent to quercetin in mg QE/g Dry Weight (DW) (Mathur and Vijayvergia 2017).

2.4 DPPH scavenging method

The antioxidant potential of crude floral waste samples and their formulated products was estimated using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging method. 0.5 mL of the stock sample solution was mixed with 2 mL of 1 mM DPPH solution in a tube. After 5 minutes of mixing, the solution was incubated in the dark for 1 hour. The absorbance was then measured in a spectrophotometer at 517nm. Ascorbic acid was used as a standard (Kouassi et al. 2016; Fanta Yadang et al. 2019), and the radical scavenging rate was calculated using the following formula:

$$\text{Radical scavenging rate (\%)} = [(A_{Control} - A_{Sample}) / A_{Control}] \text{ X } 100$$

2.5 ABTS radical scavenging method

The ABTS method offers an alternative approach to measure the antioxidant capacity of crude floral waste extracts and their formulated products by reducing the ABTS cation radical. The reaction mixture consists of equal proportions of 7 mM ABTS solution and 2.45 mM $K_2S_2O_8$ (potassium persulfate) solution, which is kept in the dark for 1-2 days. The ABTS solution is diluted in aqueous methanol at a 1:25 ratio. An aliquot of 20 µL of ten times diluted crude floral waste extract or formulated product and 2 mL of ABTS solution is added to a tube and kept at 30°C for a specific duration. The absorbance reading is then recorded at 734 nm using a spectrophotometer at 0, 10, and 20 minutes (Proestos et al. 2013; Kouassi et al. 2016; Fanta Yadang et al. 2019).

2.6 FRAP assay

The FRAP assay is a promising method for determining the reducing potential of crude floral waste extracts and their formulated products. The reaction mixture consists of 1 ml of sample solution, 2.5 ml of 0.2M PBS (pH 6.6), and 2.5 ml of 1% $K_3Fe(CN)_6$ solution. This mixture is well mixed and then heated in a water bath for 20 minutes at 50°C. After heating, 2.5 ml of 10% trichloroacetic acid (TCA) is added. The solution is centrifuged at 3000 rpm for 10 minutes, and 2.5 ml of the supernatant is collected. An equal volume of distilled water is added to the collected supernatant, followed by adding 0.5 ml of 0.1% $FeCl_3$ solution. Finally, the absorbance is measured at 700 nm using a spectrophotometer after 10 minutes of incubation. Ascorbic acid is the standard for comparison (Vijayalakshmi and Ruckmani 2016).

2.7 Estimation of TAC

The total antioxidant capacity (TAC) of the crude floral waste extracts and their formulated products was determined using the phosphomolybdenum method. A mixture was prepared by combining $(NH_4)_6Mo_7O_{24}$ (4mM), H_2SO_4 (0.6M), and Na_3PO_4 (28mM). Next, 0.1 mL of the sample solution was mixed with 1 mL of the abovementioned mixture, which was then placed in a water bath at 95°C for 90 minutes, then cooling to room temperature. The absorbance was measured at 695 nm using a spectrophotometer, and ascorbic acid was used as the standard (Re et al. 1999).

2.8 Agar well diffusion method

The antibacterial activity of bio-colours extracted from crude floral waste was assessed using the well diffusion method against two Gram-positive bacteria, *Staphylococcus aureus* (ATCC25923-0360P) and *Bacillus subtilis* (ATCC11774-0269P), as well as two Gram-negative bacteria, *Escherichia coli* (ATCC35218-0495P) and *Salmonella typhi* (ATCC14028-0363P). Ampicillin and chloramphenicol were used as positive controls. Each bacterial strain was cultured in 20mL of nutrient broth medium and then incubated for 24 hours to optimize growth. After incubation, 100 µL of the bacterial inoculum was spread over the agar surface. Wells were created in the agar media plates using a cork borer, and

the antibacterial agents and test extract solutions were added to these wells. The plates were then incubated at 37˚C for 24 hours. The antibacterial activity of the test crude floral waste extracts was evaluated by measuring the inhibition zone diameter (Mathivanan and Suseem 2016; Naqvi et al. 2020).

2.9 Product formulation

Initially, 50ml of water and 5 grams of sugar syrup were combined in a falcon. Next, 100 microliters of bio-colourant, extracted from crude floral wastes, was added to the mixture and thoroughly mixed using a mixer grinder. The solution was then poured into an ice cream maker, covered with a lid, and left to incubate overnight at 4˚C until the product formed. In this process, four different types of ice popsicles were produced: (1) Untreated ice popsicle A (Control, without any bio-colour), (2) Ice popsicle B (formulated with Rosella-derived bio-colour), (3) Ice popsicle C (formulated with Butterfly pea-derived bio-colour), and (4) Ice Popsicle D (a store-bought ice cream named SLICE)

2.10 Estimation of the Melting rate

The sample's melting rate was assessed by placing the ice popsicle at room temperature on the laboratory balance to measure the mass of the melting ice popsicles. The amount of melting was observed at 5-minute intervals until the structure completely melted. Ice popsicles with minimal overruns often melt faster than those with large overflows. The percentage of melted weight was plotted against time in minutes (Yeon et al. 2017; Martins et al. 2018).

2.11 Analysis of Organoleptic Properties

The prepared ice popsicles were assessed for taste, texture, appearance, colour, and overall preference and then stored overnight at 4°C. One hundred regular ice popsicle consumers, with an average age range of 17-55 years, including 50 males and 50 females, participated in the study. They were requested to evaluate the ice popsicles using a 9-point structured hedonic scale (Martins et al. 2018). The ice popsicles were presented to the participants randomly on coded opaque plastic plates. All participants received prior instructions about the test.

2.12 Statistical evaluations

Each experiment was repeated three times. Data was presented as Mean ± SD and analyzed statistically using one-way and two-way Analysis of Variance with the GraphPad Prism5 software V5.03 (San Diego, USA).

3 Results

3.1 Total phenolic content (TPC)

The total phenolic content (TPC) was significantly higher in the crude floral waste extract of *H. sabdariffa* derived bio-colour (47.16 mg GAE/g DW) compared to its formulated product, which had a TPC value of 25.53 mg GAE/g DW (Figure 1). Similarly, the crude floral waste extract of Butterfly pea (*C. ternatea*) derived bio-colour exhibited a higher TPC value than its formulated product, as depicted

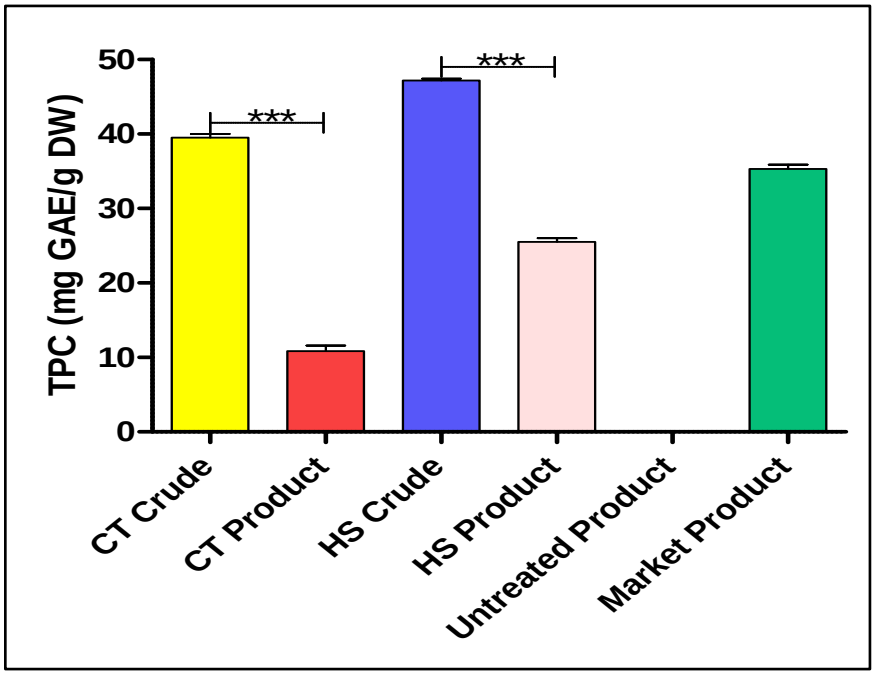

Figure 1 Comparing TPC crude floral waste samples and the formulated products (ice popsicles) expressed as GAE/g DW, the graph represents data in Mean ± SD (n=3) with ***P ≤ 0.001, **P≤0.01, *P≤0.05, CT – *C. ternatea;* HS – *H. sabdariffa*

in Figure 1. In the formulated products, the TPC content was lower than that of their crude samples, but the highest TPC content was found in *H. sabdariffa* derived bio-colour and its formulated product.

3.2 Total flavonoid content (TFC)

Rosella's crude floral waste extract shows almost six times higher total flavonoid content (TFC) than its formulated product, similar to TPC. Similarly, the crude floral waste extract of Butterfly pea exhibits a higher TFC value than its formulated product, as shown in Figure 2.

3.3 FRAP assay

The ferric ion reduction potential (FRAP) of a crude floral waste extract of *H. sabdariffa* was 9.31 µg/ml, significantly higher than its formulated product's FRAP value (4.45 µg/ml). Similarly, the crude floral waste extract of Butterfly pea showed a FRAP value of 8.02 µg/ml, higher than its formulated product (3.12 µg/ml), as depicted in Figure 3. This suggests that the ferric ion reduction potential of the crude sample of Rosella and its formulated product was slightly higher than that of the Butterfly pea derived bio-colour and its formulated product.

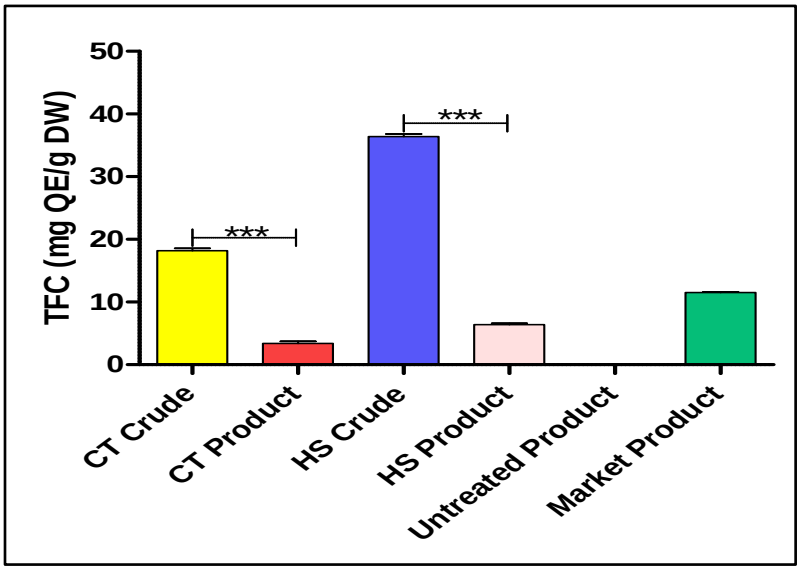

Figure 2 Comparison of TFC of crude floral waste samples and the formulated products (ice popsicles) expressed as QE/g DW, the graph represents data in Mean ± SD (n=3) with ***P ≤ 0.001, **P≤0.01, *P≤0.05, CT – *C. ternatea; HS – H. sabdariffa*

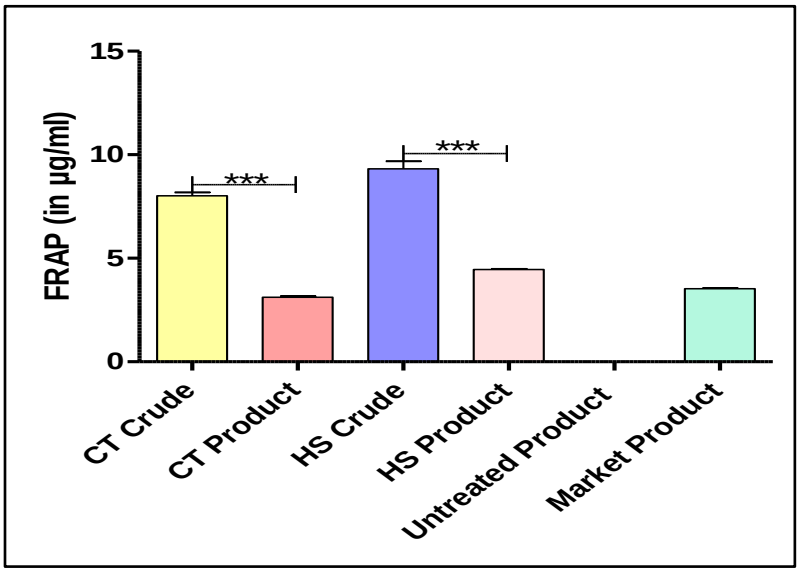

Figure 3 Result of the FRAP assay of crude floral waste samples and the formulated products (ice popsicles), the graph represents data in Mean ± SD (n=3) with ***P ≤ 0.001, **P≤0.01, *P≤0.05; CT – *C. ternatea; HS – H. sabdariffa*

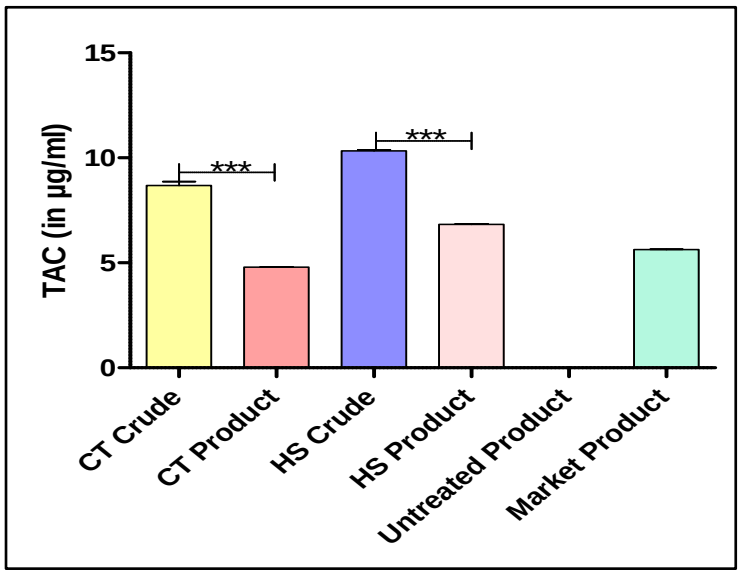

Figure 4 Result of the total antioxidant capacity of the crude floral waste extracts and the formulated products (ice popsicles); the graph represents data in Mean ± SD (n=3) with ***P ≤ 0.001, **P≤0.01, *P≤0.05; CT – *C. ternatea; HS – H. sabdariffa*

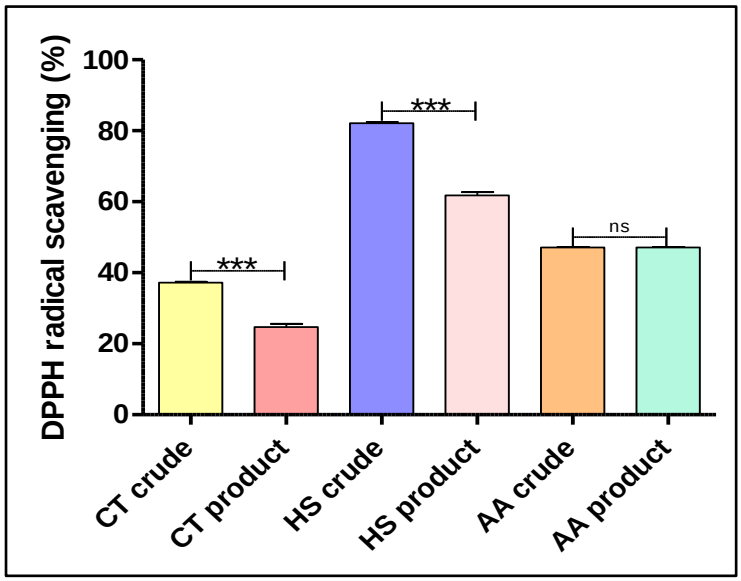

Figure 5 Comparison of the DPPH method of crude floral waste samples and the formulated products (ice popsicles) of *C. ternatea* and *H. sabdariffa*, the graph represents data in Mean ± SD (n=3) with ***P ≤ 0.001, **P≤0.01, *P≤0.05, CT – *C. ternatea; HS – H. sabdariffa,* AA – Ascorbic Acid.

3.4 TAC assay

The results of the total antioxidant capacity (TAC) of the floral wastes and their formulated products are shown in Figure 4. Similar to other parameters, the TAC value of the crude floral waste extract of *H. sabdariffa* was higher than that of its formulated product. The crude floral waste extract of *C. ternatea* exhibited almost double the total antioxidant capacity of its formulated product. The TAC value was significantly lower in the formulated product compared to the crude samples.

3.5 DPPH assay

The antioxidant content of the crude floral waste extracts and their formulated products was assessed based on their DPPH radical scavenging capacity (Figure 5). Rosella's crude floral waste extract exhibited a higher DPPH radical scavenging potential at 82% compared to its derived formulated product, which showed 61% scavenging potential. Similarly, the DPPH inhibition potential of the crude floral waste extract was 37%, exceeding the DPPH inhibition potential of its derived formulated product (24%).

3.6 ABTS assay

The results of percent ABTS inhibition are presented in Figure 6. In the ABTS radical scavenging assay, Rosella's crude floral waste extract and its formulated product showed the highest percent inhibition of ABTS radical after 10 and 20 minutes. For the 0-10 minute interval of the assay, the percent inhibition potential of the crude floral waste extract of Rosella was 57%, which was significantly greater than its derived formulated product, which had a rate of 37% (Figure 6a). In the case of the Butterfly pea crude floral waste, the ABTS percent inhibition value was 52% for 0-10 minutes, which was higher than its derived formulated product (25%), as shown in Figure 6a. As time passed, the percent inhibition decreased because the antioxidants of the plant-derived bio-colourants scavenge the cation radical $ABTS^{\bullet+}$. For 10-20 minutes, the floral waste of *H. sabdariffa* and its derived formulated product exhibited ABTS percent inhibition values of 51% and 31%, respectively (Figure 6b). The crude floral waste of Butterfly pea exhibited 49% ABTS inhibition for 10-20 minutes, which was higher than its derived product (20%), as shown in Figure 6b.

3.7 Antibacterial activity

The results of the antibacterial activity of the two crude floral waste extracts of Butterfly pea and Rosella are represented in Figure 7. Ampicillin and chloramphenicol were used as positive controls, showing maximum inhibition zone. The Butterfly pea

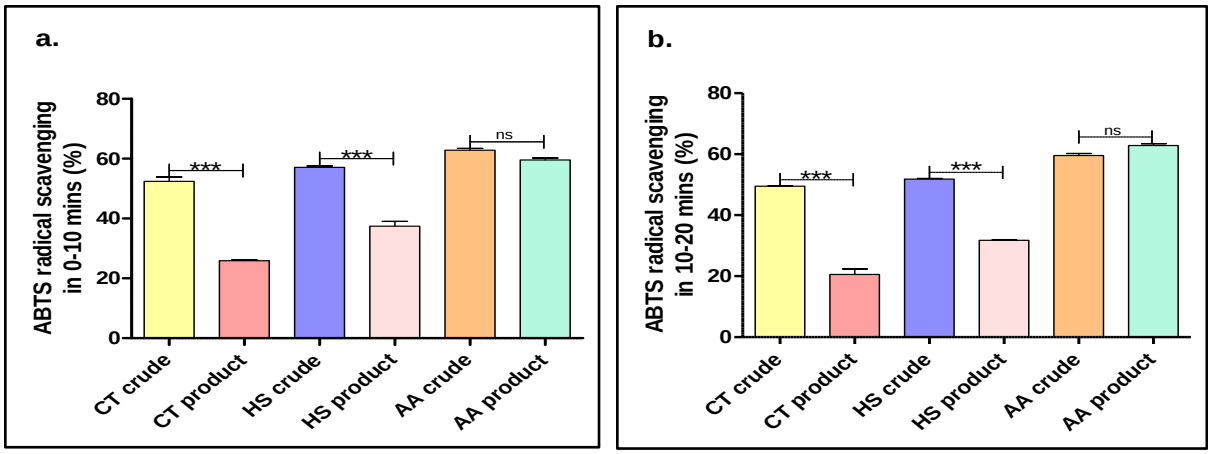

Figure 6 Comparison of the ABTS radical scavenging potential of crude floral waste samples and formulated products (ice popsicles) after (a.) 10 minutes & (b.) 20 minutes of *C. ternatea* and *H. sabdariffa*, the graph represents data in Mean ± SD (n=3) with ***P ≤ 0.001, **P≤0.01, *P≤0.05, CT – *C. ternatea; HS – H. sabdariffa*, AA – Ascorbic Acid

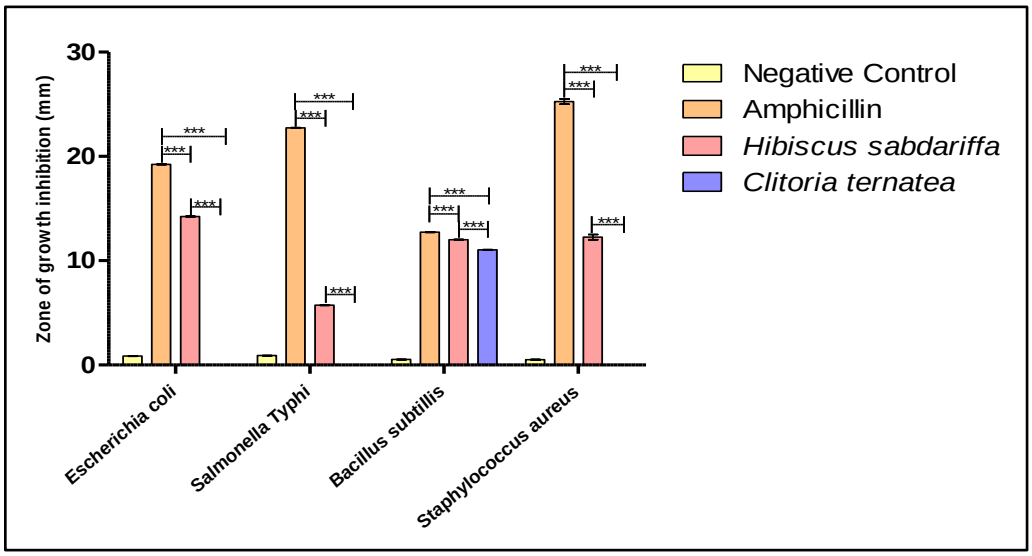

Figure 7 Bar graph showing the zone of growth inhibition (in mm) by the crude floral waste extracts against different Bacterial Strains, the values are represented as Mean ± SD (n=3) with *P≤0.05, **P≤0.01, ***P≤0.001, here, the inhibition of bacterial strains was compared with that of Ampicillin.

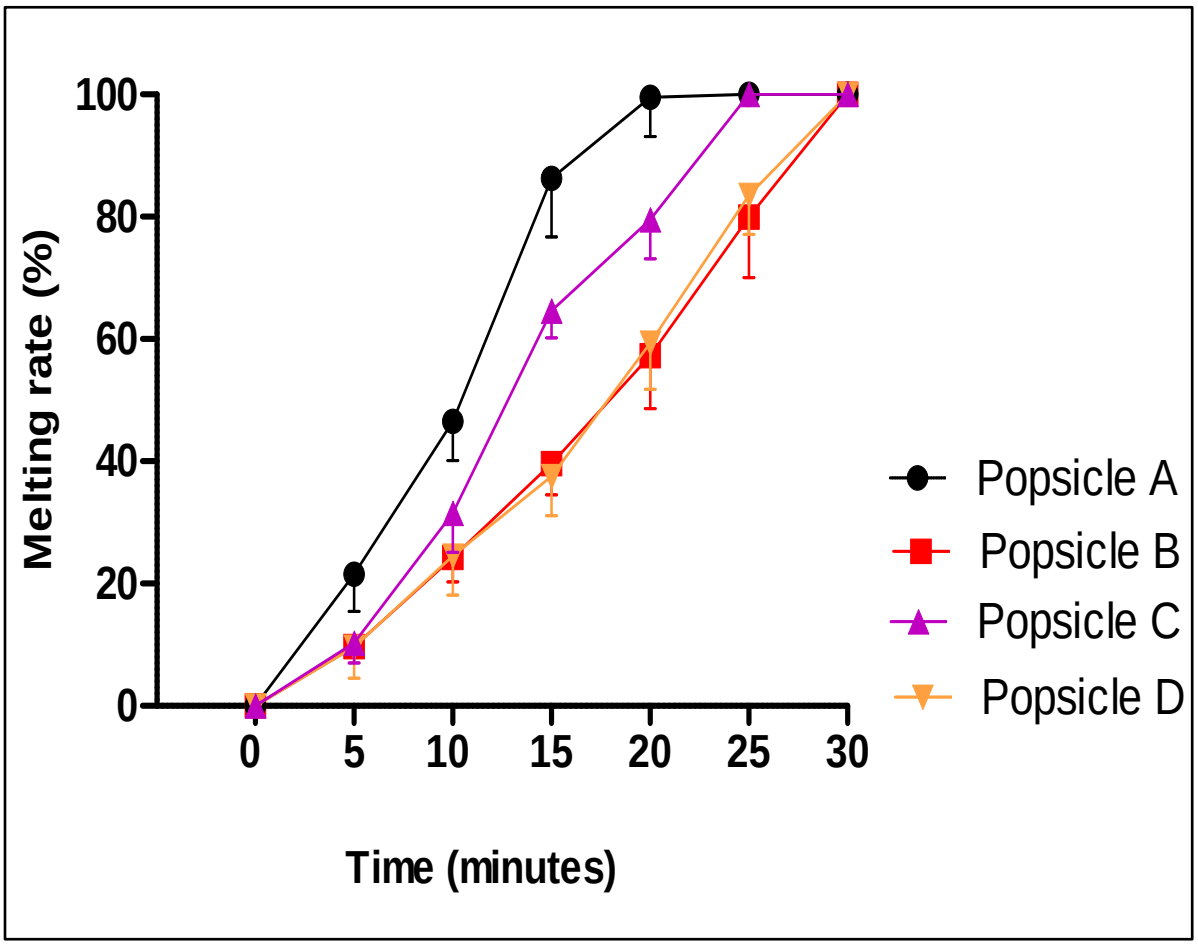

Figure 8 Melting quality of formulated Ice popsicles by observation after 5 minutes intervals, here, Ice popsicle A is Control i.e., without added bio-colour, Ice popsicle B is formulated with Rosella derived bio-colour, Ice popsicle C is formulated with Butterfly pea derived bio-colour added in it, Ice Popsicle D is popsicle ice-cream of brand name SLICE brought from Local market, The graph represents data in Mean± SD (n=3) with ***P ≤ 0.001, **P≤0.01, *P≤0.05.

flower extract exhibits antimicrobial activity only for *B. aureus* (11.035 mm). However, Rosella showed bacterial growth inhibitory effect against four different bacterial strains with diameter ranges from 5.735 mm for *S. typhi*, 12 mm for *B. subtilis*, 12 mm for *S. aureus* and 14 mm for *Escherichia coli*.

3.8 Melting quality

Ice popsicles of good quality are less likely to melt when exposed to room temperature for 10-15 minutes. Ice popsicle A (Control) melted after 20 minutes, ice popsicle C entirely melted after 25 minutes, and ice popsicle B thoroughly melted after 30 minutes. Ice popsicle B maintained its appearance longer than ice popsicles A and C. Ice popsicle D, purchased from the local market, also melted after 30 minutes, indicating a similarity in melting time between the formulated and commercial ice popsicles (Figure 8). The dishes' melted substance should conge into a uniform, homogeneous, smooth liquid.

3.9 Analysis of Organoleptic properties

The results shown in Figure 9 demonstrate the sensory characteristics of the three different ice popsicles created. The sensory properties were rated on a 9-point structured hedonic scale (Martins et al. 2018) and scored between 6.07 and 8.25. There were significant differences (≤0.05) among the three ice popsicles in colour, taste, texture, appearance, and overall rating. The ice popsicle made with Rosella-derived bio-colour had an attractive red colour, while the one made with Butterfly pea derived bio-colour had an appealing blue colour. The mean scores for colour ranged from 6.07 (Popsicle D) to 8.25 (Popsicle B). For taste, the mean score ranged from 6.92 (Popsicle C) to 7.72 (Popsicle B), and for texture, the scores ranged from 6.81 (Popsicle D) to 8.21 (Popsicle B). Regarding appearance, the mean scores ranged from 7.18 (Popsicle C) to 7.22 (Popsicle D). Consequently, the mean overall rating ranged from 7.38 (Popsicle C) to 8.02 (Popsicle B). All 100 consumers reported no health issues within 24 hours after consumption.

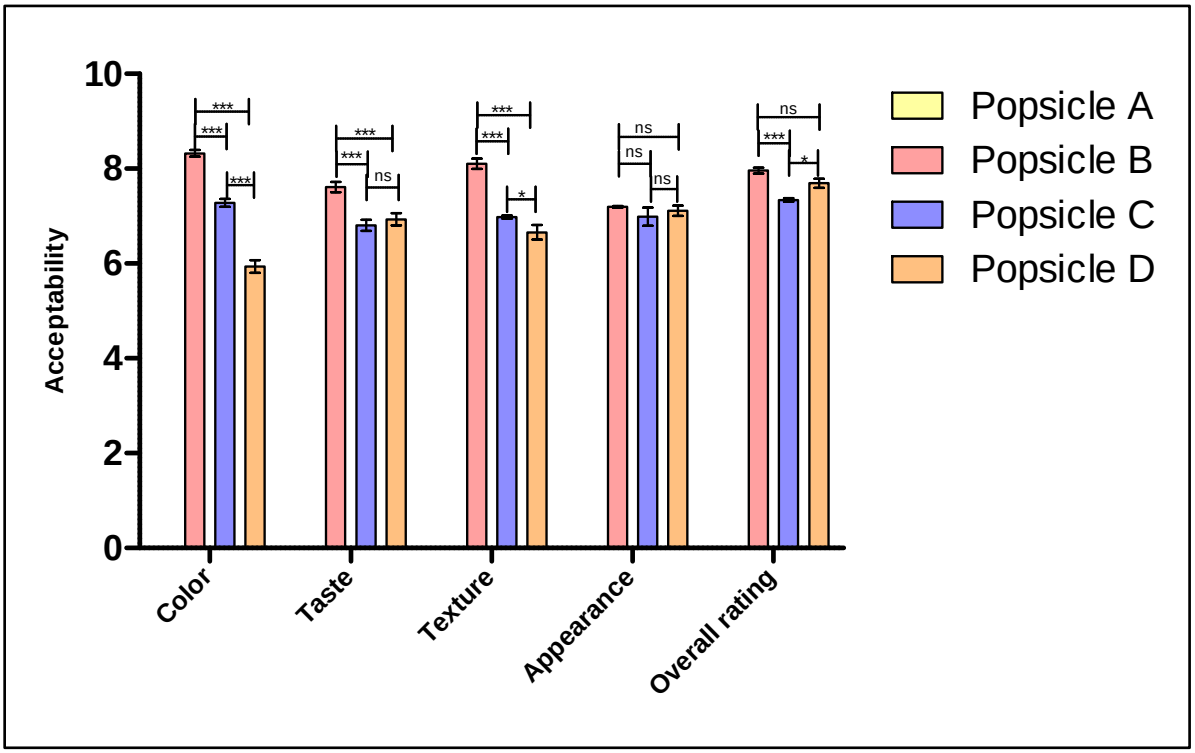

Figure 9 The graph showed consumers' acceptance of untreated ice popsicles and ice popsicles formulated with *H. sabdariffa* and *C. ternatea* derived bio-colour, the values were shown as Mean ± Standard deviation (n=3) with ***P ≤ 0.001, **P≤0.01, *P≤0.05 and compared with sensory attributes of the untreated sample. Mean data from 100 consumers based on a 9-point structured hedonic scale, Formulations of Popsicle A, i.e. Control is without added bio-colour, Popsicle B - Rosella derived bio-colour, Popsicle C - Butterfly pea derived bio-colour, Popsicle D - Popsicle ice cream brought from Local market.

4 Discussion

Food colour is a crucial factor affecting consumers' acceptance of food items. For decades, synthetic colourants have been widely used in food processing. However, recent reports have highlighted the harmful effects of these synthetic food colourants, such as hyperactivity, depression, hives, asthma, Attention Deficit Hyperactivity Disorder (ADHD), and brain tumours (Khanavi et al. 2012; Dey and Nagababu 2022; John et al. 2022). As a result, scientists have been developing new cost-effective and nutritious food colourants that enhance food flavours and are safe to use. Bio-colourants are increasingly preferred over synthetic colourants due to their easy availability, cost-effectiveness, and lack of side effects (Rymbai et al. 2011; Ghosh et al. 2022; Nabi et al. 2023).

A recent study evaluated various bio-colourant properties extracted from natural crude floral waste of H. sabdariffa and C. ternatea. The high total antioxidant capacity (TAC) and total flavonoid content (TFC) values of Rosella's crude floral waste extract can be attributed to higher phenolic and flavonoid content. Results from the ferric ion reduction potential (FRAP) and TAC assay showed that Rosella's crude floral waste extract exhibited maximum reducing capacity and antioxidant potential. The DPPH and ABTS assay results also confirmed the high antioxidant capacity of the Rosella flower's crude floral waste extract. The superior antioxidant properties of the Rosella flower extracts may be associated with the higher presence of polyphenols, flavonoids, anthocyanins, ascorbic acids, organic acids, and hibiscus acid, in the extract (Prenesti et al. 2007; Aurelio et al. 2008; Cisse et al. 2009; Abou-Arab et al. 2011). Additionally, the crude floral waste extract of Rosella demonstrates good antibacterial potential against different bacterial strains due to its high phenolic content.

The red colour of the petals of *H. sabdariffa* and the deep blue colour of *C. ternatea* petals are governed by anthocyanin (Cisse et al. 2009; Pasukamonset et al. 2016; Goh et al. 2021). These floral pigments are commonly used as food colourants and are FDA-approved (Aurelio et al. 2008; Abou-Arab et al. 2011; Goh et al. 2021, Jeyaraj et al. 2021). Rosella juice, derived from water extract of fresh or dried Rosella flowers, is a popular soft drink in various countries such as Nigeria and Thailand (Abou-Arab et al. 2011). Additionally, previous reports have suggested that rosella calyces can serve as food colourants and emulsifiers in the food industry (Duangmal et al. 2004). Surmani et al. (2022) found that administering rosella flower petal ethanolic extract capsules can stimulate erythropoiesis in female anaemic

adolescents without side effects. Furthermore, Srichaikul (2018) and Jeyaraj et al. (2021) reported that oral administration of aqueous ethanol extract of *C. ternatea* flower (2000 mg/kg body weight) in mice did not cause acute toxicity, suggesting its safety for consumption.

In this study, to investigate the acceptability and quality of bio-colour extracted as a food additive, ice popsicles were prepared using bio-colourants derived from *H. sabdariffa* and *C. ternatea*. A locally bought ice popsicle served as the quality control. Ice popsicles are popular globally regardless of age, culture, and economic status (Balthazar et al. 2017) and can be made from dairy or non-dairy ingredients. Although dairy-based ice popsicles are considered nutritious, their quality is sometimes compromised (Bahram-Parvar 2015). Therefore, this study also tested the antioxidant and antibacterial potential of the formulated ice popsicles, as these parameters are directly associated with their quality. The study also assessed the physical and sensory properties of the formulated ice popsicles. The results revealed that ice popsicles formulated with these two bio-colours possess significant polyphenol and flavonoid content, contributing to their antioxidant potential, almost similar to locally bought ice popsicles. Furthermore, the formulated ice popsicle maintained physical and sensory properties better than the locally bought one, indicating that these two crude floral wastes can be used as a functional food bio-colourant in the food industry. Although natural bio-colourants are environmentally friendly, less toxic, non-carcinogenic, facilitate incorporation into aqueous food systems, and promote protection against diverse chronic diseases (Clinton 1998; Siva 2007), there are some limitations to using these bio-colours in the food industry, such as lack of proper knowledge of extracting bio-colour, difficulty in sample collection, decolourization, sensitivity to light, temperature, oxygen, pH, and associated allergic reactions (Francis and Markakis 1989; Hallagan et al. 1995; Duangmal et al. 2004).

Conclusion

The crude floral waste extract of Rosella (*H. sabdariffa*) exhibited higher total phenolic and total flavonoid content, greater reducing capacity, more substantial radical scavenging potential, and better antibacterial activity compared to the Butterfly pea (*C. ternatea*). This trend was also observed in their respective formulated food products. Ice popsicles made with Rosella-derived bio-colour maintained physical and sensory properties better than those bought from the local market, indicating that Rosella-derived bio-colour can be used as a functional food colouring in the food industry. This discovery paves the way for utilizing floral agro-waste to produce valuable food additives, thereby addressing agro-waste-related environmental pollution. Further research is needed to investigate the therapeutic potential of this bio-colour in disease prevention.

Abbreviations

TPC - Total Phenolic Content, TFC - Total Flavonoid Content, DPPH – 2,2-Diphenyl-1-picrylhydrazyl, ABTS - 2,2′-azinobis-(3-ethylbenzothiaziline-6- sulfonic acids), FRAP - Ferric Reducing Antioxidant Power Assay, CT - *Clitoria ternatea*, HS - *Hibiscus sabdariffa L*,PBS- Phosphate Buffer Solution**.**

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