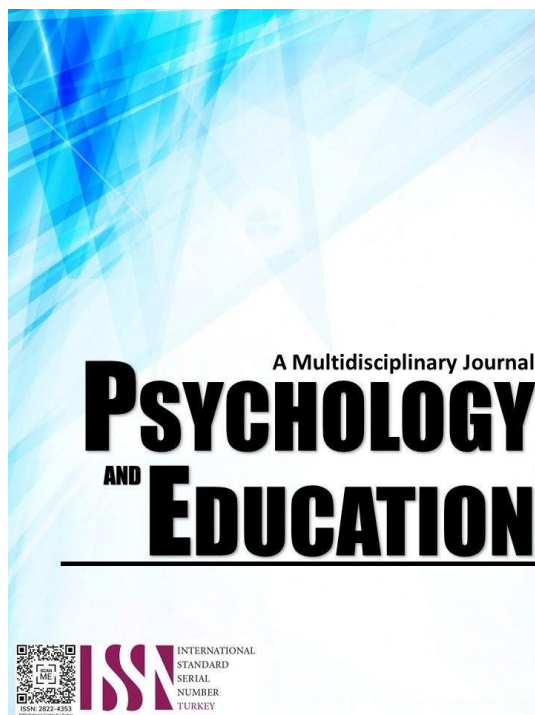


# ANTIOXIDANT PROPERTY OF NATURAL FOOD COLORANTS FROM CAROTENOIDS AND ANTHOCYANINS USING DPPH FREE RADICAL SCAVENGING METHOD



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## Antioxidant property of Natural Food Colorants from Carotenoids and Anthocyanins using DPPH Free Radical Scavenging Method

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### Abstract

This study explores the potential of natural food colorants derived from *Basella alba* (Malabar spinach) seeds and *Cucurbita* (squash) flesh, focusing on their antioxidant properties and viability as eco-friendly substitutes for synthetic food dyes. Specifically, it aims to extract and process anthocyanins from *Basella alba* and carotenoids from *Cucurbita* through dehydration and powdering, then evaluate their efficacy as both colorants and antioxidants. The first objective is to analyze their antioxidant activity using the DPPH free radical scavenging method, a widely accepted assay for assessing antioxidant capacity. The study also aims to determine the color intensity and stability of the pigments under different conditions, including variations in light, heat and oxygen exposure. Also, it incorporates the processed pigments into food matrices to assess their practical application and visual effectiveness. Findings revealed that *Basella alba* extract exhibited moderate antioxidant activity ( $EC_{50} = 187.8 \pm 1.4$  ppm), significantly stronger than *Cucurbita* extract ( $EC_{50} = 771.2 \pm 1.4$  ppm) but both were less potent compared to ascorbic acid ( $EC_{50} = 1.8 \pm 0.1$  ppm). Despite stability concerns, *Basella alba* showed potential as a functional additive due to its dual antioxidant and coloring properties, whereas *Cucurbita* was more suitable as a colorant alone. Both extracts offer promising, sustainable alternatives as artificial food dyes.

**Keywords:** *natural food colorants, basella alba, cucurbita, antioxidant activity, eco-friendly dye alternatives*

### Introduction

Cooking plays a vital role in Filipino culture, serving as a cornerstone of family life, a symbol of love and hospitality, and a reflection of their rich culinary heritage, with food being a central aspect of community and social gatherings. Heritage refers to an aspect of the culture of a specific society, encompassing traditions, languages, or structures that were established in the past and remain historically significant. A significant aspect of a community's heritage is food, commonly referred to as culinary or gastronomic heritage. In a cultural community or within a particular society, culinary traditions are woven into the essence of the cuisine that characterizes the individuals residing in specific locations. In recent years, there has been a vibrant discussion regarding the connection between culinary heritage and sustainability. The conventional method of transmitting cultural cuisine across generations revitalizes the identity of a specific family or community; hence, food is regarded as an Intangible Cultural Heritage (ICH).

However, there are difficulties in preserving this heritage. For instance, the rising prices and limited availability of conventional ingredients can pose challenges in maintaining their use in recipes handed down over time. Additionally, the younger generation might be less interested in acquiring traditional cooking techniques, resulting in a decline of culinary expertise. Another issue is the rivalry from mass-produced, processed foods that frequently include artificial additives and lack the cultural significance and nutritional value of traditional meals. The dependence on artificial colorants in processed foods raises health issues because of their possible harmful effects on consumer well-being.

Color is among the most essential characteristics of food, taking into account quality measures and acceptability. The acceptability of products by consumers is greatly influenced by their color, flavor, and taste. Currently, consumers have shown a preference for coloring food items with natural dyes. These items have garnered significant attention from consumers regarding the aesthetic, nutritional, and safety features of food (Islam, et.al.,2023).

According to Hamad et.al,2021 the color intensity of turmeric and annatto powder was discovered to be highly effective as natural colorants in both the production and preservation of fish tocino. The mixture of powdered Turmeric and Annatto serves as a natural food colorant incorporated into the production process of Sillago (locally referred to as Asuhos). Fish Tocino received the highest level of approval from consumers. Its natural dyes were utilized to substitute synthetic colors since artificial coloring contains various chemicals that may adversely affect consumers' health. Additionally, utilizing the natural colorants present in these herbs can help mitigate consumers' health issues and the risks associated with artificial colors in products from food.

Natural pigments from plants, including carotenoids, anthocyanins, and chlorophylls, not only improve the looks of food items but also provide health advantages to consumers, including antimicrobial, anticancer, antioxidant, and anti-inflammatory effects. These pigments have been positively studied for their antioxidant capabilities, which provide defense against damaging free radicals and may reduce the probability of cancer and heart disease. Natural foods are increasingly often favored over artificial alternatives due to their notable health benefits. Their rich antioxidant content makes them particularly appealing, especially when promoted as visually appealing foods with significant nutritional value. The growing preference for plant-based additives over synthetic ones is gradually transforming the food industry in a positive direction stated by (Zhang et al., 2023).

In the study conducted by Stanca, L. et al. (2024), anthocyanins belong to a class of antioxidants commonly found in fruits and vegetables with red, purple, or blue pigmentation. As a subgroup of flavonoids, they share similarities with antioxidants present in tea, wine, and dark chocolate. These compounds are believed to contribute to overall health by combating oxidative stress and inflammation, potentially lowering the risk of conditions such as heart disease, cancer, and age-related cognitive decline.

Anthocyanins are natural colorants that give plants their rich purple, red, and blue shades. They belong to the flavonoid family, which is a varied collection of flowers, plant-derived compounds found plentifully in fruits, seeds, sprouts, and foliage. These pigments are essential for plant reproduction as they lure pollinators and function as a defense against environmental stressors such as ultraviolet (UV) radiation, drought, and low temperatures. Free radicals are naturally produced during metabolism but can also originate from external sources such as pollution, cigarette smoke, alcohol, sunlight, and harmful chemicals. Among the approximately 6,000 learned flavonoids, anthocyanins are classified into six main types: Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, and Petunidin, (Alkadi, H. 2020).

On the contrary, carotenoids are bioactive substances commonly found in natural fruits and vegetables. Growing studies have validated that carotenoids exhibit several biological functions, including antioxidants, anti-cancer, anti-diabetic, anti-aging, and anti-inflammatory effects. Generally, the intake and absorption of carotenoids assist in combating oxidative stress and lowering the risk of various chronic ailments, such as cardiovascular and neurological issues, type 2 diabetes, and various cancers (Crupi, P. et al., 2023).

Carotenoids are pigments classified as tetraterpenes that are crucial to the human diet, significantly influence health, and exhibit various properties including antioxidant, cytoprotective, and photoprotective capabilities, allowing them to contribute to the prevention of numerous diseases like cancer, diabetes, cardiovascular issues, obesity, and neurodegenerative conditions (Jirí, B. et al., 2024).

In this study, the researchers will concentrate on sustainable methods for pigment extraction, exploring the elements that affect the extraction process, their uses, and how acceptable they are to consumers. Natural sources can produce nearly every color in the rainbow; these pigments offer functional advantages like antioxidant features, increased shelf life, and enhanced food quality.

### Research Objectives

This study generally aims to process and study anthocyanins from *Basella alba* seeds and carotenoids from *Cucurbita* through dehydration and powdering to utilize them as natural pigments with food applications in the food industry as green solutions. Specifically:

1. To analyze the antioxidant activity of anthocyanins and carotenoids after dehydration and powdering using relevant analytical techniques.
2. To determine the intensity of the colors and evaluate their stability after dehydration and powdering under different factors.
3. To incorporate the processed pigments into food products and determine their effectiveness as natural food colorants.

### Methodology

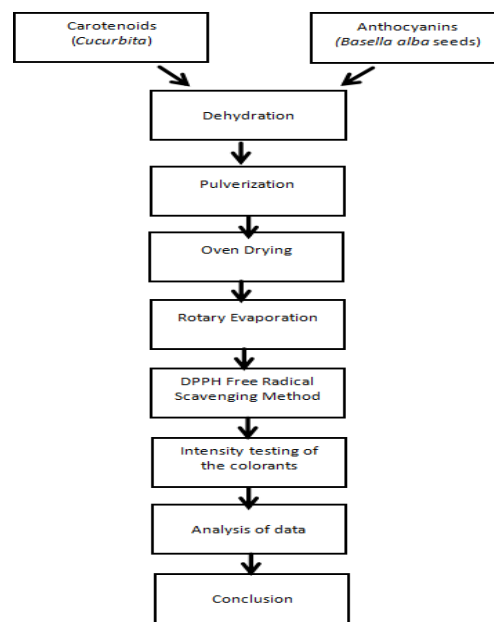


Figure 1. Experimental Procedure for the Extraction and Evaluation of Natural Colorants from *Cucurbita* Flesh and *Basella alba* Seeds

Figure 1 shows the initial stage in generating natural colorant involves the collection of samples of Cucurbita and Basella alba seeds after the collection it will undergo the dehydrating process. In this study, researchers sliced the Cucurbita into thin pieces and put it in the dehydrator for five hours. On the other hand, Basella alba seeds were dried using direct sunlight for a duration of two days to remove the liquid content and put it inside the dehydrator for 4 hours.

Following the dehydration of the Basella alba seeds and Cucurbita, the researchers proceed to pulverization process. Employing a blender and a pulverizer, researchers crush the sample into a fine powder. Pulverization during sample preparation aims to decrease the particle size of a material to a fine powder, which is essential for obtaining a uniform and representative sample for precise analysis and testing.

Once the sample is ready, the researcher subsequently delivers it to the laboratory. The sample was subjected to oven drying to eliminate moisture and volatile compounds, allowing precise measurement of moisture levels and aiding in further analysis or storage. The process of oven drying begins with weighing the sample prior to drying. Subsequently, put the sample in the oven at a designated temperature and for a fixed duration. Next, take out the sample and let it cool down. Reweigh the sample to assess the moisture level. Finally, determine the moisture content expressed as a percentage of the initial weight.

Rotary evaporation, commonly known as "rotovap," is employed in sample preparation for the effective and gentle removal of solvents and concentration through mild heat, vacuum, and rotation, facilitating accurate solvent separation from samples. Initially, the sample, dissolved in a solvent, is put into a round-bottom flask. Subsequently, the flask is spun in a heated water bath, forming a thin layer of the solvent on the inside of the flask, thereby enhancing the evaporation surface area. Next, a vacuum is introduced, lowering the pressure and permitting the solvent to evaporate at a reduced temperature. Ultimately, the solvent vapor is cooled using a cold-water condenser and gathered into a different flask.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging technique is a commonly utilized, straightforward, and quick spectrophotometric test for assessing the antioxidant potential of samples such as plant extracts, foods, and drinks, by evaluating their capability to neutralize free radicals. The DPPH assay requires combining the powdered samples with a DPPH solution, allowing the mixture to incubate, and subsequently measuring the solution's absorbance with a spectrophotometer.

Intensity testing of colorants, referred to as tinting strength or color strength testing, assesses the amount of color a pigment or dye can generate at a specific concentration, effectively gauging the "strength" or "brightness" of the color. In this section, researchers seek to establish the "coloring power" or the capacity of natural colorants derived from Anthocyanin and Carotenoid to generate a particular color intensity.

Following the production and examination of the natural colorants, the researcher subsequently evaluates the data collected from laboratory tests. Examining data utilized for a sample entail applying statistical techniques to recognize patterns, trends, and connections within that segment of a broader population, with the goal of inferring insights about the entire population. After examining the data collected from the laboratory, The researcher conducted visual sensory evaluation, involving 15 respondents from San Miguel National High School. The respondents were selected through a purposive sampling to ensure unbiased representation. The evaluation utilized a 4-point Likert scale to assess their perception on color intensity, where each participants rated the samples based on their observations.

## Results and Discussion

After all the experiments were done, this was the data collected:

### *Antioxidant Activity of Anthocyanins and Carotenoids*

This section discusses the antioxidant activity of Basella alba and Cucurbita seed extracts, focusing on their ability to scavenge free radicals as measured by the DPPH scavenging assay. The assay evaluates the extracts/ effectiveness in neutralizing DPPH radicals, with higher scavenging activities.

#### **DPPH SCAVENGING ASSAY**

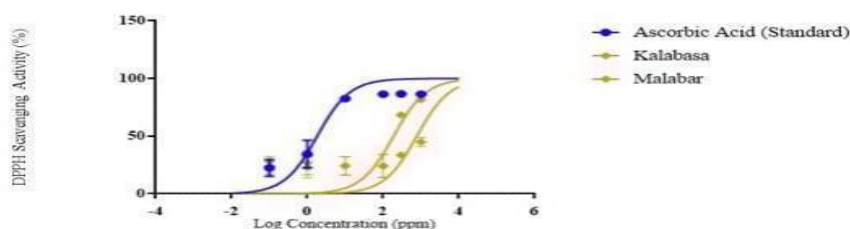


Figure 2. Dose-Response Curve Showing the DPPH Scavenging Assay Testing for Cucurbita and Basella alba seeds

The DPPH scavenging assay is responsible for measuring the extract's ability in scavenging the DPPH radicals. Higher scavenging

activities indicate stronger antioxidant properties. The results are presented as a graph showing the relationship between log concentration (in ppm - parts per million) of the extracts and the percentage of the DPPH scavenging activity.

In the graph, it shows that Ascorbic Acid (a standard antioxidant) has the highest scavenging activity at lower concentrations. Both Cucurbita and Basella alba seeds extracts show antioxidant activity, but at significantly higher concentrations compared to Ascorbic Acid.

Based on the reference classification for antioxidant activity ( $EC_{50} \leq 50$  ppm were considered as very strong, 50–100 ppm considered as strong, 101–150 ppm considered as medium and  $>150$  ppm considered as we

The DPPH scavenging assay results indicate that ascorbic acid exhibits the highest activity of antioxidant but have the lowest  $EC_{50}$  with the value of  $1.8 \pm 1.3$  ppm, making it the most effective in scavenging radical among the tested samples. Basella alba seeds extract demonstrated a moderate antioxidant activity, with an  $EC_{50}$  of  $187.8 \pm 1.4$  ppm, suggesting some potential for antioxidant applications but significantly weaker than ascorbic acid. In contrast, Cucurbita extract showed the weakest antioxidant capacity, with an  $EC_{50}$  of  $771.2 \pm 1.4$  ppm, indicating that it requires a much higher concentration to achieve similar scavenging effects. Overall, these findings suggest that Basella alba seeds extract possesses some antioxidant potential, while Cucurbita extract has limited efficacy in free radical scavenging.

These findings align with previous literature emphasizing the antioxidant potential of natural pigments such as carotenoids and anthocyanins. According to Wang Lu et al. (2021), these compounds are widely recognized for their antioxidant properties and are considered vital secondary metabolites in plants systems.

Additionally, Choo et al. (2022) highlighted that carotenoids and anthocyanins possess antioxidant activity that may contribute to health benefits, including protection against vision- related disorders.

### DPPH Scavenging Assay Raw Data

This Section presents the raw data from the DPPH scavenging assay, which measures the ability of Cucurbita and Basella alba seed extracts to neutralize free radicals. The data includes the concentration of the extracts at various levels and their corresponding DPPH scavenging activity, expressed as the percentage of radical inhibition. The results provide a detailed overview of the extracts' antioxidant potential, serving as the foundation for the further analysis.

Table 1. Raw Data of the DPPH Scavenging Assay Testing for Cucurbita and Basella alba seeds

Concentration (ppm)	Absorbance @ 517 nm			% DPPH Scavenging		
	R1	R2	R3	R1	R2	R3
Ascorbic Acid	R1	R2	R3	R1	R2	R3
0.1	0.98	0.84	0.84	14.11	26.82	26.64
1	0.91	0.67	0.66	20.60	40.93	41.81
10	0.22	0.18	0.19	80.37	84.05	83.26
100	0.16	0.15	0.15	85.98	86.77	86.50
300	0.17	0.14	0.15	85.54	87.55	86.85
1000	0.16	0.15	0.15	85.63	87.12	86.59
Kalabasa Extract	R1	R2	R3	R1	R2	R3
0.1	0.87	0.78	0.81	24.01	31.99	28.92
1	0.99	0.79	0.85	12.88	30.76	25.94
10	0.97	0.82	0.80	15.16	28.05	29.71
100	1.00	0.81	0.79	12.71	28.92	31.11
300	0.79	0.73	0.75	31.20	35.76	34.44
1000	0.67	0.58	0.64	41.72	49.43	44.17
Malabar Extract	R1	R2	R3	R1	R2	R3
0.1	0.955	0.857	0.818	16.30	24.89	28.31
1	0.966	0.74	0.794	15.34	35.14	30.41
10	0.968	0.821	0.802	15.16	28.05	29.71
100	0.996	0.811	0.786	12.71	28.92	31.11
300	0.364	0.36	0.201	68.48	68.63	68.60
1000	0.205	0.22	0.201	82.03	80.42	80.83

This table presents raw data from a DPPH radical scavenging assay. The assay measures the antioxidant capacity of different substances by neutralizing DPPH radicals. The data is organized into three sections: Ascorbic Acid (a standard antioxidant), Basella alba seeds and Cucurbita extract.

The data columns show, the Concentration of the substances tested in parts per million (ppm). The absorbance reading at a wavelength

of 517nm, DPPH has a strong absorbance at this wavelength and a decrease in absorbance indicates that DPPH radicals have been scavenged by the antioxidant. The percentage of DPPH radicals scavenged at each concentration; this is calculated based on the absorbance readings. The R1, R2, and R3 represent three replicate measurements.

The table shows the absorbance and scavenging activity at different concentrations for each substance. Lower absorbance values and higher scavenging percentages indicate stronger antioxidant activity.

### ***Evaluation of Intensity of Colors after Dehydration and Powdering***

This section presents the results of the color intensity tests for two natural pigments: Basella alba and Cucurbita. The color intensity was evaluated at varying concentrations of each pigment, mixed with water, and the results shows how the color changes with the increasing concentration of the respective samples.

**Table 2. *Basilla alba* seeds Color Intensity Testing**





<i>Grams (g)</i>	<i>Liquid</i>	<i>Color Intensity</i>	<i>Color</i>
1g	1 ml		lavender
2g	1 ml		plum
3g	1 ml		grape
4g	1 ml		wine

Table 2. shows the results of a Basella Alba seeds color intensity test. The table displays the colour intensity of Basella Alba seeds samples at different concentrations; 1g with 1 ml water, 2g with 1 ml water and 3g. with 1 ml water and 4 g with 1 ml water. The color shows a darker shade as the concentration of the Basella Alba seeds increases. The lighter color is lavender, to plum, to grape and the darker color is wine. This table demonstrates the relationship between the amount of Basellia alba seeds and resulting colour intensity.

Sadowska-Bartosz and Bartosz (2024), explained that anthocyanins, a group of polyphenolic O-glycosides, are responsible for the vibrant red, purple and blue colors found in many fruits and vegetables, making them valuable as natural food colorants. Their stability in food applications can be enhanced through natural plant defense mechanisms such metal ion binding.

Also, Janiszewska-Turak et al. (2016) observed that natural pigments are widely used as food additives to enhance the appearance, and occasionally the taste of food products. These pigments restore or intensify color lost during food processing and help with product identification and flavor perception.

**Table 3. *Cucurbita* Color Intensity Testing**





<i>Grams (g)</i>	<i>Liquid</i>	<i>Color Intensity</i>	<i>Color</i>
1g	1 ml		lemon
2g	1 ml		classic
3g	1 ml		sunshine
4g	1 ml		mustard

Table 3. shows the results of a Cucurbita color intensity test. The table displays the colour intensity of Cucurbita samples at different concentrations; 1g with 1 ml water, 2g with 1 ml water and 3g. with 1 ml water and 4 g with 1 ml water. The color shows a darker shade of orange-brown as the concentration of the Cucurbita increases. The lighter color result to lemon, to sunshine, to classic and the darker color is mustard. This table demonstrates the relationship between the amount of Cucurbita and resulting colour intensity.

This pattern reflects the findings of Ghosh et al. (2022) and Prajapati and Jadeja (2022), Who emphasized that color intensity in natural pigments such as carotenoids is concentration dependent.





Similarly, Nabi et al. (2024) and Ravindra et al. (2024) noted that increasing pigment levels not only enhance visual appeal but also supports the functional use of natural extracts in food and other products

### ***Mean Visual Acceptability Rating of Food Samples incorporated with Cucurbita and Basella alba Pigment***

These present the mean visual acceptability ratings of food samples, specifically rice and water, that were infused with natural colorants

derived from cucurbita (yellow pigment) and Basella alba (purple pigment). The samples were prepared by adding varying concentrations of the respective pigments (1g to 4g) to the rice and water. The color intensity, ranging from lighter to darker shades, was evaluated by respondents based on their visual preferences.

Table 4. Mean Visual Acceptability Rating of Rice with Varying Cucurbita Pigment

Grams (g)	Liquid	Color Intensity	Color	Mean	VI
1g	1 ml		lemon	3.45	Highly Acceptable
2g	1 ml		classic	3.10	Acceptable
3g	1 ml		sunshine	3.30	Highly Acceptable
4g	1 ml		mustard	2.95	Acceptable
Grand Mean				3.20	Acceptable





Legend: 4.00–3.26: Highly Acceptable; 3.25–2.51: Acceptable; 2.50–1.76: Moderately Acceptable; 1.75–1.00: Not Acceptable.

Table 4 shows the intensity of color in yellow rice as evaluated by the respondents. Among the yellow-colored rice samples, the sample with 1 gram pigment concentration, which exhibited a “lemon” color intensity, obtained the highest mean rating of 3.45, interpreted as “Highly Acceptable.” In contrast, the sample with 4 grams, showing a “Mustard” color intensity, garnered the lowest mean rating of 2.95, interpreted as “Acceptable” the computed grand mean across all yellow rice samples was 3.20, which is verbally interpreted as “Acceptable.”

The result implies that the incorporation of processed yellow pigments into rice products was generally favorable among respondents. However, variations in color intensity slightly influenced their visual acceptability, where lighter and brighter tones such as lemon were preferred over deeper shades like mustard

This finding is supported by Lee et al. (2013), who emphasized that consumers tend to associate lighter and more vivid food color with freshness and better-quality perception, thereby affecting their overall acceptability ratings.

Table 5. Mean Visual Acceptability Rating of Water with Varying Basella alba Pigment

Grams (g)	Liquid	Color Intensity	Color	Mean	VI
1g	1 ml		lavender	3.60	Highly Acceptable
2g	1 ml		plum	3.25	Acceptable
3g	1 ml		grape	3.50	Highly Acceptable
4g	1 ml		wine	3.15	Acceptable
Grand Mean				3.38	Highly Acceptable

Legend: 4.00–3.26: Highly Acceptable; 3.25–2.51: Acceptable; 2.50–1.76: Moderately Acceptable; 1.75–1.00: Not Acceptable.

This table shows that among the rice samples incorporated with Basella alba pigment, the sample with 1 gram concentration, yielding a “Lavender” color intensity achieved the highest mean rating of 3.60, interpreted as “Highly Acceptable.” Conversely, the sample with 4 grams, producing a “Wine” color intensity, recored the lowest mean rating of 3.15, still interpreted as “Acceptable.” The grand mean for all water samples was computed at 3.38, corresponding to a verbal interpretation of “Highly Acceptable.”

The results imply that the processed Basella alba pigments incorporated with water were highly acceptable to the respondents, suggesting that softer and pastel-like hues such as “Lavender” were more visually preferred over deeper and darker tones like “Wine.”

This result aligns with the study of Spence (2015), who noted that color brightness significantly influences consumer expectations and preferences, where lighter hues often evoke perceptions of cleanliness, freshness and enhanced palatability.

## Conclusion

The DPPH scavenging assay showed that Malabar extract had a moderate antioxidant activity with an EC<sub>50</sub> value of 187.8 ± 1.4 ppm, while Cucurbita extract exhibited weaker antioxidant activity with an EC<sub>50</sub> value of 771.2 ± 1.4 ppm. Ascorbic acid, used as a standard,

demonstrated significantly stronger antioxidant potential with an EC<sub>50</sub> of  $1.8 \pm 0.1$  ppm. The EC<sub>50</sub> value represents the concentration needed to scavenge 50% of DPPH radicals using the required extract from *Basella alba* seeds and *Cucurbita*, indicating that *Basella alba* extract is more effective than *Cucurbita* in neutralizing free radicals, although both are less potent compared to ascorbic acid. Malabar Extract: EC<sub>50</sub> =  $187.8 \pm 1.4$  ppm, moderate antioxidant activity, better than Kalabasa extract. Kalabasa Extract: EC<sub>50</sub> =  $771.2 \pm 1.4$  ppm, weaker antioxidant activity compared to Malabar and ascorbic acid (EC<sub>50</sub> =  $1.8 \pm 0.1$  ppm).

The stability of antioxidant compounds in Malabar and Kalabasa extracts was assessed through their DPPH scavenging performance across different concentrations. Malabar extract, likely rich in anthocyanins, displayed improved scavenging activity at higher concentrations, achieving 68–82% DPPH scavenging at 300–1000 ppm, indicating relatively stable performance. Kalabasa extract, containing carotenoids, showed a slower increase in scavenging efficiency, with a peak of 41–49% scavenging at 1000 ppm, suggesting its carotenoids are more prone to degradation or less reactive compared to anthocyanins. Malabar Extract: Contains anthocyanins with moderate stability but sensitive to oxygen, heat, and light. Kalabasa Extract: Rich in carotenoids, offering vibrant color but prone to oxidative degradation.

Malabar extract's moderate antioxidant activity and anthocyanin-rich composition make it a dual-purpose candidate as both a natural colorant and functional additive in food products. Its vibrant color, however, is sensitive to environmental factors like heat and light. Kalabasa extract, while weaker in antioxidant activity, could still serve as a natural food colorant due to its carotenoid-based bright pigments. Its primary application would be for coloring rather than functional health benefits. Malabar Extract: Offers both color and functional antioxidant properties; needs stability improvements for broader applications. Kalabasa Extract: Primarily a colorant due to weaker antioxidant activity; suitable for color-focused uses.

Both extracts have potential as eco-friendly and sustainable alternatives to artificial food dyes, particularly in applications that prioritize natural ingredients. Malabar extract shows better promise as a substitute, given its dual antioxidant and coloring properties. However, both extracts require process optimization, including encapsulation techniques or stabilizers, to enhance pigment and bioactive stability during storage and food processing. Such improvements are essential to ensure these natural pigments can effectively compete with synthetic dyes in terms of stability and cost efficiency. To conclude, both extracts show promise as natural alternatives to artificial dyes, supporting sustainability. Optimized processing is necessary to enhance stability and bioactive yield for food industry applications.

Alternative antioxidant tests for *Cucurbita*/pumpkin extract should be conducted—since the DPPH test showed weak results, other tests such as FRAP and ABTS should be performed to determine whether it has antioxidant activity by different means. Refinement of the extraction technique should be explored, with further studies focusing on altering solvents, temperature, and time to optimize anthocyanin and carotenoid extracts from fruits and vegetables for food use. Investigation of other claims should be conducted to examine whether these natural pigments possess additional functions besides being antioxidants, such as anti-inflammatory, antimicrobial, or UV-protective properties. Lastly, processing should be optimized by enhancing the processing of Malabar and other plant extracts to improve pigment and bioactive stability. Exploring encapsulation and stabilizers will improve their stability during storage and processing.

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