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A Natural Food Colourant from Dioscorea alata (Dandila) in Sri Lanka: Development, Storage Stability and Bio-active Properties

W.A.E.M.P. Menike[1], J.W.A. Sajiwanie[2], R.M.U.S.K. Rathnayaka[2]
[1]*Faculty of Graduate Studies, Sabaragamuwa University of Sri Lanka, Belihuoya, 70140, Sri Lanka*
[2]*Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, Belihuoya, 70140, Sri Lanka*
[*]Corresponding Author: erandipremachandra@gmail.com

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ABSTRACT

Though anthocyanins are popular as natural food colourants with additional functional properties, their instability limited their utilization in the industry. Therefore, this study aimed to formulate a natural food colourant by the microwave-assisted encapsulation of Dandila (Dioscorea alata) anthocyanins to enhance the storage stability of the pigments in ambient conditions and to study the storage stability and the bio-active properties of the formulated colourant. Acidified water was used as the solvent to extract the anthocyanins from Dandila yams. Maltodextrin and gum Arabic were tested as wall materials at three different ratios to encapsulate the extracted pigments. Storage stability, Total Phenolic content, anti-oxidant activity and anti-bacterial activity were determined using appropriate procedures. The colourant formulation encapsulated with maltodextrin (30 mg/ml) was selected as the best encapsulation treatment for Dandila anthocyanin. Anthocyanin degradation was not significant during the tested 12 months of storage period. Noteworthy values were obtained for Total Phenolic Content and anti-oxidant activity. Among the tested strains in the antimicrobial assay, the natural colourant showed inhibitory activities against Escherichia coli and Streptococcus pneumoniae. The results confirm the successful development of a natural colourant rich in bio-active properties from Dandila anthocyanin while ensuring its storage stability in ambient conditions.

Keyword: Anthocyanin, Bio-active properties, Dandila, Dioscorea alata, Encapsulation, Microwave-assisted

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1. Introduction

Recently, the demand for naturally sourced food colorants has increased and is now higher than that for synthetic colorants [1]. As natural antioxidants and sweeteners, additional health benefits provided by natural colourants and the reported toxicity of synthetic colourants are the main reasons for this increased demand. Therefore, the food industry is much interested in coloured crops as the source of natural pigments [2].

Among the variety of naturally sourced pigment groups, anthocyanins are one of the important groups which have been traditionally used as a natural food colourant since ancient time. Blue, red, or purple colour anthocyanin pigments are found in plants including flowers, fruits and tubers and are possible for use in food, nutraceutical and pharmaceutical preparations. In addition to their colourant properties, anthocyanins have been associated with a wide range of biological, pharmacological, anti-inflammatory, antioxidant, and chemoprotective properties [3]. As a natural source of antioxidants, anthocyanin acts as a free radical scavenger and plays a role in aging, cancer and degenerative illness prevention [4]. Moreover, no adverse effects of anthocyanin derivatives were reported, even after the ingestion of very high doses [5].

However, the use of anthocyanin-rich extracts as food colourants and healthy foods is limited because of the low stability of anthocyanins under the environmental conditions (heat, oxygen, and light among others) experienced during processing and/or storage [6] since the degradation of anthocyanins will impair the colour, functionality, and overall anthocyanin-contained product's sensory attributes [7]. Therefore, anthocyanin stability improvement has been considered an important requirement [8]. Co-pigmentation and encapsulation are the two most commonly used techniques for anthocyanin stabilization, where encapsulation shows potential for enhancing anthocyanin stability by building a functional barrier between the anthocyanins and external environmental factors such as oxygen, light, temperature, water, enzyme, and reactive compounds [7].

Purple yam (*Dioscorea alata*), locally known as Dandila, is an underutilized yam variety in Sri Lanka with an attractive purple colour flesh. Among the different kinds of yams, purple yam contains anthocyanins, which are responsible for their red-to-purple colour [9]. Therefore, due to the potential of natural anthocyanins as a healthy alternative to synthetic food colours, purple yam could be a valuable source to extract these compounds. Although several studies have been carried out to extract and identify the anthocyanin pigments from yams, the formulation of natural colourant to extend the stability of the extracted pigments was not studied well. Therefore, this study aimed to formulate a natural colourant by the microwave-assisted encapsulation of anthocyanin pigments from Dandila to enhance the storage stability of the pigments in ambient conditions and to study the storage stability and the bio-active properties of the formulated colourant.

2. Method

1.1 Extraction of Natural Pigments

Collected Dandila yams were cleaned and cut into small pieces. Anthocyanins were extracted using acidified water as the solvent in a ratio of 1:3 (W/V) in a microwave oven at 80% microwave power for 15 minutes. The extraction process was repeated six times. The extract was filtered through a cotton cloth and the filtrate was centrifuged at 3000 rpm for 5 minutes to remove extracted cell debris.

1.2 Encapsulation of Natural Pigments

Encapsulation is a process where a continuous thin coating is formed around solid particles, liquid droplets, or gas cells that are fully contained within the capsule wall. Extracted pigments were encapsulated with the microwave-assisted encapsulation technique using maltodextrin and gum arabic as wall materials. Pigment extracts were mixed with the wall materials in three ratios of 10, 20, and, 30 mg per milliliter of pigment extract and dissolved completely. The mixed solution was poured into petri dishes and dried in a microwave oven at 80% power for 15 minutes. Dried and encapsulated pigments were scraped with a spatula and grounded using a mortar pestle to obtain the powdered colourant. Pigment powders are ground up colors, sort of like posdered colored chalk.

A. Encapsulation Efficiency (EE)

Encapsulation efficiencies of developed colourant powders were determined using the method described by Idham *et al*. in [10] with slight modifications.

Total Anthocyanin (TA) content and Surface Anthocyanin content of the microcapsules were determined after the encapsulation process. For the TA determination, 100 mg of samples were weighed. About 1 mL of distilled water was added and then the samples were ground to destroy the microcapsule membrane. Then 9 ml ethanol was added and the samples were extracted for 5 minutes and then filtered.

For the determination of surface anthocyanins (SA), 100 mg of samples were directly extracted with 10 mL ethanol in a vortex for 30 s, followed by centrifugation at 3,000 rpm for 10 minutes. After phase separation, the clear supernatant was collected and filtered through Whatmann filter paper.

Anthocyanins content for TA and SA values were determined by measuring the absorbance at 520 nm wavelengths using a spectrophotometer. The efficiency of encapsulation (EE) was calculated as the percentage of anthocyanins encapsulated throughout the process using equation (1).

$$EE=\frac{(\text{Total anthocyanin content-Surface anthocyanin content})}{\text{Total anthocyanin content}}\times 100 \qquad (1)$$

B. Statistical analysis

Data were analysed using Analysis of Variance (ANOVA) with Minitab 19 statistical software. In all cases, statistical significance is quoted at the 5% significant level ($p < 0.05$).

The treatment combination with the highest encapsulation efficiency value was selected for further studies.

1.3 Storage studies

A. Moisture content

Initial and final moisture contents of the encapsulated anthocyanin powder were calculated using the oven dry method.

B. Anthocyanin stability

Anthocyanin retention was determined at each month during the storage period, following the method described by Sipahli in [11]. For that, 1 g of encapsulated anthocyanin powder was dissolved in 10 ml of distilled water. The mixture was centrifuged at 3000 rpm for 2 minutes. The absorbance of the purified extract was measured at 520 nm and 700 nm. The absorbance was calculated using equation (2) and the anthocyanin retention was calculated using equation (3).

$$\text{Absorbance} = A520nm - A700nm \quad (2)$$

$$\text{Anthocyanin retention (\%)} = \frac{\text{Absorbance at the each month}}{\text{Initial absorbance at the production}} \times 100 \quad (3)$$

1.4 Bio-active Properties

A. Total Phenolic Content (TPC) and Anti-oxidant Activity

For the extract preparation, 0.25 g of Encapsulated Dandila anthocyanin pigments sample was extracted with 10 ml of 50 % methanol and filtered through Whatman No.1 filter paper. The filtrate was made up to 10 ml with 50 % methanol.

The total phenolic content of the methanolic extract was determined by the Folin-Ciocalteu assay as described by Salawu *et al*. in [12]. 2 ml of distilled water and 500 µl of Folin reagent were added to 100 µl of the extract. Then 1.5 ml of 7.5% Na_2CO_3 was added to the solution, and the volume was made up to 10 ml with distilled water. The mixture was kept for 2 hours, and the absorbance was measured at 760 nm using a UV-visible spectrophotometer. Total Phenolic Content was calculated using a Gallic acid standard curve with the equation (4) where GAE is the Gallic acid equivalent, V is the total volume of the extract (ml), DF is the Dilution factor and W is the weight of the sample. The result was expressed as milligrams of Gallic acid equivalents in 1 g of the colourant.

$$\text{Total Phenolic Content} = \frac{\text{GAE} \times \text{V} \times \text{DF}}{\text{W}} \quad (4)$$

Anti-oxidant activity of the extract was determined using the DPPH assay method as described by Salawu *et al*. in [12] with modifications. The extract (300 µl) was allowed to react with 2,700 µl of 0.1 mM DPPH solution for 6 h in the dark. Then the absorbance was measured at 517 nm using a UV- visible spectrophotometer. Methanol was used as the blank. The control sample consisted of 300 µl of methanol and 2700 µl of 0.1 mM DPPH solution. The percentage of scavenging the DPPH free radical by the colourant extract was calculated using the equation (5) where Ac is the absorbance of the control sample and As is the absorbance of the testing sample solution.

$$\text{Free radical scavenging activity (\%)} = (\text{Ac-As}) / \text{Ac} \times 100 \quad (5)$$

Trolox equivalent anti-oxidant activity of the colourant extract was calculated using a Trolox standard curve using the equation (6) where TE is the Trolox equivalent, V is the Total volume of the extract (ml), DF is the Dilution factor and W is the weight of the sample. The results were expressed as milligrams of Trolox equivalent in 1 g of colourant.

$$\text{Trolox equivalent anti-oxidant activity} = \frac{\text{TE} \times \text{V} \times \text{DF}}{\text{W}} \quad (6)$$

B. Anti-bacterial activity

Anti-bacterial activity of Encapsulated Dandila anthocyanin pigments was determined using the agar well diffusion method for bacteria strains, *Escherichia coli, Streptococcus pneumoniae, Staphylococcus aureus, Listeria monocytogenes, Shigella dysenteriae* and *Salmonella typhi*. For the preparation of extract, 1 g of Encapsulated Dandila anthocyanin pigments sample was extracted with 20 ml of 70 % ethanol and filtered through Whatman No.1 filter paper. The extract was concentrated to 2 ml by evaporation in a water bath at 50 ^{0}C.

Hundred microlitres of each of the test organisms from the 48-hour-old culture was poured into different sterile Petri dishes. About 20ml of sterile Nutrient Agar media was poured into each dish. The dishes were gently rocked together for proper mixing and allowed to solidify. Wells were made in the plates using a sterilized cork borer. A sample of 200 µl from the prepared extracts as filled into each well and incubated at 37^0C for 24 hours. The diameters of the inhibition zones were measured. The test was performed in triplicates with a control sample of 70 % ethanol.

3. Result

1.5 Extraction of Natural Pigments

Conventional solvent extraction is the most commonly used extraction method for recovering anthocyanins from plants, but this process is time-demanding and requires large volumes of solvents [13]. Thus, this leads to the degradation of bioactive compounds during the extraction process. Further, the conventional extraction method usually uses organic solvents together with mixing and heating [8].

Recently, microwave-assisted extraction (MAE) has become one of the most popular extraction processes for organic pollutants, pesticides, phenols, polymers, pharmaceuticals, and natural products [13]. MAE is recognized as a green technology with the advantage of quicker heating, reduced thermal gradients, reduced equipment size, and increased extract yield [14]. As explained by Jafari *et al*. in [15], during MAE, heat transfer occurs from the material to the bulk solvent in MAE and is distributed volumetrically throughout the irradiated sample and in this phenomenon, heat is created inside the microwave-absorbing substances and is transferred to the extracting medium. Therefore, MAE has become a promising alternative to conventional extraction.

In this study, 0.187 ± 0.025 mg of anthocyanin pigments were able to extract from 1 g of Dandila yam using the Microwave-assisted extraction method with the water as the solvent. Anthocyanin pigment content was measured as Total monomeric Anthocyanin Content, expressed as mg cyanidin-3-glucoside equivalent (CGE) per 1 g yam.

1.6 Encapsulation of Natural Pigments

Encapsulation, by which coats an active compound or acts as a wall material which gives lots of advantages is functioning as an effective barrier towards environmental parameters including oxygen, light, and free radicals is the most efficient method to protect anthocyanin from harsh environments [16][17]. Among the encapsulation techniques, microwave-assisted encapsulation is an alternative technology that uses microwave radiation to stimulate molecular motion and a constant dipole to rotate the molecules to generate volumetric heating [18]. Microwave-assisted encapsulation is regarded as an economical method and green technology for preserving the natural colourant, promoting good quality and easy handling, and contributing to low water activity end-products [19]. Further, time and energy consumption can be reduced significantly during microwave treatment due to its uniform heat distribution onto the material surfaces [18].

Previously, several scientists utilized maltodextrin and gum arabic as wall materials to encapsulate anthocyanin pigments in their studies. Therefore, these two wall materials were tested for Encapsulation efficiency. Among these two wall materials, maltodextrin and gum Arabic, the Encapsulation Efficiency of maltodextrin (94.38 ± 2.50%) was significantly higher compared to that of gum arabic (88.25 ± 5.28%). Encapsulation Efficiency values obtained for the ratios of maltodextrin 10, 20, and 30 were 94.35%, 92.15%, and 96.65% respectively while; encapsulation efficiencies of ratios of gum arabic 10, 20, and 30 were recorded as, 91.85%, 90.60%, and 82.30%. However, there were no significant differences among the Encapsulation Efficiency values obtained for ratios of maltodextrin and also among the encapsulation efficiencies of ratios of gum arabic.

In addition to the recorded higher encapsulation efficiency values, maltodextrin was reported as a less expensive, excellent textural modifier with water-soluble characteristic and delicate flavour and mouthfeel. Therefore, the colourant formulation encapsulated with maltodextrin at the 30 mg/ ml ratio was selected as the best encapsulation treatment for Dandila anthocyanin and was subjected to further analysis.

In a previous study, Liew *et al*. in [20], observed encapsulation efficiency values of *Clitoria ternatea* flowers as 60.22%, 63.38% and 95.74% for ultrasonic spray drying (outlet temperature 100°C), convection oven drying (80°C), and freeze-drying (-80°C), respectively. In there, the highest EE value was recorded by freeze drying which is considered a more sophisticated technique. However, in that study, even with freeze drying, the highest EE value of the present study could not be achieved. A similar study was carried out by Ahmad *et al*. in [17] for black mulberry extract using MAE with a combination of gum arabic and maltodextrin as the

wall material and EE was observed as 90.14 % for optimum encapsulation. However, the value was slightly lower than 96.65 ± 1.20 % which was the EE value obtained in the present study for Dandila anthocyanin with maltodextrin at 30 mg/ ml ratio.

However, somewhat higher EE values were observed by Idham *et al*. in [10] for spray-dried *Hibiscus sabdariffa* L. where the combination of maltodextrin and gum arabic gave the highest encapsulation efficiencies (99.87 ± 0.04%) of anthocyanins, followed by maltodextrin (99.69 ± 0.06%), gum arabic (98.4 ± 0.11%) and soluble starch (96.7 ± 0.35%). Though they observed higher EE values the technology used was quite expensive and sophisticated compared to microwave technology.

Table 1 Encapsulation Efficiency of wall materials

Wall material	Ratio of wall material (mg/ ml of pigment extract)	Encapsulation Efficiency (%)			
Maltodextrin	10	94.35 ± 1.06[a]	±	94.38 ± 2.50[a]	±
	20	92.15 ± 2.90[a]	±		
	30	96.65 ± 1.20[a]	±		
Gum Arabic	10	91.85 ± 1.91[b]	±	88.25 ± 5.28[b]	±
	20	90.60 ± 4.38[b]	±		
	30	82.30 ± 2.97[b]	±		

Mean values with different letters in the same column are significantly different P<0.05

1.7 Storage studies

Commercial applications of anthocyanins as colourants and pharmaceuticals have been limited due to their instability under pH, temperature, light, metal ions, enzymes, oxygen, ascorbic acid, sugars, and substances that lead to degradation [21].

However, in our study, there was no significant difference observed between the initial (at 0 months) and final (after 12 months) moisture contents of the Encapsulated Dandila anthocyanin pigments recorded as 6.50±0.51 % and 7.25±0.42 % respectively during the storage.

Further, a significant difference was not observed between the initial and final anthocyanin contents of encapsulated pigments during the storage period of 12 months and the anthocyanin retention was recorded as 96.27 % confirming the storage stability of encapsulated Dandila anthocyanin. The recorded percentage of anthocyanin degradation during the storage was around 4 % and that value was not significant.

However, in a similar study on microencapsulation of anthocyanin pigments of black carrot (*Daucus carota* L.) by spray drier, Ersus and Yurdagel in [22] observed degradation of 33 % of anthocyanin content in encapsulated powders at the end of 64 days storage period at 25 ^{0}C. Mansour *et al*. in [23] carried out a study for the microencapsulation of anthocyanin extract from red raspberry using a freeze-drying technique involving ultrasonication of soy protein isolate, gum arabic, and their combination and anthocyanin retention was observed as only up to 48 % during storage at 37 ^{0}C for 60 days for the combination of soy protein isolate and gum arabic as the best treatment. The retention of anthocyanin for encapsulated copigmented Black rice (*Oryza sativa* L. indica) anthocyanin samples was observed within 74% to 83% at the end of the storage period of 04 weeks by Raharjo *et al*. in [24].

These reductions were much higher compared to the degradation percentage observed in encapsulated Dandila anthocyanin pigments. Therefore, these findings confirmed the higher and more effective storage stability of Encapsulated Dandila anthocyanin pigments for up to 12 months in ambient conditions when compared with the natural colourants developed with a variety of techniques including more advanced technologies.

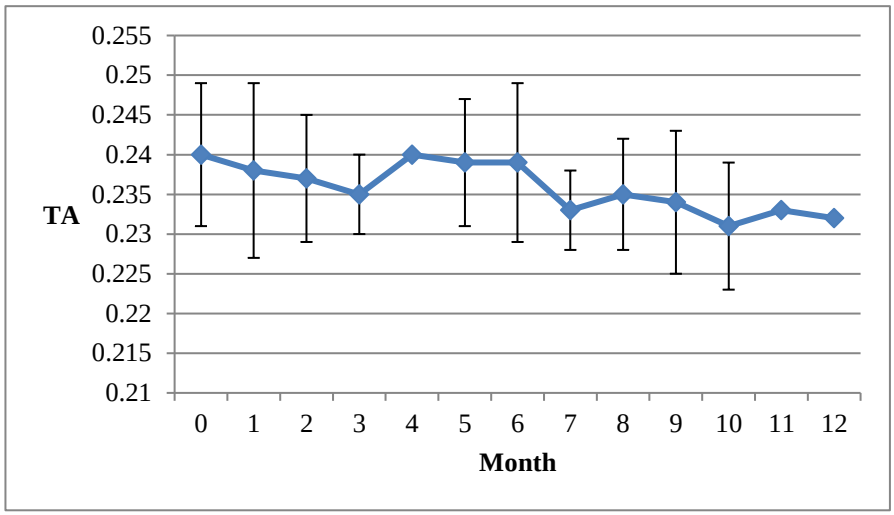

Figure 1. Change in the total anthocyanin content of the natural colourant during the storage period

1.8 Bio-active Properties

A. Total Phenolic Content (TPC)

TPC of encapsulated Dandila anthocyanin pigments was recorded as 5.27±0.05 mg GAE/ g which was 10 times higher compared to the TPC value (54.42 – 55.03 mg GAE/100g) observed for natural food colourant from *Hibiscus sabdariffa* by Sipahli in [11]. The TPC of the micro-encapsulated raspberry juice powder with freeze drying using gum arabic, maltodextrin and waxy starch in a study by Nthimole *et al*. in [25] ranged from 292.00 (Gum arabic) to 339.36 mg GAE/100 g (Waxy starch) and the value was slightly lower than that of encapsulated Dandila pigments. As reported by Azarpazhooh *et al*. in [26], the Total Phenolic Content of freeze-dried pomegranate peel anthocyanin encapsulated with maltodextrin ranged between 59.71 ± 0.83 and 73.13 ± 0.83 mg GA/kg, were much lower compared to that of natural colourant in the present study. Robert *et al*. in [27] carried out a study on the encapsulation of polyphenols and anthocyanins from pomegranate (*Punica granatum*) by spray drying and observed TPC of 1.53 – 2.75 mg GAE/ g. therefore, considering these findings, it appears that encapsulated Dandila anthocyanin contains a higher amount of phenolic compounds.

Phenolic compounds act as one of the most significant groups among the bio-active compounds found in plant-based foods, providing anti-oxidant, anti-carcinogenic, anti-mutagenic, and anti-inflammatory properties [28]. Protection of cells from oxidative damage by acting as anti-oxidants is considered as the key function of the phenolic compounds. Further, several studies have shown that increased dietary intake of natural phenolic antioxidants correlates with decreased coronary heart disease [29]. Therefore, the observed high TPC value of encapsulated Dandila anthocyanin pigments will be beneficial to use in functional foods and pharmaceuticals since this natural colourant provides additional health benefits.

B. Anti-oxidant activity

Anthocyanins are flavonoid pigments widely distributed in coloured fruits and flowers. Along with other phenolic compounds, they are potent scavengers of free radicals, although they can also behave as pro-oxidants [30]. One of the best-known properties of flavonoids, in general, is their strong antioxidant activity in metabolic reactions due to their ability to scavenge oxygen radicals and other reactive species and this feature makes flavonoids a potential tool for use in studies on oxidative stress, the ageing process, and cancer, especially since it has been reported that anthocyanins inhibit the growth of cancer cells and act as chemotherapeutics for numerous diseases [31]. Further, due to their antioxidant activity, anthocyanins contribute to the prevention of heart disease, cancer, and inflammatory disease [32].

Encapsulated Dandila anthocyanin pigments recorded antioxidant activity as 4.00±0.02 mg TE/g. Azima *et al*. in [33] observed DPPH radical scavenging activity of *Garcinia mangostana* peel, *Syzigium cumini* and *Clitoria ternatea* extracts which contain anthocyanins. The antioxidant capacity of anthocyanin extract from purple sweet potato was reported by Jiao *et al*. [34]. These studies showed the antioxidant activity of anthocyanin extracts which confirms the results of the present study. According to Konczak and Zhang in [30], because of their diverse physiological activities, the consumption of anthocyanins may play a significant role in preventing lifestyle-related diseases such as cancer, diabetes, and cardiovascular and neurological diseases [30] and the health and therapeutic effects of anthocyanin are mainly contributed by its antioxidative activities [35]. Therefore, the observed antioxidant properties of encapsulated Dandila

anthocyanin pigments may be a clue of their additional health benefits other than their main role as a natural food colourant.

C. Anti-bacterial activity

The health-beneficial properties of anthocyanin-rich foods were examined in numerous in vivo and in vitro studies [36]. Anthocyanins play a role in plant pest resistance and have anti-bacterial properties [37] and recent studies using purified anthocyanins or anthocyanin-rich extracts on in vitro experimental systems have confirmed the potential potency of these pigments and demonstrable benefits include antimicrobial activities [30]. There are different mechanisms that can explain the antimicrobial activity of anthocyanins, as they can cause localized disintegration of the bacterial outer membrane, leaking of cytoplasm (with the presence of significant amounts of cytoplasmic material and membrane debris outside the cells), and irregular shape [38].

Among the tested strains, Encapsulated Dandila anthocyanin pigments showed inhibitory activities against *Escherichia coli* and *Streptococcus pneumoniae* with the diameters of the inhibitory zones respectively as 11.00±1.00 mm and 10.50±0.71 mm. However, the natural colourant did not show any anti-bacterial effects against *Staphylococcus aureus*, *Listeria monocytogenes*, *Shigella dysenteriae*, *Salmonella typhi*. Jeyaraj *et al*. in [39] observed similar anti-bacterial effect of anthocyanins extract from *C. ternatea* flower against *E. coli* and *K. pneumonia*. The results of the present study confirmed the anti-bacterial activity of Encapsulated Dandila anthocyanin pigments which make them an ideal natural food colourant with additional health benefits of protection against common illnesses caused by *Escherichia coli* and *Streptococcus pneumonia*.

Table 2 Inhibition zone diameter of bacteria strains

Bacteria strain	Inhibition zone diameter (mm)
Escherichia coli	11.00±1.00
Streptococcus pneumoniae	10.50±0.71
Staphylococcus aureus	0.00±0.00
Listeria monocytogenes	0.00±0.00
Shigella dysenteriae	0.00±0.00
Salmonella typhi	0.00±0.00

4. Conclusion

The findings of this study confirm the successful development of a natural colourant from Dandila (*Dioscorea alata*) anthocyanin using the microwave-assisted encapsulation technique with maltodextrin as the wall material while ensuring its potential use and storage stability in ambient conditions. A noteworthy amount of total phenols contained in the natural colourant, observed inhibitory effects against *Escherichia coli* and *Streptococcus pneumoniae* and the scavenging activity of DPPH radicals reveals the potential utilization of the encapsulated Dandila anthocyanin in functional food formulations. Therefore, even using a domesticated technology without sophisticated instruments Dandila anthocyanin can be extracted and encapsulated to be used as a natural food colourant as well as a functional food ingredient with 12 months of storage stability.

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