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Bioactivity of anthocyanin, polyphenols from *Canna indica* and use as additive to improve stirred yogurt

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Abstract

This research studies the effect of crude anthocyanin extract from *Canna indica* on improving the nutritional value of stirred yoghurt. Also, The chemical composition, antioxidant, anti-inflammatory and antimicrobial activities of *Cannes indica* extract were evaluated. The crude extract contained anthocyanin (463.67µg/g), total phenolic (172.32±1.34µg/g), flavonoids (14.86±0.11 µg/g) and tannin (2.53±0.01 µg/g). The highest concentrations of phenolic compounds identified by HPLC were chlorogenic acid, ellagic acid, gallic acid, and apigenin. In addition, the crude anthocyanin extract strongly inhibited Gram-positive bacteria with a clear area zone of 13-18 mm, followed by efficacy against Gram-negative bacteria and fungi. The antioxidant potency ranged from 54.76 to 94.44% at a concentration of 75 µg/mL with strong anti-inflammatory activity in an in vitro study. During storage for 15 days, the yield of yogurt mixed with 0.2 and 0.4 mg crude anthocyanins/100 ml milk was evaluated chemically and microbiologically and compared to the control group. The growth of the probiotic *Lacticaseibacillus rhamnosus* reached its maximum on the 15th day. Treatments were fungi- and yeast-free for up to 15 days, and low fungi-yeast counts and primarily psychosomatic counts emerged for control. Moreover, the protein, fat, and ash content of treatments and control treatments increase with a progressive cold storage period.

Keywords: Anthocyanin; Polyphenols; Anti-inflammatory; Antimicrobial; Probiotics; stirred yogurt; physiochemical analysis.

1. Introduction

Synthetic additives are controversial and banned in many countries for use in food products due to safety concerns and health risks such as allergens, irritants and carcinogens (Amchova et al., 2015). At present, natural colors are one of the recent trends for many researchers in food application, as it is a safe and cheaper source of human health. Natural additives are safer even at higher doses compared to synthetic compounds (Ghosh et al., 2022).The red *Canna indica* flower contains antioxidant compounds such as phenols, flavonoids and anthocyanins. The color extracted from the petals of the Canna flower can be used as a food dye (Vankar & Srivastava, 2018).

Anthocyanins are a family of flavonoids, soluble in water and responsible for the purple, red, and blue colour of most flowers, berries, and fruits (Cooper-Driver, 2001). They are very prevalent, found not only in colorful flower petals but also in roots, stems, tubers, leaves, fruits and seeds (Liu et al., 2020). The anthocyanins extract contain powerful anti-inflammatory and antioxidants and reduce the risk of cancer. Their extracts from plants had an anti-proliferative effect against different leukaemia cell lineages (Vascellari et al., 2021). Also, it has multiple benefits such as reducing the risk of various diseases such as obesity, improving memory and age-related deficiencies, or improving the immune system due to its chemical and physical properties. (Shipp& Abdel-Aal, 2010).

Dairy products are measured as a major food group that has been supplemented with probiotic bacteria, such as cheese, yogurt and ice cream (Castro et al., 2015 & El-Sayed & El-Sayed, 2020). The food matrix was considered the perfect rote to transfer bioactive substances to humans. Stirred yogurt has been one of human's favorite dairy products because it is a good source of protein, vitamins, and minerals. Stirred yogurt can be used as a delivery system to transport vital substances and probiotic cells in sufficient amounts to humans to donate vital therapeutic effects (El-Sayed et al, 2022). This action points extracted anthocyanins with a phenolic compound from *Canna indica* flower and evaluated their antioxidant, antimicrobial and anti-inflammatory activities. The effect of curde anthocyanins added to stirred yogurt on the development of probiotics, improvement of chemical and microbiological properties during storage for 15 days.

1. Materials and methods

1.1. Materials

The red variety of *C. indica* flowers and fresh cow's full cream milk were taken from Farm National Research Centre.

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1.2.Microbial strains used in this study

The pathogenic strains used to evaluate the antimicrobial activity as *Escherichia coli* ATCC 8739, *Listeria monocytogenes* ATCC 5980, *Salmonella typhimirum* ATCC 14028, *Staphylococcusaureus* ATCC 6538, *Bacillus cereus* B-3711and *Aspergillus flavus* ATCC 9643. Additionally, the starter cultures and probiotic strains for stirred yogurt as *Streptococcus thermophilus* CH-1, *Lactobacillus bulgaricus* Lb-12 DRI-VAC, and *Lacticaseibacillus rhamnosus* NRRL B-442 (Strains from Dairy Department, National Research Centre).

1.3. Extraction of crude anthocyanins with phenolic compounds

The crude anthocyanins with phenolic compounds were extracted from red canna flowers using ethanol/water/HCl (70/30/1 (vol/vol/vol)) at room temperature, the mixture was filtered over a Buechner funnel, and the collected extract was dried using a rotary evaporator at 40°C and stored at -10 until analysis**.**

1.3.1. Total anthocyanin content from red canna flower extracted

Total anthocyanins were determined from the canna flower extracted by the method of Di Stefano et al (1989). The samples were diluted with an ethanol/water/HCl mixture solvent (70/30/1) and their absorbance was measured at 540 nm. Total anthocyanin contents were expressed in malvidin-3-glucoside equivalents using the following equation: TA = A540 nm × 16.7 × d - dilution.

1.3.2. Total phenolic content

The extracted red canna flower (0.1 g) was dissolved in 5 mL of ethanol for the determination of phenolic compounds. The ethanol extract and gallic acid standard (serial concentrations in the ranges of 1–10 µg/mL) were determined using the Folin-Ciocalteu reagent. The reaction mixture was prepared by mixing 0.5 ml of ethanol extract, 2.5 ml of 10% Folin-Ciocalteu'sreagent dissolved in water and 2.5 ml7.5% $NaHCO_3$. The mixtures were incubated in a thermostat at 45ºC for 45min. Next, the absorbance of the sample and standard was measured at 760 nm by spectrophotometer (Singleton et al., 1999).

1.3.3. Total flavonoid content

The study was used quercetin as a reference to determine the total flavonoid concentration by the aluminium chloride technique. The extracts or standard solutions (quercetin, 1–12 g/ml) were combined with 0.3 ml of $NaNO_2$ (5%). After incubating for 5 minutes, 0.3 mL of $AlCl_3$ (10%) was added. This was followed by 6 minutes of incubation and the addition of 2 mL of NaOH (1 mol/mL). A spectrophotometer was used to measure the absorbance of the solutions at 510 nm in comparison to a prepared reagent blank after the solutions had been well mixed (Saenkod et al., 2013).

1.3.4. Total tannins content

According to Polshettiwar et al., (2007), total tannin content (TTC) was assessed using the Folin-Ciocalteu reagent assay. An ethanol extract or standard solution (tannic 20–120 mg/L) was added to 7.5 mL of distilled water,. Next, 0.5 mL of Folin reagent and 1 mL of sodium carbonate solution (35% concentration) were added. A spectrophotometer (Unicom UV e300) was used to detect absorbance at 775 nm in comparison to the prepared reagent blank in a volume of 10 mL of distilled water. The amount of total tannin in the sample was calculated as mg tannic acid equivalent (TAE)/g dry weight. Triplicate samples of each were examined.

1.3.5. The HPLC condition for identification the extracted compounds

The Ethanol sample (5 µl) and phenolic compounds standard were filtered and injected in an Agilent model 1260 (Agilent, USA) high-performance liquid chromatography equipped with Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (a) and 0.05% trifluoroacetic acid in acetonitrile (b) at a flow rate of 0.9 mL/min. The column temperature was maintained at 40°C. mobile phase was successively programmed in a linear gradient as follows: 0 min (82% a); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A); 15-16 min (82% A) and 16-20 (82%A). The detector was monitored at 280 nm.

1.3.6. Antioxidant activity

1.3.6.1. *In vitro* DPPH free radical-scavenging assay.

Scavenging for free radicals (DPPH) using an ethanol extract of *Canna indica*. Ye et al. (2009) provided the assessment of free radicals. 3.0 ml of 0.1 mM ethanolic DPPH solution was mixed with samples at various concentrations. Following 30 minutes in complete darkness, the absorbance was measured at 517 nm. Using the following equation, the percentage of scavenged DPPH was determined:

Scavenging% = [1 – (A sample – A blank/A control)] x 100.

1.3.6.2. *In vitro* total antioxidant capacity assay

One ml of ethanol extract of *Canna indica* and standard (ascorbic acid) (100 to 400 µg/mL) was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate, and 4 mM ammonium molybdate). Tubes were incubated at 95°C for 90 min. After cooling, the absorbance of each sample was measured at 695 nm (Prieto et al., 1999).

1.3.7. *In-vitro* anti-inflammatory activity

Anti-inflammatory activity was evaluated according to Rahman et al., (2015). Bovine albumin serum (0.45ml) was added to the sample or standard drug diclofenac sodium (50, 100, 150µg/ml) and incubated at 37°C for 20 minutes. Then, they are heated at 57°C for 3 minutes, and 2.5 mL of PH 6.3 phosphate solution is added to the samples after cooling. The absorbance was measured using a UV-visible spectrophotometer at 255nm.

1.3.8. The antimicrobial activity of anthocyanin extracted from Canna.

The procedure described by BSAC, (2007) was used to assess the antibacterial activity of anthocyanin. The colonies of pathogens were cultured at 30ºC for 48 hours for fungi and 35ºC for 24 hours for bacteria using a nutrient agar medium. The typical colony was selected, placed in 10 ml of tryptone soy broth, and incubated at 35ºC for bacteria or 30ºC for fungi until detectable turbidity reached 0.5 "McFarland" standard solution. Then, 25 ml of Mueller-Hinton agar was put into several sterilized plates, and 0.1 ml of each of the tested strains was added as an inoculant. The plates were rested after the inoculum was placed on the surface of the agar medium using sterile wipes. With a cork borer, the wells in the agar media had a 6 mm diameter. After being dissolved in DMSO to a predetermined concentration of approximately 100 l/ml, 100 l of anthocyanin were added to each well. After being incubated overnight at 25ºC for fungus and 35ºC for bacteria, all plates' inhibitory zone diameters (mm) were measured.

1.4. The production of stirred yogurt fortified with anthocyanin

According to Fayed et al. (2019), fresh cow's milk was cooked for 15 minutes at 80°C and then swiftly cooled

to 42°C. The milk was inoculated with two percent each of the *L. bulgaricus*, *S. thermophilus*, and 2% *L. rhamnosus* cultures. After the infected milk had coagulated for three hours at 40 ºC, the yogurt was produced and left in a cold environment for the night. After that, the yogurt was separated into three parts, the first serving as the control (C), the second serving as the anthocyanin supplement with 0.2% (T1), and the third serving as the anthocyanin supplement with 0.4% (T2), and finally the result yogurt was swirled with a glass rod. In 50 mL plastic cubes, each therapy was transferred separately. The made stirred yogurt treatments were refrigerated (7 ºC) while being analyzed for chemically and microbiologically.

1.4.1. Microbiological evaluation of stirred yogurt treatments

Ten grams from each treatment were added individually and homogenized in 90 ml of sterile saline solution (0.9% NaCl) for one minute. For each treatment, decimal serial dilution was carried out, and the appropriate dilution was put in sterilized plates to assess: On M17 agar, the *S. thermophilus* counts were determined after a 48-hour aerobic incubation (APHA, 1994). Using MRS agar with 10% sorbitol and incubated anaerobically at 37 ºC for 48 hours to count *L. bulgaricus* (Saccaro et al., 2011). The *L. rhamnosus* was counted using MRS agar supplemented with 50 L-vancomycin solution (added to 100 ml of melted MRS agar prior to pouring) and anaerobically incubated at 37 ºC for 48 hours (Saccaro et al., 2011). Plate count agar incubated at the count was used to find the psychrotrophic count. Using chloramphenicol rose Bengal medium (Baggerman, 1981) and aerobic incubation at 25 ºC for four days, the counts of mold and yeast were detected. The total number of microbes was calculated as log CFU/mL values.

1.4.2. Chemical analysis of stirred yogurt treatments

Stirred yogurt treatments were analyzed for total solids, protein, fat, ash, and acidity as lactic acid by the method described by AOAC (2012).The pH values were measured for yogurt from the different treatments using a digital pH meter (Hanna, Germany). The carbohydrate values were obtained by calculation. All chemical characteristics were determined for 15 days of cold storage.

1.5. Statistical analysis

The results were analyzed by the SAS Statistical Analysis System User's Manual (SAS Institute, Inc., USA) and differences were measured to be significant at $p < 0.05$ (SAS, 2004).

2. Results and Discussion

2.1. Chemical composition of *Canna indica* flower extract

The chemical composition of the *Canna indica* flower extract was identified by a spectrophotometer. The ethanolic extract of *Canna indica* flower contained the content of anthocyanins (463.67 µg/g), total phenols (172.32 ± 1.34 µg/g), flavonoids (14.86 ± 0.11 µg/g) and tannins (2.53 ± 0.01 µg/g). The study by Vankar, & Srivastava (2010) found total phenol and flavonoid content of *Canna indica* flower were 0.96 mg GAE/ 100 g and 19.89 mg QE / 100 g, respectively. The other study by Srivastava & Vankar (2010) found the major crude anthocyanin (20.4 mg/g) and it was determined by HPLC. Four types of crude anthocyanin were separated as Cyanidin-3-O-(6`` -O-a-rhamnopyranosyl) -b-galactopyranoside (10.12mg/g), Cyanidin-3-O-(6`` -O-a-rhamnopyranosyl)-b-glucopyranoside (10.12mg/g), Cyanidin-O-b-galactopyranoside (3.45 mg/g), and Cyanidin-3-O-b-glucopyranoside (4 mg/g), respectively. The structure of anthocyanins is the glycosylated form of anthocyanidins (aglycones). Types of anthocyanidins consist of hydroxylation of the flavylium cation backbone at different positions of carbon (C_3, C_5, C_6, C_7, $C_{3'}$, $C_{4'}$ and $C_{5'}$) (Figure 1). The anthocyanin molecule contains an oxonium group in its structure, and its flavonoid structure maintains its periodic name with a charged oxygen atom on the C ring. (Castañeda-Ovando et al., 2009).

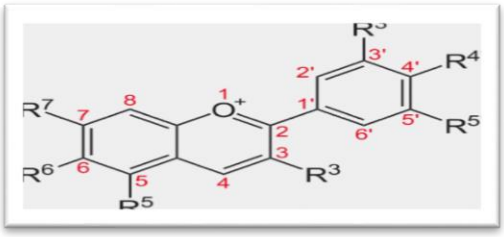

Figure 1: Structure of Anthocyanin from *Canna indica* flower extract

2.2. The phenolic compounds of *Canna indica* flower extract

The phenolic compound of the Canna indica flower was identified by HPLC as shown in Table (1). The main compound was chlorogenic acid (33872.35 µg/ml) followed by ellagic acid (7995.44), gallic acid (4073.83) and apigenin (3014.13 µg/ml), respectively.

Table 1: The phenolic compounds of *Canna indica* flower extract

Compounds	Conc. (µg/ml)
Gallic acid	4073.83
Chlorogenic acid	33872.35
Catechin	334.87
Methyl gallate	396.70
Coffeic acid	1257.90
Syringic acid	115.07
Rutin	710.56
Ellagic acid	7995.44
Coumaric acid	59.36
Ferulic acid	148.77
Naringenin	411.15
Daidzein	77.35
Querectin	506.17
Cinnamic acid	247.48
Apigenin	3014.13
Hesperetin	103.25

2.3. The antioxidant activities of the compounds extract

2.3.1. Antioxidant activity assessed by DPPH

The scavenging activity against 2, 2 di-phenyl-picryl-hydrazyl ($DPPH^{\cdot}$) is shown in Table (2). All concentrations of the extract showed the highest activity in DPPH radical removal compared to vitamin C**.**

2.3.2. Antioxidant activity assessed by ammonium molybdate

This test is based on reducing the phosphomolybdate ion in the presence of antioxidants, which leads to the formation of the green phosphate complex / MoV, which is measured by spectrophotometry (Prieto et al., 1999). The maximum reduction of phosphomolybdate ion was recorded in concentration at 75 µg/ml

(66.92±0.22%). The phytochemicals of *Canna indica* flower extract have high scavenging activity against free radicals and can be a good source of food additives (Vankar, & Srivastava 2010). Anthocyanins have twice the antioxidant capacity of other well-known antioxidants, such as (+)-catechin and vitamin E. The apparent ability of hyperpolarized anthocyanins to regenerate lipophilic antioxidants such as vitamin E may be due to them possessing similar properties to vitamin C, such as protecting biofilms from oxidation, by effectively trapping peroxyl radicals (Kuskoski et al., 2004). Also, the activity of flavonoids as antioxidants is due to the inhibition of a variety of redox systems such as cyclooxygenase, 5-lipoxygenase, monooxygenase, or xanthine oxidase (Tinoi et al., 2006).

Table 2: Antioxidant activity of the compounds extract

	Inhibition %	
concentration	DPHH	phosphomolybdic acid
25µg	80.27^{c}	50.15^{f}
50	92.27^{b}	57.29^{e}
75	94.69^{a}	66.29^{d}
Control (vit. C)25µg	40.56^{e}	80.26^{c}
50	55.92^{d}	92.17^{b}
75	92.35^{b}	94.36^{a}

Data indicated the mean of three replicates. Means with the same lowercase uppercase letters indicate slight difference strains

2.4. Anti-inflammatory activity of the compounds extract

It is reported that inflammation is caused due to the denaturation of proteins. The ability of anthocyanins to inhibit heat-induced albumin denaturation has been studied (Table 3). Significant changes in the anti-inflammatory activity of anthocyanin at different concentrations were observed (50-150 ug/mL).The Maximum inhibition (51.5±0.29%) was observed from Anthocyanin at 150 ug/mL. Ahmad et al., (2015) found that bilberry extract anthocyanins have a protective effect on visual function during retinitis. Anthocyanins can inhibit inflammatory effects by inhibiting NF-κB translocation, activator protein 1, cAMP response element-binding protein, and enhancer-binding protein/CCAA (Taverniti et al., 2014).

Table 3: Anti-inflammatory activity of the compound extract

concentration	Inhibition %
50µg	24.71^{f}
100	37.80^{e}
150	52.13^{d}
Diclofenac sodium 50µg	81.34^{c}
100	85.42^{b}
150	88.41^{a}

Data indicated the mean of three replicates. Means with the same lowercase uppercase letters indicate slight difference strains.

2.5. The antimicrobial activity of the compounds extract

Anthocyanin, once thought to be a collection of substances, is a member of the polyphenol flavonoid family of phytochemicals that are widely found in plant-based meals. Therefore, natural anthocyanins may be able to limit microbial development while also giving food its natural color. Consequently, the good diffusion method was used to assess the antimicrobial effectiveness of the natural anthocyanin derived from *Canna indica* against various bacterial foodborne strains, and fungi as the findings presented in Table (4). Evidently show that the tested anthocyanin had inhibitory action against microbial species, particularly against Gram-positive bacteria, with the diameter of the inhibition zone of Gram-positive bacteria ranging between 13 and 18. B. cereus (18mm) was the strain that suffered the most damage. The 10 to 11 mm wide inhibitory zones were applied to the tested Gram-negative bacterium *E. coli* and *S. typhimirum*, respectively. Additionally, the tested *A. flavus* was resistant to the anthocyanin at a concentration of 100 µg/mL, with an inhibitory zone that measured around 12 mm in diameter. According to Veerapandi et al., findings in (2021), anthocyanin derived from various plant sources exhibited the strongest antibacterial effects against *S. aureus*, *E. coli*, and *Klebsiella pneumonia*. Additionally, Farias-Cervantes et al. (2018) reported on the impact of an increase in anthocyanin concentration in powders that have been encapsulated and have antibacterial capabilities.

Table 4: The antimicrobial activity of the compounds extract

Test microbes	100 µg/mL (Inhibition zone, mm)	Photos of Zones
S. aureus	15 ^{b}±0.48	
L. monocytogenes	13 ^{c}±0.40	
B. cereus	18 ^{a}±0.82	
E. coli	10 ^{d}±0.39	
S. typhimirum	11 ^{d}±0.48	
A. flavus	12 ^{c}±0.60	

Data indicated the mean of three replicates ± SD. Means with the same small letter superscripts indicate insignificant difference strains.

2.6. Microbiological evaluation of stirred yogurt treatments during storage

2.6.1. Lactic acid bacteria

In stirred yogurt treatments, the effect of anthocyanin on the activity of the starting culture and *L. rhamnosus* during storage is displayed in Table (5). *S. thermophilus* counts steadily grew but slight, and counts did so while maintaining

the same log cycles, particularly after 10 and 15 days of cold storage. *S. thermophilus* counts were 6.98, 7.22, and 7.30 log CFU/mL for control, T1, and T2, respectively, at zero time. *S. thermophilus* counts improved at 10 days, recording 7.49, 7.90, and 7.99 log CFU/mL for control, T1, and T2, respectively. Additionally, the T2 followed by T1 had a higher *S. thermophilus* count than the control.

L. bulgaricus counts experienced the same outcomes. Where, for control, T1, and T2, respectively, the viable counts of *L. bulgaricus* at zero time were measured at 6.40, 6.74, and 6.88 log CFU/mL. Particularly after 10 days of storage, there was a small rise in these counts in the T1 and T2 compared to the control. After 15 days of storage, the *L. bulgaricus* counts in each treatment were slightly reduced to 7.20, 8.10, and 8.40 log CFU/mL for the control, T1, and T2, respectively.

Furthermore, it is evident from Table (5) that *L. rhamnosus* counts considerably increased with time in a progressive manner, with the highest growth observed at 10 days of storage in all treatments. T2 had a rather high viable count (9.23 log CFU/mL), which was followed by T1 (9.00 log CFU/mL). The control group had the lowest count (8.89 log CFU/mL) at 10 days, on the other hand. After 15 days of storage, the number of *L. rhamnosus* cells was somewhat reduced, recording 8.40, 8.84, and 8.95 Log CFU/mL for control, T1, and T2, respectively. This slight drop can be explained by progress of acidity during storage, as noted by previous writers El-Shafei et al., (2018). According to Cheng et al. (2016), *S. thermophilus* GIM 1.321 and *L. plantarum* GIM 1.35 had strong-glucosidase production capacities and able to degrade anthocyanins by 46.17% and 43.62%, respectively. Additionally, Wang et al.'s review in (2022) showed that anthocyanins can be utilize as prebiotic products, that they play an essential role for the growth of probiotics, that they prevent the growth of harmful bacteria, and that they enhance the environment in the intestine.

Table 5: Lactic acid bacteria counts in stirred yogurt during storage period

Storage (days)	*S. thermophilus*			*L. bulgaricus*			*L. rhamnosus*		
	Control	T1	T2	Control	T1	T2	Control	T1	T2
Fresh	6.98 ±0.12	7.22 ±0.18	7.30 ±0.30	6.40 ±0.30	6.74 ±0.40	6.88 ±0.28	7.50 ±0.40	7.6 ±0.44	7.8 ±0.40
5	7.33 ±0.12	7.45 ±0.19	7.60 ±0.28	7.33 ±0.33	8.20 ±0.46	8.55 ±0.20	8.22 ±0.45	8.50 ±0.37	8.92 ±0.37
10	7.49 ±0.28	7.90 ±0.22	7.99 ±0.33	7.95 ±0.28	8.67 ±0.33	8.93 ±0.22	8.89 ±0.33	9.00 ±0.48	9.23 ±0.55
15	7.00 ±0.20	7.50 ±0.28	7.82 ±0.20	7.20 ±0.30	8.10 ±0.30	8.40 ±0.30	8.40 ±0.28	8.84 ±0.42	8.95 ±0.40

Data indicated the mean of three replicates ± SD. **C**: Control yogurt; Yogurt with 0.2% natural anthocyanin dye; **T2**: Yogurt with 0.4% natural anthocyanin dye.

2.6.2. Detection of other microbial counts in stirred yogurt treatments

By counting molds and yeasts and identifying other bacteria as psychotrophic, the shelf life of the stirred yogurt treatments was determined. It's possible that the hygienic standards that were upheld throughout the production of stirred yogurt are what made all treatments free of mold and yeast during cold storage. Additionally, treatments were shown to contain counts of psychrotrophic bacteria throughout storage, as seen in Figure (2). The figure shows that the counts of psychrotrophic bacteria at time zero were 3, 2, 80, and 2, 43 log CFU/mL for control, T1, and T2, respectively. Following that, the numbers of psychrotrophic bacteria raised over the storage period, although at the conclusion, a higher number (4.50 log CFU/mL) was found in the control group than in the other treatments (3.15 and 3.10 log CFU/mL for T1 and T2, respectively).These results were related to the antimicrobial activity of anthocyanin that confirmed before and with other authors (Salamon et al., 2020; Veerapandi et al., 2021). Anthocyanins are typically progressively added to food and beverage items as a food colorant, a functional food, or a nutritional supplement. Anthocyanins will play a more beneficial function in human health as they grow into more stable compounds with longer shelf life (Shipp & Abdel-Aal, 2010). This will expand their use in food applications and overall consumption. To be more specific, Zhu et al. (2018) found that supplementing with anthocyanins caused the pH to drop while probiotic counts increased greatly. A variety of factors could be to blame for these outcomes.

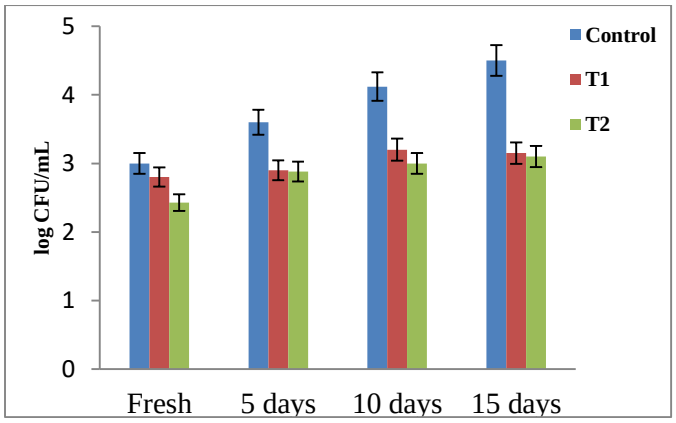

Figure 2: the psychrotrophic bacteria in stirred yogurt treatments during storage period. **C**: Control yogurt; Yogurt with 0.2% natural anthocyanin dye; **T2**: Yogurt with 0.4% natural anthocyanin dye.

2.7. Chemical composition of stirred yogurt treatments

Table (6) shows the changes in the chemical composition of the control yogurt and the other yogurt fortified with anthocyanin. The total solids content of yogurt increased with the addition of anthocyanins compared to the control yogurt. The fat, protein, and ash contents of the control yogurt were slightly higher compared with the anthocyanin fortified yogurt. Carbohydrate was significantly higher in the yogurt treatments fortified with (0.2 and 0.4 %) natural anthocyanin dye (5.26 %) and (5.82 %) compared with the control yogurt (5.05 %).

Table 6: Chemical compositions of different yogurt treatments

Treatments	T.S%	Fat%	Protein%	Ash%	Carbohydrate%
Control	14.02 ±0.02	3.70 ±0.29	4.15 ±0.08	1.12 ±0.06	5.05 ±0.16
T1	14.19 ±0.16	3.70 ±0.10	4.12 ±0.01	1.11 ±0.02	5.26 ±0.12
T2	14.62 ±0.05	3.65 ±0.06	4.10 ±0.10	1.05 ±0.05	5.82 ±0.19

Data indicated the mean of three replicates ± SD. C: Control yogurt; Yogurt with 0.2% natural anthocyanin dye; T2: Yogurt with 0.4% natural anthocyanin dye.

3.8. The acidity of the milk during the storage period

Figure (3) presents the changes in the pH of yogurt, as affected by supplementation with different proportions of the anthocyanin and the storage period. The acidity of all yogurts treatments increased significantly during 15 days of cold storage. The development of acidity was significantly higher in the anthocyanin-supplemented yogurt than in the control treatment. Thus, our studies show that yogurt fortified with 0.2 and 0.4% anthocyanins had a good effect on the activity of starters. On the contrary, the pH values were significantly lower in the anthocyanin - supplemented yogurt than in the control yogurt.

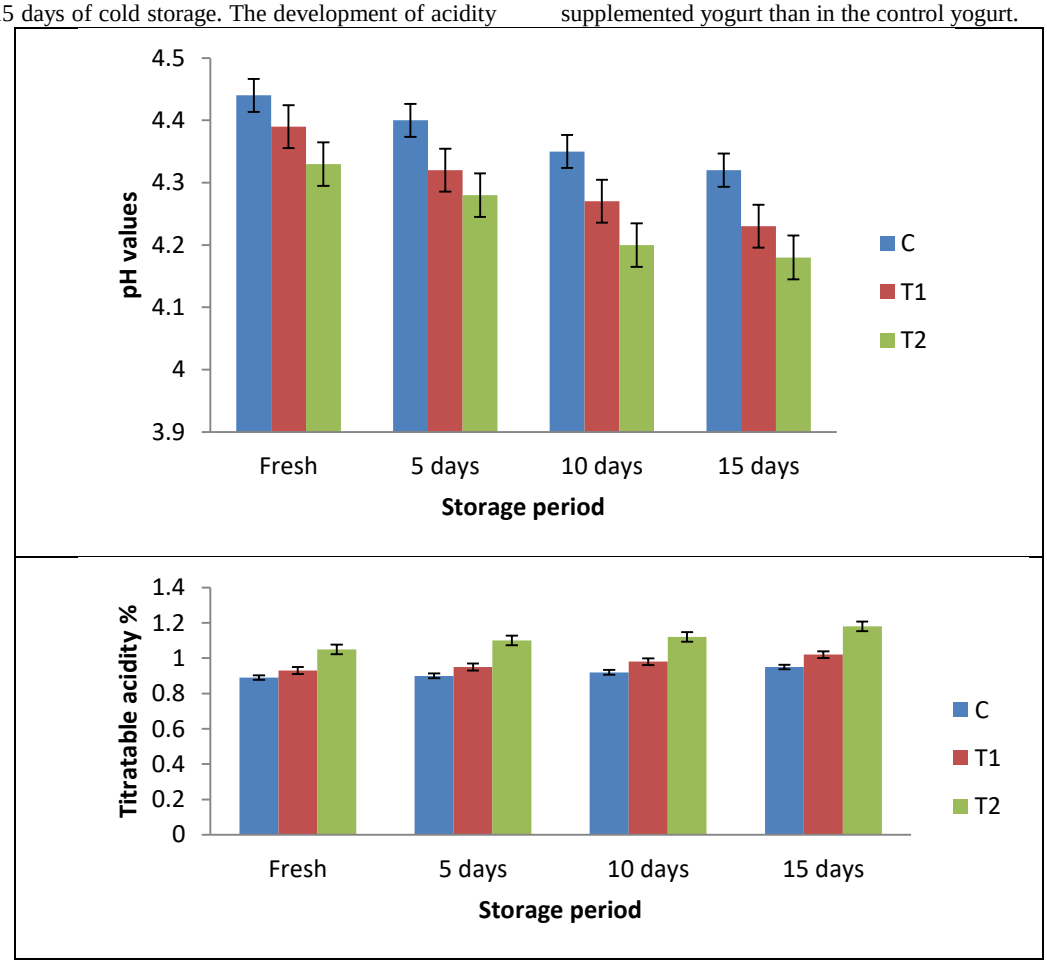

Figure 3: Changes in acidity and pH of yogurt during storage period.

C: Control yogurt; Yogurt with 0.2% natural anthocyanin dye; **T2**: Yogurt with 0.4% natural anthocyanin dye.

3. Conclusion

Anthocyanin is a safer pigment and a powerful antioxidant functional food. Crude anthocyanin extract inhibited Gram-positive bacteria with an apparent area of 13-18 mm, followed by efficacy against Gram-negative bacteria and fungi, while it has strong anti-inflammatory activity in vitro. The overall acceptability of the blended stirred yogurt fortified with raw anthocyanins at 0.2 and 0.4% was approximately closer than that of the control stirred yogurt and the percentage of raw anthocyanins did not have deterministic effects on the flavor scores, color and appearance during storage. Anthocyanins from *Canna indica* are recommended as a new value-added beneficial functional ingredient to the food industry as natural additives that will be healthier than synthetic ones.

Disclosure statement

There are no conflicts of interest by the authors.

Authors' contributions

Regarding the experiment design, the experimental work, the preparation and revision of the manuscript before submission, all authors made an equal contribution to it.

Data Availability Statement

The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author/s.

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