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Bioactive Compounds, Amino Acids and Activity of Phycobiliprotein Extracted from *Spirulina* (*Arthrospira platensis*) and their effect on Jelly Candy

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A GROWING number of consumers are interested in jelly candy goods that have new ingredients added to their classic recipes to make them healthier and more nourishing. The purpose of this work is create a jelly candy that comprises phycobiliprotein color from cyanobacterium*Spirulinaplatensis* extract (PES) after researching its antioxidant and antibacterial characteristics, as well as the make-up of the amino acids it contains and bioactive components. It was evident from the data that PES had stronger antibacterial effects than penicillin 10 against *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Pseudomonas aeruginosa*. Its IC50 value was also greater at 47.07µg/mL. Food Agriculture Organization (FAO) reported thatthe total amount of necessary amino acids was 33.74%. A protein efficiency ratio (PER) and a biological value (BV) of (PES) were1.32 and 62.98, respectively. Protein, fiber and, ash content of jelly candies recorded 27.09, 0.43, and 1.88%, respectively with adding PES up to 10%, compared to the control (0.1% artificial green color). l. It was discovered that the amounts of total polyphenols, total flavonoids, vitamin C and carotene were 0.20 mg GAE/g, 0.27 mg QE/g, 17.06 mg/100 mL and 3.01 mg/100 mL, respectively. Without altering the taste, flavor, color, or general acceptance of the jelly candy, phycobiliprotein colouring can be added up to a concentration of 5%.Phycobiliprotein of *S. platensis* extract can be used as a natural colorant in jelly candy.

Keywords: Bioactive compounds, amino acids, phycobiliprotein, *Spirulina*, jelly candy.

1. Introduction

Cyanobacteria are photoautotrophic, gram-negative prokaryotes, found in aquatic habitats (Afreen and Fatma, 2018). One of the significant cyanobacteria, *Spirulina* (*Arthrospira platensis)*, thrives in warm-climate water naturally and is primarily grown in ponds and small lakes. It can withstand greater alkaline pH levels (Fabrcio et al.2011). It was widely exploited as a source of high nutritional, medical metabolites, and health value (Al Hinai et al.2019). More than 3,000 tons of *Spirulina* are grown annually for human consumption and the manufacturing of other high-end goods chemicals (Brennan and Owende, 2010). In recent years, humans used the *Spirulina* as a tablet and powder. In addition, spirulina protein is easilydigestible and is recommended for malnourished children (Chaiklahan et al.2011) due to its relatively high protein content (60–70%), which also provides eighteen amino acids, including all necessary amino acids in equal ratio (Kim and Kang, 2011), as well as carbohydrates, fat, dietary fibres, sugars, and vitamins like vitamin A, C, E, B12, thiamine, and nicotinic acid (Chopra and Bishnoi, 2008). Lee et al. (2015) decided that, *Spirulina* is used in a variety of foods, including juice smoothies, candy, baked sweets, doughnuts, pasta, tomatoes juice, desserts, snack foods like popcorn and crackers, breakfast cereals, soups and instant meals. The ability of phycobiliproteins (PBP), which have been isolated from a variety of cyanobacterial species, to function as free radical scavengers justifies their usage in the nutrition, beauty products, pharmaceutical and biomedical research. It also has positive benefits including anticancer, neuroprotective, anti-inflammatory and anti-allergic (Liua et al. 2015).

Gummy jelly candies make up about 50% of the market for candies (Garcia, 2000). One of the most popular snacks among individuals of all ages is candy. Typically, the varieties, forms, tastes and colours of candy vary (Oktavianti, 2003). Because

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they are simpler to swallow and taste better than other candies, jelly candy is increasingly popular (Moghaddas kia et al.2020). The biggest draw for kids is the variety of jelly candies and their appealing hues. They are also ingested by children and the elderly. Foods with clear labels and no additives are more in demand these days. Therefore, a contemporary trend is the creation of confectionary items based on nutritional components in order to obtain new and healthier products (De Moura et al.2019).

Consequently, the aim of this work is to extract the phytobiliproteins from *Spirulina* and investigate their antimicrobial and antioxidant properties. Additionally, it will estimate the amount of amino acids present in the protein and add it to jelly candy to determine how it affects the candy's sensory qualities, chemical composition, mineral content and phytochemical content.

2. Materials and methods

Raw materials:

Fresh cyanobacteria (*Spirulina platensis*) were obtained from cyanobacteria Lab., Microbiology Dept., Soil, Water and Environment Institute, Sakha Agricultural Research Station, Kafr EL-Sheikh city, Kafr EL-Sheikh Governorate, Egypt.

Other ingredients: sugar, glucose syrup (30-32DE), mint flavor and gelatin powder were obtained from the local market, Kafr EL-Sheikh Governorate, Egypt.

Chemicals

All chemicals were obtained from El- Gomhoreia Company for drugs and chemicals, Tanta city, EL-Gharbia Governorate, Egypt.

Extraction of Phycobiliproteins from *A. platensis*

The phycobiliproteins extract was isolated and designated as crude extract from *A. platensis* according to Hussein et al. (2017).

Purification of phycobiliprotein extract

The obtained crude extract was stored overnight at 4°C for 65% ammonium sulphate precipitation (Chakdar and Pabbi, 2012).

Antioxidant activity by DPPH radical scavenging method of phycobiliprotein extract from *A. platensis*

Different phycobiliprotein extracts from *A. platensis* were tested for their capacity to scavenge free radicals using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay by Brand-Williams et al. (1995).

Antimicrobial activity of phycobiliprotein extract from *A. platensis*

Modifications agar diffusion method as described by Fouad et al. (2015), was used to measure the antibacterial activity of extracted phycobiliprotein. Using the agar-well diffusion method as described by, the antifungal spectrum activity was assessed (Ghazy et al. 2021). By using the proper dilutions, the number of fungal spores in the sample was increased to $1x10^6$ spores/ml (Kelmanson et al.2000).

Determination of free amino acids of phycobiliprotein extracts from *A. platensis*

The free amino acids determination of A. platensis powder phycobiliprotein extracts was conducted by Biancarosa et al. (2017).

Amino acid score:

According to FAO (1993), the chemical score is defined as follows:

(Mg of essential amino acid in 1g test protein)/ (Mg of essential amino acid in 1g reference protein) ×100

The amino acid that shows the lowest score was taken as the first limiting amino acid.

Protein efficiency ratio (PER):

PER was calculated using the equation suggested by Alsmeyer et al. (1974) as follows: PER = - 0.468 + 0.454 (Leucine) – 0.105 (tyrosine)

Biological value (BV):

Biological value (BV) of protein samples was calculated as described by Oser, (1959) following the next equation: BV = 49.09 + 10.53 (PER)

Where: BV = Biological value, PER = protein efficiency ratio

Essential amino acid index (EAAI):

Essential amino acid index was calculated according to Labuda et al. (1982).

Preparation of jelly candy supplemented with phycobiliprotein extracts from *A. platensis*

With some modifications, the traditional jelly candy recipe from (Garcia, 2000) was used to create the jelly candies. 29g of glucose syrup, 30g of sugar and 14g of water were combined and cooked in a brass pan for the cooking process. By combining

gelatin with warm water (45°C), 13g of gelatin solution was created. The previously produced glucose syrup solution was then added, and while mixing to prevent burning, the gelatin solution was added. The liquid was heated to 116°C before 3g of citric acid was added and combined. By adding some water, the mixture's brix value was changed to 68–75%. After completing this stage, the hot mixture was flavored with mint. The mixture's temperature was then lowered to 40°C. Similar steps were used to prepare the control sample, which was colored using 0.10g of artificial color. The typical jelly candy was blended with phycobiliprotein from *A. platensis* at different concentrations of 2.5, 5, 7.5 and 10%. Before the slurry was put into a mould, some air bubbles were eliminated. The samples were cooled overnight, then they were cut into 2 cm cubes and sent to analysis.

Proximate chemical composition of jelly candy samples prepared with phycobiliprotein extracted *A. platensis*

The chemical composition of jelly candy samples including moisture, ether extract; according to the method of A.O.A.C. (2016), the crude fiber and ash were measured. The total carbohydrates were determined by accounting subtraction using the following equation: 100 - (protein + fat+ fiber+ ash).

Bioactive compounds of jelly candy prepared with phycobiliprotein extracted *A. platensis*

Determination of total polyphenols content.

Using the Folin Ciocalteau reagent as described by (Singleton and Rossi, 1965).

Determination of total flavonoids content

Using a slightly modified version of the aluminium chloride colorimetry method developed by Chang et al. (2002).

Determination of carotenoids content

Carotenoid in jelly candy has been analyzed by (Chang et al. 2002).

Determination of vitamin (C) content

Utilizing the 2,6-dichlorophenol-indophenol titration method outlined in A.O.A.C (2016) to determine the amount of vitamin (C).

Organoleptic properties of jelly candy prepared with phycobiliprotein extracted from *A. platensis*

The organoleptic properties of jelly candy samples supplemented with levels of phycobiliprotein extract were performed according to (Barylko-Pikielna, 1975).

Statistical analysis

The sensory evaluation and chemical composition data were recorded as means and examined using (SPSS) Windows (Ver.20). To identify differences between various treatments, Duncan comparisons and one-way analysis of variance (ANOVA) were tested.

3. Results and discussion

3.1. Antimicrobial and antioxidant activity of Phycobiliprotein extract from *Spirulina platensis* (PES)

Phycobiliprotein extracted from *Spirulina platensis* (PES) and Pencillin 10 showed varying degree of antibacterial and antifungal activity as can be seen in Table (1). *S. platensis* extract showed inhibition of growth of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Penicillium digitatum* ATCC 10030, *Candida albicans* ATCC 14053 and *Saccharomyces cerevisiae* ATCC 9763 but no regions of inhibition against *Listeria monocytogenes*, and *Yersinia enterocolitica*.

The mean diameter of inhibition zone against *Staphylococcus aureus, Bacillus cereus, Escherichiacoli, Salmonella typhi* and *Pseudomonas aeruginosa* was 23.00, 19.00, 20.12, 18.43 and 22.00 mm, respectively in *S. platensis* extract (PES). These results agree with Athbi, (2014) who found that, the inhibition zones diameters revealed by S. platensis extract were greater reaching to 22mm and 21mm for *E. coli* and *Staph. Aureus*, respectively. PES extract gave a higher antibacterial effect against *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* than that of Penicillin 10. PhS extract gave a higher antifungal activity *against Penicillium digitatum* ATCC 10030 (15.12 mm) compared to Penicillin10 (13.00 mm). The antimicrobial activity of PES extract may be due to antibacterial activity of phycocyanin (Murugan et al. 2012; Mohamed et al. 2018 and Safari et al. 2020).

The data in the same Table (1) show the antioxidant activity of S. platens extracted using IC50. It is clear that the IC50 value for the DPPH radical scavenging activity was 47.07µgmL^{-1}. These results in line with the results obtained by Ko et al. (2014); Wang et al. (2015) and Babita et al. (2017) whose found that, the activities of DPPH radical scavenging increased by increasing the concentration. The antioxidant protective effects are mediated by phycocyanin, carotene and other vitamins and minerals found in Spirulina (Lü et al.

2010).The highest antioxidant activity of Spirulina due to its contents of flavonoids, tannins and phenolic compounds (Gaber et al. 2021).

3.2. Amino acids and nutraional value of Phycobiliprotein extracted from (PES) *Spirulina platensis*

Amino acids are very important due to help build muscles, blood and internal organs (Al Hinai et al. 2019). The data in Table (2) show the percentage of amino acids content of *A. plantesis*. It is clear that it followed by valine whilst the lowest value was noted for tryptophan.From the same table it was clearly indicated that, *S. platensis* extract appear higher values of the essential amino acid than FAO (1993) Except for Leucine, lysine, Tryptophan and Valine were found in low concentrations in S. platensis extract compared to FAO pattern. S. platensis extract contain a higher total essential amino acid (33.74%) than those in FOW (32.47%)

Non-essential amino acids play an important role in promoting the synthesis of proteins and hormones, remove the toxin in the kidneys, healing wounds, and increase the healthy immune system and the production of glucose and other amino acids. The glutamic acid gave the highest value (3. 82%), followed by aspartic acid (3.4%) and alanine (2.6 %) as non-essential amino acids. The lowest value was has nine essential amino acids and nine non-essential amino acids. In part of essential amino acids, Isoleucine (4.8%) gave the maximum value followed by Leucine and Therionine (4.2%).

Histidine represents the same value (4.2%) it was very important amino acid for children. Farg et al. (2021), *S. platensis* powder has eight essential amino acids; histidine, lysine, methionine, tryptophan, phenylalanine, isoleucine, leucine and arginine, respectively. Bashir et al. (2016) found that essential amino acids in *Spirulina*; leucine in large proportion noted for tyrosine (1.14%).

Quality of protein depends on its essential amino acids (EAA). There are several ways to determine the quality of proteins. The most admitted and approved methods are the amino acid scores (AAS), protein efficiency ratio (PER) and biological value (BV) of protein.

Amino acid scores (AAS %) can be used generally to detect protein nutritional value for human. The amino acid scores in *Spirulina platensis* extract given in the same Table (2) indicate that, Lysine is the first limiting amino acid, while valine was the second limiting amino acid in FPC. Finally, the third limiting amino acid was Leucine and tyrosine.

The protein efficiency ratio, biological value and amino acid index in *Spirulina platensis* extract represented 1.32%, 62.98 % and 0.97%.

Table 1. Antimicrobial and antioxidant activity of Phycobiliprotein extracted from *Spirulina platensis*.

Test strains	Zone of inhibition (mm)	
	***Spirulina platensis* extract**	**Pencillin 10**
Staphylococcus aureus	23.00±2.00	35.00±1.00
Bacillus cereus	19.00±1.00	10.00±0.00
Listeria monocytogenes	ND	25.00±1.00
Escherichia coli	20.12±1.08	10.00±1.00
Salmonella typhimurium	18.43±1.01	21.00±0.00
Pseudomonas aeruginosa	22.00±2.00	10.00±1.00
Yersinia enterocolitica	ND	20.00±0.00
***Penicillium digitatum* ATCC 10030**	15.12±1.03	13.00±1.00
***Candida albicans* ATCC 14053**	13.00±0.00	20.00±0.01
***Saccharomyces cerevisiae* ATCC 9763**	12.19±1.08	13.00±0.00
IC_{50}	**47.07ug/mL**	

Data is presented as mean ± standard deviation. * denotes significance at $p \leq 0.05$
ND = Not detected

Table 2. Amino acids and nutraional value of Phycobiliprotein extracted from (PES) *Spirulina platensis*.

Name of Amino Acids	Value (%)	FAO	Amino acid scores (%)
Essential amino acids			
Histidine	4.2	1.90	221.05
Isoleucine	4.8	4.2	114.28
Leucine	4.2	4.8	87.5
Lysine	2.2	4.2	52.38
Methionine	2.8	2.2	127.27
Phenylalanine	4.00	2.8	142.86
Thereonine	4.2	4.00	105
Valine	4.1	4.2	97.62
Tryptophane	3.24	4.1	79.02
Total essential amino acids (EAA)	33.74	32.47	
Non-essential amino acids			
Alanine	2.60		
Arginine	1.96		
Aspartic	3.4		
Cystine	1.71		
Glutamic	3.82		
Glycine	1.82		
Proline	1.17		
Serine	1.22		
Tyrosine	1.14		
Protein efficiency ratio (PER)	1.32		
Biological value (BV)	62.98		
Amino acid index	0.97		

3.3. Chemical composition of jelly candy prepared with different levels of phycobiliprotein extracted from *Spirulina platensis*

The moisture, protein, fat, fiber, ash and total carbohydrate contents of jelly candy prepared with S. platensis determined in Table (3). Moisture was increased significantly by increasing the concentration of phycobilioprotein from 35.82% in 2.5% PES to 38.33% in 10% PES compared to control sample (30.66%). The highest moisture content observed in 10% PES.

There were no significant differences ($p<0.05$) in protein content between jelly candy containing phycobilioprotein extracted from *Spirulina platensis* in all concentration (2.5, 5, 7.5 and 10%) (25.32-27.09%) as it gave the highest concentration compared to the control (14.68%).

Children (2–3 years old, all genders) need 13 grammes of protein per day, whereas women (19–30 years old) need 46 grammes. Males aged (19–30 years old) need 56 g of protein per day in their everyday diets. (DRI, 2005). Each 100g of jelly candy with 10% phycobilioprotein extracted from *S. platensis* covered 208.38 for child, 58.89 % for female and 48.37% for male of daily protein intake.

No significant differences were found in the fat content of all jelly candy samples, which recorded2.12% in 2.5% PES compared to 3.57% in control. Fiber content increased significantly by adding phycobilioprotein with all concentration compared to control jelly candy (0.09%). Fiber content increased by increasing PES levels from 0.23% in jelly candy with 2.5% PhS to 0.43 Jelly candy with 10% PES. Jelly candy contain phycobilioprotein extracted from *S. platensis* gave ash content ranged from 0.69% to 1.88% that was higher than ash content in control jelly candy (0.47%). These results may be due to the high content of fiber and ash contents in *S. platensis*. Total carbohydrate decreased by increasing the concentration of phycobilioprotein extracted from S. platensis. The highest total carbohydrate content appeared in control jelly candy (81.19%), while the PES jelly candy at concentration 7.5 and 10% that was 68.39 and 67.68.These results agree with Bahlol, (2018) and (Paternina et al. 2022) whose found that, *S. platensis* led to increased protein, fat, fiber and ash contents while it decreased total carbohydrate.

Table 3. Chemical composition of jelly candy prepared with different levels of phycobiliprotein extracted from *S. platensis.*

Components %	Control	PES %			
		2.5	5	7.5	10
Moisture	30.66±1.0^{d}	35.82±1.00^{b}	36.43±1.0^{b}	36.90±1.0^{ab}	38.33±1.00^{a}
Protein	14.68±1.0^{e}	25.32±1.00a	26.24±1.0^{a}	26.55±1.0^{a}	27.09±1.00^{a}
Fat	3.57±1.00^{a}	2.12±1.00^{a}	2.14±1.00^{a}	2.87±1.00^{a}	2.92±1.00^{a}
Fiber	0.09±0.01^{f}	0.23±0.01^{c}	0.32±0.01^{b}	0.32±0.01^{b}	0.43±0.01^{a}
Ash	0.47±0.01^{b}	0.69±0.51^{b}	1.02±1.00^{ab}	1.87±1.00^{a}	1.88±1.00^{a}
Total carbohydrates	81.19±1.0^{a}	71.34±1.00^{e}	70.28±1.0^{e}	68.39±1.0^{f}	67.68±1.00^{f}

Data is presented as mean ± standard deviation. * denotes significance at p ≤ 0.05.PES = phycobiliprotein extracted *from Spirulina platensis*

3.4. Bioactive content of jelly candy prepared with phycobiliprotein extracted from *Spirulina platensis*

The phytochemicals in jelly candy, which include total polyphenols (mg GAE/g), total flavonoids (mg QE/g), vitamin C (mg/100 ML) and carotene (mg/100ML) were estimated in Table (4).

The results in this table illustrate that, there were significant differences of phytochemicals in jelly candy prepared with phycobiliprotein extracted from *S. platensis* compared to control jelly candy. Total poly phenols in jelly candy increased with increasing the level of phycobiliprotein dye addition, reaching to 0.20 mg GAE/g, in jelly candy prepared with 10% phycobiliprotein extracted from *S. platensis* (PES) compared to control (0.04 mg GAE/g). From the same table, samples containing phycobiliprotein extracted from *S. platensis* (PES) showed the highest values of total flavonoids, which ranged between 0.15 mg QE/g in 2.5% PES jelly candy and 0.27 mg QE/g 1n 10% PES jelly candy. The vitamin C content increased by adding the extracts from 6.40 mg/100 ML in the control to 17.06 mg/100 ML in the jelly candy with 10% PES which represented the highest values of vitamin C in all the jelly candy samples. Finally, it is clear from the same table that, the carotenoids increased with increasing the concentration of the added extracts compared to the control (0.87mg/100ML). The highest content of carotenoid observed in 10 % PhS jelly candy which gave 3.01 mg/100 ML. Similar results were obtained by (Abd El Baky et al.2015; Batista et al. 2017).

3.5. Sensory analysis of jelly candy prepared with different levels of phycobiliprotein extract from *Spirulina platensis* and *Nostoc linckia*

Sensory evaluation of jelly candy prepared with different levels of phycobiliprotein extract from S. platensis and control jelly candy with industrial color observed in Table (5). There were no significant differences ($p<0.05$) in consistency parameter between all jelly candy samples except 10 % PESwhich represent the lowest value of consistence (9.11) and 10% PES samples which represent the lowest value of consistence (7.89).

No significant differences observed of clarity in control and jelly candy contain phycobiliprotein extracted from *S. platensis* (PES) at all concentration. It is noticed from given data in the table that the jelly candy samples containing a concentration of 2.5 and 5 % PES were significantly in agreement with the control in sensory parameters of taste, flavor, color and General acceptance. Therefore, we conclude that phycobiliprotein dye can be added to the jelly candy up to a concentration of 5% without any change in taste, flavor, color and general acceptance. These results were agree with Abd El Baky et al. (2015) and Lucas et al. (2018) whose found that adding Spirulina to this food products was significantly acceptable as control for main sensory characteristics.

Table 4. phytochemicals content of jelly candy prepared with phycobiliprotein extracted from *S. Platensis.*

Phytochemicals	Control	PES %			
		2.5	5	7.5	10
Total polyphenols (mg GAE/g)	0.04±0.01^{g}	0.08±0.01^{cd}	0.09±0.01^{bc}	0.10±0.01^{b}	0.20±0.01^{a}
Total flavonoids (mg QE/g)	0.11±0.01^{f}	0.15±0.01^{d}	0.23±0.01^{c}	0.25±0.01^{b}	0.27±0.01^{a}
Vitamin C (mg/100 ML)	6.40±1.00^{cd}	8.12±1.00^{c}	11.32±1.0^{b}	15.40±1.00a	17.06±1.0^{a}
Carotene (mg/100ML)	0.87±0.01^{b}	1.14±1.00^{b}	1.22±1.00^{ab}	2.01±1.00^{ab}	3.01±1.00^{a}

Data is presented as mean ± standard deviation. * denotes significance at p ≤ 0.05.PES = phycobiliprotein extracted *from S. platensis*

Table 5. Sensory analysis of jelly candy prepared with different levels of phycobiliprotein extract from *S. platensis.*

Sensory properties	Control	PES %			
		2.5	5	7.5	10
Consistence	8.67±1.00^{ab}	8.22±1.09^{ab}	8.17±1.28^{ab}	8.00±1.50^{ab}	7.89±1.27^{b}
Clarity	8.89±0.93^{a}	8.89±0.42^{a}	8.56±0.58^{ab}	8.72±0.44^{ab}	8.67±0.50^{ab}
Taste	9.00±0.50^{a}	8.44±0.53^{ab}	8.61±0.49^{ab}	8.39±0.49^{ab}	8.22±0.67^{b}
Flavor	8.78±0.67^{a}	8.83±0.35^{a}	8.61±0.49^{a}	8.39±0.70^{ab}	8.39±0.78^{ab}
Color	8.56±0.88^{abc}	8.83±0.61^{ab}	8.56±0.85^{abc}	8.11±0.42^{bc}	8.06±0.64^{c}
General acceptance	8.78±0.51^{abc}	8.62±0.36^{abcd}	8.50±0.50^{bcd}	8.31±0.51^{cd}	8.28±0.55^{cd}

Data is presented as mean ± standard deviation. * denotes significance at p ≤ 0.05.PES = phycobiliprotein extracted *from S. platensis*

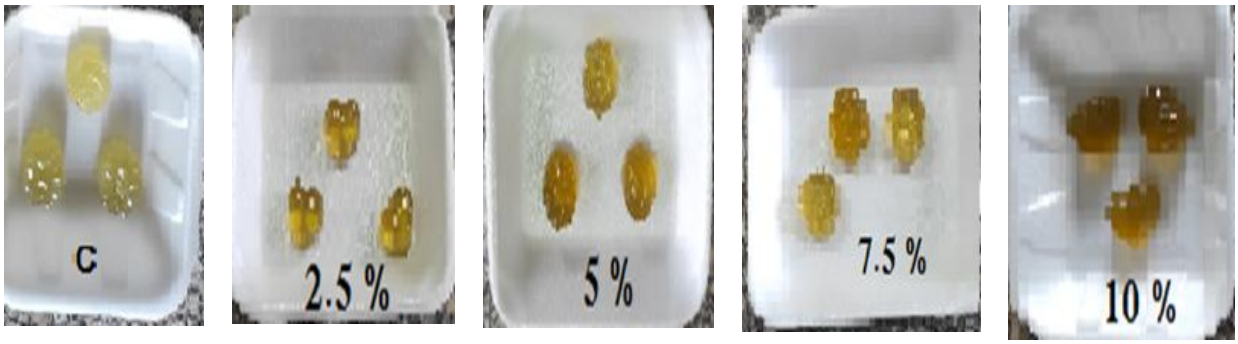

Fig.1 Jelly candy prepared with phycobiliprotein extracted from *Spirulina platensis* and control with industrial dye.

Conclusion

When compared to penicillin 10, phycobiliprotein from *S. platensis* demonstrated antioxidant, antibacterial, and antifungal activities. When the amino acids were estimated, It was shown that the proportion of all necessary amino acids was higher than the proportion of FAO-recognized amino acids. Also having bioactive components. As a result, we can draw the conclusion that phycobiliprotein dye can be used as a natural colorant in jelly candy. It was added at rates of 2.5, 5, 7.5, and 10%, and this improved the jelly candy's chemical composition, phytochemical compounds, and sensory qualities compared to the control, which contained a synthetic green colour.

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