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PARTIAL REPLACEMENT OF KOSARIC MEDIUM WITH BANANA LEAF ASH EXTRACT FOR THE CULTURE OF *SPIRULINA PLATENSIS*

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Abstract

The use of banana leaf ash extract (BLAE) as a replacement of Kosaric medium (KM) to grow spirulina (*Spirulina platensis*) was investigated. KM was partially replaced with more affordable and locally available BLAE at 0, 10, 30, 50, and 70% concentrations. The spirulina was grown for 18 days at room temperature in the experimental media with artificial lighting and aeration. By replacing KM with BLAE, the 30% replacement produced the highest biomass of *S. platensis*. It was observed that replacing of KM by 30% with BLAE produced biomass of 1.22 g/L, which was similar to that of 1.33 g/L with control ($P < 0.05$). However, replacing more than 50% KM by BLAE failed to promote growth relative to the control. Moreover, chlorophyll *a* concentrations in the control were found comparable in up to 30% of BLAE concentrations. Protein, lipid, and fiber contents of *S. platensis* were found to be 57.67 ± 0.58, 11.59 ± 0.28, and 7.35 ± 0.37%, respectively, in 30% replacement of KM, compared to corresponding values of 58.02 ± 1.16, 12.25 ± 0.39, and 7.53 ± 0.32%, respectively, in control KM. At the 50% KM replacement level, these values dropped to 52.65 ± 0.98, 9.97 ± 0.51, and 7.25 ± 0.29%, respectively. In conclusion, cultivation of *S. platensis* with BLAE up to 30% replacement of KM may be viable without undue effect on the plant's growth, pigmentation, or biochemical makeup.

Keywords: Spirulina, microalgae, Kosaric medium, chlorophyll, banana leaf ash.

Introduction

Spirulina platensis is a multicellular and filamentous cyanobacterium that may colonize in freshwater, brackish lakes, and certain marine settings, primarily alkaline saline lakes. It is a species of the group spirulina and a member of the phylum Cyanophyta (Vonshak, 1997). Its name is derived from the characteristics of its filaments, which are left-handed open helixes with cylindrical, multicellular trichomes (Jung *et al.,* 2019). This blue-green microalga is a potent source of protein (up to 70%), along with high amounts of essential fatty acids and amino acids, minerals, vitamins (particularly B_{12}), powerful water-soluble antioxidant (phycobiliproteins and carotenoids), and polysaccharides (Jaime-Ceballos *et al.*, 2006). Because of its high nutritional content, *S. platensis* is one of the most promising microalgae for culture. Due to its various features, it is currently one of the most investigated microalgae (Moraes *et al.,* 2011).

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Spirulina is being produced on a large scale for use in pharmaceuticals, animal feed, and food supplements for humans and other species. It is used as a feed additive in a variety of fish species to boost growth, feed efficiency, carcass quality, and disease resistance (Valente *et al.*, 2006; Ergun *et al*., 2008; Batista *et al.,* 2016; Sarr *et al.,* 2019). Additionally, it is the most abundant algal source of gamma-linolenic acid (GLA), a precursor to prostaglandins, a biologically active substance required for the development of the immune system in shrimp larvae (Habib *et al.*, 2008; Yuan-Kun *et al.*, 2003). The overwhelming evidence supports its potential medicinal use in addition to its high nutritional value (Belay *et al.,* 1993; Belay, 2002). Despite having immense nutritional value, research to explore the benefits of *S. platensis* and its potential as a culture organism still needs to be more conclusive.

Many factors influence the growth of *S. platensis* and the composition of biomass produced, the most important of which are nutrient availability, temperature, and light intensity (Cornet *et al.,* 1992). By contrast, Kosaric medium (KM) is the most commonly used medium for *S. platensis* culture (Phang and Chu, 1999). Although, KM gives higher growth, it requires expensive chemicals as a source of nutrients, which substantially raises the production cost (Akter *et al.,* 2019). Moreover, the mass culture cannot afford the chemicals utilized in KM. In order to produce *S. platensis* in large quantities, it is necessary to develop an alternate nutrient supply that is efficient, less expensive, and easily accessible. In this context, it is a challenge for researchers to develop a low-cost medium for the mass production of this microalga. As a result, efforts were made to identify a low-cost organic medium that contains the essential nutrients for *S. platensis* cultivation. By using inexpensive and easily accessible resources, the input costs for the commercial production of *S. platensis* can be decreased without impacting production efficiency. It has been demonstrated that decomposed organic and inorganic nutrients are reliable sources of nutrients for the cultivation of *S. platensis* (Habib *et al.,* 2019; Jain and Singh, 2013; Sharker *et al.*, 2007). It was found to be extremely cost-effective to modify the KM medium by substituting fertilizer grade MOP-K for potassium in commercial grade chemicals K_2SO_4 (Akter *et al.*, 2019).

Furthermore, *S. platensis* requires highly alkaline waters with a pH of 9-11 (Murugan and Rajesh, 2014). As a result, the successful cultivation of this species requires organic resources with high base metal content. One of the oldest and most popular commercial fruits in the world is the banana, which is a member of the Musaceae family (Kumar *et al.,* 2012). It is now grows in all tropical countries, with Brazil, India, the Philippines, Ecuador, Thailand, Indonesia, Mexico, Honduras, Columbia, and Panama being the main producers. In the year of 2018, almost 114.11 million metric tons of banana were produced worldwide (Chandru *et al.*, 2021). Toyub *et al.* (2005) evaluated the growth performance of *spirulina platensis* in banana leaf ash medium with added jackfruit seed powder and urea. High levels of base metals like potassium, sodium, calcium, and other elements are present in the banana plant *Musa sapientum* (Anhwange, 2008; Tiisekwa *et al.*, 1999). As a result, choosing BLAE as the culture medium for *S. platensis* is a reasonable

choice. Khatun *et a*l. (2019) observed that BLAE can effectively be utilized as source of $NaHCO_3$ and micronutrients for *S. platensis* culture. From their findings it is evident that there is a potentiality of using BLAE for culture of *S. platensis*. Therefore, the aim of the present study was to investigate the effects of replacing KM with BLAE on growth performance, pigmentation and nutrient composition of *S. platensis*.

Materials and Methods

Collection and maintenance of *S. platensis*

The mother culture of *S. platensis* was obtained from the department of Aquaculture, Bangladesh Agricultural University, Mymensingh. Following collection, the stock culture was maintained in KM in the Live Food Culture Laboratory of the Department of Aquaculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. On every alternate day, the growth of *S. platensis* was monitored, and its purity was examined under a microscope.

Preparation of KM

KM is the most widely used medium for *S. platensis* culture. Table 1 shows the composition of KM.

To prepare KM, the amounts of chemicals from no. 1 to 8 in Table 1 were weighed with the help of electric balance and taken into a 1.0 L conical flask. Then 0.5 mL of micronutrient solution was pipetted into the flask, and added distilled water to make the volume 1.0 L. optical.

Preparation of BLAE

The old-aged banana leaves were collected from the banana farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University. The leaves were then primarily sun-dried before being oven-dried overnight at 40 °C. Dried banana leaves were heated at 550 °C and burned for six h. After cooling, 100 g of dried banana leaf ash was dissolved in 1000 mL of sterilized distilled water (10% concentrated solution; wt./v) and left for

Table 1. Composition of KM for *S. platensis* culture

Serial No.	Chemicals/compounds	Concentration in the stock solution
1	$NaHCO_3$	9.00 g/L
2	K_2HPO_4	0.25 g/L
3	$NaNO_3$	1.25 g/L
4	K_2SO_4	0.50 g/L
5	NaCl	0.50 g/L
6	$MgSO_4.7H_2O$	0.10 g/L
7	$CaCl_2$	0.02 g/L
8	$FeSO_4.2H_2O$	0.005 g/L
9	Micronutrients solution*	0.5 ml/L

*Composition of micronutrient solution (g/L): i) H_3BO_3-2.86; ii) $MnCl_2.4H_2O$-1.81; iii) $ZnSO_4.7H_2O$-0.22; iv) $CuSO_4.5H_2O$-0.08; MoO_3-0.01; $CoCl_2.6H_2O$ -0.01.

seven days. After seven days, the solution was filtered twice: once with fine mesh to remove any remaining impurities and once with filter paper with a 0.45-micrometer pore size to get a clear solution. The extract was then thoroughly mixed and autoclaved at 121°C for 15 min. with moist heat and was used as a stock solution. For mineral determination, the banana leaf ash sample was digested in nitric acid-perchloric acid, and mineral analysis was performed utilizing techniques identical to Hossain and Furuichi (2000) using an atomic absorption spectrophotometer (PinAAcle 900F, Perkin Elmer, USA). The mineral composition of banana leaf ash is presented in Table 2.

Experimental culture of *S. platensis*

The experiment was conducted for 18 days in a completely randomized design, with five treatments, each with three replications. KM was used as a control medium and replaced at 10, 30, 50 and 70%, respectively, with BLAE. Before the initiation of *S. platensis* culture, the pH of all media was adjusted at 9.0 by incorporating either 0.1 N HCl or 0.1 N NaOH, depending on the pH condition of the selected medium. The culture was conducted in a series of Erlenmeyer flasks (1000 mL) containing only KM in control (0% BLAE) and four different concentrations of BLAE in the others (10%, 30%, 50% and 70% BLAE). Each culture flask received an inoculation of *S. platensis* to create a culture containing 10% suspension (optical density,OD at 620 nm=0.20) (Habib *et al*., 2008). The flasks were kept under fluorescent light (PHILIPS-CHAMPION, 36W, 1200mm, F-50Hz, Bangladesh) in light: dark (12h:12h) conditions and continuous aeration was provided using electric aerators (Sobo, Aquarium pump, SB-348A). The samples were taken from each flask at every three days interval to monitor cell dry weight, OD, chlorophyll *a* contents, and physico-chemical characteristics.

Table 2. Mineral composition of banana leaf ash

Sl. No.	Element	Value (mg/g)
1	Calcium	46.20
2	Phosphorus	3.10
3	Potassium	58.0
4	Sodium	71.0
5	Magnesium	0.63
6	Iron	0.41
7	Cupper	0.03
8	Manganese	0.41
9	Zinc	1.50

Measurement of optical density (OD)

Optical density was measured during sampling at 620 nm using a UV/Vis spectrophotometer (DR 6000, HACH). The samples of *S. platensis* grown in different treatments were taken in a cuvette and placed in the spectrophotometer and the OD of the samples was recorded.

Measurement of chlorophyll *a*

For the estimation of chlorophyll *a*, optical densities of the samples were determined at 664 nm, 647 nm and 630 nm using a UV/Vis spectrophotometer (DR 5000, HACH). A blank with 100% acetone was run simultaneously. Chlorophyll *a* content was calculated by the following formula (Clesceri *et al.*, 1989):

Chlorophyll *a* (mg/L) = 11.85 (OD 664) - 1.54 (OD 647) - 0.08 (OD 630).

Estimation of cell weight (dry basis)

The sample, which contained 50 ml of *S. platensis* suspension, was filtered through a filter paper (Whatman GF/C filter paper of 0.45 µm mesh size and 47 mm diameter) which was then dried in an oven for 24 h at 70°C and weighed prior to the filtration. When the sample was being filtered, it was washed with 20 ml acidified water (pH = 4) to remove insoluble salts. The filter papers were then placed in a glass Petri dish and heated to 70°C overnight in the oven. Filter papers were weighed after the Petri dish had been cooled for 20 min. in a desiccator. Then the dry weight of *S. platensis* cells was calculated as follows:

$$W = \frac{\text{FFW-IFW}}{\text{Amount of the sample taken for filtration (ml)}} \times 100$$

Where W= Cell dry weight in g/L; FFW= Final filter weight in g; and IFW= Initial filter weight in g.

Specific growth rate (SGR)

The specific growth rate (SGR, µ/day) of *S. platensis* was calculated by the following equation (Clesceri *et al.*, 1989).

$$SGR\ (\mu/day) = \ln (X_1 - X_2)/ t_2 - t_1$$

Where,

X_1 = Biomass concentration at the end of selected time interval,

X_2 = Biomass concentration at the beginning of selected time interval, and

t_2-t_1= Elapsed time between selected time in days.

Determination of physico-chemical properties of the culture media

The water temperature, dissolved oxygen (DO) and pH of the culture media were measured on the sampling day by a thermometer, a dissolved oxygen meter (HQ40d multi) and an electric pH meter (sensIONTM+ PH3), respectively. Light intensity (lux/m^2/s) was measured during sampling days using a lux-meter (LX-9621).

Statistical analysis

One-way analysis of variance (ANOVA) in a completely randomized design of mean of cell dry weight, chlorophyll *a* content, and optical density and specific growth rate of *S. platensis* cultured under different treatments was done to find out whether there was any significant difference among treatment means, while LSD test was used to compare the treatment means (Hofmann, 2008) by using Statistix 10 software.

Results and Discussion

Physico-chemical parameters

Environmental factors such as water temperature, dissolved oxygen, pH and light significantly affect microalgal growth (Khoeyi *et al.*, 2012). In the present study, the mean temperature values in different treatments during the experimental period ranged from 28.15 to 33.53 ºC (Table 3). On the day of sampling, no discernible difference in the temperatures of the various treatments was observed. According to Colla *et al*. (2007), 25–35 °C is the optimal temperature for spirulina cultivation. Torzillo and Vonshak (1994) reported that a temperature range of 28–35ºC was the best for the production of

Table 3. Temperature and dissolved oxygen content of the different culture media

Treatment		Parameters (range)	
		Temperature (°C)	Dissolved oxygen (mg/L)
T1	*KM	28.15±0.11- 33.22±0.20	3.83±0.35 - 4.47±0.15
T2	10%	28.71±0.10 - 33.22±0.10	3.53±0.21 - 4.57±0.15
T3	30%	28.19±0.22 - 33.53±0.20	3.67±0.15 - 4.33±0.06
T4	50%	28.25±0.11- 33.31±0.04	3.87±0.21 - 4.33±0.12
T5	70%	28.15±0.13- 33.22±0.09	3.43±0.15 - 4.23±0.15

*KM indicates Kosaric medium, and 10% to 70% indicate different concentrations of banana leaf ash extract. Values are presented as mean ± SE.

S. platensis. Kumar *et al.* (2011), in a study with *S. platensis*, found a maximum biomass concentration at 35 °C. These findings imply that the temperature range found in the current study was appropriate for the growth of *S. platensis*.

The range of DO levels for *S. platensis* culture in this study was 3.43 to 4.57 mg/L (Table 3). According to several studies, the ideal DO level for spirulina cultivation ranged from 3.1 to 5.5 mg/L (Chen and Lee, 2012; Rahman, 2005). Maintaining an ideal DO level never became a limiting issue in this experiment because artificial aeration was provided continuously.

The pH of the culture medium is the most crucial chemical component for growing *S. platensis*. The pH range in this investigation was varied from 9.0 to 10.62 in all the treatments, that was optimum for the growth of *S. platensis* (Fig. 1a). For *S. platensis* cultivation, it is best to maintain a pH greater than 9.5 in order to avoid contamination by other algae (Kebede and Ahlgren, 1996). The pH was adjusted to 9.0 initially, but as the culture days progressed, the bicarbonate-carbonate balance shifted in favor of carbonate, causing the pH to rise to 10.62. Finally, the pH started falling during the culture period's last phases. The decreasing trend of pH at the death phase might be due to dead cells and other organic loads. The increasing trend of pH up to the stationary phase favored the growth of *S. platensis.* Even though there were some differences of the pH levels in various treatments on the day of the sampling, high pH in all treatments favored the development of *S. platensis*. According to Joshi *et al*. (2013), *S. platensis* cultures should have a pH range between 8.0 to 10.0 for optimal growth, CO_2 absorption, and amino acid contents.

Microalgae require optimal lighting conditions for efficient photosynthesis (Baidya *et al.*, 2021). The light intensity recorded with different treatments was more or less similar during the culture period. The range of light intensity was 2200 to 2280 lux/m^2/s in the present study. Kebede and Ahlgren (1996) observed that *S. platensis* grown in modified Zarrouk's medium and exposed to a range of light intensities of 2000-2500 lux/m2/s showed a maximum specific growth rate. It is evident from the results of the present study that temperature, pH, dissolved oxygen, and

light intensity were within the suitable range for *S. platensis* culture.

Optical density (OD)

The mean values of optical density were 0.93±0.06, 0.87±0.02, 0.83±0.07, 0.79±0.01 and 0.72±0.01 mg/L in KM, and in 10, 30, 50 and 70% replacement, respectively (Fig. 1b) at day 15. The exponential phase was continued up to the 15th day from the beginning, and after the 15th day, the optical density started to decline. At the end of the experiment, maximum OD was found in KM and more or less similar OD was observed up to 30% replacement of KM with BLAE. However, 30% KM replacement might create a medium that was quite similar to that of KM and did not alter the culture medium constituent that made favorable growth conditions for *S. platensis*. These findings agree with the results reported by Akter *et al.* (2012), who studied the growth performance of *S. platensis* in papaya skin extract media, where maximum OD of 0.31 mg/L was observed in 0.6 g/L papaya skin extract medium. In another study, Alam (2005) observed the

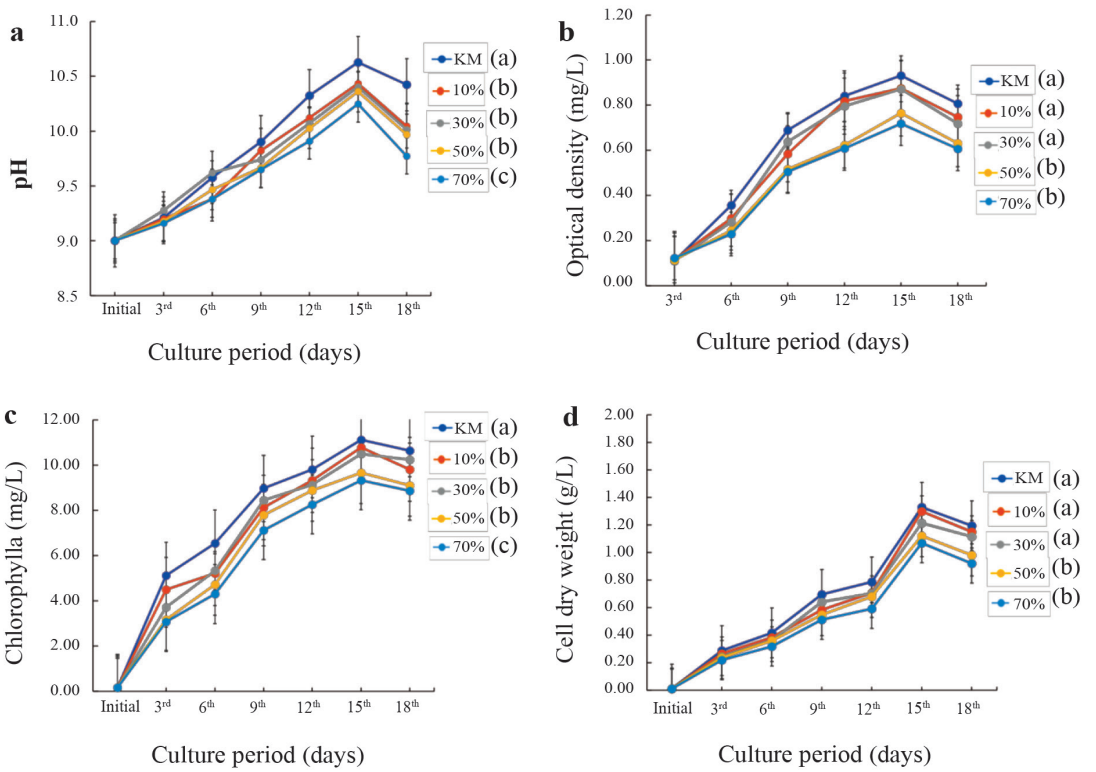

Fig. 1. Effects of KM and banana leaf ash extract at the concentration of 10-70% on (a) pH, (b) optical density, (c) chlorophyll *a*, and (d) cell dry weight of *S. platensis* up to the 18th day of the culture period. Vertical bars indicate the LSD value of different treatment variables (n=3). Different letters represent significant differences among the treatments (P< 0.05).

growth performance of *S. platensis* in different concentrations of fermented pond bottom soil in the laboratory and recorded the highest OD of 1.151 mg/L in KM and 0.991 mg/L in 120 g/L fermented pond bottom soil medium. The aforementioned research findings support the findings of the present study.

Chlorophyll *a*

The maximum chlorophyll *a* content of *S. platensis* (11.11 mg/L) was found in KM, whereas the lowest chlorophyll *a* content (9.32 mg/L) was in 70% KM replacement at the end of exponential phase, i.e., on 15th day (Fig. 1c). Furthermore, nearly similar results were observed in 30% concentrations of BLAE, indicating 30% KM can be replaced with BLAE without affecting the chlorophyll *a* concentration. The variation in chlorophyll *a* might have happened for the composition of various media and differences in nutrient concentration. Jabber (2005) observed that the chlorophyll *a* content of the inoculated *S. platensis* was 0.12 mg/L which attained a maximum of 11.01 mg/L with KM and 10.01 mg/L in 40% sesame meal medium. Parvin *et al.* (2008) observed a maximum of 13.95 mg/L of chlorophyll *a* content in KM and 13.50 mg/L in 25% digested pineapple juice medium on the 10th day of culture. In addition to have enough light and water, the plants need nitrogen, magnesium, iron, and manganese to generate chlorophyll. However, BLAE is a good source of the nutrients mentioned above (Table 2), which may be the reason *S. platensis* grown in various BLAE media has a significant amount of chlorophyll *a* (10.31 mg/L in 30% BLAE).

Cell dry weight

At the end of the exponential phase, the mean value of cell dry weight was found to be 1.33 g/L in KM, whereas 1.29 and 1.22 g/L in 10 and 30% KM replacement with BLAE, respectively (Fig. 1d). Cell dry weight in 10 and 30% concentrations of BLAE were almost similar to the cell dry weight found in KM which indicated that up to 30% KM could be replaced with BLAE (Fig. 1d). On the other hand, cell dry weight in 50 and 70% concentrations of BLAE were significantly lower than that of *S. platensis* cultured in KM ($P>0.05$), which indicated a further reduction of micronutrient in 50 and 70% media and reduced *S. platensis* production. It might be due to the differences in nutrients contents in the media.

To reduce the cost of *S. platensis* production, replacing nutrients in media with inexpensive ingredients is crucial. Rizal *et al.* (2017) were able to replace 50% sodium nitrate-nitrogen in KM with urea-nitrogen without compromising the growth. Toyub *et al.* (2005) found that the combination of 7.2 mg/L banana leaf ash, 0.4 mg/L jackfruit peel powder, and 0.2 g urea was ideal for the growth of *S. platensis*. Dey (2004) grew spirulina in mustard oil cake medium and found maximum growth at 0.5 mg/L concentration. Toyub *et al.* (2011) studied the growth performance of *S. platensis* in different concentrations of papaya (*Carica papaya*) skin powder media (PSPM), and maximum cell dry weight was found in control KM followed by 0.40 g/L of PSPM. Jain and Singh (2013) carried out an experiment to formulate a low-cost medium using different concentration gradients of cow dung ash medium (10-60%) and found 1.21

g/L dry biomass having 20% cow-dung ash supplemented with 80% prescribed medium. The results of above studies were consistent with the current findings, which point out the potential of BLAE comparing KM to reduce the cost of medium for *S. platensis* culture.

Specific growth rate (SGR) and proximate composition of *S. platensis*

The specific growth rate (SGR, µ/day) of *S. platensis* on the basis of biomass content was recorded in the range of 0.32 to 0.47 µ/day for all the treatments (Fig. 2). Although the SGR on the basis of biomass content was higher in KM among all the treatments, there were no significant differences of SGR in 0, 10 and 30% KM replacement ($P<0.05$). Toyub *et al.* (2005) cultured *S. platensis* in different concentrations of BLAE with 0.4 g/L jack fruit seed powder and 0.2 g/L urea and reported SGR ranging from 0.41 to 0.49 µ/day on the basis of chlorophyll *a,* which somehow agreed with the present findings. In another study, Toyub *et al.* (2011) found SGR of *S. platensis* on the basis of cell weight and chlorophyll *a* content in the ranges of 0.43 to 0.50 µ/day when cultured with papaya skin powder media. In another study, Khatun *et al.* (2019) reported that the growth rate and chlorophyll *a* content of *S. platensis* cultured with medium containing 50% sodium bicarbonate with BLAE was similar to that cultured in only KM, suggesting 50% $NaHCO_3$ replacement can be done without affecting the growth performance of this species. The outcomes of those studies were in line with those of the current study, which suggests the possibility of utilizing BLAE in KM to lower the price of medium for *S. platensis* culture.

Proximate compositions of cultured *S. platensis* were studied to know the nutritional values. The highest protein content of *S. platensis*

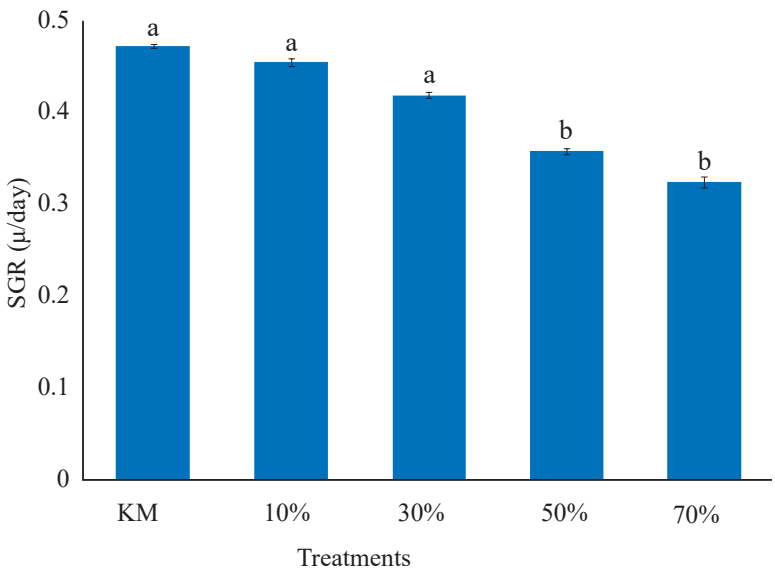

Fig. 2. Specific growth rate (µ/day) of *S. platensis* in different media prepared with partial KM replacement with BLAE. Vertical bars indicate the LSD value of different treatment variables (n=3). Means with different letters are significantly different ($P<0.05$).

(58.02%) was recorded in KM, followed by 57.87, 57.67, 52.65 and 51.40% protein in 10, 30, 50 and 70% BLAE medium, respectively (Table 4). In general, *S. platensis* contains a range of 50-70% protein (Colla *et al.*, 2007), depending mainly on the quality of nutrients present in the medium. In present study, the protein content of *S. platensis* up to 30% replacement of KM with BLAE was similar to the protein found in the control medium, indicating the presence of quality nutrients in BLAE for *S. platensis*. Further increase of BLAE in the medium decreased protein production. The lipid content of *S. platensis* up to 30% replacement of KM with BLAE gave better results (Table 4). Further increase of BLAE concentration in KM decreased lipid production of *S. platensis*. A similar trend was also found in the case of ash content (6.48 to 8.41%). The crude fiber content was recorded in the range of 7.14 to 7.84% in different treatments (Table 4). The maximum fiber content was found in 10% BLAE medium (Table 4). On the other hand, the moisture content was recorded in the range of 8.03 to 8.73% among all the treatments (Table 4). Although the lowest moisture content was found in a 30% BLAE medium, a trend of increased moisture content of *S. platensis* was observed with the increase of BLAE concentrations in KM. Toyub *et al.* (2011) recorded protein, lipid, ash, fiber and moisture content in the range of 51.49 to 58.42%, 10.44 to 12.25%, 6.46 to 8.51%, 7.04 to 7.83% and 8.83 to 8.12%, respectively in all the treatments those are almost similar to the findings of the present study. Ungsethaphand *et al.* (2009) studied the production of *S. platensis* using dry chicken manure supplemented with urea and sodium bicarbonate and noted 53.32% protein, 4.53% lipid, 20.77% carbohydrate, 11.22% moisture and 9.49% ash content. Soni *et al.* (2012) observed that the percentage of protein and carbohydrate contents in *S. platensis* were 59.00% and 15.95%, respectively. The findings of Toyub *et al.* (2011), Ungsethaphand *et al.* (2009), and Soni *et al.* (2012) are similar to those of the current study regarding the chemical composition of S. *platensis*. However, in present study, the cultured microalga was nutritionally rich, and the proximate composition of *S. platensis* was observed better up to 30% replacement of KM with BLAE. Therefore, as a nutrient source, BLAE can be used partially with KM for the culture of *S. platensis.*

Table 4. Proximate composition (%) of *S. platensis* cultured in different media

Treatment	Proximate composition (%)				
	Protein	Lipid	Fiber	Moisture	Ash
*KM	58.02±1.16^{a}	12.25 ±0.39^{a}	7.53±0.32^{ab}	8.03±0.24^{b}	8.41±0.40^{a}
10%	57.87±1.29^{a}	12.23±0.32^{a}	7.84±0.31^{a}	8.53±0.23^{ab}	8.29±0.32^{a}
30%	57.67±0.58^{a}	11.59±0.28^{ab}	7.35±0.37^{b}	8.17±0.24^{b}	7.41±0.31^{b}
50%	52.65±0.98^{b}	9.97±0.51^{c}	7.25±0.29^{b}	8.51±0.27^{ab}	7.33±0.37^{b}
70%	51.40±0.83^{c}	10.04±0.32^{c}	7.14±0.25^{b}	8.73±0.26^{a}	6.48±0.33^{c}

*KM indicates Kosaric medium, and 10 to 70% indicate different concentrations of banana leaf ash extract. Each value is presented as mean ±SE (n=3). Means within each column with different letters (a, b, and c) differ significantly ($P<0.05$).

In the present study, the growth rate and chlorophyll *a* content of *S. platensis* cultured in medium containing 30% BLAE were relatively similar to those of *S. platensis* cultured in KM; however, as BLAE is available at a very low-cost, the production cost of 30% BLAE supplemented media was significantly lower than that of KM. According to the current findings, replacing 30% of KM with BLAE may be feasible for *S. platensis* biomass production.

Conclusion

The high cost is the major concern to large-scale *S. platensis* production. Although KM gives optimum growth, it contains a higher amount of chemicals that significantly increase the cost. For scale-up economical production, either the expensive chemicals of commercial media need to be replaced with inexpensive ingredients or cheap alternative media with all necessary nutrients need to be developed. Banana leaf is a good source of base metal, high alkalinities and the presence of micro nutrients in BLAE that favor the growth of *S. platensis.* The partial replacement of KM medium with BLAE was possible in current study. The results of the present study led to the conclusion that BLAE could be used as a cheap source of nutrients for the cultivation of *S. platensis*.

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