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Isolation and Cultivation of Green Alga, *Pediastrum* spp. for Nutritional Value Study

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Green microalgae *Pediastrum* spp. from Chiang Mai Moat, Chiang Mai province, northern Thailand were isolated in Jaworski's medium (JM) at 25 ºC under continuous light. Dominant species in this study were selected for optimal study of media, pH and temperature. Cell density was determined spectrophotometrically at a wavelength of 665 nm, cell counts by whole counts method and biomass productivity as dry weight. It was found that *P. duplex*, *P. simplex* and *P. tetras* grew best in JM followed by the growth in Bold's Basal Medium (BBM) and Algal Medium (AM) media respectively. They grew better at pH 8.0 in JM and exhibited highest growth at room temperature. The protein content (36.45 ± 3.75 g/100 g) was highest in *P. duplex* and highest carbohydrate (43.86±1.75 g/100g) was found in *P. tetras*. Protein and carbohydrate were the major components in these algae which can be applied as food supplement in human and animal feed and pharmaceutical industries. Carbohydrate value was interesting as polysaccharide for sources of antioxidant. *Pediastrum* spp. have a large size which is easy for the cells to be harvested.

Keywords: Green microalgae, *Pediastrum*, Isolation, Cultivation, Nutritional Value.

Algae are the most important producer in the aquatic ecosystem. They are rich sources of carbohydrate, protein, enzyme and fiber. Besides, many vitamins and minerals e.g. vitamin A, C, B1, B2, B6, magnesium and calcium are abundantly found in algae [1]. Besides, it is environmentally friendly and can be effective in regulating global warming and climate change by controlling the level of pollution. One of the most promising strategies is using algae to mitigate the amount of carbon dioxide emitted into the atmosphere [2]. The coenobial freshwater algae, *Pediastrum* are members of the family Hydrodictyaceae which is placed within the order Chlorococcales of the Chlorophyta [3]. Their dominant characteristics are disc shape or stellate coenobia. Cell wall of *Pediastrum* is composed of thick inner layer of cellulose derivatives and the outer layer is composed of sporopolenin combined with silicon oxide which makes them highly resistant to decay [4]. Preliminary data indicated that *Pediastrum* spp. grow significantly faster than other algae and has a high protein content up to 46% [5]. There have been only a few previous studies on *Pediastrum* spp. in Thailand. In addition, the applications of *Pediastrum* spp. have not yet been fully studied due to lack of basic information. Study on the diversity, new and rare taxa of *Pediastrum* spp. in some freshwater resources in Thailand found 60 taxa consisting of 26 species and 22 taxa were newly recorded [6]. Three species, namely *Pediastrum duplex* var. *duplex* Meyen, *P. tetras* (Ehrenberg)

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Ralfs and *P. simplex* var. *simplex* Meyen were the dominant species. However, the conditions for culturing in laboratory have not yet been fully studied. The main objective of this research was to isolate and cultivate dominant species of *Pediastrum* spp. from Chiang Mai moat, Chiang Mai province of Thailand. Moreover, these dominant species were selected for optimal study of media, pH, temperature and nutritional analysis.

MATERIALS AND METHODS

Pediastrum spp. were collected by filtering 10 liters of water samples from sampling site in Chiang Mai moat, Chiang Mai province with 10 µm pore size plankton net. The samples were kept in a cool box for isolation and cultivation in the laboratory [7].

Isolation of *Pediastrum* spp.

Colonies of dominant species of *Pediastrum* spp. (*Pediastrum duplex*, *P. tetras* and *P. simplex*) in the water samples were studied under a microscope and single colonies were isolated with a glass micropipette. Each colony was washed at least five times with sterile medium and cultivated in the 12 multi well cell culture plate containing Jaworski's medium (JM). The alga was then purified by streak plate method on JM. Sub-culture was carried out until monoculture was obtained and transferred to JM broth for using as stock culture. The culture was incubated at 25 ºC under continuous 10.8 µmol.m^{-2}.s^{-1} illumination by florescent light[5].

Cultivation

Pediastrum spp. were cultivated in 3 media: algal broth (AM), bold basal medium (BBM) and Jaworski's medium (JM) for comparison and were washed with each sterile medium before being transferred to all media. They were cultivated at pH: 6.5, 7.0, 7.5 and 8.0 and finally incubated at 25 ºC and room temperature under continuous 10.8 µmol.m^{-2}.s^{-1} illumination with florescent light and aeration by bubbling air-line in 500 ml erlenmeyer flasks containing 300 ml of medium (Fig. 1).

Cell density was determined spectrophotometrically at 665 nm and cell counts by whole counts method [8]. When the growth reached the stationary phase, the cells were



Fig. 1. Cultivation of *P. duplex*, *P. simplex* and *P. tetras* in AM, BBM and JM media



Fig. 2. Cultivation of *P. duplex*, *P. simplex* and *P. tetras* in 20 L JM medium

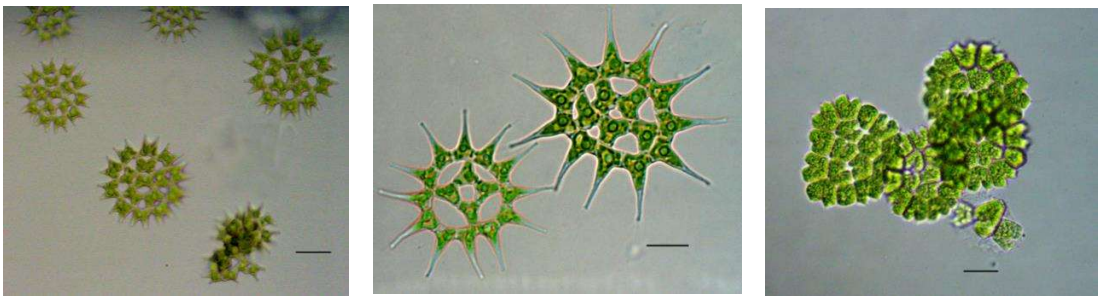


Fig. 3. *Pediastrum* spp. under compound microscope; (A) *P. duplex*, (B) *P. simplex* and (C) *P. tetras*. Scale bar =10 µm

harvested by centrifugation and dried at 60°C for 48 hr [9], and the biomass productivity (P) was calculated as maximum productivity (mg/L/d) [10].

$$P = 1000.\frac{(X1 - X0)}{(t1 - t0)}$$

X0 is the initial biomass (g/L) at time t0 (d)
X1 is the final biomass (g/L) at any time t1(d)

After the optimal condition for growth was identified, cultivation was scaled up to 20 L media (Fig. 2). Ten percent (V/V) of each stock culture was inoculated to 20 L of medium cultivated at room temperature with continuous illumination and aeration by bubbling air-line in plastic carboy tank. When the growth reached the stationary phase, the cells were harvested by sedimentation and siphoned supernatant away and centrifugation then dried at 60°C for 48 hr.

Nutritional analysis

At the end of cultivation, the biomass of the dominant species of *Pediastrum* were selected and subjected to nutritional analysis i.e. protein analysis by micro kjeldahl method [11], fatty acid analysis by acid hydrolysis method [11], fiber analysis by acid detergent method, ash analysis by burned at 450 °C 1 hour, carbohydrate analysis by calculating from

Carbohydrate (%) = 100 – (protein + fat + moisture + ash) [11]

Statistical analysis

Growth of *Pediastrum* in each experiment was analyzed as mean ± S.D. Statistical comparison between groups was determined by one way ANOVA followed by Tukey's post hoc test at $p<0.05$.

RESULTS AND DISCUSSIONS

Isolation and Cultivation of *Pediastrum* spp.

The dominant species of *Pediastrum* i.e. *P. duplex, P. simplex* and *P. tetras* were selected for optimal study on media, pH and temperature (Fig. 3).

Effect of media

The growth of the three dominant species of *Pediastrum* was compared in 3 media: Algal medium (AM), Bold basal medium (BBM) and Jaworski's medium (JM). Cell density was determined spectrophotometrically at a wavelength of 665 nm and cell counts by whole counts method. It was shown that *P. duplex, P. simplex* and *P. tetras* grew best in JM with OD_{665} at 0.87, 0.80 and 0.74 respectively with cell number of 94×10^6, 78×10^6 and 45×10^6 cell/mL respectively (Figs. 4 and 5). The

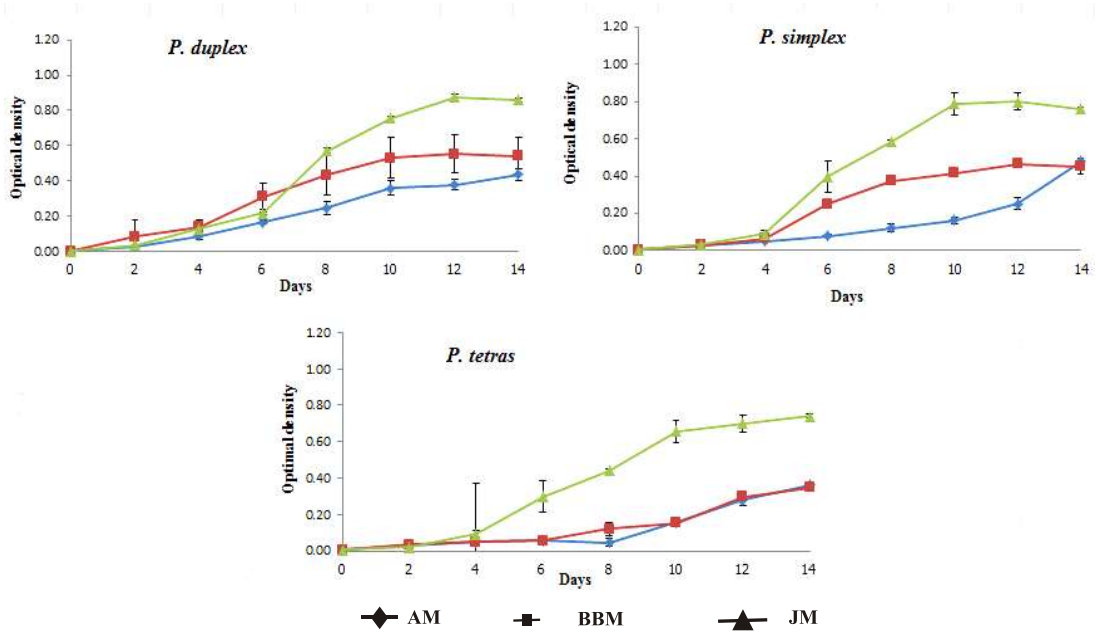


Fig. 4. Growth (optical density) of *P. duplex, P. simplex* and *P. tetras* in AM, BBM and JM

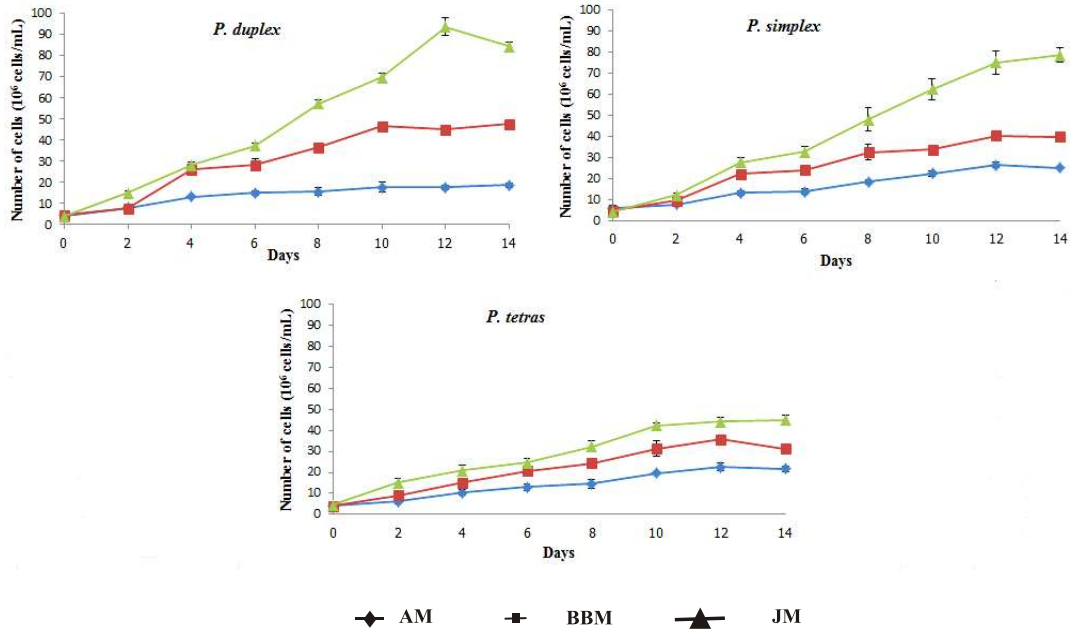


Fig. 5. Growth (cell number) of *P. duplex, P. simplex* and *P. tetras* in AM, BBM and JM

biomass productivity of the three species was studied using AM, BBM and JM. There was no significant difference ($p<0.05$) between the productivity in BBM and JM but significant difference ($p<0.05$) JM and AM (Fig. 6). Cultivation in JM and BBM gave higher growth than in AM because JM and BBM contain many macronutrients and micronutrients. Macronutrients are required in large quantities which are used generally as building materials [12]. Whereas, micronutrients comprise mainly of vitamins and minerals which are required in minute quantities. However, both macronutrients and micronutrients are essential for algal growth [13]. The elements required for the growth of green algae are N, P, K, Mg, Ca, S, Fe, Cu, Mn, and Zn. These elements are added in the form of salts [14]. These results are similar to the base data in natural water resource [6]. They were found in the meso-eutrophic status. Consequently, they had a tendency to be used in the assessment of water quality for the meso-eutrophic status. The two most important nutrients, nitrogen (N) and phosphorus (P) are derived from soluble forms such as nitrate nitrogen, ammonium nitrogen and soluble reactive phosphorus. These conditions promote the growth and reproduction of phytoplankton [14]. The lack of micronutrients in AM gave the lowest growth.

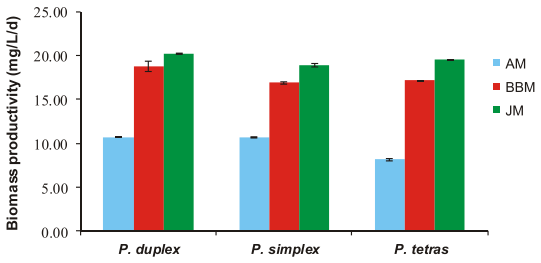


Fig. 6. Biomass productivity as dry weight (mg/L/d) of *P. duplex*, *P. simplex* and *P. tetras* in AM, BBM and JM

Effect of pH

The dominant species of *Pediastrum* were cultivated at different pH: 6.5, 7.0, 7.5 and 8.0. *P. duplex, P. simplex* and *P. tetras* exhibited highest growth at pH 8.0 in JM and highest OD_{665} at 0. 897, 0.715 and 0.870 with cell number at 87×10^6, 74×10^6 and 76×10^6 cell/mL, respectively (Figs. 7 and 8). The biomass productivity of *P. duplex, P. simplex and P. tetras* was significantly different ($p<0.05$) in the productivity between pH 8.0 and 6.5, 7.0, 7.5 but no significant difference ($p<0.05$) between pH

6.5, 7.0, 7.5 (Fig. 9). Most microalgal species favored neutral pH, whereas some species are tolerant to higher pH e.g. *Spirulina platensis* at pH 9 or lower pH e.g. *Chlorococcum littorale* at pH 4 [15]. The pH range for most cultured algal species is between 7 to 9 depending on the species and media [16]. pH is the major determinant of relative concentrations of carbon dioxide, carbonate and bicarbonate ions in water and could affect the availability of carbon for algal photosynthesis in intensive cultures [15]. These results are similar to the report that *Pediastrum* spp. were found at pH value around 8.0- 8.2 [6].

Effect of temperature

The dominant species of *Pediastrum* were cultivated at 25 °C and room temperature. The minimum temperature was 26.0-28.5 °C and maximum temperature was 29.0-33.0 °C. *P. duplex, P. simplex* and *P. tetras* were found to exhibit highest growth in JM at pH 8.0 at room temperature with OD_{665} at 0.87, 0.70 and 0.77 and highest cell number at 86×10^6, 74×10^6 and 77×10^6 cell/mL, respectively (Figs. 10 and 11). The biomass productivity of *P. duplex, P. simplex and P. tetras* in JM was studied at 25 °C and room temperature. There was significant difference ($p<0.05$) in the productivity between 25 °C and room temperature (Fig. 12). Temperature is an important factor for algal growth. It strongly influences cellular chemical composition, uptake of nutrients, carbon dioxide fixation and growth rate. It is known that the growth rate increases with the increase in temperature up to its optimum and decreases drastically. Most commonly cultured species of microalgae tolerate temperatures between 16 and 27°C. Temperatures lower than 16°C slow down the growth, whereas those higher than 35 °C are lethal for a number of species [17]. The optimum temperature for *Chlorella vulgaris* ranges from 25 to 30°C and for *Scenedesmus sp.* is between 20 and 40°C [18]. Thailand is situated in the tropical zone and the average temperature is 26.0-28.5 ºC which is suitable for algal growth [19]. The benefit of room temperature is that it is not controlled temperature which can reduce production costs.

Nutritional value of dominant species of *Pediastrum* spp.

At the end of cultivation, the biomass of the dominant species of *Pediastrum* were collected for nutritional analysis. The composition of protein in *P. duplex*, *P. simplex* and *P. tetras* was found to be in range of 30-36 g/100g. The protein content of

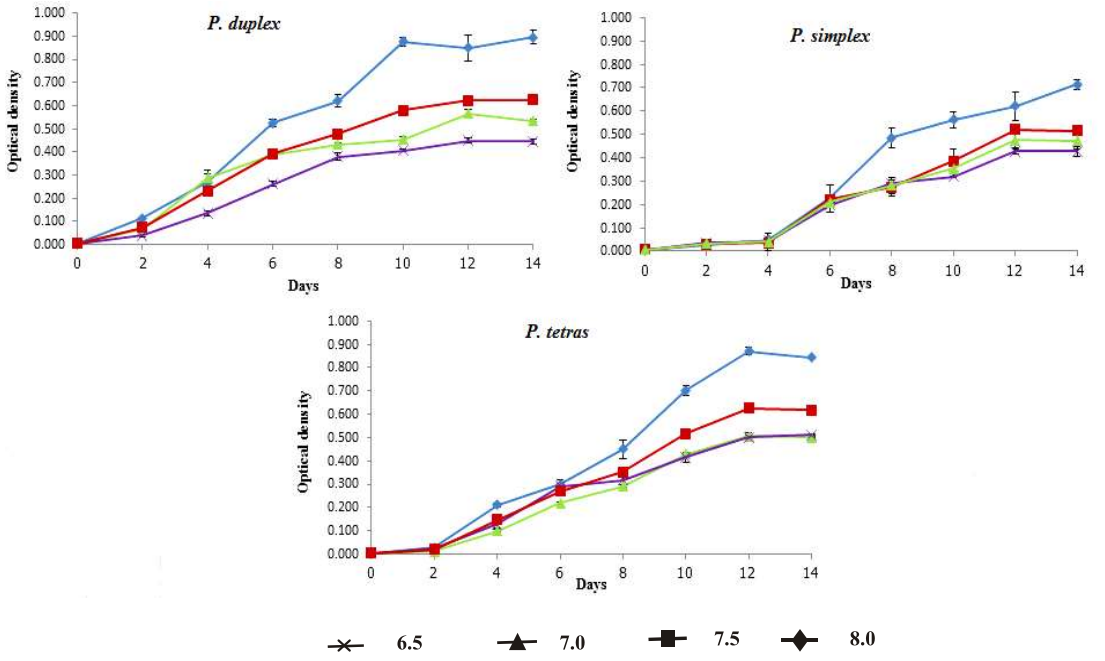


Fig. 7. Growth (optical density) of *P. duplex, P. simplex* and *P. tetras* in JM at different pH

P. duplex was higher than *P. tetras* but did not significantly differ from *P. simplex* ($p<0.05$). The Carbohydrate content of *P. tetras* was higher than *P. duplex and P. simplex (*$p<0.05$). The composition of fat, ash and moisture in *P. duplex, P. simplex and P. tetras* did not significant ($p<0.05$) (Table 1).

The composition of protein in *P. duplex* in this study was less than that previously reported by Lee *et al*. [5] i.e. 46.3 g/100g. However, carbohydrate, fat and moisture content in this study were higher than those previous reported i.e. carbohydrate 30.4 g/100g, lipid 2.4 g/100g and

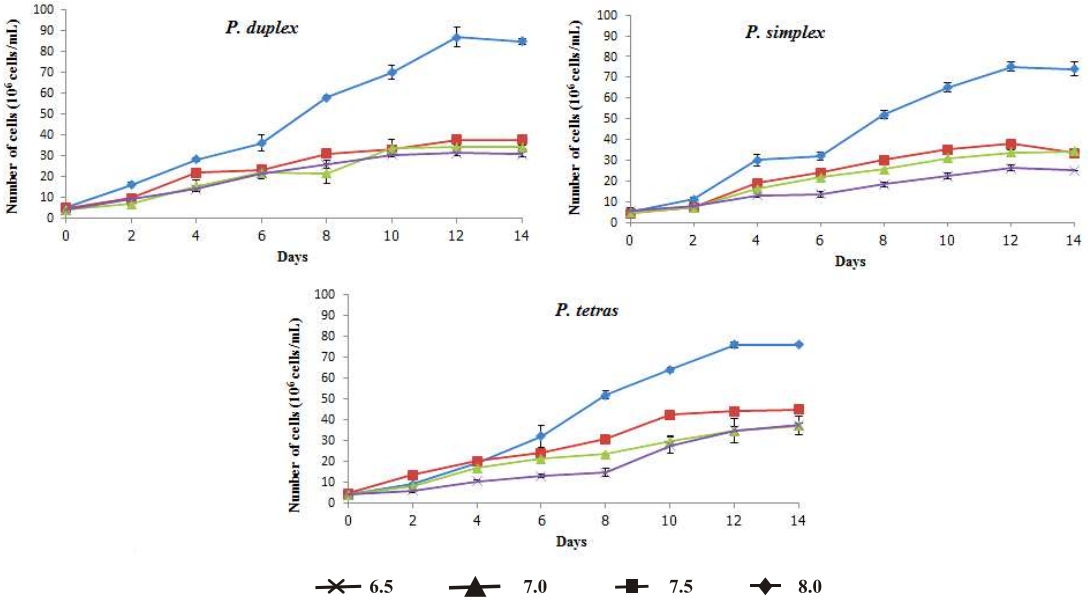


Fig. 8. Growth (cell number) of *P. duplex*, *P. simplex* and *P. tetras* in JM at different pH

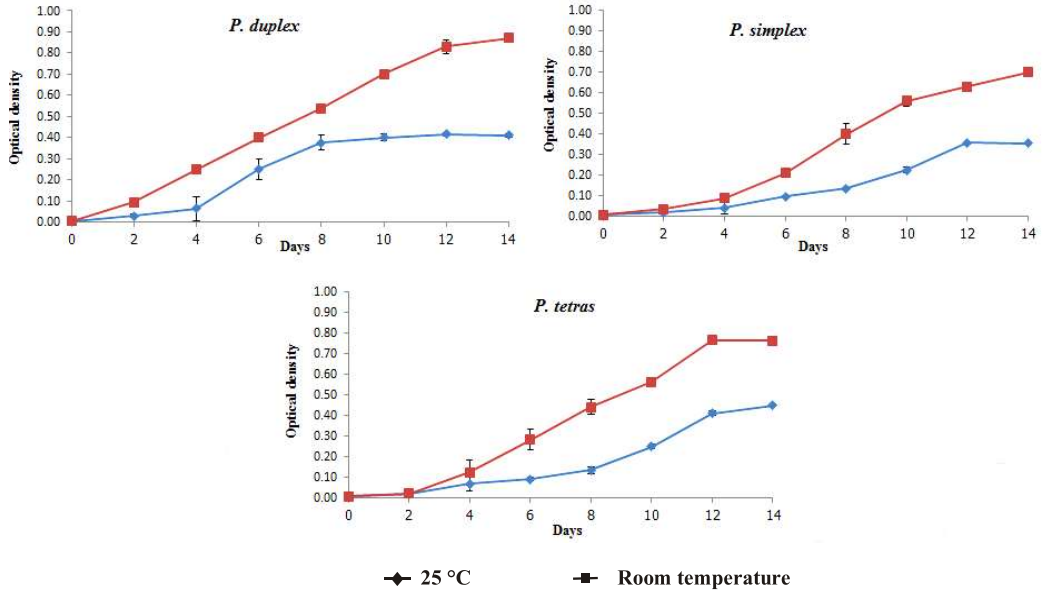


Fig. 9. Growth (optical density) of *P. duplex, P. simplex* and *P. tetras* in JM at different temperatures

moisture 6.1 g/100g due to the difference in culture condition and strain. Protein and carbohydrate were the major components in these algae that provide high protein close to that found in *Chlorella* sp. and *Spirulina* sp. They were known to provide higher protein than other natural food [4]. They can be applied as food supplement for human and animal feeds. Carbohydrate value was interesting as polysaccharide for sources of antioxidant which can be applied in food and pharmaceutical industries. Moreover, in this study, it was found that after inoculation of the three dominant species of *Pediastrum* spp. in fresh medium, the growth showed the lag phase less than five days. The maximum density was 15 times more cells than at the beginning when the culture was 12 days. Afterward the culture was relatively decline in agreement with Rojo *et al*. [8]. Moreover, cells of *Pediastrum* spp. have large size which was easy for harvest. So, they are suitable for cultivation and application in food and pharmaceutical industries.

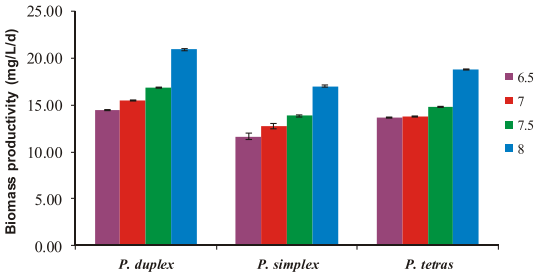


Fig. 10. Biomass productivity as dry weight (mg/L/d) of *P. duplex, P. simplex* and *P. tetras* in JM at different pH

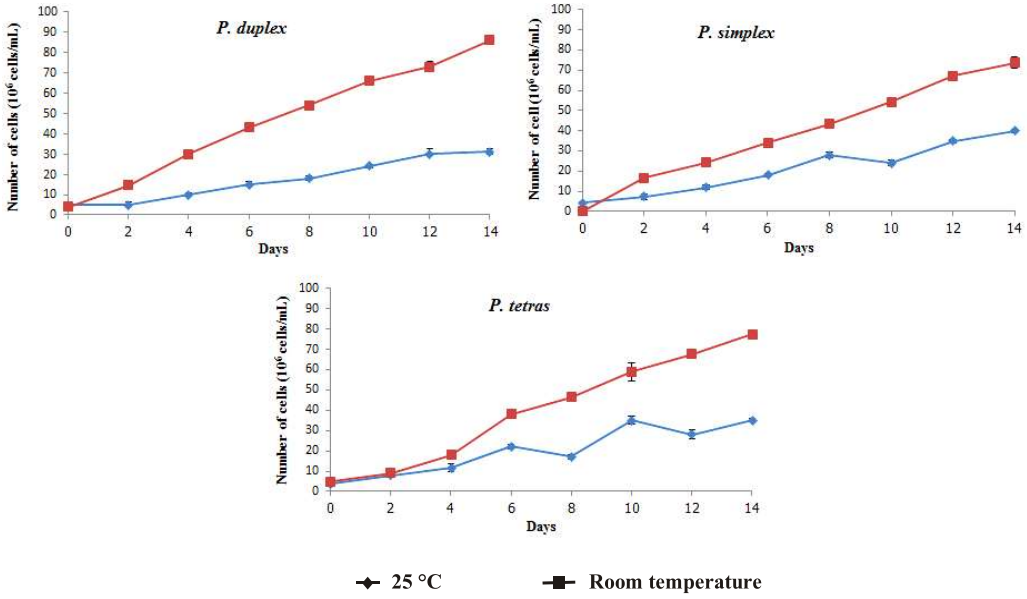


Fig. 11. Growth (cell number) of *P. duplex, P. simplex* and *P. tetras* in JM at different temperatures

Table 1. Proximate composition of dominant species of *Pediastrum* spp

Taxa	Protein g/100g	Carbohydrate g/100g	Fat g/100g	Ash g/100g	Moisture g/100g
P. duplex	36.45±3.75b	32.88±3.60b	14.06±2.89a	8.74±3.25a	7.87±3.35a
P. simplex	35.93±3.90b	34.41±2.55b	13.56±3.56a	8.73±4.14a	7.73±4.79a
P. tetras	30.44±2.35a	43.86±1.75a	10.29±2.98a	6.50±2.87a	8.91±4.02a

Data are expresses as the mean ± standard deviation (SD) of three replicates. Different letters represent the statistical comparisons between group in each column by using ANOVA and post hoc Tukey's b test ($p<0.05$)

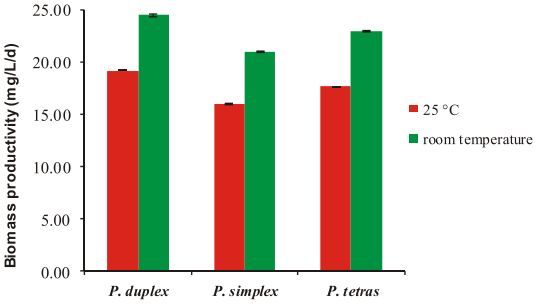


Fig. 12. Biomass productivity as dry weight (mg/L/d) of *P. duplex, P. simplex* and *P. tetras* in JM at different temperatures

CONCLUSIONS

Dominant species of *Pediastrum* spp. i.e. *P. duplex, P. simplex* and *P. tetras* in this study were selected for studying the optimal of media, pH and temperature. They grew best in JM followed by the growth in BBM and AM respectively. The three algae species grew better at pH 8.0 in JM and exhibited highest growth at room temperature as shown by optical density, cell number and biomass productivity in term of dry weight. The algae were cultivated and scaled up to get higher biomass for nutritional value determination. Protein and carbohydrate were the major components in these algae which can be applied as food supplement for human and animal feed.

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