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Pumpkin Rind and Seeds Extracts Inhibit α - Glucosidase and Nitric Oxide Production and Promote *Chlorella ellipsoidea* Growth in Culture

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Abstract

The objective of this work was to investigate the potential of pumpkin rind and seed on antidiabetic and anti-inflammatory activities and the underlying mechanism. Therefore, this work was carried out to determine the antidiabetic activity using inhibitory activities of pumpkin rind and seeds extracts on α - glucosidase and the anti-inflammatory activity via inhibitory activity on nitric oxide production. And also, the potential of the pumpkin rind and seeds on culture of *Chlorella ellipsoidea* was determined. Determination of inhibitory activity on α - glucosidase was performed using α - glucosidase assay, while the Griess assay was employed for the inhibition on Nitric oxide (NO) -production. The pumpkin seed extract inhibited α - glucosidase more potent than the pumpkin rind extract (IC_{50} of 321.36 vs. 326.18 µg/mL). However, the activities of the extracts were less potent than that of Acarbose (IC_{50} of 317.26 µg/mL). Application of the extracts at the highest concentration, 500 µg/mL, the pumpkin seed extract displayed the inhibition of NO production higher than the pumpkin rind extract did (18.01 ± 1.57 % vs. 14.99 ± 1.94 %). Study on the effects of pumpkin rind and seeds on *C. ellipsoidea* culture revealed that the optimum media was the 7th medium (NPK+ mixture of pumpkin seed water and Blue-Green Medium (BG-11), 1:4) which generated the growth of *C. ellipsoidea* for 28 days with the Optical Density (OD) value of 1.43 ± 0.01 followed by BG-11 medium OD value of 1.41 ± 0.02) and the 3rd medium (NPK+ mixture of pumpkin rind water and BG-11, 1:1, OD value of 1.32 ± 0.01), respectively. The chemical contents of *C. ellipsoidea* cultured in 7th medium contained 54.25 ± 0.06 % protein, 15.95 ± 0.87 % carbohydrates, 9.35 ± 0.05 % fat, and 20.30 ± 0.33 % ash, meanwhile 53.37 ± 0.77 % protein, 12.83 ± 0.62 % carbohydrates, 17.80 ± 0.23 % fat, and 23.30 ± 1.33 % ash in the 3rd medium. The results obtained from this investigation indicate that pumpkin rind and seeds not only can be exploited for the antidiabetic and anti-inflammatory components but also can be applied instead of the conventional medium for the culture of *C. ellipdoidea*. Therefore, bio-waste from pumpkin could be potentially utilized as the source of natural antidiabetic inhibitors, anti-inflammatory drugs and the effective components of *C. ellipdoidea* culture media.

Keywords: Pumpkin rind, Pumpkin seeds, α - Glucosidase, Nitric oxide, *Chlorella ellipsoidea*, Algae culture, Anti-inflammatory activities

Introduction

Pumpkin belonging to family Cucurbitaceae is a well-known edible plant, cultivated and abundantly used as herbal medicine and functional food [1]. It has been reported to be a rich source of nutrients, especially carotenoids and carbohydrates [2], a good source of potassium, vitamin C, folate, fiber, and numerous phytochemicals [3]. It is rich in carbohydrates, protein, crude fiber and crude oil and many unsaturated fatty acids [4], essential vitamins, and minerals [5]. Vitamins and minerals in pumpkin are clear in boosting immunity during and after the COVID-19 pandemic [6]. The fruit of pumpkin including the flesh, seed, and peel are a rich source of primary and secondary metabolites, including proteins, carbohydrates, monounsaturated fatty acids, polyunsaturated fatty acids, carotenoids, tocopherols, tryptophan, delta-7-sterols [7], total phenolic, total flavonoid, total carotenoid and mineral contents [1]. Fresh pumpkin sprouts are good sources of antioxidant components and contain amounts of phytonutrients [8]. Pumpkin leaves, flowers, seeds and pulp also contain antioxidant components [9]. Pumpkin is used as

a medicine for its anti-inflammatory, antioxidant, antiviral, and antidiabetic properties in many countries in Europe, Asia, and Africa [10]. Pumpkin is a rich source of nutrients, especially carotenoids and carbohydrates [11], potassium, vitamin C, folate, fiber, and numerous phytochemicals [12]. It is used as nutraceuticals and food supplements and folk medicines [13]. As it is rich in carbohydrates, protein, crude fiber and crude oil and many unsaturated fatty acids [4], therefore it has high potential application for nutraceuticals and food supplements, folk medicines and pharmaceutical industries [1,12,14].

Pumpkin waste, including rind (peel) and seeds cannot be used in human consumption, but it can be used as animal feed in livestock feeding. The studies regarding the use of pumpkin seeds as animal feed has been conducted in laying hens [15], broiler chickens [16], turkey [17] and lamb [18]. Pumpkin seeds exhibit antidiabetic and hypoglycemic activities [19]. Its seed oil exhibits protective effect against hepatotoxicity and nephrotoxicity in rats administered with high doses of Aspartame [20]. Although pumpkin seeds were considered a waste, their nutritional value might play a role in the food supply without any negative impact on human health [21]. Interestingly, pumpkin peel flour can be used at different replacement levels with wheat flour, to develop biscuits by improving physical and sensory characteristics [22].

Chlorella ellipsoidea (Gerneck) (Chlorophyceae), a microalga, is rich in nutrients especially protein, lipid and minerals [23], which possess high productivity and suitability for value-added products for animal feed supplements, biodiesel production, and assortment of natural products, thus it is often considered in microalgae cultivation [24]. Optimal culture medium has been found to be one of the important factors in the microalga culture system. The effective conventional media for *Chlorella* culture, such as Blue Green Medium (BG-11) [25], and Bold Basal Medium (BBM) are expensive. Some agro-industrial wastes, fertilizer factory waste and agriculture products have been used as an indigenous inexpensive ingredient for culture of microalgae instead of the conventional media.

Although the utilization of pumpkin is increasing and high nutritional value and some biological properties of the bio-waste from pumpkin rind and seeds has been documented. However, information on the antidiabetic and anti-inflammatory activities and also utilization of pumpkin rind and seeds is scanty. This study was, therefore undertaken to investigate the inhibitory activities of pumpkin rind and seeds extracts on α - glucosidase and nitric oxide (NO) production. And also, to see whether bio-waste from pumpkin can be utilized as the optimum substrate for cultivation of microalgae. Therefore, study utilizing the pumpkin rind and seeds in the *C. ellipsoidea* culture media was conducted.

Materials and methods

Preparation of ethanol plant extracts

Fresh ripe fruits of pumpkin (*Cucurbita moschata* Duchesne ex Poir) purchased from the local market in Maha Sarakham province, Northeastern Thailand were used in this work. The seeds were washed and peeled. The rind and seeds were isolated and oven dried at 50 °C for 3 days. After drying, they were grinded into fine powder. The powder sample was extracted using maceration method. The amount of 400 g of rind and seed powder was separately macerated in 1,000 mL of 80 % ethanol for 72 h at room temperature with occasional stirring. After maceration, the mixture was filtered using Whatman No. 1 filter paper [26]. The filtrate was concentrated using a rotary evaporator at 40 - 50 °C. The obtained extracts were kept in the airtight bottles and maintained at −20 °C until be used.

Determination on α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity was determined as described previously [27], with slight modifications. A volume of 60 µL of sample solution and 50 µL of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U/mL) was incubated in 96 well plates at 37 °C for 20 min. After pre-incubation, 50 µL of 5 mM p-nitrophenyl- α-D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37 °C for another 20 min. The reaction was terminated by adding 160 µL of 0.2 M $NaCO_3$ into each well. The absorbance readings were recorded at 405 nm using micro-plate reader and compared to control which had 60 µL of buffer solution in place of the extracts. Following formula is used to calculate percent inhibition:

$$\% \text{ Inhibition} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

Abs is absorbance/optical density (OD). Acarbose was used as a positive control of α-glucosidase inhibitor. The concentration of the extract required to inhibit 50 % of *α*-glucosidase activity under the assay conditions was defined as the IC_{50} value and determined graphically.

Determination on inhibition of nitric oxide production

Determination on inhibition of nitric oxide production was carried out to clarify the pumpkin rind and seeds can be used for the treatment of inflammations. Determination on inhibition of nitric oxide production was performed according to a previous study [27], with some modifications. The inhibitory activity of pumpkin rind and seeds extracts on NO production was investigated in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells. Since, NO is very unstable and rapidly oxidizes to nitrite (NO_2^-), thus the measurement of nitrite is used as an index of NO production. MTT assay was employed to reveal the concentrations of the extracts which produced cell viability of the RAW 264.7 cells more than 80 % and considered non-toxic to the cells.

The MTT assay, the RAW 264.7 cells (1×10^5 cells/well) were cultured in 96-well plate containing Dulbecco's modified Eagle's medium (DMEM), supplemented with 5 % fetal bovine serum and 1 % antibiotics penicillin-streptomycin. The plate was incubated for 24 h at 37 °C in a 5 % CO_2 incubator. After incubation, the cultured medium was removed and replaced with a fresh medium containing LPS (1 µg/mL). To accomplish this experiment, cells were simultaneously treated different concentrations of extract (25, 50, 100, 150, 200, 250, 500, 750 and 1,000 µg/mL) in a volume of 200 µL/well and incubated in a 5 % CO_2 incubator at 37 °C for 24 h. After incubation, the cultured medium was replaced with a fresh medium. Subsequently, a volume of 50 µL/well of 5 mg/mL MTT solution in PBS was added into each well and incubated at 37 °C for 24 h. The solutions in the plates were removed followed by the addition of 100 µL of DMSO. Finally, an absorbance was measured at 570 nm using the micro-plate reader. The experiments were carried out in triplicate. The percentage of cell viability is calculated as follows:

% Cell viability = $(Abs_{sample} / Abs_{negative\ control})\times100$, where $Abs_{negative\ control}$ is the absorbance of negative control (PBS) and Abs_{sample} is the absorbance of the extract.

Determination on nitrite concentration

The nitrite concentration was measured in the cell culture supernatant after the cells were treated with the different non-cytotoxic concentrations (25 - 800 µg/mL) of extracts using Griess assay [26]. The RAW 264.7 cells were cultured in DMEM medium with PBS in 96-well plate. LPS (1 µg/mL) was added to the plate prior to adding the extracts. Then the plate was incubated in a 5 % CO_2 incubator at 37 °C for 24 h. After incubation, the free cell cultured medium was transferred to a new plate followed by the addition of Griess reagent. The mixture was incubated at room temperature for 30 min. After incubation, the absorbance was measured at 570 nm using the microplate reader. The experiments were performed in triplicate. Diclofenac (20 µg/mL), a non-steroidal anti-inflammatory drug was used as a positive control. The percentage of NO production is calculated by using the following formula:

% NO production = $(Abs_{sample} / Abs_{negative\ control})\times100$.

Abs_{sample} is the absorbance of the extract. $Abs_{negative\ control}$ is the absorbance of negative control (PBS).

The potentials on culture of Chlorella ellipsoidea

Isolation and purification of C. Ellipsoidea

The *C. ellipsoidea* was isolated from natural freshwater collected nearby the campus of Mahasarakham University, Thailand. The isolated alga was subjected to purification by serial dilution followed by streaking and routine microscopic examination. Pure stock cultures were conducted and maintained routinely on both liquid and agar slants of BG-11 medium by regular sub-culturing at 15 days intervals. Pure stock cultures of *C. ellipsoidea* retained at the Department of Biology, Faculty of Science, Mahasarakham University were used.

Culture media and experimental conditions

Pumpkin rind and seed water were prepared by isolation the pumpkin rind and seeds from the fresh and clean ripe pumpkins. They were cleaned, minced and soaked in sterilized deionized water for 18 h at a ratio of 1:14 (w/v). After soaking period, the mixture was filtered using filter cloth to obtain the pumpkin rind and seed water.

Thirteen culture media for the culture of *C. ellipsoidea* were prepared by adding the mixture of pumpkin rind or seed water and Blue-Green Medium (BG-11) into the standard medium, inorganic fertilizer (0.6 g/L N-P-K, 15-15-15) [28]. The different ratios (1:4, 2:3, 1:1, 3:2, 4:1 and 5:0, v/v) of pumpkin rind or seed water and BG-11 medium were mixed. The 1st - 6th media, pumpkin rind water was mixed with BG-11 medium at the ratios of 1:4, 2:3, 1:1, 3:2, 4:1 and 5:0, respectively. The 7th - 12th media, pumpkin seed

water was mixed with BG-11 medium at the ratio of 1:4, 2:3, 1:1, 3:2, 4:1 and 5:0, respectively. The 13th was BG-11, the control medium [29]. The different cultural media are presented in **Table 1**.

The BG-11 medium contained 1.5 g $NaNO_3$, 0.04 g $K_2HPO_4.3H_2O$, 0.2 g $KH_2PO_4.3H_2O$, 0.001 g disodium EDTA, 0.001 g Fe ammonium citrate, 0.006 g citric acid, 0.02 g Na_2CO_3, and 1 mL of trace metal solution per liter, pH 7.3. The trace metal solution contained 2.85 g H_3BO_3, 1.8 g $MnCl_2.4H_2O$, 0.02 g $ZnSO_4.7H_2O$, 0.08 g, $CuSO_4.5H_2O$, 0.08 g $CoCl_2.6H_2O$, and 0.05 g $Na_2MoO_4.2H_2O$ per liter.

Table 1 The composition of the different media for the culture of *C. ellipsoidea*.

Culture media	Composition
1st	N-P-K+ pumpkin rind water and BG-11 mixture 1:4
2nd	N-P-K + pumpkin rind water and BG-11 mixture 2:3
3rd	N-P-K + pumpkin rind water and BG-11 mixture 1:1
4th	N-P-K + pumpkin rind water and BG-11 mixture 3:2
5th	N-P-K + pumpkin rind water and BG-11 mixture 4:1
6th	N-P-K + pumpkin rind water and BG-11 mixture 5:0
7th	N-P-K + pumpkin seed water and BG-11 mixture 1:4
8th	N-P-K + pumpkin seed water and BG-11 mixture 2:3
9th	N-P-K + pumpkin seed water and BG-11 mixture 1:1
10th	N-P-K + pumpkin seed water and BG-11 mixture 3:2
11th	N-P-K + pumpkin seed water and BG-11 mixture 4:1
12th	N-P-K + pumpkin seed water and BG-11 mixture 5:0
13th	BG-11

The cells of *C. ellipsoidea* with OD_{560} = 0.06 ± 0.001 were inoculated into each 200 mL Erlenmeyer flask containing the different culture media. The culture was conducted at a temperature of 28 ± 2 °C, continuous aeration under a light intensity of 3,500 - 4,000 lux in light: Dark (16:8) for a period of 28 days in the laboratory conditions [28,29].

The growth of *C. ellipsoidea* was monitored every 7 days by monitoring the culture absorbance at 560 nm (OD_{560}). The biomass *C. ellipsoidea* of was harvested and centrifuged at 7,000 rpm for 15 min to separate the microalga. After centrifugation, the separated microalga was washed twice with distilled water and dried at 80 °C for the analysis of proximate compositions [30,31].

The dried biomass of *C. ellipsoidea* was analyzed in triplicate for proximate compositions, specifically moisture, ash, proteins, fat, carbohydrates and dietary fiber using analysis methods of the Association of Official Analytical Chemists (AOAC) [32], with minor modifications. Moisture content was determined by drying the sample at 105 °C according to the AOAC method 925.10. Ash content was determined by incineration at 600 °C according to the AOAC method 942.05. Total nitrogen (N) was determined by the Kjeldahl method, AOAC method 950.36, and protein was calculated as total N×6.25. Fat content was determined using the Soxhlet apparatus with hexane, following AOAC 963.15 method. The carbohydrate content was calculated by difference: 100 – (fat + protein + crude fiber). Crude fiber content was determined using a fiber digester according to the AOAC method 973.18.

Statistical analysis

Data were calculated using the IBM SPSS Statistics software package and represented as means ± standard error means (SEM). Statistical difference between groups was calculated using a one-way analysis of variance (one-way ANOVA) followed by Duncan's New Multiple Range Test. The p-value < 0.05 was considered to be statistically significant.

Results and discussion

Determination on α-glucosidase inhibitory activity

The α-glucosidase inhibitory assay demonstrated that the pumpkin rind and seed extracts, 100, 200, 400 and 800 µg/mL, inhibited α-glucosidase in a concentration-dependent manner. At the highest concentration 800 µg/mL, the pumpkin seed extract showed a percentage α-glucosidase inhibitory activity of 80.24 ± 1.71 %, which was slightly higher than that of the pumpkin rind extract (79.89 ± 1.02 %). However, the inhibitory activity of the pumpkin rind and seed extracts was less than that of Acarbose (91.45 ± 1.34 %) as presented in **Table 2**. The result obtained from the α-glucosidase inhibitory assay in this study is similar to a study which was discovered that the polysaccharide extracted from *C. moshata* at concentrations of 0.7 - 0.9 mg/mL caused a significant inhibition of α-glucosidase [33]. Interestingly, raw pumpkin (*C. moschata*) leaf extracts showed higher *in vitro* α-glucosidase inhibitory effects than the cooked leaves [34], while raw and roasted pumpkin (*C. moschata*) seeds exhibit higher antidiabetic activity, via inhibition of α-glucosidase, than the raw seed extracts [35].

Table 2 α - Glucosidase inhibitory activity (% inhibition of α-glucosidase) of the pumpkin rind and seed extracts (Acarbose was tested as the positive control), n = 3.

Concentration (µg/mL)	% Inhibition of α-glucosidase		
	Pumpkin rind extract	Pumpkin seed extract	Acarbose
0	0	0	0
100	19.98 ± 1.64^{a}	21.28 ± 1.87^{a}	23.39 ± 1.16^{b}
200	43.56 ± 1.95^{a}	46.01 ± 1.94^{a}	48.09 ± 1.71^{b}
400	71.64 ± 1.13^{a}	72.19 ± 1.13^{a}	79.23 ± 1.97^{b}
800	79.89 ± 1.02^{a}	80.24 ± 1.71^{a}	83.45 ± 1.34^{b}
IC_{50}	326.18^{b}	321.36^{ab}	317.26^{a}

The values are expressed as mean ± SEM of 3 independent experiments a, b indicating the significant difference at *p*-value less than 0.05, between groups.

According to inhibition of α-glucosidase can inhibit the digestion and absorption of carbohydrates and causes a reduction in the rate of glucose absorption and consequently lowers postprandial blood glucose, thus α-glucosidase inhibitors can be utilized in the management or prevention of diabetes. In this study, the pumpkin rind and seed extracts inhibited α-glucosidase activity indicating the extracts possessed antidiabetic activity.

Pumpkins and their derivatives have been reported to have antidiabetic properties such as pumpkin seeds powder and oil can control blood sugar levels in diabetic rats [14], and ethanol extracts of *C. moschata* flesh and seeds exhibited significant antidiabetic activity in STZ-induced mice [33]. Nevertheless, determination on hemoglobin and glucose levels conducted in 24 male BALB/c mice by giving the methanol extracts of pumpkin seeds and skin from *C. moschata* for 14 days revealed that there is no difference in glucose levels [34].

Inhibition on NO production

The pumpkin rind and seed extracts were evaluated for the inhibition of NO production in the LPS-stimulated RAW 264.7 cells culture medium after the cells were activated by LPS. The NO production was measured as nitrite concentration. Prior to the measurement of nitrite concentration, the MTT assay was performed to verify for the noncytotoxic concentrations of the extracts and found that after exposure to the different extracts, the % viability of the LPS-stimulated RAW 264.7 about 24.76 cells was not different and was decreased with increasing concentration of the extracts. The extracts 25 up to 750 µg/mL showed noncytotoxic to the LPS-stimulated RAW 264.7 cells as shown in **Table 3**. Therefore, they were considered to be safe and used further for the determination of NO production.

Table 3 Cell viability of RAW 264.7 cells (%) after administration of various concentrations of the pumpkin rind and seed extract.

Concentrations (µg/mL)	% Cell viability	
	Pumpkin rind extract	Pumpkin seed extract
0	0	0
25	107.26 ± 1.82[a]	106.71 ± 1.06[a]
50	101.49 ± 1.61[a]	98.69 ± 1.87[a]
100	96.17 ± 2.01[a]	95.23 ± 1.66[a]
150	93.06 ± 2.12[a]	89.58 ± 1.59[a]
200	89.13 ± 2.05[a]	85.91 ± 2.01[a]
250	85.37 ± 1.42[a]	81.07 ± 1.84[a]
500	81.15 ± 2.14[a]	80.96 ± 2.09[a]
750	76.87 ± 1.76[a]	75.11 ± 1.57[a]
1,000	71.24 ± 2.02[a]	71.37 ± 1.42[a]

The values are expressed as mean ± SEM of 3 independent experiments. The values with different superscript letters [(a,b)] in the same row indicate the significant difference at *p*-value less than 0.05.

The determination of NO production revealed that the pumpkin rind and seed extracts failed to inhibit NO production in the LPS-stimulated RAW 264.7 cells when 25 and 50 µg/mL were administered. The inhibitory effects of the extracts on NO in LPS-stimulated RAW 264.7 cells were found when the pumpkin rind and seed extract 100 - 500 µg/mL were applied. The extracts inhibited NO production in the LPS-stimulated RAW 264.7 cells in a concentration-dependent manner. At the concentration of 500µg/mL, the pumpkin seed extract showed the % inhibition of NO production (18.01 ± 1.57 %) slightly higher than that the pumpkin rind extract did (14.99 ± 1.94 %). Diclofenac 20 µg/mL exhibited NO production inhibitory activity by 19.65 ± 1.72 % which was observed to be higher than that of the extracts (**Table 4**). The result of MTT cell viability assay revealed that the inhibitory effect of the pumpkin rind and seed extracts (25 - 500 µg/mL) on NO production was not due to the cell damage as the viability of the RAW 264.7 cells was higher than 80 %.

Table 4 Inhibitory effect of pumpkin rind extract, pumpkin seed extract and Diclofenac on the NO production in LPS-stimulated RAW 264.7 cells.

Concentrations (µg/mL)	Inhibition of NO production (%)		
	Pumpkin rind extract	Pumpkin seed extract	Diclofenac (20 µg/mL)
0	0	0	
25	NI	NI	
50	NI	NI	
100	2.97 ± 1.58[a]	3.12 ± 1.67[a]	19.65 ± 1.72[*]
150	6.75 ± 1.29[a]	7.24 ± 1.22[a]	
200	9.86 ± 1.38[a]	10.21 ± 1.81[a]	
250	12.87 ± 2.01[a]	13.06 ± 2.12[a]	
500	14.99 ± 1.94[a]	18.01 ± 1.57[b]	

The values are expressed as mean ± SEM of 3 independent experiments a, b indicate the significant difference at *p*-value less than 0.05, between groups. Note, [*]is the % inhibition on NO production of Diclofenac at 20 µg/mL.

Inflammation is associated with pathogenesis of various diseases. Application of synthetic anti-inflammatory drugs has been found to cause several side effects. The natural products with anti-inflammatory property could be considered as the safe biological sources. Pumpkin and its derivatives have been reported to possess pharmacological properties including anti-inflammatory property, such as pumpkin (*C. moschata*) skin polysaccharide-zinc (II) (PSP-Zn) complex exhibited a good anti-inflammatory activity in zebrafish larvae induced by copper sulphate [36]. Combined oral and topical application of pumpkin (*C. pepo* L.) attenuates inflammation [37].

According to the finding results, the pumpkin rind and seed extracts can be used for the alleviation of inflammation and to be a good candidate inhibitor for developing effective functional ingredient in the anti-inflammatory drug.

The potential on culture of C. *ellipsoidea*

The growth of C. ellipsoidea

The growth of *C. ellipsoidea* in different culture media monitored on day 7, 14, 21 and 28 revealed that the highest growth was found in the 7th medium followed by in the BG-11 medium and the 3rd medium, respectively, with the OD values of 0.45 ± 0.01, 0.68 ± 0.01, 1.00 ± 0.05 and 1.43 ± 0.01 for the 7th medium, 0.37 ± 0.00, 0.65 ± 0.00, 0.96 ± 0.01 and 1.41 ± 0.02 for the BG-11 medium and 0.23 ± 0.00, 0.67 ± 0.01, 0.95 ± 0.00 and 1.32 ± 0.0 for the 3rd medium. The growth of *C. ellipsoidea* was evidently better in the medium composed of pumpkin seed water than that of pumpkin rind water, indicating the growth performance is likely influenced by media type. The variation of production of *C. ellipsoidea* might be due to the composition of the broth [38]. However, the growth of *C. ellipsoidea* in 4 different media, viz, medium I (inorganic), medium II (organic, whole pulse powder extract), medium III (organic, whole lentil powder extract) and medium IV (organic, whole gram powder extract) were found not significantly different. These might be due to the algal culture media are similar quality [39]. Since, the growth of *C. ellipsoidea* cultured in the 7th and the 3rd medium was not different from that cultured in BG-11 medium, indicating the medium composed of pumpkin rind and seed water can be used instead of the BG-11 medium due to pumpkin rind and seeds contain high nutritive values as the chemical compositions of pumpkin seeds, including water, ash, fat, protein, and carbohydrate were 7.67 ± 0.05, 5.18 ± 0.04, 28.49 ± 0.04, 19.23 ± 0.06 and 39.51 ± 0.05 %, respectively, meanwhile they were 9.64 ± 0.08, 4.95 ± 0.07, 2.44 ± 0.07, 8.05 ± 0.04, 75.04 ± 0.08 %, respectively for pumpkin rind.

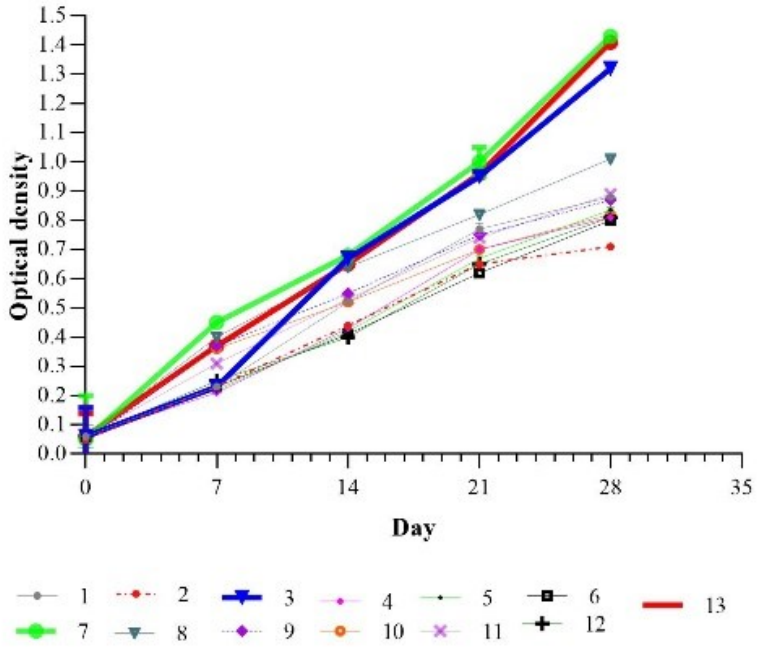

Figure 1 The growth of *C. ellipsoidea* in different cultural media. Error bars represent standard error of the mean (n = 5).

C. ellipsoidea cultured in the 7th medium was found to contain high levels of proteins, carbohydrates, fat, and ash compared to that cultured in the 3rd medium. The chemical contents of *C. ellipsoidea* were 54.25 ± 0.06 % protein, 15.95 ± 0.87 % carbohydrates, 9.35 ± 0.05 % fat and 20.30 ± 0.33 % ash for those cultured in the 7th medium, and 53.37 ± 0.77 % protein, 12.83 ± 0.62 % carbohydrates, 17.80 ± 0.23 % fat, and 23.30 ± 1.33 % ash for those cultured in the 3rd medium (**Table 5**). Therefore, the medium composed of pumpkin seed water could be potential utilized in enhancing the nutritional value of *C. ellipsoidea* better than the medium composed of pumpkin rind water. However, Uddin *et al.* [40], found that the amount of crude protein, crude lipids and ash of *C. ellipsoidea* grown in various concentrations of DWPP, TW and BBM varied from 23.4 - 48.11 %, 8.63 - 14.23 % and 2.66 - 4.05 %, respectively. The different amount of crude protein, crude lipids and ash of *C. ellipsoidea* found in Uddin *et al.* [40], and the present study may be influenced from the culture media.

Table 5 Proximate composition (% on dry weight) of *C. ellipsoidea* cultured in the 7th medium, the 3rd medium and BG-11 media.

Culture media	Proximate composition (% on dry weight)			
	Protein	**Carbohydrates**	**Fat**	**Ash**
The 7th medium	54.25 ± 0.06^{a}	15.95 ± 0.87^{b}	9.35 ± 0.05^{a}	20.30 ± 0.33^{a}
The 3rd medium	53.37 ± 0.77^{a}	12.83 ± 0.62^{a}	17.80 ± 0.23^{c}	23.30 ± 1.33^{b}
The BG-11 medium	53.76 ± 0.26^{a}	14.54 ± 0.17^{b}	11.48 ± 0.24^{b}	22.65 ± 0.51^{ab}

The values are expressed as mean ± SEM of 3 independent experiments. The values with different superscripts $^{(a,b,c)}$ in the same column indicate the statistical difference at *p*-value less than 0.05. The 7th medium = N-P-K +mixture of pumpkin seed water and BG-11 mixture 1:4, the 3rd medium = N-P-K +mixture of pumpkin rind water and BG-11 mixture 1:1 and the BG-11 medium = conventional medium, as a control medium.

Conclusions

The present findings demonstrate that the pumpkin rind and seeds possess antidiabetic and anti-inflammatory properties and they can be applied for the C. *ellipdoidea* culture medium. Further work, the active compounds which are responsible for these activities should be investigated.

Acknowledgments

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