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Phytochemical Compositions, Nutritional Contents, Cytotoxicity and Anti-Inflammatory Activity of Different Extracts from *Spirogyra neglecta* (Hassall) Kützing

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Abstract

Spirogyra neglecta (Hassall) Kützing is an edible freshwater macroalga. It is available and consumed as local food in Thailand, especially in the northern and northeastern parts. To ascertain its good benefit for health and safe for consumption, this study was, therefore carried out to determine the phytochemical compositions, nutritional contents, cytotoxicity, and anti-inflammatory activity of the different extracts from *S. neglecta*. The extracts were prepared by using different solvents, such as propylene glycol, methanol, and ethanol solutions. GC-MS analysis revealed the presence of 30, 39 and 36 phytochemical compounds in propylene glycol, methanol, and ethanol extracts, respectively. Phytol, which has been reported to have anti-inflammatory activity, was one of the predominant compositions in the extracts. The extracts contained high nutritional contents of protein, lipid, fiber, ash, moisture, and carbohydrate up to 21.68 ± 4.52, 3.53 ± 0.23, 14.77 ± 0.89, 10.73 ± 0.47, 9.79 ± 0.11 and 47.36 ± 3.89 %, respectively. The highest total protein was found in propylene glycol extract. The highest total lipid was in ethanol extract. Meanwhile, the highest fiber, ash and total carbohydrate were in methanol extract. In addition, all the extracts possess relatively low cytotoxicity, with IC_{50} values greater than 1,000 µg/mL. The extracts 1,000 µg/mL exhibited anti-inflammatory activity via inhibition Nitric oxide (NO) production by 29.87 ± 1.56 to 55.17 ± 1.91 %. The propylene glycol extracts from *S. neglecta* showed the highest inhibitory activity on NO production compared to others.

The overall results indicating the propylene glycol is the solvent that produces the best extraction performance. The extracts from *S. neglecta* exhibit anti-inflammatory activity by inhibiting NO production. The phytochemical compositions are partly responsible for anti-inflammatory activity of the extracts. The high nutritional contents complement relatively low toxic. Thus, *S. neglecta* can be used as benefit natural sources for food products, supplements, and medicinal plants.

Keywords: *Spirogyra neglecta*, Phytochemicals, Antioxidant, Muscle relief, Nutritional contents, Cytotoxicity, Anti-inflammatory activity

Introduction

Spirogyra neglecta (Hassall) Kützing is one of edible freshwater green macroalgae in the family Zygnemataceae. It has been widely consumed as local food in Thailand since ancient times, principally in northern and northeastern areas. The favorite consumption of this alga might due to its excellent nutritional properties. Its extract has been found to possess several activities/properties, including pancreatic cholesterol esterase activity [1], cancer chemopreventive [2], antioxidant, renoprotective [3], antidiabetic [4], immunomodulatory [5], hypolipidemic and hypoglycemic [6] activities. Moreover, the mixture of *S. neglecta* extract and *R. hieroglyphicum* extract possess antioxidative stress effect [7].

Attention focused on the utilization of the edible macroalgae as food products, supplements and medicinal plants is increasing. This study was attempted to verify that *S. neglecta* can provide a beneficial natural source for health and is safe for applications. Therefore, determinations of phytochemicals using Gas chromatography-mass spectrometry (GC-MS) analysis, nutritional contents using the standard methods of the Association of Official Analytical Chemists (AOAC) [9], and the method using by Crampton and Harris [10], cytotoxicity using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay,

and anti-inflammatory activity using the measurement of nitric oxide (NO) production by measuring nitrite concentrations in the cultured medium *via* Griess assay of the propylene glycol, methanol, and ethanol extracts from *S. neglecta* were carried out.

Materials and methods

Preparation of algal extracts

Spirogyra neglecta (Hassall) Kützing was collected from natural freshwater areas in Sakon Nakhon Province, Thailand during August - September 2021. The collected alga was cleaned and dried in shade followed by in hot air oven at the temperature not exceed to 40 °C. Dried samples were mechanically grinded into a coarse powder to facilitate extraction. The extraction was performed using maceration process with different extracting solvents, specifically propylene glycol, methanol and ethanol. The amount of 400 g powder was added into 1,000 mL solvent solution and allowed to stand for 7 days at room temperature with occasional stirring. After maceration, the mixtures were filtered using filter paper, Whatman No. 1. The filtrates were evaporated, concentrated using Rotary evaporator at a temperature not exceeding 60 °C and further dried at 50 °C in a hot air oven. The obtained extracts were stored at −20 °C until be used.

Determination of phytochemical compositions

The phytochemical compositions of the extracts were determined using Gas chromatography-mass spectrometry (GC-MS) analysis. In this study, a BRUKER, 450 GC gas chromatography instrument coupled to a BRUKER, 320 MS mass spectrometer was employed. The instrument utilizing was performed with following conditions viz. the Rtx-5 MS capillary column (30 m×0.25 mm, fused silica 0.25 µm) with inlet temperature and the spit ratio of 250 °C and 1:5, the initial oven temperature at 45 °C held for 2 min before ramping up to 250 at 7 °C/min, and maintained at 250 °C for 25 min. High purity helium (99.99 %) was used as carrier gas at a flow rate of 1.0 mL/min. The mass spectrometer was operated in electron impact (EI) mode with the ionization energy of 70 eV with acquisition mode scan of mass 45 - 500 amu. The temperature of the ion source and transfer line was maintained at 250 °C. The GC-MS was carried out by injecting 2 µL of the extracts in 50 mg/mL ethanol. The chemical compounds in the extracts were identified by comparison with retention times of standards, and the mass spectra obtained were compared with those recorded in the National Institute of Standards and Technology (NIST) library (NIST11-Mass Spectral Library, 2011 version) with an acceptance criterion of a match above a critical factor of 80 % according to Musharraf *et al.* [8].

Determination of nutritional contents

The nutritional contents of extracts from *S. neglecta* were determined using the following methods. Protein, lipid, fiber, moisture and ash were determined in accordance with the standard methods of the Association of Official Analytical Chemists [9]. Carbohydrate was determined according to the method of Crampton and Harris [10]. The determinations were carried out in triplicates at the Department of Biology, Faculty of Science, Mahasarakham University, Thailand.

Determination of cytotoxicity

The determination of cytotoxicity of the extracts from *S. neglecta* was carried out in the RAW 264.7 macrophage cells using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) 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, and incubated for 24 h at 37 °C in a 5 % CO_2 incubator. After incubation, the cultured medium was replaced with a fresh medium containing LPS (1 µg/mL). The cells were treated with the tested extract solution (25 - 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 and the formazan was solubilized by adding 100 µL of DMSO. Finally, an absorbance of the solubilized formazan was measured. The cell viability was determined by absorbance reading at 570 nm. The percentage cell viability is calculated using the following equation;

$$\% \text{ Cell viability} = (\text{Abs}_{\text{sample}} / \text{Abs}_{\text{negative control}}) \times 100,$$

where Abs $_{\text{negative control}}$ is the absorbance of negative control (PBS) and Abs $_{\text{sample}}$ is the absorbance of the sample (extract from *S. neglecta*). The cytotoxicity of the extracts was calculated by subtraction of background control value from each absorbance, using the following equation, and expressed as % cytotoxicity;

% Cytotoxicity = [100×(control - sample)]

Determination of anti-inflammatory activity

Nitric oxide (NO) is considered as a pro-inflammatory mediator that induces inflammation [11]. It is very unstable and rapidly oxidizes to nitrite (NO_2^-), thus the measurement of nitrite is used as an index of NO production. In this study, the anti-inflammatory activity of the extracts from *S. neglecta* was determined by measuring nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells. The measurement of NO production was performed by measuring nitrite concentrations in the cultured medium using Griess assay, a technique commonly used to quantify NO [12]. The basic reaction involves reacting of the Griess reagent, consisting of sulphaniliamide and N-1-napthylethylenediamine dihydrochloride (NED), to form a stable azo compound.

Base on the results in **Table 6**, the extracts 25 - 1,000 µg/mL produced > 80 % cell viability of the RAW 246.7 cells were used in the measurement of nitrite concentration. The RAW 264.7 cells were cultured in DMEM medium with PBS in 96-well plate. LPS (1 µg/mL) was added to each well prior to adding the extracts. The plate was incubated in a 5 % CO_2 incubator at 37 °C for 24 h. After incubation, the cultured medium was collected and transferred to a new plate followed by the addition of Griess reagent. Absorbance was measured at 570 nm using the micro-plate reader. The experiments were performed in triplicate. The percentage of NO production is calculated using the following equation;

% NO production = (Abs $_{\text{sample}}$ / Abs $_{\text{negative control}}$)×100,

where Abs $_{\text{negative control}}$ is the absorbance of negative control (PBS) and Abs $_{\text{sample}}$ is the absorbance of the sample (extract). Diclofenac (20 µg/mL), a non-steroidal anti-inflammatory drug (NSAID) was used as a positive control.

Statistical analysis

Data were calculated using IBM SPSS Statistics software package and represented as means ± standard error means (SEM). Statistical difference between groups was calculated using 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

GC-MS analysis

The GC-MS analysis revealed that the phytochemical compositions were found to be different among the different extracts. The GC-MS chromatograms of the different extracts from *S. neglecta* are depicted in **Figures 1** - **3**. They confirm the presence of various phytochemical compounds with different retention times. The compounds were identified tentatively based on the structure and molecular mass with the degree of similarity. They are also identified based on the compound structure reported in the previous reports with characteristic fragmentation patterns using a Mass Bank database. The retention time (RT), compound name, % area, and % probability of 5 predominant compounds constituted the different extracts from *S. neglecta* are tabulated in **Tables 1** - **3**.

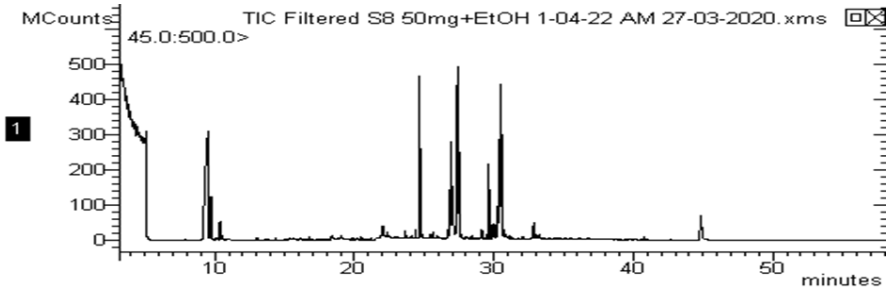

Figure 1 GC-MS chromatogram of propylene glycol extract from *S. neglecta*.

The GC-MS analysis detected 30 compounds identified in the propylene glycol extract. Five predominant compounds in this extract are 2-Propanol, 1,1'-oxybis - (24.57 %), 9,12,15-Octadecatrienal (13.96 %), Octadecanoic acid, 3-hydroxypropyl ester (13.14 %), Phytol (9.65 %), and Methyl (Z)-5,11,14,17-eicosatetraenoate (6.38 %) as presented in **Figure 1** and **Table 1**.

Table 1 Retention time (RT), compound name, % area and % probability of 5 predominant compounds in propylene glycol extract from *S. neglecta* using GC-MS analysis.

No.	RT (min)	Compound name	% Area	% Probability
1	9.51	2-Propanol, 1,1'-oxybis-	24.57	91.10
2	24.68	Phytol	9.65	83.40
3	27.39	Octadecanoic acid, 3-hydroxypropyl ester	13.14	34.80
4	27.49	Glycerol alpha-palmitate	5.92	44.80
5	30.47	9,12,15-Octadecatrienal	13.96	12.70

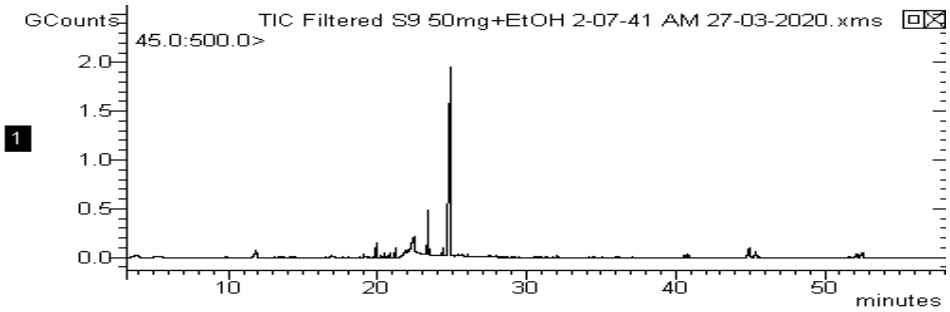

Figure 2 GC-MS chromatogram of methanol extract from *S. neglecta*.

Using GC-MS, 39 compounds were detected in the methanol extract. Five predominant compounds in this extract are Phytol (55.32 %), n-Hexadecanoic acid (15.66 %), Arachidonic acid (5.31 %), Linolenic acid (3.99 %), and gamma-Sitosterol (2.85 %) as shown in **Figure 2** and **Table 2**.

Table 2 Retention time (RT), compound name, % area and % probability of 5 predominant compounds in methanol extract from *S. neglecta* using GC-MS analysis.

No.	RT (min)	Compound name	% Area	% Probability
1	21.92	Linolenic acid	3.99	32.60
2	22.40	n-Hexadecanoic acid	15.66	71.80
3	23.38	Arachidonic acid	5.31	19.00
4	24.88	Phytol	55.32	85.40
5	44.91	gamma-Sitosterol	2.85	69.50

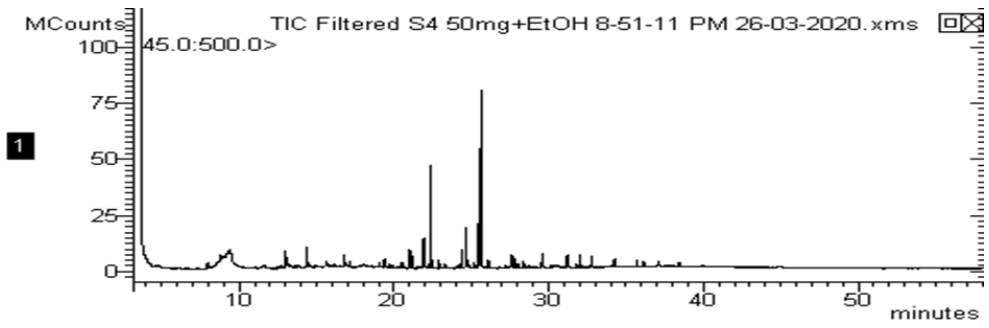

Figure 3 GC-MS chromatogram of ethanol extract from *S. neglects*.

The GC-MS analysis demonstrated 36 compounds identified in the ethanol extract. Five predominant compounds in the ethanol extract are 1, 2, 3-Propanetriol (30.33 %), Linoleic acid ethyl ester (18.20 %), Hexadecanoic acid, ethyl ester (9.07 %), Phytol (6.10 %), and Linolelaidic acid, methyl ester (4.40 %) as shown in **Figure 3** and **Table 3**.

Table 3 Retention time (RT), compound name, % area and % probability of 5 predominant compounds in ethanol extract from *S. neglecta* using GC-MS analysis.

No.	RT (min)	Compound name	% Area	% Probability
1	9.40	1,2,3-Propanetriol	30.33	92.70
2	22.37	Hexadecanoic acid, ethyl ester	9.07	56.40
3	24.64	Phytol	6.10	69.70
4	25.48	Linolelaidic acid methyl ester	4.40	16.30
5	25.62	Linoleic acid ethyl ester	18.20	38.00

Base on the % area, the most predominant compound is 2-Propanol, 1,1'-oxybis-(24.57 %) for propylene glycol extract, Phytol (55.32 %) for methanol extract and 1,2,3-Propanetriol (30.33 %) for ethanol extract. The differences are likely influenced by the extraction solvents. Similar result has been found when the phytochemical constituents in *S. longata* extracts were affected by the extraction solvents as the chemical constituents of *S. longata*, extracted with petroleum ether, methylene chloride, chloroform, acetone and methanol were varied greatly between different solvents, with the highest 1 recorded for methanol. A total of 97 compounds were identified in different extracts, the composition and mass fraction of phytochemical constituents in *S. longata* extracts were also affected by the extraction solvent, in which, the ketonehexahydrofarnesyl- acetone and the phenolic, butylated hydroxytoluene (BHT) were dominant in *S. longata* chloroform extract. Meanwhile, neophytadiene and phytol were the dominant terpenes in methylene chloride extract, while, hydrocarbons and sterols were found to maintain in *S. longata* and were mainly detected in petroleum ether extract. Moreover, Alkaloids were only detected in acetone and methanol extracts and comprise 0.399 % of *S. longata* [13].

Phytochemicals are active ingredients. They have been demonstrated to possess therapeutic properties which are considered as a medicine or drug. In this study, Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol) is the most predominant compound in methanol extract (55.32 %). It is also found in propylene glycol extract (9.65 %) and ethanol extract (6.10 %). It was extracted previously from moss and edible marine algae [14]. In addition, it can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K [15]. Interestingly, it has been reported to possess various pharmacological properties, including anti-inflammatory [16,17], anti-diarrheal activities [18], antinociceptive, antioxidant [19], and anti-diabetic [20], activities. In addition, other predominant compound found in this study, such as n-Hexadecanoic acid or Palmitic acid (CH3 (CH 2) 14COOH), a saturated fatty acid is an anti-inflammatory compound as it is found to exert anti-inflammatory effect by inhibiting phospholipase A2 (PLA 2 plays crucial roles in diverse cellular responses, host defense, and signal transduction) [21].

According to phytochemical compositions and their pharmacological properties, *S. neglecta* is possibly a potentially prolific natural source of bioactive compounds that may represent useful leads in the development of novel pharmaceutical agents.

Nutritional contents

In this study, the extracts from *S. neglecta* contained nutritional contents (as percentage in gram of dry weight) 19.71 ± 1.71 - 21.68 ± 4.52 % total protein 1.76 ± 0.24 - 3.53 ± 0.23 %, total lipid, fiber 11.44 ± 0.83 - 14.05 ± 1.74 % fiber, 8.46 ± 0.75 - 10.73 ± 0.47 % ash, 9.48 ± 0.06 - 9.79 ± 0.11 % moisture, and 38.64 ± 1.65 - 47.36 ± 3.89 % total carbohydrate. The propylene glycol extract provided the higher total protein (21.68 ± 4.52 %) compared to methanol extract (20.69 ± 2.96 %) and ethanol extract (19.71 ± 1.71 %). The extract contained the higher total lipid (3.53 ± 0.23 %) than methanol extract (3.18 ± 0.32 %) and propylene glycol extract (1.76 ± 0.24 %). The methanol extract also provided the higher fiber (14.77 ± 0.89 %) compared to propylene glycol extract (14.05 ± 1.74 %) and ethanol extract (11.44 ± 0.83 %). In addition, the methanol extract possessed the total carbohydrate (47.36 ± 3.89 %) significantly higher than propylene glycol extract (42.09 ± 3.77 %) and ethanol extract (38.64 ± 1.65 %). However, the total protein in the propylene glycol, methanol and ethanol extracts was not different. In contrast, the total lipid and the total

carbohydrate in the extracts are significantly different ($p < 0.05$). The total lipid is significant higher in the methanol and ethanol extracts than that in the propylene glycol extract. In addition, the total carbohydrate is found to be significant highest in the methanol extract in comparison to the others ($p < 0.05$), as shown in **Table 4**.

Table 4 Nutritional contents (%) of the propylene glycol, methanol and ethanol extracts from *S. neglecta*.

Parameters	Nutritional contents (%)		
	Propylene glycol	**Methanol**	**Ethanol**
Total protein	21.68 ± 4.52^{a}	20.69 ± 2.96^{a}	19.71 ± 1.71^{a}
Total lipid	1.76 ± 0.24^{a}	3.18 ± 0.32^{b}	3.53 ± 0.23^{b}
Fiber	14.05 ± 1.74^{b}	14.77 ± 0.89^{b}	11.44 ± 0.83^{a}
Ash	10.43 ± 0.01^{a}	10.73 ± 0.47^{a}	8.46 ± 0.75^{a}
Moisture	9.48 ± 0.06^{a}	9.79 ± 0.11^{a}	9.55 ± 0.72^{a}
Total carbohydrate	42.09 ± 3.77^{b}	47.36 ± 3.89^{c}	38.64 ± 1.65^{a}

Results are expressed as means ± SEM of 3 independent experiments a, b. indicate the significant difference at $p < 0.05$, between groups.

A number of macroalgae have been used as ingredients in both medicinal and food preparations, traditionally due to their rich source of biologically active metabolites and low calorie foods with high contents of minerals, vitamins, proteins and carbohydrates [22]. *S. neglecta* contained high amounts of protein, carbohydrate, fat, sulfate and dietary fiber [23]. Sitthiwong [24], found that *Spirogyra* spp. possessed the basic nutritional value and the highest values of ash, fat, moisture, protein, carbohydrate and energy by 17.37, 5.51, 14.90, 22.77, 67.13 g/100 g and 363 kcal/100 g, respectively. In addition, Tipnee *et al.* [25], reported that the extract of *S. varians* contained total content of protein (% dry weight) ranging from 12.0 to 24.4 %; carbohydrate from 42.8 to 62.0 % and lipid from 14.8 to 21.0 %.

Cytotoxicity

MTT assay demonstrated that the percentage cell viability decreased with increased the concentration of the extracts. Nevertheless, the extracts, 25 to 1,000 µg/mL, produced the percentage cell viability of the RAW 246.7 cells > 80 %, as shown in **Table 5**.

Table 5 Percentage cell viability of the RAW 246.7 cells exposed to the propylene glycol, methanol and ethanol extracts from *S. neglecta*.

Concentration (µg/mL)	% Cell viability		
	Propylene glycol	**Methanol**	**Ethanol**
0	0	0	0
25	109.87 ± 2.32^{a}	106.23 ± 2.07^{a}	110.93 ± 2.85^{a}
50	107.64 ± 2.17^{a}	102.54 ± 2.61^{a}	106.66 ± 2.11^{a}
100	104.81 ± 2.06^{b}	99.34 ± 2.97^{a}	104.92 ± 1.67^{b}
150	101.51 ± 1.98^{a}	96.11 ± 2.04^{a}	99.76 ± 1.56^{ab}
200	97.94 ± 2.87^{b}	92.65 ± 2.17^{a}	97.84 ± 2.23^{b}
250	94.35 ± 2.23^{b}	87.89 ± 2.03^{a}	95.21 ± 2.76^{b}
500	90.52 ± 2.64^{b}	85.47 ± 1.41^{a}	91.15 ± 2.37^{b}
750	87.21 ± 2.33^{a}	83.58 ± 2.35^{a}	88.19 ± 2.38^{a}
1,000	85.74 ± 1.81^{a}	82.69 ± 2.11^{a}	85.11 ± 2.76^{a}

The values are expressed as mean ± SEM of 3 independent experiments. The values with different superscripts in the same row indicate the statistical difference at $p < 0.05$, between groups.

In the meantime, the MTT assay revealed that the extracts from *S. neglecta* induced toxicity to the LPS-stimulated RAW 246.7 cells with concentration dependent manner. The methanol extract produced the higher cytotoxicity than propylene glycol and ethanol extracts significantly ($p < 0.05$). In addition, the extracts 50 - 1,000 µg/mL produced toxicity to the LPS-stimulated RAW 246.7 cells less than 20 %. Interestingly, the extracts ≤ 100 µg/mL produced non toxic to the LPS-stimulated RAW 246.7 cells, as shown in **Figure 4** indicating the extracts from *S. neglecta* possess relatively low cytotoxicity with $IC_{50} >$ 1,000 µg/mL. This result is similar to a study by Yosboonruang *et al.* [1], who found that *S. neglecta* extracts were non-toxic to primary fibroblast cells. In addition, the 50 % cytotoxicity concentration of *Spirogyra* spp. was 4,363.30 g/mL for aqueous extract, 356.57 g/mL for ethanolic extract and 250.80 g/mL for methanolic extract [26]. Furthermore, oral administration of the ethanol extracts from macroalgae, *Cladophora glomerata* and *Microspora floccosa* at a dose of 25 g/kg did not induce any toxicity sign or death in rats, and sub chronic toxicity testing indicated the non-toxicity of the extracts [26]. However, a small number of algae has been found to have highly toxic [28]. The methanol extracts of *S. aequinoctialis*, *S. pratensis* and *S. subsalsa* displayed significant phytotoxic activity against *Lemna minor* and also exhibited significant cytotoxic activity against brine shrimps larvae [29]. Interestingly, eleven freshwater green algae from various habitats of Sindh (Pakistan) displayed a significant phytotoxic activity but non-significant cytotoxic [30]. The different toxic results are probably due to the methods, algal species and the organisms tested.

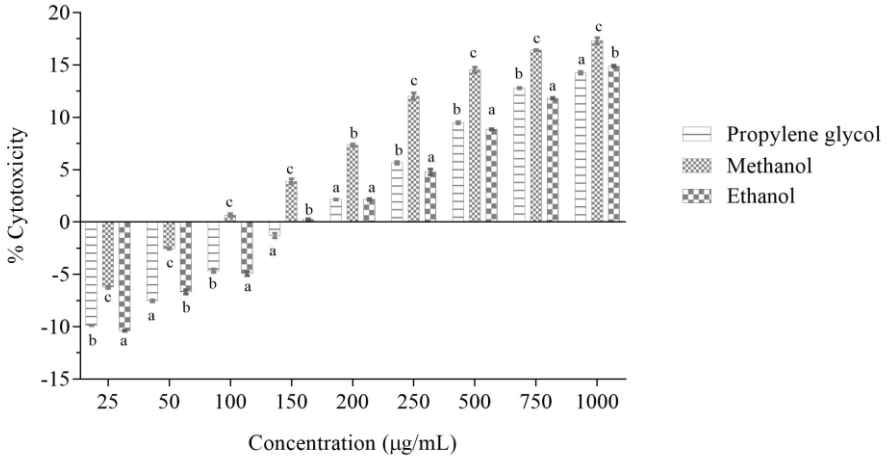

Figure 4 Percentage cytotoxicity of the propylene glycol, methanol and ethanol extracts from *S. neglecta* on the RAW 246.7 cells**,** values are expressed as mean ± SEM of 3 independent experiments a, b indicate the significant difference at $p < 0.05$.

Anti-inflammatory activity

All extracts showed low cytotoxicity in RAW 246.7 cells. Even at 1,000 µg/mL, more than 80 % of the cells survived as shown in **Table 5**, which indicating these extracts were considered to be safe. Therefore, they were used for determination of NO production.

Determination of NO production using Griess assay revealed that the extracts from *S. neglecta* 25 µg/mL failed to inhibit NO production in the LPS-stimulated RAW 246.7 cells. The methanol and ethanol extracts 50 µg/mL failed to inhibit NO production as well. The extracts inhibited LPS-stimulated NO production concentration dependently. At the concentration of 1,000 µg/mL, the propylene glycol extract showed the highest % inhibition of NO production (55.17 ± 1.91 %) compared to the methanol (29.87 ± 1.56 %) and ethanol extract (30.04 ± 1.57 %). Meanwhile, Diclofenac (20 µg/mL) inhibited NO production (23.28 ± 1.17 %) as shown in **Table 6**, which was observed to be higher than that of the extracts, as shown in **Table 5**. Similar result has been found when the sterol content of ethanol extract from *Spirogyra* sp. significantly reduced NO production in LPS-stimulated zebrafish [31].

According to the extracts from *S. neglecta* inhibited NO production, indicating the anti-inflammatory activity of *S. neglecta*. The finding in this study is in line with the study by Ontawong *et al.* [32], who revealed that *S neglecta* aqueous extract attenuates LPS-Induced renal Inflammation [4], also found that oral administration of *S. neglecta* alleviated adverse effects of diabetes on inflammatory factors in diabetic rats. Furthermore, Yosboonruang *et al.* [1], reported that *S. neglecta* possesses anti-inflammatory activity by reducing the release of TNF-α from macrophages.

Table 6 Inhibition of NO production (%) in LPS-stimulated RAW 264.7 cells exposed to propylene glycol, methanol and ethanol extracts from *S. neglecta*, and Diclofenac, a positive control.

Concentration (µg/mL)	Inhibition of NO production (%)			Diclofenac (20 µg/mL)
	Extracts from *S. neglecta*			
	Propylene glycol	Methanol	Ethanol	
0	0	0	0	23.28 ± 1.17*
25	NI	NI	NI	
50	6.47 ± 0.06[a]	NI	NI	
100	13.89 ± 0.27[b]	2.28 ± 0.01[a]	2.56 ± 0.01[a]	
150	17.65 ± 0.71[b]	6.34 ± 0.06[a]	6.33 ± 0.34[a]	
200	26.67 ± 1.16[c]	11.15 ± 1.11[b]	9.97 ± 1.27[a]	
250	39.97 ± 1.23[b]	13.82 ± 1.15[a]	13.25 ± 1.28[a]	
500	44.28 ± 1.91[c]	17.53 ± 1.56[b]	16.94 ± 1.24[a]	
750	49.61 ± 1.46[c]	20.68 ± 1.83[b]	21.61 ± 1.27[a]	
1,000	55.17 ± 1.91[c]	29.87 ± 1.56[a]	30.04 ± 1.57[b]	

The values are expressed as mean ± SEM of 3 independent experiments. The values with different superscripts in the same row indicate the statistical difference at $p < 0.05$, between groups. NI denotes no inhibition of nitric oxide production.

Phytol exhibits anti-inflammatory activity, possibly via COX-1 and 2, NF-κB, and IL-1β dependent pathways [18], and attenuates the inflammatory response by inhibiting neutrophil migration that is partly caused by reduction in IL-1b and TNF-a levels and oxidative stress [17]. In this study, Phytol was found to be higher in the methanol extract than that in the propylene glycol extract, but the propylene glycol extract inhibited NO production higher than the methanol extract did. This indicates that the anti-inflammatory activity of the extracts from *S. neglecta* is mainly via the inhibition of NO production.

Conclusions

The results indicate that the different extracts from *S. neglecta* possess different phytochemical compositions. All the extracts can exhibit the anti-inflammatory activity via inhibition NO production. The anti-inflammatory activity is partially resulted from Phytol and n-Hexadecanoic acid. Base on the phytochemical compositions, nutritional contents and pharmacological properties, the extracts from *S. neglecta* can be applied as potential sources for the management of inflammatory-related diseases and food industrial products. As the direct actions in inflammation was not determined in the present work, further studies on the mechanical of actions and the active compounds which are involved in this activity are essentially conducted.

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