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Anti-diabetic, anti-inflammatory and muscle relaxant activities of extracts from *Spirogyra varians* (Hassall) Kuetzing

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Abstract: *Spirogyra varians* (Hassall) Kuetzing is one of the edible freshwater filamentous green algae. This alga is commonly used as local food in northern and northeastern Thailand. A study on pharmacological activities of *S. varians* has not yet been extensively performed. Therefore, this study was established to investigate the anti-diabetic, anti-inflammatory and muscle relaxant activities of the extracts from *S. varians*. The *In vitro* α-glucosidase inhibition assay revealed that the propylene glycol extract exhibited the highest α-glucosidase inhibition activity compared to the methanol and ethanol extracts. However, all the extracts possess α-glucosidase inhibition activity less potent than Acarbose, an α-glucosidase inhibitor. The propylene glycol extract showed the highest inhibition of nitric oxide production in LPS-stimulated RAW 264.7 macrophage cells compared to the methanol and ethanol extracts. Nevertheless, the inhibition activity of the extracts was less potent than diclofenac, an anti-inflammatory drug. Whereas, the methanol extract exhibited the highest muscle relaxant activity on frog gastrocnemius compared to the ethanol and propylene glycol extracts. The methanol extract displayed muscle relaxant activity with a similar potent to Indomethacin, a pain relief drug. This study indicates that *S. varians* can be used as an effective novel natural resource for developing anti-diabetic, anti-inflammatory and muscle relief agents.

Keywords: *Spirogyra varians*, anti-diabetic, anti-inflammation, relaxant activity

INTRODUCTION

Spirogyra, one of the commonest green filamentous freshwater macroalgae, is named because of the helical or spiral arrangement of the chloroplasts (Krupek *et al.* 2014). The genus *Spirogyra* has recently drawn the attention of researchers due to its various biotechnological and industrial applications. It has been used as a natural source of bioactive substances and ingredients in both medicinal and food preparations, traditionally in different regions across the world (Chandini *et al.* 2008).

Spirogyra varians (Hassall) Kützing is commonly known as water silk or pond silk. It is one of the filamentous green algae found in shallow ponds, ditches amongst vegetation at the edges of large lakes, small stagnant water bodies, rivers and streams (Peerapornpisal *et al*., 2006). *S. varians* is commonly available and used as local food in northern and northeastern Thailand (Tipnee *et al*., 2015). Tipnee and coworkers investigated the biochemical and nutritional composition of *S. varians* and reported that this alga possessed an appreciable amount of pigments, dietary protein, carbohydrate and minerals content. In addition, its nutritional value including total content of protein (% dry weight) ranging from 12.0 % to 24.4%; carbohydrates from 42.8% to 62.0% and lipid from 14.8% to 21.0% was discovered (5). Previous studies on pharmacological properties of *Spirogyra* spp. have been documented. *S. neglecta* has antigastric ulcer and anti-inflammatory effects in rats (Amornlerdpison *et al*., 2012). It was able to improve hyperglycemia, hypertriglyceridemia, insulin resistance, as well as renal oxidative stress and regulation of organic anion transporter 3-Oat3 (Ontawong *et al.,* 2013). At the dose of 1.0g/kg BW, it reduced plasma glucose, triglyceride and free fatty acid in type 2 diabetic mellitus (T2DM) rats (Jitprawet *et al*., 2012). Its extracts exerted anti-inflammatory property by alleviation adverse effects of diabetes on inflammatory factors in streptozotocin-induced diabetic rats (Mesbahzade *et al*., 2018). However, the pharmacological properties and other biological effects of *S. varians* have not been intensively examined. To see whether *S. varians* possesses pharmacological activities and its activities depend on solvent extracts, therefore, this research was carried out to determine the anti-diabetic, anti-inflammatory and muscle relaxant activities of propylene glycol, methanol and ethanol extract from *S. varians*.

MATERIALS AND METHODS

Preparation of extracts

Fresh *Spirogyra varians* (Hass.) Kutzing was collected in July 2021 from natural freshwater sources in Maha Sarakham Province, northeastern Thailand. The collected alga was identified, cleaned up from epiphytes and non-living matter and washed thoroughly with running tap water. The samples were dried in shade followed by in a hot air oven at a temperature not exceed to 40^{o}C. The

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dried samples were mechanically grinded into a coarse powder to facilitate extraction. Extracts of this alga were prepared using different solvents, i.e. propylene glycol, methanol and ethanol. The extraction was performed using the maceration process by adding 400 g of powder into 1,000 mL solvent solution. The mixture was allowed to stand for 7 days at room temperature with occasional stirring. After maceration, the mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated by using a rotary evaporator at 40-50ºC (Mesbahzadeh *et al*., 2018). The extract was kept in an airtight bottle and maintained at -20°C until used.

Determination of anti-diabetic activity

The anti-diabetic activity of the extracts from *S. varians* was determined using α-glucosidase inhibitory assay. The assay was carried out according to Dong *et al*. (2012) with slight modifications. Briefly, a volume of 60µL of sample solution (100, 200, 400 and 800µg/mL) and 50µL of 0.1 M phosphate buffer containing α-glucosidase solution (0.2 U/mL) in 96-well plate was incubated at 37ºC for 20 min. After incubation, 50µL of 5mM p-nitrophenyl- α-D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.9) was added into the well and incubated at 37ºC for another 20 min. The volume of 160µL of 0.2 M Na_2CO_3 was added to terminate the reaction. The absorbance reading was recorded at 405nm using the micro-plate reader and compared to the control which had 60µL of buffer solution in place of the sample. Acarbose was assayed as a positive control of α-glucosidase inhibitor. The α-glucosidase inhibitory activity was expressed as % inhibition and was calculated using the following equation:

% inhibition = $[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$,

Where $Abs_{control}$ is the absorbance of control without test solution and Abs_{sample} is absorbance of sample with tested solution. The concentration of the extract required to inhibit 50% of α-glucosidase (IC_{50}) was determined from a plot of percentage inhibition versus sample concentrations.

Determination of anti-inflammatory activity

The anti-inflammatory activity of the extracts from *S. varians* was determined by measuring nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophage cells after treatment with the extracts. To ensure that the RAW264.7 cells were healthy and the concentrations used in the extracts were not toxic to the cells, cytotoxicity was determined using MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. The assay is based on the conversion of MTT solution into insoluble (E, Z)-5-(4, 5-dimethylthiazol-2-yl)-1,3-diphenylformazan (formazan). The tested sample was considered to be non-cytotoxic when cell viability of RAW246.7 cells in the sample treated was higher than 80% (Amornlerdpison *et al*., 2012).

Determination of cytotoxicity

In the present study, the RAW264.7 macrophage cells, purchased from the American Type Culture Collection (ATCC®TIB-71™ USA) were used. The cells (1×10^5 cells/well) were cultured in a 96-well plate containing Dulbecco's modified Eagle's medium (DMEM; ATCC® 30-2002™ USA), supplemented with 5% fetal bovine serum and 1% antibiotics penicillin-streptomycin and incubated for 24 h at 37ºC in a 5% CO_2 incubator. After incubation, the cultured medium was removed and replaced with a fresh medium containing LPS (1µg/mL). The cells were treated with the tested extract solution (25, 50, 100, 150, 200, 250, 500, 750 and 1,000µg/mL) in a volume of 200µL/well and incubated in a 5% CO_2 incubator at 37ºC for 24h. After incubation, the cultured medium was replaced with a fresh medium. Subsequently, a volume of 50µL/well of 5 mg/mL MTT solution in PBS was added to each well and incubated at 37ºC for 24h. The solutions in the plates were removed and the formazan was solubilized by adding 100µL of DMSO. Finally, the absorbance of the solubilized formazan was measured. The cell viability was determined by absorbance reading at 570 nm using the micro-plate reader (Multiskan Go, Thermo/Scientific, the Netherlands). The percentage of cell viability is calculated using the following equation,

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

Where $Abs_{negative\ control}$ is the absorbance of negative control (PBS) and Abs_{sample} is the absorbance of the sample (extract from *S. varians*).

The determination of cytotoxicity of the extracts from *Spirogyra varians* was conducted according to Buddhakala and Talubmook (2020) with slight modifications.

Determination of NO production

Nitric oxide (NO) is a signaling molecule that plays a key role in the pathogenesis of inflammation. It gives an anti-inflammatory effect under normal physiological conditions. On the other hand, it is considered as a pro-inflammatory mediator that induces inflammation due to overproduction in abnormal situations (Sharma *et al*., 2007). NO in the biological matrix 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 determination of NO production was therefore performed by measuring nitrite concentrations in the cultured medium using Griess assay, a technique commonly used to quantify NO (Meerloo *et al*., 2011). The basic reaction involves reacting of the Griess reagent, consisting of sulphanilamide and N-1-naphthyl ethylenediamine dihydrochloride (NED), to form a stable azo compound.

Base on the % cell viability of the RAW 246.7 cells in table 2, the extracts (25-1,000µg/mL) were used in the nitrite concentration analysis. The RAW264.7 cells were

cultured in DMEM medium with PBS in a 96-well plate. LPS (1µg/mL) was added to each well prior to adding the extracts. The plate was incubated in the 5% CO_2 incubator at 37ºC for 24h. 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 (Multiskan Go, Thermo/Scientific, the Netherlands). The percentage of NO production is calculated using the following equation;

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

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

The determination of anti-inflammatory activity of the extracts from *Spirogyra varians* was conducted according to Wang *et al*. and Nguyen *et al.* (2020, 2020) with slight modifications.

Determination of muscle relaxant activity

The muscle relaxant activity on electrically induced contraction of frog gastrocnemius was established to look for the evidence of changes in the frog muscle administered different solvent extracts from *S. varians*.

Healthy adult frogs (*Hoplobatrachus rugulosus* (Traijitt *et al*., 2021) weighing 90-100g purchased from a local market in Maha Sarakham Province, Northeastern Thailand where the animal used. The frogs were acclimatized for 3 days in a tank with aerated circulating water and provided with commercial frog pellets. The care and use of the animal were treated ethically according to the guidelines of the Committee Care and Use of Laboratory Animal Resource, National Research Council Thailand and performed in accordance with the advice of the Institutional Animal Care and Use Committee, Mahasarakham University, Thailand (the project approval certificate: IACUC-MSU-06/2021. After acclimatization, the frogs were pithed. The skin over the lower leg was cut and removed. Gastrocnemius was placed in a bath filled with Modified Krebs-Ringer's solution (125mM NaCl, 5mM KCl, 1mM KH_2PO_4, 1mM $MgSO_4$, 1mM $CaCl_2$, 5.5mM glucose and 20mM HEPES) at 25-27°C, aerated with O_2 containing 5% CO_2 and connected to force transducer. The contractile responses were recorded from the transducer and converted to the digital signal on a computer recording unit (LabScribe recording and analysis software package developed by iWorx. LabScribe Inc. 62 Littleworth Road Dover, NH 03820, USA). The continuous neuromuscular electrical stimulation in all the experiments was set up as 10 ms pulse width, 50 ms delay, 0.5Hz frequency, 1 of pulse and 0.4-0.6 volt. The contraction of the muscle was investigated after administration of the extracts (30, 50 and 100µg/mL) compared to that in the normal contraction (before administration). The difference in the contraction, before and after administration, was noted. The percentage relaxant activity was calculated and compared to Indomethacin, a positive control. Determination of muscle relaxant activity of the extracts from *S. varians* was conducted according to the method of Buddhakala (2007), with several modifications.

STATISTICAL ANALYSIS

Data were calculated using IBM SPSS Statistics software package and expressed as means ± standard error means (S.E.M.). Differences were evaluated by the one-way analysis of variance (ANOVA) test completed by Duncan's New Multiple Range Test (DMRT). Differences were considered significant at $p<0.05$.

RESULTS

Anti-diabetic activity

The α-glucosidase inhibitory assay in this study demonstrated that the extracts from *S. varians* (100, 200, 400 and 800µg/mL) inhibited α-glucosidase concentration-dependently. At the concentration of 800µg/mL, the propylene glycol extract exhibited the highest percentage of α-glucosidase inhibitory activity (87.79±1.86%), followed by the methanol extract (81.73±1.46 %) and the ethanol extract (79.19±1.57%), respectively. Whereas, the inhibitory activity of the positive control, Acarbose was 92.83±1.58 % (table 1).

Anti-inflammatory activity

At the same condition, the cytotoxicity study demonstrated that after exposure to the different extracts from *S. varians* the % viability of the LPS-stimulated RAW 246.7 cells was not different and was decreased with increasing concentration of the extracts. However, the extracts 25 up to 1,000µg/mL showed non cytotoxic as shown in table 2. Therefore, the extracts were considered to be safe and used further for the determination of NO production in the LPS-stimulated RAW 246.7 cells.

Determination of NO production revealed that the extracts from *S. varians* failed to inhibit NO production in the LPS-stimulated RAW 246.7 cells when 25µg/mL of propylene extract and 25-100µg/mL of methanol and ethanol extracts were administered. The inhibitory effects of the extracts on NO in LPS-stimulated RAW 264.7 cells were found when 50-1,000µg/mL of propylene glycol extract and 100-1,000µg/mL of methanol and ethanol extracts were applied. The extracts inhibited LPS-stimulated NO production concentration dependently. At the concentration of 1,000µg/mL, the propylene glycol extract showed the highest % inhibition of NO production (56.34±2.76%) compared to the methanol (19.54±2.33%) and ethanol extract (18.99±1.78%).

Table 1: Inhibitory activity of propylene glycol, methanol and ethanol extracts from *S. varians*

Concentration (µg/mL)	% inhibition of α-glucosidase			
	Propylene glycol	Methanol	Ethanol	Acarbose
100	25.79±2.11[b]	19.56±1.57[a]	18.51±2.01[a]	39.47±2.04[c]
200	64.65±1.97[b]	48.61±1.66[a]	45.87±2.02[a]	61.52±2.01[b]
400	82.31±2.04[b]	76.25±1.73[a]	73.36±1.69[a]	83.75±1.13[b]
800	87.79±1.86[b]	81.73±1.46[a]	79.19±1.57[a]	92.83±1.58[c]
IC_{50} (µg/mL)	196.94[b]	208.35[c]	212.99[c]	167.60[a]

Values are expressed as mean ± S.E.M. of three independent experiments. The values with different superscript letters in the same row indicate the significant difference at $p<0.05$. Acarbose was used as the positive control.

Table 2: Cell viability of RAW 246.7 cells (%) after exposure to various concentrations of propylene glycol, methanol and ethanol extracts from *S. varians*

Concentration (µg/mL)	% Cell viability		
	Propylene glycol	Methanol	Ethanol
25	110.16±2.22	106.60±2.69	109.58±2.45
50	108.55±2.68[b]	98.27±2.47[a]	107.31±3.13[b]
100	103.62±2.57[b]	95.65±2.95[a]	101.84±2.87[b]
150	94.72±2.74[b]	89.12±2.11[a]	93.26±2.64[b]
200	91.76±2.35	87.39±3.01	89.33±2.78
250	89.91±1.56	85.24±2.34	87.85±2.23
500	86.60±2.12	82.34±2.69	84.13±2.79
750	82.42±1.87	81.61±1.97	82.29±2.36
1,000	80.97±2.09	80.12±1.36	80.71±2.97

The values are expressed as mean ± S.E.M. of three independent experiments. The values with different superscript letters in the same row indicate the significant difference at $p<0.05$.

Table 3: Inhibition of NO production (%) in LPS-stimulated RAW 264.7 cells by propylene glycol, methanol and ethanol extracts from *S. varians* and Diclofenac a positive control.

Concentration (µg/mL)	Inhibition of NO production (%)			
	Extracts from *S. varians*			Diclofenac (20µg/mL)
	Propylene glycol	Methanol	Ethanol	
25	NI	NI	NI	20.94±1.23
50	5.87±2.29[a]	NI	NI	
100	12.84±2.34[b]	NI	NI	
150	16.08±2.70[b]	5.97±2.61[a]	4.68±1.77[a]	
200	25.36±1.85[c]	9.05±2.71$_b$	7.98±1.65[ab]	
250	40.92±2.79[d]	11.21±1.56[bc]	10.08±2.34[b]	
500	45.65±2.27[e]	15.46±2.61[c]	14.96±2.22[bc]	
750	50.21±2.58[f]	17.59±2.39[cd]	17.01±2.68[c]	
1,000	56.34±2.76[g]	19.54±2.33[d]	18.99±1.78[c]	

The values are expressed as mean ± S.E.M. of three independent experiments. The values with different superscripts in the same column indicate the statistical difference at $p<0.05$. NI denotes no inhibition of nitric oxide production.

Table 4: Contraction (Tension, g) and relaxation (%) of frog gastrocnemius to electrical stimulation in the presence of different tested solutions, Indomethacin and propylene glycol extract, methanol extract and ethanol extract from *S. varians*

Samples	Contraction (Tension, g)		% Relaxation
	Before administration	After administration	
Indomethacin	2.50±0.07[a]	1.23±0.43[a]	50.80±0.41[c]
Propylene glycol extract	2.52±0.16[a]	1.68±0.52[c]	33.33±0.46[a]
Methanol extract	2.51±0.24[a]	1.29±0.25[a]	48.61±0.22[bc]
Ethanol extract	2.50±0.33[a]	1.37±0.61[b]	45.20±0.58[b]

The values are expressed as mean ± S.E.M. of five independent experiments. The values with different superscripts (a, b, c) in the same column indicate the statistical difference at $p<0.05$.

Table 5: Contraction (Frequency, number/min) and relaxation (%) of frog gastrocnemius to electrical stimulation in the presence of different tested solutions, Indomethacin and propylene glycol extract, methanol extract and ethanol extract from *S. varians*

Samples	Contraction (Frequency, Number/min)		% Relaxation
	Before administration	After administration	
Indomethacin	3.5±0.27[a]	2.96±0.18[a]	15.43±0.20[b]
Propylene glycol extract	3.5±0.30[a]	3.14±0.02[b]	10.29±0.03[a]
Methanol extract	3.5±0.23[a]	3.02±0.13[ab]	13.72±0.15[b]
Ethanol extract	3.5±0.12[a]	3.08±0.85[b]	12.00±0.75[ab]

The values are expressed as mean ± S.E.M. of five independent experiments. The values with different superscripts (a, b,) in the same column indicate the statistical difference at $p<0.05$.

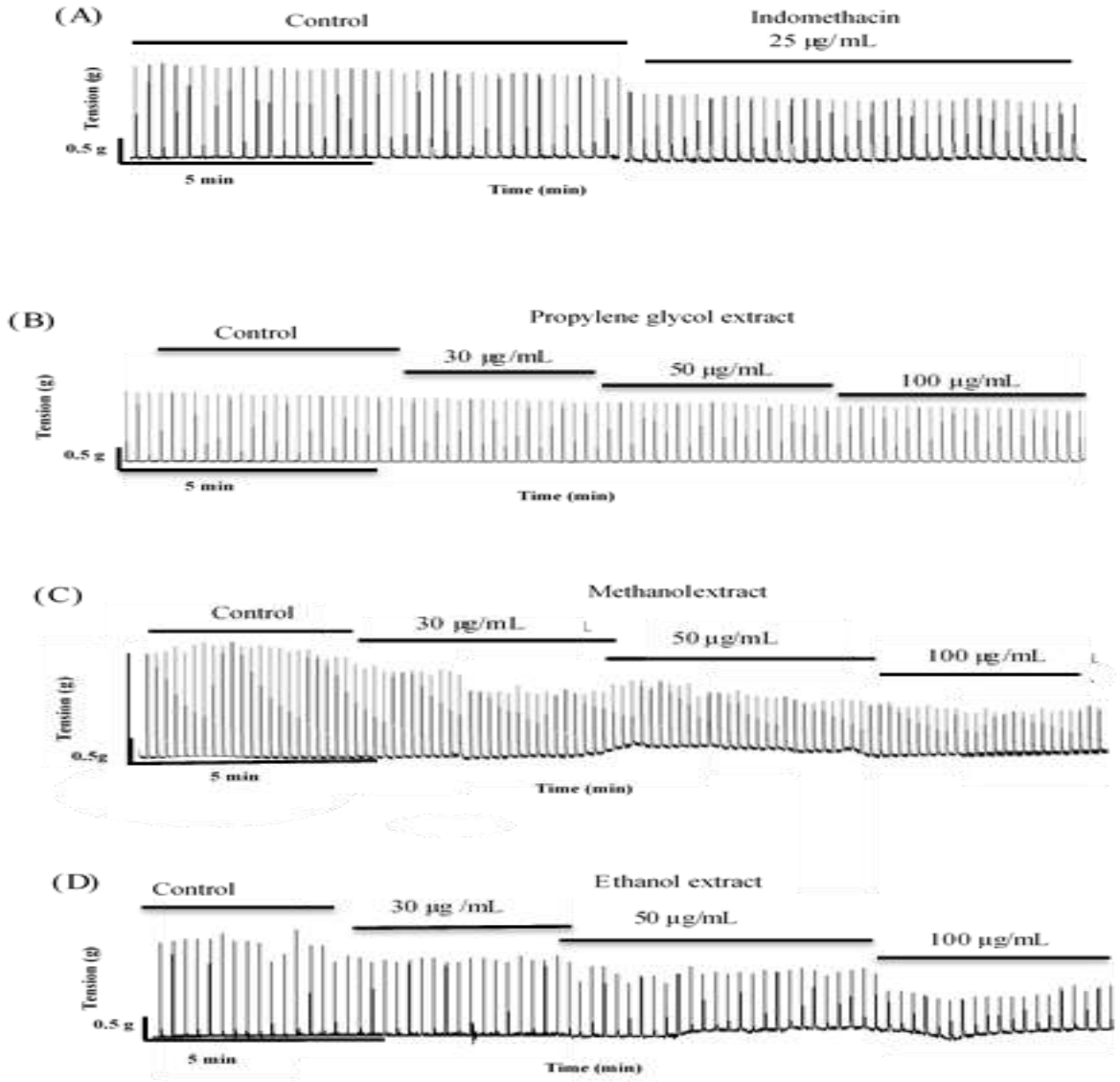

Fig. 1: Contractile response of frog gastrocnemius to electrical stimulation in the presence of Indomethacin (A) and propylene glycol extract (B), methanol extract (C) and ethanol extract (D) from *S. varians.*

Whereas, Diclofenac (20µg/mL) exhibited the NO production inhibitory activity of 20.94±1.23% which was observed to be higher than that of the extracts as shown in table 3.

Muscle relaxant activity

The extracts of *S. varians* up to a concentration of 1,000µg/mL was found to be non cytotoxic. One-tenth of this concentration which to be safe is 100µg/mL. Hence,

the accumulations of 30, 50 and 100µg/mL of each extract were used in the measurement of muscle relaxant activity.

After administration the extracts, the tension of electrical evoked contraction in frog gastrocnemius muscle was decreased compared to that before administration. The tension of the muscle was decreased with the increasing concentration (30, 50 and 100µg/mL). The highest % relaxation in tension was observed in the methanol extract (48.61±0.22%) followed by that in the ethanol extract (45.20±0.58%) and propylene glycol extract (33.33±0.46%). However, the potential muscle relaxant activities of methanol and ethanol extracts were not different. Interestingly, the relaxant activity seen in the muscle in the presence of the methanol extract was not different from that seen in the muscle in the presence of Indomethacin, a non-steroidal anti-inflammatory drug (50.80±0.41%). In line with the tension, after administration the extracts, the frequency of electrical evoked contraction in frog gastrocnemius muscle was decreased compared to that before administration. The frequency of the muscle was decreased with the increasing concentration (30, 50 and 100µg/mL).

The highest % relaxation in frequency was observed in the methanol extract (13.72±0.15%) followed by that in the ethanol extract (12.00±0.75%) and propylene glycol extract (10.29±0.03%). However, the potential muscle relaxant activities of methanol, ethanol and propylene glycol extracts were not different. Again, the relaxant activity seen in the muscle in the presence of the methanol extract was not different from that seen in the muscle in the presence of Indomethacin (15.43±0.20%) as shown in fig. 1 and table 4 and table 5).

Indomethacin has been reported to have potent antipyretic, analgesic and anti-inflammatory activity and has been effectively used in the management of mild-to-moderate pain and has demonstrated efficacy in the treatment of various other painful conditions (Nalamachu and Wortmann, 2014). Base on the result obtained, *S. varians* can be applied for muscle pain relieving. For beneficial application and pharmacological investigation, the mechanism (s) responsible for the muscle relaxant activity of the *S. varians* should be further examined.

DISCUSSION

The results from the α-glucosidase inhibitory assay demonstrated that extracts from *S. varians* exhibited α-glucosidase inhibitory activity and the propylene glycol extract displayed the highest activity compared to the methanol and ethanol extract (table 1). A similar result has been found when the crude methanolic extract of *Spatoglossum asperum*, a brown alga, at the concentration of 900 µg/mL exhibited α-glucosidase inhibitory activity of 96.75% (Pandithurai *et al*., 2015). The strongest α-glucosidase inhibitory activity was found in propylene glycol extract with IC_{50} of 196.94µg/mL compared to methanol extract (208.35µg/mL) and ethanol extract (212.99µg/mL). Nevertheless, the extracts from *S. varians* inhibited the activity of α-glucosidase comparably less than that of standard drug Acarbose (IC_{50} of 167.60µg/mL) as shown in fig. 1. The propylene glycol extract from *S. varians* showed higher potential α-glucosidase inhibition than methanol and ethanol extracts. It is likely that the higher polar solvent extracts possess the stronger α-glucosidase inhibitory activity. The higher polar solvent extracts possess the stronger α-glucosidase inhibitory activity have been found when the methanol extracts from the seeds of *Myristica fragrans* displayed anti-α-glucosidase activity (IC_{50} of 4.08±0.12µg/mL) higher than ethanol extract (IC_{50} of 11.92±0.39µg/mL). Meanwhile, n-hexane, chloroform and dichloromethane extracts showed the lowest activity with IC_{50} >200µg/mL (Li *et al*., 2020).

The activities of propylene glycol, methanol and ethanol extracts from *S. varians* were less effective than Acarbose. Whereas, the methanol extract of *S. asperum* exhibited α-glucosidase inhibition activity more effective than that of Acarbose (Pandithurai *et al*., 2015). Moreover, the crude extracts from *Rhodomela confervoides* (Huds.) Silva, *Gracilaria textorii* (Suringar) De Toni, *Plocamium telfairiae* Harv., *Dictyopteris divaricata* (Okam.) Okam, *Ulval pertusa* and *Enteromorpha intestinalis* (L.) Link show strong inhibitory activity of α-glucosidase (Xiancui *et al*., 2005). The different potential on α-glucosidase activities are more likely due to the different solvents and/or the species of algae.

The extracts from *S. varians* inhibited α-glucosidase enzyme indicating the α-glucosidase inhibitor property of the extracts. This finding corresponds with the pentagalloylglucose 3-O-digalloyl- 1, 2, 6-trigalloyl-glucose, an α-glucosidase inhibitor, was purified from the methanol extract from *S. varians* (Cannell *et al*., 1988). Inhibition on α-glucosidase activity has been reported to alleviate postprandial high glycemic levels in diabetic or prediabetic population (Liu *et al*., 2020). Therefore, the extracts from *S. varians* can be a natural source of α-glucosidase inhibitor and be the promising nutritional supplements for prevention and treatment of diabetes. Other macroalgae such as *S. neglecta, C. glomerata* and *R. hieroglyphicum* have been demonstrated to possess antidiabetic and antihyperglycemic effects (Ontawong *et al*., 2013; Srimaroeng *et al*., 2015; Janthip *et al*., 2020). *S. neglecta* has also been reported to display the alleviation adverse effects in diabetic rats (Mesbahzadeh *et al*., 2018).

Determination of NO production revealed that the extracts from *S. varians*, 50-1,000µg/mL of propylene glycol

extract and 100-1,000µg/mL of methanol and ethanol extract, inhibited LPS-stimulated NO production and the propylene glycol extract showed the highest % inhibition of NO production compared to the methanol and ethanol extract (table 3). Similar result was found when the sterol content of ethanol extract from *Spirogyra* sp. significantly reduced NO production in LPS-stimulated zebrafish (Wang *et al*., 2020). This finding indicates the anti-inflammatory activity of the extracts.

The anti-inflammatory activities of *Spirogyra* spp. have been demonstrated. Oral administration of *S. neglecta* alleviated adverse effects of diabetes on inflammatory factors in diabetic rats (Mesbahzadeh *et al*., 2018). *S. neglecta* has been shown to possess anti-inflammatory activity (Peerapornpisal *et al*., 2006).

Inflammation has been linked with pathogenesis of many diseases. Anti-inflammatory drug was recorded several side effect just like other synthetic drugs, thus, safe biological sources are now been considered. Biological sources for active compounds that have medical importance are on the increase in recent time (Kaboli *et al*., 2001). Natural compounds with anti-inflammatory activity could be good candidates for developing effective therapeutic strategies. Algae are known to have various pharmacological properties including anti-inflammatory property (Lordan *et al*., 2011). Numerous anti-inflammatory compounds have been isolated from marine algae with potential protective efficacy against neuroinflammation (Barbalace *et al*., 2019). According to the result obtained, the extracts from *S. varians* can be used for the alleviation of inflammation and a good candidate inhibitor for developing effective functional ingredient in anti-inflammatory drug.

Determination on the muscle relaxant activity demonstrated the extracts of *S. varians* possessed the relaxant activity by decreasing both tension and frequency of electrical evoked contraction of the frog gastrocnemius. This is the first study on the relaxant activity of the extracts from *S. varians*. As the extracts have been shown to have ability to decrease muscle contraction, therefore the extracts of *S. varians* can be applied to relieve muscle pain.

CONCLUSION

According to the findings in this study, it can be concluded that the extracts from *S. varians* possess antidiabetic, anti-inflammatory and muscle relaxant activities. Since, the propylene glycol extract exhibits the highest antidiabetic and anti-inflammatory activities compared to methanol and ethanol extracts. Hence, propylene glycol is the better extract for the treatment of diabetes and inflammatory-related disease. Whilst, the methanol extract is the better extract for muscle pain relief. The extracts from *S. varians* can be exploited not only for human health to use as potential target for treating diabetes, inflammatory-related disease and muscle pain, but also be an enormous biological resource, representing one of the most promising sources for new products and industrial applications. This is the first report of the *In vitro* antidiabetic and anti-inflammatory activities and *In vivo* relaxant activity of the different extracts from *S. varians*. Further investigations of the active compounds involved the activities of the extracts and also theirs other pharmacological activities and chemical components should be established.

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