



**Pigment and Nutritional Value of *Spirogyra* spp. in
Sakon Nakhon, Nakhon Phanom and Mukdahan Provinces**

Nopparut Sitthiwong

Program of Biology, Faculty of Science and Technology, Sakon Nakhon Rajabhat University,

680 Nittayo Road, Amphoe Mueang Sakon Nakhon, Sakon Nakhon 47000, Thailand

E-mail: nprstw@hotmail.com

Abstract

Edible freshwater macroalgae, *Spirogyra* spp. (Division Chlorophyta) are normally found in natural water sources and local markets, and are commonly consumed as a traditional type of food in north-eastern Thailand. *Spirogyra* spp. has been found to possess high nutritional value and contain various bioactive compounds. The focus of this research study was to examine the pigments and to assess the nutritional value of the *Spirogyra* spp. samples collected from 12 water resources of Sakon Nakhon, Nakhon Phanom and Mukdahan Provinces. The samples collected from each sampling site were dried and the pigment quantity and nutritional value were analyzed. The highest quantity of chlorophyll *a*, *b* and the carotenoid content were recorded at 4.067, 1.716 and 1.209 mg/g of cell dry weight, respectively. The samples were tested for their basic nutritional value and the highest values of ash, fat, moisture, protein, carbohydrate and energy were recorded at 17.37, 5.51, 14.90, 22.77, 67.13 g/100g and 363 kcal/100g, respectively. Thus, the results of this study support the possibility of using *Spirogyra* spp. as a food supplement and antioxidant products.

Keywords: *Spirogyra*, chlorophyll, carotenoid, nutritional value

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1. Introduction

Algae is very important and well-known as the primary producer of food web in aquatic ecosystems, as it produces oxygen via the photosynthesis pathway [1]. Algae also has many beneficial uses including as a bioindicator or as a biofuel, to purify wastewater, as a lubricant, fertilizers, cosmetics, nutraceutical, animal feed and processed food [2, 3]. Most edible algae are recognized as seaweed, either as brown or red algae. In terms of freshwater algae, the edible algae are planktons which can be cultivated to produce a variety of products and macroalgae [4, 5]. The production of macroalgae biomass are mainly produced in Asian countries such as China, the Republic of Korea, Philippines, Japan and Indonesia [6].

Freshwater macroalgae are used as ingredients in traditional food in northern and north-eastern areas of Thailand. It is often found in natural water sources, especially in rural areas and local markets. There are about 4 genera of well-known edible macroalgae; *Spirogyra* spp., *Cladophora* spp., *Microspora* spp. and *Nostochopsis* spp. These are widely used in a number of food products and supplements, and have been recognized for being medicinal plants [5]. *Spirogyra* spp. is unbranched filamentous green algae in the order Zygnematales due to the spirally coiled chloroplast. It is commonly found in stagnant or slow running water [4]. These algae

also have high nutritional compositions (carbohydrates, proteins, fats, vitamins etc.) [5, 7] and are known to possess bioactive compounds such as those that display antimicrobial and antifungal properties [8-10]. They also are known to inhibit the absorption and synthesis of cholesterol [11], inhibit gastric ulcer formation [12], and to possess immunostimulation [13], antimutagenic and antioxidant properties [14].

In terms of its macroalgae pigments; chlorophyll is composed of a porphyrin ring which is an essential structure for antioxidant activity. Additionally, the most common algal carotene is the β -carotene, which is a precursor of vitamin A and is called provitamin A [15]. Palace *et al.* [16] reported that vitamin A, carotenoids and provitamin A are antioxidants that can be effective in inhibiting the development of heart disease. Most studies of the nutritional values and pigments of *Spirogyra* spp. are evaluated from samples in northern area such as Phrae [7] and Chiang Mai province [17].

The main objective of this research was to study the pigments of *Spirogyra* spp. in certain water sources of Sakon Nakhon, Nakhon Phanom and Mukdahan Provinces. Furthermore, the nutritional value of edible macroalgae were studied. The results of this study can be used to promote the potential of natural resources that can be used as food or nutritional supplements in the future.

2. Materials and Experiment

Spirogyra spp. samples were collected from 7 sites in Sakon Nakhon Province, 2 sites in Nakhon Phanom Province and 3 sites in Mukdahan Province over a two-year period from 2016 to 2018. The samples were collected from a variety of substrates, i.e. rocks, cobbles and tree branches that were immersed in the water. The harvested macroalgae were stored in plastic boxes at low temperatures for identification in the laboratory. The morphology identification of algae was done through the use of certain relevant documents such as those by John *et al.* [18]. The samples were then photographed using an Olympus Normaski microscope.

The samples were washed and dried at 45 °C for 48 hours and then ground. The dried powder of the algae was measured 3 types of pigments and repeated in triplicate. The chlorophyll *a* and *b* samples were analyzed according to the methanol extraction method [19]. Carotenoids were analyzed according to the methods of Punyoyai [19].

The nutritional value; ash, fat, moisture and protein contents were determined by AOAC [20]. Carbohydrate and energy contents were

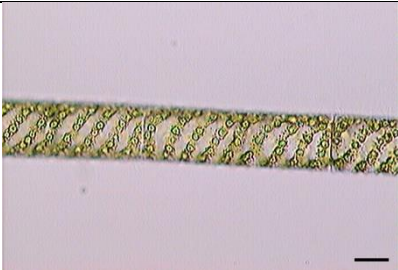
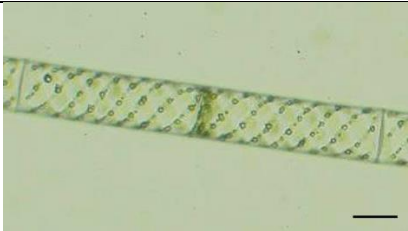
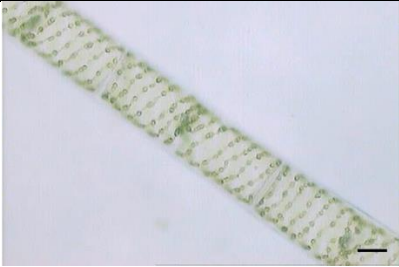
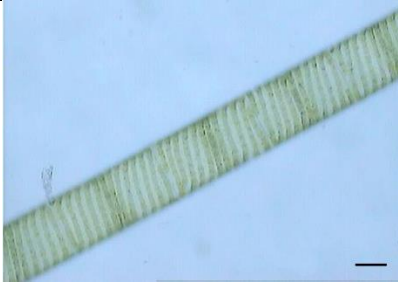
determined by a compendium of method for food analysis [21].

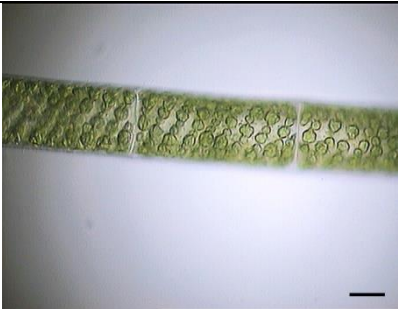
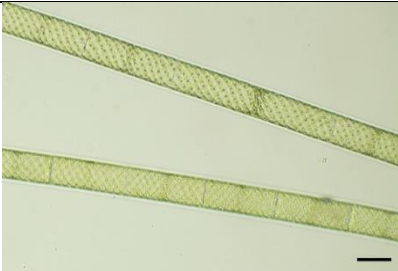
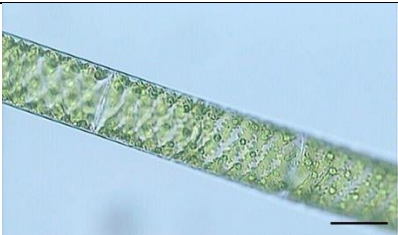
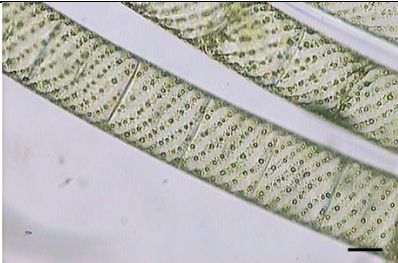
3. Results and Discussion

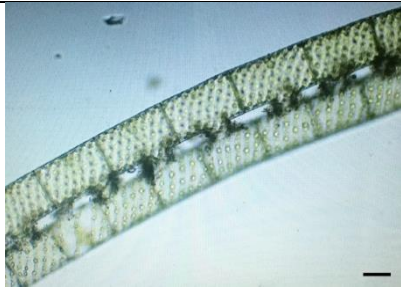
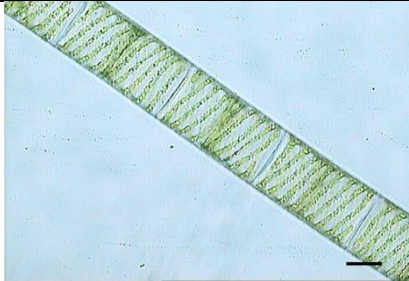
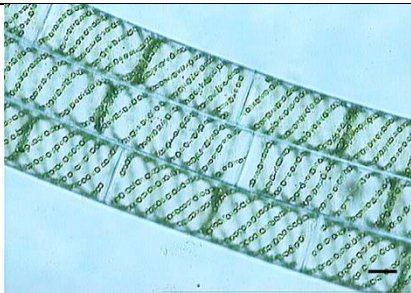

3.1 *Spirogyra* spp. diversity

The macroalgae samples obtained from 12 sampling sites in Sakon Nakhon, Nakhon Phanom and Mukdahan Provinces was recorded as 12 species of *Spirogyra* spp. There are different in diameter of filament, number of chloroplast and number of turn of chloroplast per cell (Table 1). The *Spirogyra* spp. were most commonly present during the rainy and cold seasons and the quantities were found to be sampling site-dependent. The findings were similar to those of the study Kanajan and Chueduengqi [22] at Kham Hom Waterfall, in Sakon Nakhon Province and Moosin [23] at Huay Tham Khae Reservoir, Ubon Ratchathani Province. These researchers revealed that *Spirogyra* spp. was the dominant macroalgae and occurred in the rainy and cold seasons. The reason that *Spirogyra* spp. was found to be the dominant genera of edible macroalgae is that it is present in a wide range of habitats, where it is typically attached to stable substratum. However, it can also occur as free-floating mats and has also been found in standing or slow-running water [1, 18].

Table 1 Diversity of *Spirogyra* spp. in Sakon Nakhon, Nakhon Phanom and Mukdahan Provinces

Samples	Location	Light micrographs of macroalgae
SK1	Irrigation canal, Dong Mafai, Mueang Sakon Nakhon, Sakon Nakhon	 <i>Spirogyra</i> sp.1
SK2	Nam Phung, Mueang Sakon Nakhon, Sakon Nakhon	 <i>Spirogyra</i> sp.2
SK3	Huai Diak, Mueang Sakon Nakhon, Sakon Nakhon	 <i>Spirogyra</i> sp.3
SK4	Nam Un, Nong Lat, Mueang Sakon Nakhon, Sakon Nakhon	 <i>Spirogyra</i> sp.4

Samples	Location	Light micrographs of macroalgae
SK5	Nam Phung Bridge, Tao Ngoi, Sakon Nakhon	 <i>Spirogyra sp.5</i>
SK6	Ban Pung, Na Tong Watthana, Phon Na Kaeo, Sakon Nakhon	 <i>Spirogyra sp.6</i>
SK7	Si Songkhram river, Ban Non Sang Thong, Ban Muang, Sakon Nakhon	 <i>Spirogyra sp.7</i>
NP1	Pond, Pla Pak, Nakhon Phanom	 <i>Spirogyra sp.8</i>

Samples	Location	Light micrographs of macroalgae
NP2	Songkhram river, Don Toei, Na Thom, Nakhon Phanom	
		<i>Spirogyra</i> sp.9
MK1	Phu Pasan, Ban Kho, Khamcha-I, Mukdahan	
		<i>Spirogyra</i> sp.10
MK2	Nong Khamtalad, Lao Sang Tho, Khamcha-I, Mukdahan	
		<i>Spirogyra</i> sp.11
MK3	Nong Phue, Chanot, Wan Yai, Mukdahan	
		<i>Spirogyra</i> sp.12

Scale bar = 50 μ m

3.2 Analysis of *Spirogyra* spp. pigments

In Fig. 1, the amount of chlorophyll *a* of *Spirogyra* spp. obtained from 12 sampling sites

was found to be in the range of 0.718-4.067 mg/g of cell dry weight. The highest chlorophyll *a* quantity was found in SK1 and the lowest quantity

was found in SK2. The amount of chlorophyll *b* was found in the range of 0.056-1.716 mg/g of cell dry weight. The highest amount was recorded in SK5 and the lowest amount was found in SK2. A comparison of the chlorophyll quantities of chlorophyll *a* and *b* revealed that there was a higher quantity of chlorophyll *a* when compared to chlorophyll *b*. According to Pal and Choudhury [15], it was reported that chlorophyll *a* is present in all forms of autotrophic algae, while other types of chlorophyll have more limited distribution and are known to function as accessory photosynthetic pigments. Chlorophyll *b* functions as a light-harvesting pigment that transfers absorbed light energy to chlorophyll *a*. The findings of this analysis are similarly to those of the study of Thamprawat and Srikanchai [24] who reported that chlorophyll *a* quantity was higher than that of

chlorophyll *b* and similar to the quantity of chlorophyll.

In freshwater algae, the major pigment is chlorophyll, so it is present in higher quantities than other pigments. In this study, *Spirogyra* spp. were found to possess a range of carotenoids between 0.126-1.209 mg/g of cell dry weight. The highest quantity was found in SK7 and the lowest was recorded in SK2. The quantity of carotenoids in most samples (except for the samples collected from sites SK6, SK7 and NP2) was similar to the quantity of carotenoids present in *Spirogyra neglecta* (Hassall) Kützinger obtained from Suan Khuean Sub-district, Muang, Phrae Province at 0.399 mg/g of cell dry weight [19]. Furthermore, it was found that the SK1, SK5 and SK7 samples which found high chlorophyll quantity, also found large band of chloroplast as shown in Table 1.

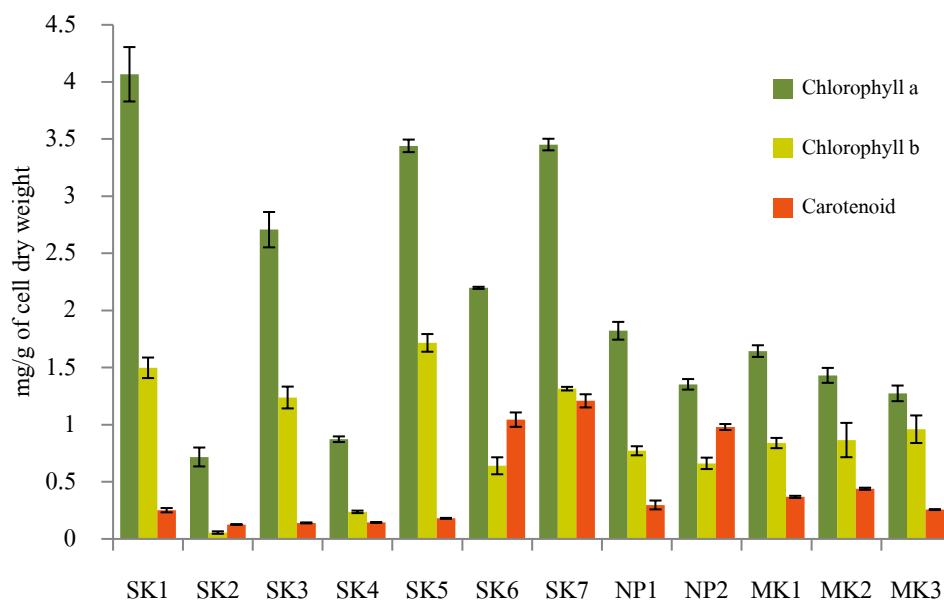


Figure 1 Chlorophyll *a*, *b* and carotenoids in *Spirogyra* spp. collected from Sakon Nakhon, Nakhon Phanom and Mukdahan Provinces

3.3 Nutritional value of *Spirogyra* spp.

In this study (Fig. 2), the *Spirogyra* spp. obtained from 12 sampling sites revealed different levels of nutritional value. In Sakon Nakhon Province, *Spirogyra* sp.2 (SK2), *Spirogyra* sp.4 (SK4) and *Spirogyra* sp.7 (SK7) reported having the highest levels of carbohydrates (67.13 g/100g), moisture (14.90 g/100g) and fat (5.51 g/100g), respectively. The *Spirogyra* sp.10 (MK1) and *Spirogyra* sp.12 (MK3) samples collected from Mukdahan Province showed the highest levels of protein (22.77 g/100g) and energy (363.61 kcal/100g). Additionally, *Spirogyra* sp.9 (NP2) was found to possess the highest level of ash (17.37 g/100g). The nutritional value of *Spirogyra* spp. in this study was similar to that of the samples of *Spirogyra neglecta* (Hassall) Kützing obtained from Suan Khuean Sub-district, Muang, Phrae

Province, which were found to be 23.37 g/100g of protein, 5.54 g/100g of fat, 55.05 g/100g of carbohydrate and 363.58 kcal/100g of energy [7]. Notably, the protein content in the SK4 samples was much less than in the samples collected from Phrae Province. The potential for using *Spirogyra* spp. as a food ingredient or as a supplement is of significant interest because the protein content present in algae is the same as in meat and some samples were found higher than eggs [25]. The Ministry of Public Health [25] reported that eggs, catfish, pork and chicken reveal protein contents of 12.3, 23.0, 19.6, and 19.5 g/100g, respectively. Additionally, *Spirogyra* spp. also revealed carbohydrate and energy levels that were higher than meat as well, but lower than rice, wheat and oat [25].

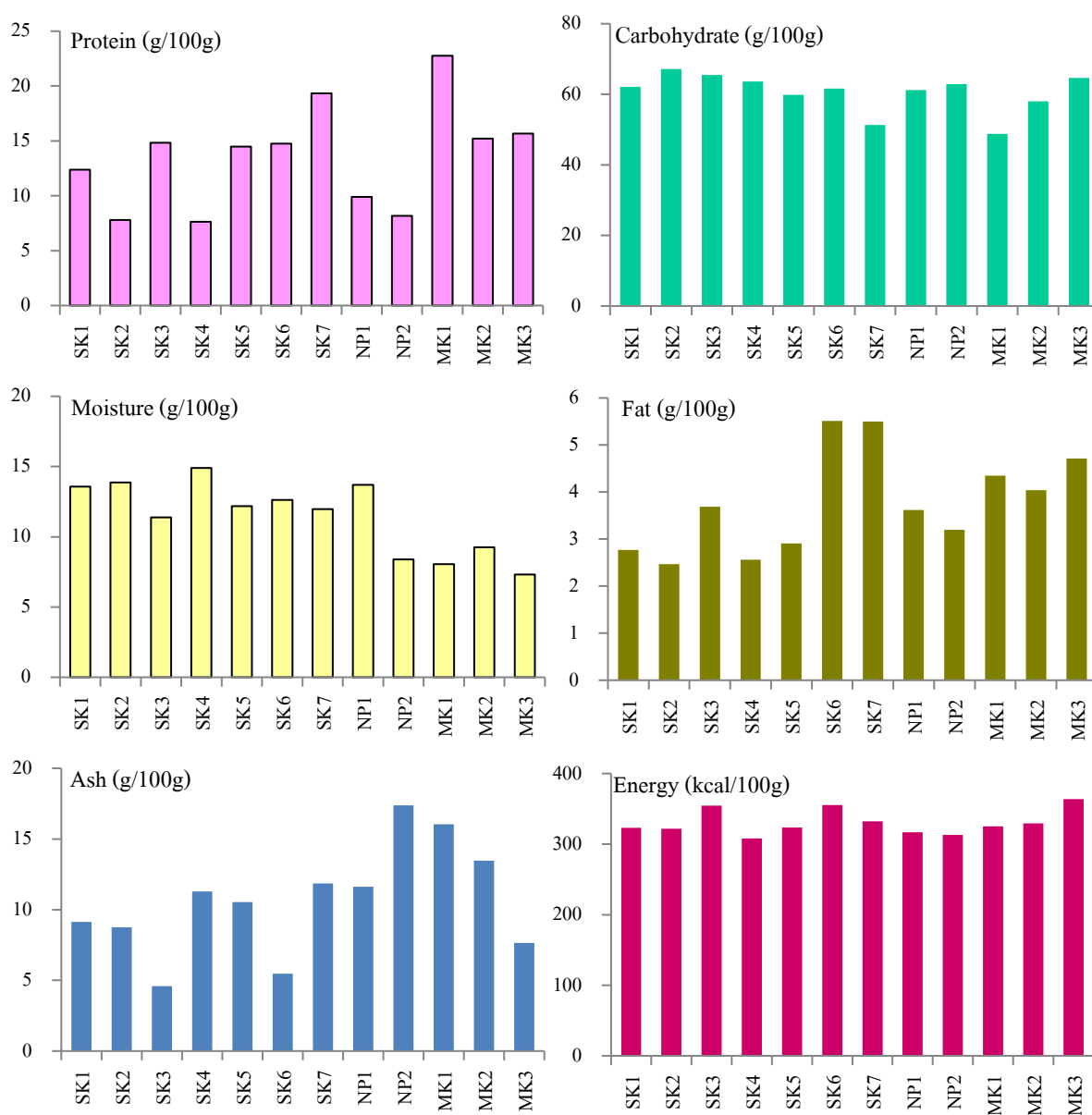


Figure 2 Nutritional value of *Spirogyra* spp. collected from Sakon Nakhon, Nakhon Phanom and Mukdahan Provinces

4. Conclusions

The study of *Spirogyra* spp. diversity was done through the collection of samples from 12 water resources of Sakon Nakhon, Nakhon

Phanom and Mukdahan Province. Twelve species of *Spirogyra* spp. were found. The period of time in which *Spirogyra* spp. was present in all sampling sites was during the rainy and cold

seasons and the quantities were found to be sampling site-dependent. All *Spirogyra* spp. specimens were found to be higher in chlorophyll *a* quantities than chlorophyll *b*. The highest chlorophyll *a*, *b* and carotenoid contents were recorded as 4.067, 1.716 and 1.209 mg/g of cell dry weight, respectively. The samples were tested for 6 types of basic nutritional value. The values were found to be between 4.59-17.37 g/100g of ash, 2.47-5.51 g/100g of fat, 8.05-14.90 g/100g of moisture, 7.63-22.77 g/100g of protein, 48.79-67.13 g/100g of carbohydrate and 308.08-363.71 kcal/100g of energy. The possibility of using *Spirogyra* spp. as a food supplement was found because the nutritional value has been found to be equal to that of meat. Additionally, the pigment quantity of *Spirogyra* spp. has been identified as an effective antioxidant.

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6. References

- [1] Bellinger E.G and Sigeo D.C. *Freshwater Algae: Identification and Use as Bioindicators*. John Wiley & Sons, Ltd., West Sussex. 2010.
- [2] Peerapornpisal Y. *Freshwater Algae in Thailand*. 3rd ed. Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai. 2013.
- [3] Kim S.K. *Handbook of Marine Microalgae Biotechnology Advances*. Academic press, Amsterdam. 2015.
- [4] Peerapornpisal, Y. *Phycology*. Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai. 2006.
- [5] Peerapornpisal Y., Pongsirikul I. and Kanjanapothi D. *Potential of Freshwater Macroalgae as Food and Medicine*. Final report submitted to Thailand Research Fund (TRF). 2005.
- [6] Carlsson A.S., van Beilan J.B., Moller R. and Clayton D. *Micro and Macro Algae: Utility for Industrial Applications*. Cpl press, Newbury. 2007.
- [7] Phinyo K. *Potentials of polysachcharides from Tao [Spirogyra neglecta (Hassall) Kützing] for use as prebiotic*. Ph.D. Thesis. Department of Biology. Chiang Mai University. 2012.
- [8] Naik Ansari A., Hemavani C. and Thippeswamy B. Evaluation of antimicrobial property of *Spirogyra* species. *International Multidisciplinary Research Journal*. 2012. 2(2) : 13-15.
- [9] Kamble S.M., Rokde A.U. and Chavan A.M. Antifungal activity of algal extracts against plant pathogenic fungi. *International Multidisciplinary Research Journal*. 2012. 2(3) : 23-24.

- [10] Snehath D. and Digamber Rao B. Antifungal activity of freshwater green-algae (Chlorophyceae). *European Journal of Pharmaceutical and Medical Research*. 2015. 2(5) : 1394-1402.
- [11] Duangjai A., Limpeanchob N., Trisat K. and Amornlerdpison D. *Spirogyra neglecta* inhibits the absorption and synthesis of cholesterol *in vitro*. *Integrative Medicine Research*. 2016. 301-308.
- [12] Amornlerdpison D., Duangjan K., Kanjanapothi D., Taesotikul T. and Peerapornpisal Y. Gastroprotective activity of *Spirogyra neglecta* (Hassall) Kützing. *KKU Sci. J.* 2012. 40(1) : 236-241.
- [13] Surayot U., Wang J.G., Lee J.H., Kanongnuch C., Peerapornpisal Y. and You S.G. Characterization and immunomodulatory activities of polysachcharides from *Spirogyra neglecta* (Hassall) Kützing. *Bioscience, Biotechnology and Biochemistry*. 2015. 79(10) : 1644-1653.
- [14] Thumvijit T., Inboot W., Peerapornpisal Y., Amornlerdpison D. and Wongpoomchai R. The antimutagenic and antioxidant properties of *Spirogyra neglecta* (Hassall) Kützing. *Journal of Medicinal Plants Research*. 2013. 7(34) : 2494-2500.
- [15] Pal R. and Choudhury A.K. *An Introduction to Phytoplanktons: Diversity and Ecology*. Springer, India. 2014.
- [16] Palace V.P., Khaper N., Qin Q. and Singal P.K. Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. *Free Radic Biol Med*. 1999. 26(5-6) : 746-761.
- [17] Tipnee S. Ramaraj R. and Unpaprom Y. Nutritional evaluation of edible freshwater green macroalgae *Spirogyra varians*. *Emer Life Sci Res*. 2015. 1(2) : 1-7.
- [18] John D.M., Whitton B.A. and Brook A.J. *The Freshwater Algal Flora of the British Isles: an identification guide to freshwater and terrestrial algae*. 2nd ed. Cambridge University Press, Cambridge. 2011.
- [19] Punyoyai T. *Antioxidant activity of Tao, Spirogyra neglecta (Hassall) Kützing*. M.Sc. Thesis. Department of Biology, Chiang Mai University. 2008.
- [20] AOAC. *Official Method of Analysis. The Association of Official Analytical Chemist*. 20th ed. Gaithersburg, Maryland. 2016.
- [21] National Bureau of Agricultural Commodity and Food Standards. *Compendium of Method for Food Analysis*. Ministry of Agriculture and Cooperatives, Bangkok. 2003.
- [22] Kanajan K. and Chueduengqi K. *Investigation of macroalgae in Kham Hom Waterfall, Amphoe Mueang Sakon Nakhon, Sakon Nakhon Province in 2015-2016*.

- Research Project in Biology. Faculty of Science, Sakon Nakhon Rajabhat University. 2017.
- [23] Moonsin P. Diversity of Macroalgae and some Water Quality Parameters in Huay Tham Khae Reservoir, Trakan Phuet Phon District, Ubon Ratchathani Province. *KMUTT Research and Development Journal*. 2016. 30(1) : 37-50.
- [24] Thamprawat J. and Srikanchai N. *Extraction of chlorophylls and carotenoid from Spirogyra* spp. Research Project in Biology. Faculty of Science, Sakon Nakhon Rajabhat University. 2017.
- [25] Ministry of Public Health. *Nutritive Values of Thai Foods*. Department of Health, Ministry of Public Health, Bangkok. 2001.