PROGRESS IN APPLIED SCIENCE AND TECHNOLOGY
Faculty of Science and Technology
Rajamangala University of Technology Thanyaburi
Prog Appl Sci Tech. 2024; 14(1):65-72
ISSN (Print): 2730-3012
ISSN (Online): 2730-3020
https://ph02.tci-thaijo.org/index.php/past

Research Article

Received: February 17, 2024
Revised: April 10, 2024
Accepted: April 18, 2024

DOI: 10.60101/past.2024.252833

Total Phenolic Compound and Antimicrobial Activity of *Spirogyra* spp.

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Abstract

Spirogyra spp. is widely distributed in freshwater habitats and is included in the category of natural resources whose significance and advantages have been researched. In this study, 6 samples of *Spirogyra* spp. were collected: *Spirogyra ellipsospora* Transeua from Bueng Kan province, *Spirogyra* sp.1 from Sakon Nakhon Province and *S. neglecta* (Hassall) Kützing, *S. ellipsospora* Transeua, *Spirogyra* sp.2, and *Spirogyra* sp.3 from Nakhon Phanom province. The highest value of total phenolic compound was measured at 18.53±0.23 mg GAE/mg in *Spirogyra* sp. 2. The evaluation of antimicrobial activity of algal extracts against fungal *Candida* sp. and bacteria *Staphylococcus aureus* and *Escherichia coli* was performed by using the disk diffusion method along with determination of minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC) and minimum bacterial concentration (MBC). The result of disk diffusion and MIC determination showed the efficiency of all extract samples against all tested strains at a concentration of 10 mg/mL. The most effective algal extract against *Candida* sp. showed with the MFC's ranged from 10 to 20 mg/mL, whereas MBC of all extracts destroyed the two tested bacterial strains ranged from 10 to 20 mg/mL within 24 hours and up to 30 mg/mL after 48 hours. The results of this study support that *Spirogyra* spp. contains bioactive compounds that are the basis for further application in cosmeceuticals.

Keywords: *Spirogyra*, Total Phenolic Compound, Antimicrobial Activity, Algae Extract

1. Introduction

Research on the utilization of local bioresources is currently gaining prevalent because researchers hope to increase the value of these resources, enabling them to be managed and planned for in the most effective and efficient ways possible. In particular, the study of the determination of bioactive compounds can be applied in various industries, including medical, food, and cosmetics (1). The active compounds in vegetables, fruits, herbs, and algae are often phenolic compounds that act as precursors in the synthesis of important substances in living organisms (2). It has antioxidant properties and inhibits oxidation reactions. More than 8,000 different phenolic compounds have been found, including simple molecules like phenolic acids, phenylpropanoids, phenol, polyphenol, and flavonoids, as well as complicated structures like lignans and tannins (3, 4). Additionally, bioactive compounds with antimicrobial properties are also widely studied to guide the use of natural extracts as a substitute for antibiotics (2, 5).

Spirogyra spp. is a freshwater macroalgae in Division Chlorophyta that people in the Northeast called "Tao". Many studies have

shown that this algae have a high nutritional value, especially with a protein content similar to meat and eggs, contains minerals and many kinds of vitamins (6, 7). It also has a pharmacological effect with antioxidants, gastroprotective activity (8), anti-inflammatory (9, 10), and antimicrobial effect (11, 12). These algae contain a variety of phytochemicals, including alkaloids, saponins, glycosides, tannins, phenols, flavonoids, terpenoids, phytosterols, and coumarins, that have been reported to have beneficial health effects (13). Furthermore, some strains of *Spirogyra* have been shown in studies to have anticancer properties (14).

This research examined the morphology of *Spirogyra* spp. from water sources in Nakhon Phanom, Sakon Nakhon, and Bueng Kan provinces, which is commonly found and widely eaten as a local food. Algal samples were taken to determine the total phenolic compounds and inhibitory effects of two bacteria: *Staphylococcus aureus* which causes skin abscesses (15) and *Escherichia coli,* which causes gangrene and severe skin inflammation (16). The fungus *Candida* sp. in this research causes skin rashes and diaper rash in children (17). The knowledge gained from this research will guide the potential of freshwater algae, *Spirogyra* spp., to inhibit microorganisms and can be used as information for the further development of pharmacological work.

2. Materials and Experiment

2.1 Sampling site and identification of algae

Spirogyra spp. was collected from water sources in Nakhon Phanom, Sakon Nakhon, and Bueng Kan provinces include 6 sampling sites as follows:

A: Nongwailum, Ban Pungkae, Tha Champa, Tha Uthen, Nakhon Phanom province (17°34'38.0"N 104°25'10.7"E)

B: He River, Ban Donhko, Pong Hai, Seka, Bueng Kan province (18°00'11.6"N 103°52'48.2"E)

C: Huaibong, Ban Kokang, Na Phiang, Kusuman, Sakon Nakhon Province (17°23'12.3"N 104°14'02.5"E)

D: Huainakham, Ban Nakham, Ban Kho, Phon Sawan, Nakhon Phanom province (17°27'13.8"N 104°14'57.3"E)

E: Huaitan, Ban Kudnamsai, Na Ngua, Na Wa, Nakhon Phanom province (17°24'52.3"N 104°03'07.0"E)

F: Nongelon, Ban Kudnamsai, Na Ngua, Na Wa, Nakhon Phanom province (17°24'40.8"N 104°03'02.7"E)

Spirogyra spp. samples were washed and photographed under a light microscope (Nikon E 600). The identification of the algae's morphology such as size, number of chloroplasts, and chloroplast turnover number was done by referencing of relevant studies, including one by John et al. (18).

2.2 Determination of total phenolic compound

Samples of *Spirogyra* spp. were dried for 24 hours at 60°C and ground to prepare the extract. Add 400 mL of distilled water to ten grams of dried samples and boil at 95°C for 1 hour and 20 minutes. Filtered the sample and evaporated the water from the extract. The extract was dissolved in distillated water and taken to a concentration of 100 mg/mL for total phenolic compounds analyzed by the Folin-Ciocalteu method (19). The phenolic content was shown as Gallic acid equivalents (GAE) of 1 g of extract. The experiment was performed in triplicate. The data of phenolic compound amount was analyzed by one-way factorial ANOVA (Analysis of Variance) and considered statically significant at $P<0.05$.

2.3 Determination of antimicrobial activity

2.3.1 Preparation of sample extracts

Add 20 g of dried and crushed *Spirogyra* spp. to 150 mL of 95% (v/v) ethanol, then give it a thorough shake. After 4 hours at room temperature, centrifuge at 6,000 rpm for 15 minutes, filter the liquid fraction, and evaporate the solvent for further analysis.

2.3.2 Antimicrobial assay

The disk diffusion method is used to evaluate the antimicrobial activity of each *Spirogyra* spp. extract by applying the method of Mostafa et al. (20) and Yosboonruang et al. (21). Potato dextrose agar (PDA) was used to cultivate *Candida* sp. TISTR 5070, while nutrient agar (NA) was used to cultivate *S. aureus* TISTR 746 and *E. coli* TISTR 073. These strains, were harvested in sterile normal saline and adjusted cell concentration using the McFarland No. 0.5 standard, then swabbed on culture media using sterile cotton swabs. Each extract from 2.3.1 was diluted with dimethyl sulfoxide (DMSO) to a concentration of 2.5, 5.0, 7.5, and 10 mg/mL, then loaded with 5 µL onto sterile filter paper

discs (6 mm in diameter) placed on the media surface. DMSO was used as a negative control. The plates were incubated at 37°C for 24 hours for bacteria and up to 72 hours for fungal respectively. The inhibition zones were measured by a vernier caliper and considered for antimicrobial activity.

2.3.3 Determination of minimum inhibitory concentration (MIC) by broth dilution test

Different concentrations of the algal extract (2.5, 5, 10, 20, 30, 40, and 50 mg/mL) were prepared as a two-fold diluted mixture by dissolving to a final volume of 2 mL of potato dextrose broth (PDB) for fungi (*Candida* sp. TISTR 5070) and NA for bacteria (*S. aureus* TISTR 746 and *E. coli* TISTR 073) respectively. Then, 18-hour-old bacterial and fungal cells grown in broth media were adjusted to the cell concentration to the McFarland No. 0.5 standard, and later transferred of 2 mL into the extract mixture. All tubes were incubated at 37°C for 24 hours for bacterial and up to 72 hours for fungi respectively. After incubation, all tubes were examined for the presence or absence of microbial growth.

2.3.4 Determination of minimum fungicidal concentration (MFC) and minimum bacterial concentration (MBC) by agar dilution test

Samples were taken from the three lowest concentrations of the algal extract tubes exhibiting invisible growth (from MIC test) and subcultures onto sterile PDA for fungal and NA for bacterial. All plates were incubated at 37°C for 24 - 48 hours for bacterial and up to 72 - 96 hours for fungal respectively. Then, examined for microbial growth in corresponding to algal extract concentration. The MFC and MBC were taken as the concentration of algae extracts that did not exhibit any microbial growth on the newly inoculated agar plates (20).

3. Results and Discussion

3.1 Morphology of *Spirogyra* spp.

The morphology of *Spirogyra* spp. from 6 sampling sites was different in length, number of chloroplasts, and chloroplast turnover number (Figure 1). The samples from sampling site A were classified as *Spirogyra neglecta* (Hassall) Kützing, B and E samples were found as *S. ellipsospora* Transeua. *Spirogyra* spp. are commonly found in shallow standing or slow-running waters, with *S. neglecta* found mainly in the north, especially in Phrae province (21-23) and in the northeast, particularly Maha Sarakham (24)] and Sakon Nakhon Province (10). *S. ellipsospora* is reported to be found in the north especially Chiang Mai (25) and Chiang Rai province (26).

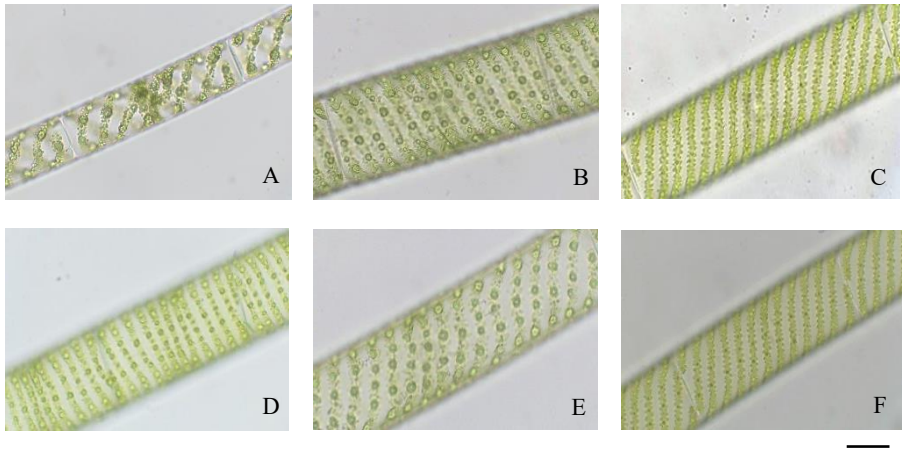

Figure 1 *Spirogyra* spp. from 6 sample sites
A: *Spirogyra neglecta* (Hassall) Kützing, B, E: *Spirogyra ellipsospora* Transeua,
C: *Spirogyra* sp.1, D: *Spirogyra* sp.2, F: *Spirogyra* sp.3.

3.2 Total phenolic compound of *Spirogyra* spp.

A study of the phenolic content of *Spirogyra* spp. extract showed that sample D, *Spirogyra* sp. 2, had the highest total phenolic compound content of 18.53±0.23 mg GAE/mg and was significantly different from other samples, followed by sample E with a value of 16.97±0.38 mg GAE/mg and sample B with a value of 15.50±0.48 mg GAE/mg. The samples A, C, and F were non-significantly different (Figure 2). The phenolic content in this study is similar to the research of Assawarachan et al. (27), which found the total phenolic compound in *Spirogyra* sp. was 14.84–17.64 mg GAE/mg. Furthermore, *Spirogyra neglecta* (Hassall) Kützing, sample A, had a phenolic content of 12.17±0.33 mg GAE/mg. This is smaller than the results of Rattanapot et al. (28) on the same species and the water extract, which showed 92.95 mg GAE/mg. The content of phenolic compounds was different, which may be due to group of substances and environmental factors such as geographic habitat, water nutrients, season, and water temperature (29-30).

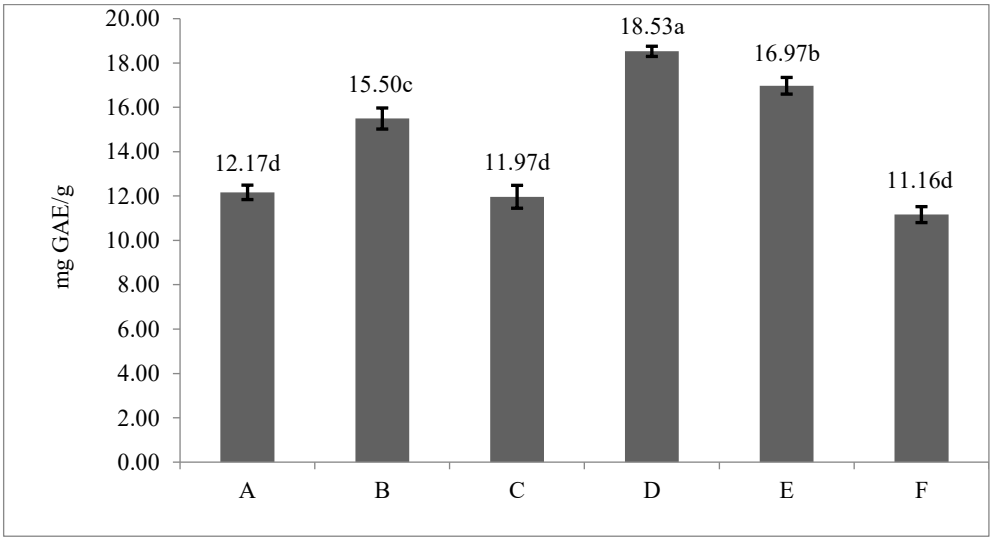

Figure 2 Total phenolic compound of 6 samples of *Spirogyra* spp. extract.
*Different letters were significantly different by Tukey HSD test at $p<0.05$

3.3 Antibacterial activity of *Spirogyra* spp. extract by the disc diffusion method

The 6 samples of *Spirogyra* spp. crude extracts were used to inhibit the growth of *Candida* sp., *S. aureus,* and *E. coli* using 4 different concentrations of 2.5, 5.0, 7.5, and 10 mg/mL, and using DMSO as a negative control. The results showed that all extract samples were effective in inhibiting the growth of *Candida* sp. at a concentration of 10 mg/mL. *S. aureus* is inhibited at concentrations of 7.5 and 10 mg/mL, while *E. coli* was inhibited by all concentrations of all samples (Table 1, Figures 3-5). Based on the result, it revealed the potential of the *Spirogyra* spp. extract to inhibit both groups of microorganisms, which corresponds to previous studies reporting that *Spirogyra* spp. contain many bioactive compounds that have potential antimicrobial agents against fungi and Gram-positive and Gram-negative bacteria (31-34).

Table 1 Inhibitory activity against *Candida* sp., *S. aureus,* and *E. coli* by *Spirogyra* spp. extract.

S.	Inhibitory zone (mm) at various extract concentrations (mg/mL)											
	Candida sp.				*S. aureus*				*E. coli*			
	2.5	5	7.5	10	2.5	5	7.5	10	2.5	5	7.5	10
A	-	-	-	6.22±0.12	-	-	6.33±0.23	6.63±0.65	6.98±0.51	7.63±0.39	7.83±0.33	10.83±0.51
B	-	-	-	6.03±0.23	-	-	6.22±0.08	6.54±0.11	6.92±0.71	7.18±0.51	7.18±0.40	9.35±0.60
C	-	-	-	5.59±0.35	-	-	6.68±0.09	6.72±0.33	7.79±0.11	8.65±0.75	9.19±0.38	13.25±0.41
D	-	-	-	6.44±0.27	-	-	6.20±0.25	6.41±0.49	6.36±0.36	6.90±0.07	7.60±0.72	10.38±0.66
E	-	-	-	6.39±0.14	-	-	6.38±0.16	6.76±0.16	7.28±0.41	7.82±0.21	9.05±0.63	11.28±0.29
F	-	-	-	5.75±0.14	-	-	6.46±0.31	6.85±0.12	8.09±0.20	8.45±0.68	8.65±0.78	11.10±0.68

Note: S. = Samples, - = no inhibition of growth

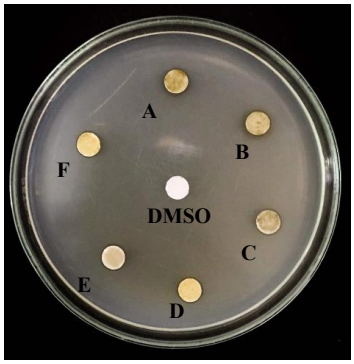

Figure 3 Inhibition zone of *Candida* sp. by six samples of *Spirogyra* spp. extract at a concentration of 10 mg/mL.

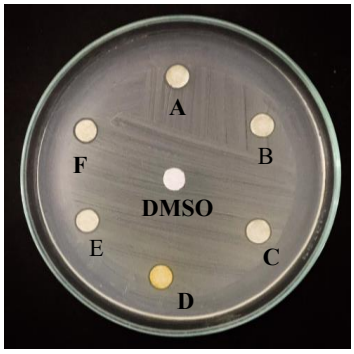

Figure 4 Inhibition zone of *S. aureus* by six samples of *Spirogyra* spp. extract at a concentration of 10 mg/mL.

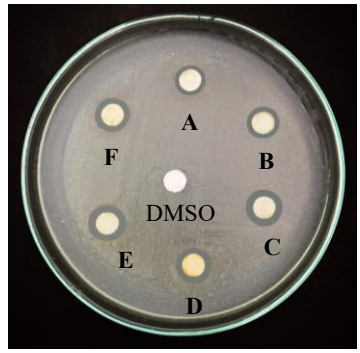

Figure 5 Inhibition zone of *E. coli* by six samples of *Spirogyra* spp. extract at a concentration of 10 mg/mL.

3.4 The minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC) and minimum bacterial concentration (MBC)

The MIC, MFC, and MBC of the most effective algae extracts were evaluated and reported in Table 2. The inhibitory effect of all samples of *Spirogyra* spp. extracts revealed that the MIC value started at 10 mg/mL can against *Candida* sp., *S. aureus,* and *E. coli.* The MFC and MBC values were confirmed by considering the absence of microbial growth of the tested strains corresponding to their lowest MIC's algal extracts by using the spread plate technique.

After incubating at 37°C for 72 and 96 hours, it was found that *Candida* sp. was destroyed at the lowest concentration (MFC) of

algal extract A-E as 10 mg/mL, while sample F was 20 mg/mL. The results are in accordance with previous study by Adrien, Gitu, Oyaro (31) and Mohammed, Al-Katib (33) which reported that algal extract from *Spirogyra* sp. could inhibit and destroy *C. albicans*. Moreover, there is also a study by Yousif, Dwish & Shafiq (35) that reports that the antifungal activity of *Spirogyra* sp. extract can inhibit other fungi (*Fusarium oxysporum*) as well.

The lowest concentration of algal extracts that can destroy *S. aureus* and *E. coli* (MBC) after 24 hours were 10 mg/mL (for samples A and C) and 20 mg/mL (for samples A, B and E) respectively. After 48 hours, the MBC value of all extract samples for the elimination of both strains was up to 30 mg/mL. This result showed that the incubation time affects the stability of the antibacterial activity of all algal extracts. When incubation time was increased, the efficiency of inhibiting bacteria also clearly decreased. As a result, this factor should be further confirmed and studied in the future.

In addition, factors affecting the efficiency of antimicrobial activity of algal extracts also depend on algae species, type of solvent extraction, environmental factors, and seasonal changes that affect algal growth, resulting in different production of secondary metabolites of algae (12).

Table 2 Minimum concentration of *Spirogyra* spp. extract that inhibits and destroys *Candida* sp., *S. aureus,* and *E. coli.*

Samples	*Candida* sp.			*S. aureus*			*E. coli*		
	MIC (mg/mL)	MFC (mg/mL)		MIC (mg/mL)	MBC (mg/mL)		MIC (mg/mL)	MBC (mg/mL)	
		72 hr.	96 hr.		24 hr.	48 hr.		24 hr.	48 hr.
A	10	10	10	10	10	30	10	20	30
B	10	10	10	10	20	30	10	20	30
C	10	10	10	10	10	30	10	30	30
D	10	10	10	10	20	30	10	30	30
E	10	10	10	10	20	30	10	20	30
F	10	20	20	10	20	30	10	30	30

4. Conclusions

Spirogyra spp. were recoverd from samples collected from 6 water sources in Nakhon Phanom, Sakon Nakhon, and Bueng Kan provinces. It was classified as *Spirogyra neglecta* (Hassall) Kützing, *S. ellipsospora* Transeua, *Spirogyra* sp.1, *Spirogyra* sp.2, and *Spirogyra* sp.3. The total phenolic compound was found to be between 11.16–18.53 mg GAE/mg. All samples of *Spirogyra* spp. extracts can act against *Candida* sp., *S. aureus,* and *E. coli.* Therefore, the results obtained from this study support the use of algae in cosmeceutical medicine or other applications of algae.

Acknowledgements

The researchers would like to thank the Program of Biology, Faculty of Science and Technology, Sakon Nakhon Rajabhat University for providing research equipment and greatly appreciate Ms. Phongsupa Chunthachaiyapoom and Ms. Onriya Sisuoy for sample collection and operation execution.

Declaration of conflicting interests

The authors declared that they have no conflicts of interest in the research, authorship, and this article's publication.

References

1. Alara OR, Abdurahman NH, Ukaegbu CI, Azhari NH. *Vernonia cinerea* leaves as the source of phenolic compound, antioxidants, and anti-diabetic activity using microwave-assisted extraction technique. Ind Crops Prod. 2018;122:533-44.
2. Balasundram N, Sundrum K, Samman S. Phenolic compound in plants and agri-industrial by products: Antioxidant activity, occurrence, and potential uses. Food Chem. 2006;99(1):191-203.
3. Alara OR, Abdurahman NH, Ukaegbu CI. Extraction of phenolic compounds: A review. Curr Res Food Sci. 2021;4:200-14.

4. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compound from medicinal plants for pharmaceutical and Medical Aspects: An Overview. Medicines. 2018;5(93):1-16.
5. Pokharkar RD, Funde PE, Pingale S, Gavshete SL, Vidhate KD. Study of antifungal activity of ethanolic extract of chlorophyta *Spirogyra* on yeasts and dermatophytes. Pharmacologyonline. 2008;3:40-5.
6. Peerapornpisal Y. Phycology: Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai; 2006.
7. Sitthiwong N. Pigment and nutritional value of *Spirogyra* spp. in Sakon Nakhon, Nakhon Phanom and Mukdahan Province. Sci. & Tech. RMUTT J. 2019;9(1):10-21.
8. Amornlerdpison D, Duangjan K, Kanjanapothi D, Taesotikul T, Peerapornpisal Y. Gastroprotective activity of *Spirogyra neglecta* (Hassall) Kützing. KKU Sci J. 2012;40(1):236-41.
9. Mesbahzadeh B, Rajaei SA, Tarahomi P, Seyedinia SA, Rahmani M, Rezamohamadi F, Kakar MA, Moradi-Kor N. Beneficial effects of *Spirogyra neglecta* extract on antioxidant and anti-inflammatory factors in streptozotocin-induced diabetic rats. BioMol Concepts, 2018;9:184-9.
10. Yongkhamcha B, Buddhakala N. Phytochemical compositions, nutritional contents, cytotoxicity and anti-inflammatory activity of different extracts from *Spirogyra neglecta* (Hassall) Kützing. Trends Sci. 2023;20(4):6528.
11. Patil KJ, Patil VA, Mahajan SR, Mahajan RT. Bio-activity of algae belonging to Bhusawal region, Maharashtra. Curr. Bot. 2011;2(1):29-31.
12. Naik Ansari A, Hemavani C, Thippeswamy B. Evaluation of antimicrobial property of *Spirogyra* species. Int Multidiscip Res J. 2012;2(2):13-5.
13. Wizi J, Ni L, Darkwah WK, Xianglan L. Analysis of bioactive compounds from different algae samples extracted with ultrasound: characterization, phytochemical contents and antioxidant potentials. Pharmacogn. Res. 2022;14(1):35-44.
14. Kumar J, Dhar P, Tayade AB, Gupta D, Chaurasia OP, Upreti DK, Tappo K, Arora R, Suseela MR, Srivastava RB. Chemical composition and biological activities of trans-Himalayan alga *Spirogyra porticalis* (Muell.) Cleve. PLoS One. 2015;10(2): e0118255.
15. Kobayachi SD, Malachowa N, DeLeo FR. Pathogenesis of *Staphylococcus aureus* abscesses. Am J Pathol. 2015;185(6):1518-27.
16. Hammond AM, Satcher KG, Bender NR, Schoch JJ, Motaparthi K. Necrotizing *Escherichia coli* skin and soft tissue infection with malakoplakia-like features mimicking pyoderma gangrenosum. JAAD Case Rep. 2021;12:1-4.
17. Janniger CK, Schwartz RA, Szepietowski JC, Reich A. Intertrigo and common secondary skin infections. Am Fam Physician. 2005;72(5):833-8.
18. John DM, Whitton BA, Brook AJ. The Freshwater Algal Flora of the British Isles: an identification guide to freshwater and terrestrial algae: Cambridge University Press; 2011.
19. Boonchum W. Antioxidant activity of some marine algae from the gulf of Thailand and the application cosmeceutical product: Chiang Mai University, Chiang Mai; 2011.
20. Mostafa AA, Al-Askar AA, Almaary KS, Dawoud TM, Sholkamy EN, Bakr MM. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi J Biol Sci. 2018;25:361–6.
21. Yosboonruang A, Duangjai A, Amormlerdpison D, Viyoach J. Screening for biological activities of *Spirogyra neglecta* water extract. Walailak J. Sci. & Tech. 2020;17(4):359-68.
22. Punyoyai T. Antioxidant activity of Tao, *Spirogyra neglecta* (Hassall) Kützing: Chiang Mai University, Chiang Mai; 2008.
23. Thumvijit T, Taya S, Punvittayagul C, Peerapornpisal Y, Wongpoomchai R. Cancer chemopreventive effect of *Spirogyra neglecta* (Hassall) Kützing on diethylnitrosamine - induced hepatocarcinogenesis in rats. Asian Pac J Cancer Prev. 2014;15(4):1611-6.

24. Insumran Y, Jansawang N, Jenakoon S, Sanwung S, Ruangchai N. Development of traditional chili paste from fresh water algae (*Spirogyra neglecta* (Hassall) Kützing). Prawarun Agr J. 2022;19(1):88-94.
25. Wangsawad P, Peerapornpisal Y. Molecular identification and phylogenetic relationship of green algae, *Spirogyra ellipsospora* (Chlorophyta) using ISSR and rbcL markers. Saudi J Biol Sci. 2014;21:505-10.
26. Waiyaka P, Prasertsin T, Pukumpuang W. Tao and Lanna Lifestyle: The institute of biodiversity & environment for local and ASEAN development Chiang Rai Rajabhat University; 2017.
27. Assawarachan R, Nookong M, Chailungka N, Amornlerdpison D. Effects of microwave power on the drying characteristics, color and phenolic content of *Spirogyra* sp. J Food Agric Environ. 2013;11(1):15-8.
28. Rattanapot T, Menhumphan K, Srimaroeng C, Junthip R, Amornlrrdpison D. Antioxidant activity of *Spirogyra* sp. and effect of its supplementation on growth performance of *Tilapia* in cage culture. J Fish Technol Res. 2012;6(2):23-43.
29. Eseberri I, Trepiana J, Léniz A, Gómez-Garcia I, Carr-Ugarte, González M, Portillo MP. Variability in the beneficial effects of phenolic compounds: A review. Nutrients. 2022;14(9):1925.
30. Besednova NN, Andryukov BG, Zaporozhets TS, Kryzhanovsky SP, Kuznetsova TA, Fedyanina LN, Makarenkova ID, Zvyagintseva TN. Algae polyphenolic compounds and modern antibacterial strategies: current achievements and immediate prospects. Biomedicines. 2020;8(9):342.
31. Adrien R, Gitu LM, Oyaro N. Mineral composition, antioxidants and antimicrobial activities of freshwater algae (*Spirogyra* genus) from Jomo Kenyatta University of Agriculture and Technology (JKUAT). World Rural Obs. 2014;6(2):86-91.
32. Thomas NV, Ghafour DD, Diyya ASM, Ismail RR, Jalal LK. Antibacterial effects of the organic crude extracts of freshwater algae of Sulaymaniyah, Kurdistan Region, Iraq. J Med Plant Res. 2021;15(4):178-87.
33. Mohammed DH, Al-Katib MA. Active and phenolic compounds in *Spirogyra* sp. PDNA1 isan antibiotic for some bacteria and fungi. Al-Kitab J Pure Sci. 2023;7(1):100-13.
34. Guleria S, Chawla P, Relhan A, Kumar A, Bhasin A, Zhou JL. Antibacterial and photocatalytic potential of bioactive compounds extracted from freshwater microalgae species (*Spirogyra* and *Ocillatoria*): A comparative analysis. Sci. Total Environ. 2024;912:169224.
35. Yousif DYM, Dwish AS, Shafiq SA. Antifungal activity of algal *Spirogyra* sp. fungal *Fusarium oxysporum*. World J Pharm. Res. 2015;4(1):1620-8.

