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Exploring the Antimicrobial Potential of *Spirogyra neglecta* against Mastitis-Inducing Pathogens

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ABSTRACT

Mastitis is an inflammation of the mammary gland that significantly threatens dairy productivity. With the emergence of antibiotic-resistant pathogens, it has become necessary to find alternative antimicrobial agents. *Spirogyra neglecta*, a filamentous green alga, is known for its diverse bioactive compounds. This study uses various solvents to investigate the antimicrobial potential of *S. neglecta* extracts against mastitis-inducing pathogens. The results show that methanol extract has the highest efficacy in inhibiting bacterial growth, with minimum inhibitory concentration (MIC) values ranging from 8.75-200mg/mL. These findings highlight the promising antimicrobial capabilities of *S. neglecta* and its potential as a natural agent in combatting mastitis-inducing pathogens. This research paves the way for further exploration and utilization of *S. neglecta* in veterinary and pharmaceutical applications.

Key words: *Spirogyra neglecta*, Mastitis, Antibacterial, Alternative therapeutics.

INTRODUCTION

Mastitis, a mammary gland inflammation, is a prevalent and economically significant disease affecting dairy cattle worldwide (Meçaj et al. 2023). Bacterial infections primarily cause it; pathogens, such as *Staphylococcus aureus*, *Streptococcus agalactiae* and Mycoplasma, are disease-causing microorganisms that can harm the human body, which can cause infections and illnesses in humans and animals. This text seems grammatically correct and contains no spelling or punctuation errors. However, it may be helpful to provide additional context or information depending on the intended purpose of the text. spp. (contagious) or *Escherichia coli*, *Enterococcus* spp., coagulase-negative *Staphylococcus* and *Streptococcus uberis* (environmental). The excessive use of antibiotics has resulted in the development of bacteria resistant to multiple drugs, necessitating the exploration of alternative therapies. (Abebe et al. 2016; Tomani´c et al. 2022). This leads to reduced milk yield and financial losses for producers. The rise in plant-based treatments for bacterial infections is attributed to their natural, safe, accessible and cost-effective nature compared to synthetic antibiotics. Additionally, these herbal remedies are more readily accepted by the populace. These factors have spurred a surge in global research efforts, leading to exploring various plants' antibacterial properties in recent years (Vaou et al. 2021). Algae have been used by humans for thousands of years, mainly because algae are rich in proteins, carbohydrates, fiber, vitamins, and minerals, valuable foods due to their low-calorie and low-fat content (Mendes et al. 2022). Many physiologically active edible and medicinal algae chemicals have anti-inflammatory, antibacterial and antioxidant properties (Khalid et al. 2018; Buddhakala and Yongkhamcha 2023). Natural compounds sourced from diverse origins exhibit potential as viable substitutes for traditional antibiotics. Among these, algae have garnered interest owing to their varied biochemical composition and bioactive attributes. *Spirogyra neglecta*, a filamentous green alga, stands out for its abundant secondary metabolites and phytochemical content, signifying its promise for therapeutic purposes (Peerapornpisal et al. 2012; Dwaish et al. 2016; Mesbahzadeh et al. 2018; Yosboonruang et al. 2020). *Spirogyra* sp. exhibits valuable curative properties, serving

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as a remedy for ovicidal, antifungal, antibacterial immunostimulant, and acaricidal activities (Ontawong et al. 2013; Thumvijit et al. 2014; Surayot et al. 2015; Arjsri et al. 2021; Yongkhamcha and Buddhakala, 2023). As Yosboonruang et al. (2020) reported, isolating an antibacterial compound from *S. neglecta* has demonstrated activity against pathogens, including *S. aureus*, *S. epidermidis*, *E. coli* and *Pseudomonas aeruginosa*. The current study assessed the antibacterial effects against mastitis-causing pathogens properties of *S. neglecta*.

MATERIALS AND METHODS

Ethical approval

Before conducting their research, the research team received ethical clearance from the Animal Ethics Committee of the Research and Development Institute, Rajabhat Maha Sarakham University. The committee reviewed the team's responses to the conditions placed upon ethical approval, which usually takes one to six months. The Animal Ethics Committee approved the research under the Animal Ethics Approval Certificate Number U1-04504-2559.

Sample preparation

S. neglecta were collected from the Kang Laungjan River in Maha Sarakham Province, Thailand (16°10′56″ N, 103°16′21′ E) and subsequently identified using both morphological techniques. These samples were then thoroughly rinsed and dried at 50°C in an oven. Following this, the dried *S. neglecta* was finely ground into a powder. A quantity of 20 grams of this finely dried algae powder was subjected to extraction with 100mL of various solvents (including ethanol, methanol, acetone, and chloroform) for 24 hours at room temperature. Subsequently, the extract underwent filtration using Whatman No. 4 filter paper. The solvent was then removed via a Rotary evaporator. The extracts were concentrated under reduced pressure and stored at -20°C until further analysis.

Microbial isolates

Milk samples were procured from dairy farms affected by mastitis in Maha Sarakham Province, Thailand. Subsequently, these samples were transported to the laboratory at the Department of Biology, Maha Sarakham Rajabhat University, Thailand, using morphological and molecular techniques. The extracted DNA sample and PCR conditions were described in our previous report (Lager et al. 2021). Purification of PCR products from bacteria using a PCR purification kit (RBC Bioscience, Taiwan) and sequences of PCR by Macrogen DNA (Seoul, Korea) and were alignment search tool (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). 16S rRNA sequencing confirmed >99.9% identity to mastitis bacteria type strains for all strains, including wild and biocide-primed (data not shown). The sequences have been deposited in GenBank and can be accessed using the accession number (Table 1).

Antimicrobial activity assays

The effectiveness of *S. neglecta* extract against bacteria was evaluated using the disk diffusion method. The bacteria were cultured in Muller-Hinton broth (MHB) at 37°C for 24 hours. The cultured bacteria were swabbed onto the surface of a 24-hour Muller Hinton Agar (MHA) dish with sterile cotton before testing. Twenty microliters of the algae extract solution at concentrations of 17.5, 25, 50, 100, and 200 mg/mL were pipetted onto each 6 mm diameter paper disk and allowed to dry. Antibiotic disks soaked with Genfloxcin and DMSO disks were positive and negative control groups, respectively. The specimens were kept in an incubator set at a temperature of 37°C for a period ranging from 24 to 48 hours. Zone diameters in millimeters were precisely measured and compared with the control group. All results were carried out in five plicates and expressed as mean ± standard deviation (SD).

Table 1: Bacterial species and their corresponding GenBank accession numbers associated with mastitis

Species	Culture accession No.	GenBank accession number
S .lugdunensis	M008011	OP217990
S. aureus	M007012	OP217991
S. epidermidis	M236021	OP217992
S. lutrae	M208011	OP217993
S. haemolyticus	M236011	OP217994
S. chromogeneus	M261021	OP217995
S. capitis	M052011	OP217996
S. condiment	M011022	OP217997
S. lugdunensis	M259011	OP217998
E. coli	M225012	OP217999
E. coli	M208012	OP218000

Minimum Inhibitory Concentration (MIC) testing

Using the microdilution approach according to Nguyen et al. (2020). A broth micro-dilution assay in the sterile tube was used to determine the values of MIC of the *S. neglecta* extract toward the four solvents. In brief, the samples were made using dimethylsulfoxide (DMSO) and then diluted in twofold serial dilutions of 500µL of 500µL Muller Hinton Broth (MHB). Afterward, 500µL of bacterial suspension (5×10^8 CFU/mL) were cultured at 37°C for 48h. After 48h incubation, 10µL of resazurin indicator solution (0.01%) was added to each tube. Post-incubation with resazurin for one hour at 37°C, the color change was assessed visually. MIC was defined as the lowest concentrations at which the blue color of the indicator remained, indicating no bacterial growth, or changed to slightly purple, indicating prominent growth inhibition. For the fidelity of the experiments, the bioassay was initially carried out with wide concentration ranges of extracts, followed by a narrower concentration series. MHB, MHB bacteria and DMSO were positive control groups. MIC was determined to be the lowest concentration of *S. neglecta* extracts with no bacterial growth in the tubes.

Analysis of volatile compounds using Gas Chromatography-Mass Spectrometry (GC-MS)

The Medicinal Plant Innovation Center at Mae Fah Luang University, Thailand, identified the compounds extracted from the algae.

RESULTS

The minimum inhibitory concentration (MIC) of *S. neglecta* varied depending on the solvent used. Methanol and crude ethanol extracts showed the highest bacteriostatic activity, with MIC values ranging from 8.75-200mg/mL. The acetone and chloroform crude extracts had MIC values

ranging from 50-200mg/mL. However, methanol extraction of *S. neglecta* produced a minimum inhibitory concentration (MIC) of 8.75mg/mL against all mastitis-causing bacteria, except for *S. condiment* M011022, against which it was not effective. *S. neglecta* from ethanol was discovered to be effective against most types of Gram-positive and Gram-negative bacteria, except for *S. condiment* M011022. It inhibited all types of bacteria with an MIC value of 25, except for *E. coli* M225012 and *E. coli* M208012, which had an 8.75 and 17.5mg/mL MIC value. An extract of *S. neglecta* obtained using chloroform and acetone solvents was effective against most Gram-positive and Gram-negative bacteria, with an MIC value of 50mg/mL. However, the chloroform extract did not inhibit the growth of *S. condiment* M011022, *S. lugdunensis* M008011, *S. aureus* M007012 and *S. epidermidis* M236021. On the other hand, the acetone extract was effective in inhibiting both Gram-positive bacteria (*S. lutrae* M208011, *S. haemolyticus* M236011, *S. capitis* M052011 and *S. lugdunensis* M259011) and Gram-negative bacteria (*E. coli* M225012 and *E. coli* M208012). However, acetone solvent in *S. neglecta* extracts was the least effective in inhibiting mastitis-causing bacteria. An extract of *S. neglecta* from chloroform and acetone solvent was effective against most Gram-positive and Gram-negative bacteria, with a MIC value of 50mg/mL. However, Chloroform did not inhibit the growth of *S. condiment* M011022, *S. lugdunensis* M008011, *S. aureus* M007012 and *S. epidermidis* M236021 While acetone solvent, it was effective in inhibiting both Gram-positive bacteria (*S. lutrae* M208011, *S. haemolyticus* M236011, *S. capitis* M052011, *S. lugdunensis* M259011) and Gram-negative bacteria (*E. coli* M225012 and *E. coli* M208012). On the other hand, using acetone solvent in *S. neglecta* extracts was the least effective in inhibiting mastitis-causing bacteria. On the other hand, using acetone solvent in *S. neglecta* extracts was the least effective in inhibiting mastitis-causing bacteria. However, it was effective in inhibiting both Gram-positive bacteria (*S. lutrae* M208011, *S. haemolyticus* M236011, *S. capitis* M052011, *S. lugdunensis* M259011) and Gram-negative bacteria (*E. coli* M225012 and *E. coli* M208012) as shown in Table 2.

We tested crude extracts of *S. neglecta* against mastitis-causing bacteria in 25, 50, 100, and 200mg/mL concentrations. Gentamicin and DMSO were used as positive controls. The results of the antibacterial test for all the solvent crude extracts of *S. neglecta* with a concentration of 100mg/mL are in Table 3. Different solvent extracts of *S. neglecta* showed varying activity levels against mastitis-causing bacteria. Methanol extracts demonstrated the highest zone of inhibition against all strains except for *S. lutrae*, *S. lugdunensis* and *E. coli*, where ethanol had the most potent inhibitory effect. On the other hand, the chloroform extract showed a minimum zone of inhibition. The methanol extract obtained from crude *S. neglata* at a concentration of 100 mg/mL exhibited a zone of inhibition of 14.00±1.41 against gram-positive bacteria *S. chromogeneus* M261021. However, the positive control gentamicin showed a higher zone of inhibition of 15.05±3.32mm. Although the zone of inhibition is lower in both cases compared to positive controls, it can still be considered as having moderate activity. On the other hand, the extract did not show any activity against *S. condiment* M011022, a gram-positive bacteria, whereas the control drug, gentamicin, exhibited a zone of inhibition of 14.15±5.38mm. (Table 3).

Table 2: The inhibitory effectiveness against mastitis pathogens of *S. neglecta* varied with different organic solvents used for extraction

Mastitis bacteria	The Minimum Inhibitory Concentration (MIC) of the extract varied with different solvents)mg/mL)			
	Ethanol	Methanol	Acetone	Chloroform
S. lugdunensis M008011	25	8.75	-	-
S. aureus M007012	25	8.75	-	-
S. epidermidis M236021	25	8.75	-	-
S. lutrae M208011	25	8.75	50	50
S. haemolyticus M236011	25	8.75	50	50
S. chromogeneus M261021	25	8.75	-	50
S. capitis M052011	25	8.75	50	50
S. condiment M011022	-	-	-	-
S. lugdunensis M259011	25	8.75	50	50
E. coli M225012	8.75	8.75	50	50
E. coli M208012	17.5	8.75	50	50
E. coli M080011	25	8.75	-	50

Table 3: The inhibitory effectiveness against mastitis pathogens of *S. neglecta* varied depending on the organic solvent used for extraction

Mastitis bacteria	Inhibition zone)mm (of an extract with different solvent)100mg(mL/					
	Ethanol	Methanol	Acetone	Chloroform	Genfloxcin	DMSO
S. lugdunensis M008011	10.33±0.57	12.66±0.57	-	-	15.26±2.20	-
S. aureus M007012	11.66±0.57	13.50±0.70	-	-	17.28±3.22	-
S. epidermidis M236021	9.33±0.57	13.50±0.70	-	-	16.45±2.60	-
S. lutrae M208011	11.00±1.00	14.00±1.00	10.50±0.70	8.00±0.00	17.15±2.92	-
S. haemolyticus M236011	9.50±0.70	13.00±1.00	9.50±0.70	9.33±0.57	17.61±2.97	-
S. chromogeneus M261021	13.00±1.00	14.00±1.41	-	10.66±0.57	15.05±3.32	-
S. capitis M052011	9.50±0.70	12.66±1.00	12.66±2.08	9.33±0.57	17.90±1.72	-
S. condiment M011022	-	-	-	-	14.15±5.38	-
S. lugdunensis M259011	12.00±0.00	11.66±0.57	10.33±0.57	8.00±0.00	17.38±2.14	-
E. coli M225012	14.50±0.70	13.00±0.00	10.66±0.57	10.00±1.00	16.65±2.34	-
E. coli M208012	10.00±1.00	13.65±0.00	11.50±0.70	8.00±0.00	14.94±6.10	-
E. coli M080011	11.00±1.00	12.66±0.57	-	9.00±0.00	15.68±4.69	-

Values (mean+SD) of five replicates. Genfloxcin: 30µg/µL

Table 4: Volatile constituents in the methanolic extract of *S. neglecta*.

Compounds	Retention Time (mins)	Area (%)	Proportion (%)
(Oxime-, methoxy-phenyl-_; C8H9NO2	5.749	1.441	0.502
(1,2,3-Propanetriol; C3H8O3	7.733	5.324	1.856
Tetradecanoic acid; C14H28O2 fatty acid	38.907	29.072	10.133
Tetradecanoic acid, ethyl ester; C16H32O2	39.875	5.799	2.021
Doconexent; C22H32O2	44.446	11.586	4.038
cis-7-Hexadecenoic acid; C16H30O2	45.026	4.577	1.595
(Tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene, trans-2,9-anti-9,10-trans-1,10-; C16H24)	45.026	3.370	1.175
7,10,13-Hexadecatrienoic acid (Z,Z,Z)-; C16H26O2)	44.834	100	8.723
n-Hexadecanoic acid; C16H32O2	45.862	14.453	46.371
Hexadecanoic acid, ethyl ester; C18H36O2	46.469	100	6.702
cis,cis,cis-6,9,12-Octadecatrienoic acid, propyl ester; C21H36O2 fatty acid	47.901	14.543	1.643
1,5,9,11-Tridecatetraene, 12-methyl- ; (E,E)-; C14H22) ester	48.077	3.542	0.543
Phytol; C20H40O)	50.051	1.037	66.40
9,12-Octadecadienoic acid (Z,Z)-; C18H32O2)	50.98	86.624	30.194
9,12-Octadecadienoic acid (Z,Z)-; C18H32O2)	51.170	18.285	15.170
9,12-Octadecadienoic acid, ethyl ester; C20H36O2)	51.587	32.715	3.219
Ethyl Oleate; C20H38O2 ester	51.714	16.792	7.787
2,4-Disilapentane, 2,4-dimethyl-; C5H16Si2	60.636	1.008	0.351
(diethylamino)-3-phenyl-propanethioic acid S-ethyl ester; C15H23NOS)	61.387	2.491	0.868

Using GC-MS, the chemical compositions of *S. neglecta* were detected in the methanol extract. Five predominant compounds in this extract are Phytol; C20H40O) 66.40% n-Hexadecanoic acid; 46.371%, 9,12-Octadecadienoic acid (Z,Z)-; C18H32O2) (30.194%), 9,12-Octadecadienoic acid (15.170), Tetradecanoic acid; C14H28O2 fatty acid (10.133%) and 7,10,13-Hexadecatrienoic acid (8.723%) as shown in Table 4.

DISCUSSION

The antimicrobial potential of *Spirogyra neglecta* against mastitis-inducing pathogens holds promise for developing alternative therapeutic strategies in managing mastitis in dairy animals. The diversity of bioactive compounds in algae opens avenues for sustainable and eco-friendly approaches to combat antibiotic resistance in agriculture. The methanol and ethanol crude extracts of *S. neglecta* exhibited commendable bacteriostatic activity, as evidenced by their notably low MIC values ranging from 8.75-200mg/mL. Meanwhile, the acetone and chloroform crude extracts also demonstrated satisfactory results in terms of bacteriostatic activity. Their MIC values ranged from 50-200mg/mL., indicating a slightly higher concentration requirement than the methanol and ethanol extracts. It is worth noting that while the methanol and ethanol extracts exhibited lower MIC values, the acetone and chloroform extracts still demonstrated effective bacteriostatic activity. *S. neglecta* can be a source of bioactive compounds with antimicrobial properties. Different solvents, such as ethanolic, methanolic, chloroform, or acetone, can extract these compounds. Previous studies by Dwaish et al. 2016; Syamsuddin et al. 2016; Uthorn et al. (2019) have shown that these extracts can effectively inhibit pathogenic bacteria that cause mastitis. It demonstrated antimicrobial activity against Gram-positive (*Staphylococcus* sp.) and Gram-negative (*E. coli*) bacteria. Methanol extracts displayed low Minimum Inhibitory Concentration (MIC) values, particularly 8.75mg/mL. against *S. aureus*. Extracts exhibiting low MIC values necessitate further fractionation to discern associated chemical groups and evaluate the potential for additional MIC reduction. We previously demonstrated that the minimum inhibitory concentration (MIC) values were 12.5mg/mL when tested against *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Enterococcus faecalis*.

This observation is consistent with a prior study indicating that *Spirogyra rhizopus*, extracted using various solvents such as ethanol, methanol, chloroform, and acetone, exhibited substantial inhibitory efficacy against *S. typhimurium*, *P. aeruginosa*, *S. aureus* and *E. coli*. (Daniel et al. 2019). Methanol extracts demonstrated the highest zone of inhibition against all strains except for *S. lutrae*, *S. lugdunensis* and *E. coli*, where ethanol had the most potent inhibitory effect. The study found that methanol and ethanol extracts have promising antibacterial activity. Chloroform and acetone extract also showed some antimicrobial activity. Kamble et al. (2012) reported that *Spirogyra plena* extract showed total inhibitory growth against plant pathogenic fungi. Methanol extraction yielded higher antimicrobial activity than hexane and ethyl acetate. Khalid et al. (2012) found that crude methanol extracts from two types of green algae, *Spirogyra hyaline* Cleve and *S. rhizoids* Randhawa, exhibited vigorous antimicrobial activity against 14 bacterial and 20 fungal species. Methanol is the best solvent for extracting phytochemicals from algae that have a strong inhibitory effect on both Gram-positive and Gram-negative bacteria, according to a 2004 study by Vijaya Parthasarathy. Differences in results from previous studies may be due to ecological and biogeographic conditions and the method used for antimicrobial testing. Algae physiology closely correlates with parameters affecting the content of active metabolites (Abdel-Aal et al. 2015; Belyagoubi et al. 2022). The extract from *S. neglecta* is renowned for its antibacterial attributes. The dominant constituents identified via GC-MS analysis included Phytol n-Hexadecanoic acid 9,12-Octadecadienoic acid 9,12-Octadecadienoic acid, Tetradecanoic acid and 7,10,13-Hexadecatrienoic acid. Phytol, terpenes were proven to be potent antimicrobial agents against drug-resistant pathogens, mainly bacteria and fungi (Mahizan et al. 2019), exert antibacterial properties via (Masyita et al. 2022)

inducing oxidative stress response in *S. aureus* and *E. coli* (Guimarães et al. 2019; Muilu-Mäkelä et al. 2022; El Fannassi et al. 2023) *P. aeruginosa* (Lee et al. 2016) *Bacillus licheniformis* (Saha and Bandyopadhyay 2020) *C.albicans*, *A. niger* and *S. aureus* (Ghaneian et al. 2015). Terpenes are natural compounds with high biological activity against various microorganisms, especially Gram-positive bacteria. They can alter the cytoplasmic stability and cell envelope of bacteria, leading to cell damage. Terpenes and their derivatives have several target sites and action methods, making them potent antimicrobial agents. They are effective against multidrug-resistant organisms like methicillin-resistant *Staphylococcus aureus* MRSA, and no microbial resistance has yet been created against them (Mahizan et al. 2019; Zhang et al. 2022; El Fannassi et al. 2023; El-Demerdash et al. 2023). It has been reported that both Hexadecanoic acid and Octadecadienoic acid demonstrate antibacterial activity, inhibiting Gram-positive and Gram-negative bacteria to some extent. (Kumar et al. 2015; Dwaish et al. 2016; Okunowo et al. 2017; Daniel et al. 2019). Hence, it can be inferred that Phytol 9,12-Octadecadienoic acid ethyl ester and Hexadecanoic acid possess potent antimicrobial properties. Additionally, Ethyl Oleate is useful as a solvent in preparing pharmaceutical drugs. (Ory et al. 1983). As reported earlier, Phaeodactylum tricornutum's polyunsaturated fatty acids - EPA and hexadecatrienoic acid (HTA), as well as monounsaturated fatty acid (palmitoleic acid, PA) - exhibit activity against MRSA. (Cepas et al. 2021). While Tetradecanoic Acids are a compound of fatty acids (FA), FA has widely distributed molecules in nature that have structural functions, are used as carbon sources, and also have bactericidal properties. Moreover, at sublethal concentrations, some FA act as signal molecules in regulating bacterial virulence (Calder 2015; Kenar et al. 2017; Cepas et al. 2021). The antimicrobial efficacy against pathogens that cause mastitis highlights the promise of *S. neglecta* as a reservoir of alternative therapeutics for mastitis treatment. Bioactive compounds are accountable for these outcomes. *S. neglecta* extracts obtained through various solvents were evaluated for their antimicrobial potential. Additionally, the antimicrobial effectiveness of these extracts was tested against clinically relevant mastitis-causing bacteria. The results showed promising levels of antimicrobial activity effects against various pathogens. This study suggests that *S. neglecta* holds potential as a candidate for the development of novel mastitis therapeutics.

Conclusion

This research furnishes compelling evidence regarding the antimicrobial capabilities of *S. neglecta* against pathogens responsible for mastitis. The results indicate that extracts from *S. neglecta* show potential as a natural alternative in creating innovative mastitis therapeutics. However, additional research is imperative to identify the precise bioactive compounds accountable for these effects and to evaluate their safety and efficacy in vivo.

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Author's contribution

Yuwadee Insumran and Weeradej Khonsuntia Carried out the practical and laboratory work and drafted the manuscript. Natchanok Jansawang, Kraijak Kaewprom, and Manakant Intrakhamhank Shared in the useful work. All authors read, revised and approved the final manuscript.

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REFERENCES

Abdel-Aal EI, Haroon AM and Mofeed J, 2015. Successive solvent extraction and GC–MS analysis for the evaluation of the phytochemical constituents of the filamentous green alga *Spirogyra longata*. The Egyptian Journal of Aquatic Research 41(3): 233–246. https://doi.0rg10.1016/j.ejar.2015.06.001

Abebe R, Hatiya H, Abera M, Megersa B and Asmare K, 2016. Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk Shed, South Ethiopia. BMC Veterinary Research 12: 270. https://doi.0rg 10.1186/s12917-016-0905-3

Arjsri P, Phetcharaburanin J, Suksawat M, Mapounga S, Subkamkaewa C, Semmarath W, Yodkeeree S and Dejkriengkraikul PL, 2021. *Spirogyra neglecta* (Hassall) Kutzing attenuates metastasis of castration-resistant human prostate cancer via the blockage of AKT signaling pathway. South African Journal of Botany 139: 26-37. https://doi.org/10.1016/j.sajb.2021.02.005

Belyagoubi L, Belyagoubi-Benhammou N, Atik-Bekkara F and Abdelouahid DE, 2022. The influence of harvest season and different polarity solvents on the biological activities, phenolic compounds, and lipid-soluble pigment contents of *Spirogyra* sp. from Algeria. Advances in Traditional Medicine 22: 359–369. https://doi.org/10.1007/s13596-021-00551-0

Buddhakala N and Yongkhamcha B, 2023. Anti-diabetic, anti-inflammatory and muscle relaxant activities of extracts from *Spirogyra varians* (Hassall) Kuetzing. Pakistan Journal of Pharmaceutical Sciences 36(1): 1–8.

Calder PC, 2015. Functional roles of fatty acids and their effects on human health. Journal of Parenteral and Enteral Nutrition 39 (1 Suppl): 18S–32S. https://doi.0rg 10.1177/0148607115595980

Cepas V, Gutiérrez-Del-Río I, López Y, Redondo-Blanco S, Gabasa Y, Iglesias MJ, Soengas R, Fernández-Lorenzo A, López-Ibáñez S, Villar CJ, Martins CB, Ferreira JD, Assunção MFG, Santos LMA, Morais J, Castelo-Branco R, Reis MA, Vasconcelos V, López-Ortiz F, Lombó F and Soto SM, 2021. Microalgae and cyanobacteria strains as producers of lipids with antibacterial and antibiofilm activity. Marin Drugs 19: 675. https://doi.org/10.3390/md19120675

Daniel E, Girma T and Venkatesan JS, 2019. Preparation of different crude extracts and antimicrobial studies of *Spirogyra rhizopus*. Asia Journal of Pharmaceutical and Clinical Research 12(7): 271-274.

Dwaish AS, Yousif YDM and Lefta SN, 2016. Use of *Spirogyra* sp. extract against multidrug-resistant bacterial pathogens. International Journal of Advanced Research 4(7): 575- 579.

El-Demerdash AS, Bakry NR, Aggour MG, Elmasry SS, Mowafy RE, Erfan A, Taha MF, El-Gmaal AAAM, Mohamed AAE, Hagag N and Samir M, 2023. Bovine mastitis in Egypt: bacterial etiology and evaluation of diagnostic biomarkers.

International Journal of Veterinary Science 12(1): 60-69. https://doi.org/10.47278/journal.ijvs/2022.161

El Fannassi Y, Gharsallaoui A, Khelissa S, El Amrani MA, Suisse I, Sauthier M, Jama C, Boudra S and Chihib NE, 2023. Complexation of terpenes for the productionof new antimicrobial and antibiofilm molecules and their encapsulation in order to improve their activities. Applied Sciences 13: 9854. https://doi.org/10.3390/app13179854

Ghaneian MT, Ehrampoush MH, Jebali A, Hekmatimoghaddam S and Mahmoudi M, 2015. Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. Environmental Health Engineering and Management Journal 2(1): 1316.

Guimarães AC, Meireles LM, Lemos MF, Guimarães MCC, Denise EC, Fronza M and Scherer R, 2019. Antibacterial activity of terpenes and terpenoids present in essential oils. Molecules 24(13): 2471. https://doi.org/10.3390/molecules24132471

Kamble S, Rokde A and Chavan A, 2012. Antifungal activity of algal extracts against plant pathogenic fungi. International Multidisciplinary Research Journal 2(3): 23–24.

Kenar JA, Moser BR and List GR, 2017. Chapter 2 - Naturally Occurring fatty acids: source, chemistry and uses. Fatty Acids, Chemistry, Synthesis and Applications 23-82. https://doi.org/10.1016/B978-0-12-809521-8.00002-7

Khalid MN, Shameel M and Ghazala B, 2012. Bioactivity and phytochemistry of two species of *Spirogyra link* (Zygnemophyceae) from Pakistan. International Journal on Algae 14(3): 237–246.

Khalid S, Shahid M, Natasha, Bib I, Sarwar T, Shah AH and Niazi NK, 2018. A Review of environmental contamination and health risk assessment of wastewater use for crop irrigation with a focus on low and high-income countries International Journal of Environmental Research and Public Health, Public Health 15(5): 895: https://doi.org/10.3390/ijerph15050895

Kumar J, Dhar P, Tayade AB, Gupta D, Chaurasia OP, Upreti DK, Toppo K, Arora R, Suseela M and Srivastava RB, 2015. Chemical composition and biological activities of trans-Himalayan Alga *Spirogyra porticalis*)Muell (.Cleve .PloS One 10:e0118255. https://doi.org/10.1371/journal.pone.0118255

Lager M, Wilhelmsson P, Matussek A, Lindgren PE and Henningsson AJ, 2021. Molecular detection of borrelia bacteria in cerebrospinal fluid-optimisation of pre-analytical sample handling for increased analytical sensitivity. Diagnostics 11: 2088. https://doi.org/10.3390/diagnostics11112088

Lee W, Woo ER and Lee DG, 2016. Phytol has antibacterial property by inducing oxidative stress response in *Pseudomonas aeruginosa*. Free Radical Research 50: 12. https://doi.org/10.1080/10715762.2016.1241395

Mahizan NA, Yang SK, Moo CL, Song AAL, Chong CM, Chong CW, Abushelaibi A, Lim SHE and Lai KS, 2019. Terpene derivatives as a potential agent against antimicrobial resistance (AMR) pathogens. Molecules 24(14): 2631. https://doi.org/10.3390/molecules24142631

Masyita A, Mustika SR, Dwi AA, Yasir B, Rahma RN, Emran TB, Nainu F and Simal-Gandara J, 2022. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. Food Chemistry: X 13: 100217. https://doi.org/10.1016/j.fochx.2022.100217

Meçaj R, Muça G, Koleci X, Sulçe M, Turmalaj L, Zalla P, Koni A and Tafaj M, 2023. Bovine environmental mastitis and their control: an overview. International Journal of Agriculture and Biosciences 12(4) 216-221. https://doi.org/10.47248/journal.ijab/2023.067

Mendes MC, Navalho S, Ferreira A, Paulino C, Figueiredo D, Silva D, Gao F, Gama F, Bombo G, Jacinto R, Aveiro SS, Schulze PSC, Gonçalves AT, Pereira H, Gouveia L, Patarra RF, Abreu MH, Silva JL, Navalho J, Varela JCS and Speranza LG, 2022. Algae as food in europe: an overview of species diversity and their application. Foods 11: 1871. https://doi.org/10.3390/foods11131871

Mesbahzadeh B, Rajaei SA, Tarahomi P, Seyedinia SA, Rahmani M, Rezamohamadi F, Kakar MA and Moradi-Kor N, 2018. Beneficial effects of *Spirogyra neglecta* extract on antioxidant anti-inflammatory factors in streptozotocin-induced diabetic rats. Biomolecular Concepts 9: 184-189. https://doi.org/10.1515/bmc-2018-0015

Muilu-Mäkelä R, Aapola U, Tienaho J, Uusitalo H and Sarjala T, 2022. antibacterial and oxidative stress-protective effects of five monoterpenes from softwood. Molecules 27: 3891. https://doi.org/10.3390/molecules27123891

Nguyen NTB, Molicotti P, Hung T, Phuc LT, Diem NTK, Chum NT, Thach LT, Hue TV and Tram NVQ 2020. Application of the resazurin microtitre assay for the detection of isoniazid and/or rifampicin resistant Mycobacterium tuberculosis clinical isolates in Central Vietnam Journal of Medicine and Pharmacy 10: 39-43. https://doi.org/10.34071/jmp.2020.7.6

Okunowo WO, Abiola OO, Josiah AI, Lukman OA and Ifeoma EU, 2017. Antimicrobial, antioxidant potential and chemical composition of the methanolic extracts of *Spirogyra setiformis* and *Navicula* species. Journal of Scientific Research and Development 17)1:(15-20.

Ontawong A, Saowakon N, Vivithanaporn P, Pongchaidecha A, Lailerd N, Amornlerdpison D, Lungkaphin A and Srimaroeng C, 2013. Antioxidant and renoprotective effects of *Spirogyra neglecta* (Hassall) Kützing extract in experimental type 2 diabetic rats. BioMed Research International 2013: 820786. https://doi.org/10.1155/2013/820786

Ory SJ, Hammond CB, Yancy SG, Hendren RW and Pitt CG, 1983. The effect of a biodegradable contraceptive capsule (Capronor) containing levonorgestrel on gonadotropin, estrogen, and progesterone levels. American Journal of Obstetrics & Gynecology 145 (5): 600-605.

Peerapornpisal Y, Panyoyai T and Amornlerdpison D, 2012. Antioxidant and anti-inflammatory activities Of *Spirogyra neglecta* (Hassall) Kutzing. KKU Science Journal 40(1): 228-235.

Saha M and Bandyopadhyay PK, 2020. In vivo and in vitro antimicrobial activity of phytol, a diterpene molecule, isolated and characterized from *Adhatoda vasica* Nees. (Acanthaceae), to control severe bacterial disease of ornamental fish, Carassius auratus, caused by *Bacillus licheniformis* PKBMS16. Microbial Pathogenesis 141: 103977. https://doi.org/ 10.1016/j.micpath.2020.103977

Surayot U, Wang JG, Lee JH, Kanongnuch C, Peerapornpisal Y and You SG, 2015. Characterization and immunomodulatory activities of polysachcharides from *Spirogyra neglecta* (Hassall) Kützing. Bioscience, Biotechnology and Biochemistry 79(10): 1644–1653. https://doi.org/10.1080/09168451.2015.1043119

Syamsuddin R, Nasmia N, Rantetondok A and Zainuddin EN, 2016 .*Caulerpa racemosa*)Chlorophyta, Caulerpaceae (the extract increases the growth and biomass production of (*Gracilaria verrucosa)* Rhodophyta, Gracilariaceae . Aquaculture, Aquarium, Conservation and Legislation. International Journal of the Bioflux Society 95 :1044-1052.

Thumvijit T, Taya S, Punvittayagul C, Peerapornpisal Y and Wongpoomchai R, 2014. Cancer chemopreventive effect of *Spirogyra neglecta* (Hassall) Kützing on diethylnitrosamine-induced hepatocarcinogenesis in rats. Asian Pacific Journal of Cancer Prevention 15: 1611-1616.

Tomani´c D, Božin B, Kladar N, Stanojevi´c J, Cabarkapa I, Stilinovi´c N, Api´c J, Boži´c DD and Kovaˇcevi´c Z, 2022. Environmental Bovine Mastitis Pathogens: Prevalence,

Antimicrobial Susceptibility, and Sensitivity to *Thymus vulgaris* L., *Thymus serpyllum* L., and *Origanum vulgare* L. Essential Oils. Antibiotics 11: 1077. https://doi.org/10.3390/antibiotics11081077

Uthorn C, Sunanta K, Luksamee V and Chadchasa TU, 2019. Antibacterial Activity of Caulerpa racemosa var. corynephora Crude Extracts against Pathogenic Bacteria of Aquatic Animals. Rajamangala University of Technology Srivijaya Research 11(1): 30-40.

Vaou N, Stavropoulou E, Voidarou C, Tsigalou C and Bezirtzoglou E, 2021. Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. Microorganisms 9: 2041. https://doi.org/10.3390/microorganisms9102041

Vijaya Parthasarathy MD, Prema M and Krishnamurthy A, 2004. Preliminary report on the antibacterial activity of extracts from *Spirogyra* sp. Zygnematacea-Chlorophyceae. Indian Hydrobiol 7: 229–233

Yosboonruang A, Duangjai A, Amorlerdpison D and Viyoach J, 2020. Screening for Biological Activities of *Spirogyra neglecta* Water Extract. Walailak Journal of Science and Technology 17(4): 359-368.

Yongkhamcha B and Buddhakala N, 2023. Phytochemical Compositions, Nutritional Contents, Cytotoxicity and Anti-Inflammatory Activity of Different Extracts from *Spirogyra neglecta* (Hassall) Kützing. Trends in Sciences 20(4): 6528. https://doi.org/10.48048/tis.2023.6528

Zhang W, Margarita GE, Wu D, Yuan W, Yan S, Qi S, Xue X, Wang K and Wu L, 2022. Antibacterial Activity of Chinese Red Propolis against *Staphylococcus aureus* and MRSA. Molecules 27: 1693. https://doi.org/10.3390/molecules27051693