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Antibacterial, Antifungal Properties and Chemical Composition of Freshwater Macroalgae, *Cladophora Glomerata*

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Abstract

The freshwater macroalgae (Cladophora glomerata), a filamentous green alga, collected from the sub-branch of the dam canal, was identified based on morpho-anatomical characters and chemical compositions. The algal biomass containing carbohydrate, protein, lipid, contents were 31.65± 1.5, 13.7± 1.5 and 2.05± 1.5 wt.%, respectively. Also, C. glomerata has chlorophylls and carotenoids pigments were 258.35±2.16 and 79.54±0.67 µg/ml, respectively. Furthermore, to evaluate the antimicrobial potential of methanolic solvent extracts derived from C. glomerata against bacteria and fungus. The feasibility of utilizing C. glomerata extracts as a potential source for the antibacterial, antifungal properties was investigated. The methanol solvent extracts of C. glomerata used in this article have revealed significant bacterial strains, namely, B. substilis, K. pneumonia, P. aeruginosa, P. vlgaris, S. aureus and E. coli. The Epidermophyton floccosum shows that higher inhibition was reached to 91.02 %. Fungal strains C. albicans, E. floccosum, M. canis, M. phaseolina, P. oedochilum, R. solani, A. flevus, N. oryzae and T. hamatum were examined with C. glomerata extracts. Staphylococcus aureus express that highed positive control Ampicillin compared to other fungus species in this article. These results suggest that the methanol extracts of C. glomerata possess an effective wide spectrum of antimicrobial and antifungal, which can assist as a remarkable source for antimicrobial compounds.

Keywords: ***Freshwater macroalgae, Antibacterial activity, Antifungal activity, Medicine, Pharmaceutical uses.***

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1. Introduction

Algae are the most important primary producer in the aquatic ecosystem [1]. These are aquatic photosynthetic organisms that were given the remarkable ecological significance and food chain for other animals [2]. Algae were mainly classified by the green, red, and brown colors and they are, belong to the group of Chlorophyta, Rhodophyta and Phaeophyta, respectively. Algae can grow in a wide range of habitats such as freshwater, marine water, deep oceans, rocky shores, and extreme conditions such as snow zone, sands, desert, hot springs. For biomass production, algae can cultivate in the open and closed ponds, photobioreactors, sewage and wastewater, desert as well as CO_2 emitting industries etc. [3]. Commonly, algae are found in moist places, water bodies, terrestrial land and aquatic environments. The eukaryotic microalgae, cyanobacteria and macroalgae biomass were produced for many types of applications [4]. Especially, macroalgae are an essential source of bioactive natural compounds; they are regarded as a source of bioactive compounds with cytostatic, antihelminthic, antiviral, antibacterial and antifungal activities, they have also been used to cure some sicknesses such as cancer, arthritis, etc.

Hence, algae provided the basis of the aquatic food chain, and they were fundamental to keep CO_2 of the carbon cycle via photosynthesis as a substantial role in biogeochemical cycles [5-7]. Most algae were photoautotrophic and contained complete photosynthesis mechanisms which, converting solar energy into chemical forms. The algal photosynthesis process is similar in higher plants and their products are molecularly equivalent to conventional agricultural crops [8]. The key benefits of producing algae biomass were applicable to get high photosynthetic yields, high oil content and to produce high concentrations of beneficial compounds such as proteins, carbohydrates, lipids and pigments [7-10]. Also, algae application is widely accepted in practice as one of the best strategies in biomedical and biochemical engineering. There are several reasons for this high approach value of algae biomass, including agriculture and industry in the broader sense feed, food, nutrition, chemical, cosmetic, pharmaceutical, fertilizer, aquaculture, biofuel, etc. as Shown in Fig. 1 [11].

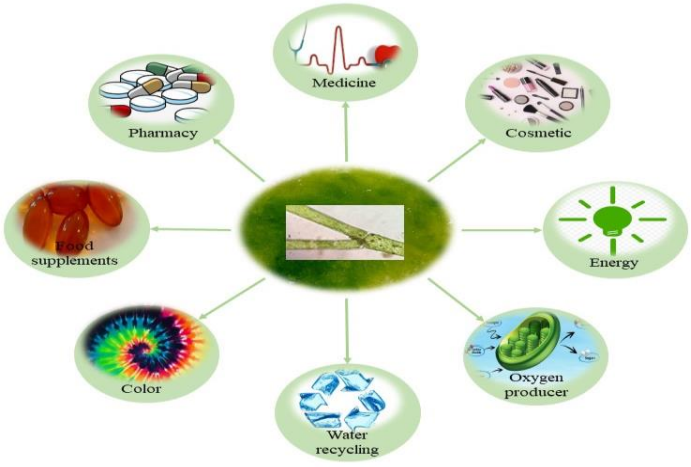

Fig.1. Benefits of freshwater macroalgae, *Cladophora glomerata.*

The literature suggested that algae are primary and hugely available natural resources and providing massive benefits from their biomass [12]. Algae are serving as food and healing agents in many regions of the world. Moreover, the algal nutritional perspective is needed in the pharmaceutical, food and cosmetics and energy industries [13-17]. Mainly,

Cladophora species are the most common in the aquatic environment. Currently, worldwide, cosmopolitan, ubiquitous filamentous green alga, widely recognized as multiplies and reproduces fiercely provided by *Cladophora glomerata.*

Moreover, *C. glomerata*, is one of the most common species of macroalgae bloom and achieve high biomass in a short time. Global abundance arising from the described features, together with distinctive structure, fosters high functional and taxonomic diversity. *C. glomerata* is having biologically active compounds, including saturated and unsaturated fatty acids, sterols, terpenoids and phenolic compounds. As well as the described chemical compounds, *C. glomerata* are the source of many other bioactive substances. Many decades in human history, humans utilize land and aquatic plant extracts and essential oil in daily life as a natural remedy against various infections. Until today, it is widely used in food preservation and pharmaceuticals alternative medicine, antimicrobial, and antifungal properties. However, further phytochemical research is needed to identify the active principles responsible for antifungal effects. Therefore, this study examined *C. glomerata* is a uniseriate branching filamentous green macroalgae chemical composition, antimicrobial, and antifungal properties.

2. Materials and Methods

2.1 Plant material collection and identification

The algae were collected from the slow running cannel at MaeTeang, Chiang Mai Province, Thailand, and transported to the School of Renewable Energy/Energy Research Canter, Maejo University, Sansai, Chiang Mai, within two h for identification and analysis. Spirogyra spp. was collected and harvested from the natural water body used with the traditional local methods. The light microscope (Nikon Eclipse 80i microscope) and an attached digital camera are used for taking the algal picture for identification. Relevant publication of Prescott [18] was referred for the identification of algal taxa and taxonomically determined with the help of authentic literature [19-21]. For the taxonomic description of taxa, dimensions were given in micrometer (µm). The measuring scales given for algae photographs were equal to 20 µm. The morphological characters, including length and width, were recorded for species confirmation.

2.2. Pigments and chemical composition analysis

Chlorophylls estimation: Ten ml of sample was taken and centrifuged at 6000 rpm for 15 minutes. Supernatants were discarded and re-suspended in a known volume of methanol, while pellets extracted with 5 ml of 96% methanol extraction. The tubes were wrapped with aluminum foil and kept in the dark. The samples were centrifuged again and the supernatants were used for measuring the optical density at 663 nm and 645 nm against 96% methanol as a blank by spectrophotometer (Spectronic Genesys 20, Thermo Fisher Scientific). After extraction chlorophyll concentration was determined spectrophotometrically and calculated chlorophyll (Chlorophyll a, chlorophyll b and total chlorophylls) and total carotene contents were computed using the following equations:

$$\text{Chlorophyll-a (µg/ml)} = \{(15.65 \times A666 - 7.340 \times A653) \times V / 50 \times W\} \times \text{dilution}$$

$$\text{Chlorophyll-b (µg/ml)} = \{(27.05 \times A653 - 11.21 \times A666) \times V / 50 \times W\} \times \text{dilution}$$

$$\text{Total chlorophyll (µg/ml)} = \text{chlorophyll-a} + \text{chlorophyll-b}$$

$$\text{Total carotene (µg/ml)} = (1000\ A470 - 2.860\ \text{Chl-a} - 129.2\ \text{Chl-b}/245)$$

Protein, carbohydrates, lipids determination procedures were adopted from Tipnee et al. [22]. Elemental composition (Carbon (C), Hydrogen (H), Nitrogen (N), Sulfur (S) and Oxygen (O)) was analyzed using the element analyzer (Perkin–Elmer 2004). ASTM Standard D 4442-07 method was used for moisture content estimation. The preliminary, phytochemical analysis was carried out by using the standard method to identify the components in the algae extracts of C. glomerata. The methanol extracts of C. glomerata was subjected to the analysis of chemical components.

2.3. Preparation of algae crude methanol

C. glomerata biomass washed thoroughly under running tap water and air-dried at room temperature for two days and ground into a fine powder. The air-dried and powdered plant material are extracted with 400 ml of 95.5% methanol, kept in the room temperature for 72 h with periodic stirring. After that, the extract was filtered by Whatman no 4. filter paper to remove the marc. The supernatant was collected and evaporated in a rotary evaporator under reduced pressure at a temperature of 64 °C (methanol bp) until a dark oily paste was formed then transferred to a water bath to allow further evaporation of methanol. The plant extracts were stored in the refrigerator at -80°C for 2 days before transferred to freeze-dry for further usage.

2.4. Microorganisms

The fungal strains were used in inoculum is prepared using 24-hour plate cultures of *Candida albicans, Epidermophyton floccosum, Microsporum canis, Macrophomina phaseolina, Pythium oedochilum, Rhizoctonia solani, Aspergillus flevus, Nigrospora oryzae* and *Trichoderma hamatum*. The bacterial strains, namely, *Bacillus substilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteous vlgaris, Staphylococcus aureus, Escherichia coli* were used as test organisms.

2.5 Fungal and bacterial strain and inoculum preparation

The strains were tested is subculture on Sabouraud dextrose agar (SDA) and Potato dextrose agar (PDA). Then, incubated for 24 h at 37ºC. Inocula obtained from the overnight agar culture. The inoculum is prepared using 24-hour plate cultures of Candida albicans, Epidermophyton floccosum, Microsporum canis, Macrophomina phaseolina, Pythium oedochilum, Rhizoctonia solani, Aspergillus flevus, Nigrospora oryzae and Trichoderma hamatum. The microbial strains and inoculum preparation proceducre and methods were adopted from Andrews **[23]**.

2.6 Antifungal assay preparation

This method used to assay the plant extracts for antifungal activity. The procedure is explained in detail later. The potency and activity of antifungal are usually determined by zone of inhibition on agar plates. Antifungal activity determined by the disc diffusion assay. Firstly, the plant extracts are dissolved in methanol. Under aseptic conditions, 0.2 mL of standardized inoculum is spread on to sterile Sabouraud dextrose agar (SDA) plates and Potato dextrose agar (PDA) to achieve a confluent growth. The plates are then allowed to dry. The 50 µL of methanolic extracts (100 mg/mL) were loaded with a 5 mm diameter on the sterile filter paper discs. The paper discs are allowed to evaporate and then placed aseptically on the surface of the inoculated agar plates. Standard Fluconazole and Nystatin (30 µg) discs for antifungal activity are used as a positive control, while methanol (30 µL/disc) is used as a negative control. Plates are then incubated at 37°C for 24 h. The experiment is performed in triplicates. After the incubation time, antifungal activity was measured using the zone of inhibition around each paper disc.

2.7 Antibacterial assay preparation

Antibacterial tests of *C. glomerata* extracts were performed using the disc diffusion method, in Petri dishes. Sterile disks of 6 mm in diameter were soaked with 25 µL of algal extract placed on agar medium surface (Mueller-Hinton Agar, pH 7.4 ± 0.2 at 25 °C). Bacterial strains were incubated at 37 °C for 24 h before inoculated. The diameters in millimeter of the inhibition halos of bacterial growth around the disk were measured to get the results. Methanol was used as the negative control (i.e., without algae extract) as well as clear halos greater than 10 mm were measured as positive results from the samples.

2.8 Statistical analyses

All experiments were determined in biological triplicate to ensure reproducibility. Experimental results were obtained as the mean value ± SD. Statistical analyses were performed using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). The statistical significance was achieved when $p<0.05$.

3. Results and Discussion

3.1 Characteristics of scientific classification

The morphology of algal specimens was studied carefully and the specimen was identified as *Cladophora glomerata*. It is a filamentous green macroalga with typically branched thalli, and the microscopic picture is shown in Fig. 2. The *C. glomerata* species is typically bimodal maxima in late spring and late summer/fall; summer decline. The taxonomic characteristics were as follows: the coverage, filament length, growth rate maximum, respiration were 100-940 g dr wt m−2, 30-50 cm, 0.77 day−1 and 0.44 day−1, respectively. It can grow up to 25 cm day−1 and alkaline conditions pH optimum 7–9; growth temperature at ~9–30°C; Optimum 300–600; suitable light compensation point is 36–104 (µmol quanta m−2 s−1); current velocity is increased photosynthesis from 0–2.1 (cm s−1) (up to 8 for small tufts) and decreased at higher levels; usually nutrient regime is eutrophic to mesotrophic.

3.1.1 Systematic classification

- Kingdom: Plantae
- Subkingdom: Viridiplantae
- Infrakingdom: Chlorophyta infrakingdom
- Phylum: Chlorophyta
- Subphylum: Chlorophytina
- Class: Ulvophyceae
- Order: Cladophorales
- Family: Cladophoraceae
- Genus: Cladophora Kütz., 1843
- Species: *Cladophora glomerata*

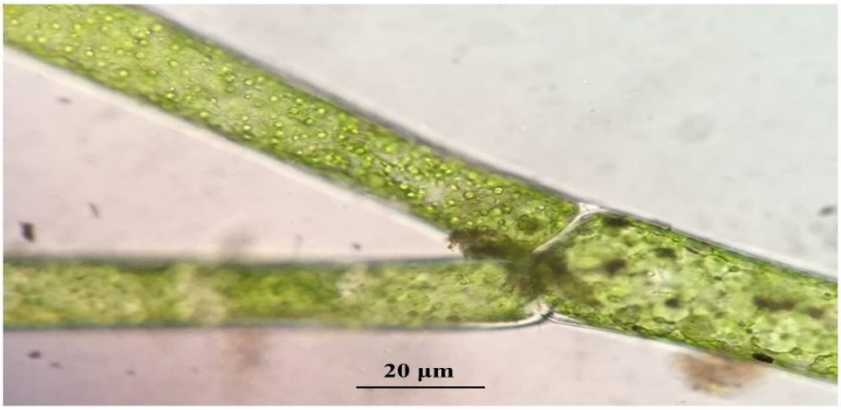

Fig 2. Microscopic picture of *Cladophora glomerata.*

3.3 Biochemical composition of *Cladophora glomerata*

Algae are deliberated prospectively significant resources due to the lipid, or oil, content in the cells, although certain human populations use algae for food, food additives, or compost, fiber due to its other valuable components [1-7] and [9-17]. In our study, lipid, protein, and carbohydrate compounds were present in *C. glomerata* samples, indicating that many of these natural compounds can be harvested from the biomass, as a source of beneficial food, medicinal and pharmaceutical products. *C. glomerata* having huge amount of macro, micro nutrients and trace elements which have supplementary bioactive ingredients. That's main reasons algae were exposed as the food products recently. The main composition of algal biomass were proteins, lipids, polysaccharides, mineral, vitamins and enzymes. The moisture, carbohydrate, protein, lipid, pigments and other content of *C. glomerata* were represented in Table 1.

Table 1: Chemical Composition of *C. Glomerata.*

Parameters	***C. glomerata***
Moisture (wt.%)	5.14
Carbohydrate	31.65± 1.5
Protein	13.7± 1.5
Lipid	2.05± 1.5
C (wt.%)	54.04
H (wt.%)	5.425
N (wt.%)	3.89
S (wt.%)	1.99
O (wt.%)	34.09
Chl a (µg/ml)	168.00 ± 1.08
Chl b (µg/ml)	90.35 ± 1.53
Total chlorophylls (µg/ml)	258.35±2.16
Total carotenoids (µg/ml)	79.54±0.67

Algae have already been utilized as food source for centuries for its high protein value. Chlorophyll is the green colored pigment present in plants, algae and cyanobacteria and it has important role in photosynthesis. Ramaraj et al. [5] stated that green algae are common as a main source of chlorophyll production and called 'Emerald food'. Because green algae were containing high content chlorophyll [5]. Carotenoids are lipid soluble natural pigments extracted from plants, algae, and photosynthetic bacteria and these pigments performed as an important component in photosynthesis process. Fabrowska et al. [24] stated that freshwater algae from the genus Cladophora are characterized by a high amount of such macroelements as: calcium, potassium and phosphorus, as well as the following microelements: magnesium, iron and zinc.

3.3 Antifungal activity of *Cladophora glomerata*

Fungal diseases represent an increasing threat to human health worldwide, which in some cases might be associated with substantial morbidity and mortality. However, only a few antifungal drugs are currently available for the treatment of life-threatening fungal infections. Furthermore, plant diseases caused by fungal pathogens represent a global economic problem for the agriculture industry. The freshwater environment continues to provide structurally diverse and biologically active secondary metabolites, several of which have inspired the development of new classes of therapeutic agents. The secondary metabolites and some compounds are containing rare antifungal activities which were isolated from the marine microorganisms, invertebrates, and algae.

Table 2. Antifungal Activity Exhibited by the Methanol Extract of *C. Glomerata*.

Fungal culture	Colony sample	Diam. (mm) control	Inhibition %	MIC g/mL Miconazole	Ketoconazole
Candida albicans	15	87	90.24	0.05	0.1-4.3
Epidermophyton floccosum	19	99	91.02	0.5-1.0	0.1-8.5
Microsporum canis	11	65	87.33	0.5-10	0.1-13
Macrophomina phaseolina	15	102	89.57	0.01	0.02
Pythium oedochilum	18	55	43.64	0.03	0.02
Rhizoctonia solani	19	74	75.74	0.01	0.02
Aspergillus flevus	20	97	88.11	0.3	0.3
Nigrospora oryzae	19	74	81.74	0.2	0.2
Trichoderma hamatum	21	98	79.43	0.1	0.1

Several decades, many natural products possessing antifungal activities have been isolated from sponges and bacteria. This research work provides a suggestion of natural products from various marine resources. These are revealed that in vitro and/or in vivo possible as antifungal agents, through their mechanism of action at whatever appropriate. The antifungal activities of algal extracts were screened against important fungal species, nine important pathogens, and one well-known antagonist. The antibacterial and antifungal activities of freshwater and marine algae as natural antibiotics reported by Chowdhury et al. [25]. Mohy El-Din and Mohyeldin [26] suggested that methanolic extracts

showed higher antifungal activity than other solvent extracts. Antifungal activity exhibited by the methanol extract of *C. glomerata* results were presented in Table 2. The current study emphasized the significance of *C. glomerata* extracts using methanol solvents, as antifungal agent. *C. glomerata* give the impression to be capable natural resources with low cost for antifungal treatment in pharmacology and medicine applications.

Fungal sensitization is widespread in bronchial asthmatic cases, and the connection with airway colonization by fungi remains uncertain. Antifungal healing letdowns rise the cost of treatment, morbidity, and mortality is higher of asthmatic cases in many countries. This study outcome emphasized the advantage in antifungal activity of the tested algal extracts. Currently, most of the compounds of algae were described as antibacterial in human medicine. It is estimated that the antifungal activity establishes through us to be done in the presence of bioactive molecules, as phenolic compounds, polysaccharides, fatty acids. These compounds structured were known as antimicrobials.

3.4 Antibacterial activity of *Cladophora glomerata*

Mostly, the extracts made applying traditional solvents performed more valuable, as the inhibition mechanisms are in portion to the hydrophobic nature of a few components, for example fatty products, and a few researches stated that polar extracts shown higher antibacterial activity [27-29]. Different researches were established that alcoholic solvents, and hydrophilic solvent blends given better results, i.e., methanol and acetone extracts were more active than those in lipophilic solvents as chloroform, methanol etc. In this study, *C. glomerata* methanolic extracts were used and results of the screening of antibacterial activities are summarized in Table 3; data presented the species showing a positive activity against strains tested.

The methanol extract of *C. racemosa* exhibited strong inhibition of *Klebsiella spp*. and *S. typhi,* with significantly higher inhibition than all other algae methanol extracts ($p < 0.05$). From this study, S. aureus was indicated that more sensitive among other bacterial strains. A similar observation was made in a methanol extract of green microalgae *U. lactuca* (200 µg/ml), which showed high inhibiting activity against *S. aureus* [29]. Hussein [30] suggested that the antibacterial activity of *C. crispate* may be attributed to the presence of bioactive compounds such as alkaloids, tannins, fatty acids, polysaccharides, amino acids, proteins, terpenes, sterols, phenolic, flavonoids, aromatic organic acids, aldehydes and ketones. Therefore, effective chemical composition and potential of *C. glomerata* as a source of compounds active against pathogenic microorganisms have been confirmed in this study.

Table 3. Antibacterial activity of *C. Glomerata* Extracts and Minimum Inhibitory Concentration Against Pathogens.

***Cladophora Glomerata* Methanol Extracts (200 µg/ml)**		
Test organism	**Positive control Ampicillin**	**Negative control Methanol**
Bacillus substilis	12.00±1.50	0.00±0.00
Klebsiella pneumonia	11.00±0.44	0.00±0.00
Pseudomonas aeruginosa	13.00±0.75	0.00±0.00
Proteous vlgaris	15.00±1.53	0.00±0.00

Staphylococcus aureus	16.00±0.50	0.00±0.00
Escherichia coli	10.00±1.38	0.00±0.00

4. Conclusion

The freshwater algae used in this article were identified as *C. glomerata*, filamentous green macroalgae. The edible macroalgae, *C. glomerata*, green algae were analyzed for its biochemical and mineral composition. The biochemical and nutritional composition of *C. glomerata* exposes that this macroalga has an appreciable amount of pigments, dietary protein, carbohydrate, and minerals content; also, nutraceutical components were much higher. These findings demonstrate that the methanol extract of *C. glomerata* demonstrated significant antimicrobial activity and thus have great potential as a source for natural health products.

5. Acknowledgement

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6. Conflict of Interests

Authors certify that there has been no conflict of interest in this research, interpretation of data, or in preparing this manuscript for publication.

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