Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Document heading doi:10.12980/JCLM.2.2014C1156

Screening of phytochemicals and antimicrobial activity of *Caulerpa scalpelliformis* collected from Manapad Coast, Tuticorin District, Tamilnadu, South India

N Karthick, M Anees Fathimal, K Ramesh, H Sridhar, M Natrajan, VV Divya, M Umavanitha, S Umamaheswari*

Department of Biotechnology, Manonmaniam Sundaranar University, Tirunelveli−627012, Tamilnadu, India

PEER REVIEW

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Dr. Shimaa MA El Shafay, Lecturer of Phycology, Botany Department, Tanta University, Egypt.
Tel: 040 3344352, 0100 9570818
Fax: 040 3305804
E−mail: sh.shfa3y@yahoo.com

Comments

In this research, the author(s) studied the phytochemical composition of *C. scalpelliformis* and tested the antifungal and antibacterial activity of *C. scalpelliformis* by using different solvents. It's concluded that the presence of chemical constituents plays a vital part in their antibacterial and antifungal activity. On evaluating the antimicrobial property of *C. scalpelliformis*, the algae proved to be an effective antimicrobial agent.
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ABSTRACT

Objective: To analyse the phytochemicals, elements and evaluate the antimicrobial activity of *Caulerpa scalpelliformis* (*C. scalpelliformis*) against different bacterial and fungal pathogens.
Methods: For the elemental analysis and the screening of phytochemicals, some common and available standard tests were done. The antimicrobial activity was done through the agar well diffusion method.
Results: In the qualitative phytochemical screening, among the five different solvent extracts of *C. scalpelliformis*, the benzene extract showed a maximum number of compounds such as tannins, flavanoids, glycosides, phenols, saponins, terpenoids, *etc*. The quantitative analysis showed the total protein, total carbohydrate and total lipid content to be (15.86±1.13)% w/w, (10.32±0.94)% w/w, and (1.05±0.08)% w/w respectively. The antibacterial activity showed a maximum zone of inhibition (15±0.18) mm and a minimum zone of inhibition (6±0.05) mm in the benzene extract of *C. scalpelliformis* exhibited against *Serratia marcescens* and *Bacillus subtilis*. The antifungal assay of *C. scalpelliformis* showed the benzene extract rendered a maximum activity (20±0.25) mm against *Aspergillus terreus* whereas a minimum activity (12±0.14) mm obtained in the chloroform extract against *Aspergillus flavus*.
Conclusions: Our findings provide the evidence that the benzene extract of *C. scalpelliformis* possesses the good antimicrobial activity and hence the algae proves to be an effective therapeutic agent.

KEYWORDS

Phytochemical, *Caulerpa scalpelliformis*, Antibacterial activity, Antifungal activity, Protein, Carbohydrate, Lipid, Therapeutic agent

1. Introduction

Seaweeds are the macroscopic marine algae found attached to the bottom in relatively shallow coastal waters. These seaweeds are classified based on their nutrition and chemical composition such as Chlorophyta (green algae), Phaeophyta (brown algae), Rhodophyta (red algae). Marine species have been used in a wide range of usual remedies and they provide a fine source of antimicrobial analysis. Many metabolites are isolated from marine algae and have been shown to possess bioactive components that exhibit biomedical and antimicrobial properties[1,2]. The seaweeds have been traditionally used in human and animal nutrition. They have rich source of bioactive compounds such as carotenoids, proteins, essential fatty acids, vitamins and minerals. Marine organisms have a number of novel and biologically active metabolites. So far, many chemically unique compounds of marine origin with different biological activity have been isolated and a number of them are under investigation. The nutrient compositions of seaweeds

*Corresponding author: S Umamaheswari, Department of Biotechnology, Manonmaniam Sundaranar University, Tirunelveli−627012,Tamilnadu, South India.
Tel: +91−9442040205
E−mail: umamsu@gmail.com
Founding Project: Supported by Tamilnadu State Council of Science and Technology (Grant No TNSCST/SPS/AR/10).

Article history:
Received 4 Jan 2014
Received in revised form 8 Jan, 2nd revised form 16 Jan, 3rd revised form 18 Jan 2014
Accepted 29 Jan 2014
Available online 28 Feb 2014

are different depending on species, habitats, maturity and environment conditions[3]. Seaweeds are often found in the list of ingredients of cosmetic items particularly in body creams or lotions. Coastal farmers applied seaweed manure to many crops as they contain good amount of nitrogen, potassium and other minerals, carbohydrates and other organic matters present in seaweeds helping in altering the nature of soil and improving its moisture retaining capacity[4]. Today, there is a growing demand for biodiversity in the screening of selecting the drugs from the natural products. Seaweeds are identified as a major source of antibiotics. The production of antimicrobial activities is considered to be an indicator of seaweeds to produce the bioactive secondary metabolites[5,6]. Most of the compounds of marine algae exhibit anti-bacterial activities[7,8]. Many metabolites isolated from marine algae have been shown to possess bioactive efforts[9-11].

The phyto constituents such as flavonoids, phenols and tannins are present in seaweeds and sea grasses, indicating a possibility that the extracts may have antioxidant property. This activity is believed to help in eradicating a number of diseases through free radical scavenging activity. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of micro organisms in *in vitro* conditions. The detection of new antimicrobial compounds from the natural resources is a promise to the rising emergency of antibiotic resistance and their side effects. Seaweeds are valuable sources of protein, fibre, vitamins, polyunsaturated fatty acids, macro and trace elements as well as important bioactive compounds[12]. Many of the seaweeds have more ash contents than terrestrial plants and animal products. Some of the trace elements in seaweeds are rare or absent in terrestrial plants[13]. The present study was undertaken to screen the phytochemical constituents, analysis the elements of *Caulerpa scalpelliformis* (*C. scalpelliformis*) and to determine the antimicrobial activity of different extracts of *C. scalpelliformis*.

2. Materials and methods

2.1. Collection and processing of algal sample

The fresh species of *C. scallpelliformis* were collected from the coastal area of Manapadu, Tuticorin District, Tamil Nadu, South India. It was thoroughly washed with distilled water to get rid off their holdfasts and epiphytes. The water was drained off from the thallus and they were spread on blotting paper to remove the excess water. The shade dried material was crushed in an electric mixer to obtain coarse powder[14].

2.2. Preparation of different extracts of C. scalpelliformis

Extracts were prepared by soaking the coarse powdered material in 100 mL of different solvents like chloroform, benzene, acetone, diethyl ether and methanol with intermittent shaking. The extracts were filtered using muslin cloth and again filtered by filter paper. The organic extracts were concentrated till solvent free by evaporation at 30 °C. The residues obtained were finally dried and dissolved in the respective solvents.

2.3. Elemental analysis

The colour, pH, sodium, potassium, magnesium, calcium, silica, chloride, sulphate, phosphorus, nitrate, iron, zinc, copper were analysed by the method described by American Public Health Association[15].

2.4. Total ash and acid insoluble ash

Total ash and acid insoluble ash have been analyzed as per the protocols given in Ayurvedic pharmacopoeia[16]. A little quantity of powdered *C. scalpelliformis* was taken in a silica crucible and incinerated by slowly increasing the heat not exceeding dull red heat (450 °C) until free from carbon, cooled and weighed. The percentage of ash was calculated with reference to air-dried drug. The process was frequent to get the constant weight. The total ash obtained was boiled for 5 min with 25 mL of (10% w/v) dilute hydrochloric acid and filtered through the ash less filter paper (Whatmann 4.1). The filter paper was ignited in the silica crucible, cooled and insoluble ash was weighed.

2.5. Phytochemical analysis

Phytochemical screening determines the biologically active compounds that are present in the different solvent extract and aqueous extract of *C. scalpelliformis*. All the extracts were tested for the presence of different phytochemicals like alkaloids, steroid, tannin, flavanoids, glycosides, phenolics, saponin, phlobotannins, terpenoids *etc*.

2.5.1. Total protein content

The total protein content was calculated in which 1 mL of the *C. scalpelliformis* extract or standard, 5 mL of alkaline copper sulphate reagent was added, mixed well and allowed to stand for 10 min[17]. Later 0.5 mL of Folins-Ciocalteau's reagent was added and mixed well. The mixture was allowed to stand under dark for 30 min. The blue colour developed was read at 660 nm using UV visible spectrophotometer (Systronics, 119, India). The protein content of the extract was calculated from the standard graph of bovine serum albumin and the results were expressed as % w/w.

2.5.2. Total carbohydrate content

The total carbohydrate content was evaluated by following the method[18]. To 0.5 mL of *C. scalpelliformis* extract or standard, 0.5 mL water was added to make the volume to 1 mL. A volume of 4 mL of anthrone reagent was added. The mixture was heated for 8 min in boiling water bath and cooled. The green colour developed was read at 630 nm using UV visible spectrophotometer (Systronics, 119, India).

The carbohydrate content of the extract was calculated from the standard graph of glucose and the results were expressed as % w/w.

2.5.3. Total lipid content

The total lipid content was estimated by the method with minor modifications[19]. About 0.1 mL of the *C. scalpelliformis* extract supernatant or standard was made up to 5 mL with working ferric chloride acetic acid reagent and the tubes were kept at room temperature for 10 min. Three millilitres of 85% concentrated sulphuric acid was added. The mixture was kept in an ice cold condition for 20 min. The pink colour formed was read at 540 nm using UV visible spectrophotometer (Systronics, 119, India). The lipid content of the extract was calculated from the standard graph of cholesterol and the results were expressed as % w/w.

2.6. Antimicrobial activity test

Four Gram negative rods such as *Escherichia coli*, *Serratia marcescens* (*S. marcescens*), *Klebsiella pneumoniae*, *Pseudomonas aeuroginosa* and two Gram positive cultures of *Staphylococcus aureus*, *Bacillus subtilis* and the fungal cultures *viz.*, *Aspergillus niger* (*A. niger*), *Aspergillus terreus* (*A. terreus*), *Candida albicans* (*C. albicans*) and *Aspergillus flavus* (*A. flavus*) were obtained from the Microbial Biotechnology Laboratory of Manonmaniam Sundaranar University, Tamil Nadu. They were stored at 4 °C at refrigerator.

The different solvent extracts of the seaweeds were subjected to antimicrobial assay by agar well diffusion method. The sterile Muller Hinton agar plates and potato dextrose agar were prepared and inoculated with respective bacterial and fungal cultures. A volume of 60 μL of the five different solvent extracts of *C. scalpelliformis* were introduced to respective wells and allowed for diffusion for 45 min. Solvents alone served as the control. The plates were incubated at 37 °C for 24 h in upright position.

2.7. Statistical analysis

The experimental results were done in triplicates and are expressed as mean±standard deviation.

3. Results

In the elemental analysis of *C. scalpelliformis* the number of macro nutrients (Na, K, Ca, N, Cl, Mg, P, and S), micro nutrients (Fe, Cu, Zn, and Si) are represented in Table 1. Among the macronutrients in *C. scalpelliformis*, chloride has the higher content (632.00 mg/L) and in the micronutrients, iron has the higher content (2.30 mg/L). The total ash content of *C. scalpelliformis* was found to be 1.8% w/w. The acid insoluble ash content was presented to be 0.8% w/w.

Table 1
Elemental composition of *C. scalpelliformis*.

Sequnce No.	Chemicals	Content (mg/L)
1	Sodium	462.30
2	Potassium	218.00
3	Magnesium	120.46
4	Calcium	187.27
5	Silica	95.68
6	Chloride	632.00
7	Sulphate	56.31
8	Phosphorous	49.52
9	Nitrate	130.67
10	Iron	2.30
11	Zinc	1.27
12	Copper	1.52

The phytochemical screening of various extracts of *C. scalpelliformis* are tabulated in Table 2. The benzene extract showed a maximum number of phytochemical compounds such as tannin, flavanoids, glycosides, phenols, saponins and terpenoids. The percentage composition of the primary metabolites of *C. scalpelliformis* extract showed the total protein, total carbohydrate and total lipid content to be (15.86±1.13)% w/w, (10.32±0.94)% w/w and (1.05±0.08)% w/w, respectively.

The antibacterial activity of *C. scalpelliformis* in different solvent extracts indicated an inhibitory zone with a maximum of (15.00±0.18) mm along with a minimum of (6.00±0.05) mm in the benzene extract against *S. marcescens* and *Bacillus subtilis* respectively and the values are represented in Figure 1. The antifungal assay of five different solvent extracts of *C. scalpelliformis* clearly showed that the benzene extract rendered a maximum activity (20.00±0.25) mm against *A. terreus* whereas a minimum activity (12.00±0.14) mm was obtained in the chloroform extract against *A. flavus*, which are tabulated in Figure 2.

Table 2
Qualitative phytochemical analysis of *C. scalpelliformis* in aqueous and five different solvent extracts.

Phytochemical constituents	Aqueous extract	A	B	C	D	M
Alkaloid	−	−	−	−	−	−
Steroid	−	−	−	−	−	+
Tannin	+	+	+	−	+	+
Flavanoid	−	−	+	+	+	+
Glycosides	+	+	+	−	+	−
Phenolic	−	−	+	−	−	−
Saponin	+	+	+	+	+	+
Phlobotannin	−	−	−	−	−	−
Terpenoids	−	+	+	+	−	+

+: Presence, −: Absence. A: Acetone, B: Benzene, C: Chloroform, D: Diethyl ether, M: Methanol.

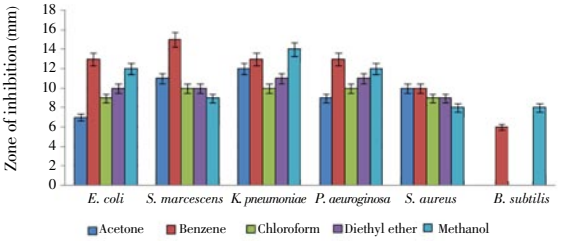

Figure 1. Antibacterial activity of *C. scalpelliformis* in five different solvents.

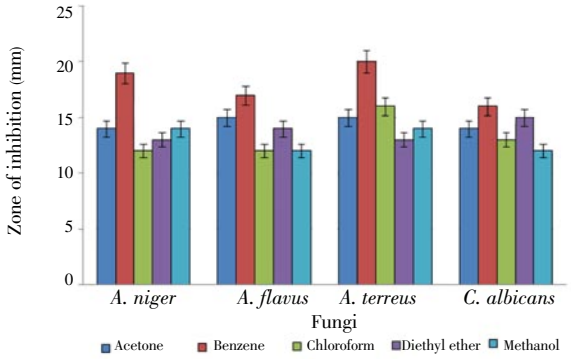

Figure 2. Antifungal activity of *C. scalpelliformis* in five different solvents.

4. Discussion

Seaweeds are great potential production of secondary metabolites which are not found in the terrestrial environment. Thus, the marine algae are among the richest source of known novel bioactive compounds[20,21]. Algae are eukaryotic organisms inhabiting in salty sea water and are recognised to synthesise several bioactive compounds which harbour antimicrobial property[22]. In addition, other substances identified as antimicrobial agents were chlorellin derivatives, acrylic microbial acid, halogenated aliphatic compounds, terpenes, sulphur containing heterocyclic compounds and phenolic inhibitors[23]. Different varieties of marine algae were reported to contain active ingredients that can cure diseases. Nowadays, higher percentage of population refers to use remedies of natural origin for curing illness as these claim to produce less side effects[24]. The present study is focused to study the elemental analysis, screening of phytochemicals and antimicrobial activity of *C. scalpelliformis* against the pathogenic bacteria and fungi. The activity of the algae against both Gram positive and Gram negative bacteria may be an indicative of the presence of broad spectrum of antibiotic compounds or simply the content of pharmacological active constituents like alkaloids, saponins, glycosides, tannins *etc*[25-27]. The phytochemical screening of different solvent extracts of *C. scalpelliformis* revealed the presence of protein in higher amount along with the phytochemical compounds such as flavanoids, tannins, glycosides, phenols, saponins are increased condition in the benzene extract.

Seaweed extracts are considered to be a rich source of phenolic compounds[28]. The large majority of these terpenes but fatty acids are also common with nitrogenous compounds. Four seaweeds extracts in four different solvents are tested against fungal pathogen (*Aspergillus niger*, *Candida albicans*, *A. flavus* and *A. terreus*)[29]. The present study differs from the previous study as the antifungal activity was evaluated using the benzene extract of *C. scalpelliformis*. Apart from this, *C. scalpelliformis* being studied individually for the first time is significantly a new concept. Results of the present study evidenced that the benzene extract of *C. scalpelliformis* possessed good antimicrobial activity. The benzene extract represented the maximum inhibition zone against *S. marcescens* and for the fungi, *A. terreus*. This clearly emphasizes that the benzene extract of *C. scalpelliformis* possessed antimicrobial activities. The benzene and diethyl ether are the suitable solvents for extracting of antibiotic principles[30].

The traditional medicine practice is recommended strongly for *C. scalpelliformis* as well as it is suggested that further work should be carried out to isolate, purify and characterize the active constituents responsible for the bioactivity study. The Chlorophyceae members showed higher antibacterial activity than the Phaeophyceae and Rhodophyceae members[31].

In conclusion, divergence between the results of present investigation and the results of other studies may be due to the production of bioactive compounds related to the organic solvents used for the extraction. The phytochemical screening of *C. scalpelliformis* indicates the presence of chemical constituents that play a vital part in their antibacterial and antifungal activity. On evaluating the antimicrobial property of *C. scalpelliformis*, the algae proved to be an effective antimicrobial agent. The findings of the study also pave the system to find out the specific active compounds responsible for the antimicrobial activity in the upcoming research.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

We are thankful to Manonmaniam Sundaranar University for providing the needed facilities for the research work. This research was funded by Tamil Nadu State Council of Science and Technology. Grant No. TNSCST/SPS/AR/10.

Comments

Background

Seaweeds are the macroscopic marine algae found attached to the bottom in relatively shallow coastal waters. Marine species have been used in a wide range of usual remedies and they provide a fine source of antimicrobial analysis. Many metabolites are isolated from marine algae and have been shown to possess bioactive components that exhibit biomedical and antimicrobial properties.

Research frontiers

It evaluates the phytochemical analysis, elemental analysis and also the antimicrobial activity of *C. scalpelliformis* against different bacterial and fungal pathogens.

Related reports

The phytoconstituents such as flavonoids, phenols and tannins are present in seaweeds and sea grasses indicating a possibility that the extracts may have antioxidant property. This activity is believed to help in eradicating a number of diseases through free radical scavenging activity. The elemental analysis and the screening of phytochemicals, some common and available standard tests are done. The antimicrobial activity was done through the agar well

diffusion method.

Innovations and breakthroughs

The qualitative phytochemical screening among the five different solvent extracts of *C. scalpelliformis*, the benzene extract showed a maximum number of compounds such as tannins, flavanoids, glycosides, phenols, saponins, terpenoids. Algal extract of *C. scalpelliformis* by benzene indicates the good antimicrobial activity and hence the algae proved to be an effective therapeutic agent.

Applications

The phytochemical screening of different solvent extracts of *C. scalpelliformis* revealed the presence of protein in higher amount and the phytochemical compounds such as flavanoids, tannins, glycosides, phenols, saponins are in increased condition in the benzene extract.

Peer review

In this research the author(s) studied the phytochemical composition of *C. scalpelliformis* and tested the antifungal and antibacterial activity of *C. scalpelliformis* by using different solvents. It's concluded that the presence of chemical constituents plays a vital part in their antibacterial and antifungal activity. On evaluating the antimicrobial property of *C. scalpelliformis*, the algae proved to be an effective antimicrobial agent.

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