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RESEARCH ARTICLE

ANTIFUNGALPHYTOCHEMICAL SCREENING AND REPELLENT EFFECT IN SEAWEED G.*edulis*

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ABSTRACT

Seaweeds are multicellular macroalgae used as potential renewable resource in the field of medical and commmerical environment. The aim of this study was to study the antifungal, phytochemical and repellency activities of the red seaweed *G.edulis* since published reports on this particular seaweed were limited.The seaweed *G.edulis* was collected from the Gulf of Mannar coast of Tuticorin in Tamil Nadu. It was shade dried and extracted using five different solvents such as methanol, ethanol, acetone, ethyl acetate and chloroform in a soxhlet apparatus. Among the solvents, the acetone extract showed highest antifungal activity against *A.flavus* (18±0.4mm) and it was followed by *A.terreus* (17±0.6mm) and *F. semitectum* (17±0.6mm). Moderate activity was observed in the ethanol extract showing inhibition zone of (13 ± 0.4mm, 13 ± 0.3mm and 13±0.6mm) against *A.niger*, *Mucor sps* and *T.viride* respectively. In the preliminary phytochemical screening 11 phytoconstituents were present out of the 14 tested.Terpenoids, phenols and flavanoids were present in all the solvent extracts. 100% repellent effects were observed in the acetone extract of 4% concentration of *G.edulis* during the fourth and fifth hour. At 4% concentration 100% repellency rate was observed with the ethyl acetate and the chloroform extracts at the fifth hour. The present study showed that the seaweed *G.edulis* may be a rich source of phytoconstituents. Acetone being a polar solvent was effective in extracting bioactive compounds which produced antifungal and repellency activities. The compound producing biological activity could be further isolated and used in the pharmaceutical industry.

INTRODUCTION

Marine life is fascinating and is considered to have great potential for its intrinsic value as well as for the development of new drugs. In recent years considerable importance is attached to the discovery of new biodynamic agents from marine sources to unearth the new sources of drugs from the sea. Marine plants and animals are reported to possess a wide spectrum of bioactive substances, which are structurally novel and biologically active substances. Research in the areas of marine natural products has grown geometrically in the recent past (Aswal *et al*, 1984; Rawiwon *et al*, 1990). The marine natural products have proved to be the potential source of pharmaceuticals, nutritional supplements, cosmetics, agrochemicals, molecular probes, enzymes and fine chemicals and each of these classes has a potential multibillion dollar market value. (Donia and Hamann, 2003). New trends in drug discovery from natural sources emphasize on investigation of the marine ecosystem to explore numerous complex and novel chemical entities. These entities are the source of new lead for treatment of many diseases such as cancer, AIDS, inflammatory condition, arthritis, malaria and large variety of viral, bacterial, fungal diseases (Prakash *et al*, 2007; Nazar *et al*, 2009). Seaweeds are marine macro algae and primitive type of plants, growing abundantly in the shallow waters of sea, estuaries and backwaters. They flourish wherever rocky, coral or suitable substrata are available for their attachment (Mishra *et al*, 1993). They are a group of aquatic autotrophic organisms that are broadly classified as Chlorophyta (green), Rhodophyta (red seaweeds) of Phaeophyta (brown seaweeds) based on the presence of photosynthetic pigments. Seaweeds are widely included in Japanese and Chinese diet and traditional medicine (Fujiwara *et al*, 1984; Chengkui *et al*, 1984) and have developed biological molecules and approaches which help them survive in their harsh and extreme environment. Seaweeds are considered as source of bioactive compounds and produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. (Lindequist and Schweder, 2001, Newman *et al*, 2003). It was not until the 1970s that large-scale screening of antimicrobial activity was carried out (Glombitza, 1970., Hornsey and Hide, 1974). More than 600 secondary metabolites have been isolated from marine algae (Faulkner, 1986). It was estimated that about 90% of the species of marine plants are algae and about 50% of the gobal photosynthesis contributes to algae (Dhargalkar and Neelam, 2005) .There are numerous reports of compounds derived from *macroalgae* with a broad range of biological activities, such as antibiotics (antibacterial and antifungal properties), antiviral diseases (Trono, 1999), antitumors and anti-inflammatories (Scheuer, 1990) as well as neurotoxins (Kobashi, 1989). Chemical structure types include sterols (Ahmad *et al*, 1993), isoprenoids amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated

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ketones and alkanes, cyclic polysulphides, fatty acids and acrylic acid can be counted (Mtolera and Semesi, 1996). Consumption of seaweeds as sea vegetables in human diets has also been the common practice in several Asian countries (Nisizawa, 2002). There are alarming reports of opportunistic fungal infections. (Singh, 2001). The infections caused by opportunistic fungi are included under new spectrum of fungal pathogens. Such fungi were earlier reported from various plants as pathogens. But now they are known to cause disease in human beings. There is an increasing awareness amongst clinicians and microbiologists pertaining to importance of infection caused by opportunistic fungi (Alex, 1998). Pharmacologist physiologists and chemists have been paying increasing attention to the marine organisms particularly on seaweeds for screening bioactive substances .Several works have been undertaken on crude and purified compounds obtained from seaweeds for evaluating their bioactive potential(Faulkner,1992). Insect infestation of stored grains and their products is a serious problem throughout the world (Irshed and Gillani 1990; Arther and Zettler 1991). There are about 200 or more insects and mites which attacks stored grains and stored products (Maniruzzaman,1981).The present trend, however, is towards alternative non-toxic control methods that pose no threat, to the health of operator or consumer and are environmentally safe. In the middle of the 17th century, pyrethrum, nicotine, and rotenone were recognized as effective insect-control agents (Silva-Aguayo, 2004). Biopesticides from marine algae have gained considerable attention as eco-friendly approach for the management of insect pests and plant pathogens. Much attention has been focused on the antibacterial activity and phytochemical analysis of various types of seaweeds but little has been studied on the antifungal activity, phytoconstituents and the repellent activities against rice weevil of the seaweed G.*edulis* .Since the finding of antibacterial and antifungal activities in many species of marine algae from different part of the world and the isolation of some active compounds the marine algae have become recognized as potential sources of antibiotic substances (Rao, 1991).The genus Gracilaria is an important component of the flora on most tropical to temperate shores. To our knowledge there has been no study on the repellent activity studied in this particular seaweed .So an attempt was made to study the antifungal activity against human fungal pathogens, phytochemical screening and to study the repellent activities against the red flour beetle (*Tribolium casteneum*).

MATERIALS AND METHODS

Collection Of The Plant And Preparation Of The Extracts

Fresh seaweed *G.edulis* were collected from Tuticorin, Gulf of Mannar southeast coast of India .The taxonomic identification of species was done by experts in these fields, using standard literature and taxonomic keys. They were brought to the laboratory in plastic bags and washed with seawater twice and then with distilled water to remove adhering sand particles and extraneous matter like epiphytes, pebbles, shells etc. They were separated and dried at room temperature. The dried seaweed was powdered by an electrical bender. About 25gms of the powdered seaweeds were subjected to solvent extraction by keeping in a mechanical shaker with low polar solvents like Methanol, ethanol, acetone, ethyl acetate and chloroform based on polarity were used to extract the bioactive compounds from the crude extract. It was then filtered using a Whatmann filter 1 paper. The filterate was evaporated and dried at 55-60° C to yield viscous dark green residues for antifungal activity.

Antifungal Activity

The seaweed extracts were screened for antifungal activity by agar well diffusion method (Perez *et al*, 1990). The cultures of 48 hours old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. The fungal cultures such as *Aspergillus niger, Aspergillus fumigates, Aspergillus flavus Aspergillus fumigates, Mucor sp, Aspergillus terreus, Tricoderma viride* and *Fusarium semitectum* were used. An aliquot (20 µl) of inoculum was introduced to molten PDA and poured in to a petri dish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method (20mg/ml), 100µl of various seaweed crude extracts were introduced into the wells. Incubation period of 24-48hours at 28°C was maintained for observation of antifungal activity of the crude extracts. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the extract. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured in diameter. Nyastin was used as the positive control and the respective solvents were used as the negative control. The experiment was done in triplicate and the values were reported as ±SD.

Phytochemical Screening

The preliminary qualitative phytochemical screening was performed following standard procedures (Sofowora, 1931; Trease and Evans, 1989).

Method Of Repellency Test

Repellency test was conducted following the method of Talukdar and Howse (1993) and Amin *et al*, (2000). The dried extracts were dissolved in the respective solvents to make solutions of different concentrations. For the experiment, solutions of four different concentrations such as 0.05% 1.0%, 2.0% and 4.0% (w/v) were used. (Whatman No.1) were marked into two portions. One-milliliter solution of each extract was applied to one half of the filter paper (treated half) and on the other half one milliliter of solvent was applied (control half). The treated disks were then air-dried and placed in a petridish. Twenty (20) insects were placed in the middle line of filter paper. Number of insects on each side was counted at one hour intervals upto the five hours after treatments. Percent repellency was calculated by using the following formula from Abbott (1925):

$$\text{Percent Repellency} = (A\text{-}B/A) \times 100$$

Here,
A = Average number of insects present on untreated portion.
B = Average Number of insects present on treated portion.
The percentages of repellency were then categorized according to the following scale by the method of Roy *et al*, (2005) and Amin *et al*, (2000).

Class Repellency Rate (%)

0 > 0.01-0.10
I - 0.10 to 20.00

II - 20.10 to 40.00
III - 40.10 to 60.00
IV - 60.10 to 80.00
V -80.10 to 100.0

RESULT

The antifungal activity of *G.edulis* is presented in Table 1. In the present study the acetone extract of *G.edulis* showed higher zone of inhibition compared to the other solvent extracts. The highest zone of inhibition was exhibited by acetone extract against *A.flavus* (18±0.4mm) and it was followed by *A.terreus* (17±0.6mm) and *F. semitectum* (17±0.6mm).The methanol extract also showed a similar zone of inhibition of (17±0.4mm) against *A.fumigatus* .The methanol and the chloroform extracts was active against *A.terreus* and showed an inhibition range of (16 ± 0.5mm) and (16 ± 0.4mm). Almost the same inhibition was observed in the acetone extracts against *A.fumigatus*, *Mucor sps*, *T.viride* .The ethyl acetate extract also showed similar inhibition zone against *A.fumigatus.* Moderate activity was observed in the ethanol extract showing inhibition zone of (13 ± 0.4mm, 13 ± 0.3mm and 13±0.6mm) against *A.niger*, *Mucor sps* and *T.viride* respectively. The lowest zone of inhibition was observed in the chloroform extract of *G.edulis* against *F.semitectum*.

Table 1 Antifungal activity of solvent extracts in *G.edulis*

Pathogens	Zone of inhibition in mm					
	Methanol	**Ethanol**	**Acetone**	**Ethyl acetate**	**Chloroform**	**Nyastin**
Aspergillus niger	15±0.4	13±0.4	15±0.6	14±0.4	15±0.6	16±0.3
Aspergillus flavus	14±0.6	16±0.6	18±0.4	15±0.6	16±0.4	18±0.4
Aspergillus fumigatus	17±0.4	15±0.7	16±0.8	16±0.8	15±0.6	15±0.3
Mucor sps	15±0.6	13±0.3	16±0.3	14±0.3	13±0.4	17±0.2
Aspergillus terreus	16±0.5	14±0.4	17±0.6	15±0.4	16±0.4	16±0.3
Tricoderma viride	14±0.6	13±0.6	16±0.4	13±0.6	14±0.6	15±0.3
Fusarium semitectum	13±0.4	14±0.4	17±0.6	13±0.7	10±0.4	18±0.4

Table 2 Phytoconstituents in solvent extracts in *G.edulis*

Phytoconstituents	**Methanol**	**Ethanol**	**Acetone**	**Ethyl acetate**	**Chloroform**
Proteins	Present	Present	Present	Moderately Present	Moderately Present
Resins	Moderately Present	Moderately Present	Moderately Present	Moderately Present	Moderately Present
Steroids	Absent	Absent	Absent	Absent	Absent
Tannins	Absent	Absent	Absent	Absent	Absent
Glycosides	Present	Moderately Present	Moderately Present	Present	Present
Reducing sugar	Absent	Absent	Absent	Absent	Absent
Carbohydrates	Moderately Present	Moderately Present	Moderately Present	Moderately Present	Moderately Present
Saponins	Moderately Present	Present	Present	Present	Moderately Present
Terpenoids	Present	Present	Present	Present	Present
Acidic compounds	Present	Present	Present	Present	Absent
Phenols	Present	Present	Present	Present	Present
Catechols	Absent	Absent	Absent	Absent	Absent
Alkaloids	Moderately Present	Moderately Present	Absent	Absent	Absent
Flavanoids	Present	Present	Present	Present	Present

The phytoconstituents were analyzed in all the solvent extracts and it is represented in Table 2.In the current study phytoconstituents such as proteins, resins, steroids, tannins, glycosides, reducing sugar, carbohydrates, saponins, terpenoids, acidic compounds,phenols,catechols,alkaloids and flavanoids were assessed.

The phytoconstituents such as flavanoids and phenols and terpenoids were present in methanol, ethanol, acetone, ethyl acetate and chloroform extracts of *G.edulis.* Resins, carbohydrates were moderately present in all the five solvent extracts.Steroids, tannins and were completely absent in all the extracts.

Moderate amounts of glycosides were present in the ethanol and acetone extracts. Alkaloids were completely absent in the acetone, ethyl acetate and the chloroform. Saponins were moderately present in the methanol and the chloroform extracts. Acidic compounds were absent only in the chloroform extracts whereas it was present in the rest of the solvent extracts. The repellent activity of the solvent extracts of *G.edulis* was done against the test insect *ie, Tribolium casteneum* and is shown in Table 3. 100% repellent effects were observed in the acetone extract of 4% concentration of *G.edulis* during the fourth and fifth hour. At 4% concentration, 100% repellency rate was observed with the ethyl acetate and the chloroform extracts at the last hour. The chloroform extract at 0.05% concentration showed 27.7% repellency rate which gradually increased and about 75% repellency effects were attained during the last hour. At 1% concentration of the ethyl acetate extract the about 66.66% repellency rate was noticed during the first hour and there was an increase to almost 88.88% during the fifth hour. The 2% concentration of the ethyl acetate extract of also had 88.88% during the first hour itself and almost 94.7% of repellency effects were achieved during the last hour. While considering the mean repellency rate the highest repellency was observed in the 4% chloroform extract showing about 95.66% and was placed in class V .This was followed by the 4% acetone extract that showed mean repellency of 94.49%.The lowest mean repellency rate was observed in the 0.05% concentration of ethanol extract of 27.27%.

DISCUSSION

Marine environment is a rich source of biological and chemical diversity. This diversity has been a unique source of chemical compounds as potentials for pharmaceuticals, cosmetics, dietary supplements and agrochemicals (Chau *et al*, 2005). Seaweeds have adapted to grow in marine environments from coast to coast, living in both tropical and temperate regions on almost every continent in the world. The genus Gracilaria is an important component of the marine flora which is present in tropical and temperate regions. *Gracilaria edulis*, a common species of *Gracilaria* has been recommended as source of agar having gel textural attributes suitable for food applications, although inferior in gel quality compared to those harvested species (Villanueva and

Montana, 1999) .The compounds derived from macroalgae are reported to have broad range of biological activities such as antibacterial (Chakraborthy *et al,* 2010) anticoagulant (Lipton and Jose, 2006) and antifouling activities (Marechal *et al,* 2004). Antifungal activities has been focused on other seaweeds rather than *G.edulis* .So an attempt was made to study the antifungal capacities of this particular seaweed against seven pathogenic fungus. In the present study the solvents such as Methanol, ethanol, acetone, ethyl acetate and chloroform extracts were used for extracting the bioactive compounds present in the seaweed. Among them the acetone extract of *G.edulis* showed high fungal inhibition against the test organisms. The highest zone of inhibition was noticed against *A.flavus* (18±0.4mm) which was equal to the positive control nyastin. Earlier reports showed that different seaweeds showed various activities in different solvents (Subba Rangaiah *et al,* 2010).

This could be attributed to the presence of various kinds of phytoconstituents such as phenol, carbohydrates, proteins, tannins, flavanoids, saponins, terpenoids etc. Zovko *et al,* (2012) studied antifungal activity against fungal strains of *C. albicans* with a high activity of algal extracts. Gao *et al,* (2011) showed that a few extracts of marine algae have not only an antifungal activity but toxicity towards cancer cells. Several extractable compounds, such as cyclic polysulfides and halogenated compounds are toxic to microorganisms and, therefore, responsible for the antibiotic activity of some seaweeds (Fenical, 1975; Wrattens and Faulkner, 1976). Kayalvizhi *et al,* (2012) reported that the acetone extract of brown seaweeds showed significant antifungal activity against five fungal species. Acetone was also the best solvent used for extracting antimicrobial compounds from the British marine algae (Hornsey and Hide, 1974). The methanol extract of *G.edulis* also had appreciable antifungal activities and are in line with reports of (Kolanjinathan and Saranraj, 2014) who observed the highest zone of inhibition of (18±0.6 mm) against *A.flavus* in the methanol extract of *G.edulis.* Moderate antifungal activities were also observed with the ethanol extracts but it was higher than the results of Hebsidah *et al*, (2011) who showed maximum antifungal activity with the ethanolic extract with the red seaweed *Gelidiella acerosa* against *C.albicans* (7mm),*C.tropicals* (7mm) and *A.niger* (7mm). Hodgson (1984) has reported antimicrobial activity of seaweeds belonging to Chlorophyta, Phaeophyta and Rhodophyta. In the present study it was observed that the individual solvents which were used as the negative control did not produce any antifungal activity against the tested fungal pathogens.

The phytoconstituents were also assessed in the crude extracts of *G.edulis* and the results showed that the phytoconstituents levels varied in the different solvent. This may be due the capacity of different solvents in extracting various phytoconsistuents from the seaweed. Out of the 14 phytoconstituents analysed about 11 were present in *G.edulis.*

There are not many reports published on the phytoconstituents of this particular seaweed. Abirami and Kowsalya (2012) observed for the presence of 12 different phytoconsituents in the underexploited seaweed *G.edulis* collected from the Mandapam region. The presence of nine phytochemicals in the ethyl acetate and the ethanol extracts of *G.edulis* were reported by Divya *et al,* (2013). There are no reports showing the presence of resins and acidic compounds in *G.edulis* but it

Table 3 Repellent activity of solvent extracts of *G.edulis* against *T. casteneum*

Methanol							
Extract conc (%)	After treatment (%)					Mean Repellency	Class Repellency
	1h	2h	3h	4h	5h		
0.05	46.1	46.1	57.1	66.66	66.66	56.52	III
1	66.66	66.66	66.66	66.66	66.66	66.66	IV
2	46.1	46.1	57.1	57.1	66.66	54.60	III
4	75	75	75	75	75	75	IV
Ethanol							
Extract conc (%)	After treatment (%)					Mean Repellency	Class Repellency
	X	2h	3h	4h	5h		
0.05	33.33	18.18	18.18	33.33	33.33	27.27	II
1	42.85	42.85	66.66	75	75	60.47	IV
2	75	75	75	75	82.35	76.47	IV
4	82.35	88.88	82.35	88.88	94.73	87.44	V
Acetone							
Extract conc (%)	After treatment (%)					Mean Repellency	Class Repellency
	1h	2h	3h	4h	5h		
0.05	18.18	18.18	18.18	18.18	46.15	34.96	II
1	42.85	42.85	66.66	75	75	60.47	IV
2	75	66.66	66.66	75	75	71.64	IV
4	88.88	88.88	94.73	100	100	94.49	V
Ethyl acetate							
Extract conc (%)	After treatment (%)					Mean Repellency	Class Repellency
	1h	2h	3h	4h	5h		
0.05	18.18	33.33	18.18	33.33	18.18	27.87	II
1	66.66	66.66	82.35	88.88	88.88	78.68	IV
2	88.88	94.7	88.88	94.7	94.7	92.37	V
4	88.88	88.88	94.7	94.7	100	93.43	V
Chloroform							
Extract conc (%)	After treatment (%)					Mean Repellency	Class Repellency
	1h	2h	3h	4h	5h		
0.05	27.7	75	50	50	75	55.54	III
1	75	75	75	75	82.35	76.47	IV
2	82.35	88.88	82.35	88.88	94.73	87.44	V
4	88.88	94.73	88.88	94.73	100	95.66	V

was reported in the present study. Phenolic toxicity to microorganisms is due to the site(s) and number of hydroxyl groups present in the phenolic compound (Scalbert, 1991; Urs and Dunleavy, 1975). Flavones, flavonoids and flavonols are phenolic structure with one carbonyl group. They are synthesized by plants in response to microbial infection (Dixon *et al*, 1983) and are often found effective in vitro as antimicrobial substance against a wide array of microorganisms (Bennet and Wallsgrove, 1994).

Biopesticides from marine algae have gained considerable attention as eco-friendly approach for the management of insect pests and plant pathogens. Thus the exploitation of pesticide of marine algae origin in agriculture will appreciably reduce the risk of human and environmental hazards associated with the persistent use of synthetic chemicals. Presently, considerable efforts are directed at exploring the potentials of biopesticides as alternatives or complimentary to synthetic chemicals. Botanicals have been shown to be environmentally safe and sustainable owing to their biodegradable and non-persistent nature. In the current study the acetone extract of *G.edulis* was able to show repellency effect from the fourth and fifth hour. The results of the present study is in agreement with the findings of Echereobia and Sahayaraj (2012) who revealed 0.4% of emulsifiable concentrations of *Caulerpa varavalensis + Ulva fasciata + Padina pavonica* exhibited repellent activity against *T. castaneum* at 10, 20, 40 and 60 minutes observation time. Appreciable levels of repellent activity was also noticed in the ethyl acetate and chloroform extracts and this is in line with the reports of (Kombiah and Sahayaraj, 2012) who reported that the crude chloroform extract highly repelled *Spodoptera litura* (API = -1.00 at 150 min after exposure) higher than *Dysdercus cingulatus* (API = -1.00 at 180 min after exposure) followed by methanol and hexane extract of *Caulerpa scalpelliformis.* It was observed that the repellent activity was time and dose dependent in some cases.

CONCLUSION

In general the seaweed *G.edulis* showed good antifungal and repellent activities. The phytoconstituents present in them could be responsible for resisting the growth of the fungus and also showing repellent activity.

Futher investigation is going on to identify the compounds responsible for producing these biological activities. Moreover, substantial amount of research regarding the toxicity aspects also need to be carried out before they could actually be used for clinical trials.If the compounds derived from natural products such as seaweeds were isolated it would be beneficial for human beings in the near future.

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