ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Antibacterial and Antifungal potential of acetone, ethanol and methanolic extract of marine red algae *Gelidium amansii*

Nagalingam M[1], Rajeshkumar S*[2], Panneerselvam A[1], Lakshmi T[2]

[1]Department of Zoology, Thiruvalluvar University, Vellore-115, Tamilnadu, India
[2]Department of Pharmacology, Saveetha Dental College and Hospitals, SIMATS, Chennai – 600077, Tamil Nadu, India

Article History:

Received on: 11.06.2018
Revised on: 02.02.2019
Accepted on: 05.02.2019

Keywords:

Gelidium amansii,
Antibacterial,
Antifungal,
Seaweed,
Red algae

ABSTRACT

The marine algae *Gelidium amansii* is one of the important edible marine red algae having many biomedical applications. In this study, the red algae of *Gelidium amansii* were analysed for their antibacterial activity and antifungal activity against organisms such as *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Klebsiella pneumonia* and *Pseudomonas aeruginosa* and *Candida albicans, Candida tropicalis, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus*. The antimicrobial susceptibility test for the isolates by agar well diffusion method using following acetone, ethanol, and methanol-toluene. The Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC) using the following extracts. The results are showing the antibacterial and antifungal activity of red algae is good.

* Corresponding Author

Name: Rajeshkumar S
Phone: +91-9629739263
Email: ssrajeshkumar@hotmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i2.374

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

Marine algae are majorly classified into three groups' red, brown and green algae. In that brown and red algae highly wide range of applications in pharmaceutical and industrial applications (Rajeshkumar *et al.,* 2013; Ponnanikajamidin *et al.,* 2014). Marine algae are considered as sources of natural bioactive compounds because they have a great variety of secondary metabolites characterized by a broad spectrum of biological activities (Malini *et al.,* 2014, Je Hyuk Lee *et al.,* 2014). Several compounds with cytostatic, antiviral (Zandi K *et al.,* 2007), anthelmintic, antifungal (Mayer A M S *et al.,* 2009) and antibacterial activities were studied in green, brown and red algae (Karabay-Yavasoglu N U *et al.,* 2007, Salvador N *et al.,* 2007 and Inci T *et al.,* 2006). There are numerous reports concerning antibacterial and antifungal activities of macroalgae against bacteria and fungi and yeast related to human diseases. Algae have been considered as a potential source of new compounds. Algae possess several biological activities, including anticancer activity. Many algae have been used for the treatment of cancer, many crude or partially purified polysaccharides from various brown, green, and red algae have been tested for their antitumor activities (Ankita Mridha *et al.,* 2017). The effect of the algal mediated silver nanoparticles from *Turbinaria conoides* and *Padina tetrachromatic* against the pathogenic fungi and bacteria is very good and it is cheap and single step synthesis process (Rajeshkumar *et al.,* 2012, Rajeshkumar *et al.,* 2012a, Rajeshkumar *et al.,* 2013a, Rajeshkumar *et al.,* 2014, Rajeshkumar *et al.,* 2016). *Gelidium amansii* extracts tested for antibacterial and antifungal activities. The ethanol extract showed the strongest activity against the bacteria and fungi tested (Zheng Yi, Chen Yin-Shan and Lu Hai-Sheng *et al.,* 2001). Antimicrobial activity was tested against ten gram-positive bacteria: *Bacillus cereus*,

Bacillus thuringensis, Bacillus subtillus, Clostridium sporogenes, Staphylococcus aureus, Mycobacterium smegmatis, Streptococcus feacalis and *Bacillus sp* on against two gram-negative bacteria : *Escherichia Coli* and *Pseudomonas sp* and against fungi *: Candida tropicalis, Candida albicans* and *Cryptococcus neoformans* (Khadija Oumaskour *et al.,* 2013). It has many biotechnological applications that make the red algae as an effective antiviral agent, an antidiabetic agent, an anti-inflammatory agent, an antimicrobial agent and good anticancerous agent (Rajasulochana *et al.,* 2015). Algae Crude extracts exhibit high antibacterial activity and low antifungal activity. Green synthesised Ag-NPs showed very high antimicrobial properties on comparing its crude extracts (Annakodi Jothirethinam *et al.,* 2015).

MATERIALS AND METHODS

Preparation of algae Powderization

The red algae were collected from Tuticorin coastal area, South India. In this study, *Gelidium amansii* were dried at normal heat for several days. After drying the plant material was nicely made into a fine powder using mixer grinder. In each step, the plant material was dried without moisture to overcome the fungal contamination. The air-dried powder of plant material was stored within an airtight container for further use.

Extraction of *Gelidium amansii*

The chemical nature and physical state of the *Gelidium amansii* material, making it difficult to dissolve in distilled water or any other solvent, so the plant materials were soaked in distilled water for 24 hrs. The 1 gm of powder was dissolved in 10 ml of ethanol, methanol, and acetone used in the soxhlet procedure in a cleaned screw cap bottles for 24 hrs. After 24 hrs dissolved extracts were collected and transferred to centrifugal tubes and centrifuged at 3000 rpm for 10 min. The centrifuged extracts were again recentrifuged and filter with Millipore filter. After a few days, the solvents dissolved chemicals were purely concentrated and stored in a refrigerator at 4ºC for antimicrobial susceptibility testing.

Qualitative Phytochemical Screening of *Gelidium amansii* Extract

The phytochemicals present in *Gelidium amansii* was detected using different biochemical methods such as Alkaloids (Mayer's test and Wagner's test), Carbohydrate (Molish's test, Fehling's test (Fehling A and Fehling B), Benedict's test and Barford's test), Saponins, Proteins and amino acids (Millon's test, Beret's test and Ninhydrin test), Phytosterols (Libermann-Burchard's Test), Phenolic Compounds (Ferric chloride test, Lead acetate test and Alkaline reagent test), Terpenoids (Salkowski Test) and Steroids (Malini *et al.,* 2014).

Antibacterial and Antifungal activity study

The main characteristics of the medium were to support the growth of the organisms normally tested and not contain antagonist of antimicrobial activity. The medium must allow free diffusion of algal extract from the well. The sterilised medium was poured into a Petri dish in a uniform thickness and kept aside for solidification. Using sterilised swabs, even distribution of lawn culture was prepared using test bacteria such as *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Klebsiella pneumonia* and *Pseudomonas aeruginosa.* The fungi like such as *Candida albicans, Candida tropicalis* in Muller Hinton Agar (MHA) plates. The other organisms like such as *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* in Potato Dextrose Agar (PDA) plates.

Determination of Minimum Inhibitory Concentration (MIC)

1 ml of *Gelidium amansii* extract (1mg/ml) was incorporated into 1 ml of nutrient broth and potato dextrose broth and was serially to obtain a concentration of 15.6 – 1000 µl respectively. 20 µl of the inoculum was added to each of the test tubes. The tube without the extract served as control. The tubes were incubated at room temperature, and readings were recorded after a period of 24 hrs for bacteria and 3 days for fungi. MIC was recorded as the lowest concentration of the extract at which no visible growth of the bacterial and fungal occurred after a period of seven days' incubation of agar.

RESULTS AND DISCUSSION

Antimicrobial activity of acetone, ethanol and methanol extracts of *Gelidium amansii*

Acetone, Ethanol and Methanol extracts of *Gelidium amansii* seaweed were tested against bacteria and fungi, *Staphylococcus aureus, K. pneumonia, Escherichia coli, Pseudomonas aeruginosa, Micrococcus luteus, Bacillus cereus* and Fungi, *Candida albicans, Candida tropicalis, Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatus*. The results are shown in table 1 to 6.

The acetone extract of *Gelidium amansii* shows good Zone of inhibition against *Bacillus cereus* shows in Table – 1. In that, the algae extracts show a good zone of inhibition against *Bacillus cereus*.

The acetone extract of *Gelidium amansii* shows good Zone of inhibition against *Aspergillus niger* and *Aspergillus flavus* shows in Table – 2.

The methanol extract of *Gelidium amansii* shows good Zone of inhibition against *Bacillus cereus* shows in Table – 3.

Table 1: Antibacterial activity of acetone extract of *Gelidium amansii*

S. No	Name of the Organisms	Zone of Inhibition (mm)	
		Control	Test
1	*Staphylococcus aureus*	No Zone	No Zone
2	*Micrococcus luteus*	No Zone	No Zone
3	*Escherichia coli*	No Zone	No Zone
4	*Pseudomonas aeruginosa*	No Zone	No Zone
5	*Klebsiella pneumonia*	No Zone	No Zone
6	*Bacillus cereus*	No Zone	12

Table 2: Antifungal activity of acetone extract of *Gelidium amansii*

S. No	Name of the Organisms	Zone of Inhibition (mm)	
		Control	Test
1	*Candida albicans*	No Zone	No Zone
2	*Candida tropicalis*	No Zone	No Zone
3	*Aspergillus niger*	No Zone	5
4	*Aspergillus flavus*	No Zone	5
5	*Aspergillus fumigatus*	No Zone	No Zone

Table 3: Antibacterial activity of methanol extract of *Gelidium amansii*

S. No	Name of the Organisms	Zone of Inhibition (mm)	
		Control	Test
1	*Staphylococcus aureus*	No Zone	No Zone
2	*Micrococcus luteus*	No Zone	No Zone
3	*Escherichia coli*	No Zone	No Zone
4	*Pseudomonas aeruginosa*	No Zone	No Zone
5	*Klebsiella pneumonaea*	No Zone	No Zone
6	*Bacillus cereus*	No Zone	7

Table 4: Antifungal activity of methanol extract of *Gelidium amansii*

S. No	Name of the Organisms	Zone of Inhibition (mm)	
		Control	Test
1	*Candida albicans*	No Zone	No Zone
2	*Candida tropicalis*	No Zone	No Zone
3	*Aspergillus niger*	No Zone	No Zone
4	*Aspergillus flavus*	No Zone	5
5	*Aspergillus fumigatus*	No Zone	No Zone

Table 5: Antibacterial activity of ethanol extract of *Gelidium amansii*

S. No	Name of the Organisms	Zone of Inhibition (mm)	
		Control	Test
1	*Staphylococcus aureus*	No Zone	No Zone
2	*Micrococcus luteus*	No Zone	No Zone
3	*Escherichia coli*	No Zone	No Zone
4	*Pseudomonas aeruginosa*	No Zone	No Zone
5	*Klebsiella pneumonaea*	No Zone	No Zone
6	*Bacillus cereus*	No Zone	6

Table 6: Antifungal activity of acetone extract of *Gelidium amansii*

S. No	Name of the Organisms	Zone of Inhibition (mm)	
		Control	Test
1	*Candida albicans*	No Zone	6
2	*Candida tropicalis*	No Zone	No Zone
3	*Aspergillus niger*	No Zone	No Zone
4	*Aspergillus flavus*	No Zone	5
5	*Aspergillus fumigatus*	No Zone	No Zone

The methanol extract of *Gelidium amansii* shows good Zone of inhibition against *Aspergillus flavus* shows in Table – 4.

The ethanol extract of *Gelidium amansii* shows good Zone of inhibition against *Bacillus cereus* shows in Table – 5.

Table 7: Phytochemical analysis of an aqueous extract of *G. amansii*

S.No.	Experiment	Observation	Inference
1	Test for tannins	No brownish green color	-
2	Test for flavonoids	No yellow color	-
3	Test for terpenoids (Salkowski test)	No reddish brown color	-
4	Test for carbohydrates (Molish's test)	Violet ring	+
5	Test for reducing sugars (Fehling's test)	No red precipitate	-
6	Test for reducing sugars (Benedict's test)	No red precipitate	-
7	Test for polysaccharides (Iodine test)	Violet color	+
8	Test for amino acids (Ninhydrin test)	No Violet color	-
9	Test for proteins (Millon's test)	White precipitate	+
10	Test for phenolic compounds ($FeCl_3$ test)	No green color	-
11	Test for saponins	No formation of an emulsion	-
12	Test for alkaloids (Mayer's test)	White precipitate	+
13	Test for alkaloids (Wagner's test)	Reddish brown precipitate	+
14	Test for phytosterols (Liebermann-Burchard test)	An array of color changes	+

+ Present; - Absent

Table 8: Phytochemical analysis of an acetone extract of *G. amansii*

S.No.	Experiment	Observation	Inference
1	Test for tannins	No brownish green color	-
2	Test for flavonoids	No yellow color	-
3	Test for terpenoids (Salkowski test)	No reddish brown color	-
4	Test for carbohydrates (Molish's test)	Violet ring	+
5	Test for reducing sugars (Fehling's test)	No red precipitate	-
6	Test for reducing sugars (Benedict's test)	No red precipitate	-
7	Test for polysaccharides (Iodine test)	Violet color	+
8	Test for amino acids (Ninhydrin test)	No Violet color	-
9	Test for proteins (Millon's test)	White precipitate	+
10	Test for phenolic compounds ($FeCl_3$ test)	No green color	-
11	Test for saponins	No formation of an emulsion	-
12	Test for alkaloids (Mayer's test)	White precipitate	+
13	Test for alkaloids (Wagner's test)	Reddish brown precipitate	+
14	Test for phytosterols (Liebermann-Burchard test)	An array of color changes	+

+ Present; - Absent

Table 9: Phytochemical analysis of a methanol extract of G. amansii

S.No.	Experiment	Observation	Inference
1	Test for tannins	No brownish green color	-
2	Test for flavonoids	No yellow color	-
3	Test for terpenoids (Salkowski test)	No reddish brown color	-
4	Test for carbohydrates (Molish's test)	Violet ring	+
5	Test for reducing sugars (Fehling's test)	No red precipitate	-
6	Test for reducing sugars (Benedict's test)	No red precipitate	-
7	Test for polysaccharides (Iodine test)	Violet color	+
8	Test for amino acids (Ninhydrin test)	No Violet color	-
9	Test for Proteins (Millon's test)	White precipitate	+
10	Test for Phenolic compounds ($FeCl_3$ test)	No green color	-
11	Test for saponins	No formation of an emulsion	-
12	Test for alkaloids (Mayer's test)	White precipitate	+
13	Test for alkaloids (Wagner's test)	Reddish brown precipitate	+
14	Test for Phytosterols (Liebermann-Burchard test)	An array of color changes	+

+ Present; - Absent

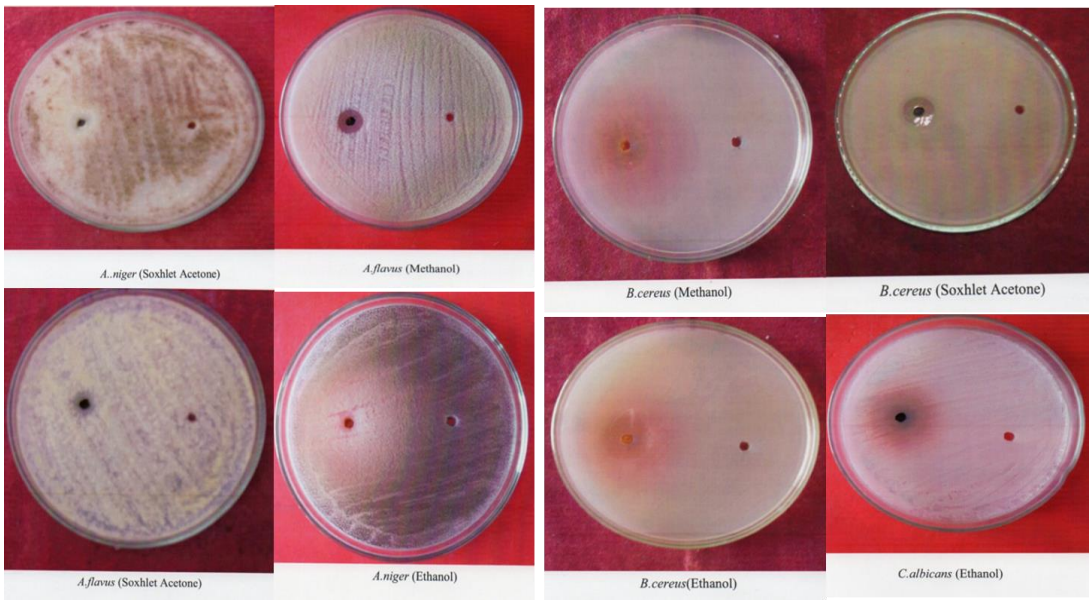

Figure 1: Zone of inhibition on Antibacterial and antifungal activity of red algae

The ethanol extract of *Gelidium amansii* shows good Zone of inhibition against *Candida albicans* and *Aspergillus flavus* shows in Table – 6.

The phytochemical analyses of Aqueous *Gelidium amansii* extract such as Carbohydrate, polysaccharides, proteins, alkaloids, and phytosterols showed in table – 7.

The phytochemical analyses of Acetone *Gelidium amansii* extract such as carbohydrates, polysaccharides, proteins, alkaloids and phytosterols showed in table – 8.

The phytochemical analyses of methanol *Gelidium amansii* extract such as carbohydrates, polysaccharides, proteins, alkaloids and phytosterols showed in table – 9.

To study the antibacterial and antifungal activity of seaweed extracts, *Gelidium amansii* was collected and using Soxhlet extraction process Acetone, Methanol and Ethanol extract was prepared. The zone of inhibition formed against different bacterial and fungal pathogens are shown in figure 1. The effective zone was formed due to the different bioactive compounds present in the algal extract (Ponnanikajamidin *et al.,* 2014).

The ethanolic extract of *Gelidium amansii* contains phytochemicals such as Carbohydrate, Polysaccharides, Proteins, alkaloids, and phytosterols. The ethanolic extract shows a good zone of inhibition against some selective bacteria and fungi may be the reason of phytochemicals present in the algae extract. (Min-Cheol Kang *et al.,* 2016) The acetone extract of *Gelidium amansii* contains phytochemicals such as Carbohydrate, Polysaccharides, Proteins, alkaloids, and phytosterols. The acetone extract shows a good zone of inhibition against some selective bacteria and fungi may be the reason of phytochemicals present in the algae extract. The methanolic extract of *Gelidium amansii* contains phytochemicals such as Carbohydrate, Polysaccharides, Proteins, alkaloids, and phytosterols. The methanolic extract shows a good zone of inhibition against some selective bacteria and fungi may be the reason of phytochemicals present in the algae extract. (Malini *et al.,* 2014).

REFERENCES

Ankita Mridha, Santanu Paul. Algae as a potential repository of anti-cancerous natural compounds. International Journal of Phytomedicine. 9; 2017(2): 181-194: 0975-0185.

Inci T, Bilge Hilal Ç, Dilek U, Atakan S. Antimicrobial Activities of the Extracts of Marine Algae from the Coast of Urla (Üzmir, Turkey). Turk J Biol 2006; 30: 171-175.

Je Hyuk Lee. Antibacterial effect of marine algae against oral borne pathogens. Res.Jou.Med.Plant 2014; 8(4): 196-203: 1819-3455.

Karabay-Yavasoglu N U, Sukatar A, Ozdemir G, Horzum Z. Antimicrobial activity of volatile components and various extracts of the red alga Jania Rubens. Phytother Res 2007; 21: 153-156.

Khadija Oumaskour, Nabila Boujaber, Samira Etahiri and Omar Assobhei. Anti-Inflammatory and Antimicrobial Activities of Twenty-Three Marine Red Algae from the Coast of Sidi Bouzid (El Jadida-Morocco). International Journal of Pharmacy and Pharmaceutical Sciences. Vol 5, (3), 2013: 0975-1491.

M. Ponnanikajamideen, M. Malini, C.Malarkodi, S.Rajeshkumar Bioactivity and phytochemical constituents of marine brown seaweed (padina tetrachromatic) extract from various organic

solvents International Journal of Pharmacy & Therapeutics, 5(2), 2014, 108-112.

Malini, M. Ponnanikajamideen, C.Malarkodi and S.Rajeshkumar Explore the Antimicrobial Potential from Organic Solvents of Brown Seaweed extracts (Sargassum longifolium) alleviating to pharmaceuticals (2014) International Journal of Pharmaceutical Sciences and Research 6(1):28-35.

Mayer A M S, Rodríguez AD, Roberto B G S *Et al.,* Marine pharmacology in 2005-2006: Marine compounds with anthelmintic, antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action. Bioch Bioph Acta; 2009. p. 283-308.

Min-Cheol Kang, Nalae Kang, Seo-Young Kim, Ines S. Lima, Seok-Chun Ko, Young-Tae Kim, Young-Bum Kim, Hee-Do Jeung, Kwang-Sik Choi, You-Jin Jeon. Popular edible seaweed, Gelidium amansii prevents against diet-induced obesity. Food and Chemical Toxicology 90 (2016) 181-187.

Rajeshkumar S, Kannan. C and Annadurai. G (2012) Synthesis and characterisation of antimicrobial silver nanoparticles using marine brown seaweed Padina tetrastromatica Drug Invention Today 4 (10), 511-513.

Rajeshkumar S, Malarkodi C, Paulkumar K, Vanaja M, Gnanajobitha G, Kannan C, Annadurai G Seaweed mediated synthesis of gold nanoparticles using Turbinaria conoides and its characterisation (2013a) Journal of Nanostructures in Chemistry 3 (44): 1-7.

Rajeshkumar S., Kannan, C and Annadurai, G (2012a). Green synthesis of silver nanoparticles using marine brown turbinaria conoides and its antibacterial activity. International journal of pharma and Bioscience (IJPBS) 3 (4) 502 – 510. Impact Factor (2014): 2.95.

S Rajeshkumar, M Vanaja, C Malarkodi, G Gnanajobitha, K Paulkumar, C Kannan, G Annadurai Development in Therapeutic importance of most sought marine algal Polysaccharide Fucoidans (2013) International Journal of Research in Biomedicine and Biotechnology 3(2): 37-43.

S. Rajeshkumar Phytochemical constituents of fucoidan (Padina tetrastromatica) and its assisted silver nanoparticles for enhanced antibacterial activity (2016) IET Nanobiotechnology doi: 10.1049/iet-nbt.2016.0099

Salvador N, Garreta AG, Lavelli L, Ribera M A. Antimicrobial activity of Iberian macroalgae. Sci Mar 2007; 71: 101-113.

Zandi K, Fouladvand M, Pakdel P, Sartavi K. Evaluation in vitro antiviral activity of a brown alga (Cystoseira myrica) from the Persian Gulf against Herpes simplex virus type 1. Afr J Biotechnol 2007; 6: 2511-2514.

Zheng Yi, Chen Yin-Shan and Lu Hai-Sheng. Screening for Antibacterial and Antifungal Activities in Some Marine Algae from the Fujian Coast of China with Three Different Solvents" Chinese Journal of Oceanology and Limnology. Vol.19, (4), P.327-331; 2001