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Research Article

INVITRO SCREENING OF SECONDARY METABOLITES AND ANTIMICROBIAL ACTIVITIES OF ETHANOL AND ACETONE EXTRACTS FROM RED SEAWEED *GELIDIUM ACEROSA*

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ABSTRACT

The present study is aimed at the *invitro* screening of secondary metabolites like, alkaloids, carbohydrates, saponins, glycosides protein and Amino acids, Phytosterol, Phenolic compound, flavonoids, terpinoids, Tannins, Antimicrobial activities of ethanolic and acetone extracts of *Gelidium acerosa* against human pathogens like *Staphylococcus aureus, Bacillus aureus, Micrococcus leutus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa and* Fungi like *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, C.albicans, C.tropicalis*. Ethanolic extracts of *Gelidium acerosa* presented the highest antimicrobial activity and was effective against all pathogens tested than acetone extracts. *Gelidium acerosa* of ethanolic extracts shows presence of maximum number of secondary metabolites, than acetone Extract.

Keywords: Seaweeds, Antimicrobial activities, Secondary metabolites.

INTRODUCTION

The genus *Gelidium acerosa* is an excellent sources of Agar.(1,2) But, *Gelidium acerosa* also contain many components of therapeutic value (3,4) , *Gelidium acerosa* now very popular in developing countries on account of improved knowledge on secondary metabolites (phytochemicals), and it has been investigated as a source of medicinal agents.

It is also proved that the phytochemicals have antimicrobial activities and therefore used for the treatment of various bacterial and fungal infections (5). Nowadays there is an urgent need for discover of new antimicrobial compounds with chemicals structure as effective drug against microbial infections (6, 7), so *Gelidium acerosa* is one of the red algae, which with more components of phytochemicals and strong antimicrobial activity has been taken for the study.

This study is aimed at determining the efficiency of ethanol and acetone extracts from red seaweed which are available in Tamil Nadu region-India to obtain highly active biological substances which are active against the human pathogens.

MATERIALS AND METHODS

The sample *Gelidiella acerosa (forssbai)* was purchased from the Mandabam region, Tamil Nadu, India and identified by SNAP Natural and Alginate Products Pvt Ltd, SIPOT, Ranipet, Tamilnadu, India. It was cleaned and epiphytes and necrotic parts were removed. The samples were rinsed with sterile water to remove associated debris, and kept under sun shade for 7 days. After drying it was ground thoroughly to powder and used for the analysis.

Extracts preparation

Dry algal material (10g) was extracted with ethanol and acetone in a soxhlet apparatus for 8hrs (8). The extract was concentrated under reduced pressure at 60^0C using rotary evaporator, then filtered, washed with about 25ml distilled water and dried and stored in the dark at 4^0C (9) for phytochemical analysis.

The phytochemical analysis were carried out for the presence of Alkaloids (Evans, 1977), Carbohydrates (Ramakrishna et al., 1994), Saponins (Kokate, 1999) Glycosides (Ramakrishna et al, 1994), Protein and Amino acids (Fisher, 1968), Phytosterol (Finar, 1986), Phenolic compounds, Flavins, Terpinoids, Tannins (Mace 1963, Evans 1997, Harburn 1988) using the modified procedures (10, 11, 12)

Antimicrobial assay

The tested organisms like *S.aureus, B.cereus, M. leutues, K. pneumonia, E.coli, P. aeroginosa, A.flavus, A.niger, A.fumigatus, C.albicans, C.tropicalis* were obtained from the Micro labs, Institute of Research and Technology Arcot, Vellore District, Tamil Nadu. The cultures were maintained in the appropriate agar slant at 4^0C throughout the study and used as test microorganism in the appropriate medium. The microbial assay was done by Agar well diffusion method (13). Each microbial isolate was suspended in Brain Heart Infusion (BHI) broth and diluted to approximately 105 colony forming unit (CFU) / ml and inoculated on BHI agar. 5mm, diameter wells were cut and 1ml of sample solutions were placed in the well. The plates were incubated for 18hrs at 37^0C.

The antimicrobial activity was evaluated by measuring zone of inhibition against test microorganism. Ethanol was used as solvent control for Ethanol extracts, and acetone was used as solvent control for acetone extracts.

Table 1: The phytochemical analysis of ethanol and acetone algal extracts with *Gelidium acerosa* brown algae

S. No	Phytochemicals	Ethanol Extract	Acetone Extract
1.	Alkaloids	+	+
2.	Carbohydrates	+	+
3.	Saponins	+	+
4.	Glycosides	-	-
5.	Protein and amino acids	+	-
6.	Phytosterols	+	+
7.	Phenolic compounds	+	-
8.	Flavonoids	+	+
9.	Terpinoids	-	-
10.	Tannins	+	+

(+) positive (-) negative

Table 2: Antimicrobial assay of ethanol and acetone extracts of *Gelidiella acerosa* against human pathogens

S.No	Organisms	Zone of Inhibition (mm) Control	Ethanol extracts	Acetone extract
	Bacteria			
1	*S.aureus*	No zone	7	5
2..	*M.luteus*	No zone	6	5
3.	*E.coli*	No zone	No zone	No zone
4.	*P.aeruginosa*	No zone	No zone	No zone
5.	*K.pneumonia*	No zone	5	No zone
6.	*S.cereus*	No zone	6	7
	Fungi			
1.	*C.albicans*	No zone	7	6
2.	*C.tropicals*	No zone	7	No zone
3.	*A.niger*	No zone	7	No zone
4.	*A.flavus*	No zone	5	5
5.	*A.fumigatus*	No zone	No zone	No zone

In the phytochemical screening test, the ethanol extract of *Glidiella acerosa* showed many secondary metabolites qualitatively than acetone extracts **(Table - 1)**. Phytochemicals like, alkaloids, carbohydrates, saponins, protein and amino acids, phytosterols, phenolic compounds flavinoids, and Tannins, were identified, and showed positive result in ethanol extracts. Terpinoids and Glycosides were absent. In acetone extract glycosides, proteins, phenolic compounds and terpinoids were absent and other metabolites were present.

RESULTS AND DISCUSSION

The Antimicrobial activity (Bacteria and Fungi) of Ethanol and Acetone extracts *Gelidiella acerosa* of and as ethanol of acetone are summarized in Table 2. In this study the zone of inhibition of various human pathogens ranged from 4mm to 12mm.

The Ethanol extract of *Gelidiella acerosa* showed maximum activity against pathogens like *S. aerius* (7mm) and minimum activity in *K.pneumoniae* (5mm) and moderate activity against *M.luteus* (6mm) and *B.cereus* (6mm), no activity against bacteria like *M.letus* and *E.coli.* In fungi ethanol extracts of *Gelidium acerosa* showed the maximum activity against *C.albicans* (7mm) *C.tropicals* (7mm) *A.niger* (7mm) and minimum zone formation in *A.flavus* (5mm) and no zone was observed in *A.fumigatus*. And no zone was formed in the control against all the tested microorganisms. The above results show that the G*elidila acerosa* species exhibit the high Antibacterial and Antifungal activity (14, 15, and 16) in ethanol extract when compared to Acetone extracts.

The acetone extract showed a minimum activity against *M.leutius* (5mm) and *S.aerus* (5mm) and maximum activity against the *B.cereus* (7mm) and no activity was seen against *E.coli* and *P.aeruginosa, K.pneumoniae*, and for fungi, the maximum activity of acetone extract was seen in *C.alibican,* and minimum activity against *A.flavus* (5mm) and no activity were found against *C.tropicals, C.niger, A.fumigatus*.

CONCLUSION

The seaweeds are found as important source of bioactive natural substances (17). The identification and isolation of the metabolites with biological activities has recently received significant attention for their anti oxidant properties and other related properties (18). The phytochemicals which are identified has the free radical scavenging effects (19). The phenolic compound plays an important role in antimicrobial, anti inflammatory, and anti cancer activity (20, 21, 22). A single solvent extraction may not be enough to exhaustively extract certain compounds responsible for the activity (23). The study shows that *Gelidiella acerosa* has large amount of secondary metabolites, and shows strong antimicrobial activity. The finding of the current works appears to be useful for the further investigation and this study may be extended in various aspects like anti cancer activity, anticoagulant activities fibrinolytic properties.

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