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Research Article

IN-VITRO ANTIBACTERIAL ACTIVITY AND PRELIMINARY PHYTOCHEMICAL SCREENING OF THREE ALGAE FROM VISAKHAPATNAM COAST, ANDHRA PRADESH, INDIA

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ABSTRACT

In-vitro antibacterial activity and Preliminary phytochemical screening of ethanol (70%) extracts of three marine algae (*Chaetomorpha antennina, Gracilaria corticata* and *Ulva faciata)* from Visakhapatnam coast, Andhra Pradesh, India were investigated. The marine algae which were used for present study belongs to red algae and green algae. The tested micro organisms for antibacterial activity were gram positive bacteria (*Bacillus megaterium, Staphylococcus epidermidis, Lactobacillus acidophilus*) and gram negative bacteria (*Escherichia coli, Salmonella typhi* and *Klebsiella pneumonia*). The tested three algae for phyto chemical screening showed positive results for biological active compounds like steroids, Terpinoids, alkaloids, glycoside, amino acids, carbohydrates, saponins and oils. The three algae extracts were showed good antibacterial activity against tested bacterial species. The *Chaetomorpha antennina* showed highest zone of inhibition against bacteria than other two algae.

Keywords: *In-vitro* antibacterial activity, Preliminary phytochemical screening, Visakhapatnam Coast, Red algae, Green algae.

INTRODUCTION

Sea has higher biodiversity than land. The marine organisms produce different primary and secondary metabolites for their survival, defense, signals and to protect themselves from environmental pressures, attacks. The primary and secondary metabolites produced from marine organisms were chemical compounds. The chemical compounds isolated from marine organisms were sources of metabolites for application in human health. The marine organisms were good sources of marine natural products. The main sources for marine natural products were sponges, algae, corals, mollusks, ascidians, micro organisms and others. Algae are group of marine plants and they are not only primary and major producers of organic matter in the sea but they also exert profound effects on the density and distribution of their inhabitants of the marine environment. Algae contain rich and largely entrapped sources of a vast assortment of biologically active substances[1,2]. Earlier many studies were reported on the presence of the antibacterial activity of marine algae from coastal area [3-6]. The chemical compounds already isolated from algae are providing valuable ideas for the development of new drugs against different diseases [7-9]. In this background, the present study was carried to evaluate phytochemical constituents and their antibacterial activity with three marine algae *Chaetomorpha antennina* (green algae), *Gracilaria corticata* (red algae) and *Ulva faciata* (green algae) from Visakhapatnam coast.

MATERIALS AND METHODS

Collection and Preparation of Extract

For screening of phytochemical constituents and antibacterial activity of marine algae were collected from Rrushikonda beach, Visakhapatnam Coast, Andhra Pradesh, India in the month of December and were identified by algal experts, Department of Botany, Andhra University. The collected live and healthy algae were washed with distilled water and then shade dried at room temperature for 10 days. The shade dried algae material was pulverized into courses powder and extracted by maceration process by using ethanol (70%) solvent. The extract was filtered and concentrated to crude extract in a rotary vacuum evaporator (Buchi) at 40°C. Then the extract was subjected to preliminary photochemical screening and tested for antibacterial activity.

Test organisms used

To testing antibacterial activity of selected algae six bacterial species were selected and they were collected from National Collection of Industrial Micro-Organisams (NCIM), Pune, India. The bacterial species were maintained in nutrient broth medium placing on shaker in separate culture tubes for each species. Out of six three are gram positive bacteria and three are gram negative.

Gram positive Bacteria	Gram negative Bacteria
Bacillus megaterium	*Escherichia coli*
Staphylococcus epidermidis	*Salmonella typhi*
Lactobacillus acidophilus	*Klebsiella pneumonia*

Culture media

For Anti bacterial activity of selected algae Muller-Hinton Agar media (Solid and Broth) was used. For maintaining the bacterial species Nutrient both was used.

Phyto-chemical Screening

Phyto-chemical constituent's analysis was carried out qualitatively for the presence of steroids, terpenoids, tannins, flavanoids, saponins, phenols, alkaloids, carbohydrates, glycosides, oils, amino acids etc following the described procedures [10- 12].

Anti-Bacterial Activity

The extracts of algae were tested by using cup plate method for their antibacterial activity[13,14]. Different concentrations of the extracts were prepared by reconstituting with dimethyl sulphoxide (DMSO). The prepared Muller-Hinton Agar medium was heated at 45^0c to get liquid state. The Muller-Hinton Agar (MHA) medium was cooled at room temperature. Then, 20ml of Muller-Hinton Agar medium is taken in the eight test tubes, to those test tubes subjected to testing bacterial inoculums (20µl). After adding inoculums to the test tubes were mixed well for equal distribution of Bacterial species in the MHA medium. After proper mixing, the medium was poured into the autoclaved Petri dishes. These Petri dishes were placed in undisturbed condition for solidifying the medium. After solidification of the medium wells (6mm dia) were prepared by using metal steel borer. Different concentrations of algal extract were placed in the wells of solidified Petri dishes. Then plates were incubated in incubator for 24hrs at 36^0c. After incubation the zones of inhibitions were measured in mm.

RESULTS AND DISCUSSION

Earlier many studies were reported on the presence of different bio-active compounds and their antibacterial activity of marine algae collected from the coastal area [15-18]. The tested algae contain different chemical constituents like steroids, alkaloids, terpenoids, glycosides, phenols, flavanoids, amino acids and oils. The

presence or absences of different chemical constituents in extracts were responsible for different biological activities. Many studies were reported on biological activities of algal extracts from different coastal regions around the world[19]. The tested three algae mainly contain steroids, terpenoids, alkaloids, phenols and glycosides. The *Chaetomorpha antennina* and *Ulva faciata* extracts were contain the flavanoids but *Gracilaria corticata* do not contain the flavanoids. The three algal extracts have normal sugars but *Gracilaria corticata* contain the reducing sugars and amino acids but these three extracts do not contain the tannins. In this background, the collected algae were tested for their antibacterial activity. The three algae were showed good antibacterial activity (zone of inhibition) against tested bacterial species. *Gracilaria corticata* and *Ulva faciata* showed good zone of inhibition against gram positive and gram negative bacteria than *Chaetomorpha antennina. Ulva faciata* showed highest zone of inhibition against *Lactobacillus acidophilus* but *Chaetomorpha antennina* showed lowest zone of inhibition. The results of anti bacterial activity and phytochemical constituents screening of three algae were showed in Tables 1-4. The results obtained in this present study supports that the marine algae contain biological active compounds wgich effective in resisting the growth of the pathogenic bacteria. The coastal area of Visakhapatnam is best owed with large number of pharmaceutically useful seaweeds which can be studied for investigation of drugs for many serious diseases like cancer, tumors, AIDS, and many human degenerative diseases.

Table 1: Phytochemical analysis of the extract of *Chaetomorpha antennina, Gracilaria corticata* and *Ulva faciata*

S.no.	Name of the test	*Chaetomorpha antennina*	*Gracilaria corticata*	*Ulva faciata*
1	Test for steroids			
	Salkowski Test	+	+	+
	L.B. Test	+	+	+
2	Test for terpinoids			
	Salkowski Test	+	+	+
	L.B. Test	+	+	+
3	Test for saponins			
	Foam Test	+	+	--
4	Test for Alkaloids			
	Dragen droff's test	+	+	+
	Mayer's test	+	+	+
	Wagner's test	+	+	+
	Hager's test	+	+	+
5	Test for flavonoides			
	Shinoda's test	+	--	+
	Ferric chloride test	+	--	+
	Lead acetate test	+	--	+
	Zn+ HCl test	+	--	+
6	Test for Carbohydrates			
	Bendict's test	-	+	-
	Molish's test	+	+	+
	Fehling's test	-	+	-
7	Test for Glycosides			
	Buljet test	+	+	+
	Legal test	+	+	+
8	Test for Amino acids			
	Millon's test	-	+	-
	Ninhydrin test	-	+	-
	Biruete's test	-	+	-
9	Test for tannins			
	Ferric chloride test	--	--	--
10	Test for Oils			
	Spot test	+	+	--
11	Test for phenols			
	Ferric chloride test	+	+	+

'-'Absent, '+' Present

Table 2: Antibacterial Activity of *Chaetomorpha antennina*

S. No.	Name of Organism	Zone of inhibition in mm				
		Positive Control 50µg/100 µl	50µg/100µl	100µg/100µl	150µg/100µl	200µg/100µl
1	*Bacillus megaterium*	12	6	8	12	15
2	*Staphylococcus epidermidis*	16	7	9	12	15
3	*Lactobacillus acidophilus*	12	7	10	11	13
4	*Escherishia coli*	12	8	10	13	15
5	*Salmonella typhi*	14	7	9	12	15
6	*Klebsilla pneumonia*	22	6	8	13	16

Table 3: Antibacterial Activity of *Gracilaria corticata*

S. No.	Name of Organism	Zone of inhibition in mm				
		Positive Control 50µg/100 µl	50µg/100µl	100µg/100µl	150µg/100µl	200µg/100µl
1	*Bacillus megaterium*	12	8	10	14	17
2	*Staphylococcus epidermidis*	16	8	12	15	16
3	*Lactobacillus acidophilus*	12	7	10	13	18
4	*Escherishia coli*	12	7	11	13	15
5	*Salmonella typhi*	14	6	10	12	17
6	*Klebsilla pneumonia*	22	7	11	13	16

Table 4: Antibacterial Activity of *Ulva faciata*

S.no.	Name of Organism	Zone of inhibition in mm				
		Positive Control 50µg/100 µl	50µg/100µl	100µg/100µl	150µg/100µl	200µg/100µl
1	*Bacillus megaterium*	12	7	11	15	17
2	*Staphylococcus epidermidis*	16	7	9	12	16
3	*Lactobacillus acidophilus*	12	8	12	15	18
4	*Escherishia coli*	12	7	9	12	15
5	*Salmonella typhi*	14	8	11	13	17
6	*Klebsilla pneumonia*	22	8	10	12	16

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