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ANTIBACTERIAL ACTIVITY OF MARINE MACRO ALGAE AGAINST HUMAN PATHOGENS

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Abstract

Methanol, ethanol and acetone extracts of six seaweed species from the south east coast of India were tested invitro for their antibacterial activities against bacteria. *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia, Enterococcus faecalis* with the disc diffusion method. Acetone was the best solution for extracting the antimicrobial materials from the algal species used in this experiment, with the exception of *Ulva lactua* for which ethanol was the most effective extraction solution. A significant in antibacterial activity was not observed between the ethanol and methanol extracts of each algae. In addition as a result of the comparison of dried and fresh extract of antibacterial activity, it was found that, all the test organisms were more sensitive to fresh extracts of the algae.

Key Words: *Enterococcus faecalis*; Antibacterial activity; Algae; Human Pathogens.

Introduction

More than 1,50,000 macro algae or seaweed species are found in oceans of the globe, but only a few of them were identified [1]. Secondary or primary metabolites from these organisms may be potential bioactive compounds of interest for the pharmacological industry [2]. Special attention has been reported for antiviral, anti bacterial and antifungal activities related to marine algae against several pathogens [3]. The antimicrobial compounds derived from the marine flora consist of diverse groups of chemical compounds [4]. The cell extracts and active constituents of various algae have been shown to have antibacterial activity against Gram positive and Gram negative bacteria [5].

In most previous studies only one kind of solvent was used to screen seaweeds for their antimicrobial activities. This study was aimed to determine the efficiency of methanol, ethanol and acetone for extracting antibiotics from seaweeds and to obtain species with highly active antibacterial compounds.

In this investigation, the antibacterial activities of six marine algae belonging to families such as chlorophyceae (*Ulva lactua*, *Halimedia gracilis*) Rhodophyceae (*Gracilaria edulis*, *Hypnea musiformis*) and Phaeophyceae (*Turbinaria conoides*, *Sargassum myricystum*) was studied against pathogenic bacteria like *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia and Enterococcus faecalis.*

Materials and Methods

Six species of marine algae occurring in the south east coast of India were collected. Algal samples were cleaned of epiphytes and necrotic parts were removed. Then the samples were rinsed with sterile water to remove any associated debris. Half of these cleaned fresh materials were air dried. 25 g of each fresh and air dried algal samples were extracted in 50 ml of methanol, ethanol and acetone [6].

Test organisms

The strains of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia* and *Enterococcus faecalis* were obtained from Department of Microbiology, Raja Muthiah medical college and hospital, Annamalai University, Annamalai Nagar, India and were maintained on suitable agar medium at 4°C until testing.

Antimicrobial activity

Antimicrobial activity was evaluated using agar diffusion technique in petri plates [5]. Briefly 25 µl of each extract was loaded on a sterile filter paper disc 6 mm in diameter and air dried. Indicator organisms were spread on Mueller-Hinton agar plates with sterile effusion and the discs were placed on agar plates. After incubation for 24 hours at 30°C, a clear zone around a disc was evidence of antibacterial activity. Diameter of the zones of inhibition were measured in millimeters. Each test was prepared in duplicate discs loaded with the extracting agents were tested as controls.

Results and Discussion

Antibacterial activities of six species of seaweed tested against bacteria *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia* and *Enterococcus faecalis.* The results of primary screening tests are summarized in table 1, which shows that the extracts of 6 algal species processed antibacterial activity. For some species the anti bacterial activity we observed was similar to previous screening studies [3, 4, 6].

Table 1: Antibacterial activity of the most inhibiting acetone extracts of marine algae

	Algae		*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Enterobacter Faecalis*
1.	*Ulva lactua*	FW	9	8	10	8	9
		DW	7	-	-	-	-
2.	*Halimeda gracilis*	FW	12	14	14	12	14
		DW	10	12	12	10	10
3.	*Gracilaria edulis*	FW	13	12	13	9	9
		DW	12	12	12	-	8
4.	*Hypnea musciformis*	FW	19	18	18	13	13
		DW	18	17	16	12	11
5.	*Turbinaria conoides*	FW	28	29	22	16	15
		DW	26	26	20	13	14
6.	*Sargassum myricystum*	FW	32	27	26	20	19
		DW	30	25	26	18	18

Where FW-Fresh weight; DW-Dry weight

In this study acetone was the best solution for extracting the effective antimicrobial materials from the algae species used in this experiment. A significant difference in antimicrobial activity was not found between the methanol and ethanol extracts of each alga. For instance acetone extracts of fresh *Sargassum myricystum*, *Turbinaria conoides*, *Hypnea musiformis*, *Gracilaria edulis* and *Halimedia gracilis* showed effective results against all test organisms, however the acetone extracts of *Ulva lactua* was low effective against micro organisms. This result could be related to the presence of bio active metabolites present in the Ulva, which are not soluble in acetone, but they can be soluble in methanol.

As a consequence, the acetone extracts showed most effective anti bacterial activity were selected from 6 algal species. In our study, the fresh extracted and dry extracted samples using acetone as a solvent were assayed against 3 Gram +ve and 2 Gram –ve bacteria (Table 2).

Table 1: Antimicrobial activity of different fresh extracts of marine algae

	Algae	Solvents testes	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcuc aureus*	*Klebsiella pneumoniae*	*Enterobacter Faecalis*
1.	*Ulva lactua*	Ethanol	-	-	-	-	-
		Methanol	++	+	++	+	+
		Acetone	+	-	-	-	-
2.	*Halimeda gracilis*	Ethanol	-	-	-	-	-
		Methanol	-	-	-	-	-
		Acetone	++	++	+	+	+
3.	*Gracilaria edulis*	Ethanol	+	+	-	-	-
		Methanol	-	-	+	-	-
		Acetone	++	++	+	+	+
4.	*Hypnea musciformis*	Ethanol	-	-	+	-	+
		Methanol	-	-	+	-	-
		Acetone	++	+	++	+	++
5.	*Turbinaria conoides*	Ethanol	-	-	-	+	-
		Methanol	-	-	+	-	+
		Acetone	++	++	+	++	+
6.	*Sargassum myricystum*	Ethanol	-	-	+	-	-
		Methanol	-	+	-	+	-
		Acetone	++	++	++	++	++

Where - low activity; + medium sensitivity; ++ high sensitivity

The dried extracts have less effect on bacteria in comparison to the fresh extracts. This result can be related to volatile antimicrobial compounds in the sample such as hydrogen peroxide, terpenoid and bromo ether compounds [7, 8]. Another reason might be the loss of active materials that may be present in alga, like volatile fatty acids, during the drying process.

Another significant result of the present study was that the acetone extracts of all algal species showed antibacterial activity. The antibacterial activities of the extracts from 26 algal species prepared by dichloromethane, methanol and water [9].

The hexane extract of *Gracilaria* sp inhibits only *Bacillus subtilis* [10]. Similarly our results showed that the acetone extract of algal species *Sargassum myricystum* effectively inhibited *E. coli*, *P.aeruginosa*, *Staphylococcus aureus*. The activity against G+ve bacteria was less effective compared to G-ve bacteria.

Some studies concerning the effectiveness of extraction methods highlight that methanol extraction yields higher antibacterial activity than n-hexane and ethyl acetate [11]. Whereas other report that chloroform is better than methanol and benzene [6]. It is clear that using organic solvents provides a higher efficiency in extracting compounds for antimicrobial activities compared to water based methods [11]. According to our experimental results, acetone caused better halo-zones than methanol and ethanol.

Conclusion

Finally we conclude that macro-algae from south east coast of India are potential sources of bioactive compounds and should be investigated for natural antibiotics.

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