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Antibacterial activity of seaweed (*Gracilaria species*) extracts against infectious pathogens

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Abstract

Pathogenic microorganisms currently resistant to drugs cause innumerable mortality in human population which creates need for searching new antibacterial agents from easily available unexplored natural sources such as seaweed. In this study the antibacterial activity of seaweeds (*Gracilaria verrucosa, Gracilaria foliifera, Gracilaria corticata, Gracilaria crassa and Gracilaria edulis*) was tested against human bacterial pathogens (*K. pneumoniae, P. aeruginosa, E. aerogenes, S. typhi* and *S. aureus).* Different types of extracts (Acetone, Benzene, Butanol, Chloroform, Ethanol, Ethyl Acetate, Hexane, Isoamyl Alcohol, Methanol, and Propanol) were evaluated for antibacterial activity by agar cut well diffusion methods. All the studied species of *Gracilaria* showed antibacterial activity. The best results were obtained by isoamyl extract with inhibition activity on most of the pathogens and *G. verrucosa* is slightly more effective compared to other *Gracilaria* species. The isoamyl alcohol extract showed highest (24 mm) zone of inhibition followed by Chloroform extract with highest zone of inhibition (21 mm). These results show the potential of *Gracilaria* species for screening new bioactive (antibacterial) compounds.

INTRODUCTION

Bacterial species are responsible for mortality in human population because their infection causes diseases like food borne gastroenteritis, secondary infections, mastitis and upper respiratory complications [1,2]. This problem creates need to search for new antibacterial agents from available natural sources and seaweed is one of them having a vast potential for screening of antibacterial agents to develop new antibiotics [3, 4]. Seaweeds are photosynthetic microalgae and classified as red algae, brown algae and green algae on the basis of their pigment constituents [5]. Seaweeds are considered as diverse source of secondary metabolites characterized by a broad spectrum of biological activities [6]. These compounds have diverse functions and earlier reports evaluated use of Seaweed by the pharmaceutical industries in drug development and attention has been given for antibacterial and antifungal activities of marine algae [7,8,9]. Since ancient time, thousands of plants species are being used in India to cure specific malady [10]. The marine habitat of India has diverse seaweeds, spread through inter-tidal and deep water regions of the Indian coast. It is already documented that seaweeds from southeast cost of India has antibacterial property [11]. *Gracilaria genus* has shown potential for synthesis of new natural medicines [12, 13]. Most identified active antimicrobial compounds are water insoluble and thus organic solvent extracts have been found more potent [14]. In the present study antibacterial activity of different organic solvents extract of marine seaweeds (*Gracilaria*) was examined against human pathogens (*K. pneumoniae, P. aeruginosa, E. aerogenes, S. typhi* and *S. aureus)* to search for new antibacterial agents.

MATERIALS AND METHODS

Sample collection

Seaweeds were collected by hand picking from the Rameshwaram, Ramnad District, Tamil Nadu, India. The collected seaweeds were identified in Centre for Marine Fisheries Research Institute, Mandapam, Tamil Nadu, India.

Collection of microorganisms

The pathogenic bacteria were isolated from clinical samples collected from diagnostic labs and identified on the basis of morphological, biochemical and physiological characters according to Bergey's manual of determinative bacteriology. The isolated microorganisms were found to be *K. pneumoniae, P. aeruginosa, E. aerogenes, S. typhi, and S. Aureus.*

Extract Preparation

Seaweed samples were washed with fresh water and then rinsed with distilled water to remove extraneous matter. Then samples were shade dried, cut into small pieces and powdered in a mixer grinder. Since organic solvents provides a higher efficiency [15] different solvents with increasing polarity Hexane < Benzene <Chloroform <Ethyl acetate <Acetone <Butanol <Propanol <Ethanol <Methanol and Isoamyl alcohol were used. For extraction process 5 g of the samples were extracted in 50 ml of different solvents by soaking for overnight at room temperature. After incubation the extract was filtered and then concentrated based on boiling points of the solvents. The concentrated extracts were tested for the antibacterial activity against the pathogens.

Determination of antibacterial activity

The antibacterial activity was evaluated by agar well diffusion method. The overnight grown bacterial culture was transferred to sterile MH agar plates. Wells were punched out using a sterile 6 mm cork borer into the previously inoculated MH agar plates. About 80 µL of the different *Gracilaria* extract were transferred in to the wells using sterile micropipette tips and allowed to diffuse for 2 hrs at 4^0C and then the plates were incubated at 37^0C for 24 hrs. The results were recorded by measuring the inhibition zone diameter for each well.

Determination of minimal inhibitory concentration (MIC)

The extracts that exhibited considerable activity were used for MIC determination. The extracts were tested in four dose levels of

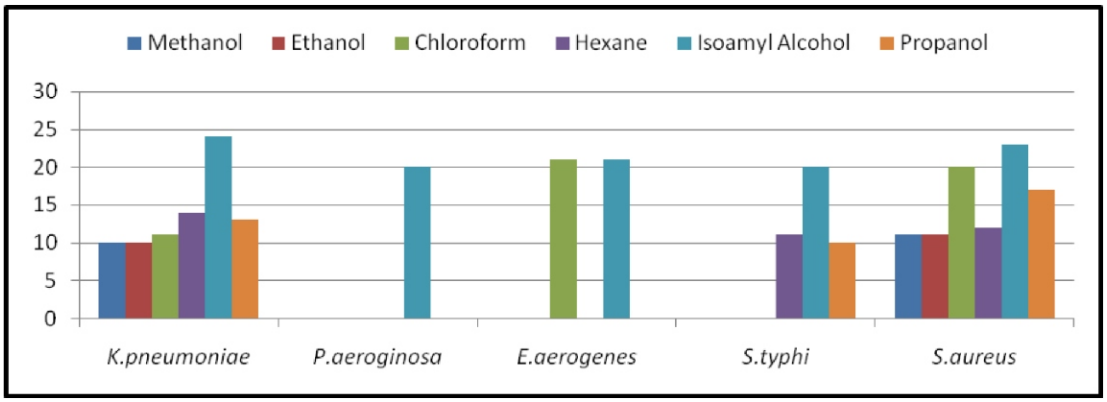

Fig. 1 : Antibacterial Activity of *G. Verrucosa*

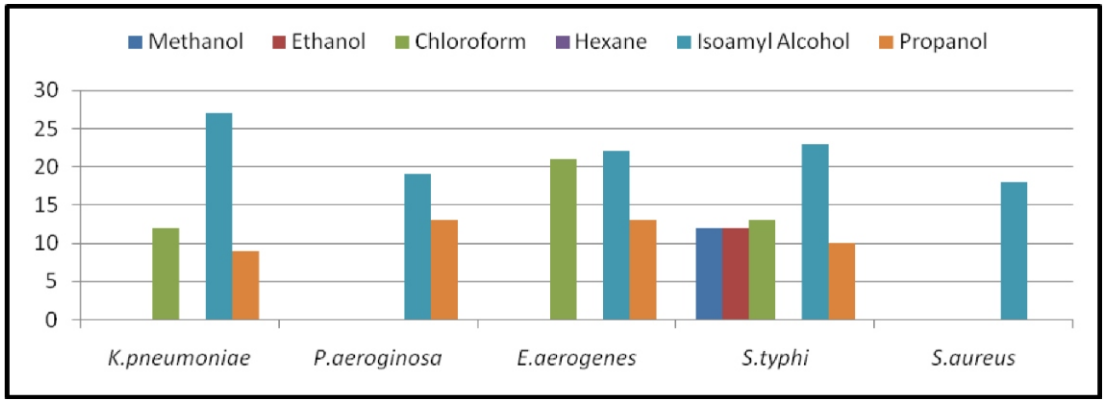

Fig. 2 : Antibacterial Activity of *G. Foliifera*

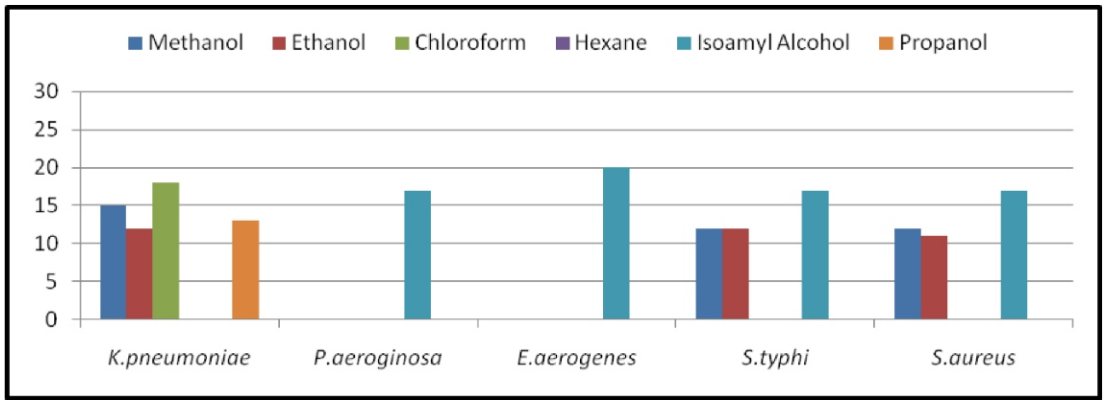

Fig. 3 : Antibacterial Activity of *G. Corticata*

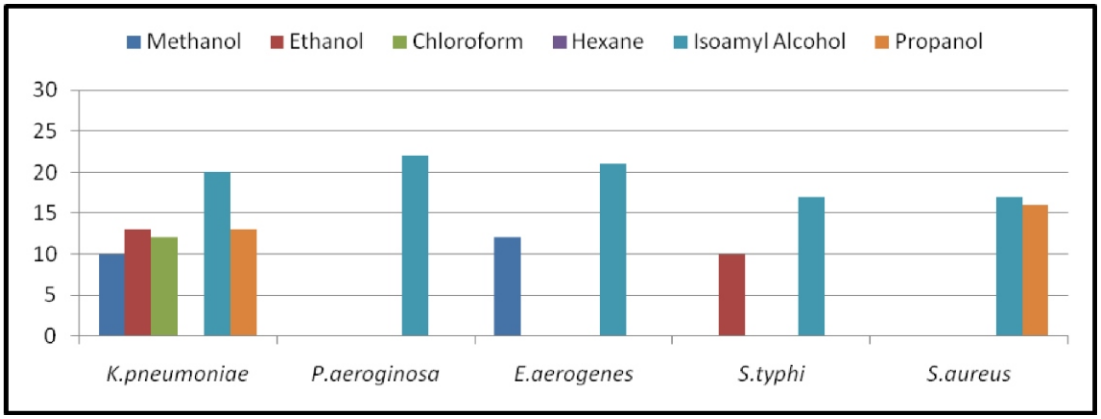

Fig. 4 : Antibacterial Activity of *G. crassa*

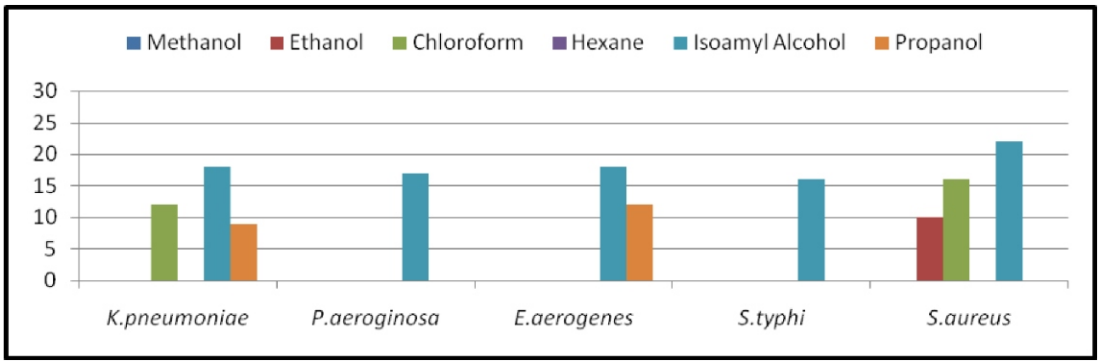

Fig. 5 : Antibacterial Activity of *G. Edulis*

Table 1. Minimum Inhibitory Concentration of *Gracilaria* extracts.

Seaweed and Pathogens	Minimum Inhibitory Concentration Extract					
	Methanol	Ethanol	Chloroform	Hexane	Isoamyl Alcohol	Propanol
G. verrucosa						
K. pneumoniae	60	80	60	60	20	40
P. aeruginosa	-	-	-	-	20	-
E. aerogenes	-	-	20	-	20	-
S. typhi	-	-	-	60	20	60
S. aureus	60	60	20	60	20	40
G. foliifera						
K. pneumoniae	-	-	60	-	20	80
P. aeruginosa	-	-	-	-	20	60
E. aerogenes	-	-	40	-	20	60
S. typhi	40	40	40	-	20	80
S. aureus	-	-	-	-	20	-
G. corticata						
K. pneumoniae	40	60	40	-	-	60
P. aeruginosa	-	-	-	-	20	-
E. aerogenes	-	-	-	-	20	-
S. typhi	80	80	-	-	20	-
S. aureus	80	80	-	-	20	-
G. crassa						
K. pneumoniae	80	80	80	-	20	60
P. aeruginosa	-	-	-	-	20	-
E. aerogenes	60	-	-	-	20	-
S. typhi	-	80	-	-	40	-
S. aureus	-	-	-	-	40	60
G. edulis						
K. pneumoniae	-	-	60	-	40	80
P. aeruginosa	-	-	-	-	20	-
E. aerogenes	-	-	-	-	40	60
S. typhi	-	-	-	-	20	-
S. aureus	-	60	40	-	20	-

20 to 80 µL. The overnight grown bacterial culture was transferred on MH agar plate and wells were punched out using a sterile 6 mm cork borer. Different amount (2080 µL) of the extract was placed in separate wells, allowed to diffuse for 2 hrs at 4^0C and then the plates were incubated at 37^0C for 24 hrs. The zone of inhibition was observed and the lowest concentration of the test sample showing zone of inhibition was recorded as the MIC.

RESULTS AND DISCUSSION

The antibacterial activity of five *gracilaria* species was determined in both gram positive and gram negative bacteria. In the preliminary assay ten different organic solvents like Acetone, Butanol, Ethanol, Ethyl acetate, Isoamyl alcohol, Methanol and Propanol (polar) and Benzene, Chloroform and Hexane (non polar) were evaluated. Only the extracts showing antibacterial activity (Ethanol, Chloroform, Isoamyl alcohol, Methanol and Propanol) were considered for further study. The zone of inhibition produced by these extracts against pathogenic microorganisms is summarized in Figure 1-5. Different species showed sensitivity for different solvents. The isoamyl extract was most active and hexane extract was least active among the solvents used for the study. The isoamyl alcohol extract was most active and showed broader zone of inhibition against most of the pathogens. The highest zone of inhibition (27mm) was produced by isoamyl alcohol extract of *G. foliifera* against *K. pneumoniae*. Chloroform was second best solvent for antibacterial activity with highest zone of inhibition (21 mm) against *E. aerogenes.* The extracts having antibacterial activity was then tested for their potency by MIC determination assay and results were summarized in table 1. The isoamyl alcohol extract was most active and showed antibacterial activity at lower concentrations.

CONCLUSION

The extracts of *Gracilaria* were effective against most of the tested pathogenic bacteria. *G. verrucosa* showed best results with inhibition activity on most of the pathogens which indicate its effectiveness compared to other *Gracilaria* species. *K. pneumoniae* and *S. aureus* were more sensitive for all the solvent extracts of *G. verrucosa*. These results indicate significant capacity of *Gracilaria* species as new bioactive compounds as antibacterial agent in the treatment of pathogenic organisms.

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