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ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF MARINE MACROALGAE *AMPHIROA ANCEPS* USING THREE SOLVENT EXTRACTS

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ABSTRACT: The antibacterial activity and phytochemical screening of red algae *Amphiroa anceps* were using three different solvents like aqueous, methanol and ethyl acetate extracts. The phytochemical screening of macroalgae showed the presence of flavonoids and terpenoids in methanol and ethyl acetate extract. The aqueous extract of *Amphiroa anceps* observed only tannins. The eight human pathogenic bacteria like *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella* sp, *Pseudomonas aeruginosa*, *Spreptococcus* sps, *Enterobacter* sps and *Neisseria* sps used for the antibacterial activity. The highest zone of inhibition was recorded in ethyl acetate extract of the *Amphiroa anceps* against the bacteria *Enterobacter* sp.

INTRODUCTION: About eight million tonnes wet seaweeds are harvested annually worldwide, and stranded seaweed on the beach constitute a considerable part of it¹. Currently, human consumption of green algae (5%), brown algae (66.5%) and red algae (33%) of high in Asia, mainly Japan, China, and Korea². The different species consumed presently a great nutritional value as a source of proteins, carbohydrates, minerals, and vitamins. These seaweeds are harvested and utilized for a variety of purposes such as feed, fertilizer and as a source of raw material for industrial production of phytochemical of commercial importance³. Many compounds in nature can inhibit the growth of microorganisms.

However, microorganisms have developed resistance to these natural compounds and microbiologists are forced to continue their search for naturally occurring antimicrobial compounds. The marine environment provides a unique and still largely unexploited source of novel bioactive compounds in the form of secondary metabolites⁴. The algal extracts were used as a curative and preventive agent for various diseases such as antibiotics, antihelminthics, and cough remedies, antitumor and antidiarrhoea. Recently, we have embarked on the chemical investigation of marine algae with a special accent on their bioactive properties⁵. Thus, the present study aims at the macroalgae *Amphiroa anceps* using three solvent extracts for preliminary detection of various phytochemical analysis and antibacterial studies.

MATERIALS AND METHODS:

Collection of Macroalgae: The macro algae *Amphiroa anceps* was collected from Chagkumugam coast. The macroalgae were cleaned with seawater three times and then

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successively with tap water and distilled water to remove the epiphytes and other wastes. Finally, these cleaned fresh materials were cut into small pieces and spread on blotting paper to remove excess water.

Solvent Extraction: The samples were shade dried for one month and then pulverized into a fine powder using mixer and grinder. Soxhlet extraction techniques did the extraction. Three different solvents like aqueous, methanol and ethyl acetate were selected for the phytochemical analysis. The extracts were evaporated to complete dryness by Vacuum distillation and stored in the refrigerator for further use.

Phytochemical Screening: All the extracts of macroalgae were subjected to various tests for the identification of phytochemicals such as Saponins, Glycosides, Steroids, Tannins, Phenol, Protein, Alkaloids, Flavonoids, Terpenoids and Amino acids, by using standard methods.

Test Microorganisms: The organisms used for the screening of antibacterial activity are as follows: *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella* sp, *Pseudomonas aeruginosa*, *Streptococcus* sps, *Enterobacter* sps, and *Neisseria* sps. The cultures were collected from in biotics, Institute of Biology of clinical research, Nagercoil.

Antibacterial Assay: The macroalgae extracts were screened against eight human pathogens. Antibacterial activity was carried out using Disc Diffusion Method^{6,7}. The Antibiotic susceptibility testing by a standardized disk Amikacin was used. Sterile Muller Hinton Agar (Hi-media) plates were prepared and allowed to set.

The cultures to be screened were swabbed on top of the solidified media. Discs impregnated with the seaweed extract were placed on the swabbed plate. The plates were incubated at 37 °C for 24 h. After incubation, the inhibition zone has measured the edge of the disc to the clear zone in millimeter.

RESULTS: The results on the phytochemical analysis of *Amphiroa anceps* are presented in **Table 1** the compounds amino acids, flavonoids and terpenoids were observed in the methanol extract. In the ethyl acetate extract of *Amphiroa anceps* showed phenol, protein, flavanoids, and

terpenoids. The aqueous extract of *Amphiroa anceps* observed only tannins.

Antibacterial Activity of Various Extracts of *Amphiroa anceps*: Table 2 and Fig. 1 demonstrates the antibacterial activity in ethyl acetate extract of *Amphiroa anceps* showed the highest activity against the bacteria *Enterobacter* sp 13.23 ± 0.14, *Klebsiella* sp 12.03 ± 0.26 and lowest zone of inhibition against the pathogen *E. coli* 8.26 ± 0.0.30. The methanol extract of *Amphiroa anceps* showed maximum activity against the bacteria *Enterobacter* sp. 12.16 ± 0.20, *Klebsiella* sp. 10.04 ± 0.40 and minimum zone of inhibition against the pathogen *E. coli* 7.43 ± 0.40.

The standard antibiotic Amikacin showed the highest zone of inhibition against the pathogen *Staphylococcus aureus* 25.36 ± 0.35 *Klebsiella* sp. 25.23 ± 0.25 and lowest zone of inhibition against the bacteria *E. coli* 20.02 ± 0.26. The aqueous extracts of *Amphiroa anceps* were most resistant in all Pathogens; it did not show any inhibition zones with an aqueous extract from the macroalgae.

DISCUSSIONS: The seaweeds are economically valuable resources, used as food, fodder, fertilizer, and medicine and thus useful to mankind in many ways. The brown algae *Sargassum wightii* showed the maximum presence of steroids in methanol, acetone, benzene, and chloroform extracts and flavonoids showed the presence of methanol, benzene, and chloroform extracts.

The phenol showed the presence of methanol and benzene extracts and flavonoids, phenol, steroids, alkaloids, saponins were absent in aqueous, petroleum ether extracts⁸. In the present study, the red algae *Amphiroa anceps* showed the presence of flavonoids and terpenoids in methanol and ethyl acetate extracts.

The present work correlated with the findings of Marimuthu *et al.*, in *Sargassum wightii*. The extracts of the test marine algae expect *Clostridium officinalis* showed the lowest inhibition against *Staphylococcus aureus* and highest inhibition activity among all the extracts was shown to *Enterobacter aerogenes*⁹.

In the current study, the extract of *Amphiroa anceps* showed highest inhibition zone against the

pathogen *Enterobacter* sp. and the extract of *Amphiroa anceps* showed the lowest activity against the bacteria *Proteus vulgaris*. The same

trend was observed in work carried out by Taskin et al., in *Clostridium officinalis*.

TABLE 1: THE PHYTOCHEMICAL ANALYSIS OF VARIOUS EXTRACTS IN AMPHIROA ANCEPS

S. no.	Phytochemicals	Methanol	Ethyl acetate	Aqueous
1	Saponins	-	-	-
2	Glycosides	-	-	-
3	Steroids	-	-	-
4	Tannins	-	-	+
5	Phenol	-	+	-
6	Protein	-	+	-
7	Alkaloids	-	-	-
8	Flavonoids	+	+	-
9	Terpenoids	+	+	-
10	Amino acids	+	-	-

Note: + (present), - (absent)

TABLE 2: ANTIBACTERIAL ACTIVITY OF AMPHIROA ANCEPS AGAINST MICROORGANISMS

Microorganisms	Zone of Inhibition (mm)			
	Ethyl acetate	Methanol	Aqueous	Amikacin
<i>E. coli</i>	8.26 ± 0.30	7.43 ± 0.40	-	20.02 ± 0.26
<i>Staphylococcus aureus</i>	9.16 ± 0.20	8.02 ± 0.20	-	25.36 ± 0.35
<i>Proteus vulgaris</i>	7.16 ± 0.15	7.26 ± 0.30	-	20.03 ± 0.30
<i>Klebsiella sp</i>	12.03 ± 0.26	10.04 ± 0.40	-	25.23 ± 0.25
<i>Pseudomonas aeruginosa</i>	11.36 ± 0.40	9.26 ± 0.25	-	20.16 ± 0.20
<i>Streptococcus sp</i>	9.26 ± 0.30	9.13 ± 0.15	-	23.23 ± 0.25
<i>Enterobacter sp</i>	13.23 ± 0.14	12.16 ± 0.20	-	25.05 ± 0.45
<i>Neisseria sp</i>	10.03 ± 0.30	9.26 ± 0.30	-	23.16 ± 0.20

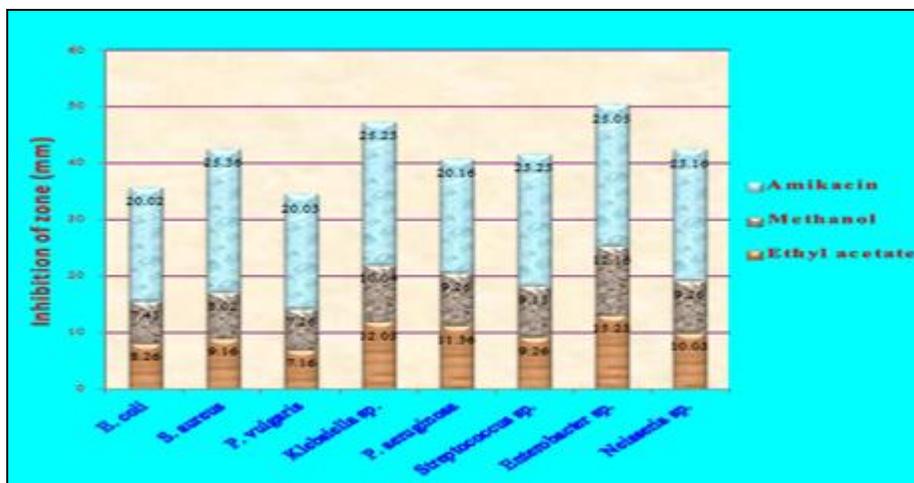


FIG. 1: ANTIBACTERIAL ACTIVITY OF AMPHIROA ANCEPS AGAINST MICROORGANISMS

CONCLUSION: These results indicate the phytochemical and antibacterial activity of different solvent extracts. The highest zone of inhibition was recorded in ethyl acetate extract of the *Amphiroa anceps* against the bacteria *Enterobacter* sp. The lowest activity was showed *Proteus vulgaris*. Further work is needed to identify the principle compound which is responsible for macroalgal extracts of antibacterial activity against pathogenic bacteria causing the human disease.

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CONFLICT OF INTEREST: Nil

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