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## ANALYSIS OF BIOACTIVE COMPOUNDS AND ANTIMICROBIAL ACTIVITY OF MARINE ALGAE *KAPPAPHYCUS ALVAREZII* USING THREE SOLVENT EXTRACTS

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Seaweeds, *Kappaphycus alvarezii*, Secondary metabolites, Antimicrobial activity

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### ABSTRACT

The seaweeds are economically valuable resources, used as food, fodder, fertilizer and medicine and thus useful to mankind in many ways. In the present study, *Kappaphycus alvarezii*, a marine alga, has been analysed for the presence of bioactive products using three solvent extracts. Antimicrobial activity was also done using the same extracts of seaweed. The results revealed that the selected seaweed has active secondary metabolites and also exhibited antimicrobial activity, mainly in the methanolic extract of *Kappaphycus alvarezii*.

**INTRODUCTION:** Economical significance of seaweed lies in their utilization in food and cosmetic industry and also in medicine and pharmacy. Genera of agar containing red seaweed, *Gelidium* Sp. and genera of carrageenan-containing red seaweed. *Kappaphycus alvarezii* are used most widely<sup>1</sup>.

The chemical composition of seaweed provides their high nutritional value contributing to human nutrients-such as proteins with all essential amino acids, minerals and vitamins.

In addition, they consist of bioactive secondary metabolites and many different compounds with health benefits<sup>2,3,4</sup>.

The chemical composition of seaweed varies, which is dependent on the type of species, habitat, time of collection, external conditions such as temperature, light and nutrient concentration in water<sup>5,6</sup>. Bioactivity of diverse secondary metabolites and other compounds extracted from seaweeds plays an

important role in prevention of different serious diseases. These biogenic compounds, also have antibacterial, antialgal, antifungal properties<sup>7,8</sup>.

The antimicrobial activity of seaweeds are associated to secondary metabolites like terpenoid derivatives, phlorotannins or phenolic lipids<sup>9,10</sup>.

Thus, the present study aims at the extraction and qualitative detection of various phytochemicals of biological importance, from the selected seaweed *Kappaphycus alvarezii* using three solvent extracts. Antimicrobial activity was also done using the same solvent extracts of the seaweed.



## MATERIALS AND METHODS:

**Collection and Identification of Seaweed:** The seaweed *Kappaphycus alvarezii* was purchased from the Mandabam region, Tamil Nadu, India and identified by SNAP natural and alginate products pvt ltd, Sipcot Ranipet, Tamil Nadu, India.

**Preparation of Plant Extracts:** The seaweed was washed thoroughly to remove epiphytes and other debris. Then they were shade dried and coarse powdered. The powder obtained (250gms) were extracted successively with 3 solvents, Ethanol, Methanol and Acetone in a soxhlet extractor for 18-20hrs.

All those extracts were concentrated at 45°C under reduced pressure using rotary flash evaporator. All the three extracts were used for phytochemical screening and the methanolic extract was used for antimicrobial studies.

1. **Phytochemical Screening:** All the extracts of seaweed (*Kappaphycus alvarezii*) were subjected to various qualitative tests for the identification of phytochemicals such as alkaloids, carbohydrates, glycosides, proteins and amino acids, phytosterols, phenolic compounds, flavanoids, terpenoids, tannins and saponins, by using standard methods<sup>11</sup>.

### 2. Antimicrobial studies:

A. **Preparation of Extract For Microbial Studies:** For microbiological studies, 1 gm of dried methanol extract was dissolved in 10 ml of the same solvent.

B. **Test Organisms Used:** The bacterial and the fungal strains that were used for the determination of antimicrobial activity of the selected seaweed are as follows,

- i. **Bacterial strains** - *Staphylococcus aureus*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *E.Coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*.
- ii. **Fungal strains** - *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Candida albicans*, *Candida tropicalis*.

**Well Diffusion Method:** Antimicrobial assay was done using Muller Hinton Agar (MHA) and Potato Dextrose Agar (PDA). Sterilized medium was poured into a petridish and was inoculated by streaking the swabs of the test organisms. After allowing the inoculum to dry at room temperature, methanol extract of *Kappaphycus alvarezii* was then loaded in the well bored in solidified agar. The plates were allowed to stand at room temperature for 1 hour for extract to diffuse into the agar and then they were incubated at 37° for 24 hours. After incubation, the zone of inhibition exhibited by the extract was measured.

## RESULTS AND DISCUSSION:

**Phytochemical Screening of *Kappaphycus alvarezii* extracts:** Table 1 demonstrates qualitative phytochemical screening of ethanol, methanol and acetone extracts of *Kappaphycus alvarezii*. The results revealed the presence of various phytochemical constituents in all the extracts used. The methanolic extract showed a maximum number of active phytochemicals compared to ethanolic and acetone extracts.

TABLE 1: QUALITATIVE PHYTOCHEMICAL SCREENING OF VARIOUS EXTRACTS OF *KAPPAPHYCUS ALVAREZII*

| Phytochemicals           | Ethanol extract | Methanol extract | Acetone Extract |
|--------------------------|-----------------|------------------|-----------------|
| Alkaloids                | +               | +                | +               |
| Carbohydrates            | +               | +                | -               |
| Saponins                 | -               | -                | +               |
| Glycosides               | +               | -                | -               |
| Proteins and amino acids | +               | +                | -               |
| Phytosterol              | -               | +                | +               |
| Phenolic compounds       | +               | +                | -               |
| Flavanoids               | +               | +                | +               |
| Terpenoids               | +               | +                | +               |
| Tannins                  | -               | +                | -               |

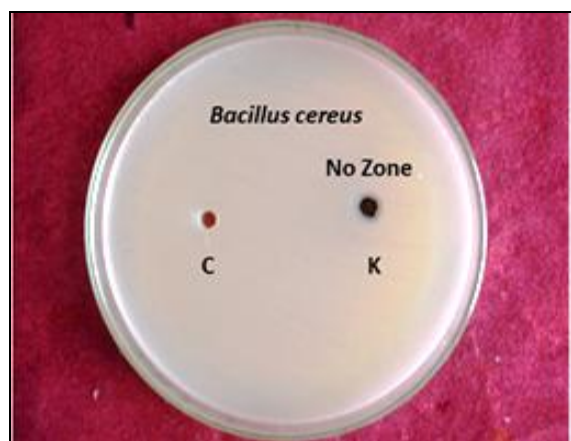
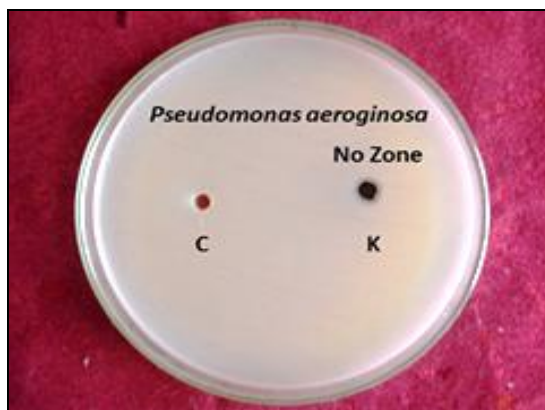
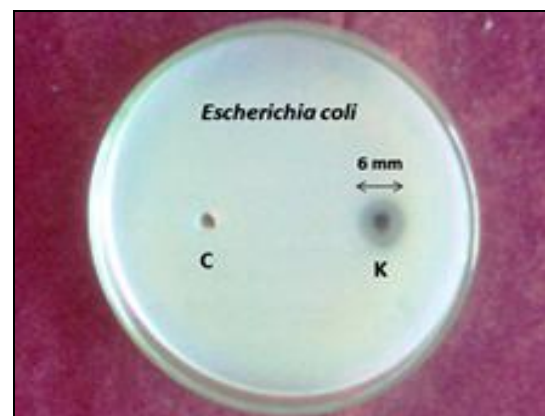
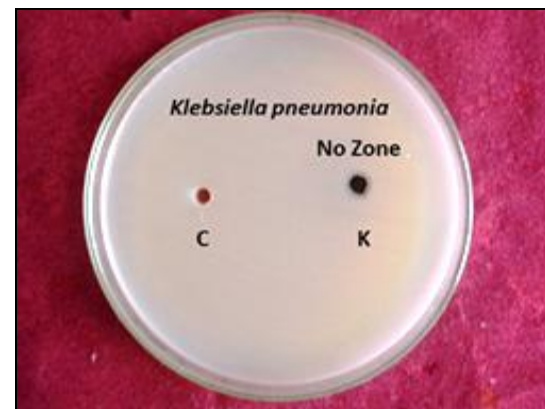
+ = Presence ; - = Absent

**Antimicrobial activity of Methanolic Extract of *Kappaphycus alvarezii*:** Antimicrobial activity of seaweed is mainly due to the presence of phenolic lipids, terpenes and phlorotannins. Since antimicrobial agents required for the antimicrobial activity were maximum present in the methanolic extract of the selected seaweed. The antimicrobial assay was done using the methanolic extract of the seaweed<sup>9</sup> (Table 2). The zone of inhibition was observed. The selected seaweed exhibited antimicrobial activity for 3 bacteria and 3 fungi out of 6

and 5 test micro-organisms used respectively (Fig. 1 and Fig. 2).

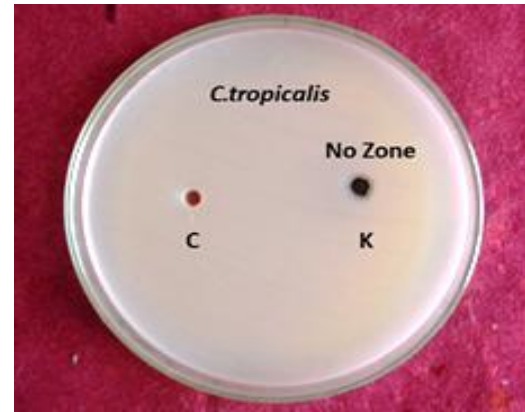
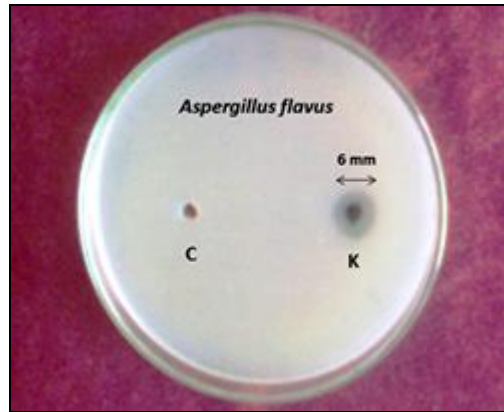
TABLE 2: ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *KAPPAPHYCUS ALVAREZII*

| Micro-organisms               | Zone of inhibition (mm) |                              |
|-------------------------------|-------------------------|------------------------------|
|                               | Control                 | <i>Kappaphycus alvarezii</i> |
| <b>Bacteria:</b>              |                         |                              |
| <i>Staphylococcus aureus</i>  | No zone                 | 5                            |
| <i>Bacillus cereus</i>        | No zone                 | No zone                      |
| <i>Micrococcus leutues</i>    | No zone                 | 6                            |
| <i>Klebsiella pneumonia</i>   | No zone                 | No zone                      |
| <i>Escherichia coli</i>       | No zone                 | 6                            |
| <i>Pseudomonas aeruginosa</i> | No zone                 | No zone                      |
| <b>Fungi:</b>                 |                         |                              |
| <i>Aspergillus flavus</i>     | No zone                 | 6                            |
| <i>Aspergillus niger</i>      | No zone                 | No zone                      |
| <i>Aspergillus fumigates</i>  | No zone                 | 5                            |
| <i>C. albicans</i>            | No zone                 | 5                            |
| <i>C. tropicalis</i>          | No zone                 | No zone                      |

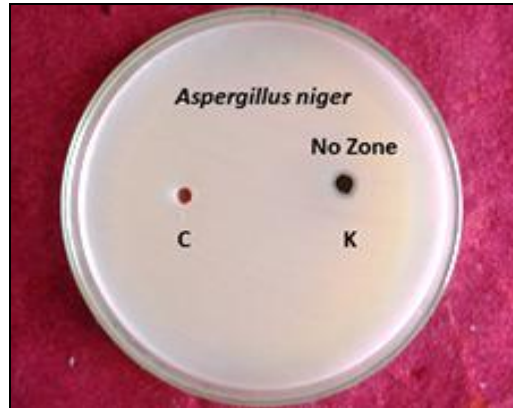


C – CONTROL ; K – *KAPPAPHYCUS ALVAREZII*

FIG. 1: ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF *KAPPAPHYCUS ALVAREZII*



C – CONTROL ; K – *KAPPAPHYCUS ALVAREZII*



**FIG. 2: ANTIFUNGAL ACTIVITY OF METHANOLIC EXTRACT OF *KAPPAPHYCUS ALVAREZII***

In summary, of the 3 solvent extracts of the seaweed, methanolic extract showed potential antibacterial activity<sup>12</sup> against gram positive and gram negative organisms, and antifungal activities against selected fungal species. Also results of phytochemical screening showed the presence of carbohydrates, proteins, sterols, phenolic compounds, tannins, terpenes etc, in the solvents used, and which act as potential antimicrobial agent, when extracted with methanol.

**CONCLUSION:** The result of this study justifies that the marine algae, *Kappaphycus alvarezii* is associated with numerous health benefits with all its bioactive compounds, and can be recommended for the development of antimicrobial drugs.

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