ANTIFUNGAL ACTIVITY OF ACANTHOPHORA SPICIFERA, PADINA TETRASTOMATICA AND CAULERPA SCALPELLIFORMIS AGAINST SOME FUNGAL PATHOGENS IN CRUDE AND FRACTIONATED FORM

1*Dr. D. Radhika and 2R. Priya

1,2PG and Research Department of Zoology, V.O.C. College, Tuticorin-8.

ABSTRACT
One representative from each group of seaweeds namely Acanthophora spicifera from Rhodophyceae, Padina tetrastomatica from Phaeophyceae and Caulerpa scalpelliformis from Chlorophyceae was chosen. Crude and fractions of the seaweeds were taken using four solvents namely water, ethanol, acetone and methanol by soxhlet extraction. They were then compared for their antifungal activity by disc diffusion technique against five fungal strains, namely Aspergillus terrus, A. fumigatus, Gibberline sp., Alternaria sp. and Ganoderma sp. A. fumigatus was the highest inhibited fungal species followed by Alternaria sp. and A. terrus. Ganoderma sp. showed the least activity against all the pathogens tested.

KEYWORDS: Seaweeds, Acanthophora, Antifungal, Aspergillus, Alternaria, Disc Diffusion.

INTRODUCTION
Biostimulant properties of seaweeds are explored for use in agriculture and the antimicrobial activities for the development of novel antibiotics. Seaweeds have some of the valuable medicinal value components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. Seaweeds are considered as source of bioactive compounds and produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Currently, multiple drug resistance of human pathogenic microorganisms is due to the indiscriminate use of the commercial antimicrobials.
commonly used for the treatment of infectious diseases.\[1\] The search for new, more specific and better adapted antimicrobial agents has been further stimulated by the occurrence of fatal opportunistic infections associated with AIDS, antineoplastic chemotherapy and transplants.\[2\]

As a consequence of the increasing demand for biodiversity in screening programs for potential therapeutic activities of natural products, there is an increased interest in marine fauna and flora throughout the world.\[3\] In this context, tropical marine algae have proven to be a rich source of bioactive compounds of potential biomedicinal interest.\[4-9\]

Hence, the present study was aimed to investigate and evaluate the antifungal activities of crude extracts and their fractions of *Acanthophora spicifera, Padina tetrastomatica* and *Caulerpa scalpelliformis*.

**MATERIALS AND METHODS**

Seaweed (*Acanthophora spicifera, Padina tetrastomatica* and *Caulerpa scalpelliformis*) samples which were healthy and fully grown and submerged underwater were collected from the tidepools from Tuticorin coast (08° 46’ 2.15”N lat; 78°11’ 16.05” E long). Representative species from each group of macro algae such as Red (*Acanthophora spicifera*) Brown (*Padina tetrastomatica*) and Green (*Caulerpa scalpelliformis*), were collected. The samples were washed with seawater and freshwater to remove salt, epiphytic microorganisms and other suspended materials. The clean algae were frozen and lyophilized. The dry material was stored at -20°C. The fractions of seaweeds were separated by column chromatography technique from extracts obtained from water, ethanol, methanol and acetone solvents.

**Media preparation**

**a. Fungal media (Potato Dextrose Agar)**

Two Hundred gram of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion. 20g of agar was added as a solidifying agent. These constituents were mixed and autoclaved.

**b. Fungal strains**

Antifungal activity of the different seaweed extract was investigated against five fungal strains *Aspergillus terrus, Aspergillus fumigatus, Gibberline* sp., *Ganoterma* sp., and *Alternaria* sp., in the present study.

**c. Disc diffusion method**
The antifungal activity of the seaweeds was carried out by disc diffusion method. The circular discs of 6mm diameter were prepared using Whatmann no.1 filter paper. The discs were then loaded with the seaweed extracts. Discs impregnated with the respective solvents acted as control. Streak plate method was performed to seed pathogenic fungal culture on the agar plates. Using the loop which had been flamed, cooled and dipped in the inoculums, continuous horizontal streaks were made in the solid agar plates. The clear labeling of sample was marked on the plate. The plates were incubated at 37±2°C for 48 hours for fungal activity. The plates were observed for the zone formation around the discs. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the disc (in mm) including the disc diameter. The readings were taken in three different fixed directions and are shown in the graphs.

RESULT
The antifungal activity of the red seaweed (Acanthophora spicifera), brown seaweed (Padina tetrastomatica) and green seaweed (Caulerpa scalpelliformis) was analysed against five fungal strains, namely Aspergillus terrus, A. fumigatus, Gibberline sp., Alternaria sp. and Ganoderma sp. The former three being animal pathogens and the latter two were plant pathogens. The crude extracts were fractionated and the activity was compared between the crude and fraction. The extracts were prepared using different solvents namely water, ethanol, acetone and methanol. The antimicrobial activity was carried out using disc diffusion method and the results are shown in fig 1-6.

In the study on A.spicifera, the ethanol extracts showed good antifungal activity (6mm) followed by acetone and then methanol extracts. Aqueous extract had the least activity. A. fumigatus was the highest inhibited fungal species followed by Alternaria sp. and A. terrus. Ganoderma sp. showed the least activity against all the pathogens tested. The fractions had a slightly higher rate of inhibition when compared to the crude extracts with the exception of ethanol extract where the crude showed a higher rate of inhibition against A. terrus and A. fumigatus and in acetone extract in which the crude extracts showed promising antifungal activity more than the fraction in fighting against the pathogen Gibberline sp. Aqueous extract had no activity against A. terrus and Ganoderma sp (Fig 1 and 2).
P. tetrastomatica had the highest inhibition against Gibberline sp. (7.5mm) and the second best against Alternaria sp. (6.5mm). A. fumigatus was also moderately inhibited by both crude and fractions of the seaweed extract. Comparing the crude and fraction, the ethanol fraction of P. tetrastomatica had the highest activity (7.5mm) followed by ethanol crude extract. Acetone and methanol extracts also showed good antifungal activity. Aqueous extract had the least activity as it showed no inhibition against four of the five pathogens tested with the exception of A. fumigatus. On comparing the inhibitory capacity of the crude and fraction extracts, the crude extracts had lesser antifungal activity with the exception of acetone extracts where the crude was more successful in curbing the pathogens. Methanol extracts also showed a similar pattern against A. terrus and A. fumigatus (Fig 3 and 4).
When the antifungal activity was carried out with the green seaweed *C. scalpelliformis*, (Fig 5 and 6) both crude and fractionated extracts showed good activity against all the fungal strains tested. *Alternaria* sp. was inhibited the most (7.5mm) succeeded by *A. fumigatus*, *Gibberline* sp., *A. terrus* and *Ganoderma* sp. All the eight different extracts inhibited *Gibberline* sp. Aqueous and methanol extracts had little or no inhibiting factor against *A. terrus* and *Ganoderma* sp. *Gibberline* sp. and *Alternaria* sp. was the most susceptible against all the seaweeds tested and *Ganoderma* sp. was the most resistant followed by *A. terrus*. Among the solvents used for extraction, ethanol proved to be a good one, having the highest inhibition rate against the fungal pathogens tested.
DISCUSSION

When the three seaweeds were tested for their antifungal activity against three animal pathogens (Aspergillus terrus, A. fumigatus and Gibberline sp.) and two plant pathogens (Alternaria sp. and Ganoderma sp) ethanol extract proved to be the most effective while aqueous extract was the least effective. This aligns with the study in the ethanol extract of L. dendroidea\(^{[11]}\) which showed the highest activity against A. flavus. Similarly the ethanol extracts of P. pavonica, Rhodomela confervoides and Ulva lactuca were very efficient against Mucor ramiannus and Candida albicans while methanol and acetone extracts were not active against C. albicans.\(^{[12]}\)
Methanolic extracts of the four species of algae: *Cystoseira tamarisciflora* (brown algae), *Padina pavonica* (brown algae), *Rhodomela confervoides* (red algae) and *Ulva lactuca* (green algae) exhibited antifungal activities against the three strains.$^{[13]}$ Similar results against fungal strains with a high activity of algal extracts against *C. albicans* were also recorded.$^{[14]}$ In this study ethanol proved to be a better solvent in the inhibition of fungal pathogens. Brown seaweed *P. tetrastomatica* showed the highest antifungal activity when compared to the other two seaweeds. *Ganoderma* sp. was the toughest to inhibit.

**CONCLUSION**

Hence we can conclude that the seaweed extracts showed differences in their antimicrobial activity depending upon the solvents used in the extraction process.

**REFERENCES**


