

In vitro antifungal activity of *Turbinaria conoides* collected from Mandapam coast, Tamil Nadu, India

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ABSTRACT

Marine macroalgae have been used as medicines or drug sources for a great many years, stretching back to the era of folk medicines. Algae have been extensively used in the traditional medicines of maritime nations for the treatment of goiter, cancer, hypertension, cough, and other diseases. The present work was carried out to find out the antifungal activity of the seaweed *Turbinaria conoides* collected from Mandapam coastal regions of Tamil Nadu. The extracts were tested against *Candida albicans*, *Candida parapsilosis*, *Fusarium* sp., *Aspergillus flavus*, and *Aspergillus fumigatus*. The hexane, chloroform, and ethanolic extracts showed a well profound inhibitory activity against *C. albicans* and *C. parapsilosis*. No inhibitory activity was found at *Fusarium* sp., *A. flavus*, and *Aspergillus fumigatus* under chloroform and ethanolic extracts.

KEY WORDS: Antifungal, cancer, *Candida albicans*, diseases, medicine

INTRODUCTION

Algae possess a wide range of beneficial effects. The growth of infectious diseases is growing rapidly nowadays. The rapid growth of infectious diseases brought a global awareness among the people toward natural medicine. Hence, the demand for natural medicine increases the importance of algae. To identify the hidden medicinal property of algae, many researchers have unearthed its beneficial aspects rigorously. The present study has been undergone in bringing the antifungal activity of the brown seed, *Turbinaria conoides*, collected from Gulf of Mannar, Rameshwaram, Tamil Nadu, India.

The increasing demand for biodiversity in the screening programs, searching therapeutic drugs from natural products, there is now a greater interest in marine organisms, especially algae. The ability of seaweeds to produce secondary metabolites of potential interest has been extensively documented (Faulkner, 1993; Scheuer, 1987). There are numerous reports of compounds derived from macroalgae with a broad range of biological activities such as antibiotics, antivirals, antitumorals, and anti-inflammatories (Scheuer, 1990) as well as neurotoxins (Kobashi, 1989).

Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of

interest in the pharmaceutical industry. Biologically active compounds present in the plants have always been of great interest to scientists working in this field. The coastal region of Tamil Nadu, South India, produces rich vegetation marine algae. Many studies have been reported a great diversity in the macroalgal community of the marine algal vegetation in the region (Manivannan *et al.*, 2011).

Pharmaceutical industries are giving importance to the compounds derived from marine organisms (Solomon *et al.*, 2008). Screening of bioactive metabolites of algal crude extracts is enforced in clinical practice, where antibacterial, antiplasmodia and cytotoxicity (Selim, 2012), antifungal (Tang *et al.*, 2002), and antiviral (Serkedjieva, 2004) activity have been accessed to these metabolites. Microalgae are rich in bioactive natural products, so it has been studied as potential biocidal and pharmaceutical agents (Rangaiah *et al.*, 2010).

MATERIALS AND METHODS

Collection and Identification of Seaweeds

The seaweed of *T. conoides* was collected in bulk quantity from the coastal area of Mandapam, Gulf of Mannar, Tamil Nadu in India. Seaweed species exposed on sand

and rocks were collected in sterile plastic bags under ice and brought to the laboratory. Each species was washed thoroughly with running water to remove epiphytes, animal castings, attached debris, and sand particles. Moreover, the final washings were done using fresh water and finally dried under shade. The seaweed sample was identified in comparison with the herbarium collection under the University of Madras.

Preparation of Solvent Extracts

The shade dried algae sample of 5 g was placed in a Soxhlet apparatus and was successively extracted using the following solvents hexane, chloroform, and ethanol. The crude extracts of the whole part of algae at different concentrations were subjected to bioassay studies.

Microbial Strains

Antifungal activity was tested against the standard culture of fungal strains such as *Candida albicans* (MTCC 183), *Candida parapsilosis* (MTCC 2509), *Fusarium sp.*, *Aspergillus flavus* (MTCC 418), and *Aspergillus fumigatus* (MTCC 343). These fungi were obtained from Microbial Type Culture Collection, IMTECH, and Chandigarh, India.

Antifungal Assay

The different solvent extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 100 mg/ml. Each fungal stain was suspended in potato dextrose broth and incubated for 16 h at 37°C. In each of these plates, wells were cut out using sterile cork borer. Using sterilized dropping pipettes, different concentrations (500, 1000, 1500, and 2000 µg/ml) of extracts was carefully added to the wells and allowed to diffuse at room temperature for 2 h. The plates were then incubated at 37°C for

18-24 h. Ketoconazole (10 µg g/disc) and clotrimazole (10 units/disc) were used as positive controls and the solvent DMSO as a negative control. The antifungal activity was evaluated by measuring the diameter of inhibition zone.

RESULTS

The hexane extract showed a well profound inhibitory activity (8.0 ± 0.0 mm) was obtained against *C. albicans* and *C. parapsilosis* at 2000 µg/ml. Under the same extract, no inhibitory activity was found against *A. flavus* and *A. fumigatus* at all concentration except at 2000 µg/ml. Under chloroform extract, a maximum inhibitory activity (8.0 ± 0.0 mm) was found against *C. albicans* at 2000 µg/ml. A minimum inhibitory activity (5.0 ± 0.0 mm) was resulted against *C. parapsilosis* at 500 µg/ml. No inhibitory activity was resulted against *Fusarium sp.*, *A. flavus*, and *A. fumigatus* at all concentrations under chloroform extract.

The ethanolic extract showed a greater activity (8.5 ± 0.7 mm) against *C. parapsilosis* at 2000 µg/ml. Against *Fusarium sp.*, *A. fumigatus*, and *A. flavus*, no inhibitory activity was resulted at all concentrations. The standard drug clotrimazole showed a highest inhibitory zone against *A. fumigatus*. Against *C. parapsilosis*, the inhibitory activity of the standard drug ketoconazole and the algae *T. conoides* taken for the present study were predominant (Table 1).

DISCUSSION

The results obtained from the present study revealed the antifungal activity of *T. conoides* under hexane, chloroform,

Table 1: Antifungal activity of *Turbinaria conoides* crude extracts against the tested pathogens

Test samples	Concentrations (µg)	Zone of inhibition diameter (mm)				
		<i>C. albicans</i>	<i>C. parapsilosis</i>	<i>Fusarium sp.</i>	<i>A. flavus</i>	<i>A. fumigatus</i>
Hexane	500	7.0 ± 0.0	7.0 ± 0.0	-	-	-
	1000	7.0 ± 0.0	7.5 ± 0.7	-	-	-
	1500	7.5 ± 0.7	8.0 ± 0.0	6.0 ± 0.2	-	-
	2000	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.1	7.0 ± 0.0	8.0 ± 0.0
Chloroform	500	7.0 ± 0.0	5.0 ± 0.0	-	-	-
	1000	7.0 ± 0.0	6.0 ± 0.0	-	-	-
	1500	7.0 ± 0.0	7.0 ± 0.0	-	-	-
	2000	8.0 ± 0.0	7.0 ± 0.0	-	-	-
Ethanol	500	7.0 ± 0.0	-	-	-	-
	1000	7.0 ± 0.0	7.0 ± 0.1	-	-	-
	1500	7.0 ± 0.0	7.5 ± 0.7	-	-	-
	2000	7.5 ± 0.7	8.5 ± 0.7	-	-	-
Clotrimazole	10	NA	NA	NA	19.7 ± 0.8	20.2 ± 0.6
Ketoconazole	10	21.7 ± 0.1	13.9 ± 0.7	-	NA	NA

C. albicans: *Candida albicans*, *C. parapsilosis*: *Candida parapsilosis*, *A. flavus*: *Aspergillus flavus*, *A. fumigatus*: *Aspergillus fumigatus*, *T. conoides*: *Turbinaria conoides*, NA: Not applicable

and ethanolic extracts. Ambika *et al.* (2014) reported that *Gymnopilus edulis*, *Caulerpa racemosa*, and *Sargassum myricocystum* reduced the fungal mycelial growth of *Alternaria porri* at increased concentrations of 30%. Prabha *et al.* (2013) revealed that *Kappaphycus alvarezii* has active secondary metabolites and also exhibited antimicrobial activity against *A. flavus*, *A. fumigatus*, and *C. albicans* mainly in the methanolic extract of *K. alvarezii*, and this may be mainly due to the presence of phenolic lipids, terpenes, and phlorotannins.

Zovko *et al.* (2012) studied antifungal activity against fungal strains of *C. albicans* with a high activity of algal extracts. Gao *et al.* (2011) showed that a few extracts of marine algae have not only an antifungal activity but also toxicity toward cancer cells. Padmakumar and Ayyakannu (1997) screened 80 species of marine algae for antifungal activities but did not find a single algal extract active against *A. flavus*. Shanmugam *et al.* (2010) reported no inhibitory activity against *C. albicans* under ethanolic extract and good activity (16 mm) against *A. flavus* under hexane extract of *T. conoides*. The present investigation, on the other hand, showed that the seaweed *T. conoides* is effective against *C. albicans* under ethanolic extract (7.5 ± 0.7 mm) and *A. flavus* under hexane extract (7.0 ± 0.0 mm). Similarly, Manivannan *et al.* (2011) reported a good inhibitory activity (18.33 ± 2.25 mm) against *C. albicans* under ethanolic extract. In *Turbinaria ornata*, ethanol extract showed a strong activity against *C. albicans* (15-20 mm) and nil activity against *A. flavus* and *Fusarium* sp. (Ibraheem *et al.*, 2012). Likewise, Rattaya *et al.* (2014) reported no inhibition against *Aspergillus niger* under the same genus. The present investigation in *T. conoides* also showed a similar result against *C. albicans* and *A. flavus*. The standard drug clotrimazole and ketoconazole were dominant in their inhibition against the fungal strain *A. fumigatus* and *C. albicans*, respectively.

CONCLUSION

T. conoides exhibits specific antifungal activity against the tested fungal pathogens. With the present inspection, the future work is needed to identify the principle compound responsible for antifungal activity against pathogenic fungi, especially those causing the human diseases.

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