Advance Research in Pharmaceuticals and Biologicals

(A Peer Reviewed International Journal for Pharmaceutical and Allied Research)

ISSN 2250-0774 USA CODEN: ARPBGZ

ESTIMATION OF ANTI-FUNGAL ACTIVITY OF AQUEOUS EXTRACTS OF BROWN SEAWEED, *SARGASSUM ILLICIFOLIUM*

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Received on 21/02/2013 Revised on 28/02/2013 Accepted on 03/03/2013

ABSTRACT:
Marine organisms are a novel source of medicines. In this regard the present study aims to evaluate the anti fungal activity of aqueous extract of Brown Seaweed, *Sargassumillicifolium* on three types of terrestrial, non- pathogenic fungi.The anti-fungal activity of aqueous extract of *Sargassumillicifolium* of different concentration such as 5%, 10%, 15%, 20%, 25% and controls both positive and negative were carried out against three types of terrestrial, non-pathogenic fungiusing Agar Diffusion Assay.The results indicated that in the negative control and lower concentrations (5%, 10%, 15%) there was no or least amount of antifungal activity which was followed by that of the positive control, Clotrimazole, and the higher concentration of 20% and 25% showed better antifungal activity than that of the patented medicine. This preliminary study indicates that the aqueous extract of *Sargassumillicifolium*, is indeed endowed with antifungal properties against the three different type of terrestrial non-pathogenic fungus.

Keywords: *Sargassumillicifolium,* Aqueous Extract, Antifungal Activity, *Trichodermaasperellum,Chrysosporiumparvum, Uncinocarpusreesii.*

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INTRODUCTION

Too much of a good thing also leads to bad consequences. This paradigm has come to a full circle in case of antibiotics that was a life-saver has lost its cutting edge due to indiscriminate usage leading to drug resistant pathogens[1]. This has lead to the development and discoveries of novel avenues of drugs from several unforeseen areas, one among them is the aquatic chiefly the marine (ocean) environment. The marine algae especially the seaweeds are seen to be unexploited source of several bio-active compounds of potential medicinal applications[2-4].In this regard the brown seaweed, *Sargassumillicifolium* was chosen to be a source of such antifungal agent. The main reason for this is that the seaweed is not exploited for the three main uses such a food, fodder and fertilizer. This relatively underused seaweed but which occurs in abundance [5] was used to look into the anti-fungal activity of this marine alga. The various bioactive compounds that occur in these algae are carotenoids, terpenoids, xanthophylls, chlorophylls, vitamins, saturated and polyunsaturated fatty acids, acelozenins, antioxidants such as polyphenols, alkaloids, halogenated compounds and polysaccharides such as sugar, carrageenan, proteoglycans, alginate, laminarins, rhamnansulphate, galactosyl glycerol and fucoidan. These compounds collectively or as even isolated ingredient can act as anti-biotic or anti-microbial activities as anti-bacterial, anti fungal, anti viral, anti inflammatory, anti helminthic, anti-oxidants etc.,. Apart from the "anti"- properties it also has several positive

factors such as nutrient supplement, beauty preparations such as facial creams / packs, epithelial scrubs, herbivore deterrent, ultra violet screening agents etc.,. Pharmaceutical industry currently has less the ten percentage of its products derived from the sea which are extensively used in case of various ailments from pain / inflammation to acquired immune deficiency syndrome (AIDS) / cancer[6]. Thus the spectrum of biomedical activity ranges from simple diseases to complex ones. In addition to these algae produce pure forms of fatty acids found in human milk that helps in proper growth of infants.

There are many studies on inhibition against bacteria, some viruses and marine fungi but it is to be stated that only a few studies of the activity of compounds or extracts from marine brown algae, Sargassumillicifolium against terrestrial non-pathogenic fungus. The three different types of terrestrial, non-pathogenic fungus (mostly facultative fungus) were selected are *Trichodermaasperellum*, *Chrysosporiumparvum* and *Uncinocarpusreesii.* These were selected because non virility means that these do not lead a parasitic / pathogenic existence but survive by themselves[7]. If they are pathogenic they cannot be easily killed because they are not dependant on very specific conditions for their survival. Secondly since they are terrestrial, it is of interest to see whether they are affected / retarded / killed by compounds obtained from marine algae. This surely helps in aiding the study to understand whether the application of marine algae derived compound can e of any assistance in treating human fungal diseases, both of topical and systemic in nature.The methodology used was Agar Diffusion Assay [8]to determine the anti fungal activity of the seaweed. The technique was selected against the more specific method of Serial Dilution Method because the extract is of aqueous in nature and thus if used in dilution it will not be appropriate to estimate the activity due to dilution effect combined with very low quantities of the compound in the well micro-plates as there may not be sufficient algae components to interact with the fungus.

The present study is an initial report of the results of screening assay foranti fungal activity of the aqueous extract of Brown seaweed, Sargassumillicifolium on the three different types of terrestrial non-pathogenic fungus. If the aim of the study in identifying novel and interesting potentially useful therapeutic activities of the seaweed is fulfilled then the tests can be applied to other pathogenic varieties of fungus in search of drugs from the marine realm.

MATERIALS AND METHODS

Seaweed Collection

The seaweed for this study was collected from Mandapam, Gulf of Mannar, Tamil Nadu, India. The collected seaweeds were transported to the laboratory in seawater itself and they were kept in the same medium until they were suitably identified.

Processing of seaweed

Seaweed that were carefully collected, isolated, and identified was processed by the following procedure. In order to preserve the volatile, labile and sublimate compounds in the seaweed, they were systematically dehydrated dried and dusted in a procedure similar to that of the herbarium preparation.

Preparation of extract from brown algae

The powdered drug (50 gm) was charged into Soxhlet apparatus each time. The Soxhlet apparatus was kept on a heating mantle, to provide constant temperature to the process. After completion of extraction the concerned solvents were removed under reduced pressure. The residual components were taken out and filtered using whatman filter paper 1.0 micron and the components were air dried to a great extent before lyophilizing. Aqueous extract was subjected to chemical tests and Pharmacological screening for antibacterial activity.

Terrestrial Non Pathogenic Fungus

The test organisms included three American Type Culture Collection (ATCC) fungal strains equivalents, namely,*Trichodermaasperellum*,*Chrysosporiumparvum* and *Uncinocarpusreesii*.

Anti Fungal Drugs and Agar Diffusion Assays

Clotrimazole was obtained as a reagent-grade powder from Hi-Media, India. The fungus in question is swabbed uniformly across a culture plate. A filter-paper disk, impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk, and will decrease as distance from the disk increases. If the compound is effective against fungus at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. Thus, the size of the zone of inhibition is a measure of the compound's effectiveness: the larger the clear area around the filter disk, the more effective the compound.

Statistical analysis

Regression analysis was carried out to test the efficacy of the drug based on increasing concentration. In addition to this ANOVA was also carried out to give clear comparison between the different groups of fungi studied.

RESULTS

The antifungal activity of the seaweed extract, *Sargassumillicifolium* on the fungal species of *Trichodermaasperellum* showed that at higher

concentrations of 20 and 25 % it was better than that of the patented medicine, namely, Clotrimazole. (Fig.-1)

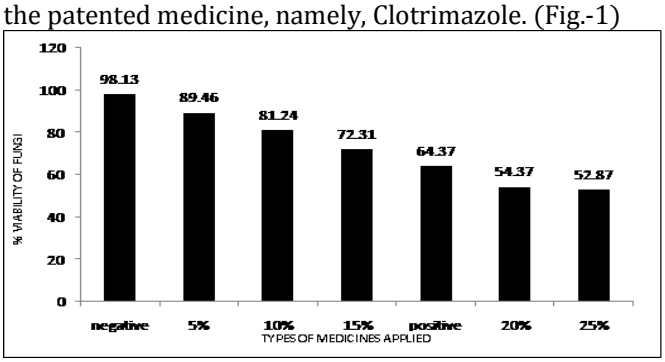

Fig.1 Antifungal activity of *Sargassumillicifolium*aqueous extract on *Trichodermaasperellum*

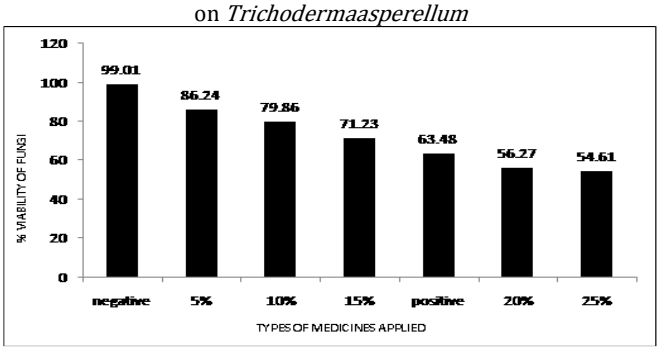

Fig.2Antifungal activity of Sargassumillicifoliumaqueous extract on Chrysosporiumparvum

Uncinocarpusreesii was tested against the extract's of Sargassumillicifolium and the results are given in Fig.3.

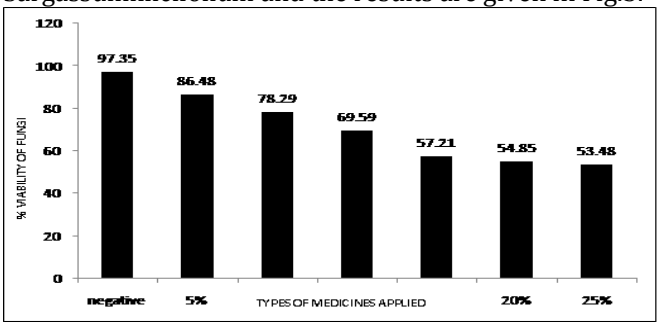

Fig.3 Antifungal activity of *Sargassumillicifolium*aqueous extract on *Uncinocarpusreesii*

Regression analysis for the effect of antifungal activity by the aqueous extract ofSargassum is given in Fig.4

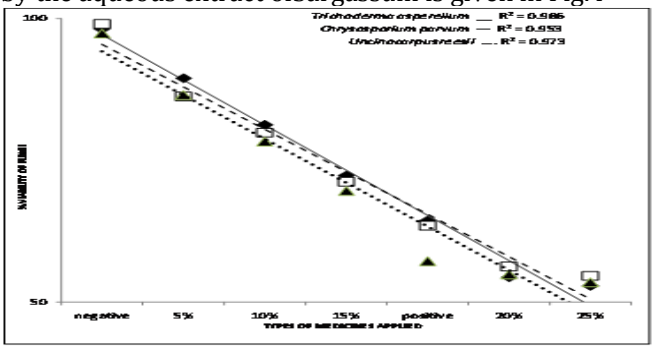

Fig.4 Regression analysis of the results obtained from various strains of fungi tested

The results of ANOVA are given in the Table – 1 for the various strains of fungus tested and the inter comparison of the different percentages of extract concentrations are also incorporated.

Table1: ANOVA for the results obtained from various strains of fungi tested

Type of assay	Anova criteria	Ss	Df	Ms	F	P	Statistical criteria
Assay 1	Between	86341.4	6	14390.23	7355	0.0001	Extremely significant
	Within	205.4399	105	1.95657			
	Total	86546.84	111	-			
Assay 2	Between	928.5324	6	154.7554	7445	0.0001	Extremely significant
	Within	2.182423	105	0.020785			
	Total	930.7148	111	-			
Assay 3	Between	3755	6	625.7609	8369	0.0001	Extremely significant
	Within	7.850718	105	0.0747687			
	Total	3762.416	111	-			

DISCUSSION

Marine macro algae are important ecologically and commercially to many regions of the world, especially in Asian countries such as China, Japan and Korea. They are a valuable food resource which contains low calories, and they are rich in vitamins, minerals, proteins, polysaccharides, steroids and dietary fibers[9]. Since as early as 3000 BC, they were also considered important as traditional remedies. The Japanese and Chinese use brown algae in the treatment of hyperthyroidism and other glandular disorders[10].

The resistance of pathogens to commonly used antibiotics has enhanced morbidity and mortality and has triggered the search for new drugs. Several species of the brown alga genus *Sargassae* are very interesting candidates as potential sources of natural products with pharmaceutical activity because they are known to produce a wide range of chemically interesting halogenated secondary metabolites[11-14]. This is an initial report of the antifungal activities of the secondary metabolites of brown seaweed,Sargassumillicifolium, collected in the state of Tamil Nadu, India against three types of terrestrial non-pathogenic fungi, *Trichodermaasperellum*, *Chrysosporiumparvum* and *Uncinocarpusreesii*.

The results all indicate to higher concentration of 20% and 25% of the extract to be effective against all the three types of fungi, as opposed to the standard antifungal drug namely, Clotrimazole. Further statistical analysis of both Regression analysis and ANOVA indicated that the results were indeed due to the superior antifungal activity of the seaweed and it was an encouragement to test these compounds in other scenarios such a pathogenic fungus.

The probable nature of antifungal activity could be due to the presence of Phlorotannins. These are restricted to brown seaweeds (Phaeophyceae) and are biosynthesized through the acetate-malonate pathway

in Golgi apparatus, in the perinuclear area of the cell. They are stored in vesicles called physodes, appearing as a colourless and refractive acidic fluid. Phlorotannins concentration in brown seaweeds can vary among species, being affected by seaweed size,age, tissue type, salinity, season, nutrient levels, intensity of herbivore, light intensity and water temperature. Their concentration can reach the maximum in temperate and tropical Atlantic (up to 20% of brown seaweed dry mass), and the minimum intropical Pacific and Indo-Pacific regions. These compounds have primarily been regarded as defense chemical agents. These results point phlorotannins extracts as potential pharmaceutical resources for combating infections with multi-etiological causes, as the same extract can cover a wide range of microorganisms and has the potential to act on inflammatory states and oxidative environment, commonly associated with microbial infections[15].

The importance of investigating the activity of crude extracts lies in identifying promising candidates for additional investigation. In addition, the activity of an extract may be due synergy between two or more components and other beneficial pharmacological or medicinal properties can be explored. For extracts that's how activity, subsequent fractionation or isolation of the individual components is necessary to obtain an understanding of the activity of each individual component and of the contribution of each component to the overall activity of the extract as a whole. In conclusion, this study suggests that aqueous extract of *Sargassumillicifolium*, possess interesting antifungal properties, which should encourage the continued search for new rugs for therapy of infectious diseases derived from marine algae.

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