EFFICACY OF *SPIRULINA PLATENSIS* ON FISH PATHOGENS

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Abstract:

Natural therapy has its inherent attraction in that there are limited side-effects. Spirulina platensis, a naturally occurring algae, has been reported to have more than one therapeutic advantage. It is also used as a source of natural protein and vitamins in many parts of the world. The present study has been focused on to investigate the role of extracts of marine algae *Spirulina platensis* against fish pathogens. The crude extract of the marine algae have shown promising activity against most of the screened pathogens. The green marine algae *Spirulina platensis* was very active against fish pathogens and could be a good source for the antifungal activity. In partitioning crude extract portioned between ethanolic and petroleum ether. The activity of each phase was screened using disc diffusion method.

Key words: *Spirulina platensis*, fish pathogens, antifungal activity, disc diffusion method.

Introduction:

Due to the occurrence of fungal resistance to synthetic fungicides, the use of chemical compounds is strictly controlled and their application is subjected to tighter regulations (Kim et al., 2018; Tetz et al., 2019). Therefore, the use of biological substances with antimicrobial properties redirected researchers for the development of novel and stable approaches that are less

inducible to antimicrobial resistance in order to replace chemical fungicides, bactericides and pesticides for food preservation and safety as well as plant crop protection. Furthermore, the growing interest in biological food additives has challenged the scientific community to innovate in alternative food preservation and plant crop protection systems. Thus, natural preservatives from sources like bacteria, plants and algae were reported to ensure food safety due to their antimicrobial activity against a wide spectrum of foodborne pathogens (Dubey et al., 2017; Mtibaa et al., 2019). In this regard, dietary antioxidants (i.e., phenolic compounds) have recently attracted extensive attention because of their ubiquity in nature and their various beneficial effects, including antimicrobial, antioxidant, antiinflammatory and antiproliferative activities (Fendri et al., 2013).

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their uses in traditional medicine. In the pre-industrial era the first generation of drugs was usually simple botanicals employed in more or less their crude form. Following industrial revolution, a second generation of drugs emerged based on scientific processing of the algal extract to isolate their "active principle" which was a finer form of the original crude extract (Noaman et al., 2004). There has been a rising interest of researchers for natural products from plants for the discovery of new antimicrobial and antioxidant agents in the last three decades (El-Sheekh et al., 2006; El-Sheekh et al., 2008; Becker et al., 1994; Hernandez-Corona et al., 2002). The cyanobacteria (Blue Green Algae) are able to produce biologically active compounds (Özdemir et al., 2000). They are a source of inspiration for novel drug compounds that can be exploited for human health and well-being, safer or more effective than synthetically produced antimicrobial agent. Pathogen resistance to synthetic drugs and antibiotics that are already in use makes search for

plants with antimicrobial activity more important, as they can substitute for synthetic antibiotics and drugs. *Spirulina platensis* produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Spirulina platensis or its extract show therapeutic properties, such as the ability to prevent cancers, decrease blood cholesterol level, reduce nephrotoxicity of pharmaceuticals and toxic metals and provide protection against the harmful effect of radiation.

Spirulina is an economically important filamentous cyanobacterium that grows in aquatic habitats. It is approved in Russia as a medicinal food for treating radiation sickness (Rabadiya and Patel, 2010). Spirulina is an ideal bio-resource due to its richness in protein, phycocyanin, essential amino acids, polysaccharides, carotenoids, minerals, vitamins, and essential fatty acids (Morist et al., 2001). It is also rich in vitamins, minerals, carbohydrates, and gamma-linolenic acid. Spirulina is gaining attention not only for its food value, but also for development of pharmaceuticals. Spirulina has therapeutic effects as a growth promoter, probiotic, and booster of the immune system in animals including fish (James et al., 2006). Phycocyanin is the principal bioactive substance in spirulina and its content ranges 10-15% of the dry weight (Becker, 1994). Spirulina species exhibit antiviral and antioxidant properties against human pathogens (Khan et al., 2006). This study evaluates the antifungal activity of Spirulina platensis (reclassified as Arthospira platensis but still commonly known as spirulina) against selected fish fungal pathogens.

Materials and methods

Collection of algae

The fresh algae of Spirulina were obtained from Parankipettai, Tamilnadu in India.The algae was collected in a sterile container and maintained at room temperature.

Extraction of algae

Preparation of the extract algae were collected from Parankipettai, India and dried. Dried algae were crushed in pestle and porter. The algae powdered (500gm) were exhaustively extracted with 70% ethanol and then successively with petroleum ether. The extracts were then re dissolved in 10% DMSO (v/v) to yield solutions containing 100.0mg of extract per ml.

Collection of fish

Labeo rohita was collected in sterile container maintained at lab conditions and excess of fish preserved in 50% of formalin solutions.

Isolation of Fungi

The fish gut region was removed carefully and then cut in to small pieces. The 1gm of gut pieces was weighed aseptically, crushed and added to the dissolved in 99ml of distilled water. From this each dilution 1ml each added in to petriplates in duplicates. The samples was poured in to petriplates and mixed the medium and allowed to solidify. After the pour plate technique the plates were incubated at 37°C for 48 hours. After incubation period the isolated colonies were streaked in to the PDA medium plates for purification and identification. The isolated colonies were transformed to rosebengal agar slants and stored for further studies.

Identification of fungi

Identification of the fungi was done based on microscopic observation. The isolated cultures were streaked onto the PDA medium and incubated at 37°C for 48 hours.

Antifungal activity

Invitro antifungal activity was performed with ethanolic and petroleum ether extracts of algae of *Spirulina* against the fish pathogenic bacteria by the disc diffusion method (Savikin et al., 2018; Singh et al., 2019). Each Petridish was inoculated with one of the fungal cultures

suitably diluted to contain above 10^6 cells/ml by spreading 0.1ml suspension of the organism with a sterile cotton swab. In each plate cups of 6mm diameter were made at equal distances using sterile cork borer. One cup was filled with 0.1 ml of standard drug. The petridish were incubated at 37°C for 48 hours. The diameter zone of inhibition in mm was recorded after incubation.

The extracts that showed antibacterial activity were subjected to minimum inhibitory concentration (MIC) assay by serial two fold dilution method (Savikin et al., 2018). MIC was interpreted as the lowest concentration of the sample, which showed clear fluid without development of turbidity.

Results

The antifungal activities of the ethanolic and petroleum ether extracts of *Spirulina* showed significant variations as shown in table 1 & Fig 1 to 5. Among the two extracts tested, ethanolic extract had greater antifungal potential, followed by petroleum ether extracts. *Aspergillus flavous* was zone of inhibition in ethanolic ether (12mm) and petroleum ether (10.21). *Aspergillus niger* was zone of inhibition in ethanolic ether (09.56mm) and petroleum ether (08.44 mm). *Penicillium chrysogenum* was zone of inhibition in ethanolic ether (07.54 mm) and petroleum ether (09.53 mm) and compare with control and standard. Control was used as DMSO and standard was used as clotrimazole.

Discussion

Spirulina sp. represents one of the most important commercial microalga for the production of biomass as healthy food and animal feed (Smaoui et al., 2018). A large number of microalgal extracts and extracellular products have been found to have antibacterial activity. The algal extracts showed antibacterial and antifungal activities, both culture filtrate and whole

culture (cells and exometabolites) have been proved to have wide spectrum antimicrobial activity, where *Bacillus sublilis* and *Candida albicans* were the most sensitive species. The antimicrobial activity of microalgae could be explained by the presence of cyclic peptides, alkaloids and lipopolysaccharides (Smaoui et al., 2011). This activity may be due to the toxins produced by its cells like a number of blue green algae that produce toxins which have potential pharmaceutical applications.

Microorganisms have developed adaptation mechanisms against the action of antimicrobial drugs (Al-Haj et al., 2009). This problem is one of the main reasons for continued research into antimicrobial compounds, including molecules from cyanobacteria and marine algae (Kim et al., 2007; Al-Wathnani et al., 2012). Much attention is being paid towards plant extracts and biologically active compounds isolated from natural resources in the present. Aquatic organisms are a rich source of structurally novel and biologically active metabolites (Ely et al., 2004). Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry (Prakash et al., 2011). Most of the secondary metabolites produced by seaweeds have bactericidal or the antimicrobial compounds derived from seaweeds consist of diverse groups of bacteriostatic properties terpenols, sterols, polysaccharides, dibutenolides peptides and proteins metabolites. Compounds with antibacterial activity have been detected in green, brown and red algae (Yuan et al., 2005; Bansemir et al., 2006; Chew et al., 2008). Lipid soluble extracts from marine macroalgae have been investigated for their antifungal and antibacterial properties.

In view of the increasing number of antibiotic resistant strains of bacteria, interest in isolating and characterizing antibacterial substances of cyanobacterial origin is growing. Cyanobacterial extracts in different solvents are effective against gram-positive and gram-

negative pathogenic microorganisms (Ghasemi et al., 2004). Likewise, in our study, extracts of A. platensis, prepared in different solvents, had similar effects on both types of organisms. Methanol, ethanol, and water are the most commonly used solvents for antimicrobial activity using plants or algae (Parekh et al., 2006). Acetone is not a commonly used solvent but was the best solvent for extract preparation in the present study, as in the study of Abedin and Taha (2008). The acetone soluble fraction (ASF) showed strong antibacterial and antifungal activity.

The antimicrobial activities of S. platensis could be attributed to different compounds belonging to a diverse range of chemical classes (Al-ghanayem et al., 2017). The antimicrobial activity found in S. platensis extracts could be due to contain γ-linolenic acid (Demule et al., 1996), active fatty acid (Xue et al., 2002), synergetic effect of lauric and palmitoleric acid (Mendiola et al., 2007). The test microorganisms differ significantly in relation to their susceptibility to S. platensis antimicrobial substances, Candida albicans was the most sensitive microorganism (MIC µ50 g ml-1) while Gram positive bacteria were more sensitive than the Gram negative bacteria. In the present study spirulina different solvent extracts resistance to selected fish fungal pathogens.

In this study, the cyanobacterium S. platensis had the most effective antifungal activity against selected fungal pathogens compared with other screened algae and these results are in agreement with the findings by Abdo et al. (2012) and Kaushik and Chauhan (2008). The collected fractions of the methanol extract of S. platensis using silica gel chromatography showed a high inhibitory activity against A. niger and A. flavous. Physical and chemical characterizations of the most active fractions were applied. From UV analysis, maximum absorption spectrum at 285 nm was observed. In fact, this antifungal activity was similar to standard synthetic antifungal agent (Amphotericin B). Similarly, Al-Ghanayem, 2017, reported

the inhibition growth of Fusarium oxysporum followed by Aspergillus flavus and Aspergillus niger by Spirulina platensis organic extract. Furthermore, Kumar et al. in 2011 reported the inhibitory effect of hexane and methanolic extracts of Spirulina platensis against Aspergillus spp. (Kumar et al., 2011). The activity of the alga could be due to the intracellular and extracellular metabolites that have antifungal properties (Usharani et al., 2015). In general, it was demonstrated that the most antifungal compounds in Spirulina are mainly polyphenols along with polysaccharides that inhibit microbial growth, or directly by destroying the living structures of fungi (López-Malo. et al., 2002). In our present study, a wide range of fish pathogenic microorganisms were examined, the fish pathogenic bacteria. This may indicate that the *Spirulina* extracts have broad inhibitory activities to pathogenic microorganisms and promising to act as potential antifungal agents from natural plant sources.

Conclusion:

Due to the fast that the marine algae *Spirulina* is very useful found by above mentioned reports and there is a need to find out more about the potentially of this algae as an antimicrobial agent. The present study is, therefore, designed to assess the potency of ethanolic and petroleum ether extracts of *Spirulina* on some fish pathogens.

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Table 1: Antifungal activity of different extracts of algae of *Spirulina*

Micro-organism	Zone of Inhibition (mm)			MIC (µg/ml)	
	Ethanolic Extract	**Petroleum Ether**	**Clotrimzole**	**Ethanolic Extract**	**Petroleum Ether**
Aspergillus flavus	15.34±1.00	13.65± 0.61	19	12.24	18.43
A. niger	10.76 ± 0.44	12.11±0.89	14	13.53	23.53
Penicillium chrysogenum	11 ± 0.20	13.54±0.53	12	15.66	25.19

Fig 4: Antifungal activity of different extracts of algae of *Spirulina*

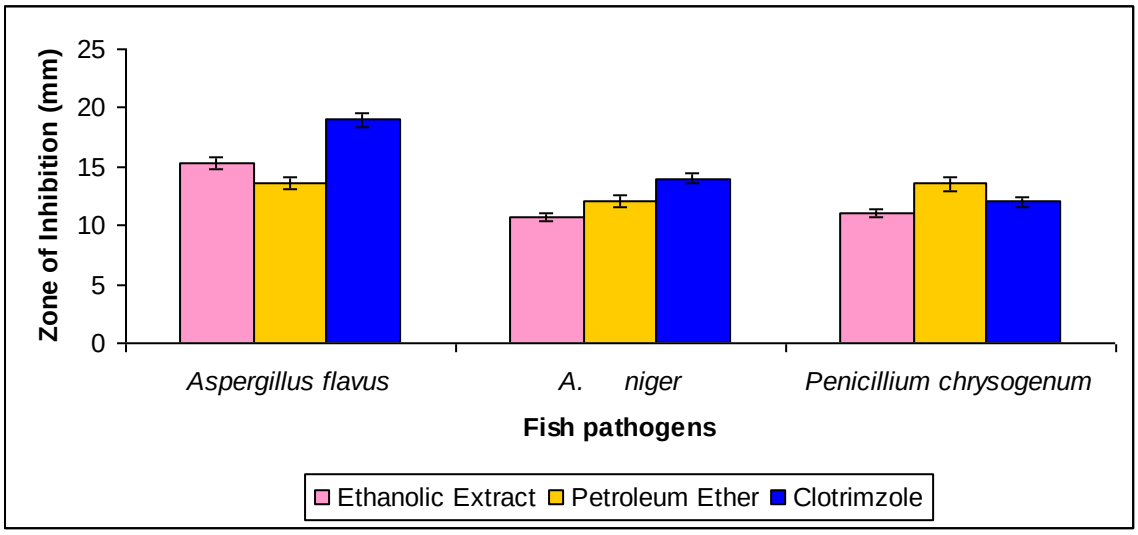

Fig 5: Minimum inhibitory concentration of different extracts of algae of *Spirulina*

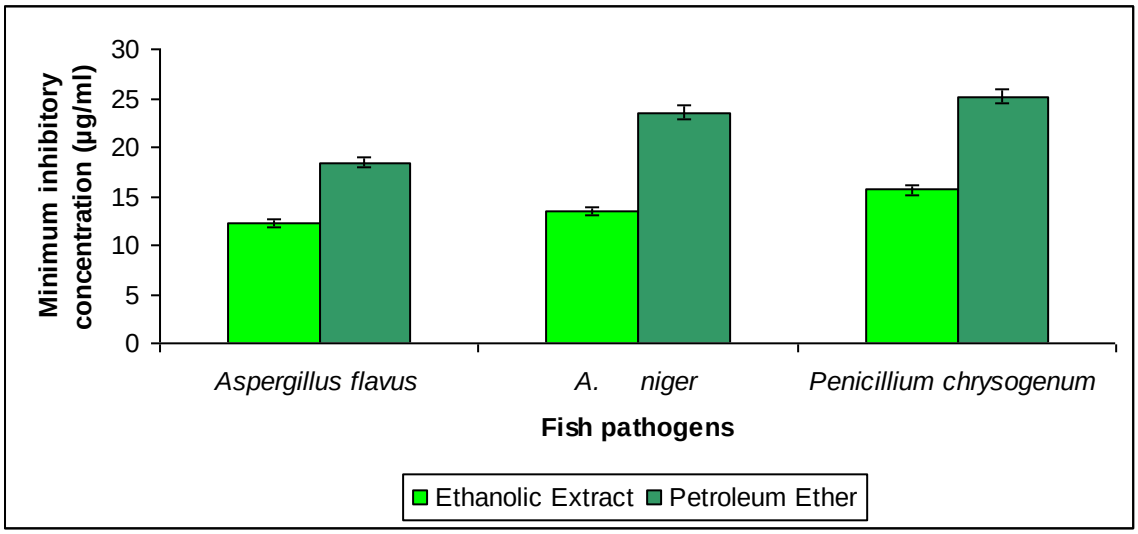

Fig 1: Antifungal activity of different extracts of algae of *Spirulina* against *Aspergillus flavus*

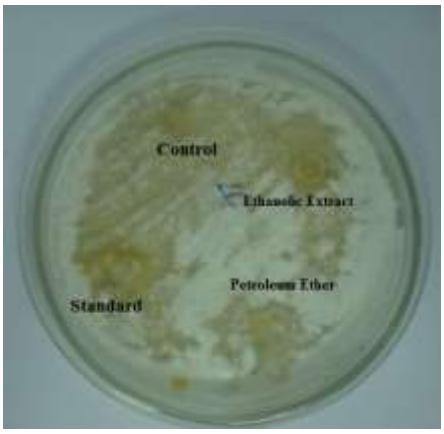

Fig 2: Antifungal activity of different extracts of algae of *Spirulina* against *Aspergillus niger*

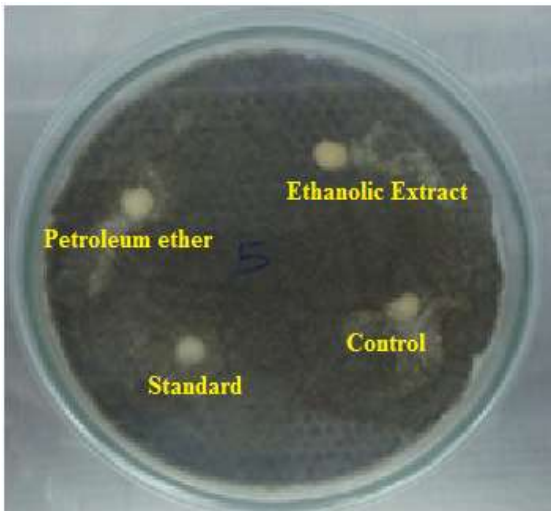

Fig 3: Antifungal activity of different extracts of algae of *Spirulina* against *Penicillium chrysogenum*