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ANTIPATHOGENIC ACTIVITY OF *SPIRULINA* POWDER

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Abstract

Spirulina is a microscopic blue-green alga in the shape of a spiral coil, living both in sea and fresh water. In this study, dried *Spirulina* was extracted at different solvents such as Hexane, Ethyl acetate, Ethanol, Butanol, Acetone, Methanol and chloroform. That extracts were stored in an airtight glass bottles in a refrigerator. Antimicrobial activity analysis from different pathogens and using well diffusion methods. Butanol extract of *Spirulina* also gave the highest antimicrobial activity of 19 mm against *Staphylococcus epidermids*, 18mm against *Staphylococcus aureus* and *Aeromonas liquefaciens,* 13mm against *Candida glabrata,* 12mm against *Enterococcus feacalis,* 11mm against *Campylobacter coil and Vibrio cholerae* and low activity against 5mm *Salmonella typhi*. Remaining solvents extracts antimicrobial activity low compare to butanol.

Keywords: Blue-green alga; *Spirulina* sp; Solvents, Pathogens

Introduction

Spirulina is microscopic blue - green alga that exists as a single celled organism turning sunlight into life energy. It is one of the first life forms designed by nature more than 3.6 billion years ago. *Spirulina* contains billions of years of evolutionary wisdom in its DNA and is an offspring of earth's first photosynthetic life forms. Genus *Spirulina* has gained an importance and international demand for its high phytonutrients value and pigments which have applications in healthy foods, animal feed, therapeutics and diagnostics (Becker, 1994; Vonshak and Tomaselli, 2000). *Spirulina* has been used as food and nutritional supplements since long time (Dillon *et al*., 1995). It is generally a rich source of protein, vitamins, essential amino acids, minerals, essential fatty acids such as ã-linolenic acid and sulfolipid (Mendes *et al*., 2003). Moreover in addition to ϕ-3 and ϕ-6-poly unsaturated fatty acids, it has also phycocyanin and other phytochemicals (Chamorro *et al*., 2002). Some *Spirulina* species exhibit antibacterial (Ozdemir *et al*., 2004), antiplatelet (Hsiao *et al*., 2005), antihepatoxic (Mohan *et al*., 2006) and antiviral activities (Hernandez-Corona *et al*., 2002). Several algal species contain natural bioactive compounds that act as potent antimicrobial agents *Spirulina species,* for example, have some valuable antiviral and antioxidant compounds (Ozdemir *et al.,* 2004 and Khan *et al.,* 2006). Huang *et al*. 2007 reported that *Spirulina platensis* could be used as a matrix for the production of selenium-containing compounds and proved to be successful in transforming inorganic selenium to organic selenium *in vivo* when cultivated in selenium-rich medium (Li *et al.* 2003). The antioxidant activities of selenium-containing phycocyanin and its different aggregates (monomer, trimer, and hexamer) against free radicals of superoxide, hydrogen peroxide, and 2,2-diphenyl- 1-picryl-hydrazyl (DPPH) were found to be variable. Various organic and aqueous extracts of Spirulina platensis were screened for their antibacterial activities (Mala et al., 2009). In the present study conformed that antimicrobial activity of spirulina by using different solvent extraction.

Materials and Methods

Collection of *Spirulina* sp

The algae *Spirulina* sp powder was purchased from Red jungle brand, Made in U.S.A

Preparation of *Spirulina* extracts

Freshly dried powder form *Spirulina* was mixed with Hexane, Ethyl acetate, Ethanol, Butanol, Acetone, Methanol and chloroform (150ml solvent/100g of *Spirulina*) in soxhlet apparatus and extracted for 60 minutes. The extracts were filtered and the solvent was removed using rotary evaporator. Sequential extraction was performed with all solvents in the order, Hexane, Ethyl acetate, Ethanol, Butanol, Acetone, Methanol and chloroform. Antimicrobial activity analysis from well diffusion methods.

Shigella flexneri, Vibrio paraheamolyticus, Bacillu subtilis, Salmonella typhi, Campylobacter coil, Enterococcus feacalis, Pseudomonas aeruginosa, Vibrio cholerae, Staphylococcus aureus, Escherichia coli, Staphylococcus epidermids, Aeromonas liquefaciens, Candida albicans, Candida glabrata and *Cryptococcus neoformans.* All these strains were

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colleted from Government hospital, Tiruchirappalli, Tamil Nadu.

Microorganisms tested

Strains of human pathogenic microorganisms used in this study were as follow:

Preparation of 24 hours pure culture

A loop full of each of the microorganisms was suspended in about 10 ml of physiological saline in a Roux bottle. Each of these were streaked on to the appropriate culture slants and incubated at 37ºC for 24 hours.

Antimicrobial activity using disc diffusion method

Antimicrobial activity was checked by well diffusion method. The pathogenic cultures were grown in nutrient broth and incubated at 37°C for 24 h. After incubation period prepared Sterile Mueller-Hinton agar medium and it was poured into sterile petri plates and allowed to solidify. After solidification each plates swab different pathogens and cut the well by using gel puncher (diameter 6mm) and cured extracts were pour different concentration. Those plates were incubated from 24 hours at room temperature. After incubation period the zone of inhibition was measured.

Result and Discussion

Spirulina is a microscopic blue-green alga in the shape of a spiral coil, living both in sea and fresh water. Spirulina is the common name for human and animal food supplements produced primarily from two species of cyanobacteria: *Arthrospira platensis*, and *Arthrospira maxima* (Vonshak 1997). The cyanobacteria represent a large group within the prokaryotic kingdom. They are the oldest oxygenic photosynthetic organisms known so far and they also serve as a rich source of novel bioactive metabolites, including many cytotoxic, antifungal and antiviral compounds (Patterson *et al*. 1994). *Spirulina platensis*, a blue green microalga, has been used since ancient times as a source of food because of its high nutritional value (Dillon *et al.* 1995). The cyanobacterium *Spirulina platensis* is rich in nutrients, such as proteins, vitamins, minerals, carbohydrates, and γ-linolenic acid. It is gaining more and more attention, not only for the foods aspects but also for the development of potential pharmaceuticals (Quoc & Pascaud 1996). *Spirulina platensis* was also reported to present antimicrobial activity (Demule *et al.* 1996; Ozdemir *et al.* 2004) as well as to inhibit the the replication of several viruses, such as Herpes simplex and HIV-1 (Ayehunie *et al.* 1998; Hernández-Corona *et al*. 2002). Extracts of *Spirulina platensis*, obtained by different solvents exhibited different degrees of antimicrobial activity on both Gram-positive and Gram-negative organisms (Raina et al., 2008, Ozdemir et al., 2001). In the present study, Spirulina extracted from seven different solvents such as, Hexane, Ethyl acetate, Ethanol, Butanol, Acetone, Methanol and Chloroform by using antimicrobial activity analysis. Most antimicrobial active components that have been identified are not water soluble and organic solvent extracts have been found to be more potent (Geissman, 1963).

Water extract of *Spirulina platensis* showed maximum antimicrobial activity of 18.0 mm against *Klebsiella pneumoniae* (NCIM2063) and a minimum activity of 10.0 mm against *Proteus vulgaris (*NCIM202*)* (Mala et al., 2009).

Acetone extract of *Spirulina platensis* also gave the highest biological activity of 17.0 mm against *Klebsiella pneumoniae* (NCIM2063), moderate activity of 11.0 mm against *Salmonella typhi* (NCIM2080), and 10.0 mm againest *Pseudomonas aeruginosa* (NCIM2076), *Escherichia coli* (NCIM2065) and *Staphylococcus aureus* (NCIM2079). (Mala et al., 2009)

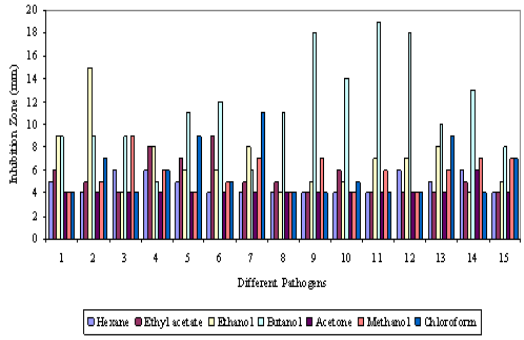
Hexane extract of *Spirulina* also gave the highest antimicrobial activity of 6mm against *Bacillu subtilis, Salmonella typhi, Aeromonas liquefaciens* and *Candida glabrata* and low activity against 4mm *Vibrio paraheamolyticus, Enterococcus feacalis, Pseudomonas aeruginosa, Vibrio cholerae, Staphylococcus aureus, Escherichia coli, Staphylococcus epidermids* and *Cryptococcus neoformans* (Fig. 1).

Ethyl acetate extract of spirulina also gave the highest antimicrobial activity of 9 mm against *Enterococcus feacalis*, 8mm against *Salmonella typhi* remaining moderate activity, low activity against 4mm *Bacillu subtilis, Staphylococcus aureus, Staphylococcus epidermids, Aeromonas liquefaciens, Candidaalbicans* and *Cryptococcus neoformans* (Fig.1).

Ethanol extract of spirulina also gave the highest antimicrobial activity of 15mm against *Vibrio paraheamolyticus* , 9mm against *Shigella flexneri,* 8mm against *Salmonella typhi, Pseudomonas aeruginosa* and *Candidaalbican* and low activity against 4mm *Bacillu subtilis, Vibrio cholerae* and *Candida glabrata* (Fig. 1).

Butanol extract of spirulina also gave the highest antimicrobial activity of 19 mm against *Staphylococcus epidermids*, 18mm against *Staphylococcus aureus* and *Aeromonas liquefaciens,* 13mm against *Candida glabrata,* 12mm against *Enterococcus feacalis,* 11mm against *Campylobacter coil and Vibrio cholerae* and low activity against 5mm *Salmonella typhi* (Fig. 1).

Fig. 1. Antimicrobial activity of Spirulina



Acetone extract of spirulina also gave the low level activity (4mm) maximum pathogens (Fig.1).

Methanol extract of spirulina also gave the highest antimicrobial activity of 9mm against *Bacillu subtilis* and low activity against 4mm Bacillu *subtilis, Campylobacter coil, Vibrio cholerae, Escherichia coli* and *Aeromonas liquefaciens* (Fig.1).

Chloroform extract of spirulina also gave the highest antimicrobial activity of 11mm against Pseudomonas *aeruginosa* and low activity against 4mm *Bacillu subtilis, Bacillu subtilis, Vibrio cholerae, Staphylococcus aureus, Staphylococcus epidermids, Aeromonas liquefaciens* and *Candida glabrata* (Fig .1).

Diethyl ether showed a marked activity against *Klebsiella pneumoniae* (NCIM2063) followed by *Shigella shigae* (NCIM2064) exhibiting 20.3 mm and 20.1 mm of inhibition zone. The same results were also reported by other workers (Tuney, et al., 2006, Moreau et al., 1988, Ozdemi et al., 2004 and Mala et al., 2009).

Petroleum ether showed a marked activity against *Shigella shigae* (NCIM2064 followed by *Klebsiella pneumoniae* (NCIM2063) exhibiting 23 mm and 20 mm of inhibition zone (Mala et al., 2009)

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