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Antibacterial Activity of Crude Extracts of *Spirulina Platensis* Against Some Pathogenic Bacteria and Fungi Isolated from Different Sites on Human Body

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Abstract

Spirulina platensis are filamentous, undifferentiated, non-toxigenic cyanobacteria that have been used as food since ancient times. There have been numerous studies on its antioxidant and antimicrobial actions. *Spirulina* as many other cyanobacteria species have the potential to produce a large number of antimicrobial substances, so they are considered as suitable organisms for exploitation as biocontrol agents of plant pathogenic bacteria and fungi. In the present study, antimicrobial activity of *Spirulina platensis* solvent extracts in serial dilution was investigated against pathogenic bacteria and fungi. The antimicrobial activity of *Spirulina platensis* was determined against pathogenic bacterial and fungal isolates. The methanol extract of *Spirulina platensis* showed maximum zone of inhibition against all the bacterial and fungal isolates**.**

Keywords *: Spirulina platensis, Inhibition zone*

Introduction

Early interest in *Spirulina* was focused mainly on its potential as a source of protein, vitamins, especially vitamin B12 and provitamin A (β- carotene), and essential fatty acids like γ - linolenic acid (GLA). Recently more attention has been given to study its therapeutic effects, which include reduction of cholesterol and nephrotoxicity by heavy metals, anticancer properties, protection against radiation, and enhancement of the immune system[(1)]. *Spirulina* also possesses other biological functions such as antiviral, antibacterial, antifungal, and antiparasite activities[(2).]

Microalgae, such as *Ochromonas* sp., *Prymnesium parvum*, a number of blue green algae produce toxins that may have potential pharmaceutical application. 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, of which many are based on their uses in traditional medicine[(3).] *S. platensis* produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. 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[(4).] Characteristics of plants that inhibit microorganisms have been investigated in laboratories since 1926. The past decade has witnessed a significant increase in the prevalence of resistance to antibacterial and antifungal agents. Resistance to antimicrobial agents has important implications for morbidity, mortality and health care costs in U.S. hospitals, as well as in the community. These developments and the associated increase in bacterial infections intensified the search for new, safer, and more efficacious agents to combat serious bacterial infections [(5).]

Materials and Method

Preparation of biomass and harvesting

A prepared flask containing 100 ml of Bg-11 culture media and transfer 25 ml of isolated algae then incubated for 14 days; transfer this culture growth to 500 ml of culture media and incubate again for 14 days, then transfer this culture growth to 1000 ml of Finally, the culture of growth is transferred to glass basins of 4L dimensions (50 cm long, 40 cm wide and 30 cm high) for biomass culture[(6)]. These pools were covered with a piece of gauze and the air was supplied with rubber. With bubble stone[(7)].

The 20-day biomass culture was harvested by centrifugation at 4000 rpm for 10 minutes[8]. The samples were washed with sterile water and dried in the oven with 38-40 ° C. These samples were then weighed and stored in the refrigerator[9].

Preparation of organic extract of the spirulina platensis

According to[10] with some modifications, were followed to prepare the crude extracts of algae as follows: One gram of spirulina platensis powder was extracted with 250 ml of 97% ethanol using a Soxhlet extraction device at 76 C for 3-4 hours until the solvent becomes insoluble the color. The raw extract was dried by rotary evaporator at 40 °

C. The residues (raw extracts) were collected and stored at -20 ° C until use again. The extract was weighed and the percentage of the extraction yield was calculated in terms of the primary algae material used in extraction.

The method of posting agar well:

Antimicrobial and fungal activities from the crude extracts of Spirulina Platens were tested using the Agar method for good propagation. Four different concentrations were prepared(10-20_30_40 µg/mL). Nutrient agar plates were fortified with 100 ml in a 24-hour broth culture of tested bacteria or 100 ml of Dextrose Sabouraud`s culture soup 5 days of tested fungus. Four wells (6 mm) were manufactured and filled with 100 ml extract. The dishes were incubated for 24 hours at 37 ° C for bacteria or for 3 days at 30 ° C for fungus. The diameter of the region was measured to discourage recorded results[11]. In addition, antimicrobial activity was compared with the standard.

Analysis by Gas Chromatography –Mass Spectrometry(GC-MS)

For GC-MS analysis, a high-temperature column (Inert cap 1MS; 30 m × 0.25 mm id × 0.25 µm film thickness) was purchased from Agilent Technologies (SHIMADZU—Japan), by employing a high-temperature column. Derivatization of each sample was eliminated. The injector and detector temperatures were set at 280 °C while the initial column temperature was set at 100 °C. A 5 µL sample volume was injected into the column and ran using split (1:10) mode After 1 min, and the oven temperature was raised to 225 °C at a ramp rate of 12.5 °C/min (hold time 4 min). The oven temperature was then raised to 300 °C at a ramp rate of 7.5 °C/min (hold time 5 min). The helium carrier gas was programmed to maintain a constant flow rate of 17.5 mL/min and the mass spectra were acquired and processed using both Agilent GC-Mass. Solution (SHIMADZU—Japan) and postrun software. The compounds were identified by comparison of their mass with NIST library search and authentic standards.

Statistical Analysis

The Statistical Analysis System- SAS (2012)[12] program was used to effect of difference factors in Inhibition Zone Diameter . Least Significant Difference-LSD test was used to significant compare between means in this study.

Result

The present study included bioactivity of crude extracts of *Spirulinaplatensis* on some pathogenic organisms isolated from different site as presented in the table (1).

The result showed the different sensitivity to the series of extraction on inhibition zone to an microorganism under study ,table (2) .

The zone of inhibition of *Spirulina platensis* extracts against bacteria was ranged between (0-13 mm at 10 mg/l and 8mm - 15mm, 8- 13mm and 7mm-15mm at 20mg/l, 40mg/l and 50mmg/l respectively.

The zone of inhibition of *Spirulina platensis* extracts against fungi was ranged between (0-9 mm at 10 mg/l and 7mm - 8mm, 0-7mm and 9mm-13mm at 20mg/l, 40mg/l and 50mmg/l respectively.

Table (1): pathogenic isolation site

No	Gram Positive Bacteria (+ ve)	Source of infection
1	Staphylococcus epidermidis	Skin
2	Staphylococcus aureus	Sputum (Chest infection)
No	Gram Negative Bacteria (- ve)	Source of infection
1	Klebsiellasp	Urine (UTI)
2	E.Coli	Urine
No	Fungi	Source of infection
1	Candida albicans	skin ulcers
2	Aspergillsniger	Ear

Table (2) Antimicrobial Activityof crude extractsof *Spirulinaplatensis* as presented by inhibition zone diameter (mm)

Microbes	Control	10 mg/l	20mg/l	30mg/l	40mg/l	LSD value
E. coli	0	6	10	8	7	2.07 *
Klebsiella	0	0	10	8	9	2.88 *
St. epidermis	0	8	8	11	8	3.19 *
St aureus	0	13	15	13	15	3.64 *
c. albicans	0	0	8	7	13	2.72 *
Asperniger	0	9	7	0	9	2.16 *
LSD value	-	2.69 *	3.44 *	2.89 *	2.96 *	---
* (P<0.05).						

The *St aureus* bacteria show more sensitivity and inhibition zone which reach a significant level (P<0.05) in all dilution were compare with other microorganisms , while *Asperniger* was less sensitive in compare with other organisms as shown figure (1) as well as the result show a significant differences in any dilution between all pathogenic organisms inhibition zones , furthermore there are found a significant difference between serial dilution against the same pathogen under probability levels (P<0.05).

Discussion

The antibacterial activity of algal compounds extracted from algae depends upon the type of solvent used for extraction

Spirulina has been studied because of its therapeutic properties and the presence of bioactive compounds[13] . The occurrence of antimicrobial compounds in plants was well documented and these compounds are known to possess antimicrobial activity in biological systems. But, the antioxidant characteristics of algae and cyanobacteria are less well documented, although decreased cholesterol levels have been reported in hypercholesteremic patients fed *Spirulina* and the antimicrobial activity of phycobiliproteins extracted from *Spirulina platensis* has also been demonstrated.

Many investigations mentioned that the methanol extracts of *Nostoc muscorum* revealed antibacterial activity on *Sclerotinia sclerotiorum* by[14]. Also the methanolic extract of a blue green alga has been investigated by[15] for *in vitro* antimicrobial activity against *Proteus vulgaris, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger, Aspergillus* flavus and *Rhizopus nigricans* using agar cup diffusion method. The antimicrobial activity of methanolic extract of *S. platensis* was also explained

by[16] due to the presence of γ- Linolenic acid and compound was also present in the methanol extract in the present study as observed by GC-MS analysis. Previous publications reported that the compounds such as 1-Octadecene, 1-Heptadeceane were found in both algae and plants show anticancer, antioxidant and antimicrobial activity[17,18]. Antimicrobially active lipids and active fatty acids are present in a high concentration in this alga. It was hypothesized by [19] that lipids kill microorganisms by leading to disruption of the cellular membrane as well as bacteria, fungi and yeasts because they can penetrate the extensive meshwork of peptidoglycan in the cell wall without visible changes and reach the bacterial membrane leading to its disintegration. Present investigations is contradictory with the results of other studies[20,21] may be due to the production of bioactive compounds related to the seasons, method, organic solvents used for extraction of bioactive compounds.

Conclusion

It is concluded from the study that extracts of algal strain used in the present investigation showed better antibacterial activity against the pathogens used, but further researches should be made to identify and purify natural product against antibacterial and antifungal. The enhanced antibacterial activity expressed in sequential extraction might be due to the fact that both hydrophobic and hydrophilic bioactive compounds were extracted. An improved knowledge of the composition, analysis, and properties of *S. platensis* with respect to antimicrobial compounds would assist in efforts for the pharmaceutical application of this cyanobacteria.

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