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ORIGINAL ARTICLE

Antimicrobial activity of *Spirulina platensis* extracts against certain pathogenic bacteria and fungi

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ABSTRACT
Ethanol, methanol and aqueous extracts of the algal plant Sprirulina plantensis were evaluated for their antimicrobial activity against four types of Gram positive bacteria namely Staphylococcus aureus, Streptococcus pneumoniae, Bacillus cereus and Enterococcus faecalis. Six types of Gram negative bacteria were also tested. They were Pseudomonas aureginosa, Proteus vulgaris, Salmonella typhi, Enterobacter cloacae, Klebsiella pneumoniae and Escherichia coli. Four species of Candida sp. were also bioassayed their response when the extracts were used. All of these algal extracts irrespective of their types inhibited the growth of all microbes to varying degrees. Methanol extract showed strong and superior antibacterial activity against all bacterial strains especially with regard to Gram positive bacteria (Staphylococcus aureus, Streptococcus pneumoniae, Bacillus cereus and Enterococcus faecalis) as compared to ethanol or aqueous extracts. Less or no activity was observed against Penicillium sp. (5±0.41) and Candida parapsilosis (08±0.44) in aqueous extract with 50mg/mL concentration. The minimum inhibitory concentration value of ethanol and methanol extract ranged between 5-100mg/mL for different strains tested. It was clearly noticed that Spirulina had a broad spectrum activity against bacteria and fungi in all the extracts tested. These findings support the traditional use of Spirulina as probiotic agent or in the treatment of different infections in area.
Key words: Antibacterial, Antifungal, Minimum inhibitory concentration, Spirulina platensis,

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INTRODUCTION

Spirulina (*Spirulina platensis*) - a blue green fresh water algae, the first single- celled organism responsible for converting high levels of carbon dioxide in atmosphere to life sustaining oxygen through photosynthesis. The United Nations stated *Spirulina* as the best for tomorrow [1]. No additional treatment such as cooking is needed for *Spirulina* to increase the available protein. This serves as a significant advantage for both simplicity in production and preservation of valuable vitamins and fatty acids in the processed materials [2]. This alga is well studied not only as an alternative food source but also gaining more attention in preparation of non toxic pharmaceuticals against tumor, anemia and malnutrition [3]. It is now one of the most researched super foods today because of its renowned flavor and powerful nutritional benefits. It is a rich source of protein, polysaccharide, lipids, essential amino and fatty acids, dietary minerals and vitamins. In addition to nutritional benefits, pharmacological activities such as antimicrobial, anticancer, metallo-protective, as well as immune-stimulant and antioxidant activities have been reported [4]. Therefore, these blue green algae have an international demand in healthy food, feed, therapeutic and diagnostic industries [5].

Spirulina is also known to nourish our body with numerous benefits including boosting immune support, providing essential amino acids, fights sugar cravings, support neurons, improves eyesight, maintains hemoglobin etc., [6,7,8,9]. From ancient days nature was providing the remedies for diseases and thousands of impressive modern drugs have been isolated from natural sources of which they have been used as traditional medicines. For the past decades or so, a significant increase in prevalence of multidrug resistant strains of human pathogens against antibiotics. This will lead complications such as morbidity, mortality and increase in health care costs. Spirulina has been reported to produce a large number of

bioactive compounds that are potentially important with medicinal properties. There are many reports on *S. platensis* showing anticancer, decreasing high blood cholesterol, stimulating immune system, reduce nephrotoxicity, reduce metal toxicity and reduces the harmful effects of radiation [10]. The presence of phycobiliprotein, phenoloic compounds, carotinoids, organic acids, sulphated polysaccharides spirulan, polyunsaturated fatty acids contributes for biological activities [3]. Spirulina extracts have also shown higher antioxidant properties compared to the other algal products [2]. Apart of nutritional aspects Spirulina has been reported for antimicrobial activities especially against plant pathogens [3]. A significant effort has been made to explore Spirulina for extracting pharmaceutical products and food additives. The plant Spirulina has been cultivated in small farms in Saudi Arabia by Arabian Agricultural service Company (ARASCO) [11]. The heavy metal concentration of the Spirulina grown locally was determined and within at expected limit [12].

Some preliminary studies on *Spirulina plantensis* have shown effective antimicrobial properties. From the available literature antimicrobial activities shown by Spirulina has to be investigated at a deep level to develop antibiotics against multidrug resistant pathogens. Thus, there is an urgent need to develop safe and biodegradable antimicrobial agent which could be promising with less side effects. Multidrug resistant pathogenic strains make a new way to detect plant-based antibiotic substances that can substitute synthetic drugs which are safer and having high efficiency towards serious bacterial infections. Therefore, the objective of the present work was to test *Spirulina platensis* collected from different parts for antimicrobial properties against different groups of microorganisms.

MATERIALS AND METHODS

Algal Material

Spirulina platensis plant materials used in the present study were obtained from different locations of Saudi Arabia. Identification and authentication were done by the Department of botany and microbiology, King Saud University, Riyadh and deposited in departmental herbarium for reference.

Bacterial test organism

The bacterial isolates used in the present study were ATCC culture and two wild strains obtained from the departmental culture collection. The bacterial strains tested were *Staphylococcus aureus (*ATCC 29213), *Streptococcus pneumoniae* (ATCC 49619), *Pseudomonas aureginosa (*ATCC27584), *Proteus vulgaris (*ATCC8427), *Enterobacter cloacae* (ATCC23355), *Klebsiella pneumoniae* (ATCC700603), *Enterococcus faecalis (*ATCC29212), *Escherichia coli* (ATCC25922), *Bacillus cereus* (wild strain), *Salmonella typhi* (wild strain). *All of the cultures were maintained in nutrient agar slants.*

Fungal test organism

The fungal cultures used were *Candida albicans* (ATCC10231), *Candida krusei (*ATCC14243*), Candida parapsilosis* (ATCC 22019) and *Candida tropicalis* (ATCC66029). Some strains of filamentous hyphal fungi were also used to detect the antifungal activity such as *Aspergillus flavus, Aspergillus niger, Penicillium sp. and Fusarium oxysporum f. sp. vasinfectum. All of the cultures were maintained in Sabouraud* Dextrose *Agar (SDA) and incubated at 25^{0}C.*

Preparation of *Spirulina platensis* Extract

Spirulina platensis materials were ground to a fine powder (50g) extracted successively with 200 ml of solvents (ethanol, methanol and water) in Soxhlet extractor. The extracts were filtered and the solvents were evaporated [13]. Extracts were stored in airtight glass bottles in refrigerator until use. For testing antimicrobial activity the extracts were mixed with Dimethyl sulfoxide (DMSO) and adjusted to a final concentration of 1, 10 and 100 mg/mL.

Bioassay for antimicrobial activity

Antibacterial activity was tested using well diffusion plate method with different concentration of extracts. The test microorganisms were grown in nutrient broth and incubated for 24h at 37^{0}C. The bacterial culture turbidity was standardized with McFarland standards to get a cell density of 10^8 CFU/mL. Mullen Hinton agar plates were prepared and the test culture was inoculated with the help of sterile cotton swab. Wells were made by using a cork borer and the different concentration of extracts were filled in each wells. Plates were sealed and incubated at 37^{0}C for 24 h. In another set of experiments for fungal bioassay sterile filter paper discs of 6 mm diameter were impregnated with 0.1mL/disc of extract which have been dissolved in dimethyl sulphoxide (DMSO) and placed in duplicates onto SDA plates seeded with 0.1 mL of fungal suspension. The plates were then incubated at 37^{0}C for 24-48 h for *Candida* and 25^{0}C for 5-7 days for other hyphal fungal strains [14, 15]. The zone of inhibition around each disc was measured and compared to that with synthetic antifungal antibiotics, such as Amphotericin B and Nystatin (100 µg/mL).

The Minimum Inhibitory Concentration (MIC)

Spirulina platensis extracts were tested for minimum inhibitory concentration (MIC) against bacterial isolates by broth macrodilution method [16]. Isolates were grown in nutrient broth and the extracts were dissolved in DMSO to get 50, 100 and 150 mg/mL concentrations. The diluted extracts (0.5mL) were then mixed with 0.5mL of nutrient broth and inoculated with tested bacterial strains. Control tube was left without extracts inoculation. The culture tubes were incubated at 37^0C for 18-24 h and the lowest concentration which does not show any growth were plated in nutrient agar plates. The plates which did not contain any bacterial colonies were determined as the minimum inhibitory concentration (MIC).

Antibiotic sensitivity test

In an attempt to compare efficacy of the extract with the commonly used commercial antibiotics, sensitivity test was done using standard disc diffusion method of Kirby – Bauer [17]. Antibiotics used were Tetracycline (30 µg), Erythromycin (15 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg) and Gentamicin (10 µg).

RESULTS AND DISCUSSION

The present study showed that *Spirulina platensis* has an antimicrobial activity. Table 1 showed that the extract was active against Gram positive bacteria. Ethanol and methanol extracts of *Spirulina platensis* at 10 and 100 mg/ml concentration showed different ranges of inhibition zones. When the concentration of the extract was increased to 100 mg/ml an increased inhibitory activity was observed. The highest inhibitory activity was observed with methanol extract towards *Streptococcus pneumoniae*, at a concentration of 100 mg/mL, whereas *Enterococcus faecalis* was inhibited by ethanol extract at 100mg/mL dose. *Bacillus cereus* and *Staphylococcus aureus* were also inhibited at high concentration of methanol and ethanol extracts respectively. The low concentration of both ethanol and methanol extracts showed less inhibitory activity against tested bacterial strains. However, aqueous extracts of *S. platensis* did not show any inhibitory activity towards all of the tested bacterial strains. The antibacterial activity of ethanol and methanol extracts against Gram negative bacteria was shown in Table 2. Comparatively the extracts were less active towards Gram negative bacteria tested when compared with Gram positive bacteria (Table 1). Ethanol extract at a concentration of 100 mg/ml showed inhibitory activity against *Salmonella typhi*.

Table 1. Antibacterial activity of *Spirulina platensis* **extracts against certain Gram positive bacteria***

Isolate	*Bacillus cereus*		*Staphylococcus aureus*		*Streptococcus pneumoniae*		*Enterococcus faecalis*	
Conc. (mg/ml)	10	100	10	100	10	100	10	100
Ethanol extract	15	18	11	17	14	19	18	21
Methanol Extract	12	19	15	20	17	21	17	20

*Data are means of three replicates

Table 2. Antibacterial activity of *Spirulina platensis* **extracts against certain Gram negative bacteria***

Isolate	*Pseudomonas aureginosa*		***Proteus vulgaris***		*Salmonella typhi*		***Enterobacter cloacae***		*Klebsiella pneumoniae*		*Escherichia coli*	
Conc. (mg/ml)	10	100	10	100	10	100	10	100	10	100	10	100
Ethanol extract	9	15	8	13	16	20	12	16	16	19	13	17
Methanol Extract	10	17	15	18	14	19	14	19	11	17	11	15

*Data are means of three replicates

The MICs of the Spirulina platensis extracts were studied (Table 3). Among Gram positive bacteria studied *Streptococcus pneumoniae* and *Enterococcus faecalis* that were completely inhibited by 50mg/mL of the ethanol extract. Whereas, other bacterial strains such as *Staphylococcus aureus* and *Bacillus cereus* were inhibited by 100mg/mL concentration of ethanol extract. *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella typhi* were inhibited by 50mg/mL of the ethanol extract among the Gram negative bacteria (Table 2). However, *Pseudomonas aureginosa* and *Enterobacter cloacae* might require a higher concentration of extracts for complete inhibition. There are numerous reports on *Spirulina* platensis

solvent extracts which inhibited both Gram negative and Gram positive bacteria [16, 17]. Due to the increase in antibiotic resistance in bacteria, a search for antibacterial drug from cyanobacteria has increased worldwide [18].

Table 3. Minimum inhibitory concentration (MIC) values (mg/mL) of *Spirulina plantensis* for different bacterial isolates

Isolate	Ethanol	Methanol
*Staphylococcus aureus (*ATCC 29213),	100	50
Streptococcus pneumoniae (ATCC 49619),	50	50
*Pseudomonas aureginosa (*ATCC27584),	100	50
Proteus vulgaris *(*ATCC8427),	100	100
Enterobacter cloacae (ATCC23355),	100	50
Klebsiella pneumoniae (ATCC700603),	50	100
Enterococcus faecalis (ATCC29212),	50	50
Escherichia coli (ATCC25922),	50	50
Bacillus cereus (wild strain),	100	100
Salmonella typhi (wild strain).	50	50

Vinay kumar *et al.,* [19] has reported the antibacterial activity of algal extracts towards Gram negative bacteria *Salmonella typhimurium* and Gram positive bacteria i.e., *Staphylococcus aereus*. In case of methanol extract most of the bacterial strains were inhibited by 50mg/mL of the extracts. However 100mg/mL concentration was required for *Klebsiella pneumoniae* and *Proteus vulgaris* among Gram negative strains and *Bacillus cereus* among Gram positive strain for complete inhibition. There are many documented reports on *Spirulina platensis* methanol extracts that contain phenolic compounds which inhibits the growth of Gram positive bacteria [20]. Antibiotic sensitivity pattern of the test pathogens were studied with commercially available antibiotics and the results are shown in Table 4.

On the other hand, antifungal activities of aqueous and methanol extracts of *Spirulina platensis* against *Candida* spp. were tested and the result is shown in Table 5. In the present study 150 mg/mL of extract inhibited *Candida tropicalis*, whereas, the least inhibitory activity was observed towards *Candida parapsilosis*. Methanol extract showed high inhibitory activity towards all the *Candida* isolates when compared to aqueous extracts. *Candida albicans* was inhibited (24±0.11) by 150mg/mL which was almost equal (24±0.31) to the antifungal activity of Nystatin 100mg/mL. The inhibitory activity of extracts was directly proportional to the concentration of the extract used in the study. There was no inhibitory activity in the blind controls where DMSO was used in the wells (Table 6).

The aqueous extract inhibited the growth of *Fusarium oxysporum* followed by *Aspergillus flavus* and *Aspergillus niger*. Methanol extract was more active towards *Aspergillus flavus* when compared to that of the aqueous extract. The antifungal activity of extracts was compared with standard synthetic antifungal agents such as Nystatin and Amphotericin B. From the results it is clear that the antifungal activity of extracts is effective against pathogenic and filamentous fungi. Hexane and methanolic extracts of *Spirulina platensis* has been reported in previous studies for the inhibitory effect on *Aspergillus* spp. [21]. Our results are in close agreement with that of Kaushik and Chauhan [18] and Usharani *et al.,* [3] against pathogenic bacteria elsewhere. The activity of the alga might be due to the intracellular and extracellular metabolites that have antibacterial [22, 23] and antifungal activities [24].

The present study is based on crude extracts of different solvents, thus the compound or antibacterial or antifungal component in the extract is not identified. However, the results indicate a broad spectrum activity of *Spirulina platensis* extract against both bacteria and fungi. Further studies are needed to elucidate the structure of bioactive component responsible for antimicrobial activity, and this study will help to know the potential use of *Spirulina* in pharmaceutical industry as antimicrobial drug and in aqua feed as antimicrobial agent.

Table 4. Antibiotic sensitivity test pattern showed by the test isolates against commercial antibiotics.

Bacterial strains	Zone of inhibition in (mm)				
	Tetracycline (30 µg)	Erythromycin (15 µg)	Chloramphenicol (30 µg)	Ciprofloxacin (5 µg)	Gentamicin (10 µg)
*Staphylococcus aureus (*ATCC 29213)	18	14	20	15	10
Streptococcus pneumoniae (ATCC 49619),	15	18	26	17	9
*Pseudomonas aureginosa (*ATCC27584)	19	11	27	25	14
Proteus vulgaris *(*ATCC8427)	20	16	30	23	13
Enterobacter cloacae (ATCC23355)	25	14	27	24	15
Klebsiella pneumoniae (ATCC700603)	24	14	24	21	6
Enterococcus faecalis (ATCC29212)	23	17	28	26	15
Escherichia coli (ATCC25922)	20	20	22	20	17
Bacillus cereus (wild strain),	21	13	21	26	13
Salmonella typhi (wild strain)	18	20	23	10	11

Table 5: Antifungal Activity of *Spirulina platensis*, against different *Candida spp* *.

Extract type	Concentration (mg/mL)	Fungal isolates			
		Ca	*Ck*	*Cp*	*Ct*
Aqueous	50	09±0.12	09±0.23	08±0.44	10±0.70
	100	11±1.24	10±1.18	09±0.13	11±0.22
	150	16±0.13	16±1.87	14±1.24	18±1.99
Methanol	50	10±1.16	10±0.55	09±0.16	11±1.32
	100	15±0.32	11±0.12	10±1.31	12±1.14
	150	24±0.11	19±1.12	19±1.13	20±1.42
Nystatin	100ug/mL	24±0.31	21±0.23	21±0.33	21±0.62
Amphotericin B	100ug/mL	27±0.14	24±0.20	24±0.21	24±0.33

*Data presented as mean ±SD zone of inhibition(mm);inhibition zones are the means of three replicates; *Ca= Candida albicans*, *Ck=C. krusei*, *Cp=C. parapsilosis*, and *Ct=C. tropicalis*.

Table 6: Antifungal Activity of *Spirulina platensis*, against different fungal isolates*.

Extract type	Concentration (mg/mL)	Fungal isolates			
		Af	*An*	*P*	*Fo*
Aqueous	50	06±0.45	06±0.23	05±0.41	07±0.60
	100	08±1.11	07±1.08	06±0.12	08±0.12
	150	13±0.63	13±1.67	11±1.34	15±1.19
Methanol	50	07±1.08	07±0.35	06±0.46	08±1.12
	100	12±0.32	08±0.22	07±1.31	09±1.24
	150	21±0.11	16±1.15	16±1.43	17±1.22
Nystatin	100ug/mL	21±0.31	18±0.27	18±0.23	18±0.52
Amphotericin B	100ug/mL	24±0.12	21±0.21	21±0.31	21±0.53

*Data presented as mean ±SD zone of inhibition(mm);inhibition zones are the means of three replicates;*Af=Aspergillus flavus,An=Aspergillus niger,P = Penicillium sp.,Fo=Fusarium oxysporum*.

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