INNOVARE JOURNAL OF HEALTH SCIENCES

Vol 5, Issue 1, 2017

ISSN - 2347-5536

Research Article

ANTIBACTERIAL ACTIVITY OF VARIOUS SOLVENT EXTRACTS OF *SPIRULINA PLATENSIS* AGAINST HUMAN PATHOGENS

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Received: 24 September 2016, Revised and Accepted: 26 September 2016

ABSTRACT

Objective: *Spirulina* has been used as human food supplement over the last century. It contains high protein content, vitamins (A, D, E, K, and B complex vitamins), beta-carotene, manganese, zinc, copper, iron, selenium, and gamma linolenic acid. Numerous studies reported that *Spirulina* contains biological properties such as immunomodulation, antioxidant, anticancer, antimicrobial, and probiotic effects. In this study, phytochemical analysis and *in vitro* antibacterial activity of four different solvent extracts (methanol, acetone, chloroform, and hexane) of *Spirulina platensis* was examined.

Methods: Agar well diffusion technique and paper disc diffusion technique were used to analyze the antimicrobial against human bacterial pathogens, *viz., Escherichia coli*, *Salmonella typhi, Proteus mirabilis, Vibrio vulnificus,* and *Cellulomonas cellulans*.

Results: Results cleared that methanol and chloroform extracts of *S. platensis* showed maximum zone of inhibition against *E. coli* followed by *C. cellulans* and *P. mirabilis* at 100 µg concentration. Acetone extracts showed moderate biological activity against all tested organisms and least activity was recorded in hexane extracts at 10 µg concentrations. The presence of phytochemical compounds such as protein, carbohydrates, flavonoids, phenols, terpenoids, and steroids exhibits the relation to the antimicrobial activity of *S. platensis* against human pathogens.

Conclusion: Nutritional composition and antimicrobial activity analyses of this study help in increasing the importance of utilizing *S. platensis*.

Keywords: Antimicrobial activity, Phytochemical compounds, Human pathogens.

INTRODUCTION

Therapeutic properties of *Spirulina platensis* have the ability to prevent cancer, decrease blood cholesterol level, reduce nephrotoxicity of pharmaceuticals and provide protection against the harmful effect of radiation. In recent years, the antimicrobial activity of cyanobacteria has gained importance due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms [1]. Cyanobacteria found to be a rich source for various products for commercial and pharmaceutical interest. It also contains primary metabolites such as proteins, fatty acids, vitamins, pigments [2,3] and various secondary metabolites with different bioactivities such as antifungal, antiviral, antibiotic, and other properties [4,5]. As many other cyanobacteria species, *Spirulina* has the potential to produce a large number of antimicrobial substances, so they are considered as suitable organisms for exploitation as biocontrol agents of pathogenic bacteria and fungi [6].

Spirulina ingestion shows the preventive effect against the skeletal damage under exercise-induced oxidative stress. In count, *Spirulina* has been shown to have protective effects against oxidative stress induced by lead acetate in the liver and kidney of rats. Utilization of *S. platensis* also reduces hepatoxicity induced by cadmium in rats, and the effect is suggested to be mediated through its antioxidant properties. There have been very few studies on the antioxidant activity of *Spirulina* using cell-based assays. One of such studies used neutrophils to assess the antioxidant and anti-inflammatory activities of *S. platensis* preparations [7].

METHODS

Algal source

The blue-green algae, *S. platensis* was collected from Annamalai Nagar Lake, Annamalai Nagar and was maintained in Zarrouk's medium at 30°C. Samples were then sun dried and ground in pulverization to get coarse powder. Subsequently, the powdered samples stored in refrigerator.

Extraction of *S. platensis*

Sun dried *S. platensis* sample was extracted by soxheletation using four different solvents (methanol, chloroform, acetone, and hexane) separately at the ratio of 0.5:10 w/v. 50 g of dried *Spirulina* packed separately in Soxhlet apparatus containing 300 ml of solvents (methanol, chloroform, acetone, and hexane) and extracted continuously for 72 hrs. The resulting extracellular extracts of *S. platensis* from these different solvents were covered with aluminum foil and stored at 40°C until dried. Dried extracellular extracts were diluted in various concentration levels using dimethyl sulfoxide (DMSO), such as 2, 4, 6, 8, and 10 µg.

Collection of bacterial pathogens

In vitro antibacterial studies were carried out against four bacterial pathogens, *viz., Salmonella typhi*, *Escherichia coli*, *Proteus mirabilis*, *Vibrio vulnificus*, and *Cellulomonas cellulans* which were obtained from the RMMCH, Annamalai Nagar, Chidambaram, India. Bacterial cultures were emulsified in normal saline. Antibacterial activity by disc diffusion method [8] and agar well diffusion methods were observed.

Disc diffusion technique [8]

Sterile Mueller-Hinton agar plates were prepared and swabbed with test cultures. Sterile empty discs purchased from HiMedia were saturated with different solvent extracts at various concentrations of extracellular extracts were prepared and placed on Mueller-Hinton agar medium supplemented with the test organism. DMSO serves as negative control. The plates were incubated at 27°C and were observed for the zone of inhibition after 24 hrs.

Agar well diffusion technique

Agar well diffusion technique was determined by following the method of Shanmuga [9]. Different concentrations of methanol, chloroform,

acetone, and hexane extracts of *S. platensis* were tested against selected bacterial pathogens. They were cultivated in Mueller-Hinton agar media for bacteria. Using a sterile cork borer, wells were cut in MHA. *S. platensis* extracts of different solvent extracts in various concentrations were added in respective wells. Then incubated at 37°C for 24 hrs and after incubation, the zone of inhibition around the well was observed and tabulated.

Qualitative phytochemical screening

The homogenized biomass was then extracted in different solvents for an hour each in an all-glass filtration chamber, using 100 ml ethanol (90%; for flavonoids, alkaloids, phenols, tannins, and saponins), 100 ml methanol (80%; for aldehydes, sterols, and terpenes), and 100 ml n-hexane (polyunsaturated fatty acids), following different methods referenced in Table 1.

RESULTS AND DISCUSSION

Disc diffusion technique

In the case of the methanol extracts at 10 µg concentration showed more potent antibacterial activity than other extracts tested. Maximum zone of inhibition was observed against the *E. coli* of 26.0 mm, followed by *P. mirabilis* of 22.0 mm, and the least was recorded in *C. cellulans*. From the chloroform extracts, *V. vulnificus* showed maximum antimicrobial activity of 23.0 mm zone of inhibition followed by *P. mirabilis* of 19.0 mm and the least was recorded in *E. coli* of 16.0 mm. In acetone extracts, *P. mirabilis* showed highest antibacterial activity of 21.0 mm zone of inhibition, followed by *V. vulnificus* of 17.0 mm zone of inhibition, and the least was recorded in *E. coli* and *S. typhi* of 15.0 mm zone of inhibition.

In hexane extract, *V. vulnificus* and *C. cellulans* organisms showed maximum antibacterial activity of 17.0 mm zone of inhibition, followed by *S. typhi* of 14.0 mm zone of inhibition and the least was recorded in *E. coli* of 11.0 mm zone of inhibition. Ampicillin at 10 µg concentration was used as positive control zone of inhibition observed for the clinical pathogens were tabulated. *E. coli* showed 15 mm zone of inhibition, *S. typhi* and *C. cellulans* showed 12 mm zone of inhibition, *P. mirabilis* and *V. vulnificus* showed 14 mm zone of inhibition against the positive control ampicillin (Fig. 1).

Agar well diffusion technique

The methanol extract of *S. platensis* showed maximum antibacterial activity than other solvent extracts at 10 µg of 27.0 mm against *E. coli* and an adequate activity of 24.0 mm against *S. typhi*, 23.0 mm against *P. mirabilis*, 22.0 mm against *V. vulnificus*, 21.0 mm against, and 8mm against *C. cellulans.* Next to methanol, chloroform extracts at 10 µg of *S. platensis* showed maximum antibacterial activity of 24.0 mm against *E. coli,* 19.0 mm against *S. typhi*, 21.0 mm against *P. mirabilis*, 20.0 mm against *V. vulnificus*, 19.0 mm against, and 8 mm against *C. cellulans.*

Following chloroform, acetone extracts showed maximum antibacterial activity at 10 µg against *E. coli* and *S. typhi* of 18.0 mm, 17.0 mm against *P. mirabilis,* and 16.0 mm against *C. cellulans.* Least antibacterial activity was observed in hexane extracts of *S. platensis* at 10 µg. Inhibition zone of 16.0 mm against *E. coli*, 15.0 mm against *S. typhi* and *V. vulnificus*, and 14.0 mm against *P. mirabilis* and *C. cellulans*. Ampicillin at 10 µg concentration was used as positive control and the zone of inhibition observed for the clinical pathogens were tabulated. *E. coli* showed 15 mm zone of inhibition, *S. typhi* and *C. cellulans* showed 12 mm zone of inhibition, *P. mirabilis* and *V. vulnificus* showed 14 mm zone of inhibition against the positive control ampicillin (Fig. 2).

Table 1: Different types of phytochemical compounds were screened using the following assays

Phytochemicals	Methods	References
Alkaloids	Mayer's and Wagner's reagent	Scholz and Liebezeit [10]
Flavonoids	Shinoda test	Scholz and Liebezeit [10]
Phenols	Folin–Ciocalteau	LeBlanc *et al*. [11]
Tannins	Gelatin – Salt Block test	Scholz and Liebezeit [10]
Saponins	Frothing test	Scholz and Liebezeit [10]
Aldehydes	Schiff's and Fehling's test	Scholz and Liebezeit [10]
Sterols	Liebermann–Burchard test	Stadtman [12]
Terpenes	Salkowski test	Harborne [13]

Phytochemicals in *S. platensis*

The phytochemical compounds in *S. platensis* were screened using various methods. The presence of these phytochemicals effectively increases the antibiotic efficacy of *S. platensis*. The results showed the presence of high level of protein (68.42 µg/g), carbohydrates (10.73 µg/g), ash (09.15 µg/g) and low lipid content (2.05 µg/g), phenols, triterpenoids, steroids, flavonoids, and saponins.

DISCUSSION

Extracts from *S. platensis* inhibited the growth of *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *S. typhi,* and *Klebsiella pneumoniae*. They used hexane, ethyl acetate, dichloromethane, and methanol to obtain the phenolic extracts, and the methanolic extracts exhibited maximum activity [14]. Parisi *et al.* [15] also found high antimicrobial activity of phenolic compounds in methanol extracts from *S. platensis* against *S. aureus.* The antibacterial effects of methanol extracts *S. platensis* against *S. aureus* and *Salmonella typhimurium* using both agar well diffusion method and paper disc diffusion method at concentrations from 250 up to 7000 ppm and observed all the tested bacteria showed inhibition [16].

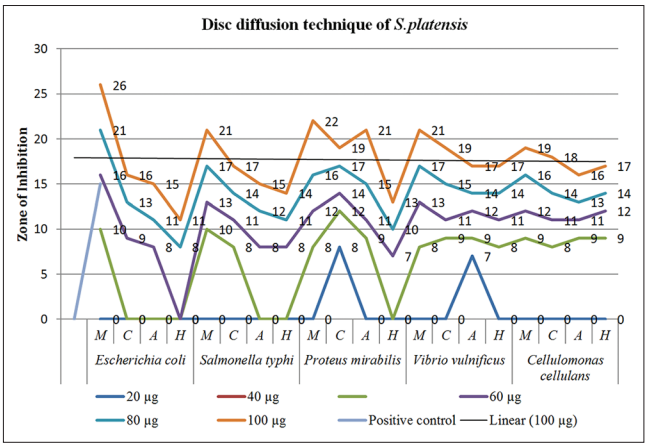

Fig. 1: Disc diffusion technique at various concentrations. M: Methanol extract; C: Chloroform extracts; A: Acetone extracts; H: Hexane extracts, Positive control: Ampicillin 10 µg

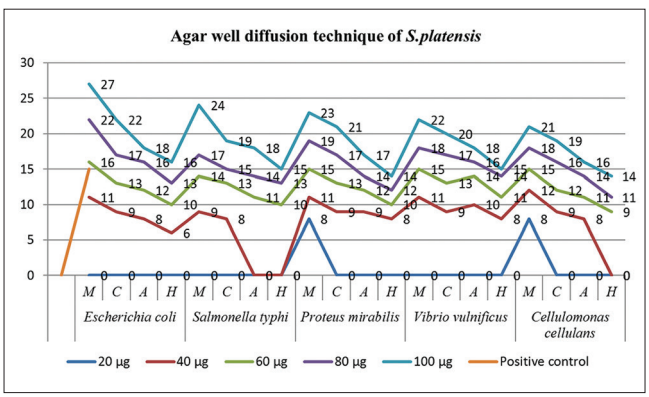

Fig. 2: Agar well diffusion method. M: Methanol extract; C: Chloroform extracts; A: Acetone extracts; H: Hexane extracts, positive control: Ampicillin 10 µg

The methanolic extract of a blue-green alga has been investigated [17] for *in vitro* antimicrobial activity against *Proteus vulgaris*, *Bacillus cereus*, *E. coli*, *Pseudomonas aeruginosa, Aspergillus niger*, *Aspergillus flavus,* and *Rhizopus nigricans* using agar well diffusion method with best activity.

Agustini *et al.* [18] analyzed the proximate composition of phytochemicals and reported that dried samples of *Spirulina* contain high protein and ash content when compared with fresh samples. Likewise in our study, also these phytochemicals were screened at high rates in dried form. The presence of these compounds stimulates the immune system against pathogenic bacteria.

CONCLUSION

Results of this analysis show antimicrobial properties of *S. platensis* against pathogenic bacteria. Improved analysis of bioactive compounds with respect to antimicrobial activity would assist in efforts for the pharmaceutical application of *S. platensis*.

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