ORIGINAL ARTICLE

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In vitro antibacterial activity of *Spirulina platensis* extracts against clinical isolates of *Salmonella enterica* serovars Typhi and Paratyphi (SUBP03)

Sunjukta Ahsan[1*], Md. Shamsul Arefin[1], John Litol Munshi[2], Mst. Nadira Begum[2], Maisha Maliha[1], Sahida Rahman[1], Anindita Bhowmik[3] and Md. Shahidul Kabir[4]

[1]Department of Microbiology, University of Dhaka, Dhaka 1000; [2]Bangladesh Council of Scientific and Industrial Research, Dr. Qudrat-i-Khuda Road, Dhanmondi, Dhaka-1205; [3]Department of Microbiology, Jagannath University, Dhaka, Bangladesh, [4]Department of Microbiology, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217

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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. In the present investigation, the antimicrobial activity of *S. platensis* against clinical isolates of *Salmonella enterica* serovar Typhi (n=17) and Paratyphi (n=3) was investigated. Ethanolic and chloroform extracts of *S. platensis* (40 mg/mL) were investigated for antimicrobial effect. Variable zones of inhibition were observed for the former extract whereas the latter did not show any inhibitory effect on growth of the test organism. Diameters of the zones of inhibition were 9.7-14.0 mm for *S*. Paratyphi and 9.5-16.0 mm for *S*. Typhi. The presence of zones of inhibition at the concentration of 40 mg/ml of the extract used is promising towards developing a natural remedy against infections with *S. enterica* serovars Typhi and Paratyphi.

Key words: *Spirulina platensis*; Algal extract; Antibacterial activity

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 (1). 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 (2-5). The cyanobacteria (Blue Green Algae) are able to produce biologically active compounds (6). 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 (7).

The aim of the present work was to study the antimicrobial activity of cell extracts of various strains of *Spirulina platensis* against clinical isolates of *Salmonella enterica* serovar Typhi (*S*. Typhi) and *S. enterica* serovar Paratyphi. To our knowledge, this is the first report of the antimicrobial activity of *S. platensis* extract on clinical isolates of *S*. Typhi and *S*. Paratyphi.

MATERIALS AND METHODS

Bacteria. A total of 17 *S. enterica* serovar Typhi and 3 Paratyphi were used in this study. The bacteria used in antibacterial assays were provided by a local hospital which were isolated from patients suffering from typhoid and paratyphoid fever. *Salmonella enterica* serovar Typhimurium ATCC 10428 was used as control.

Preparation of extracts. The blue-green alga, *S. platensis* was collected from Bangladesh Council of Scientific and Industrial Research (BCSIR) as dried powder. It was kept at room temperature. Samples were then shade dried and pressed to get coarse powder. Subsequently, the powdered samples were stored in the refrigerator. *Spirulina* powder was resuspended in ethanol and chloform separately to prepare solutions with strengths of 40 mg/mL. From each of this preparation, 20 µl was used to soak each filter paper disc.

Preparation of pure culture. A loopful of each of the microorganisms was suspended in about 10 ml of physiological saline (0.85% NaCl) in a roux bottle. It was streaked on Xylose lysine deoxycholate (XLD) agar medium and incubated at 37 °C for 24h. Discrete colonies were selected on the basis of their colony morphology and biochemical profile. Standard biochemical tests: Kligler Iron Agar (KIA), Motility Indole Urea (MIU), citrate agar were used for identification of *Salmonella* spp.

[*]Corresponding Author: Mailing address**.** Dr. Sunjukta Ahsan, Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh. Email: Sunjukta@du.ac.bd.

Paper-disc diffusion method. Antibacterial activity was observed by disc diffusion method. Whatmann Filter paper discs, saturated with 20µl of ethanolic extract were aseptically placed on different culture medium supplemented with the test and control organisms. Discs were fed with solvent alone to serve as control. The plates were incubated at 37 °C and observed for zone of inhibition after overnight incubation. A control assay was conducted using Ciprofloxacin discs (5 µg).

RESULTS

Pure culture of *S. enterica* serovar Typhi and Paratyphi were subcultured on XLD agar and following colony morphologies were observed (Table 1).

TABLE 1. Load of various microorganisms in the pear samples

Microorganism	Culture media	Colony characteristics
Salmonella Typhi	Xylose lysine deoxycholate (XLD) agar	Black colonies
Salmonella Paratyphi	Xylose lysine deoxycholate (XLD) agar	Black colonies

Biochemical properties of the test bacteria. All the clinical isolates of *Salmonella* Typhi and Paratyphi were re-confirmed by a number of biochemical tests following primary confirmation at the hospital from where they were collected. In KIA, all isolates produced blackening of butt with evidence of gas formation and red color of slant. They were motile and produced indole. They lacked urease activity and were unable to use citrate as sole carbon source.

Antimicrobial activity of the ethanol extract of *Spirulina platensis.* Variable antimicrobial activity was exhibited by the ethanolic extract of *Spirulina platensis* against the test organisms. Diameters of the zone of inhibition were 9.7-14 mm for *S.* Paratyphi and 9.5-16 mm for *S.* Typhi. With reference to ciprofloxacin, no conclusion could be drawn between sensitivity to ciprofloxacin and zone diameters obtained with ethanolic extract of *S. platensis* i.e. isolates that were resistant to ciprofloxacin did not always produce small zone sizes with *Spirulina* extract and *vice versa*. Figure 1 depicts the zone sizes produced by the clinical isolates of *S.* Typhi and *S.* Paratyphi when treated with Ciprofloxacin (5 µg) and ethanolic extract of *S. platensis*.

Antimicrobial activity of the chloroform extract of *Spirulina platensis.* The chloroform extract of *S. platensis* was found to have no effect on the test bacteria.

DISCUSSION

Spirulina platensis is one of the most important micro-alga showed antimicrobial activity against many pathogenic bacteria and fungi (7). *Spirulina* is one of the several algal genera that have attracted special attention due to their importance as human foodstuff and there *in vitro* or *in vivo* functional properties. It has been extensively cultivated to obtain a protein rich material of

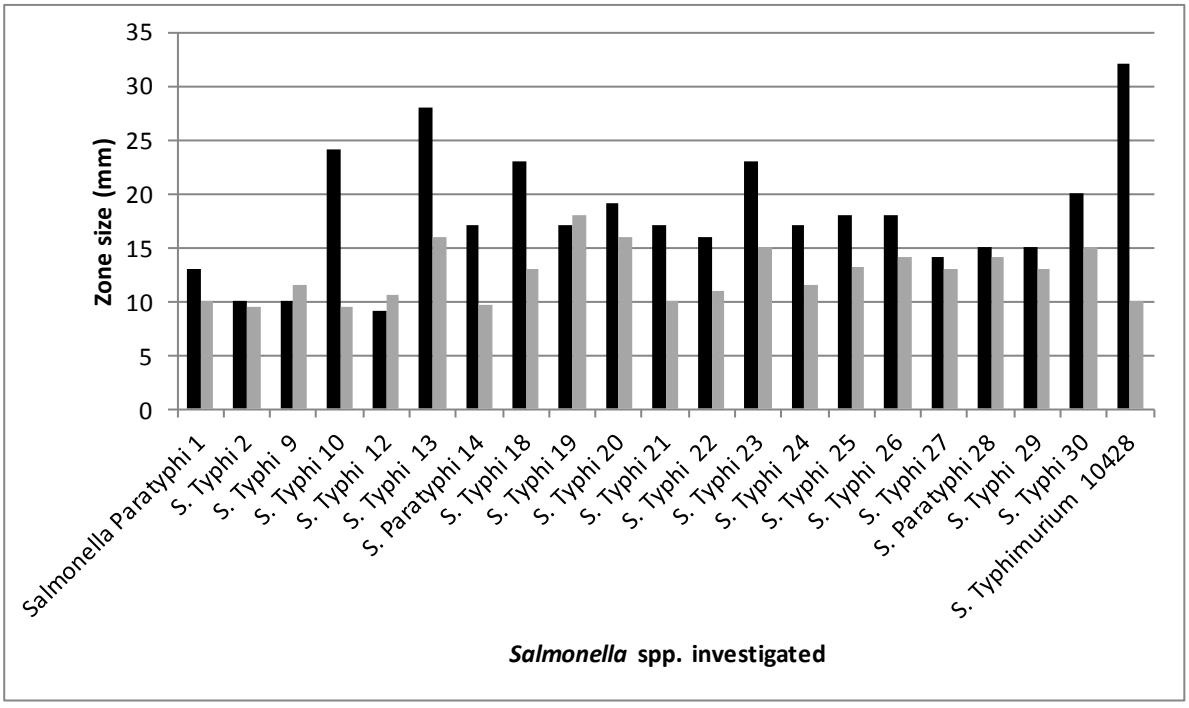

FIG. 1. Zone diameters obtained with treatment of the clinical isolates of *S.* Typhi and *S.* Paratyphi with ciprofloxacin (5 µg) and 20 µl crude extract of *Spirulina platensis*. The black bars indicate ciprofloxacin treated samples and the grey bar represent zone diameters obtained when treated with ethanolic extract of *S. platensis*.

nutritional or industrial use (blue pigment).

It has been recommended by medicinal experts for better health (7). In the present investigation, the antimicrobial activity of the ethanolic extract of *Spirulina platensis* was tested against clinical isolates of *S*. Typhi and *S*. Paratyphi. The extract was found to have variable effect on the test isolates with some isolates showing greater sensitivity to the crude ethanolic extract than others. In contrast the chloroform extract showed no antimicrobial activity against the test isolates. In earlier studies, the methanol extract was found to be more effective against *Staphylococcus aureus* and *Salmonella typhimurium* than extracts with other solvents (7). Extracts with methanol, dichloromethane, petroleum ether and ethylacetate were found to have antibacterial and antifungal (against *C. albicans*) activity against four Gram positive and six Gram negative bacteria (8). In another study (9) the ethanol extract of *S. platensis* was found to be antibacterial against *S. aureus* and *E. coli* and antifungal against *A. niger* and *C. albicans*.

In another study, extracts with acetone, ethanol and diethyl ether were found to exert antibacterial activity against reference strains of *Klebsiella pneumoniae*, *Enterobacter* spp., *E. coli* and *S. typhi* (10). Similar findings were reported earlier (11) using hexane, ethyl acetate, dichloromethane and methanolic extracts on *E. coli*, *S. typhi*, *P. aeruginosa* and *K. pneumoniae*. Both antibacterial and antifungal activities were found in the extracts with diethyl ether, acetone, ethanol and methanol against. These extracts were active against the bacteria *Bacillus subtilis*, *Pseudomonas aeruginosa*, *E. coli*, and *S. aureus* and against the fungi *A. flavus*, *F. moniliforme*, *C. albicans* (12). Mala et al., (13) have reported on the antibacterial activity of methanol, ethanol, propanol and aqueous extracts of *S. platensis* against *K. pneumoniae*, *Proteus vulgaris*, *E. coli* and *S. aureus*. *S. aureus*, *E. coli* and *P. aeruginosa* were also found to be inhibited by the methanolic extract of *S. platensis* (14). Other reports of the antimicrobial activity of *S. platensis* include effect against *S. typhi* (control strain), *Shigella frexneri*, *E. coli* and *C. albicans* by ethanolic extract (15), by methanolic extract against against *A. flavus* (16), by hexane, ethyl acetate, ethanol, butanol, acetone, methanol, chloroform extracts against *S. epidermis*, *A. liquefaciens* and *C. glabrata* (17), by ethanol extract against *S. aureus*, *E. coli*, *P. aeruginosa*, *Klebsiella* sp. (18), by methanol extract against *B. subtilis*, *E. coli*, *P. vulgaris* and *C. albicans* (19), by ethanol and aqueous extracts against the bacteria *Vibrio alginolyticus*, *Pseudomonas fluorescens*, *P. aeruginosa*, *Aeromonas hydrophila* and *A. salmonicida* and the fungi *Aspergillus niger*, *Penicillium javanicum*, *Candida albicans* and *Trichoderma viride* (20), by methanol, ethanol and aqueous extracts against *P. fluorescence*, *P. aeruginosa*, *P. putida*, *V. alginolyticus*, *Vibrio flavalis*, a *V. fisheri* and *E. coli* (21).

The present study differs from previous studies in that it was conducted on clinical isolates of *S*. Typhi and *S*. Paratyphi. The sensitivity of the isolates was compared to ciprofloxacin as a control assay. There was no correlation between the sensitivity of the isolates to the antibiotic and the zone diameters obtained with the ethanolic extract of *S. platensis*. Moreover, the sizes of the zone of inhibition were not comparable to that obtained for ciprofloxacin. At 40 mg/ml concentration, the ethanolic extract generated zones of inhibition significantly smaller than that obtained with ciprofloxacin. Future studies need to be carried out with increased concentration of the extract to obtain increased zones of inhibition. Other solvents like methanol, essential oil, water may be used for extraction of chemical ingredients imparting antimicrobial activity from *S. platensis*. Active ingredients may be separated and tested for designing new generation antibiotics. However, the finding from the present investigation is highly promising in view of the fact that the ethanolic extract was successful to inhibit the growth of the clinical isolates of the tested bacteria. Further research carried out along this line may prove to be fruitful in bringing out a natural solution against infection with *S*. Typhi and *S*. Paratyphi.

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