Antibacterial Activity of Freshwater Microalga *Scenedesmus* sp. on Foodborne Pathogens *Staphylococcusaureus* and *Salmonella* sp.

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Abstract

Microalgae possess promising bioactive compounds with a wide range of activities. In this study, crude pigment extract of *Scenedesmus* sp. a freshwater microalga was screened against two known foodborne pathogens *Staphylococcus aureus* (ATCC 25923) and *Salmonella* sp. (ATCC 14028) to evaluate its antimicrobial properties. The crude pigment of the microalga was extracted with 90 % acetone whereas antibacterial screening was done by agar well diffusion method. In addition, the MIC of crude pigment was determined following the macrobroth dilution method. Concentrations ranging from 0.35mg/ml – 3.48 mg/ml demonstrated highest ($P < 0.05$) inhibitory activity against *Staphylococcus aureus* (ATCC 25923). The MIC was achieved at 0.08 mg/ ml. On the other hand, there was no inhibitory activity at any concentration of crude extract against *Salmonella* sp. (ATCC 14028). Results of this study suggest that the inhibitory activity may be limited to gram positive foodborne pathogen *Staphylococcus aureus.*

Keywords: antibacterial activity; microalga; foodborne pathogens

1. INTRODUCTION

Globally, the incidence of foodborne diseases has a significant level of morbidity as it causes many people to fall ill and even die as a result of ingesting certain microorganisms or their toxins through unsafe foods. A wide variety of etiological agents such as bacteria, viruses and parasites have been associated with food poisoning [1]. *Staphylococcus aureus* (*S.aureus*) has been reported to be a significant source of foodborne illnesses worldwide due to its ability to produce several heat resistant enterotoxins [2, 3].

These days, the safety aspects of chemical or synthetic food additives are being increasingly questioned thereby leading to a demand for naturally occurring food preservatives worldwide. Biologically active substances from several microalgae species are been extracted both as cell extracts and extracellular products which have been found to possess antimicrobial activities and these include carotenoids, lipids, polysaccharides, terpenoids and chlorophyll [4,5]. These activities can be antibacterial, antifungal, antialgal and antiprotozoal. Although for several of these activities, the constituents are not yet known as their structures and identities are yet to be discovered [6]. The unique diversity of microalgae is responsible for its capability to be a valuable source of compounds with biotechnological potentials [7, 8]. These valuable compounds are natural substances that attract the attention of both scientists and industrialists due to their use in the development of biotechnology [9,10]. Novel compounds with antibacterial, antiviral, and antifungal properties have been intensively investigated in microalgae over the last years [e.g 5, 11, 12, 13, 14 & 15]. Likewise *Scenedesmus* sp. have been used in many biotechnological applications due to their high nutritional content and bioactivities [16, 17].

This study was carried out to evaluate the antibacterial activities of pigment extract from freshwater microalga *Scenedesmus* sp., against two known foodborne pathogens *Staphylococcus aureus* (*S.aureus*) and *Salmonella* sp. In addition, the minimum inhibitory concentration of the extract was also determined.

2. MATERIALS AND METHOD

2.1 Pigment Extraction

Prior to extraction, the biomass of the microalga *Scenedesmus* sp. was concentrated by centrifugation. The concentrated biomass of was placed in a tissue grinder and was covered with about 2-3 ml 90 % acetone. It was then macerated for 1 min. The slurry was transferred to a screw cap centrifuge tube, and the tissue grinder was rinsed with a few ml of 90 % acetone which was added to the slurry. The volume was adjusted to 10 ml with 90 % acetone and steeped for at least 2 h at 4 ℃ in the dark after which it was centrifuged [18].

2.2 Preparation of Bacteria Suspension

Prior to preparation of bacterial suspension, *Staphylococcusaureus* (ATCC 25923) and *Salmonella* sp. (ATCC 14028) were cultured on mannitol salt agar (MSA) and eosin methylene blue agar (EMB) for 24 hrs. Thereafter, using the direct colony suspension, 3-5 well isolated colonies of *S.aureus* and *Salmonella* sp. were picked from MSA and EMB plates and transferred into a tube of TSB with a sterilized wire loop. The bacteria was mixed well in the broth to dissolve the colonies and the turbidity of the bacterial suspension was standardized to match that of a 0.5 Mc Farland standard which corresponds to approximately 1.5×10^8 CFU/ml. The adjusted innocula was used within 15 minutes of preparation [19].

2.3 Antibacterial Activity Testing Against *S.aureus* and *Salmonella* sp.

Ten different concentrations of crude pigment extract corresponding to ten treatments in triplicates were prepared. In addition, a positive (tetracycline) and negative (solvent) controls were also prepared. The concentrations of individual volume of crude pigment extracts from 10µl – 100 µl are as follows (0.35 mg/ml, 0.70 mg/ml, 1.04 mg/ml, 1.39 mg/ml, 1.74 mg/ml, 2.09 mg/ml, 2.44 mg/ml, 2.78 mg/ml, 3.13 mg/ml & 3.48 mg/ml).
A sterile cork borer was used to punch 4 mm holes (wells) into a prepared Mueller Hinton agar (MH). A sterile cotton swab was dipped into the previously prepared bacterial suspension and streaked over to the surface of the MH agar for even distribution. After which different volumes of crude pigment extracts were loaded into the wells and allowed to stand until they were well absorbed. The plates were then inverted and incubated at 37℃ for 24 hrs. The inhibition zones were measured after incubation.

2.4 Preparation of Bacterial Suspension for Minimum Inhibitory Concentration (MIC) testing

A direct broth suspension of *S.aureus* colonies selected from a 24 hour agar plate was made in a 5ml MH broth. This was adjusted to achieve a turbidity that is equivalent to 0.5 McFarland turbidity standard. It resulted in a suspension containing approximately 1×10^8 CFU/ml of bacteria. This inoculum was diluted in a 1:2 dilution to bring the final inoculum concentration to 5×10^5 CFU/ml of bacteria.

2.5. MIC of Crude Pigment Extract

Determination of MIC values follows [20]. Two sets of eight (8) sterile capped test tubes containing 2 ml final volume of MH broth plus crude extract of concentration ranging from (173 mg/ml, 57.67 mg/ml, 19.22 mg/ml, 6.4 mg/ml, 2.14 mg/ml, 0.71 mg/ml, 0.24 mg/ml & 0.08mg/ml) were prepared prior to the testing. Two sets of sterile tubes l containing only the MH broth at final volume were used as control. After which 1ml of the previously prepared bacterial suspension were added to each of the tubes. All the tubes were then incubated at 35 ℃ for 24 hrs. After incubation, each tube was examined for bacterial growth.

3. RESULTS

The crude pigment extract of *Scenedesmus* sp. showed significant antibacterial activity against foodborne pathogen *S. aureus* (ATCC 25923). The highest zone of inhibition was observed at concentrations 2.78 mg/ml, 3.13mg/ml, 3.48mg/ml with mean zones of inhibition at 12.67±0.67 mm, 14.00±2.00 mm and 17.33±1.67 mm, respectively (Table 1). Whereas the lowest zone of inhibition was observed in 0.35 mg/ml with mean zone of inhibition at 4.67±0.33 mm (Table 1).

Table 1: Antibacterial activity of *Scenedesmus* sp. pigment extracts evaluated using agar well diffusion assay on *S.aureus* (mm).

Volume of crude pigment extract (µl)	Concentration of pigment in individual volume of extract (mg/ml)	Zones of bacterial inhibition (mm) Mean±SE
10	0.35	4.67±0.33**
20	0.70	5.67± 0.33
30	1.04	6.00± 0.00
40	1.39	7.33± 0.33
50	1.74	8.67±0.67
60	2.09	9.00± 1.00
70	2.44	10.33± 0.88*
80	2.78	12.67± 0.67*
90	3.13	14.00± 2.00*
100	3.48	17.33± 1.67*
10	0.001tetracycline (positive control)	8.67±0.67
90% acetone (negative control)	-	0.00.±0.00**

*Values in column with asterisks is significant at P< 0.05 level, **Values in column with asteriksis significant at P< 0.01 level

The minimum inhibitory concentration value was obtained at the concentration 0.08±0.00 mg/ml. Thus antibacterial activity of Scenedesmus sp. pigment extract against S.aureus was observed in the plates with increasing concentration of the extract (Figure 1).

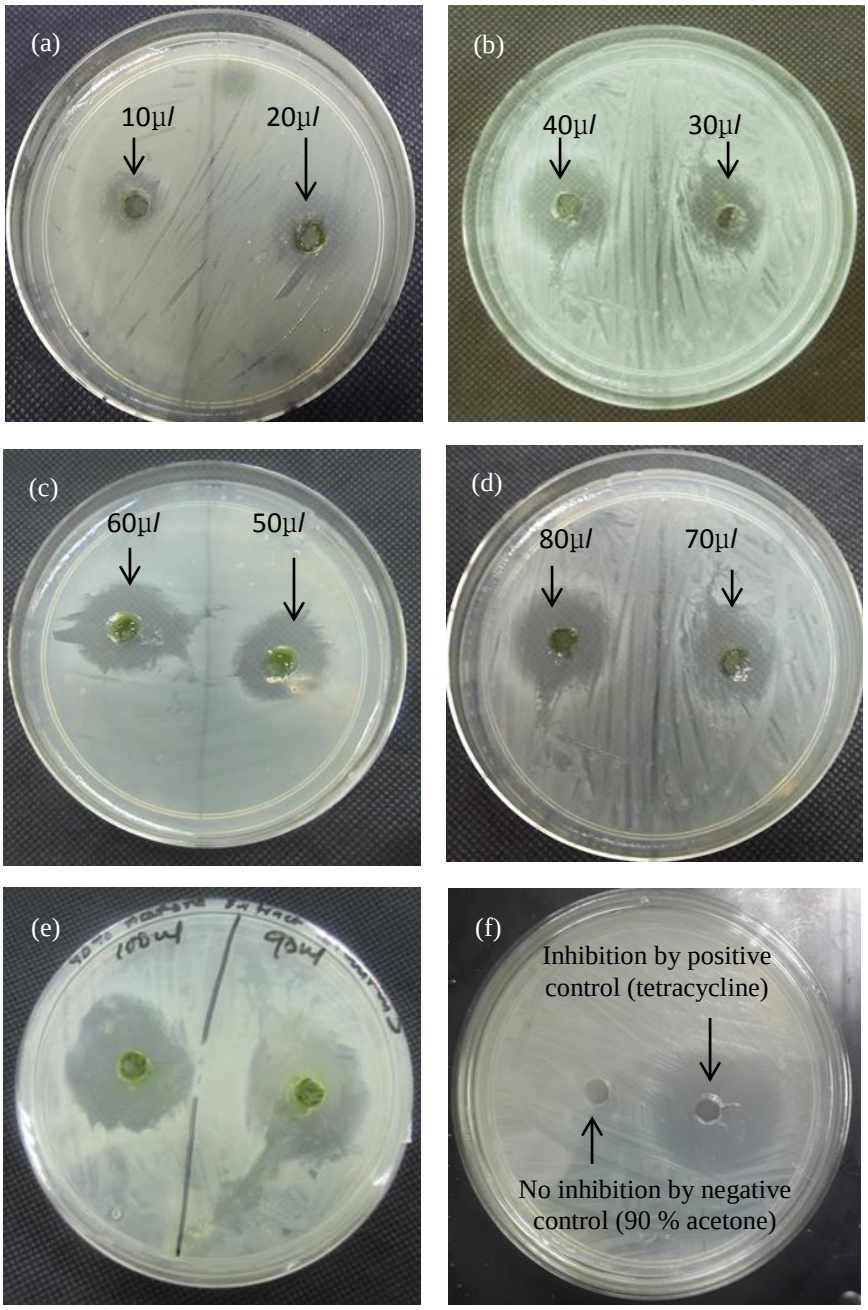

Figure 1 (a – f): Inhibitory activities of *Scenedesmus* sp. pigment extract on *S. aureus* and *Salmonella sp.* observed on Mueller Hinton agar plates after 24 hours incubation. (a) Zones of inhibition by 10 µl and 20 µl pigment extract on *S.aureus*; (b) Zones of inhibition by 30 µl and 40 µl pigment extract on *S.aureus*; (c) Zones of inhibition by 50 µl and 60 µl pigment extract on *S.aureus*; (d) Zones of inhibition by 70 µl and 80 µl pigment extract on *S.aureus*; (e) Zones of inhibition by 90 µl and 100 µl pigment extract on *S.aureus* (f) Positive and negative controls

Meanwhile, there was no inhibitory activity against *Salmonella* sp. (ATCC 14028) in any concentration of crude pigment extract as well as positive control (Table 2). Thus antibacterial activity of *Scenedesmus* sp. pigment extract had no effect on the Salmonella sp. (Figure 2).

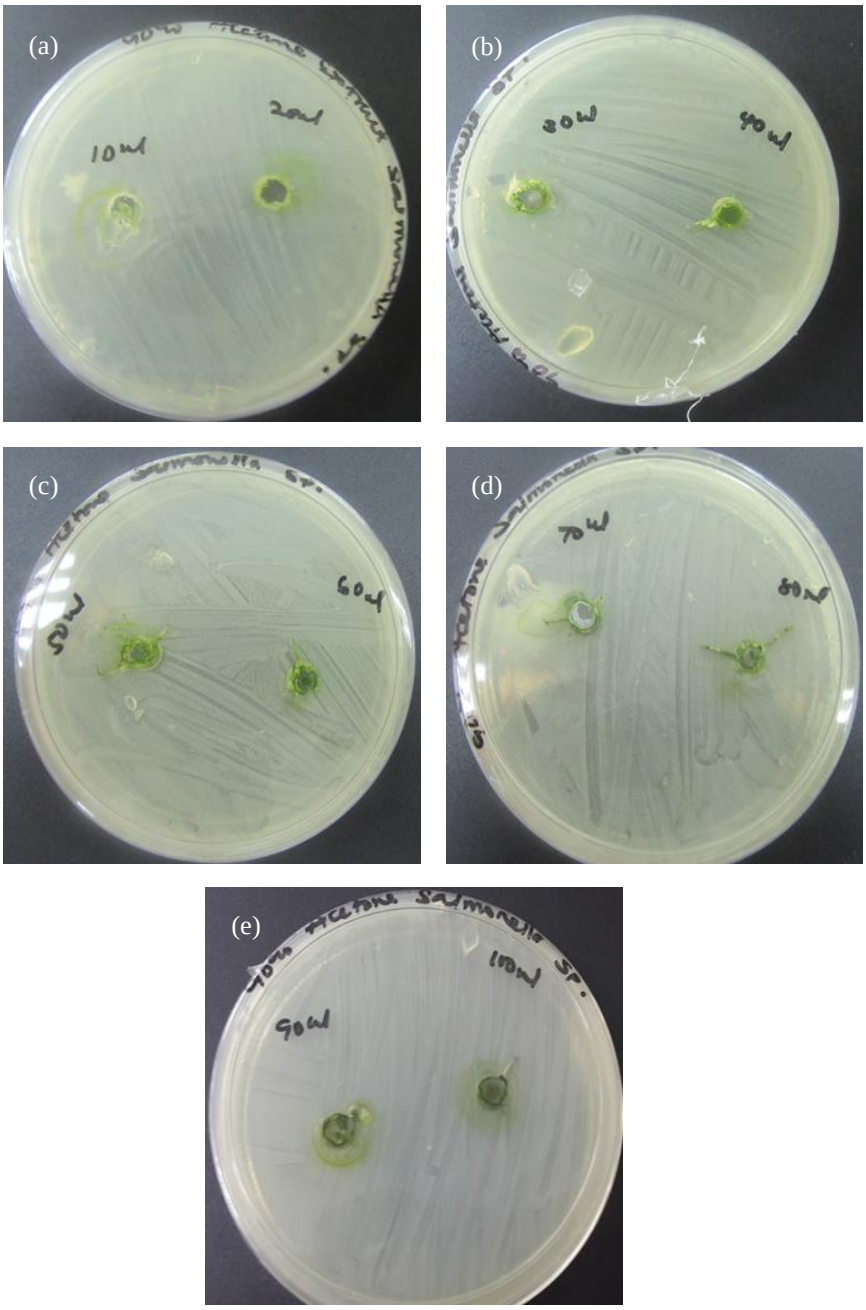

Figure 2 (a – e): (a) No inhibitory action by 10 µl and 20 µl pigment extract on *Salmonella* sp.; (b) No inhibitory action by 30 µl and 40 µl pigment extract on *Salmonella* sp.; (c) No inhibitory action by 50 µl and 60 µl pigment extract on *Salmonella* sp.; (d) No inhibitory action by 70 µl and 80 µl pigment extract on *Salmonella* sp.; (e) No inhibitory action by 90 µl and 100 µl pigment extract on *Salmonella* sp.

Table 2: Antibacterial activity of *Scenedesmus* sp. pigment extracts evaluated using agar well diffusion assay on *Salmonella* (mm).

Volume of crude pigment extract (µl)	Concentration of pigment in individual volume of extract (mg/ml)	Zones of bacterial inhibition (mm) Mean±SE
10	0.35	0.00±0.00
20	0.70	0.00±0.00
30	1.04	0.00±0.00
40	1.39	0.00±0.00
50	1.74	0.00±0.00
60	2.09	0.00±0.00
70	2.44	0.00±0.00
80	2.78	0.00±0.00
90	3.13	0.00±0.00
100	3.48	0.00±0.00
10	0.001tetracycline (positive control)	8.67±0.67
90% acetone (negative control)	-	0.00.±0.00

The positive and negative control shows the inhibition or lack of it is due to the crude pigment extract of the *Scenedesmus* (Tables 1 and 2).

4. DATA ANALYSIS

The obtained data were analyzed using one-way analysis of variance. Significant differences among individual inhibitory effects were determined using Duncan's multiple range test at 0.05 level of probability. Statistical analysis was performed using computer software Statistical Packages for Social Sciences (SPSS) version 20.

5. DISCUSSION

The search for antimicrobials from natural sources has recently received much attention and efforts are on to identify compounds that can act as suitable antimicrobial agents to replace synthetic ones. These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts of various microalgae for the discovery of new antimicrobial compounds [17, 21, 22, 23 & 24]. To date, microalgae extracts and bioactive compounds have found their way into pharmaceuticals, nutraceutical and food supplements. The crude pigment extract of *Scenedesmus* sp. was found to have inhibitory activity against the foodborne pathogen *S. aureus*. Similar findings were reported by [17, 21, 23, 25, 26 & 27]. According to [28, 29]) antimicrobial activity depends on both algal species and the solvents used for their extraction. The antimicrobial activity of algae extracts is generally assayed using various organic solvents which always provide a higher efficiency in extracting compounds for antimicrobial activity compared to inorganic solvents [30, 31].

Chlorophylls and β –carotene are major pigments present in microalgae that are known to act effectively as microbial growth inhibitors [22]. Pigment from microalgae with antibacterial effect on certain bacteria which includes *S.aureus* was also reported [32, 33]. In a similar way, antibacterial activity exhibited by *Scenedesmus* sp. was due to the chlorophyll contained in its cell. According to [34] the Chlorophyta, or green algae, which includes *Scenedesmus* contains chlorophyll a, b and several carotenoids.
The lack of inhibition by the negative control (90 % acetone) being the solvent used for the extraction of the pigment, shows that it was not the cause of inhibitory action or lack of it against the two organisms. The positive control (tetracycline) a known antibiotic exhibited antibacterial activity similar to the pigment extract to a certain degree (10µg/ml). This suggests that the pigment extract, contains bioactive compounds with antibacterial activities. The MIC was defined as the lowest concentration of the antibiotic or extracts that completely inhibited visible growth of the test organism [35]. According to [36], antimicrobial activity of an extract is considered to be good if its MIC is less than 100.0 µg/ml. In the present study, the MIC value of the crude pigment extract was 0.08 mg/ml which is considered a good MIC value. On the other hand, the lack of inhibitory activity of the pigment extract against *Salmonella* sp. perhaps suggest that it's potency is limited to gram positive bacteria such as *S. aureus*. According to [25], the resistance of *Salmonella* against the pigment extracts may be due to their more complex multilayered cell wall structure. In addition, the presence of lipopolysaccharides on the outer cell wall of *Salmonella* prevents the penetration of the active compounds [25]

6. CONCLUSION

This work suggests that *Scenedesmus* sp. can serve as a potential antibacterial agent against foodborne pathogen *S. aureus.* However, further work is required to asses it's potency against other foodborne pathogens in order to propose it for use as an additive in food.

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