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INHIBITORY EFFECT OF PIGMENT EXTRACT FROM SCENEDESMUS SP. ON FOOD SPIKED WITH FOODBORNE STAPHYLOCOCCUS AUREUS

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ABSTRACT

Foodborne diseases are a recognized problem worldwide and the search is on to discover novel antimicrobial agents from natural sources that can replace the use of synthetic antimicrobial compounds. One of such sources are microalgae. The antibacterial efficacy of pigment extract from Scenedesmus sp. was tested against foodborne Staphylococcusaureus. The extract was prepared using 90% acetone and varying amounts was added to food suspension spiked with a known volume of the bacteria. Antibacterial activity was observed and evaluated through bacterial colony counts. Uncountable bacterial colonies were observed in the sample with 0.41 mg/ml and 0.81 mg/ml concentration of pigment extract as they were TMTC and there was no growth of bacterial colonies in samples with 2.83 mg/ml - 4.05 mg/ml concentration of pigment extract.The results of this study suggest that Scenedesmus sp. is a potential source of antibacterial compounds for food applications as an additive.

1. INTRODUCTION

Foodborne diseases involve a wide spectrum of illnesses which are caused by bacterial, viral, parasitic or chemical contamination of food. The most common causes of foodborne diseases worldwide are food infection and intoxication. Foodborne pathogens find their way into foods through cross contamination, improper handling and temperature abuse. Staphylococcus aureus and Salmonella sp. are among the common foodborne microorganisms that cause infection and intoxication [1-2]. Chemical preservatives which act as antimicrobial compounds are used to inhibit the growth of undesirable microorganisms in food. However, the demand for minimally-processed, extended shelf life foods and reports of chemical preservatives having potential toxicity led to the search for alternative sources of antimicrobial compounds by food manufacturers [3-4].

Food manufacturers involved in the production of novel functional foods often employ the addition of one or more interesting bioactive compounds usually referred to as functional ingredients to a traditional food, thereby developing and commercializing several functional foods. Microalgae are the basis of the food chain in aquatic ecosystems; with the aid of solar energy, they can use water and carbon dioxide to synthesize complex organic compounds—and subsequently accumulate and/or secrete many primary and secondary metabolites of interest [5-7]. Scenedesmus belongs to the order Sphaeropleales of the family Scenedesmaceae which is frequently dominant in freshwater lakes and rivers [8-9]. Scenedesmus sp. can exist as unicellular organisms and are also often found in coenobia of four or eight cells [10] (Fig 1).

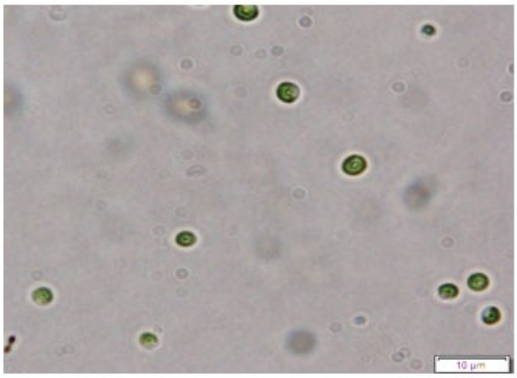

Figure 1 morphology of Scenedesmus sp. (100x magnification).

Many species of this genus are being used worldwide for various purposes due to their ability to adapt to harsh environmental conditions, ability to grow rapidly and ease of cultivation and handling [11-12]. Likewise Scenedesmus sp. have been used in many biotechnological applications due to their high nutritional content and bioactivities [7,13].The potential of microalgae in biotechnological applications range from nitrogen fixation, production of bioactive and pharmaceutical compounds, health foods, and aquaculture feeds [14]. This study was carried out to evaluate the antibacterial activities of Scenedesmus sp. pigment extract on food spiked with foodborne pathogen S. aureus.

2. EXPERIMENTAL

2.1 Microalga culture

Scenedesmus sp. was obtained from the food technology and microbiology laboratory of Universiti Tun Hussein Onn Malaysia (UTHM) and maintained on Bold Basal Medium (BBM). The cultures were grown in Erlenmeyer flasks containing 500ml of BBM respectively and incubated at room temperature under light illumination from sunlight during the day. Although no aeration was provided throughout, the cultures were shaken manually few times every day to allow dispersal as well as prevent cells from sinking to the bottom.

2.2 Extraction of microalgal pigment

Scenedesmus sp. biomass was harvested following the method described [15] through centrifugation. Briefly, the concentrated biomass was placed in a tissue grinder and covered with about 2-3 ml 90 % acetone and macerated for 1 min. The slurry 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.

2.3 Estimation of chlorophyll a and b content

Pigment extract was transferred into 1 cm cuvette and the absorbance was measured at 750 nm, 664 nm and 647 nm and 630 nm using a spectrophotometer (Biomate 3S, Thermofisher Scientific USA). The absorbance reading at 750 nm was subtracted from the readings of 664 nm, 647 nm and 630 nm. The chlorophyll a and b content were then estimated using the following equations:

$$Ca = 11.85(A664) - 1.54(A647) - 0.08(A630)$$

$$Cb = 21.03(A647) - 5.43(A664) - 2.66(A630)$$

Ca and Cb = concentration of chlorophyll a and b in µg/ml [15]

2.4 Estimation of Carotenoid content

The method of [16] was used to determine the carotenoid content present in the same pigment extract. The extract was measured using spectrophotometer (Biomate 3S, Thermofisher Scientific USA) at 480 nm. The carotenoid content was calculated thus;

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Carotenoid (µg/ml) = A480 + (0.114 X A663) – (0.638 X A645) Where, A = Absorbance at respective wave length.

2.5 Determination of pigment concentration extracted

It is important to calculate and know the concentration of a working substance or solution to allow for new solutions to be created for further use in experiments. This also aids in dilution if the actual concentration of a solution is known. Below are calculations showing how the pigment extract concentration was evaluated. To calculate the concentration, weight per unit volume was used [17].

Conc. of pigment obtained = Weight of Scenedesmus sp. Obtained / Total volume of 90 % acetone used for extraction

2.6 Bacterial inoculum

Using the direct colony suspension, 3-5 well isolated colonies of S. aureus cultured on Tryptic soy agar (TSA) were picked and transferred into a tube of sterilized Tryptic soy broth (TSB) with a sterilized wire loop. The bacteria were mixed well in the broth to dissolve the colonies. The turbidity of the bacterial suspension was standardized to match that of a 0.5 McFarland standard and this corresponds to approximately 1.5 ×〖10〗^8 CFU/ml. Turbidity of the actively growing broth culture was adjusted with sterile saline or broth to obtain turbidity comparable to that of the 0.5 McFarland standard. The inoculum was compared against the 0.5 McFarland standard against a card with a white background and contrasting black lines. The adjusted innocula was used within 15 min of preparation [18].

2.7 Preparation of fried rice

The fried rice used in this study was prepared in the laboratory. The ingredients used in the preparation of the food include; rice, cooking oil, meat, vegetables, onions, salt, and seasoning. The raw ingredients were washed thoroughly prior to cooking. The prepared food was put into a sterile bag and then homogenized to a paste form with a stomacher.

2.8 Preparation of food samples for antibacterial activity

A modification of the method by [19-20] was used in spiking the food with S. aureus. Food suspension was made by weighing 10g of the homogenized fried rice into 100ml of sterile distilled water and re-homogenized again with a homogenizer. Thereafter, 1ml of the food homogenate was pipetted into labeled sterile tubes and 10 µl of S. aureus inoculum was added to the tubes and mixed thoroughly. Different volumes of pigment and lipid extracts (10 µl – 100 µl) at different concentrations were separately pipetted into the mixture and shaken thoroughly. The mixtures were incubated at 37 ⍰ for 2 hours and then poured into sterile plates for pour plate method using prepared Mannitol salt agar. After solidification of the samples in the agar, the plates were incubated at 37 ⍰ for 24 hours. Antibacterial activity was determined after 24 hours by counting the colonies grown on the plates using a colony counter. Positive (tetracycline) and negative (solvents) controls were used and a sterility check was also carried out on the food. Counts were expressed as CFU/ml

3. RESULTS

The presence of three major pigments were determined in the pigment extract and their concentrations evaluated individually. Chlorophyll 'a' (39.97±1.103) µg/ml, chlorophyll 'b' (13.58 ±1.482) µg/ml and carotenoid (2.25±0.357) µg/ml was recorded in the freshwater microalgae Scenedesmus sp. (Fig 2).

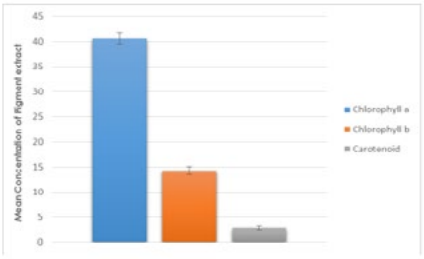


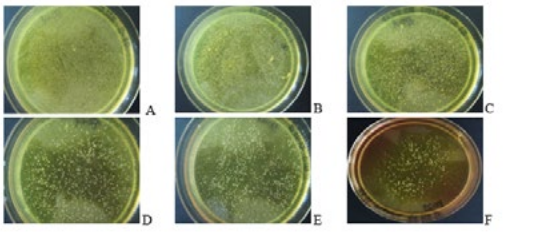
Figure 2 Mean Concentrations of photosynthetic pigments present in Scenedesmus sp.

The results revealed that the pigment extract of Scenedesmus sp. exhibited antibacterial activities against the test bacterial isolate S. aureus (ATCC 25923) when spiked in food. The highest (P< 0.05) bacterial count was observed in the treatment with 0.41 mg/ml and 0.81 mg/ml pigment extract as they were TMTC(Table1).On the other hand, No (P< 0.05) bacterial counts between the samples were observed in the treatments with 2.83 mg/ml – 4.05 mg/ml pigment extract (Table 1). Thus higher (P< 0.05) inhibitory activity of Scenedesmus sp. pigment extract was observed in the plates with 70 – 100 µl (2.83 mg/ml – 4.05 mg/ml) of the extract against S. aureus (Figure 3).

Table 1 Bacterial colony count (CFU/ml) for pigment extract in food spiked with *S.aureus*. Values are mean±SE

Volume of pigment extract used in treating food samples spiked with S. aureus (µl)	Concentration of individual volume of extract (mg/ml)	Bacterial colony count (CFU/ml)		
		Mean	Minimum	Maximum
10	0.41	TMTC**	TMTC	TMTC
20	0.81	TMTC**	TMTC	TMTC
30	1.21	9.08×10^4	8.04×10^4	9.89×10^4
40	1.39	8.01×10^4	7.66×10^4	8.28×10^4
50	2.02	3.87×10^4	3.52×10^4	4.06×10^4
60	2.43	4.50×10^3	2.80×10^3	6.5×10^3
70	2.83	0.00±0.00*	0.00	0.00
80	3.24	0.00±0.00*	0.00	0.00
90	3.64	0.00±0.00*	0.00	0.00
100	4.05	0.00±0.00*	0.00	0.00
100 µl 90% acetone		TMTC**	TMTC	TMTC
10	10µg/ml tetracycline	2.31×10^4	2.04×10^4	2.30×10^4
Food only (sterility checking)		0.00	0.00	0.00

*Mean difference is significant at 0.05 level, TMTC: too many to count **Values in column with asterisks are significant at P< 0.01 level



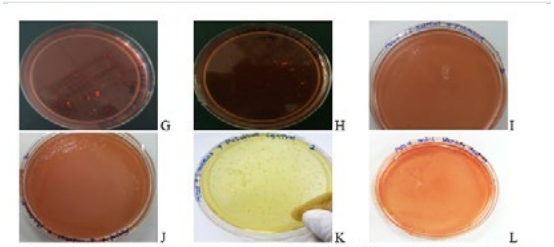


Figure 3 (a-l) Bacterial colonies of S. aureus spiked in food showing inhibitory activities of Scenedesmus sp. pigment extract observed on Mannitol salt agar plates after 24 hours incubation at 37 °C. (a) S. aureus growth on plate with 0.41 mg/ml pigment extract; (b) S. aureus growth on plate with 0.81 mg/ml pigment extract; (c) S. aureus growth on plate with 1.21 mg/ml pigment extract; (d) S. aureus growth on plate with 1.39 mg/ml pigment extract; (e) S. aureus growth on plate with 2.02 mg/ml pigment extract; (f) S. aureus growth on plate with 2.43 mg/ml pigment extract; (g) No S. aureus growth on plate with 2.83 mg/ml pigment extract; (h) No S. aureus growth on plate with 3.24 mg/ml pigment extract; (i) No S. aureus growth on plate with 3.64 mg/ml pigment extract; (j) No S. aureus growth on plate with 4.05 mg/ml pigment extract; (k) positive control; (l) food only(sterility checking).

4. DISCUSSION

Scenedesmus sp. have been reported to produce antimicrobial substances which from the pharmaceutical's point of view, are a good source of new bioactive compounds [21]. Scenedesmus sp. is reported as being among the few members of the green algae to produce antimicrobial substances and have active and prominent antibacterial properties that inhibited the growth of several pathogenic strains of bacteria when tested against them [7]. Chlorophyll is one of the most valuable bioactive compounds that are being extracted from the microalga biomass. Due to its strong green pigment content and consumers growing preference for natural foods, since many foods tend to lose their original colors due to the chemical processes they undergo, chlorophyll is gaining importance as a food additive [22]. The exhibition of antibacterial activity was considered to be an indicator of the capability of the freshwater microalga to synthesize bioactive secondary metabolites. Chlorophylls and β – carotene are major pigments present in microalgae that are known to act effectively as microbial growth inhibitors [23]. [24 & 25] reported that pigments from microalgae had antibacterial effect on certain bacteria which includes S. aureus. According to [26], the Chlorophyta, or green algae, which includes Scenedesmus contains chlorophyll a, b and several carotenoids. The main objective of this study

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was to evaluate the ability of the microalga pigment extract to inhibit the growth of foodborne Staphylococcus aureus.

The antibacterial analysis of pigment extracts of Scenedesmus sp. in food spiked with bacteria showed good inhibitory activity. The antibacterial activity of the extract was evident in the reduction of the bacterial colony counts formed on the agar plate as the concentration of the pigment was increased. The antibacterial activity and efficacy of pigment extract of Scenedesmus sp. was in conformity with earlier tests done on the solid agar in which the zones of inhibition were measured [7, 27-29]. Comparisons between studies are often complicated because different authors have used a variety of methodological approaches to determine and measure potency of antibiotics with considerable variation in the inoculum, solvent and incubation conditions. It is also important to note that the solvent must be separated from the final extract, especially if the product is to be used in food applications [30]. Consumer demand has renewed the use of natural food antimicrobial agents for food preservation [31]. Many medicinal plants incorporated into food exhibit antibacterial activities which limits the growth of foodborne pathogens, thereby increasing food safety. In many cases, when an extract is mixed into food the antimicrobial effect is reduced by reaction or interaction with food components. Therefore, a greater concentration of the extracts are needed to achieve the same effect in food as it is in microbiological media [32]. The intrinsic properties of food such as; fat/protein, antioxidants, water activity, pH, salts, other additives as well as external determinants such as; temperature, packaging as well as the characteristic of the microorganism can also influence bacteria sensitivity to the plant extracts [33-34].

5. CONCLUSION

Based on the data obtained, it is clear that the pigment extract shows great prospects as a source of natural antibacterial compound that can be used as food additive to inhibit the growth of foodborne pathogen S. aureus. This information can prove to be very helpful in further research and discovery of new drugs in antimicrobial therapy.

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