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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *CHLORELLA VULGARIS* BEIJERINCK

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ABSTRACT

Aim: In the last few decades, increased resistance of bacterial strains to drugs including antibiotics has been a major factor for increasing morbidity, mortality and healthcare costs to bacterial infections. With this concern, effort has been made to investigate the phytochemicals and antibacterial potency of chloroform, acetone, ethanol and aqueous extract from *Chlorella vulgaris* Beijerinck (Chlorellaceae). **Method:** Mass cultivation of algae was carried out in outdoor and chloroform, acetone, ethanol and aqueous extracts were extracted directly from the biomass. The extracts were subjected to phytochemical screening and antimicrobial activity against test organisms; *E.coli*, *P.vulgaries*, *S.aureus*, *P.aeruginosa* and *B.subtilis* by agar well diffusion assay and Minimum Inhibitory Concentration of all the four extracts were determined. **Result:** Phytochemical screening revealed the presence of alkaloid, flavonoid, phenol, tannin, terpenoids, saponin and glycosides. Acetone and ethanol extracts were potential antimicrobial agent when compared to chloroform and aqueous extracts. Aqueous extract showed very less inhibitory potency against test organisms. **Conclusion:** Daily supplementation of food with *C. vulgaris* Beijerinck will not only nourish in the body growth but it will also serve as immense source to defend against bacterial infection.

Keywords: *Chlorella vulgaris*, mass cultivation, phytochemicals, agar well diffusion, Minimum Inhibitory Concentration.

INTRODUCTION

Chlorella vulgaris Beijerinck is a single-celled fresh-water algae super-food and is thought to be one of the planet's earliest life-forms. Since the 1960's *Chlorella* has been popular in Japan as a multi-supplement taken to maintain health through optimal nutrition. It is the most researched of all the algae. Initially research was focused on improving our understanding of photosynthesis, but since the 1970s the health benefits of *Chlorella* have been documented in vast collection of studies[1]. *Chlorella* users report to experience more energy, improved physical appearance and protection from disease and illness. Infectious diseases are the leading cause of death world-wide and antibiotic resistance has become a global concern[2]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens[3]. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Unmatched availability of chemical diversity among natural products either as pure compounds or

standardized plant extracts, provide unlimited opportunities for new drugs to lead in pharmaceutical industries. With an urgent need to discover new antimicrobial compounds, there is also need for the developing novel mechanisms of action for new and re-emerging infectious diseases[4]. In recent years, marine natural product search has yielded a considerable number of drug candidates[5]. The chemical compounds responsible for the antibacterial activity in algae have been variously identified as bromophenols, carbonyls, halogenated aliphatic compounds, terpenes, isoprenylated and brominated hydroquinones, as well as phlobatannins[6]. In this regard, the present study is focused on the phytochemical analysis and antibacterial potency of chloroform, acetone, ethanol and aqueous solvent extracts of *Chlorella vulgaris* Beijerinck.

MATERIALS AND METHODS

Culturing and collection of algal mass

C.vulgaris Beijerinck (Chlorellaceae) was cultured in Bold Basal medium (BBM) and incubated for 15 days at 24 ± 1 ºC in a thermostatically controlled room and illuminated with cool inflorescence lamps (2000 lux in a 12: 12 h L/D) regime. The log-phase culture was used as feeder culture for outdoor cultivation and was carried out at terrace in wide mouthed plastic tubs by inoculating 1500 mL of culture in 15.0 L of medium; pH was adjusted to 8.5. Biomass was harvested and collected after 15 days.

Preparation of algal extract

Ten gram of collected fresh algal biomass which was equal to 1g of dried weight[7] was completely homogenized and soaked (w/v; 1:10) in 100 ml of chloroform, acetone, ethanol and water; and kept for 72 hours. This process was repeated until the extracted solvent became colorless except with that of water. The extracts were pooled together, filtered through Whatman no. 40 and chloroform, acetone, ethanol extract were condensed under vacuum at 50˚C using rotary evaporator while water extract was lyophilized. The condensed chloroform, acetone, ethanol extracts were dried at room temperature and stored in an air tight amber vial at 4˚C while aqueous extract was stored in desiccator, until further analysis to determine phytochemicals and antimicrobial activity against test organisms.

Phytochemical screening

Standard phytochemical test[8] was carried out on the chloroform, acetone, ethanol and aqueous extracts of *C.vulgaris* Beijerinck to determine presence of Alkaloids, steroids, flavonoids, phenols, tannin, terpenoids, saponin and glycosides.

Antimicrobial susceptibility studies

Inhibition of microbial growth was tested by agar well diffusion assay. Standard aseptic microbiological methods were followed throughout this antibacterial study. Bacterial inoculums were prepared as per 0.5 McFarland standard[9] with test organisms that were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Chandigarh ,India includes: *Escherichia coli* MTCC -1687, *Proteus vulgaries* MTCC-742, *Staphylococcus aureus* MTCC -96, *Pseudomonas aeruginosa* MTCC -1688 and *Bacillus subtilis* MTCC -441 in Muller Hinton broth (MHB). Dried crude chloroform, acetone, ethanol and aqueous extracts were dissolved in 100% Dimethyl Sulfoxide (DMSO) at mg/mL concentration. From this 25 µL, 50 µL, 75 µL and 100 µL extracts were used to load in agar wells.

Agar well diffusion assay

Antimicrobial activity of chloroform, acetone, ethanol and aqueous extract of *C.vulgaris* Beijerinck was determined by agar well diffusion technique[10] using Muller Hinton agar. Bacterial culture of 10^8cells/mL was swabbed on sterile agar surface and well was cut using cork borer of 9 mm diameter. Wells were loaded with

different extracts, negative control DMSO and positive control, Streptomycin (50 µL at mg/mL in sterile water). Loaded plates were incubated at 37˚C for 24 hrs and after incubation, diameter of inhibition zone was measured in mm using ruler and experiment was repeated thrice.

Minimum Inhibitory Concentration (MIC)

MIC of chloroform, acetone, ethanol and aqueous extract of *C.vulgaris* Beijerinck was determined by the standard method[11]. Nutrient broth was prepared and sterilized using autoclave. One mL of the prepared broth was dispensed in to the test tubes numbered 1-9. A stock solution containing 25 mg/mL of each extract was prepared. Then 1 mL of the solution was dispensed into the tubes numbered 1. Subsequently, from tube 1, serial dilution was carried out and 1 mL from tube 1 was transferred up to tube number 7 and 1 ml from the tube 7 was discarded. Tube 8 was control for sterility of the medium and tube 9 for viability of the organisms. An overnight culture (inoculums) of each of the test organism was prepared in sterile nutrient broth. 1 mL of the inoculum was transferred into each tube from tube 1 to tube 9 with exception of tube 8, to which another sterile nutrient broth was added. The final concentration of the algal extract in each of the test tubes numbered 1-7 after dilution 25, 12.5, 6.25, 3.125, 1.563, 0.78 and 0.39 mg/mL, were incubated at 37°C for 24 hrs and examined for growth. The last tube in which growth failed to occur was the Minimum Inhibitory Concentration tube.

RESULT

Preliminary phytochemical screening of chloroform, acetone, ethanol and aqueous extract of *C.vulgaris* Beijerinck revealed the presence of alkaloids, flavonoids, phenol, tannins, terpenoids, saponins and glycosides (Table 1).

Antimicrobial activity

All the four extracts of *C.vulgaris* Beijerinck; chloroform, acetone, ethanol and aqueous extract exhibited varying degree of antimicrobial activities against test microorganisms, *E.coli*, *P. vulgaries*, *S. aureus*, *P. aeruginosa* and *B. subtilis* (Table 2, 3, 4, 5, 6). *S. aureus* showed low susceptibility to all the four extracts whereas *E. coli* was showed moderate susceptibility to acetone and ethanolic extract. Wide range of antimicrobial activity was exhibited by all the extracts between the range 50-100 µg/mL.

The Minimum Inhibitory Concentration of acetone, ethanol, chloroform and aqueous extract of *Chlorella vulgaris* in this study against the test organisms ranged between 25 to 1.5 mg/mL (Table 7). Ethanol extract was more potential in inhibiting test bacteria at minimum concentration when compared to other extracts.

DISCUSSION

The variation in the presence and absence of phytochemicals with respective solvents attributed to the solubility of the active component in different solvents[12]. To a large extent, the chronological growth phase, percentage humidity of the harvested material, situation and time of harvest, and the method of extraction were possible sources of variation for the chemical composition, toxicity and bioactivity of the extracts[13].

It was observed that antibacterial effectiveness increased with increasing concentration of extracts and higher concentrations of antimicrobial substances shows appreciable growth inhibition to microorganisms[14]. *B.subtilis* showed susceptibity to acetone and ethanol extract. Though chloroform exhibited low antimicrobial activity against most test organisms, it showed potential inhibitory activity against *P.aeruginosa.* Aqueous extract showed low antimicrobial activity when compared to other extracts. Acetone and ethanol

extract are potential antimicrobial agents, effectively inhibiting the growth of *B. subtilis, P.vulgaris* and *P.aeruginosa.* Although the positive control, streptomycin showed significant growth inhibitory activity on all the bacteria tested, chloroform extract was found to be more effective on *P.aeruginosa* at concentration of 100 µg/mL (Table 2) while the ethanolic extract was found to show similar activity against *S.aureus* at same concentration (Table 4).Antimicrobial agents with low activity against an organism have a high MIC while a highly active antimicrobial agent gives a low MIC.

Tannins, alkaloids saponins and phlobatannins have been reported for their antibacterial and antiviral activity[15]. Furthermore, alkaloids and saponins are classes of compounds that are known to be effective for the treatment of syphilis and other venereal diseases[16]. The presence of these constituents has been reported to account for the exertion of antimicrobial activity by plants[17].

CONCLUSION

After evaluating the phytochemicals and antimicrobial potency of different extracts of *C.vulgaris* Beijerinck, it can be concluded that *C.vulgaris* Beijerinck, not only serve as extreme food supplement, beside it is good wound healer, antioxidant and from the above study it is a good antimicrobial agent also. Further studies can be carried out to isolate the pure compound responsible for antimicrobial activity.

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Table 1: Qualitative phytochemical screening of *Chlorella vulgaris* Beijerinck.

S.No	Phytochemicals	Acetone extract	Ethanol extract	Chloroform extract	Aqueous extract
1.	Alkaloid	+	+	+	-
2.	Steroid	-	-	-	-
3.	Flavonoid	+	+	-	+
4.	Phenol	-	+	-	+
5.	Tannins	+	+	+	+
6.	Terpenoids	+	+	+	+
7.	Saponins	-	-	+	+
8.	Glycosides	+	+	+	+

Note: + Presence, - Absence.

Table 2: Antibacterial activity of Chloroform extract of *Chlorella vulgaris* Beijerinck

S.No	Concentration (µg/mL)	Zone of inhibition (mm ±SD)				
		E.coli	*P.vulgaries*	*S.aureus*	*P.aeruginosa*	*B.subtilis*
1	25	0.0±0.0	9.5±0.32	0.0±0.0	12.0±0.27	0.0±0.0
2	50	0.0±0.0	11.0±0.17	0.0±0.0	15.0±0.24	9.5±0.0
3	75	11.0±0.16	13.0±0.23	12.0±0.28	20.0±0.35	13.0±0.23
4	100	13.0±0.30	14.0±0.25	13.0±0.21	25.0±0.30	16.0±0.27

Table 3: Antibacterial activity of Acetone extract of *Chlorella vulgaris* Beijerinck

S.No	Concentration (µg/mL)	Zone of inhibition (mm ±SD)				
		E.coli	*P.vulgaries*	*S.aureus*	*P.aeruginosa*	*B.subtilis*
1	25	0.0±0.0	10.0±0.24	0.0±0.0	11.0±0.25	12.0±0.18
2	50	9.5±0.21	12.0±0.18	0.0±0.0	13.0±0.21	15.0±0.21
3	75	13.0±0.30	14.0±0.35	11.0±0.16	14.0±0.17	18.0±0.26
4	100	15.0±0.35	17.0±0.26	13.0±0.23	16.0±0.24	20.0±0.0

Table 4 Antibacterial activity of Ethanol extract of *Chlorella vulgaris* Beijerinck

S.No	Concentration (µg/mL)	Zone of inhibition (mm ±SD)				
		E.coli	*P.vulgaries*	*S.aureus*	*P.aeruginosa*	*B.subtilis*
1	25	10.0±0.28	11.0±0.35	12.0±0.25	11.0±0.23	12.0±0.28
2	50	12.0±0.30	13.0±0.19	12.0±0.23	14.0±0.27	16.0±0.30
3	75	15.0±0.24	14.5±0.25	13.5±0.32	16.0±0.21	20.0±0.26
4	100	20.0±0.21	16.0±0.16	15.0±0.25	23.0±0.0	24.0±0.22

Table 5: Agar well diffusion assay of aqueous extract of *Chlorella vulgaris* Beijerinck

S.No	Concentration (µg/mL)	Zone of inhibition (mm ±SD)				
		E.coli	*P.vulgaries*	*S.aureus*	*P.aeruginosa*	*B.subtilis*
1	25	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2	50	0.0±0.0	0.0±0.0	0.0±0.0	9.5±0.0	0.0±0.0
3	75	0.0±0.0	12.0±0.26	0.0±0.0	13.0±0.21	0.0±0.0
4	100	11.0±0.27	14.0±0.23	9.5±0.25	15.0±0.0	0.0±0.0

Table 6: Antimicrobial activity of Streptomycin

SNo	Concentration (µg/mL)	Zone of inhibition (mm ±SD)				
		E.coli	*P.vulgaries*	*S.aureus*	*P.aeruginosa*	*B.subtilis*
1	50	20.0±0.34	18.0±0.25	15.0±0.30	24.0±0.36	27.0±0.30

***Note: All values are mean of triplicates repeated thrice and standard deviation (SD).**
SD- Standard Deviation
mm- Millimeter

Table 7: Minimum Inhibitory Concentration of *Chlorella vulgaris* Beijerinck

S.No	Microorganisms	Minimum Inhibitory Concentration (mg/mL)			
		Acetone extract	Ethanol extract	Chloroform extract	Aqueous extract
1	*Escherichia coli*	6.25	3.12	12.5	-
2	*Proteus vulgaries*	3.12	12.5	6.25	-
3	*Staphylococcus aureus*	-	25	25	-
4	*Pseudomonas aeruginosa*	3.12	1.5	3.12	12.5
5	*Bacillus subtilis*	12.5	3.12	6.25	6.25