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Research Article

Antioxidant and Antimicrobial activity of Crude Sterol from *Chlorella*

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ABSTRACT

The study investigates the antioxidant and antimicrobial activities of crude sterol extracted from *Chlorella*, a green microalga known for its potential therapeutic properties. Sterols are one of the important compounds obtained from Plant oils. Microalgae can be an alternative source of the sterols in future. Sterols have proven records of hypocholesterolemic properties. *Chlorella* is a well-known microalga for its multiple health benefits and properties. These study findings suggest that *Chlorella*-derived sterols possess better antimicrobial activities than antioxidant properties, supporting their potential use in pharmaceutical and food industries. The current paper study focused to explore the biochemical properties of sterols from *Chlorella* which may increase the significance of the Sterols in commercial market. Further research is required to isolate specific compounds responsible for these activities and to evaluate their safety and efficacy.

INTRODUCTION

Microalgae are minute algae found in both marine and freshwater ecosystems. Microalgae produce natural products and are being explored for biotechnological applications (1) *Chlorella* is one of the most well-known microalgae genera. *Chlorella* is a freshwater, unicellular organism with bioactive properties including colours, vitamins, proteins, sterols, and long-chain polyunsaturated fatty acids (2)

Plant sterols, also known as phytosterols, are naturally occurring chemicals present in plant-based foods such as Vegetable oils, Nuts, Seeds, Cereals, and Legumes in tiny quantities(3). Sterols are isoprenoid lipids present in eukaryotic membranes. They have important functions in regulating membrane Fluidity and Permeability. Plant oil is the primary source of Phytosterols nowadays. Microalgae might be a more sustainable source of Sterols compared to Plant and Shark Oils (4). In mammals, Cholesterol is the primary Sterol required for numerous biological

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functions. Ergosterol is the most common Sterol found in fungus. Plant sterols, also known as Phytosterols, share physiological and structural similarities with Cholesterol (4). Phytosterols are known for lowering Cholesterol, their Antioxidant properties are less studied. It states that, Phytosterol chemically works as Antioxidant and it is a moderate radical Scavenger(5). *Chlorella spp*. extracts exhibit Antibacterial activity against *Bacillus subtilis, Pseudomonas aeruginosa, and Escherichia coli. C. vulgaris'* Antibacterial activity is linked to its Cyclic Peptides, Terpenoids, Alkaloids, Steroids, and Tannins. Algae are a valuable source of Antimicrobial compounds used in laboratory experiments to suppress disease-causing microorganisms(2). Antibiotic resistance in pathogens is rising due to overuse, leading to multidrug-resistant microorganisms. There is a significant need to identify novel antipathogenic compounds with minimal negative effects (2) The growing number of documented cases of Antibiotic resistance is a global issue. Researchers have explored the antibacterial properties of few microalgae extracts (6). The purpose of the current study to address this issue, by screening new Antibiotics or Chemicals with Antibacterial characteristics from *Chlorella*.

MATERIAL AND METHODS

Extraction of Crude Sterols from *Chlorella pyrenoidosa and Chlorella vulgaris*

Weight the 1 gm *Chlorella spp* fine powder harvested from lab cultivation. Saponification of Sterols was achieved by adding 30 ml of 0.2 M Methanolic Potassium hydroxide (KOH) solution and reflux it for next 30 minutes on same temperature. Then add 50 ml Hexane and shake vigorously for 1 minute. The hexane layer will be at upper level in separating funnel. Separate the hexane in 250 ml Crucible and allow it to evaporate.

DPPH Anti-Oxidant Assay

100µl of the test concentration (Crude Sterol) was incubated with 100µl DPPH solution. The mixture was incubated at room temperature under dark conditions for 30 minutes. After the incubation period, the absorbance of the mixture was read Spectrophotometrically at 517nm. Appropriate color blanks were prepared which contained 100µl of test concentration and 100µl of Methanol. Negative control or Control consisted of 100µl DPPH reagent and 100µl of Methanol. The Absorbance of Control is used to calculate DPPH free radical Scavenging antioxidant potential of samples. IC50 was calculated from Dose response curve.

Note: Lower the IC50 Value better is the antioxidant potential of sample under study.

$$\text{Percent Anti Oxidant Potential} = 100 - \frac{(\text{Absorbance of Test} - \text{Absorbance of Colour Blank})}{(\text{Absorbance of Zero Control})} \times 100$$

Antimicrobial Activity:

Preparation of *E. coli* and *S. aureus* culture: -

E. coli and *S. aureus* cultures were prepared by inoculating a loopful of each organism from their respective Sterile Nutrient Agar Slants into Sterile Nutrient Broth. The inoculated broths were incubated at 37°C overnight. The Minimum Inhibitory Concentrations (MICs) of crude Sterols extracted from *Chlorella pyrenoidosa* and *Chlorella vulgaris* were determined against both bacterial species.

Agar Cup Method:

Antibiotics are placed in wells on agar plates seeded with bacteria like *E. coli* or *S. aureus*. After incubation at 37°C for 24 hours, the inhibition zones diameters are measured. Sterile Nutrient Agar is used, with cultures inoculated, and 0.1 ml of sample solutions or controls placed in wells.

Pre-diffusion occurs for 30 minutes before incubation and zone measurements.

Paper Disc Method:

The disc diffusion method tests antibiotic effectiveness by placing antibiotic-soaked discs on agar plates inoculated with *E. coli* or *S. aureus*. As the antibiotic diffuses, a concentration gradient forms, creating a zone of inhibition. Disc soaked in Cefotaxime and test samples, along with controls (Antibiotic and Ethanol), are placed on Nutrient agar. After 24 hours of incubation at 37°C, the inhibition zones' diameters are measured and interpreted to assess Antimicrobial activity.

Minimal Inhibitory Concentration:

MIC is the Minimum Concentration of the antibiotic that inhibits the growth of the organism.

Addition table for MIC

Table I. Experiment setup for Minimum Inhibitory Concentration

Microcentrifuge tube number	St. Nutrient broth in ml	Sample in ml	Culture *(E. coli and S. aureus)*
1	0.2	0.8	0.1
2	0.4	0.6	0.1
3	0.6	0.4	0.1
4	0.8	0.2	0.1
5	-	1.0	0.1
Positive control	1.0	-	0.1
Negative control	1.0	-	-

Three sample were used for each organism, three sets for *E. Coli* culture and three sets for *S. aureus* culture. Incubate the tubes at 37°C for 24 hours. MIC is interpreted as the least dilution which inhibits growth judged by lack of turbidity in the tube.

Minimal Lethal Concentration:

The Minimum Lethal Concentration (MLC) is the lowest drug concentration that kills a pathogen. The positive control tube, with no sample, is sub-cultured and incubated at 37°C for 24 hours. Tubes from the MIC test showing no growth are sub-cultured, and results are observed after 24 hours. Outcomes may include similar colonies (Bacteriostasis), fewer colonies (partial Bactericidal activity), or no growth (complete kill). The highest dilution showing ≥99% inhibition is the MLC.

Results and Discussion:

Extraction of Crude Sterols:

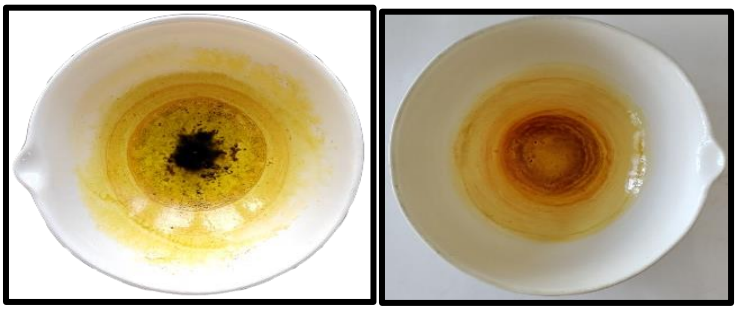

Figure I & II. Extract of Crude Sterols from *C. Pyrenoidosa* (Fig. I) and *C. vulgaris (Fig. II)*

Table II. Yield of Crude sterol Extract from both C. pyrenoidosa and C. vulgaris

Sr. No.	Heads	Extract Weight/1 gm	Yield %
1.	*Chlorella pyrenoidosa* crude sterols	90 mg	9 %
2.	*Chlorella vulgaris* crude Sterols	50 mg	5 %

Crude sterols were extracted from *C. pyrenoidosa* and *C. vulgaris* using the saponification method. *C. pyrenoidosa* showed a higher yield (9%) compared to *C. vulgaris* (5%), with both considered superior yields for commercial applications.

Antioxidant Activity through DPPH Assay:

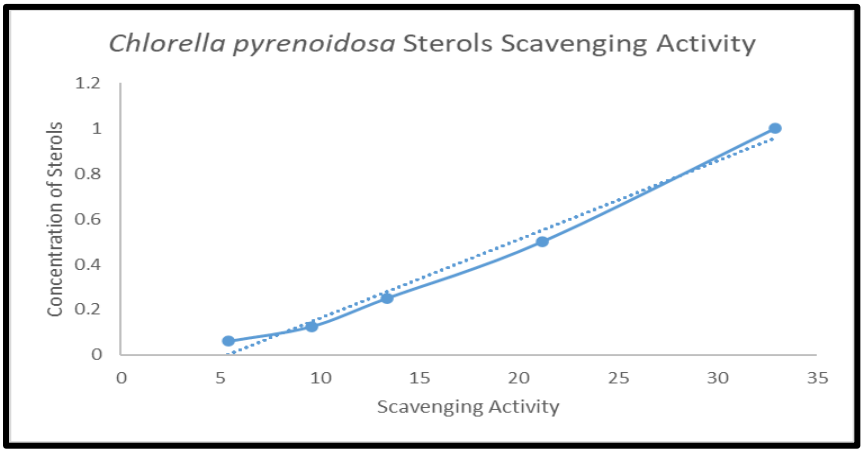

Figure III: Scavenging Activity of crude sterols from *C. pyrenoidosa* (Fig III)

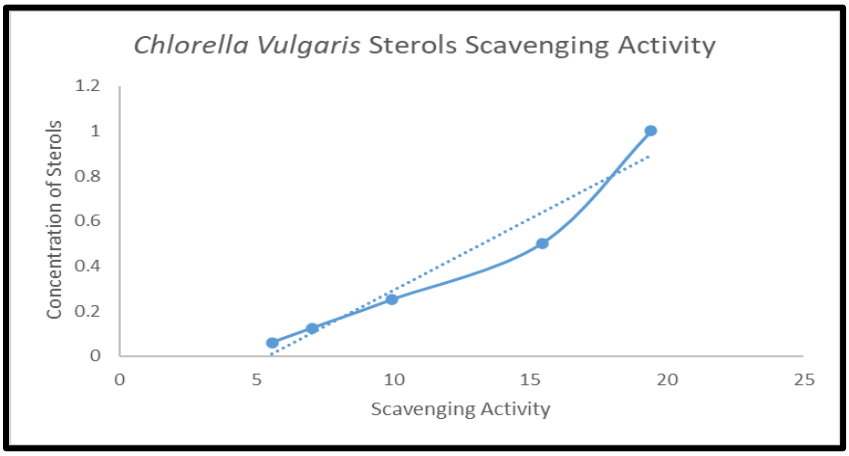

Figure IV: Scavenging Activity of crude sterols from *C. vulgaris*

The crude sterol extracted from *Chlorella's* both strains have low Scavenging activity as per DPPH biochemical assay. Each extract shows scavenging activity below 50% which restricts to calculate the IC_{50} value.

Anti-Microbial Activities:

Agar Cup Method:

Table III. Results of Agar Cup Method

	E .coli		*S. aureus*	
	CV	CP	CV	CP
Zone of Inhibition in mm	30	26	12	19
Ethanol	11	14	12	13

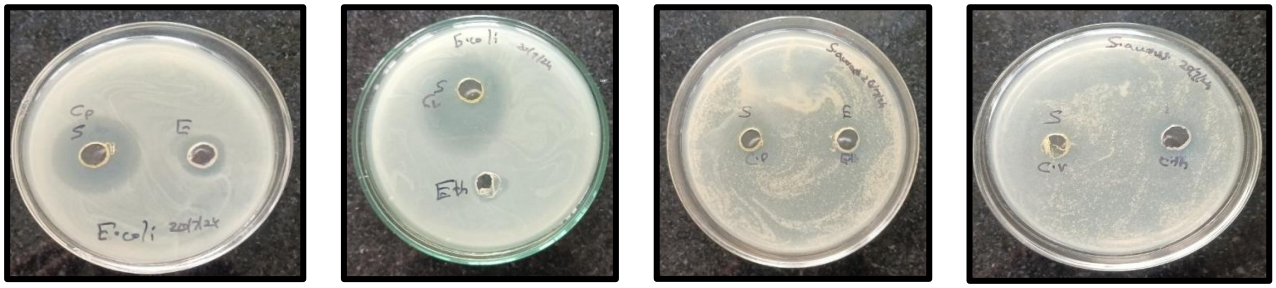

Figure V. and VI. *E. Coli* Agar cup method

Fig VII and VIII *S. aureus* Agar Cup Method

The antimicrobial activity of crude Sterols from *C. pyrenoidosa* and *C. vulgaris* was tested against *E. coli* and *S. aureus* using the agar cup method. Against *E. coli*, the inhibition zones were 26 mm for *C. pyrenoidosa* and 30 mm for *C. vulgaris*, while ethanol (positive control) showed 14 mm and 11 mm, respectively. For *S. aureus*, the inhibition zones were 19 mm for *C. pyrenoidosa* and 12 mm for *C. vulgaris*, with ethanol showing 13 mm and 12 mm. Both samples showed moderate Antibacterial activity, *C. vulgaris* more effective against *E. coli* and *C. pyrenoidosa* more effective against *S. aureus*.

Disc Diffusion Method:

Table IV. Results of Disc Diffusion Methods

	E. coli		*S. aureus*	
	CV	CP	CV	CP
Zone of Inhibition in mm	27	26	6	7
Cefotaxime	36	36	30	30
Ethanol	00	00	00	00

Figure IX, X, XI. Results of *E. coli* (IX), *S. aureus (X)* and Control (XI) Disc Diffusion Method

The Antimicrobial activity of crude Sterols from *C. pyrenoidosa* and *C. vulgaris* was tested against *E. coli* and *S. aureus* using the disc diffusion method. Against *E. coli*, the inhibition zones were 26 mm and 27 mm for *C. pyrenoidosa* and *C. vulgaris*, respectively, while the positive control (Cefotaxime) showed 36 mm for both strains. Ethanol showed no inhibition (0 mm). Against *S. aureus*, the inhibition zones were 7 mm and 6 mm, with the positive control showing 30 mm. Both strains demonstrated moderate Antibacterial activity. Higher inhibition in the agar cup method compared to the disc diffusion method suggests more efficient diffusion or higher concentrations of antimicrobial agents in the wells. The findings indicate that *C. vulgaris* crude bioactive compounds effectively inhibited both *Gram-negative* and *Gram-positive* bacteria.

Minimum Inhibitory Concentration (MIC):

The MIC results for *C. pyrenoidosa* and *C. vulgaris* against *E. coli* and *S. aureus* showed turbidity at 0.1 to 0.4 concentrations, while the 0.5 concentration had clear broth without turbidity, indicating effective antimicrobial activity at 0.5 concentration.

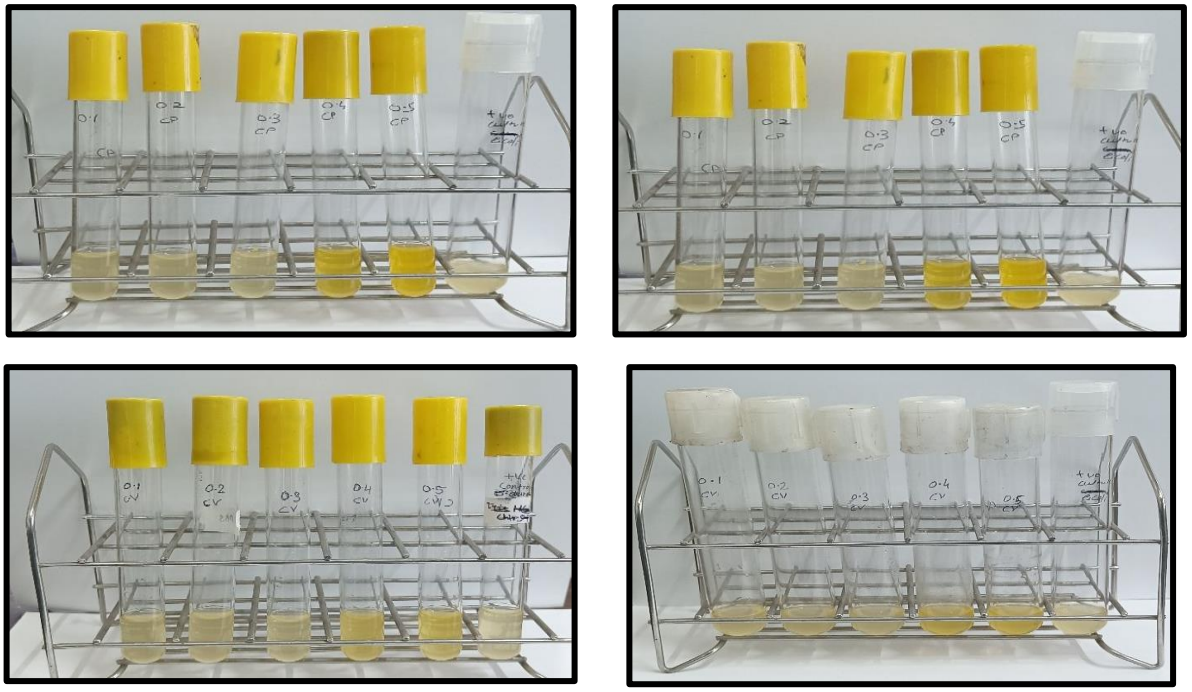

***Figure XII-XV: E.coli* (Fig. XII & XIII) and *S. aureus* (Fig. XIV & XV) results on Minimum Inhibition Concentrations (MIC)**

Minimum Lethal Concentration (MLC):

As per MIC results the 0.5 concentrations are non-turbid and clear. Therefore, we conducted MLC on 0.5 concentrations of *C. pyrenoidosa* and *C. vulgaris* against *E.coli* and *S. aureus*. The microbial growth was observed on both extracts against *E.coli* and *S.aureus*.

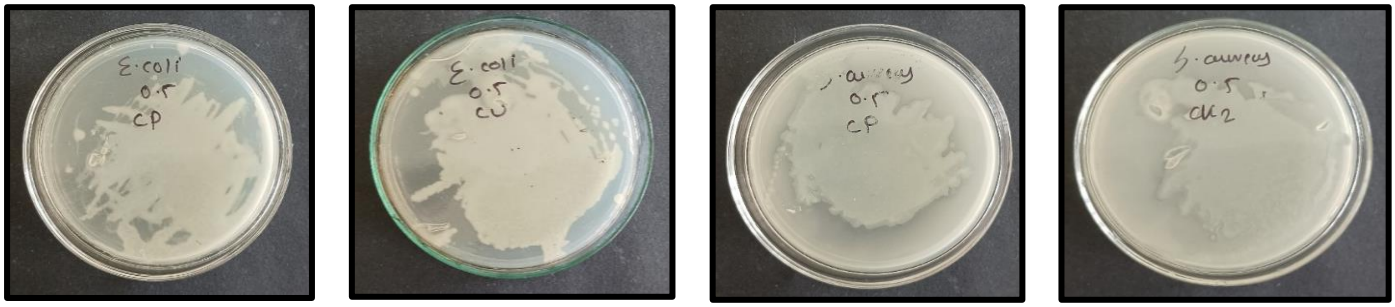

Figure XVI-XIX: Results of Minimum Lethal Concentration on *E.coli* (Fig. XVI & XVII) and *S. aureus* (Fig. XVIII & XIX)

CONCLUSION:

The *C. Pyrenoidosa* gives higher crude Sterol extract yield than *C. vulgaris*. The crude Sterols from *C. pyrenoidosa* and *C. vulgaris* shown negligible Scavenging activity which concludes no Antioxidant property to the crude Sterol extract. The Agar cup and Disc diffusion method both follows moderate zone of inhibition. The sample was dissolved in Ethanol, therefore, disc diffusion method shown exact results of crude Sterol extract. However, the crude Sterol extracts unable to give an inhibition results in MIC. It concludes that, *E. coli* (*Gram-negative* bacterium) gets inhibited effectively compared to the *S. aureus* (*Gram-positive* bacterium) by both strains crude Sterol extracts. The *C. pyrenoidosa* shown effective inhibition on *S. aureus* than *C. vulgaris* in both Agar cup and Disc diffusion method. The MIC results shown clear inhibition in 0.5 concentrations which followed by MLC. The microbial growth was observed on all plates of 0.5 concentrations of MLC. This concludes the crude Sterol extract have an activity of Bacteriostatic.

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