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ORIGINAL ARTICLE

Study of phytochemical screening, biochemical composition and antibacterial activity of various solvent extract of freshwater microalgae *Scenedesmus* sp.

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ABSTRACT
Scenedesmus sp is a freshwater microalgae that belongs to the genus of green algae. Because of its high productivity, it's also extensively used in biofuel production, aquaculture feed, and waste water treatments. Their biochemical characterization becomes critical for future study. As a result, the present study was designed to determine the close composition and antimicrobial activities of Scenedesmus sp. extract in various solvents (Chloroform, Ethanol, Diethyl ether, and Methanol) was screened for determine the antagonistic towards human pathogens (Vibrio cholera, Pseudomonas, Streptococcus, Escherichia coli and Klebsiella pneumonia) by the method of agar well diffusion. The ethanol extract shows the maximum inhibition on E. coli (13mm) and leaser in Pseudomonas (8mm).In methanol the maximum inhibition on V. cholera (12mm) and minimum in Klebsiella pneumonia (6mm). In chloroform the maximum inhibition on Klebsiella pneumonia (12mm) and minimum in Vibrio cholera (7mm). In diethyl ether the maximum inhibition on Streptococcus (13mm) and minimum in V. cholera (6mm).
Keywords: *Microalgae, Scenedesmus, Biochemical, Antimicrobial activity, Phytochemical.*

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INTRODUCTION

Microalgae are the heterogeneous group that comes under the prokaryotic and eukaryotic photosynthetic microscopic organism. They have a diverse habitat and accelerated growth in fresh water and marine water and also present in hyper saline environment as an extremophiles. They are structurally unicellular and morphologically may be exist in individually or whether in chains or either in groups they utilize the solar energy into chemical energy by photosynthesis
[1].

In the ocean, microalgae served as a main source for numerous bioactive substances like proteins, minerals and other organic matter [2]. among all of the them, some investigated microalgae strains were grouped as excellent sources for their biochemical composition (proteins, lipids and carbohydrates) and that can be utilized for food and feed added substateces, for over 40 years. The main limitation in indoor or outdoor growing is the little quantity of biomass obtained (c.a. 0.1 percent (w/w), which might make the collecting procedure exhausting and costly. The priority of microalgae in fish farming is tremendous since it is a primary producer in the ecosystem as well as a major source of feed. To considering the rich protein source of *Scenedesmus sp* produced about 30% of the current algal biomass turnover is sold for fulfill the need of animal feed [3].

The microalgae *Scenedesmus* sp and *Chlorococcum* sp are used extensively in the biofuel and aquaculture industries. [4]. The optimal cultural conditions including media composition, external condition are already evaluated for culturing of *Scenedesmus* sp, *Dunaliella* sp and *Nannochloropsis* sp It makes the promising for adequate and in proliferation of nutraceutical biomass [5].

Scenedesmus sp., and *Nannochloropsis* sp., has recently been recommended for nutrient supplemental application because of their groupings of the sustaining unsaturated fat eicosapentaenoic corrosive (EPA), and nutrients and fundamental minerals, individually [6].

The excellent antimicrobial activities were found in different microalgal extracts [14]. And it has the various phytochemical compounds in their biomass.

The screening of antimicrobial [7], antiviral [8] and antifungal [9] was achieved by various microalgal extracts. In the purpose of control, the pathogens, the drug discovery and development are most remarkable and fruitful accomplishments in current science and Technology [10]. The phenolic compounds of the algal extracts could be ascribed the antimicrobial impacts. Appropriately, which drugs derived from natural resources like plant and marine organisms have the crucial important in pharmaceutical industries [11].

Therefore, the present investigation was conducted on the antimicrobial potential and phytochemical screening of *Scenedesmus* sp.

MATERIAL AND METHODS

Laboratory culture of Scenedesmus sp.

Scenedesmus pure culture was obtained from Bharathidasan University, Tiruchirappalli's Department of Marine Science. For cultivation, the acquired culture was cultured in BG 11 medium [25].The algal biomass was cultured under the laboratory condition and exposed to suitable condition of physical parameter. Such as, light (16:8-hour light: dark), temperature (25°C), Salinity (28o/oo) and aeration. When the *Scenedesmus* culture reaches it stationary phase (14 days) is ready to harvest the wet biomass and dried by using the hot plate at 50 ˚C. During growth period, Daily samples were taken and the optical density was measured to monitor the growth at 680 nm [26].

Microalgal extracts preparation

For the purpose of to carry out the antibacterial and phytochemical screening, microalgal extract were prepared by using various solvents: ethanol, diethyl ether, methanol and chloroform. By using the pestle and mortar the dried microalgal biomass were well crashed. The microalgal biomass was weighted about 1g and suspended in centrifuge tube which containing 10ml of each solvent. Then it was allowed to centrifuge at 4000 rpm for 10 minutes, after centrifugation the supernatant was obtained and used for further works. The resulting crude extracts were kept in 4˚c for else work [12].

Analysis of biochemical composition

Evaluation of lipid

In 50 ml test tube, the 1g powdery biomass was taken and mixture with the 19 ml combination of chloroform, methanol and water at the ratio of 0.26:0.53:0.21. And then it was allowed to vortexed at 10 min with consequently in the inclusion of 10 ml combination of chloroform and water (1:1) and vortexed for 2 min. It was allowed to centrifuge at 2500 rpm for 15 min and resulting into three layers [19]. The pellet containing lipid extract with chloroform and the supernatant which containing methanol and water layer could be discarded. Some leftover biomass may be present in the center layer, above procedure is repeated to collect them. By allowing the entire lipid in the sample to evaporate in an extract in a pre-gauged aluminum dish, the total lipid in the sample was measured gravimetrically.

The total lipid was calculated as follows:

Complete lipid (percentage weighted average) = A/B100

Where A is the amount of lipids in the resulting extract and B is the amount of dried microalgae biomass utilized in the extraction.

Evaluation of carbohydrate

The carbohydrate content of microalgae was estimated using the phenol sulfuric acid technique. [21]. Glucose was utilized as a control in this experiment. Aqueous content of 100mg of microalgal sample was break by 1 mL of hydrochloric acid at 100 °C for around 2 hours. Combine 200 L of hydrolysis process with 200 L of aqueous phenol solution (five percent v/v) in a test tube. The mixture was then added 1000 L of concentrated tetraoxosulfate (vi) acid and left to settle for 10 minutes before being vortexed for 30 minutes.A UV-visible spectrophotometer was used to detect the resultant combination at 490nm (UV vis 1800, shimadzu, japan). The glucose standard was used to determine total carbohydrate.

Evaluation of protein

The 0.1 microalgae sample was hydrolyzed with 100 µl 2N NaOH and then incubated at 100°C for 10 minutes in a water bath. After the mixture has cooled, add 1ml of the freshly mixed complex framing reagent. Allow the mixture stand for 10min at the room temperature. Finally, add 0.1 ml of folin reagent mix with the sample and vortex. For 60 minutes, the mixture should be kept at room temperature.

Evaluate the result 750 nm absorbance and the outcome was then corresponded with the bovine serum albumin (BSA) standard curve [20].

Screening of Phytochemical compounds

Contents of total phenols (CTP) estimation

The mixture of 500mL of Folin–Ciocalteu's phenol reagent and 1ml of H_2O were added to the 50 ml of microalgae extract. The mixture was then treated with 2.5mL of a 20% Na2CO3 solution before resting in the dark for 45 minutes at ambient temperature. The absorbance of the resulting mixing was estimated to be 735 nm when assessed against a blank. Gallic acid was applied to create a standard curve (0.025–0.6mgmL-1). The estimate was given in milligram of gallic acid equivalents (GAE) per gram of extract (dw) [22].

Contents of total Flavonoids (CTF) estimation

After 6 minutes of mixing an aliquot (250 mL) of each extract or standard solution with 1.25 mL of H2O and 75 mL of 5 percent NaNO2 solution, 150 mL of 10% AlCl3 H2O solution was then added [23]. After 5 minutes, add 0.5mL of 1M NaOH solution, followed by H2O to create the entire volume 2.5mL. At 510 nm, the absorbance was read against a blank after fully mixing the solution. The standard curve was established with catechin (0.05–0.5 mg/mL-1). The findings was expressed in milligrams of catechin equivalents (CE) per gram of catechin extracted (dw).

Contents of condensed tannins (CCT) estimation

1.5 mL of 4 percent vanillin (produced with MeOH) and 750 mL of HCl were added to a 50 mL volume of extract or standard solution. For 20 minutes, The well-mixed formulation was incubated at room temperature in the dark (22˚ C). The absorbance was read at 500 nm in comparison to a blank. The standard curve (0–1 mgmL-1) was produced utilizing (þ)-catechin. The values were presented in milligram of catechin equivalents (CE) per gram of catechin extracted (dw) [22].

Content of Pigment

500 mg of microalgal powder were suspended in 10 mL of 80% acetone and centrifuged (3000 rpm, 15 min). The pellet was resuspended with acetone at an 80% concentration (5 mL) and centrifuged several times until it was colorless. The pigments were measured using the supernatant (Arnon 1949). The absorbance of chlorophyll (645 and 663 nm) was read by a spectrophotometer (UV-1800, Shimadzu) as follows [24]:

Where A = absorbance at the corresponding wavelength, Chlorophyll a (g/mL) = 12.7 (A663) 2.69 (A645), and Chlorophyll b (g/mL) = 22.9 (A645) 4.68 (A663).

The amount of carotenoid was also determined in a spectrophotometer (UV-1800,Shimadzu) using the Kirk and Allen (1965) technique at 480 mg. fresh wt. carotenoids = A480+ (0.114 A663) (0.638 A645),

where A = absorbance at the surface wavelength.

The Antibacterial activity

Microorganisms are used in the testing

Various extracts of *Scenedesmus* sp were tested for antimicrobial activity against five species of pathogenic bacteria (*Vibrio cholera, Pseudomonas, Streptococcus, Escherichia coli* and *Klebsiella pneumonia*) were bought from Raja Muthaiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, India.

Method of diffusion using agar wells

The agar well diffusion method is being used for the bioactivities of various Scenedesmus solvent extracts against all of the tested bacterial species were investigated. Muller Hinton Agar medium was prepared and poured onto Petri plates before solidifying. Agar plates were punched with a 4 mm sterile cork borer. In the bore, 100 µlof microalgal extract solvents (chloroform, ethanol, diethyl ether, and methanol) were poured with a micropipette. For 24 hours, the plates were incubated at room temperature. The antibacterial effect was observed as crescent-shaped inhibition zones [13].

RESULTS AND DISCUSSION

Growth rate

The growth rate of the culture was monitored from the lag phase to the death phase. The optimal growth conditions are given to the *Scenedesmus* sp have its growth rate in 14 days. It can be measured by the optical density by spectrophotometer [26]. The highest growth in stationary phase was observed at 12^{th} day (0.69 cells $\times 10^5$) (Graph.1). The biomass was harvested by filtration and the wet biomass and dry biomass was weighed and recorded in table 1.

Estimation of Biochemical composition and pigment analysis:

Previous studies [27, 28] have proven similar results to this one. *Scenedesmus sp* was analyses for the presence of biochemicals namely carbohydrate, protein, and lipid in a laboratory-culture. In *Scenedesmus*

sp, carbohydrate content is 34.56±1.28 percent, protein content is 40.75±3.09 percent and lipid content is 37.59±0.88 percent (Graph. 2). When *Scenedesmus sp*. is cultured, it is analyses for the presence of pigments like chlorophyll a, b, and carotenoid. There are 4.16±0.05 and 3.96±0.08 g/ml of chlorophyll a and b, respectively, and 2.30.89 g/ml of carotenoids (Graph. 3).

Screening of phytochemical compounds

The efficiency of antibiotics which are derived from microalgae is influenced by their several phytochemical compounds as active secondary metabolites. This study assesses phytochemical compounds, namely total phenols, total flavonoids, and condensed tannins in various solvent (ethanol, methanol, chloroform and diethyl ether) extract of *scenedesmus sp*. Each *scenedesmus sp* extract contains an adequate amount of phenolic compounds. Its findings are respectively 26.78±0.215, 39.16±0.200, 46.87±0.080 & 37.72±0.260 (Graph. 4). As same as above solvents are used in flavonoid screening and their results are 23.08±0.03, 30.56±0.02, 36.25±0.02 & 32.44±80.02 respectively. The results were expressed in mg/g.

Because of their low side effects and ability to remain active for an extended period of time, microalgae are gaining popularity as a therapeutic agent. In the phytochemical analysis, two bioactive compounds (phenols and flavonoids) were abundant, but tannin was present in such small amounts that it was undetectable in all samples.

In prior reports, phytochemicals derived from *Scenedesmus sp* were used for pharmacological purposes [29]. Screening of phytochemical compounds in *Chlorella sp, Scenedesmus sp* and *Spirulina sp* was done by earlier investigations [30].

Analysis of antibacterial activity:

Graph. 4 *Scenedesmus* sp extracts were used to evaluate the 5 pathogenic microbes. The ethanol extract of *Scenedesmus* sp had the greatest zone of inhibition against E. coli (13mm) and the minimum activity in *Pseudomonas aeruginosa* (8mm).In *V.cholera* (12mm), the methanol extract demonstrated the greatest zone of inhibition, where as *Klebsiella pneumonia* showed the least (6mm). Chloroform extract studies revealed a maximum zone of inhibition (12mm) against *Klebsiella pneumonia* and a minimum zone of inhibition in *V. cholera* (7mm).And finally, the results of diethyl ether extract showed highest zone of inhibition against *Streptococcus* (13mm) and lowest activity was measured in *Vibrio cholera* (6mm) (Figure. 1).

In previous study, *Scenedesmus sp* crude extract showed adequate antibacterial action against the food poisoning pathogen *Staphlococcus aureus*. As a result, the antibacterial activities of the *Scenedesmus sp.* pigment extracts are not effective on Salmonella sp. [14]. *Scenedesmus quandricanda* methanolic extract has antibacterial on against *E.coli, Bacillus cereus*, and *Staphylococcus aureus*, whereas ethanol, acetone, and diethyl ether extracts exhibit antibacterial activity against *B.cereus* and *S.aureus. P.aeruginosa,* on the other hand, is resistant to *S.quandricanda* extracts namely ethanol, acetone, diethyl ether, and methanol [15].

Microalgae as a Potential Source of New therapeutics was investigated by earlier reports [16], [17], [18]. They come to the conclusion that tiny molecular secondary metabolites can be used as priority structures in the creation of novel antibiotics.

Hence, Drug resistance in medical pathogens can be overcome by citing new citations on active principles from microalgae. To develop a new class of drugs, a commercially viable strategy that is high throughout the drug screening system should be developed. Despite the fact that microalgae have been shown to have antibacterial activity, these substances have only been used in a few cases for pharmaceutical purposes.

Table 1: Optimum growth factors of *Scenedesmus* sp.

S.no	Growth factor	Values
1.	pH	7.6-8.3
2.	Temperature	24ºC
3.	Light	2500 lux
4.	Wet weight	4.93 g/L
5.	Dry weight	1.68 g/L

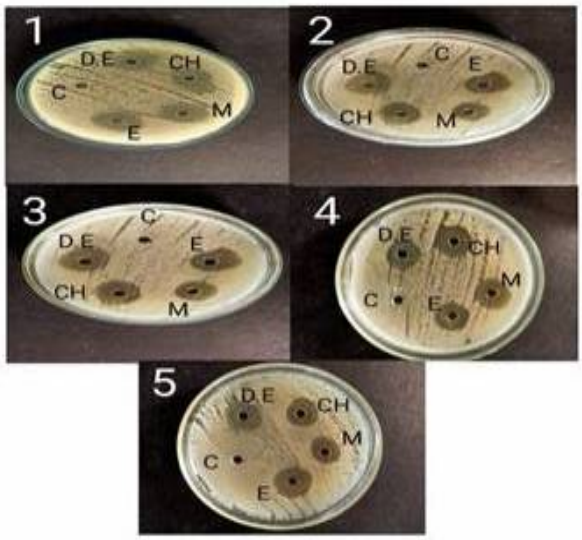
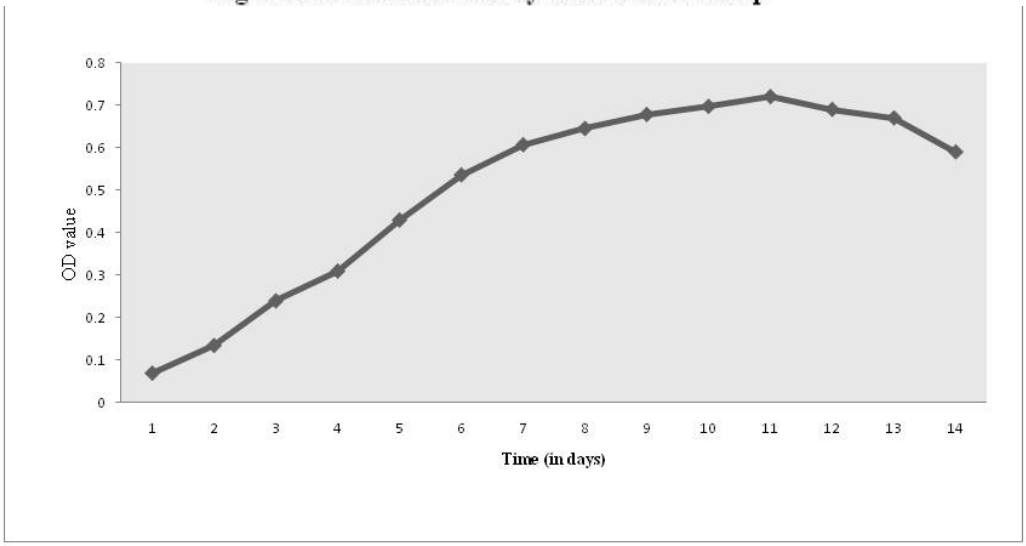
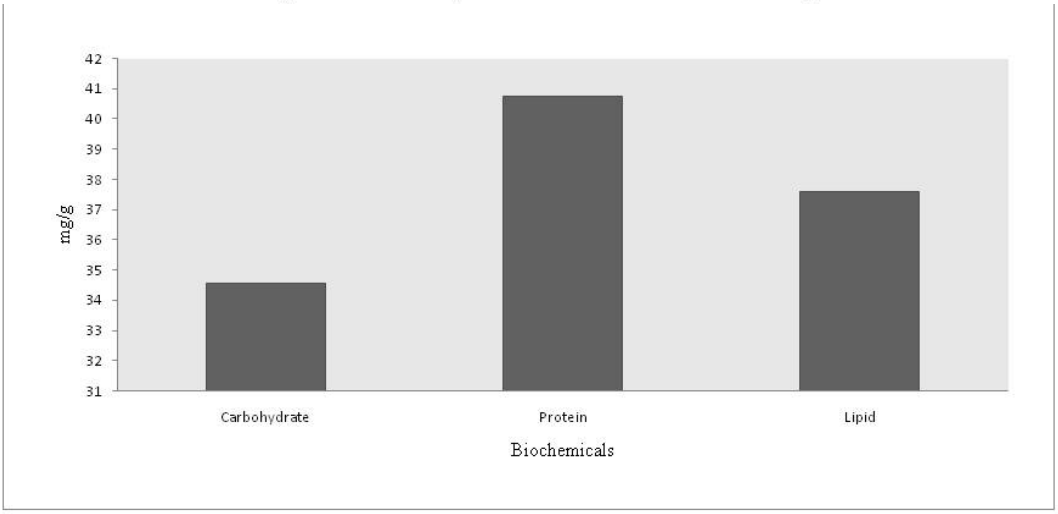


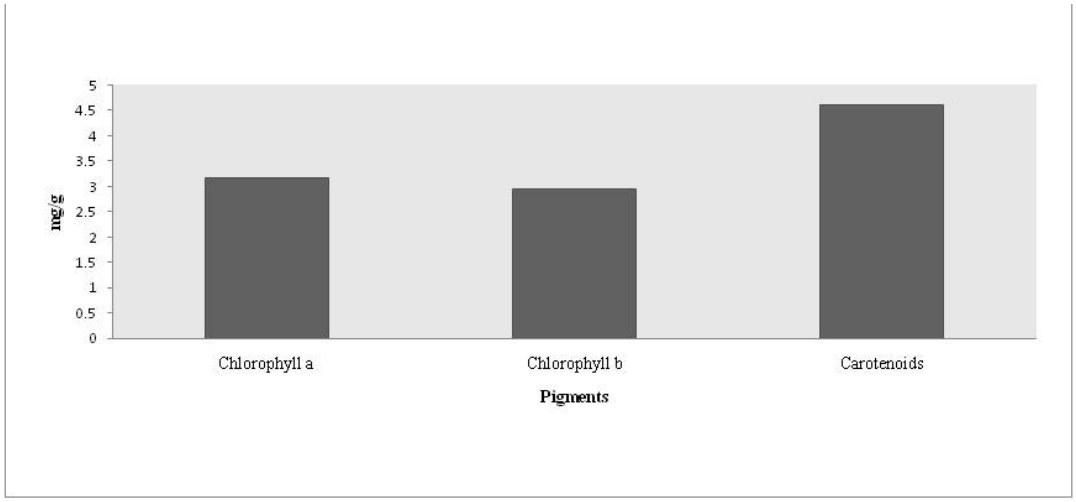
Fig 1. Antibacterial activity of *Scenedesmus* sp



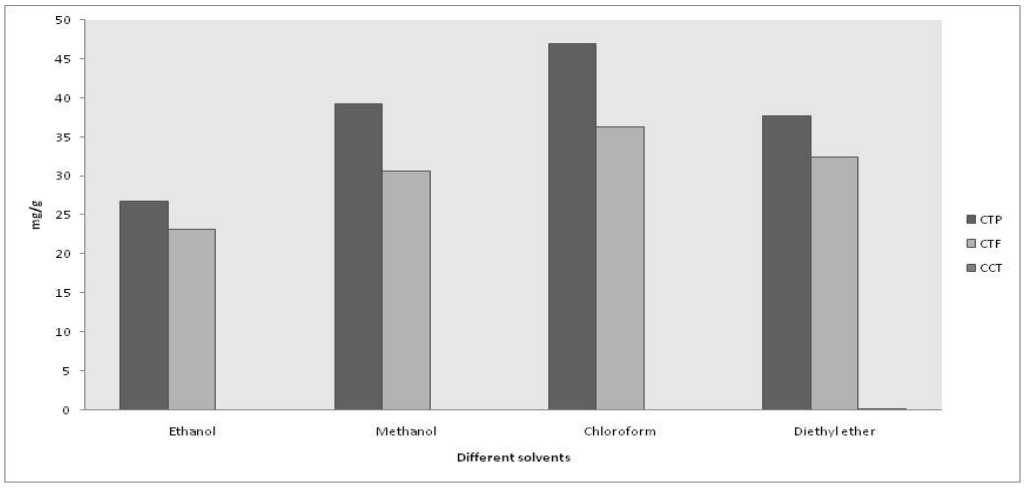
Graph 1: Growth performance of *Scenedesmus sp*



Graph 2: Biochemical analysis of *Scenedesmus sp*

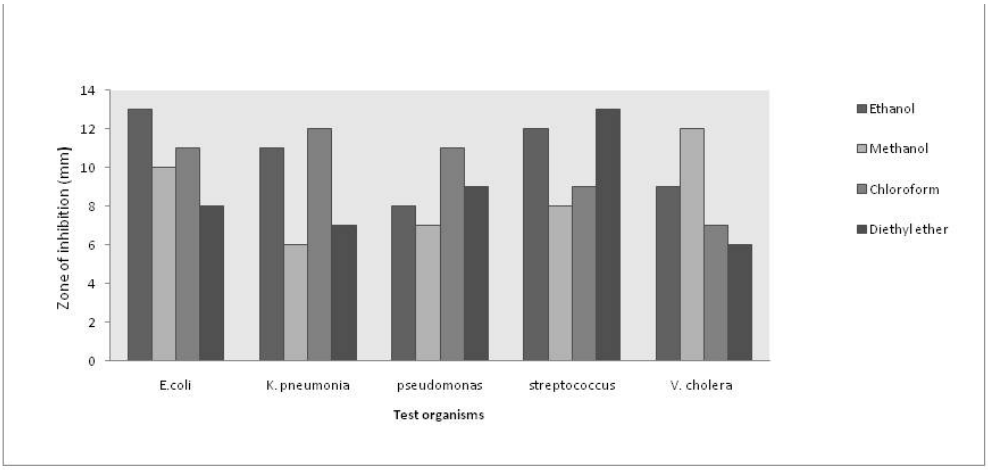


Graph 3: Pigments analysis of *Scenedesmus* sp



Graph 4: Phytochemical screening of *Scenedesmus* sp

CTP – Contents of total phenols, CTF – contents of total Flavonoids and
CCT – contents of condensed tannins



Graph 5: Antibacterial activities of *Scenedesmus sp*

CONCLUSION

The study's goal was to look into the best growth factors for *Scenedesmus* sp. biomass production. It also concentrates on the estimation of pigments such as chlorophyll a, b, and carotenoids, as well as biochemical compounds such as carbohydrate, protein, and lipid content. The disc diffusion technique was used to test the antibacterial activity against *Klebsiella pneumoniae*, *E.coli*, *Pseudomonas*, *Streptococcus,* and .*V.cholerae*.

The relevance of *Scenedesmus* sp species in reducing pathogenic bacteria that pose a hazard to human health has been established in this study. This scientific information may be utilized to provide the groundwork for the creation of low-cost, safe and effective natural medications.

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ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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