Unveiling the Therapeutic Potential: "Invitro Assessment of Antioxidant and Anticancer Properties in Blue-Green Algae Extract"

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Abstract

The abstract aims to explore the therapeutic potential of blue-green algae through in vitro assessment, specifically focusing on antioxidant and anti-cancer properties. Utilizing laboratory techniques, we assess the algae's ability to neutralize oxidative stress and inhibit cancer cell growth. Preliminary findings suggest promising outcomes, indicating significant antioxidant activity and potential anti-cancer effects. For understanding the anti-cancer and anti-oxidant characterization of blue-green algae, the following tests were analysed, MTT Assay, IC_{50}, % of inhibition, DPPH, FRAP, NO_2, H_2O_2. The MTT assay is commonly used for characterizing blue-green algae to assess their metabolic activity and viability. The determination of IC_{50} (half maximal inhibitory concentration) for blue-green algae is often performed to assess the effectiveness of various substances, such as inhibitors or toxins, on their growth or metabolic activities. The percentage of inhibition (% inhibition) is often used to characterize blue-green algae to assess the effectiveness of inhibitors or treatments on their growth or activity. DPPH is a purple stable free radical that delocalizes electron patterns and prevents dimerization in the whole molecule of DPPH and is also responsible for the molecule's color. The FRAP assay is commonly used in the characterization of blue-green algae to assess their antioxidant capacity. Hydrogen peroxide (H_2O_2) is often used in the characterization of blue-green algae to assess the oxidative stress response and overall physiological status of the algae. Nitrogen dioxide (NO_2) is often used in the characterization of blue-green algae to study their response to nitrogen-based signaling molecules. This investigation not only contributes to the scientific understanding of blue-green algae but also holds implications for future drug development and the development of complementary therapies for cancer treatment. The detailed exploration of its properties provides a foundation for potential applications in the realm of health and medicine.

Keywords: Blue-green algae, antioxidant, anticancer, DPPH, FRAP, H2O2, NO2, MTT Assay

1. INTRODUCTION

Lung

The lungs are essential organs vital for oxygenating blood and expelling carbon dioxide through the process of respiration. Found in the chest, these spongy, air-filled organs are pivotal in the respiratory system, facilitating the exchange of oxygen and carbon dioxide between the air and the bloodstream.

Upon inhalation, air flows into the lungs through the trachea, progressing into smaller tubes known as bronchi and bronchioles. Culminating at these airways are minuscule air sacs called alveoli. During this intricate process, oxygen traverses the thin alveolar walls into the bloodstream, while carbon dioxide, a metabolic byproduct, moves from the blood into the alveoli to be expelled during exhalation.

Lung Cancer

Lung cancer manifests as a form of malignancy originating in the lungs, typically affecting the cells lining the air passages. Although closely linked to smoking, it's noteworthy that non-smokers can also develop lung cancer. Recognizable symptoms encompass a persistent cough, chest pain, breathing difficulties, and weight loss. The two primary classifications of lung cancer are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Treatment modalities vary based on cancer type and stage, encompassing options such as surgery, chemotherapy, radiation therapy, targeted therapies, or immunotherapy. Timely identification plays a pivotal role in enhancing outcomes. Hence, individuals displaying risk factors or symptoms should promptly seek medical attention. Strategies like smoking cessation and minimizing exposure to lung carcinogens contribute to lowering the risk of developing lung cancer.

Algae

Algae form a diverse group of photosynthetic organisms, ranging from small single-celled entities to large multicellular structures. Flourishing in different environments such as freshwater, saltwater, and damp terrestrial areas, algae play a crucial role in the ecosystem. They contribute significantly by producing oxygen through photosynthesis and serving as foundational components in aquatic food chains.

Blue-Green Algae

Blue-green algae, or cyanobacteria, offer both advantages and potential drawbacks. On the positive side, they are nutrient-rich, containing proteins and vitamins, which makes them a valuable food source for specific organisms. Some cyanobacteria contribute to nutrient cycles through nitrogen fixation, while certain species actively participate in the bioremediation of polluted water. In the field of research, cyanobacteria are being explored for various biotechnological applications. However, caution is essential due to the potential toxicity of certain cyanobacteria, posing health and environmental risks,

especially during visible algae blooms. Achieving a balance between their potential benefits and associated risks is crucial for the responsible management of aquatic ecosystems.

Medical benefits of blue-green algae

Blue-green algae, also known as cyanobacteria, present a range of potential medical benefits. These microorganisms boast a nutrient-rich composition, including proteins, vitamins, and minerals, making them valuable for addressing nutritional deficiencies. Moreover, blue-green algae exhibit antioxidant properties, aiding in neutralizing free radicals and reducing oxidative stress. Certain compounds within these algae showcase anti-inflammatory effects, suggesting potential applications for conditions associated with inflammation. In addition, some studies suggest neuroprotective effects, contributing to brain health. Blue-green algae may support the immune system, enhance certain immune functions, and show promise in weight management due to their nutrient density. Preliminary research also explores their potential cardiovascular benefits, influencing factors like cholesterol levels. Furthermore, certain species are investigated for their role in detoxification, assisting in the elimination of toxins from the body.

Incorporation of blue-green algae for cancer treatment

Blue-green algae, particularly spirulina, have been explored for their potential contributions to cancer treatment through several key mechanisms:

1. Immunomodulation: Spirulina's ability to regulate the immune system has been under investigation. Studies suggest that it may enhance specific immune functions crucial for identifying and eliminating cancer cells.

2. Antioxidant Properties: The presence of antioxidants in blue-green algae, such as spirulina, is noteworthy. These antioxidants play a role in combating oxidative stress, which is closely associated with cancer development and progression. By neutralizing free radicals, they promote overall cellular health.

3. Anti-Inflammatory Effects: Chronic inflammation is implicated in cancer development, and the anti-inflammatory properties of blue-green algae, including spirulina, have been subject to study. Research indicates that these properties may have a positive impact on reducing inflammation, potentially influencing the advancement of cancer.

4. Apoptosis Induction: Blue-green algae compounds have been explored for their potential to induce apoptosis, or programmed cell death, in cancer cells. This natural process helps eliminate damaged or abnormal cells, including cancerous ones, and research aims to determine whether blue-green algae compounds can facilitate this process in cancer treatment.

Problem Statement

Finding bioactive compounds having antimicrobial, antioxidant, and anticancer properties from blue-green algae extract.

Problem Solution

Immunomodulation along with the antioxidant properties of blue-green algae make it an ideal candidate for lung cancer treatment. The immunomodulation helps to modulate, identify, and eliminate cancerous cells. The antioxidant property helps it combat oxidative stress and neutralizes the free radicals. Another important feature of blue-green algae is that it helps apoptosis or helps to eliminate damaged cells which result in abnormal growth and lead to cancer. It is one of the few first efforts to study the effect of algae on cancer treatment rather than using harmful radiation and chemotherapy. Radiation and chemotherapy can have various side effects and have negative results on the person.

2. OBJECTIVE OF RESEARCH

The main objective of this project is to produce blue-green algae (spirulina) and its extraction for treating lung cancer. The ideal variety for producing spirulina is selected. A microalgae suspension is prepared as the media for growth. In which the concentrated microalgae slurry and water are taken in a 1:4 ratio and kept at 6°C.The growth time for spirulina is 2-3 weeks. It is agitated for almost 30 minutes. After that centrifugation is done at 4000 rpm for 2 mins. Then ultrasonication is done for cell disruption. From this, the pellet is collected cleaned, and powdered. This sample is filtered using inline sivtek vibro sifter. The filtered sample is then used for further processing.

2.1 Antimicrobial activity: The Blue-green algae, particularly spirulina, have emerged as promising contenders in the realm of natural antimicrobial agents, showcasing substantial activity against various microorganisms. Central to this antimicrobial prowess is the presence of phycocyanin, a pigment within spirulina, known for its inhibitory effects against both Gram-positive and Gram-negative bacteria. Additionally, chlorophyll, peptides, and proteins originating from spirulina contribute to its antimicrobial repertoire, exhibiting inhibitory activity against a diverse spectrum of microorganisms, including fungi. Lipopolysaccharides and fatty acids found in blue-green algae further contribute to their antimicrobial potential, showcasing efficacy against various pathogens. While laboratory studies have unveiled encouraging findings, the practical application of blue-green algae, especially spirulina, as a standalone treatment for microbial infections in humans remains an evolving area of research. The effectiveness may be contingent on factors such as the specific strain of blue-green algae and the targeted microorganism. Integrating blue-green algae into a well-balanced diet may offer potential health benefits, yet consulting with healthcare professionals is imperative, particularly for individuals with underlying health conditions or those undergoing medication regimens.

2.2 Antioxidant activity: Blue-green algae, notably spirulina, exhibit remarkable antioxidant activity owing to the presence of various bioactive compounds. Central to this capability is phycocyanin, a vibrant blue pigment abundant in spirulina, known for its efficacy in neutralizing free radicals and mitigating oxidative stress. Additionally, the beta-carotene content in spirulina contributes to its antioxidant prowess, scavenging free radicals and supporting cellular health. Enzymes such as superoxide dismutase (SOD) further enhance the antioxidant defense by converting harmful superoxide radicals into less destructive forms. The inclusion of vitamin E in spirulina complements its antioxidant profile, safeguarding cell membranes from oxidative damage.

2.3 Anticancer activity: Research into the potential anticancer effects of blue-green algae, specifically spirulina, has yielded promising insights on multiple fronts. Spirulina's abundance in antioxidants, including phycocyanin and beta-carotene, equips it with the capability to neutralize free radicals associated with cellular damage and cancer development. Moreover, the algae exhibit anti-inflammatory properties, a significant factor in impeding cancer progression. Spirulina's potential in immunomodulation, enhancing specific immune functions crucial for recognizing and eliminating cancer cells, adds to its appeal in cancer research. Studies indicating the ability of spirulina to induce apoptosis, or programmed cell death, in cancer cells present a compelling aspect of its anticancer potential. Animal studies reinforcing the algae's capacity to inhibit tumor growth contribute to the growing body of evidence. Despite these encouraging findings, a cautious approach is essential, emphasizing the need for further clinical research to ascertain the safety and efficacy of spirulina as a complementary or alternative strategy in cancer management. Individuals contemplating such interventions should seek professional guidance from healthcare providers before integrating them into their treatment plans.

3. MATERIALS AND METHODS

Sample collection

The blue-green algae were collected from the outskirts of Coimbatore and washed in water to remove dust. Then the algae were shade-dried for about 10 – 15 days.

Solvent extraction

The dried blue-green algae were coarsely powdered using an electric mixer and then subjected to extraction using the Soxhlet apparatus. The extract was then stored and used.

3.1 PHYTOCHEMICAL ANALYSIS

In conducting qualitative phytochemical analysis, various standard tests were employed on the extracts.

3.1.1 Test for Alkaloids (Wagner’s test):

A few milliliters of blue-green algae extract underwent treatment with 4-5 drops of Wagner’s reagent. Confirmation of the presence of alkaloids was indicated by the formation of a reddish-brown precipitate.

3.1.2 Test for Phenol (Ferric chloride test):
Approximately 2ml of the extract was subjected to 10% ferric chloride solution, and the observation for the formation of a deep blue/black color was noted.

3.1.3 Test for Reducing Sugars (Fehling's Test):
The extract (1 ml) received a few drops of Fehling's reagent and underwent boiling in a water bath for 10 minutes. The appearance of a blue color signified the presence of reducing sugars.

3.1.4 Test for Saponins (Foam test):
2 ml of the blue-green algae extract was mixed with 6 ml of water in a test tube, shaken vigorously, and observed for the formation of persistent foam, confirming the presence of saponins.

3.1.5 Test for Flavonoids: The addition of a few drops of 10% ferric chloride solution to 2ml of blue-green algae extract resulted in the formation of a green or blue color, indicating the presence of flavonoids.

3.1.6 Test for Phytosterols (Salkowski's Test):
Treating 1 ml of blue-green algae extract with 2 ml of chloroform and adding acetic anhydride followed by concentrated sulfuric acid resulted in a bluish-green color, confirming the presence of phytosterols.

3.1.7 Test for Amino Acids and Proteins (Ninhydrin test):
A few milliliters of blue-green algae extract, when mixed with a small amount of Ninhydrin reagent, displayed a purple or violet color, indicating the presence of amino acids and proteins.

3.1.8 Test for Steroids:
Combining 2 ml of chloroform and 0.2 ml of concentrated sulfuric acid with 1 ml of flower extract led to the formation of a red color precipitate, confirming the presence of steroids.

3.1.9 Test for Tannin:
1ml of blue-green algae extract, upon the addition of dilute ferric chloride solution, displayed a dark green or blue color, confirming the presence of tannin.

3.1.10Test for Glycosides:
The addition of concentrated sulfuric acid to 1 ml of blue-green algae extract resulted in the formation of a red color, indicating the presence of glycosides.

3.2 ANTIOXIDANT ACTIVITY

3.2.1 DPPH radical scavenging activity

The assessment of free radical scavenging activity in the methanolic extract of a plant involved using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Following the method outlined by Braca et al. (2001), 3 ml of 0.004% DPPH solution in methanol was mixed with 0.5 to 2.5 µl of plant extract/ascorbic acid at different concentrations. After vigorous shaking, the mixture reached a steady state at room temperature for 30 min, and decolorization of DPPH was gauged by measuring absorbance at 517 nm. A control, substituting plant extract/ascorbic acid with 0.1 ml of the respective vehicle, was prepared.

The percentage inhibition of DPPH radicals by the extract/compound was determined by comparing absorbance values between the control and experimental tubes.

$$\text{Scavenging activity \%} = \frac{\text{A518 (control) - A518 (sample)=045}}{\text{A518 (control)}} \times 100$$

3.2.2 Ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacity of algal extract samples was assessed following the protocol outlined by Benzie and Strain (1996) with modifications by Pulido et al. (2000). Freshly prepared FRAP reagent (900 µl), incubated at 37 ºC, was combined with 90 µl of distilled water and 30 µl of the test sample or acetone (for the reagent blank). Incubation at 37 ºC for 30 minutes in a water bath ensued for both the test samples and reagent blank. The FRAP reagent comprised 2.5 ml of 20 mmol/l TPTZ solution in 40 mmol/l HCl, along with 2.5 ml of 20 mmol/l FeCl3.6H2O and 25 ml of 0.3 mol/l acetate buffer, pH 3.6 (Benzie and Strain, 1996). Post-incubation, absorbance readings were promptly recorded at 593 nm using a spectrophotometer. A calibration curve was prepared using known Fe (II) concentrations ranging from 100 to 2000 µmol/l (FeSO4.7H2O). The Equivalent Concentration (EC1) was defined as the antioxidant concentration with ferric-TPTZ reducing ability equivalent to 1 mmol/l FeSO4.7H2O. EC1 was calculated based on the absorbance increase in the FRAP assay, matching the theoretical absorbance value of a 1 mmol/l Fe (II) solution using the corresponding regression equation.

$$\text{Scavenging activity \%} = \frac{\text{A518 (control) - A518 (sample)=0.422}}{\text{A518 (control)=0.459}} = 0.91 \times 100 = 91\ \%$$

3.2.3 Nitric oxide (NO) scavenging activity

The assessment of NO scavenging activity for the sample followed the methodology outlined by Garrat DC. in 1964. Briefly, 400 µL of 100 mM sodium nitroprusside, 100 µL of PBS (pH 7.4), and 100 µL of varying concentrations of algal extract were mixed and incubated at 25°C for 150 minutes. Following this, 0.5 mL of the resulting solution was combined with 0.5 mL of Griess reagent (0.1 mL of sulfanilic acid and 200 µL of naphthyl ethylenediamine dichloride at 0.1% w/v).

After a 30-minute incubation at room temperature, absorbance was measured at 540 nm. All experiments were carried out in triplicates, and the percentage inhibition was calculated using the specified formula.

% Inhibition = (A control-A sample) /A control) X 100

3.3 ANTIBACTERIAL ACTIVITY

To perform antimicrobial activity using various bacterial and fungal species were selected viz., Escherichia coli, staphylococcus aureus, pseudomonas species, bacillus species, klebsiella species; proteus species are bacterial cultures.

Media and culture condition:

Muller-Hinton Agar (MHA), Nutrient Broth (NB) and Luria Britani (LB) were used throughout the study for determining the antibacterial assay. The media was adjusted to the pH and autoclaved at 121°c for 15 minutes.

Preparation of the bacterial inoculum

Maintaining stock cultures at 4°C on nutrient agar and potato dextrose agar slopes, active cultures for experiments were prepared by transferring a loop full of cells from the stock to 50ml test tubes with nutrient broth. Bacterial cultures were incubated with agitation at 37°C for 24 hours in a shaking incubator, while fungal cultures were incubated at 27°C for 3-5 days. Subsequently, each test organism's suspension was streaked out on nutrient agar and potato dextrose agar. Bacterial cultures were then incubated at 37°C for 24 hours, and fungal cultures were incubated at 27°C for 3-5 days. A single colony was transferred to nutrient agar slants, incubated at 37°C for 24 hours, and potato dextrose slants were incubated at 27°C for 3-5 days. These stock cultures were stored at 4°C. For experiments, a loop of each test organism was transferred into 50ml nutrient broth and incubated separately at 37°C for 18-20 hours for bacterial cultures.

Well Diffusion method:

The antibacterial and antifungal activities of the crude extracts were assessed using the Well Diffusion method (Bauer et al., 1996). Molten Mueller Hinton Agar (MHA) plates were prepared by pouring 20ml of the medium into sterile petriplates, which solidified subsequently. A bacterial inoculum suspension (20-25µl) was uniformly swabbed onto the plates. Sterile paper discs dipped in the required solvents, were placed on the agar plates, and 10-50 µl of algal extract was poured into the wells. Following an incubation at 37°C for 24 hours, the assay was performed in triplicates, with control plates maintained. The zone of inhibition was measured from the well edge to the zone in mm. For the fungal assay, the tested cell suspension was spread on Muller Hinton Agar and Potato Dextrose Agar plates. Wells were placed into the agar medium using sterile forceps, and algal extracts were poured onto the wells. After incubating at 37°C for approximately 24 hours, including control plates, the zone of inhibition was measured from the clear zone in mm.

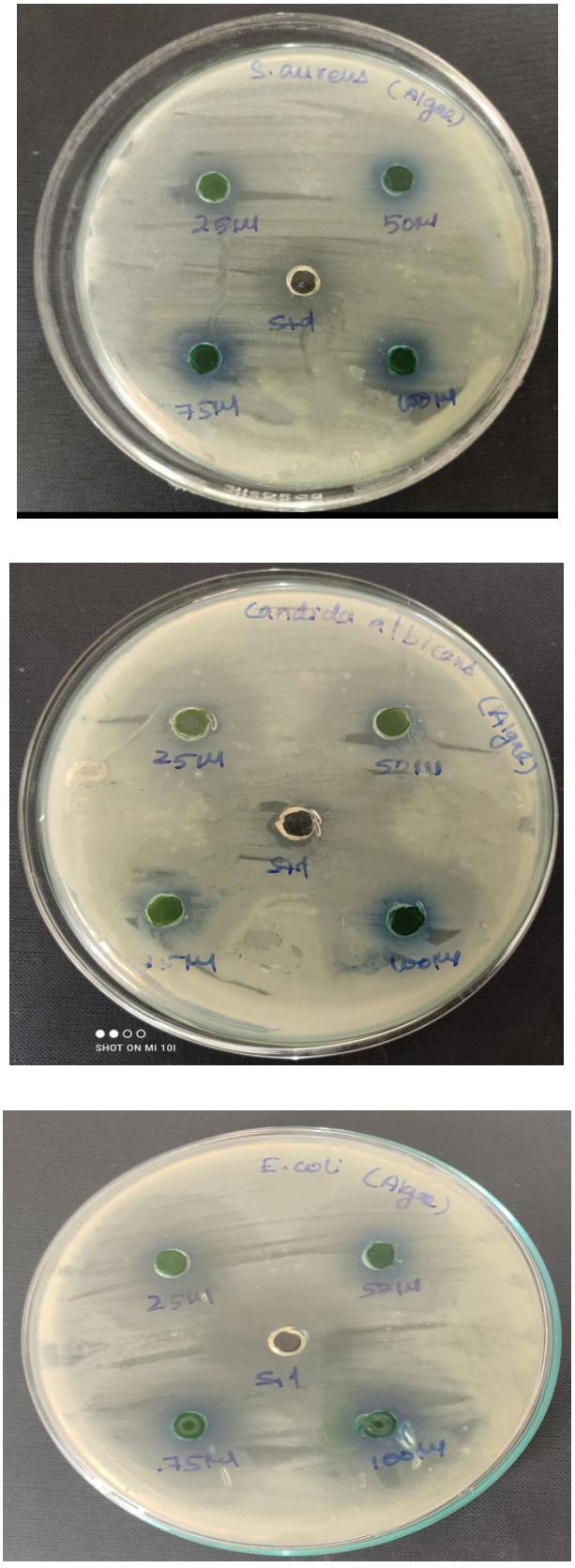


Fig 1: Antibacterial activity of the blue-green algae extracts against S. aureus, E. coli and Candida albicans

3.4 IN-VITRO ANTICANCER ACTIVITY

Cell line:

The Hep-G2 human liver cancer cells were acquired from the National Centre for Cell Science (NCCS), Pune, and cultivated in Eagles Minimum Essential Medium supplemented with 10% fetal bovine serum (FBS). The cells were cultivated at 37°C, in an atmosphere of 5% CO2, 95% air, and 100% relative humidity. Weekly passages were performed for maintenance cultures, and the culture medium was refreshed twice a week.

Cell treatment procedure:

For cell treatment, monolayer cells were detached using trypsin-ethylene diamine tetra acetic acid (EDTA) to create single-cell suspensions. Viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to achieve a final density of 1x105 cells/ml. Subsequently, 100 microliters per well of the cell suspension were seeded into 96-well plates at a plating density of 10,000 cells/well. The plates were incubated for cell attachment at 37°C, 5% CO2, 95% air, and 100% relative humidity. After 24 hours, the cells underwent treatment with serial concentrations of the test samples. These samples were initially dissolved in neat dimethylsulfoxide (DMSO), and an aliquot of the solution was diluted to twice the desired final maximum test concentration with serum-free medium. Four additional serial dilutions were prepared to achieve a total of five sample concentrations. Aliquots of 100 µl from these different sample dilutions were added to the appropriate wells containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 hours under conditions of 37°C, 5% CO2, 95% air, and 100% relative humidity. Medium without samples served as the control, and triplicates were maintained for all concentrations.

3.4.1 MTT assay

MTT, a yellow water-soluble tetrazolium salt, undergoes conversion to an insoluble purple formazan by the mitochondrial enzyme succinate dehydrogenase in living cells. This reaction, indicative of viable cell count, was initiated by adding 15µl of 5mg/ml MTT in PBS to each well after 48 hours of incubation at 37°C. Following a 4-hour incubation, the MTT-containing medium was removed, and formazan crystals were dissolved in 100µl of DMSO. Absorbance at 570 nm was then measured using a microplate reader.

Cell viability was calculated as,

% Cell viability = [A] Test / [A] Control x 100

and % Cell Inhibition was determined by,

100 - Abs (sample)/Abs (control) x 100

The nonlinear regression graph of % Cell Inhibition versus Log concentration was plotted, and IC50 was determined using Graph Pad Prism software.

4. RESULTS

4.1 PHYTOCHEMICAL ANALYSIS

Standard tests were conducted in the qualitative phytochemical study of the extracts. The confirmation of Alkaloids was indicated by the formation of a reddish-brown precipitate, while the presence of reducing sugars was evidenced by the appearance of a blue color. Saponins were confirmed by the formation of persistent foam, and the presence of flavonoids was denoted by a green or blue color. Phytosterols were identified by a bluish-green color, amino acids and proteins by a purple or violet color, and steroids by a red color precipitate. Tannin presence was confirmed by a dark green or blue color, while the formation of a red color indicated the presence of glycosides.

TABULATION OF PHYTOCHEMICAL ACTIVITY

PHYTOCHEMICAL TEST	REAGENTS USED	RESULTS
Alkaloids	Wagners reagent	Reddish Brown(+++)
Flavanoids	NaOH(2 drops)	Green(+++)
Steroids	Chloroform+H2So4	Red Color precipitate(++)
Ancinoacids&proteins	Ninhydran+waterbath	Negative(-)
Phytosteroids	1 drop H2So4	Bluish Green(+++)
Tanin	1% Ferric chloride	Blue colour(+++)
Reducing sugar	Fellin's solution	Blue colour(+++)
Phenol	Ferric chloride (10%)	Deep blue colour(+++)
Saponins	water	Present foam(++)

Table No 1: Tabular column showing the presence of different phytochemicals in the blue-green algae extract

4.2 ANTIOXIDANT ACTIVITY

4.2.1 DPPH Assay:

The decolorization of DPPH was assessed by measuring absorbance at 517 nm. A control was established using 0.1 ml of the respective vehicle instead of the algal extract/ascorbic acid. To determine the percentage inhibition of DPPH radicals by the extract/compound, absorbance values of the control and experimental tubes were compared.

Sample	DPPH Solution	Methanol	Result
20 µl	500	480	S1=0.652
40 µl	500	460	S2=0.535
60 µl	500	440	S3=0.316
80 µl	500	420	S4=0.237
100 µl	500	400	S5=0.119
Control	500	500	0.791

Table 2: Tabulated results of the antioxidant activity of the sample at different concentrations using the DPPH assay

4.2.2 FRAP Assay:

The antioxidant concentration demonstrated a ferric-TPTZ reducing ability equivalent to 1 mmol/l FeSO4.7H2O. EC1 was determined as the antioxidant concentration that resulted in an absorbance increase in the FRAP assay, comparable to the theoretical absorbance of a 1 mmol/l Fe (II) solution, calculated using the corresponding regression equation.

Sample	FRAP Reagent	H20	Result
20 µl	900	80	S1=0.844
40 µl	900	60	S2=0.744
60 µl	900	40	S3=0.679
80 µl	900	20	S4=0.457
100 µl	900	0	S5=0.254
Control	900	100	0.928

Table 3: Tabulated results of the antioxidant activity of the sample at different concentration using FRAP assay

4.2.3 Nitric oxide (NO) scavenging activity:

The assessment of antioxidant activity in the algal extract involved nitric oxide scavenging activity using varying concentrations of 0.2µl, 0.4µl, 0.6µl, 0.8µl, and 1.0µl. Absorbance readings were taken at 540 nm. All reactions were conducted in triplicates, and the percentage inhibition was determined using the following formula.

Sample	Reagent	pbs	Griss	Result
20 µl	400	480	100	S1=0.533
40 µl	400	460	100	S2=0.415
60 µl	400	440	100	S3=0.359
80 µl	400	420	100	S4=0.233
100 µl	400	400	100	S5=0.124
Control	400	500	100	0.848

Table 4: Tabulated results of the antioxidant activity of the sample at different concentration using NO scavenging activity

4.2.4 Hydrogen peroxide scavenging activity (H_2O_2)

In the evaluation of hydrogen peroxide scavenging activity, the absorbance of the reaction was measured at a wavelength of 230 nm. This specific wavelength is commonly used to assess hydrogen peroxide levels in a solution. As a benchmark, BHT (butylated hydroxytoluene) was employed as the positive control, ensuring a comparative reference for the antioxidant activity of the tested sample. The experimental procedure was conducted in triplicates, involving the repetition of the entire process three times to enhance reliability and accuracy of the results. This practice helps minimize the impact of variability and ensures a more robust assessment of the hydrogen peroxide scavenging activity.

Following the reactions, the percent inhibition of the assay was calculated. This calculation involves determining the extent to which the tested sample, in comparison to the positive control, inhibits or neutralizes the hydrogen peroxide present in the system. The percentage inhibition provides a quantitative measure of the antioxidant capacity of the algal extract, shedding light on its effectiveness in scavenging hydrogen peroxide radicals.

Extract/Sample	Reagent	Result
20 µl	980	S1=0.490
40 µl	960	S2=0.440
60 µl	940	S3=0.310
80 µl	920	S4=0.281
100 µl	900	S5=0.126
Control= 1 µl	999	0.902

Table 5: Tabulated results of the antioxidant activity of the sample at different concentrations using hydrogen peroxide scavenging activity

4.3 ANTI-CANCER ACTIVITY

4.3.1 IN-VITRO ANTICANCER ACTIVITY

Cell line

The human Cancer cell was obtained from the National Centre for Cell Science (NCCS), Pune, and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passed weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single-cell suspensions and viable cells were counted using a hemocytometer and diluted with a medium containing 5% FBS to give a final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at a plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethyl sulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum-free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

4.3.2 MTT ASSAY

In hydrostatic testing, MTT is a yellow-soluble tetrazolium salt that contains three trimethylthiazol-2-yl components (4,5-dimethylthiazol-2-yl). The mitochondrial enzyme succinate-dehydrogenase converts MTT into insoluble purple formazan, which cannot be dissolved by water. In this way, the amount of formazan produced is directly related to how many viable cells are present.

After 48 h, 15l of MTT (5mg/ml) in phosphate-buffered saline (PBS) was added to each well, which was incubated for 4 hours at 370C. Using a microplate reader, the absorbance at 570 nm was measured after flicking off the MTT medium and solubilizing the formamide crystals in 100l of DMSO.

The percentage cell viability was then calculated with respect to control as follows;

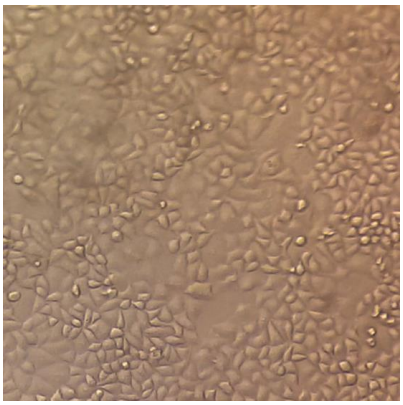
% Cell viability = [A] Test / [A]control x 100

The % cell inhibition was determined using the following formula.

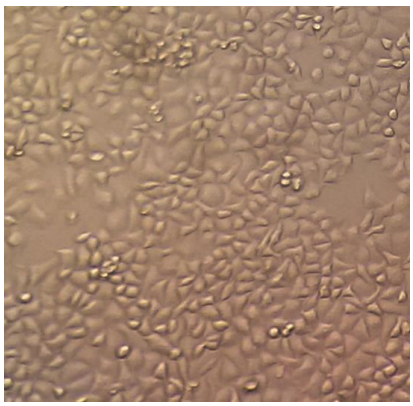
% Cell Inhibition = 100- Abs (sample)/Abs (control) x100.

A nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using GraphPad Prism software.

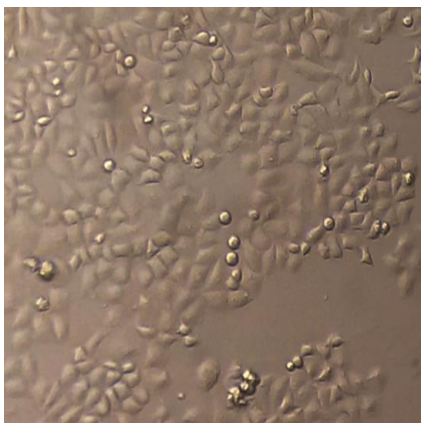
Cancer Cell Line

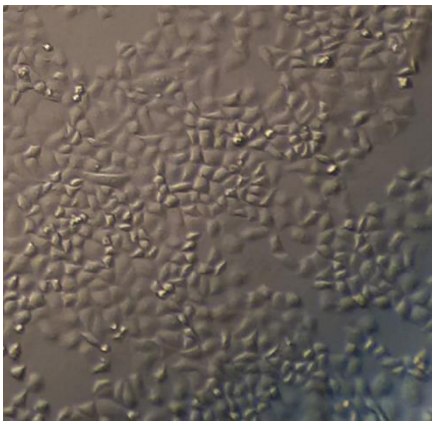


18.25 µl



37.5 µl





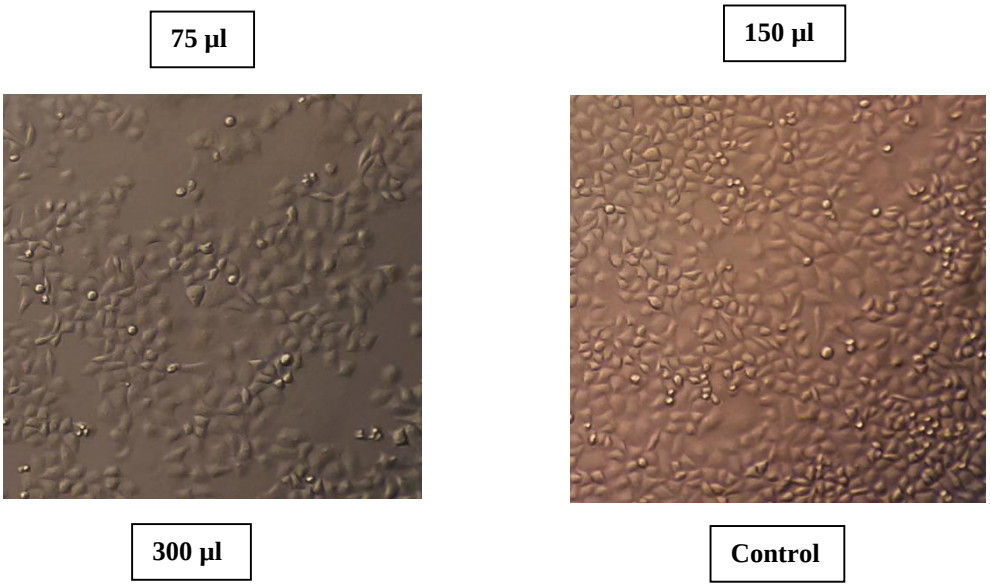


Fig 2: Human cancer cell lines grown in Eagles minimum essential medium containing 10% FBS obtained from NCCS treated with blue green algae extracts at different concentrations

Conc (µg/ml)	% cell inhibition	IC50
18.75	9.376114	
37.5	18.36007	
75	33.29768	
150	45.24064	**227.37 µg/ml**
300	53.29768	

Table No 6: Table showing different concentration of blue green algae extract and their percentage of cell inhibition

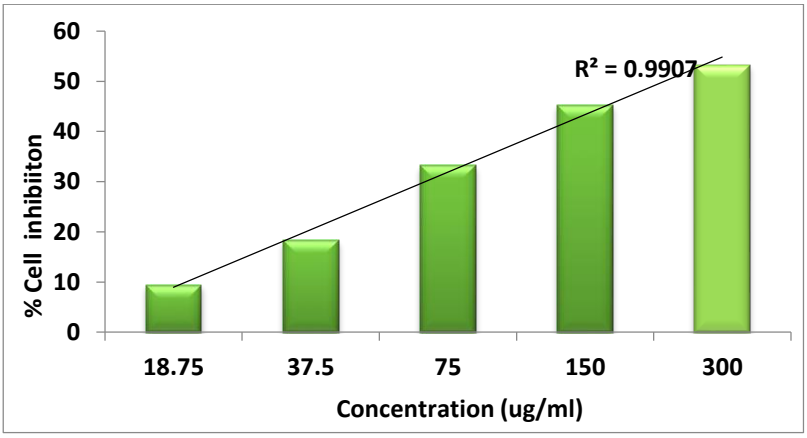


Fig No 3: Graph showing the percentage of cell inhibition at different concentrations of Blue-green algae extract

5. CONCLUSION

In summary, the in vitro evaluation of antioxidant and anti-cancer properties in blue-green algae extract has uncovered substantial therapeutic promise. The study has yielded crucial insights into the extract's capacity to neutralize free radicals, as evidenced by robust antioxidant activity, notably demonstrated through methods like the DPPH assay. This underscores its effectiveness in alleviating oxidative stress, a critical factor in the development of various diseases. Moreover, the investigation into anti-cancer properties has yielded promising outcomes, spotlighting the extract's impact on cancer cell lines. The identified cytotoxicity and clarified mechanisms of action suggest that blue-green algae extract may serve as a source of bioactive compounds with specific toxicity against cancer cells, paving the way for the development of innovative anti-cancer therapeutics. The comprehensive knowledge gained from this project not only contributes to the scientific understanding of blue-green algae but also emphasizes its potential as a valuable asset in the pharmaceutical sector. The bioactive compounds uncovered in this study could form the basis for creating antioxidant supplements and anti-cancer drugs, providing sustainable and nature-derived alternatives to address health challenges. In essence, the revealed therapeutic potential from this in vitro assessment positions blue-green algae extract as a promising avenue for further exploration and development in the quest for innovative and efficacious healthcare solutions.

6. DISCUSSION

The investigation into the therapeutic potential of blue-green algae extract represents a compelling research focus, particularly centered on in vitro evaluations of its antioxidant and anti-cancer properties. Renowned for its rich repository of bioactive compounds, blue-green algae emerge as a promising reservoir for innovative therapeutic agents. The in vitro assessment of antioxidant characteristics, employing methodologies such as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, provides valuable insights into the extract's proficiency in scavenging free radicals. This capability signifies its potential efficacy in addressing disorders associated with oxidative stress, a pivotal factor in various diseases, including cancer. Furthermore, the exploration of the blue-green algae extract's anti-cancer properties opens new avenues for cancer therapeutics. Conducting in vitro studies in a controlled environment facilitates the observation of the extract's impact on cancer cell lines, offering preliminary data on cytotoxicity and potential mechanisms of action. Unraveling these anti-cancer properties holds the promise of identifying bioactive compounds with selective toxicity against cancer cells. This project not only enriches the field of natural product research but also aligns seamlessly with the growing interest in discovering sustainable and bioactive compounds for therapeutic purposes. The outcomes of this study bear significant implications for the development of antioxidant supplements and anti-cancer drugs derived from blue-green algae, underscoring its potential as a valuable resource within the realms of healthcare and pharmaceuticals.

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