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# ANTICANCER, ANTIOXIDANT ACTIVITY AND GC-MS ANALYSIS OF SELECTED MICRO ALGAL MEMBERS OF CHLOROPHYCEAE

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**Keywords:**

Anticancer, Antioxidant, GC-MS Analysis and Microalgae

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**ABSTRACT:** Microalgae have been widely used as novel source of bioactive substances. These substances exhibit various biological actions including, antioxidant, antimicrobial, antiviral, antitumoral, anti-inflammatory and anti-allergy effects. In the present investigation algal biomass in methanol extracts of chlorophyceae member of three micro algae of *Chlorella vulgaris*, *Desmococcus olivaceous* and *Chlorococcum humicola* was screened. The antioxidant property of the methanolic extracts of these green microalgae was evaluated by measuring the free radical scavenging activity by DPPH assay method. The algal extracts were then evaluated for their suppressive effect on tumor cell growth (MCF7) by using MTT assay and further analysed using GC-MS to determine the profile of specific molecules or compounds. Antioxidant activities of the microalgal methanol extracts were studied by their ability to scavenge DPPH at various concentrations. The DPPH radical scavenging activity was found to be higher in *Chlorella vulgaris* (RSA 53.96%) at a concentration 20µg compared to standard ascorbic acid (RSA 59.42%). Anticancer property of the methanol extracts of *Chlorella vulgaris*, *Desmococcus olivaceous* and *Chlorococcum humicola* in MCF7 breast cancer cell was studied. In this assay the micro algal *Chlorella vulgaris* inhibited the growth of tumor cells (MCF7) when compared to *Desmococcus olivaceous.* GC-MS analysis of methanol extracts of *Chlorococcum humicola* showed the presence of five compounds which are reported to be involved in antioxidant and anticancer properties.

**INTRODUCTION:** Microalgae produce metabolites in order to survive under varying conditions of salinity, temperature, pressure etc. These metabolites are usually secondary, which could be carbohydrate, or proteins or enzymes or pigments or antibiotics or even toxic compounds. These metabolites are found to have pharmaceutical properties such as antiviral, antimicrobial, anti-inflammatory, antifungal, neuroprotective, antihelmintic, cytotoxic, immunological, and enzyme inhibitory [1].

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**Antioxidant activity of algae:** The health benefits of algae seemed to be due to different biochemical mechanisms. Microalgal species such as *Chlorella*, *Spirulina* and *Dunaliella* have been used in nutraceuticals, pharmaceuticals, cosmetics, nutrition and other functional quality of foods. In 2006, World Health Organization described

*Spirulina* as one of the greatest super-foods on earth which examplified the potential of microalgae and reviewed the current and future uses of microalgae as novel source of health promoting compounds [2]. However, not all groups of microalgae can be used as natural sources of antioxidants. Reports on the antioxidant activity of microalgae are limited, especially concerning the relationship between their phenolic content and antioxidant capacity. Therefore, it was desirable to identify some rich sources of antioxidants from a large group such as of microalgae to evaluate and validate the relationship between these two parameters [3].

*Chlorella* contains all eight amino acids and impressive amounts of Vitamin C, beta-carotene (pro-Vitamin A), B-1, B-2, B-6, B- 12, niacin, pantothenic acid, folic acid, biotin, choline, inositol, 4-Aminobenzoic acid (PABA), Vitamin E and Vitamin K. It also includes minerals such as phosphorous, potassium, magnesium, sulphur, iron, calcium, manganese, copper, zinc, iodine and cobalt [4].

An important and well-known class of antioxidants from microalgae is the carotenoids. Carotenoids play an important role in quenching reactive oxygen species (ROS) generated during photosynthesis, especially the singlet oxygen. Several studies have demonstrated that carotenoids contribute significantly to the total antioxidant capacity of microalgae [5, 6]. Polyphenols for instance, use their phenol rings as electron traps for free radicals [7].

**Bioactive compounds:** polyphenols, catechin, flavonols, glycosides, and phlorotannins recovered from methanol extract of red, green and brown algae have been reported to have uniqueness in their molecular skeleton and structures contributing to the strong antioxidant activity [8]. *Chlorella vulgaris* is a microalgae that produces proteins, vitamins and other phytonutrients with high efficiency, utilizing light and carbon dioxide [9]. Numerous studies in relation to the health benefits of food have proved that the increased intake of green and yellow vegetables is associated significantly with a decrease in chronic and age related diseases. Nutritional studies of *Chlorella* have revealed that these algae produced many intracellular phytochemicals namely carotenoids, chlorophyll, tocopherols, ubiquinones, proteins and others. The antioxidant properties of *C. vulgaris* are attributed to these phytochemical [10].

Antioxidants are substances that may protect cells from damage caused by unstable molecules known as free radicals. Antioxidants interact and stabilize free radicals and prevent some of the free radical mediated damage. Antioxidants thus act as cell protectors. Oxygen, an essential element for life, can create damage by it's by- products during normal cellular metabolism. Antioxidants, during normal cellular metabolism, counteract these cellular by-products which are free radicals, before they can cause cellular damage. Antioxidants exist in a variety of non-enzymatic forms, which include Vitamin C, Vitamin E, and Carotenoids. Thus, antioxidants play an important role in the protection of cells against oxidative damage caused by Reactive Oxygen species (ROS) [11]. ROS, such as superoxide-anion, hydroxyl radicals and hydrogen peroxide are generated by general physiological processes and by various exogenous factors that initiat peroxidation of membrane lipids as well as a wide range of other biological molecules through a process that is believed to be implicated in the etiology of several disease conditions including Coronary heart disease, Stroke, Rheumatoid arthritis, Diabetes and Cancer.

Antioxidants play an important role in the inhibition and scavenging of the free radicals thus providing protection to humans against infections and degenerative diseases. However, the two most commonly used synthetic antioxidants, butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) have been restricted because of their toxicity and DNA damage induction. Therefore, natural antioxidants from plant and algal extracts have attracted attention due to their safety. Recent researches have been in focus in finding novel antioxidants to combat and or to prevent ROS mediated diseases [12]. Microalgae have been known to be a useful source of health foods rich in antioxidants [13].

**Anticancer activity of algae:** Cancer, a class of diseases in which a cell or a group of cells cause uncontrolled growth (i.e. division beyond the normal limits), invasion (i.e. intrusion on and

distortion of adjacent tissues), and metastasis (spread from one part to another part in the body through lymph or blood). These three malignant properties of cancer differentiate cancer from benign tumors, which are self-limited, and do not invade or metastasize, while malignant tumors are not self-limited and metastasize. Cancer is a human tragedy that affects people at all ages with the risk for most types increasing with age. It caused about 13% of all human deaths in 2007 (7.6 million) [14].

Cancer is one of the most serious threats to human health in the world and chemotherapy is still a standard method of treatment. Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells and cause immunotoxicity which affects not only tumor development, but also prolongs patient's recovery. The discovery and identification of new anti-tumor drugs with low side-effects on immune system has become an essential goal in immunopharmacology [15]. Regarding the reduced side-effects of plants and other natural compounds, scientists are interested in working on them to find new formulations.

The medicinal value of cyanobacteria was appreciated as early as 1500 BC, when strains of *Nostoc* were used to treat gout, fistula and several forms of cancer (Cyanobacteria are a rich source of potentially useful natural products) [16]. Over 40 different Nostocales species, the majority of which are *Anabaena* and *Nostoc* spp. produce over 120 natural products (Secondary metabolites) having activities such as, anti-cancer, anti-fungal, anti-malarial and anti-microbial.

Studies of the active components in algae grew in line with the success of isolated natural products. Researchers have reported the use of algae as agents for anti-bacterial, anti-helmintic, anti-cancer, anti-ulcer, lowering blood pressure, lowering cholesterol, prevention of stroke, goiter, iron deficiency, and blood related diseases [17].

Until now, there is no effective remedy for the treatment of liver cancer besides surgery. Numerous studies have been done in the past to find alternative medications in the treatment of liver cancer. Herbs have been widely used in traditional medicine for the treatment of various cancers including cancers of the liver.

There has been increased attention towards natural compounds obtained from plants or seaweeds for their medicinal properties. The anti-tumor activity was one of the most important activities in drugs, sourced from marine ecosystem. A large species of algae and their metabolites have been shown to have potent cytotoxicity. These metabolites have played an important role in synthesizing new pharmaceutical compounds from algae for anti-tumor drugs [18].

Studies have focused on water soluble anti-tumor substances from various marine algae, however, most anti-cancer agents have not been used clinically because of their undesirable side effects on normal cells [19]. Microalgae, being microscopic, diverse and having evolved their own defense mechanisms by synthesizing secondary metabolite, which are explored in anticancer studies [20, 21].

**GC-MS Analysis of Algae:** Soltani *et al.,* in their study, obtained pressurized liquid extracts which were chemically characterized by GC-MS and HPLC-DAD. Different fatty acids and volatile compounds with antimicrobial activity were identified, such as phytol, fucosterol, neophytadiene or palmitic, palmitoleic and oleic acids [22]. Based on the results obtained, ethanol was selected as the most appropriate solvent to extract active compounds from the natural sources. Crude extract analysis of the algal species using gas chromatography-mass spectrometry (GC-MS) had revealed several important organic volatile compounds as fatty acids. In their study, aqueous, petroleum ether, and methanol extracts from 76 microalgae were examined for anti-microbial properties against four test bacteria and two test fungi. Of the total microalgae, 22.4% (17 cyanobacteria) exhibited anti-microbial effects. Fresh water microalgae *Chlorella vulgaris* was grown in media amended with acenaphthene and fluoranthene. *Chlorella vulgaris* was identified as tolerating and effectively degrading polycyclic aromatic hydrocarbons that may be toxic in the environment. Based on $LC_{50}$ value three different concentrations were selected for study. Decrease in total chlorophyll and protein content with increasing time and concentration showed the impact of PAHs on *C. vulgaris*. GC/MS analysis explained the degradation of these compounds by *C. vulgaris* and converted both acenaphthene and

fluoranthene into non-toxic form *C. vulgaris* completely degraded acenaphthene on the 16th day of experiment with all three $LC_{50}$ concentrations, while in fluoranthene 93% reduction was seen at 6 mg $L^{-1}$.

**Scope of the Present Investigation:** In the present investigation algal biomass in methanol extracts of three microalgae *viz. Chlorella vulgari, Desmococcus olivaceous* and *Chlorococcum humicola* was screened for the study was undertaken to evaluate antioxidant properties of methanolic extracts of green microalgae by measuring free radical scavenging activity by DPPH assay method. The algal extracts were then evaluated for their suppressive effect on tumor cell growth (MCF7) by using MTT assay and finally analyzed using GC-MS to determine the profile of specific molecule or compound.

## MATERIALS AND METHODS:

### Algal Source:

**Microalgae:** *Chlorella vulgaris, Chlorococcum humicola* and *Desmococcus olivaceous* were obtained from the culture collections of the PG and Research Department of Botany, Ramakrishna Mission Vivekananda College (Autonomous), Chennai – 600 004.

**Preparation of algal inoculum:** The inoculums of the algal cultures to be used for the mass cultivation have been prepared under laboratory conditions. Microalgae; *Chlorella vulgaris, Chlorococcum humicola* and *Desmococcus olivaceous* were grown in CFTRI medium [23].

**Growth conditions for Microalgae:** Cultivation of microalgae was carried out at 24±1 ºC in a thermostatically controlled room and illuminated with cool white fluorescent lamps (Philips 40w, cool daylight, 6500k) at an intensity of 2000 lux in a 12:12 light dark regime, for 30 days. The purity of cultures was examined microscopically during cultivation and harvested on 30$^{th}$ day.

**Preparation of Algal extract:** Dried algal material (0.6g) was ground in pestle and mortar with a methanol solvent. The extract was centrifuged at 3000rpm for 10min and the supernatant was collected in a vial. The pellet was then re-centrifuged and the supernatant was collected. The extract was concentrated under room temperature upto 1ml and stored in the eppendorf tubes. The extract was used for the bacterial susceptibility, anti-oxidant potential and cytotoxicity arrays.

**Culture medium for Algae:**

**CFTRI-Medium was prepared using the following compositions (g/l).**

| **Chemical Composition** | | **g/L** |
|---|---|---|
| $NaHCO_3$ | - | 4.5 |
| $K_2HPO_4$ | - | 0.5 |
| $NaNO_3$ | - | 1.5 |
| $K_2SO_4$ | - | 1.0 |
| NaCl | - | 1.0 |
| $MgSO_4.7H_2O$ | - | 0.2 |
| $CaCl_2$ | - | 0.04 |
| $FeSO_4$ | - | 0.01 |
| pH of the medium | - | 10 |
| $DH_2O$ | - | 1000ml |

**DPPH Assay**: **(2, 2-diphenyl-1-picrylhydrazyl):** Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay with minor modifications [24]. Different concentrations of sample 20,40,60,80 and 100µg/ml of the extracts were taken in the test tubes 3.0ml of 0.1mM DPPH in ethanol was added to each tube. The mixture was vortexed thoroughly. The setup was left in the dark at room temperature for 20 minutes. The absorbance was read at 517nm using UV-visible spectrophotometer. Ascorbic acid was used as a standard. The % of inhibition (I %) was calculated using the formula.

$$\%\ \text{of DPPH Radical Scavenging Activity (\% RSA)} = \frac{\text{Abs. control} - \text{Abs. sample}}{\text{Abs. control}} \times 100$$

**Anti-cancer in cell lines:**

**MCF7 Cell Line:** Human breast adenocarcinoma (MCF7) cell line was obtained from National Centre for Cell Science, Pune (NCCS). MCF7 cells were maintained in Rose well Park Memorial Institute medium (RPMI) containing 10% FBS, supplemented with penicillin (250 U/ml), streptomycin (250 Units/ml), gentamycin (100µg/ml) and amphotericin B (1mg/ml) at 37$^{o}$C under 5% $CO_2$ in air.

**Reagents:** RPMI, Fetal bovine serum (FBS), Acridine orange, methyl thiazolyldiphenyl-tetrazolium bromide (MTT), and MCF 7 Dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich, St. Louis, USA.

***In vitro* assay for Cytotoxicity activity (MTT assay):** In this study, cancer cell growth inhibition activity was measured by using MTT assay [25]. MCF7 was seeded at a density of $5\times10^3$ cells/well in 96-well plates for 24 h, in 200µl of RPMI with 10% FBS. The monolayer of cells washed twice with RPMI media with FBS to remove the dead cells and excess FBS. To the washed cell sheet, 1ml of medium (with FBS) containing concentration of the test compound (10ng - 100µg/ml) was added in respective wells of the 96 well titre plates and incubated for 48 h. To the control wells, 1 ml RPMI with FBS was added. Plates were incubated at 37 ºC in 5% $CO_2$ environment and observed for cytotoxicity using inverted microscope. In each well add 200µl of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) concentration (10µl, 5mg/well) was added and then incubated for cytotoxicity at 37 ºC for 4 hours. Acridine orange in DMSO in each well was added and mixed and kept for 1 hour. Any viable cell, if present, it shows orange colour formation. The suspension is then transferred into the cuvette of scanning multi-well spectrophotometer and an OD value was read at 595nm by taking DMSO as a blank. Viability curve is plotted by taking concentration of the drug on X axis and relative cell viability on Y axis.

Measurements were taken and the concentration required for a 50% inhibition of viability ($IC_{50}$) was determined graphically. The effect of the samples on the proliferation MCF7 cells was expressed as % cell viability, using the following formula:-

Cell viability (%) = (Average test OD/Control OD) x 100

**Data analysis:** The $IC_{50}$ value (concentration at which 50% of cells were death) was reported as mean values of six independent experiments. The $IC_{50}$ value against the human cancer cell lines was calculated for the solvent extracts inhibiting at least 50% inhibition at different concentrations.

**GC-MS Analysis of *chlorococcum humicola*:** The Clarus 500 GC analyser with a fused silica column packed with Elite-1 (100% dimethyl poly siloxane, 30 nm × 0.25 nm ID × 1µm df) was used. The components were separated using Helium as carrier gas at a constant flow of 1 mL/min. The sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th minute of GC extraction process, the oven was maintained at a temperature of 1100 ºC with 2 minutes holding. The injector temperature was set at 2500 ºC (mass analyzer). The parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet line temperature: 2000 ºC; Source temperature: 2000 ºC). Mass spectrum was taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 450 D.

**Identification of components:** Interpretation of mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST), which has more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained from the data base.

## RESULTS:

**Antioxidant Activity:** The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. DPPH is a compound that possesses nitrogen free radicals and is readily absorbed by free radical scavengers. The algal extracts in methanol displayed greater antioxidant potential in all assays when compared to ethanol and acetone extracts of green microalgae: *Desmococcus olivaceous* and *Chlorococcum humicola* [26].

DPPH radical scavenging activity of the microalgae *Chlorella vulgaris, Desmococcus olivaceous* and *Chlorococcum humicola* exhibited higher radical scavenging activity in extracts prepared in methanol when compared to ascorbic acid. The three algae species, whose methanol extracts were analysed at different concentrations 20, 40, 60, 80 and 100µg with ascorbic acid as the standard.

DPPH radical scavenging activities (%) of extracts in methanol of three microalgae are presented in **Table 1, 2** and **3**. All these microalgae extracts possess the ability to scavenge DPPH at various degrees of concentration, the DPPH radical scavenging activity was found to be higher in

*Chlorella vulgaris* (RSA 53.96%). *Chlorococcum humicola* showed moderate level of DPPH radical scavenging activity (RSA 35.21%). Among the three Algae tested, the lowest DPPH radical scavenging activity was exhibited by *Desmococcus olivaceous* (RSA 35.21%) at a concentration of 20µg when compare to standard (Ascorbic acid RSA 59.42%).

**TABLE 1: DPPH RADICAL SCAVENGING ACTIVITY (RSA) OF *CHLORELLA VULGARIS***

| Sl. No | Concentration (µg) | OD | RSA % | Standard % |
|---|---|---|---|---|
| 1 | 20 | 0.383 | 53.96 | 59.42 |
| 2 | 40 | 0.372 | 55.28 | 64.51 |
| 3 | 60 | 0.354 | 57.45 | 68.76 |
| 4 | 80 | 0.297 | 64.30 | 74.49 |
| 5 | 100 | 0.266 | 68.02 | 77.45 |

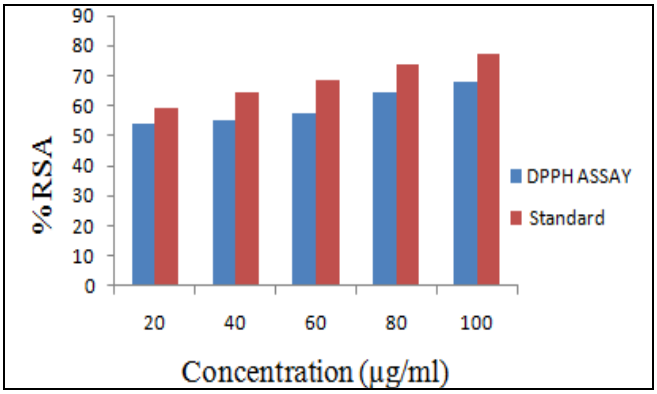



**FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY (RSA) OF *CHLORELLA VULGARIS***

**TABLE 2: DPPH RADICAL SCAVENGING ACTIVITY (RSA) OF *DESMOCOCCUS OLIVACEOUS***

| Sl. No | Concentration (µg) | OD | RSA % | Standard % |
|---|---|---|---|---|
| 1 | 20 | 0.624 | 25.00 | 59.42 |
| 2 | 40 | 0.610 | 26.68 | 64.51 |
| 3 | 60 | 0.438 | 47.35 | 68.76 |
| 4 | 80 | 0.402 | 51.68 | 74.49 |
| 5 | 100 | 0.317 | 61.89 | 77.45 |

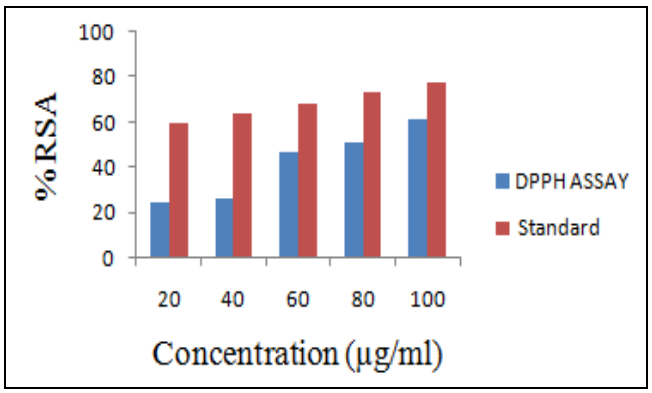



**FIG 2: DPPH RADICAL SCAVENGING ACTIVITY (RSA) OF *DESMOCOCCUS OLIVACEOUS***

**TABLE 3: DPPH RADICAL SCAVENGING ACTIVITY (RSA) OF *CHLOROCOCCUM HUMICOLA***

| Sl. No | Concentration (µg) | OD | RSA % | Standard % |
|---|---|---|---|---|
| 1 | 20 | 0.539 | 35.21 | 59.42 |
| 2 | 40 | 0.511 | 38.58 | 64.51 |
| 3 | 60 | 0.472 | 43.26 | 68.76 |
| 4 | 80 | 0.429 | 48.43 | 74.49 |
| 5 | 100 | 0.361 | 56.61 | 77.45 |

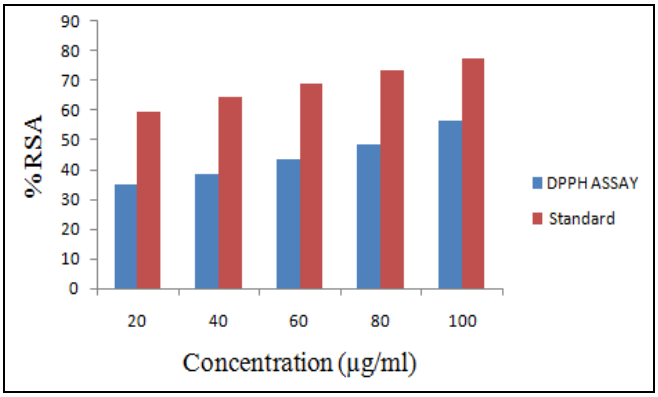



**FIG. 3: DPPH RADICAL SCAVENGING ACTIVITY (RSA) OF *CHLOROCOCCUM HUMICOLA***

**Cytotoxicity activity (MTT assay):** Anticancer properties were studied from the extract of *Chlorella vulgaris*, *Desmococcus olivaceous* and *Chlorococcum humicola* in MCF7 breast cancer cell line. The assays consisted counting of viable cells after staining with MTT. The extracts of *Chlorella vulgaris*, *Desmococcus olivaceous* and *Chlorococcum humicola* were also tested for their proliferation-inhibition ability in MCF7 cancer cell lines. The cytotoxic effects of extracts in methanol of three microalgae: *Chlorella vulgaris*, *Desmococcus olivaceous* and *Chlorococcum humicola* using MCF7 cancer cell lines *in-vitro* are presented in **Table 6** and **Fig. 5**. The microalgae extract exhibited cytotoxic effects under *in-vitro* condition at clinically acceptable concentrations ($IC_{50}$ values≤ 50 mg L-1) used by MTT method. The cytotoxic effect of microalgae extracts was determined using concentrations ranging 10ng to 100µg/ml for 48 hrs.

After an exposure of 48 hrs, the extracts induced concentration-dependent cytotoxic effects in MCF7 cell lines with 100µg/ml of cell viability with *Chlorella vulgaris* (84.11%) and also showed higher dead counts when compared to control DMSO; *Desmococcus olivaceous* with 86.27% and *Chlorococcum humicola* with 88.61% viability in MCF7 cells **Fig. 6**.

This assay proves that the microalga *Chlorella vulgaris* inhibits the growth of tumor cells (MCF7) when compared to *Desmococcus olivaceous* and *Chlorococcum humicola.*

**TABLE 4: CYTOTOXICITY ACTIVITY OF MICROALGAE**

| Sample | Concentration | OD1 | OD2 | Average | t/c | t/c*100 |
|---|---|---|---|---|---|---|
| Control | Control | 0.898 | 0.902 | 0.9 | 1 | 100 |
| Solvent control | Solvent control | 0.881 | 0.891 | 0.886 | 0.984444 | 98.44444 |
| | 10ng | 0.849 | 0.852 | 0.8505 | 0.945 | 94.5 |
| | 100ng | 0.805 | 0.81 | 0.8075 | 0.897222 | 89.72222 |
| | 1µg | 0.791 | 0.794 | 0.7925 | 0.880556 | 88.05556 |
| | 10µg | 0.778 | 0.779 | 0.7785 | 0.865 | 86.5 |
| *Chlorella vulgaris* | 100µg | 0.753 | 0.761 | 0.757 | 0.841111 | 84.11111 |
| | 10ng | 0.863 | 0.863 | 0.863 | 0.958 | 95.88 |
| | 100ng | 0.813 | 0.816 | 0.8145 | 0.905 | 90.5 |
| | 1µg | 0.799 | 0.805 | 0.802 | 0.891111 | 89.11111 |
| | 10µg | 0.787 | 0.789 | 0.788 | 0.875556 | 87.55556 |
| *Desmococcus olivaceous* | 100µg | 0.771 | 0.782 | 0.7765 | 0.862778 | 86.27778 |
| | 10ng | 0.875 | 0.878 | 0.8765 | 0.9738 | 97.38 |
| | 100ng | 0.826 | 0.813 | 0.8195 | 0.910556 | 91.05556 |
| | 1µg | 0.771 | 0.865 | 0.818 | 0.908889 | 90.88889 |
| | 10µg | 0.803 | 0.816 | 0.8095 | 0.899444 | 89.94444 |
| *Chlorococcum humicola* | 100µg | 0.801 | 0.794 | 0.7975 | 0.886 | 88.61 |

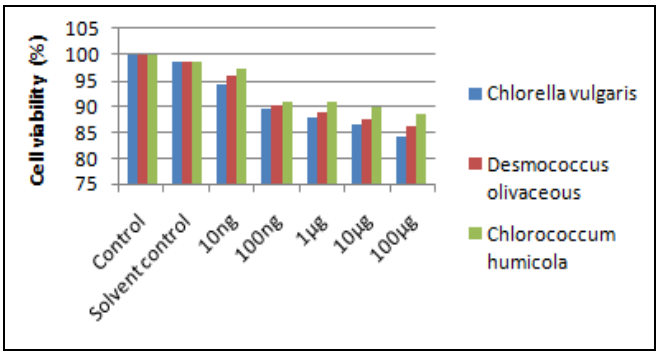



**FIG. 4: CYTOTOXICITY ACTIVITY OF MICROALGAE**

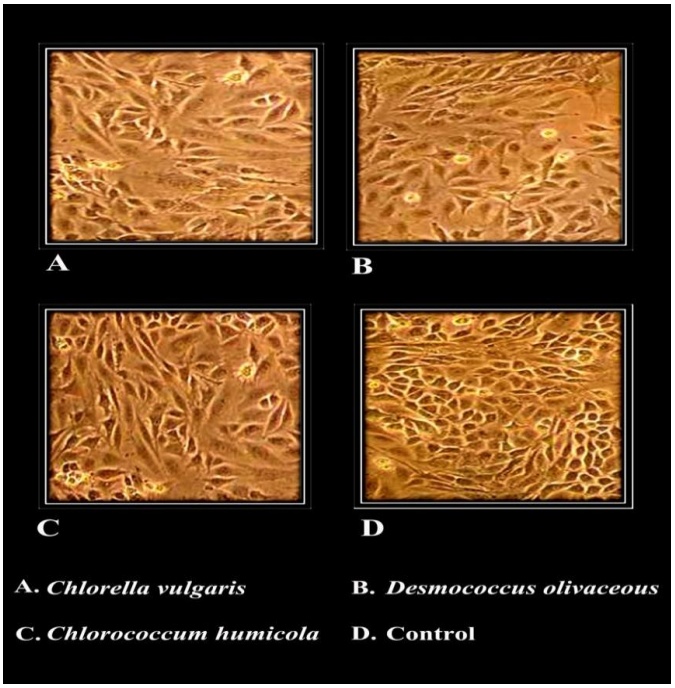



**FIG. 5: CYTOTOXICITY OF MICROALGAL EXTRACTS AT 100mg/ml CONCENTRATION**

**GC-MS analysis:** The antibacterial activity of *Chlorococcum humicola* was superior compared to *Desmococcus olivaceous* and *Chlorella vulgaris.* The latter showed moderate level of DPPH radical scavenging activity. Based on the above observation the alga *C. humicola* was selected for further GC-MS analysis.

Gas chromatography-Mass spectrometry (GC-MS) is a valuable tool for reliable identification of bioactive compounds. In the present study, five compounds have been identified from the methanol extract of the microalga, *Chlorococcum humicola* by Gas Chromatography-Mass Spectrometry analysis. The identified bioactive compounds are 2-Pentadecanone 6, 10, 14-trimethyl, Pentadecanoic acid 14-methyl-methyl ester, Benzeneaceticacide a, 3-bis(acetyloxy) methyl ester, Phytol and 1,2-Benzenediol 4-(2-aminopropyl).

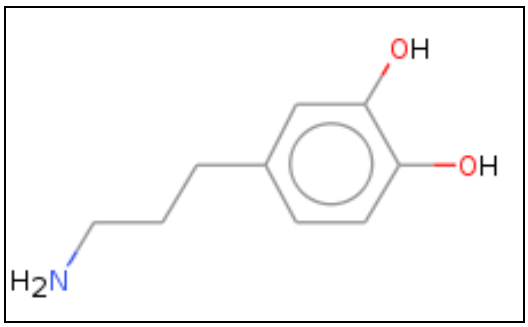
GC-MS analysis of the *Chlorococcum humicola* showed the presence of five major peaks. The respective retention times (Rt) of individual peak recorded were (Rt12.12), (Rt15.17), (Rt16.28), (Rt17.13) and (Rt18.97). The major phyto-constituents in the fraction were 2-Pentadecanone 6, 10, 14-trimethyl, Pentadecanoic acid 14-methyl-methyl ester, Benzeneaceticacide a, 3-bis(acetyloxy)methyl ester, Phytol and 1,2-Benzenediol4-(2-aminopropyl). The results are showed in **Table 5** and **Fig. 6-11**.

The mass spectra of the identified compounds are compared with those in the pubchem database. The chemical compounds identified are given in **Table 5**.

**TABLE 5: GC-MS ANALYSIS OF *CHLOROCOCCUM HUMICOLA***

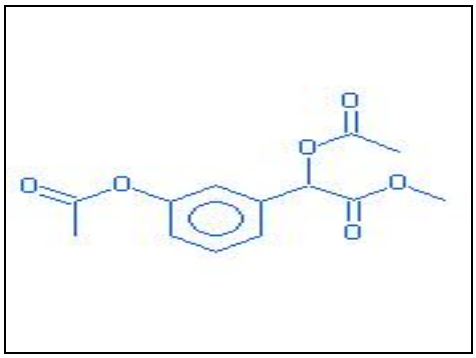
| S. NO | RT(min) | Name of Compound | Molecular Formula | Molecular Weight (g/mol) |
|---|---|---|---|---|
| 1 | 12.12 | 1,2-Benzenediol,4(2-aminopropyl) | $C_9H_{13}NO_2$ | 167.20502 |
| 2 | 15.17 | Benzeneacetic acid,a,3bis(acetyloxy)methyl ester | $C_{13}H_{14}O_6$ | 266.24666 |
| 3 | 16.28 | 2-Pentadecanone 6,10,14-trimethyl | $C_{18}H_{36}O$ | 268.4778 |
| 4 | 17.13 | Peantadecanoic acid,14-methyl-methyl ester | $C_{17}H_{34}O_2$ | 270.4507 |
| 5 | 18.97 | Phytol | $C_{20}H_{40}O$ | 296.531 |

**1, 2-Benzenediol,4 (2-aminopropyl):**

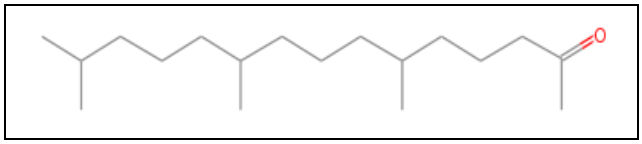



Benzenediols or dihydroxybenzenes are organic chemical compounds in which two hydroxyl groups are substituted onto a benzene ring. These aromatic compounds are classed as phenols. There are three isomers of benzenediol: 1,2-benzenediol (the ortho isomer) is commonly known as catechol, 1,3-benzenediol (the meta isomer) is commonly known as resorcinol, and 1,4-benzenediol (the para isomer) is commonly known as hydroquinone.

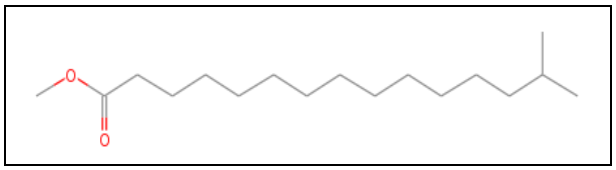
**Benzeneacetic acid, a, 3-bis(acetyloxy)methyl ester:** Antimicrobial activity



**2-Pentadecanone 6, 10, 14-trimethyl:** Cancer-preventive



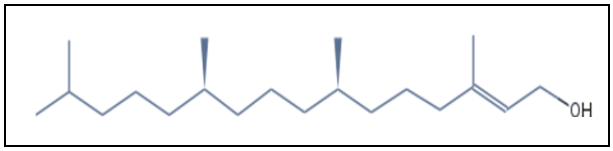

**Peantadecanoic acid, 14-methyl-methyl ester:** Antifungal, Antimicrobial activity.




Pentadecanoic acid is a saturated fatty acid. Its chemical formula is $CH_3(CH_2)_{13}COOH$. It is rare in nature, and found at a level of 1.2% in milk-fat from cows. The butter-fat in cow's milk is its major dietary source, and it is used as a marker for butterfat consumption. Pentadecanoic acid also occurs in hydrogenated mutton fat.

**Phytol:** Phytol is an important component of all plants used in cosmetics, shampoos, toilet soaps, household cleaners as it is known to possess antimicrobial, anticancer, antidiuretic activity. Interestingly, phytol shows high antimicrobial potency against the food borne pathogens. Phytol is important in the processing of glucose and can activate enzymes that have strong positive effects on insulin level within the body. This means that phytol in the human diet could possibly help restore the metabolic functions.

Phytol is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of Vitamin E and Vitamin $K_1$. In ruminants, the gut fermentation of ingested plant materials liberates phytol, a constituent of chlorophyll, which is then converted to phytanic acid and stored in fats.



Phytol is known to possess antimicrobial, anticancer, anti-inflammatory, anti- diuretic, immunostimulatory and anti-diabetic properties. It is also antifungal against *Salmonella typhi*, and anti-malarial.

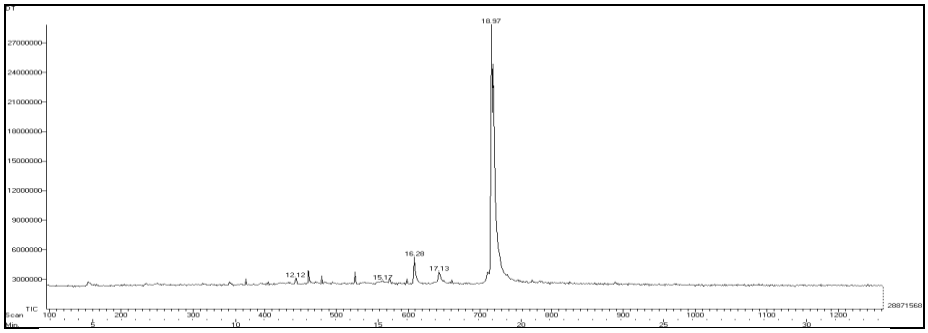



**FIG. 6: RETENTION TIME AND PEAK LEVEL BY GC-MS ANALYSIS**

| RT (min) | Name of Compound |
|---|---|
| 12.12 | 1,2-Benzenediol,4 (2-aminopropyl) |
| 15.17 | Benzeneacetic acid,a,3 bis(acetyloxy)methyl ester |
| 16.28 | 2-Pentadecanone 6,10,14-trimethyl |
| 17.13 | Peantadecanoic acid,14-methyl-methyl ester |
| 18.97 | Phytol |

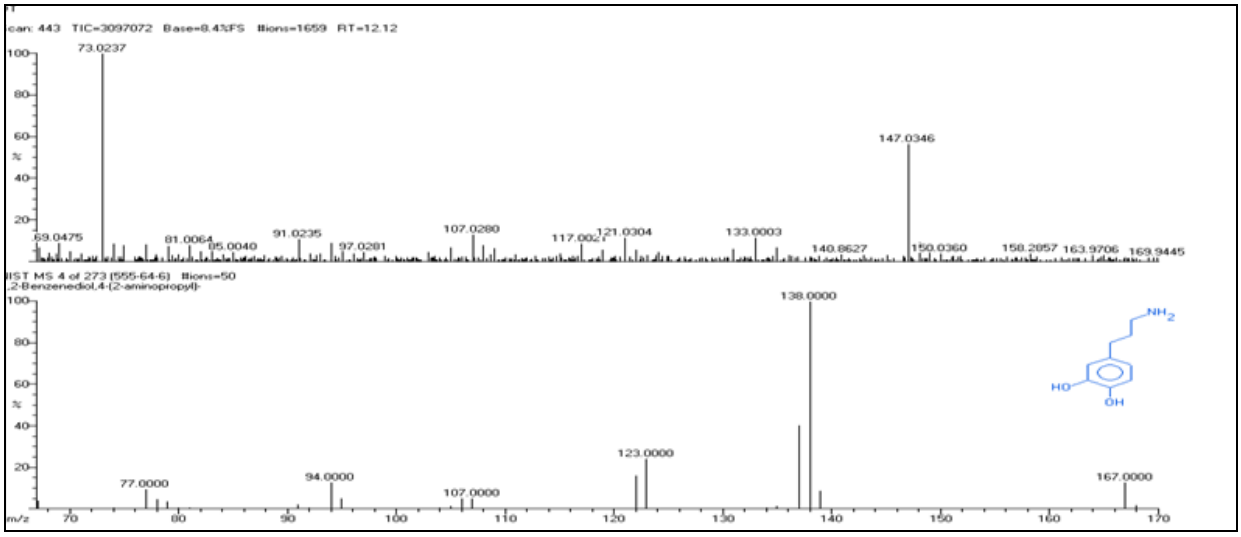



**FIG. 7: 1, 2-BENZENEDIOL,4 (2-AMINOPROPYL)**

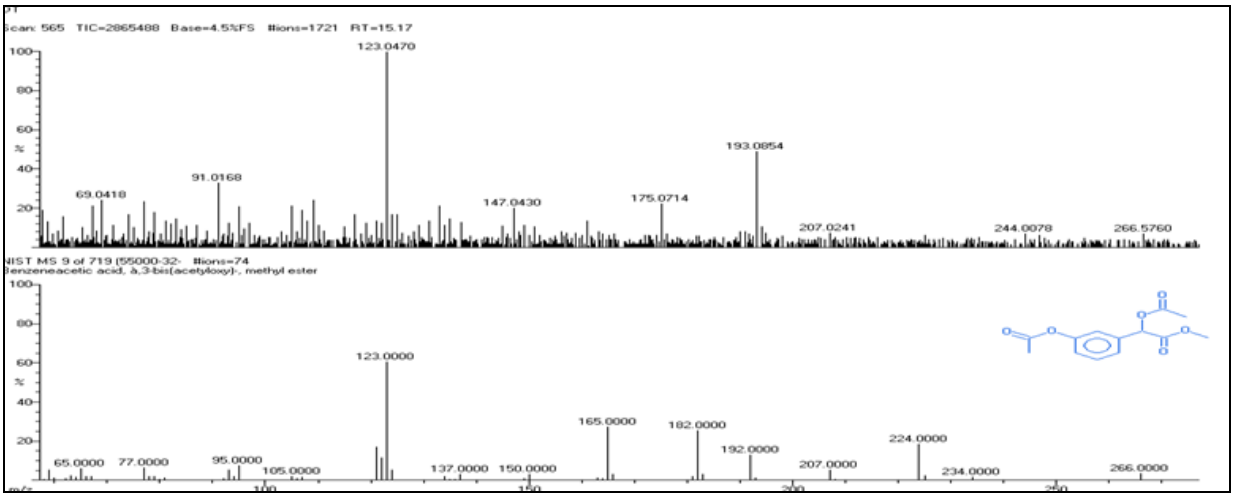



**FIG. 8: BENZENEACETIC ACID, a,3 BIS(ACETYLOXY)METHYL ESTER**

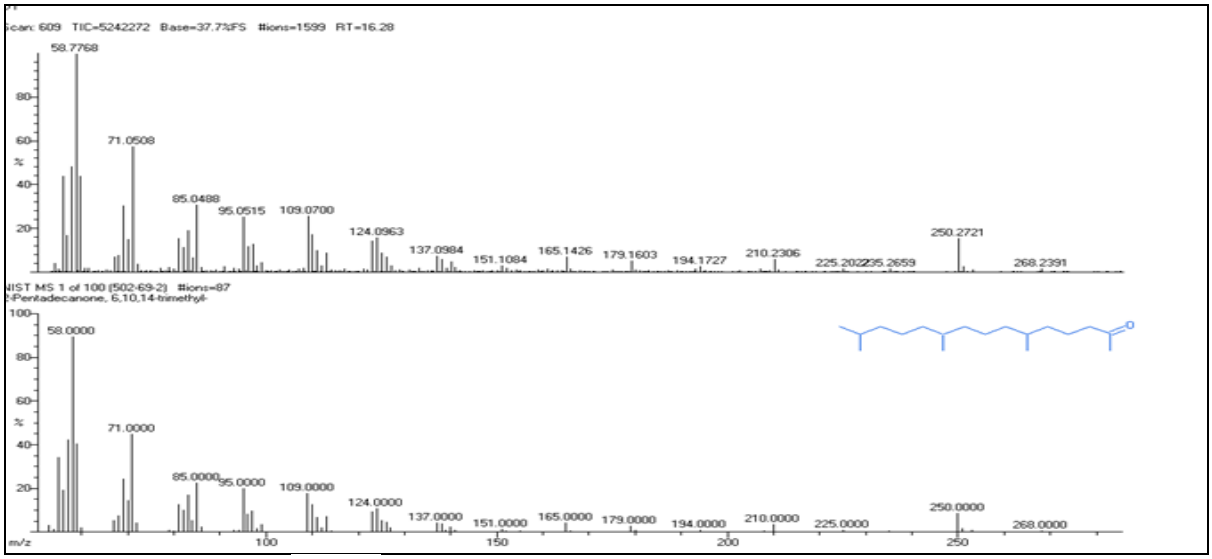



**FIG. 9: 2-PENTADECANONE 6, 10, 14-TRIMETHYL**

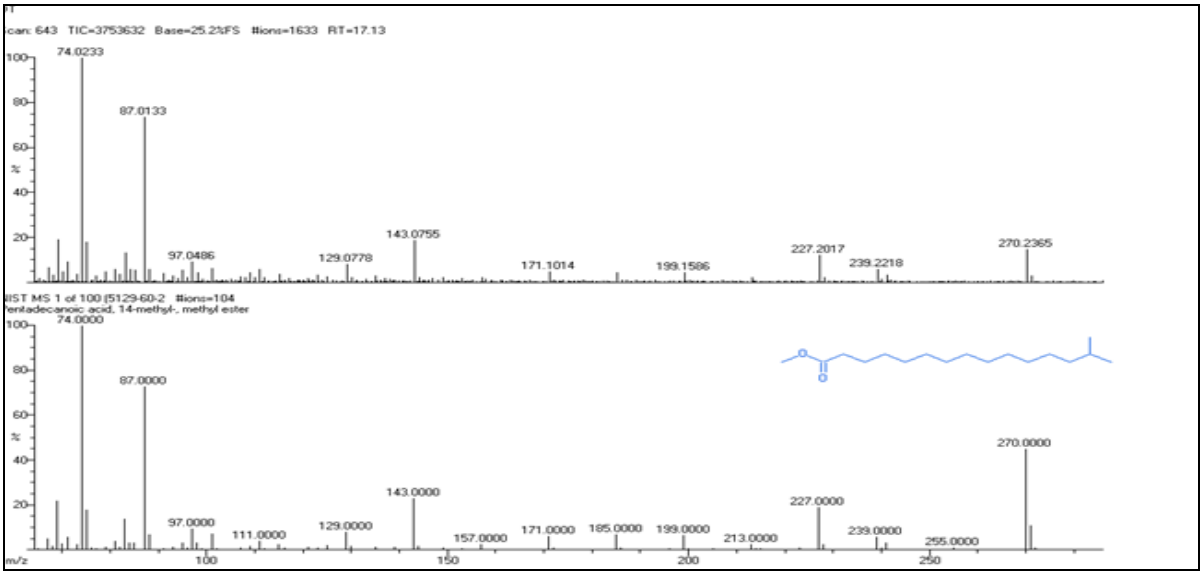



**FIG. 10: PEANTADECANOIC ACID, 14-METHYL-METHYL ESTER**

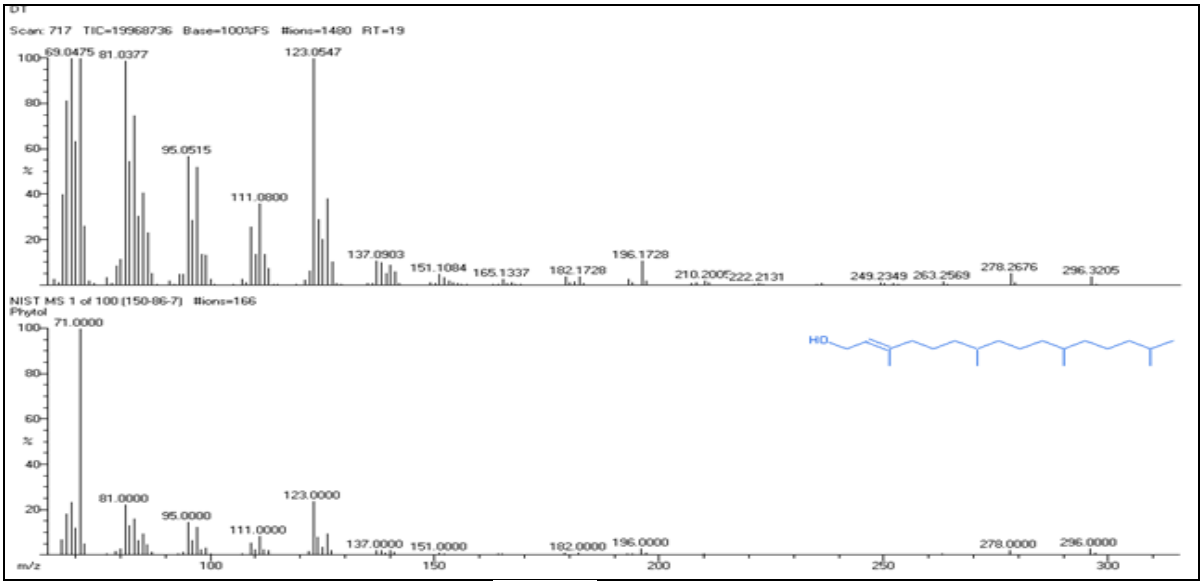



**FIG. 11: PHYTOL**

## DISCUSSION:

**Antioxidants Activity:** In a study the beta-glucan extract of *Chroococus turgidus* was shown to have significant radical scavenging activity at a higher dosage. At a concentration of 250µg, the scavenging activity of *Chroococus turgidus* was 78 %. The results indicated that, activity of the SOD levels of *Chroococus turgidus*, was found to be 98% super oxide inhibition at a concentration of 500 µg/ml [27]. The dose-response curve of DPPH radical scavenging activity of the *Oscillatoria terebriformis* at a concentration of 250µg, of methanol extract of was 58.1% [28]. The 2, 2-diphenyl-2-picryl hydrazyl (DPPH) radical was widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic and anthocyanins or crude mixture such as the ethanol extract of plants. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The electrons become paired off and the solution loses colour stochiometrically depending on the number of electrons taken up [29].

Among the most relevant compounds found in the algae, antioxidants are probably the substances that have attracted major interest. Antioxidants are considered key- compounds in the fight against various diseases (e.g. cancer, chronic inflammation, atherosclerosis and cardiovascular disorder) and ageing [30]. Polyphenols in marine brown algae are called phlorotannins and are known to act as potential antioxidants. Of the three microalgae studied, the highest antioxidant activity was seen in *Chlorella vulgaris* (RSA 53.96%). The lowest activity was noticed in *Desmococcus olivaceous* (RSA 25%). *Chlorococcum humicola* showed moderate level of DPPH radical scavenging activity (RSA 35.21%) at a concentration of 20µg when compared to standard ascorbic acid (RSA 59.42%). The results are given in **Table 1, 2** and **3** and represented in **Fig. 1, 2** and **3**.

**Anticancer activity:** The glycoprotein derived from *Chlorella vulgaris* showed immunoactive anti-tumor activity [31]. *C. vulgaris* is green algae with high nutritive value and has rich potential in pharmaceutical use. The effects of *Chlorella vulgaris* extract on certain diseases have also been reported [32].

In the present investigation, cytotoxicity assay showed *Chlorella vulgaris* exhibiting maximum cytotoxicity effect. *Desmococcus olivaceous* with moderate level and low effect was recorded in *Chlorococcum humicola* at a concentration of 100µg/ml. The results are given in **Table 4** and represented in **Fig. 4** and **5**.

**GC-MS analysis:** The experimental test results and the spectra were confirmed with MS library. The higher concentration of Dodecanoic acid methyl ester, Tetradecanoic acid methyl ester, Hexadecanoic acid methyl ester was recorded in the present study. The important peak was identified at m/z 74.05, which is formed due to carbomethoxy ions and β-ion expulsion [33].

The volatile components were divided into five classes: fatty acids, hydrocarbons, cholesterol, aromatic dicarboxylic acid ester and others. Oleic acid (14.58%) and n-hexadecanoic acid (24.73%) were the major compounds detected, along with the minor compounds; octadecanoic acid, hexadecanoic acid, Z-11-, Cholestane-3, 6-dione, (5,17,20S)-,1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester, heneicosane, nonacosane and hexacosane. Results of our study matched with a composition study of *Acanthophora spicifera* from Pakistan in respect of the major compounds detected; octadecadienoic acid (36.05%) and hexadecanoic acid (8.30%) [33].

The GC–MS profile of *Phormidium fragile* showed the presence of 27 compounds, which includes by 8-Octadecenoic acid methyl ester (31.30%) followed by 9-hexadecanoic acid methyl ester (Z) (14.83%), Hexadecanoic acid methyl ester (13.78%) and 24 compounds were distributed in different quantities [34].

The isolated beta-glucan from *Chroococcus turgidus* revealed the presence of active constituents. In GC-MS analysis 12 bioactive phytochemical components were identified in the isolated beta-glucan from *Chroococcus turgidus*. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula. Compounds such as atropine, Octadecanoic acid, 2, 3-dihydroxypropyl ester have antimicrobial and anticancer properties [35].

GC–MS chromatogram of the bioassay guided column fraction of *S. isoetifolium* showed the presence of five major peak. The respective retention times (Rt) of individual peaks recorded were 0.00–16.050, 0.00–17.531, 0.00–20.651, 0.00–20.778 and 0.00– 27.960 min. The major phytoconstituents observed in the active fraction were 2-pentadecanone, 6,10,14-trimethyl, hexadecanoic acid methyl ester, 9,12-octadecadienoic (Z,Z)-methyl ester, 9,12,15-octadecatrienoic acid methyl ester (Z,Z,Z) and 1,2-benzenedicar- boxylic acid diisooctyl ester. The antibacterial and antifungal activity of 2-pentadecanone 6, 10, 14- trimethyl, were well documented [36].

In the present study GC-MS analysis of *Chlorococcum humicola* revealed the presence of five major phycoconstituents such as 2-Pentadecanone,6,10,14-trimethyl, Pentadecanoic acid, 14-methyl-, methyl ester, Benzeneaceticacide, a,3-bis(acetyloxy)-,methyl ester, Phytol and 1,2-Benzenediol,4-(2-aminopropyl). The chemical compounds identified from GC-MS analysis have been reported to have high anti-microbial, anti-oxidant and anti-cancer potential. The results are given in **Table 5** and represented in **Fig. 6 - 11**.

**CONCLUSION:** Antioxidant analysis of the three microalgae *Chlorella vulgaris, Desmococcus olivaceous* and *Chlorococcum humicola* was studied using methanol extracts. They possessed the ability to scavenge DPPH at various degrees. The DPPH radical scavenging activity was found to be highest in *Chlorella vulgaris* (RSA 53.96%) at a concentration 20µg when compared to standard ascorbic acid (RSA 59.42%). Anticancer property of the methanol extracts from *Chlorella vulgaris, Desmococcus olivaceous* and *Chlorococcum humicola* in MCF7 breast cancer cells were studied. In this study, the micro algae *Chlorella vulgaris* inhibited the growth of tumor cells (MCF7) when compared to the inhibition of *Desmococcus olivaceous.* The GC-MS analysis of methanol extracts from *Chlorococcum humicola* shows the presence of five compounds, which are reported to possess antioxidant and anticancer properties.

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**CONFLICTS OF INTEREST:** Nil

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