Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (9) Issue (3) (2024), (187-196) **online: ISSN 2356–976x**
http://bjas.bu.edu.eg

Antioxidant, antimicrobial, and anticancer activity of an extract of two pollution-tolerant green microalgae

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Abstract

Microalgae are a potentially valuable source of structurally different and biologically active compounds experiencing pharmaceutical and nutraceutical value. Here we compared the antioxidant, *in vitro* antimicrobial activity, and anticancer potential of the widely investigated green microalga *Tetradesmus obliquus* and the less-studied unicellular species *Myrmecia bisecta.* Based on our findings, *T. obliquus* extracts had much more outstanding antioxidant potentials than those of *M. bisecta* where IC_{50} values of $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ were 16.18 and 23.35 µg.ml^{-1} in *T. obliquus vs.* 20.21 and 26.05 µg.ml^{-1} in *M. bisecta*, respectively. Interestingly, the antibacterial activity of *M. bisecta* extract against the tested human pathogenic bacterial strains was more powerful than those of *T. obliquus*, i.e., 45.2% and 25.3% inhibition *vs.* 32.3% and 14.20% inhibition against *Escherichia coli* and *Staphylococcus aureus*, respectively. Both microalgal species showed significant cytotoxic effects against the HepG2 cell line with IC_{50} values of 36.44 and 40.12 µg.ml^{-1} for *T. obliquus* and *M. bisecta*, respectively. Also, both of the two microalgal species showed significant cytotoxic effects against the WI-38 cell line with IC_{50} values of 65.63 and 61.74 µg.ml^{-1} for *T. obliquus* and *M. bisecta*, respectively. Based on our results, both *T. obliquus* and *Myrmecia bisecta* are reasonable candidates for future applications in pharmaceutical manufacturing.

Keywords: antioxidant; anticancer; antimicrobial activity; green microalgae

1. Introduction

Growing water use in Egypt results in the production of a significant amount of municipal wastewater, which is full of nitrogen, phosphorus, and other organic and inorganic pollutants [1]. The simultaneous removal of inorganic nutrients as well as organic matter and suspended solids from wastewater has been achieved through the development of multiple treatment methods (primary and secondary) [2-5]. However, these methods are effective but still unsatisfactory for regular uses of water because they are still rich in nitrogen and phosphorus which may cause health and environmental problems [6,7]. Hence, efforts have been made to enhance the efficiency of remediation technologies. Using microalgae-based systems in wastewater treatment plants is one of the most significant tertiary treatment technologies currently. Where sunlight is the source of energy

for microalgae, capture carbon dioxide, and provide an opportunity to restore valuable biomass [8]. Microalgae have been shown to biodegrade pollutants at the lowest cost and significant environmental advantages (e.g., release O_2 and mitigate CO_2 concentration) [9-11]. Microalgae are highly capable of removing nutrients, organic pollutants, and heavy metals [12-14]. By harvesting microalgal biomass in large quantities, biotechnological applications like biofuels, pharmaceutical products, and biofertilizers can be utilized. Additionally, there is a growing demand for microalgae to be utilized in nutraceuticals, pharmaceutical, and cosmetic industries because they are rich in pigments, phenols, polysaccharides, proteins, essential fatty acids, vitamins, mineral oxides, and other valuable, biologically active compounds that may operate as the generators of na tural antioxidant sources [15]. Secondary metabolites formed by microalgae are highly beneficial to human health due to their biological activity and ability to generate chemicals with antibacterial, antioxidant, and anticancer properties. [16,17].

Oxidative stress molecules can be inhibited or reduced by antioxidants, which are biological macromolecules [18]. Consequently, it is essential to take an exogenous antioxidant molecule supplement [19]. An extensive variety of substances are known as exogenous antioxidants, and these include antioxidant minerals, carotenoids, polyphenols, and vitamins [20]. Moreover, it has been noted that synthetic antioxidants raise safety issues because they may have mutagenic and carcinogenic properties [21]. Thus, global interest has grown in the search for novel exogenous natural antioxidants that can replace synthetic antioxidants that are strong, safe, cheap, and environmentally acceptable. Several research on the antioxidant activity of microalgae has revealed that they contain compounds with a high antioxidant capacity.

Because of their rapid growth and high biodiversity, microalgae have appeared as a highly desirable source of antibacterial and offer several benefits for antimicrobial research [22]. It has been demonstrated that the active components and cell extracts of several microalgae exhibit antibacterial properties against both Gram-positive and Gram-negative bacteria [23]. Because antibiotic resistance is increasing, there is a sustained need to find new antimicrobial substances [24,25]. Numerous studies

suggested that microalgae might produce a range of biochemical compounds with unique biological properties. These compounds can either suppress or eliminate hazardous germs and other microbes [26-29].

Cancer is typically treated with chemotherapy as the first line of treatment, such drugs can kill malignant cells, but they have some negative side effects, hence, it is important to look into novel anticancer agents from a variety of sources, including microalgae, which are a vital source of conventional and therapeutically useful medications for treatment of various types of cancer [30]. Algae typically create a large number of natural anticancer metabolites [31]. Numerous studies have focused on the biological activities of phytochemicals obtained from plants, but chemicals made from microalgae are preferred over those derived from plants because of the variations in phenolic classes and the higher levels of carotenoid and chlorophyll that microalgae contain when compared to certain plants [32,33]. Many beneficial byproducts, including polysaccharides, vitamins, lipids, proteins, and antioxidants, which are used in medicine, and pharmaceuticals, can be obtained from microalgae [34,35].

This study was focused on two microalgal species isolated from local municipal wastewater which aimed at evaluating the capabilities of the harvested *Myrmecia bisecta* and *Tetradesmus obliquus* microalgal biomass for various biotechnological applications as antioxidant, anticancer, and antimicrobial agents.

2. Materials and methods

Microalgae isolation, and maintenance

A wastewater sample was collected from the Qaha wastewater treatment plant, Qalubia in Egypt. Shortly after being collected, the sample was transported to the laboratory in a sterile bottle. The sample was enriched using Bold's basal medium (BBM) [36]. After incubation for 2 weeks at 25 ± 1 °C with continuously illuminated cool-white fluorescent light. Fresh agar media was used to subculture single microalgal colonies. The inoculated plates were further incubated for 2 weeks, and subculturing was applied to obtain pure cultures. Identification was done by observation under bright field microscope. After being examined under a microscope, the isolated microalgae were transferred from agar plates into slants and 50 milliliters of liquid BBM for preservation.

Microalgal biomass production and harvesting

The two isolated microalgae were grown in 100 % wastewater for biomass production in the aforementioned growth conditions, after reaching the stationary growth phase. For three days, the biomass was allowed to naturally settle in the flask. Subsequently, two distinct layers developed, one at the bottom with concentrated microalgae biomass and one at the top with water-containing suspended microalgae cells. The bottom layer was moved to a 500-mL beaker and dried at 60 °C for 24 hours, while the top layer was carefully decanted. After being removed from the beaker, the dried microalgae biomass was stored in an empty container for subsequent use.

Extraction of microalgal biomass

The dried powdered biomass was subjected to extraction by mixing with different solvents, shaken well and each mixture was subjected to sonication for 15min. Using frequent agitation, the algal powder and solvent were kept in contact for seven days in a stopper container. In the extraction procedure, 30 mL of hexane, chloroform, methanol, ethanol, and an aqueous solution were used to extract 3 g of dry algal mass. This involves extraction with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent (aqueous) to ensure that a wide polarity range of compounds can be extracted. Then, solvents are centrifuged, filtered, and evaporated at 40–50 °C in a rotary evaporator. The quantity of crude extract was represented as a proportion of the dried biomass weight used (mg extract/g dry biomass weight). [37].

- **Antioxidant activity**

DPPH radical scavenging assay

The method for evaluating the studied microalgal extract's scavenging effect was by Desmarchelier et al. [38], and IC50 was calculated. The experiment was repeated three times at each concentration.

ABTS scavenging assay

Free radical scavenging potentials were evaluated by ABTS (2,2′-Azino-bis-3 ethylbenzothiazoline-6-sulfonic acid) cation radical scavenging technique based on the method outlined by [39].

- **Antimicrobial activity**

Gram-negative bacteria *(Escherichia coli)*, Gram-positive bacteria *(Staphylococcus aureus)*, and a fungus (*Aspergillus niger*) were used as test organisms. The test was performed in 96-well flat polystyrene plates. Test extracts containing 10 µl (final concentration of after addition 250 µg/ml) to 80 µl of lysogeny broth (LB broth), 10 µl span class='highlighted color-6'>span> of bacterial culture suspension (log phase) were added, and the plates were incubated at 37°C for the entire night. After incubation, thewells exhibited an obvious sign of theirpositive antibacterial activity; in contrast, the growth media in the wells of the compounds that had no effect on the bacteria seemed opaque, the control is the pathogen without any treatment. Using a Spectrostar Nano Microplate Reader (,Allmendgrun, Germany), the absorbance was measured at OD600 after roughly 20 hours.

Anticancer activity

Cell lines and culture media

Breast tumor cell lines WI-38 and liver tumor cell lines HepG2 were obtained from (Sigma-Aldrich, USA). The cells were cultivated by the supplier's instructions in high glucose RPMI 1640 medium (Thermo Fisher Scientific Inc., USA) in addition to 10% FBS (Thermo Fisher Scientific Inc., USA), penicillin (100 U/mL) and streptomycin (100 µg/mL) and incubated at 37°C in a humid environment (5% CO_2).

Cytotoxicity MTT cell viability assay

Cellular viability and morphological form were analyzed to indicate the cytotoxic profile of Aluminum nanoparticles using the 3-(4, 5-Dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide (Sigma-Aldrich, USA), this colorimetric MTT assay was according to modified Van-de et al. [40]. To determine the data as an estimate of cell viability, triplicate plates with 96 wells were examined and the fractions dissolved in DMSO were tested against cell lines Then, to measure the absorbance at 570 nm, an enzyme-linked immunosorbent assay (ELISA) plate reader (BioTeck, Bad Friedrichshall, Germany) was used concerning cellular density. Using GraphPad prism 8.2.4, the half-maximum inhibitory concentration (IC50) was calculated.

% Cell viability = Abs (Sample)/Abs (control)*100

Cell morphology was recorded using an inverted microscope with a digital camera (Nikon, Japan). The experiments were implemented in triplicate.

3. Results and discussion

Isolation and identification

According to the characteristics of the cells designated in the current study, BBM was chosen as a control medium and for isolation because it is frequently utilized as a successful growth medium for several freshwater microalgae. Isolation was carried out by the serial dilution method to isolate the two microalgal species. In the present study, classical morphology-based methods were used for the identification of *Myrmecia bisecta* and *Tetradesmus obliquus* which were isolated from wastewater. It was confirmed using a light microscope which revealed that *Myrmecia bisecta* appeared as unicellular non-motile coccoid cells having a parietal and cup-shaped chloroplast (fig. 1) and *Tetradesmus obliquus* appeared as spindle-shaped cells with a plate-shaped chloroplast (fig. 2).

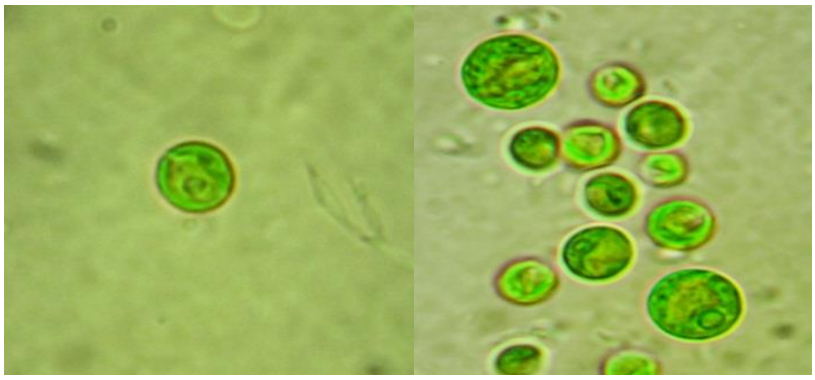

Fig. (1) Microscopic photograph of *Myrmecia bisecta*

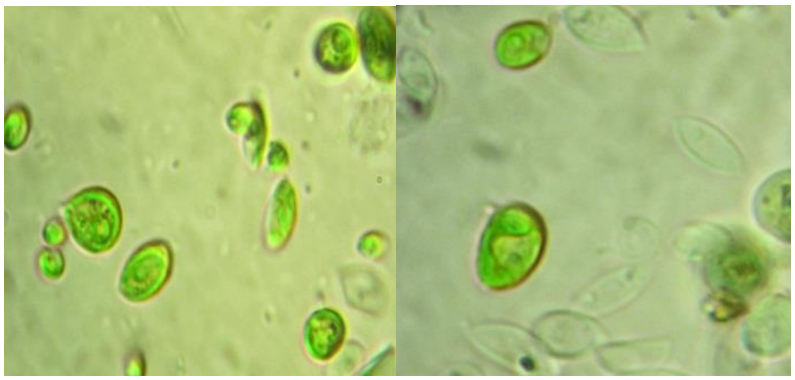

Fig. (2) Microscopic photograph of *Tetradesmus obliquus*

- Antioxidant activity

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical process that has the ability to generate free radicals, which can damage cells through a series of reactions. Microalgae have antioxidant activity due to producing metabolites that balance cell metabolism by eliminating oxidative stress and these metabolites are vital for the cellular protection of human health [41,42]. In the present study, two methods were used to evaluate the total antioxidant capacity versus vitamin C of extracts of *Tetradesmus obliquus* and *Myrmecia bisecta* were evaluated for their antioxidant activity by DPPH assay and ABTS assay. Antioxidant activity tests were performed by measuring IC_{50} between different antioxidant tests (DPPH IC_{50} and ABTS IC_{50}), they are shown in Tables (1&2). According to IC_{50} values, both two organisms can produce active compounds that act as *obliquus* showed lower antimicrobial activity. It showed a 32.33% inhibition against *Escherichia coli* and a 14.20% inhibition against *Staphylococcus aureus.* The results indicate that *Tetradesmus obliquus* is less potent compared to *Myrmecia bisecta* in inhibiting bacterial growth. In contrast, *ciprofloxacin* at a dosage of 10 µg/ml demonstrated potent antimicrobial activity. The inhibition percentages were notably high, with 97.24% against *Escherichia coli* and 98.24% against *Staphylococcus aureus*. *Ciprofloxacin*, a known antibiotic, acted as a reliable control and performed better than the natural compounds tested.

antioxidants, However, *Tetradesmus obliquus* activity exceeded *Myrmecia bisecta* activity because antioxidant activity is highly potent when the IC_{50} value of the test is low, and vice versa. [43].

Carotenoids play a role in the cosmetic industry because they serve as natural antioxidants and pigments [44]. Specifically, because of their bioactivity and possible benefits for human health, carotenoids are the most researched antioxidant compounds [45]. Numerous carotenoids have strong antioxidant properties found in microalgae [46]. Owing to their outstanding characteristics as oxygen removal, UV barrier, antioxidants, antibacterial, and limited environmental effects, a mixture of microalgal antioxidant substances that exist naturally and inorganic nanoparticles were common in active food packaging [47,48].

- *Antibacterial activity*

The antibacterial results against *Escherichia coli* and *Staphylococcus aureus* for *Myrmecia bisecta*, *Tetradesmus obliquus*, and *ciprofloxacin* (cipro) are presented in Table (3) and Fig (3). The inhibition percentages indicate the efficacy of each substance at the specified concentrations. *Myrmecia bisecta* exhibited moderate antimicrobial activity against *Escherichia coli*, it achieved a 45.20% inhibition, while against *Staphylococcus aureus*, the inhibition was 25.26%. This suggests a relatively stronger effect on *Escherichia coli* compared to *Staphylococcus aureus*. Conversely, *Tetradesmus*

Table (1) Comparison between IC_{50} between two antioxidant tests of *Tetradesmus obliquus versus* vitamin C

	DPPH IC_{50}	**ABTS IC_{50}**
Tetradesmus obliquus	16.18±1.44 µg/mL	23.35 ± 1.22 µg/mL
Vitamin C " standard"	25.31 ±0.85 µg/mL	11.70± 0.27 µg/mL
P-value	0.048	0.041

Table (2) Comparison between IC_{50} between two antioxidant tests of *Myrmecia bisecta versus* vitamin C

	DPPH IC_{50}	**ABTS IC_{50}**
Myrmecia bisecta	20.21±1.02 µg/mL	26.05 ± 2.08 µg/mL
Vitamin C " standard"	16.23 ±0.54 µg/mL	22.27± 1.32 µg/mL
P-value	0.17	0.24

Table (3) Presented the antimicrobial results against *Escherichia coli* and *Staphylococcus aureus* for *Myrmecia bisecta*, *Tetradesmus obliquus*, and ciprofloxacin (cipro).

	Escherichia coli	***Staphylococcus aureus***
Myrmecia bisecta	45.2	25.3
Tetradesmus obliquus	32.3	14.2
cipro (10 µg/ml)	97.2	98.2

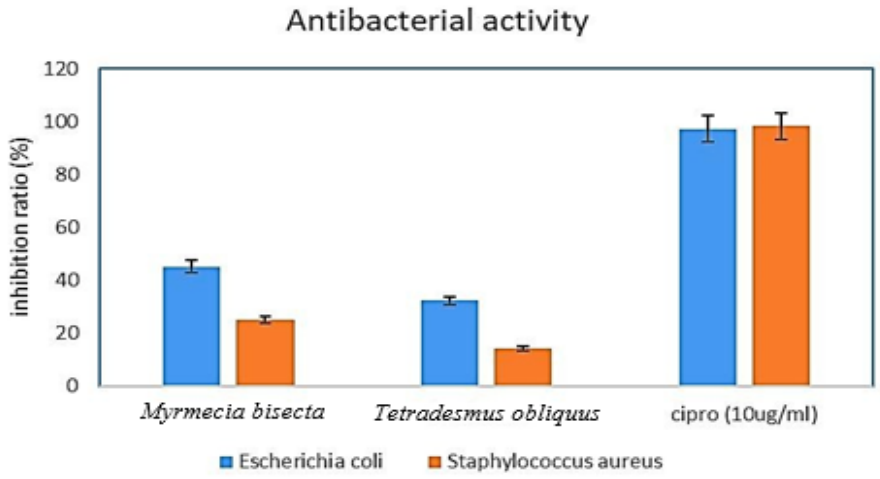

Fig. (3) Presented the antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* for *Myrmecia bisecta, Tetradesmus obliquus,* and *ciprofloxacin* (cipro).

- *Antifungal activity*

The antifungal results against *Aspergillus niger* for *Myrmecia bisecta*, *Tetradesmus obliquus*, and *nystatin* (Nys) at a dosage of 10 µg/ml are elucidated below. in the table (4) and fig (4). *Myrmecia bisecta* exhibited a moderate level of antimicrobial activity against *A. niger*, with an inhibition percentage of 21.02%. It demonstrates that there may be some possibility for *Myrmecia bisecta* to suppress the growth of *A. niger*. In contrast, *Tetradesmus obliquus* exhibited less antifungal efficacy against *A. niger*, with an inhibition percentage of 10.24%. Based on the results, it sounds like *Tetradesmus obliquus* may restrict *A. niger* growth less effectively than *Myrmecia bisecta*.The effectiveness of *Tetradesmus obliquus* against *A. niger* might benefit from further exploration and optimization. *Nystatin* (Nys) at a concentration of 10 µg/ml emerged as a highly potent antifungal agent, displaying an impressive inhibition percentage of 98.42% against *A. niger*. *Nystatin*, a well-known antifungal control, reaffirms its efficacy in suppressing the proliferation of *A. niger*. The high level of inhibition underscores the robust antifungal properties of Nys.

Table (4) Presented the antifungal results against *A. niger* for *Myrmecia bisecta*, *Tetradesmus obliquus,* and *nystatin* (Nys)

	Aspergillus niger
Myrmecia bisecta	21.0
Tetradesmus obliquus	10.2
Nys (10 µg/ml)	98.4

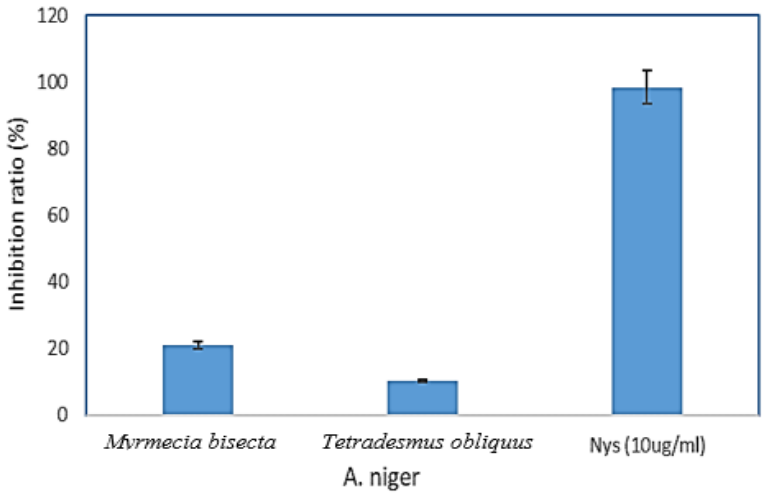

Fig. (4) Presented the antifungal activity against *A. niger* for *Myrmecia bisecta, Tetradesmus obliquus*, and *nystatin* (Nys)

- *Anticancer activity*

Cytotoxicity assay (MTT assay)

The MTT assay is based on the reduction of MTT by mitochondrial dehydrogenase by purple formazan product. In vitro model systems are commonly used to evaluate the cytotoxic effects of various toxic substances and plant extracts on cancer cell lines. MTT assay results shown the cytotoxic effect of the tested microalgal extracts against HepG2 cells and control with IC_{50} (half inhibitory concentration) values. As shown in Fig (5) microalgal extracts IC_{50} values of *Tetradesmus obliquus* and *Myrmecia bisecta* were 36.44, 40.12, respectively. The cytotoxic effect of both microalgal extracts were tested against WI-38 cells and controlled with IC_{50} values. According to Fig. (6) microalgal extracts IC_{50} values were 65.63 and 61.74, respectively. A significant cytotoxic effect against the HepG2 and WI-38 cellline is suggested via an IC50 value of less than 100 µg/mL [49], and this implies that the two have powerful cytotoxic impacts on the HepG2 cell line. It can be said that extract *Tetradesmus obliquus* has a more cytotoxic effect with 36.44 µg/mL IC_{50} value. Microalgae enhance human body defenses by decreasing the growth of cancer cells, increasing natural killer-cell activity, and activating the immune system. [50-52]. The possible inhibition of cell growth by microalgae extracts can be ascribed to the solvent action of several bioactive components, the presence of phenolic compounds, and the availability of further antioxidant agents [37]. The MTT assay was utilized to evaluate the extract's cytotoxicity. The MTT experiment's result showed an encouraging correlation between the anticancer properties of the microalgae extracts and the quantities used. Figures 7 and 8 show the morphology of the HepG2 and WI-38 cell lines treated and control cells. When exposed to microalgae extract, the treated cells typically lose their desmosomes, cellular appendages, and circular shape while also becoming less viable. The study revealed that the application of microalgae extract resulted in the reduction in the presence of normal spindle intact cells in the treated cell lines, as compared to the untreated control cells, whereas detached cells showed ruptured membranes and irregular cell membranes (Figs. 7&8). A similar trend was observed in the cytotoxicity activity [53-55].

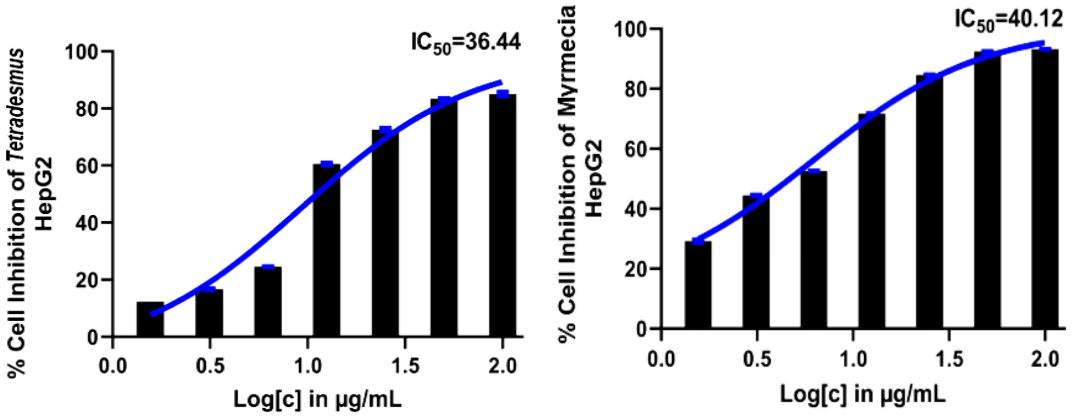

Fig. (5) Treatment of HepG2 cell line with *Tetradesmus obliquus* and *Myrmecia bisecta* extract at various doses

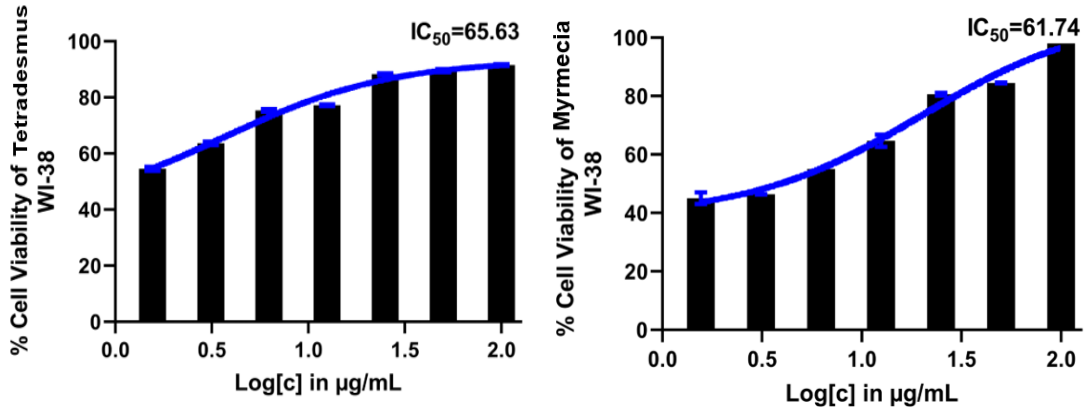

Fig. (6) Treatment of WI-38 cell line with *Tetradesmus obliquus* and *Myrmecia bisecta* extract at various doses

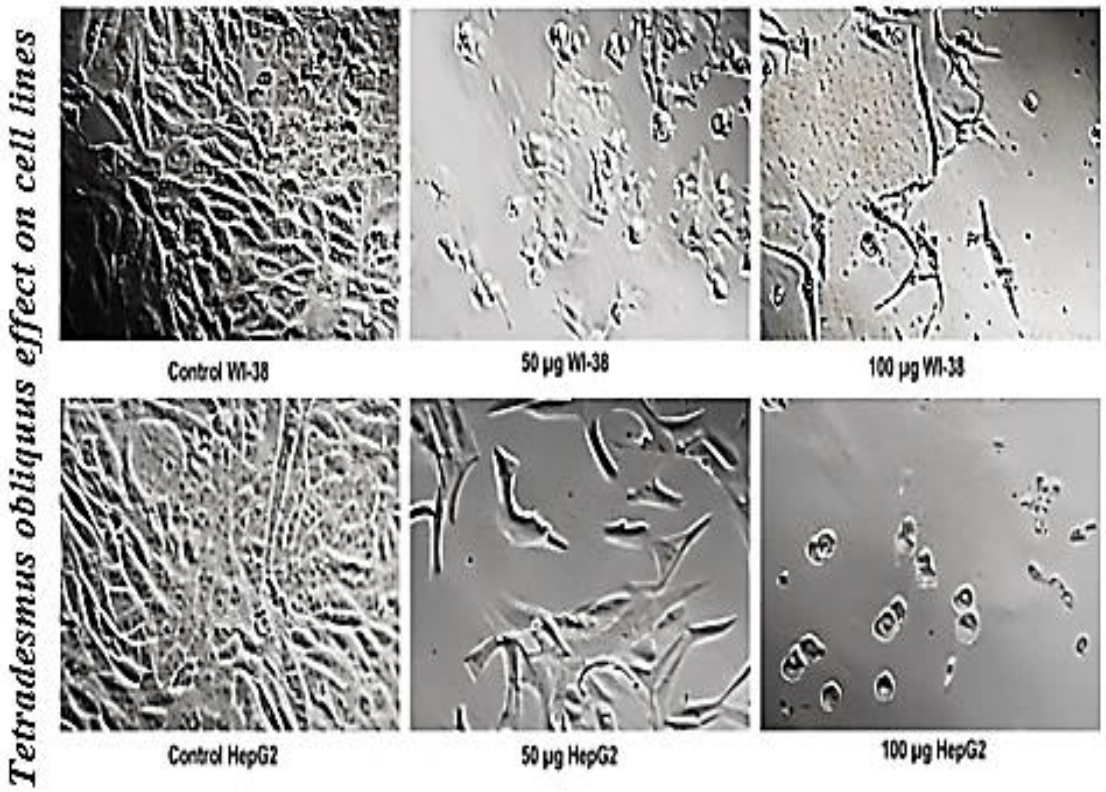

Fig. (7) Morphological alterations demonstrating the suppression of the cell lines at various doses of *Tetradesmus obliquus* extract

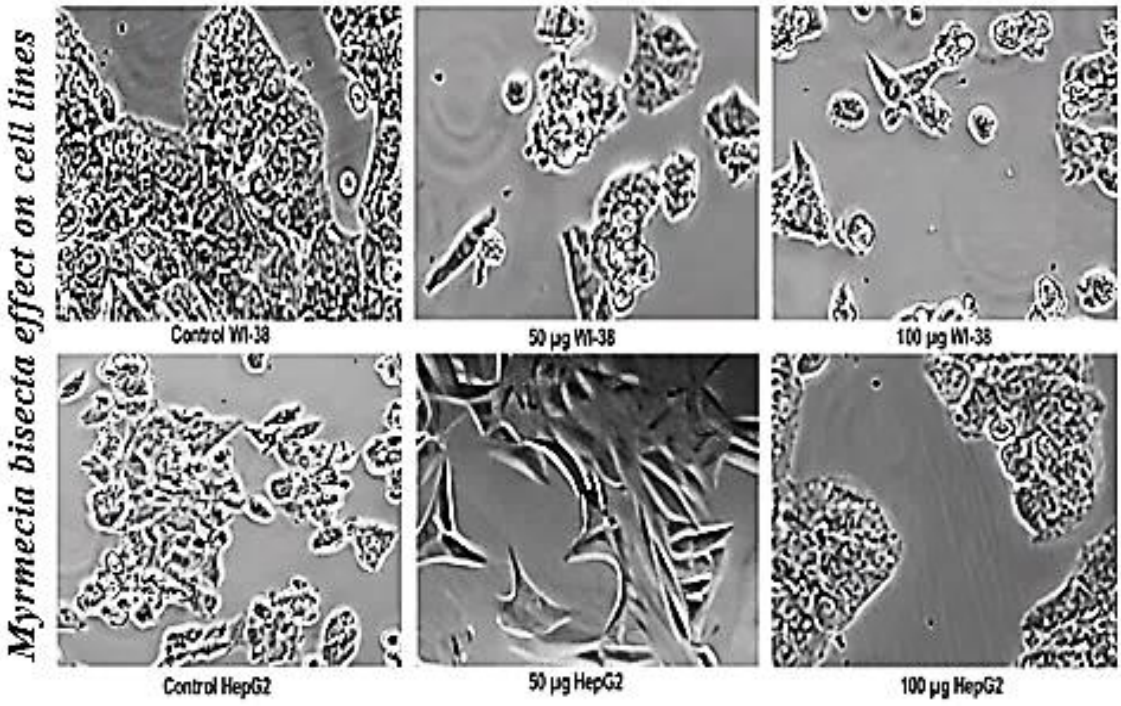

Fig. (8) Morphological changes showing the cell lines' suppression at different *Myrmecia bisecta* extract dosages

Conclusion

As a result of the current investigation, *Myrmecia bisecta* and *Tetradesmus obliquus* microalgae, were isolated, cultivated, and the dried microalgal biomass performed solvent extraction. Numerous secondary metabolites with powerful antibacterial, anticancer, and antioxidant effects have been found to be prevalent in the microalgae. The study provides scientific evidence to support further research, examine the main compounds found in both of the two microalgae.

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