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Enhancement of Anticancer, Antibacterial, and Acetylcholinesterase Inhibition Activities from *Oscillatoria sancta* under Starvation Conditions

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Abstract: The growth response and biological activity of the cyanobacterium *Oscillatoria sancta* were investigated in starvation conditions. *Oscillatoria sancta* growth potential was examined on BG11 and Zarrouk's media. Zarrouk's medium supported the maximum growth of the test cyanobacterium. Zarrouk's medium composition was modified by excluding $CaCl_2 \cdot 2H_2O$, NaCl, EDTA (Na), micronutrients, and replacing sodium nitrate with urea. Using Zarrouk's medium and three different concentrations of modified Zarrouk's media (Treatments 1–3), the growth response of *Oscillatoria sancta* (MZ366482) was examined and compared. Zarrouk's medium and modified Zarrouk's medium at 12.5% nutrient concentration had non-significant differences in both the dry weight biomass and total protein of *Oscillatoria sancta*. *Oscillatoria sancta* crude biomass extracts grown on Zarrouk's and modified Zarrouk's media (T3) inhibited human breast cancer, pathogenic bacteria, and acetylcholinesterase activity. *Oscillatoria sancta* grown on T3 showed the most potency against MDA-MB-231 cells with an IC_{50} of 165.2 µg mL^{-1}, antibacterial activity only against *Bacillus cereus* (17.2 mm) and *Staphylococcus aureus* (15.3 mm), and acetylcholinesterase inhibition activity by 60.7%. Thus, it is advisable to use the 12.5% nutrient concentration of modified Zarrouk's medium as a reduced-cost medium for mass cultivation of *Oscillatoria sancta* with potential anticancer, antibacterial diseases, and anti-Alzheimer purposes.

Keywords: acetylcholinesterase; antibacterial; nutrient stress; MDA-MB-231 cell line; *Oscillatoria sancta*, Modern technology

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1. Introduction

Microalgae are rich sources of secondary metabolites that are naturally synthesized and can be used in the pharmaceutical, cosmetic, and healthcare industries [1]. Depending on stress conditions, different suites of microalgal secondary metabolites with significant structural variations can be produced. As cyanobacterial strains directly relate to eukaryotic photosynthetic organisms, such as plants, they can serve as a model to investigate the stress response in this context [2,3].

Cyanobacteria can produce different suites of secondary metabolites in response to the different biotic and abiotic stresses to defend their cells against grazers and predators, as well as antioxidant, chemosensory, and photoprotection functions [4]. Under nutrient stress conditions, cyanobacteria undergo significant alterations and develop sophisticated coping mechanisms that allow them to endure stress using a "stand-by" energy metabolism [5]. Cyanobacteria synthesized poly-b-hydroxybutyrate (PHB) inclusions, cyanophycin, and phycobiliproteins under nitrogen, phosphorus, and sulfur starvation conditions, exhibiting anti-oxidant, anti-inflammatory, neuroprotective, hepatoprotective, and anticancer effects [6–11].

Furthermore, L-valine, L-tryptophan, and methionine were synthesized in response to carbon stress, and are efficient against *Staphylococcus* and *Clostridium* bacteria. However, phycobiliprotein production and antioxidant activity increased under nitrogen stress [12–16]. Anatoxins, flavodoxin, and iron chelators are also produced by cyanobacteria when there is a lack of iron, which increases their antimicrobial action [17,18].

Additionally, an extensive list of indole alkaloids' secondary metabolites can be synthesized by cyanobacteria to maintain their survival under starvation conditions such as norharmane (9H-pyrido(3,4-b) indole), which, when excreted by *Nodularia harveyana*, exhibited high algicidal activity [19]. Nostocarboline from *Nostoc* 78–12A and anatoxin-a from *Nostoc* sp. could be used as an acetylcholinesterase inhibitor for the treatment of neuronal diseases like Alzheimer's [20,21]. Furthermore, hapalindole synthesized from *Hapalosiphon fontinalis* exhibits antibacterial and anti-tuberculosis effects [22]. Phenolics, bromophenols, terpenoids, cyclic dep-sipeptides, lipoprotein, and isonitrile-containing indole alkaloids are produced by different strains of cyanobacteria and are efficient as antifungal [23].

They also produce several effective compounds against cancer progression by stimulating apoptotic death, such as synthadotin [24], cryptophycin, and curacin [25]. Some authors [26,27] reported using marine blue-green algae for anticancer compounds. Numerous researchers have investigated the bioactive components of cyanobacteria and found that they have pharmacological characteristics. However, more in vivo and in vitro investigations employing various animal models and clinical studies will be necessary to bring cyanobacteria and their active components into translational mode [28]

The present work investigates the effect of nutrient deficiency on the biochemical composition and activity of *Oscillatoria sancta* crude extract against MDA-MB-231 cell line, some pathogenic bacteria, and AChE inhibition to evaluate the possible activity against breast cancer, Alzheimer's disease, and bacterial diseases.

2. Materials and Methods

2.1. The Isolate

The cyanobacterium used in this study was obtained from the culture collection of the phycology laboratory, Faculty of Science, Mansoura University, Egypt. Cultures were maintained in blue–green (BG11) nutrient medium at 28 ± 2 °C under continuous illumination of 50 µmol photons m^{-2} s^{-1} [29].

2.2. Molecular Identification

The test isolate's genomic DNA was isolated using the Thermo Scientific GeneJET Genomic DNA Purification Kit (Thermo Scientific Sciences, Hilden, Germany). Polymerase chain reaction (PCR) amplification was performed for purified DNA slightly modified from that used by [30]. Specific primers targeting part of the 16S rRNA gene: (forward primer) CYA106F (5′ CGG ACG GGT GAG TAA CGC GTGT 3′) and (reverse primer) CYA781R (5′ GAC TAC TGG GGT ATC TAA TCC CAT 3′), were used. The PCR reaction was performed in a total volume of 25 µL reaction mixtures containing the following: 3 µL from forward and reverse primer (10 pmol/µL from each), dNTPs 2.5 µL, Taq DNA polymerase 0.2 µL (Fermentas, Hilden, Germany), 10× Taq buffer 5 µL, 8 µL nuclease-free water, and 1 µL template DNA (equivalent to 10 ng). The mixture was subjected to the following amplification conditions: an initial denaturalizing step of 4 min at 94 °C, 40 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 48 °C, and extension for 1 min at 72 °C, ending with a final extension step at 72 °C for 7 min. Amplified DNA fragments were estimated using a DNA ladder of 2 kb. The PCR products were electrophoresed on 2% (*w*/*v*) agarose gel in 0.5× TBE buffer at 90 mV for 3 h 30 min, using the 2 kb plus DNA Ladder (Gibco-BRL) as a molecular size marker. The target band was excised and purified with a DNA gel Extraction Kit (Fermentas, Hilden, Germany). The BLAST tool and MEGA ver. 5 software were used to perform multiple alignments and phylogenetic analyses on the gene sequences of *Oscillatoria* strains (16S rRNA) [31].

2.3. Impact of Various Nutrient Media on the Growth Potential of Oscillatoria sancta

The growth of *Oscillatoria sancta* was assessed using the BG11 medium [29] and Zarrouk's medium [32] to compare the growth potential of *Oscillatoria sancta* in the two distinct nutritional media. In this research, we employed sterile cultures with a pH of 8 and maintained the temperature at 28 ± 2 °C for two weeks while the cells were exposed to 50 µmol photons m^{-2} s^{-1}. The optical density of the inoculum at the start was 0.18 (OD_{682}).

2.4. Modification of the Chemical Composition of Zarrouk's Medium

The experimental results in this study indicated that Zarrouk's medium supported the maximum biomass production of *O. sancta*. Thus, it was chosen for further growth experiments. Modified Zarrouk's medium was devoid of $CaCl_2 \cdot 2H_2O$, EDTA (Na), NaCl, and micronutrients. Sodium nitrate ($NaNO_3$) was substituted by urea ($CO(NH_2)_2$) (0.8 g L^{-1}) (Table 1). Three-grade nutrient concentrations (100%, 50%, and 12.5%) of modified Zarrouk's medium representing T1, T2, and T3 were prepared for testing and comparing growth with standard Zarrouk's medium (control). The initial inoculum was comparable to 0.05 g L^{-1} dry wt. The goal was to examine the growth potential of *O. sancta* under starvation conditions.

Table 1. The modified Zarrouk's medium chemical composition (g L^{-1}).

Constituents	Zarrouk's Medium (g L^{-1}) *	Modified Zarrouk's Medium (g L^{-1})		
		100% (T1)	50% (T2)	12.5% (T3)
$NaNO_3$	2.5	-	-	-
Urea	-	0.8	0.4	0.1
$CaCl_2 \cdot 2H_2O$	0.04	-	-	-
NaCl	1.0	-	-	-
$FeSO_4 \cdot 7H_2O$	0.01	0.01	0.005	0.00125
EDTA (Na)	0.08	-	-	-
K_2SO_4	1.0	1.0	0.5	0.125
$MgSO_4 \cdot 7H_2O$	0.2	0.2	0.1	0.025
$NaHCO_3$	16.8	16.8	8.4	2.1
K_2HPO_4	0.5	0.5	0.25	0.0625
Micronutrients (H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 4H_2O$ Na_2MoO_4, $CuSO_4 \cdot 5H_2O$)	1.0 mL	-	-	-

Note(s): * standard Zarrouk's medium (control).

2.5. Growth Assessment

The growth of *O. sancta* was evaluated using a combination of gravimetric dry-weight biomass and measurements of the chlorophyll a concentration [33]. The established *O. sancta* culture volume was filtered through a dry pre-weighed GF/C filter to estimate the dry weight. To obtain an average dry weight, we dried *O. sancta* biomass filters for 12 h in an oven at 105 °C before cooling them in a desiccator and weighing them (g L^{-1}).

2.6. Growth Rate

A formula was used to calculate the *O. sancta* cultures' specific growth rates according to Anderson [34] using the following Equation (1):

$$\mu = \frac{\ln X_2 - \ln X_1}{t2 - t1} \quad (1)$$

where µ is the specific growth rate and X_1 and X_2 are the chlorophyll a concentration (mg L^{-1}) at times t1 and t2 in this experiment, respectively.

The divisions per day (Dd^{-1}) were calculated following Equation (2):

$$Dd^{-1} = \frac{\mu}{\ln 2} \quad (2)$$

2.7. Biomass Harvesting

Known volumes of *Oscillatoria sancta* cultures were collected after 14 days of growth and centrifuged at 2688× *g* for 10 min. Glass-distilled water was used to thoroughly clean the pellets before they were placed in a freezer and kept at −20 °C overnight. After the algal pellets had been frozen, the freeze-dried biomass was weighed using a four-decimal point balance [35].

2.8. Biochemical Properties

Biochemical properties include many parameters, such as the determination of total protein, carbohydrate, and lipid contents. Protein concentration was determined using the Bradford methods [36] and updated by Stoscheck [37]. Carbohydrate concentration was determined using the Hedge and Hofreiter method [38]. Lipid extraction was performed according to the exhaustive Soxhlet process of Sadasivam and Manickam [39].

2.9. Crude Extraction with Methylene Chloride/Methanol (1:1) v/v

Soxhlet extractors were used to extract algal biomass from a sample of 5 g freeze-dried powder wrapped in filter paper and gently squeezed [39].

2.10. Biological Activities of Oscillatoria sancta Crude Extract

2.10.1. Antibacterial Screening Assay

The antibacterial activity of *Oscillatoria sancta* methylene chloride was investigated using agar well diffusion; methylene chloride/methanol crude extracts were used [40]. *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Escherichia coli* were collected from the bacteriology laboratory of the Faculty of Science at Mansoura University, Egypt. Then, 8 mm wells on nutrient agar plates were made using a sterile cork borer and filled with 100 µL of *O. sancta* crude extracts (10 mg mL^{-1}). Methylene chloride was employed with methanol serving as a negative check. At 37 ± 2 °C, the diameters of clean zones were assessed for antibacterial activity after 24 h.

2.10.2. In Vitro Acetylcholinesterase Activity Test

The activity of acetylcholinesterase (AChE) in the presence of *O. sancta* crude extracts was determined according to the method proposed by Refaay [41]. The assay was performed in a 96-well microplate containing 200 µL assay mixture containing 0.75 mM of 5, 5_-dithio-bis-[2-nitrobenzoic acid] (DTNB) and electric-eel AChE (0.0004 µg mL^{-1}) in 50 mM Tris-HCl (pH 8). Then, 1.5 mM of acetylthiocholine iodide (ATCI) was added to the reaction mixture to start the reaction. The developed yellow color of 5-thio-2-nitrobenzoate (TNB) was measured at 405 nm. Then, 5 µL (10 µg mL^{-1}) of *O. sancta* crude extracts were pre-incubated with the enzyme for one minute before initiating the reaction. The AChE activity was also performed in the presence of methylene chloride/methanol as the negative control and donepezil (10 µg mL^{-1}) as the positive control. The AChE inhibition percent was calculated according to the following equation:

$$\text{Inhibition}(\%) = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

where A_0 is the absorbance in the presence of the solvent only and A_1 reflects the absorbance when the tested extract was utilized instead.

2.10.3. Evaluation of Anticancer Activity

Cell Line

The American Type Culture Collection (ATCC) breast cancer cell line (MDA-MB-231) was grown on Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, penicillin, and streptomycin, and then incubated at 37 °C in a 95% relative humidified environment with 5% CO_2 for 30 min.

In Vitro MTT Assay

The cytotoxicity of crude extracts of *Oscillatoria sancta* was examined utilizing the Mosmann method [42]. MDA-MB-231 cells were plated in 96-well microplates containing different doses of extract (0.125, 0.25–0.5, 0.5, 1, and 3 mg mL^{-1}) at a concentration of 3 × 103 cells/well (including 2% antibiotics and 2% FBS). MDA-MB-231 cells cultured in a medium were used to create a parallel monitor. During a 72 hr. incubation period at 37 °C, wells were filled with MTT (5 mg mL^{-1}) solution and incubated for another 4 h at 37 °C until violet (formazon) crystals emerged. Dimethyl sulfoxide (DMSO) was used to dissolve 50 µL of Formulazan crystals at 37 °C for 30 min. Using an ELISA reader, we determined an absorbance of 570 nm. For each extract, the MDA-MB-231 cells' growth rate inhibition % was calculated according to the following equation:

$$\text{The inhibition rate \%} = 100 - \left[\left(\frac{\text{Absorbance of treated cells}}{\text{Absorbance of Control Cells}}\right) \times 100\right]$$

Chart Pad Prism 8 software was used to calculate the IC_{50} value (the concentration that affects 50% of cell death) using data on the cell growth inhibition rate.

2.11. GC-MS Analysis

Gas chromatography (Aglient 6890) equipped with an Aglient mass spectrometric detector was used to determine the chemical composition of *O. sancta* crude extract. An electron impact ionization mode detector was used to accomplish mass spectrophotometric scanning between 50 and 500 m/z. Here, 70 e.v. was the ionizing power. The electron multiplier's EM voltage was kept constant at 1250 V using auto-tune. The instrument was tuned by hand with perfluorotributyl amine (PFTBA). The mass spectral database Wiley and Wiley NIST was used to identifying the peaks.

2.12. Statistical Analysis

One-way analysis of variance (ANOVA) was performed on the data at $p \leq 0.05$ to determine the significance of the findings using SPSS Ver. 20.

3. Results

3.1. Phylogenetic Analysis and Placement of Oscillatoria sancta

The dendrogram of phylogenetic analysis (Figure 1) indicated that the Egyptian isolate *Oscillatoria sancta* HSDM1 falls into a clade together with *Oscillatoria sancta* strain VMRJHK012020 (accession No. MW364271), maintaining a 100% bootstrap value and in conjunction with the help of the local pairwise orientation (http://www.ebi.ac.uk/Tools/psa/emboss_water/nucleotide.html) (accessed on 20 May 2022) using the Smith–Waterman algorithm of the two sequences. The test findings demonstrate that the two sequences are 99% similar. The *Oscillatoria sancta* HSDM 1 sequencing product was placed in the GenBank database with the entry number MZ366482.

3.2. Growth Assessment of Oscillatoria sancta on Different Nutrient Media

Oscillatoria sancta growth on BG11 and Zarrouk's nutritional media was assessed using chlorophyll a as a concentration variation. Growth curves are illustrated in Figure 2. *O. sancta* maintained the highest chlorophyll a content at the end of the sixth day on Zarrouk's medium and at the end of day eight on the BG11 medium at 1554 ± 31.6 and 889 ± 27.9 mg L^{-1}, respectively.

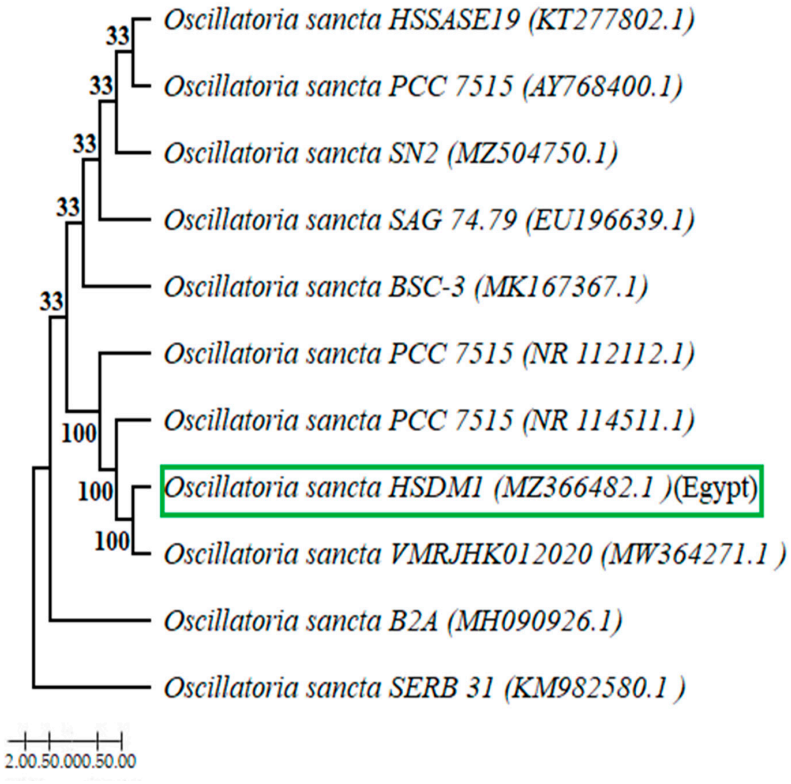

Figure 1. Phylogenetic tree dendrogram based on *O. sancta* 16S rRNA gene sequence using the bootstrap test of the maximum-likelihood (ML) technique.

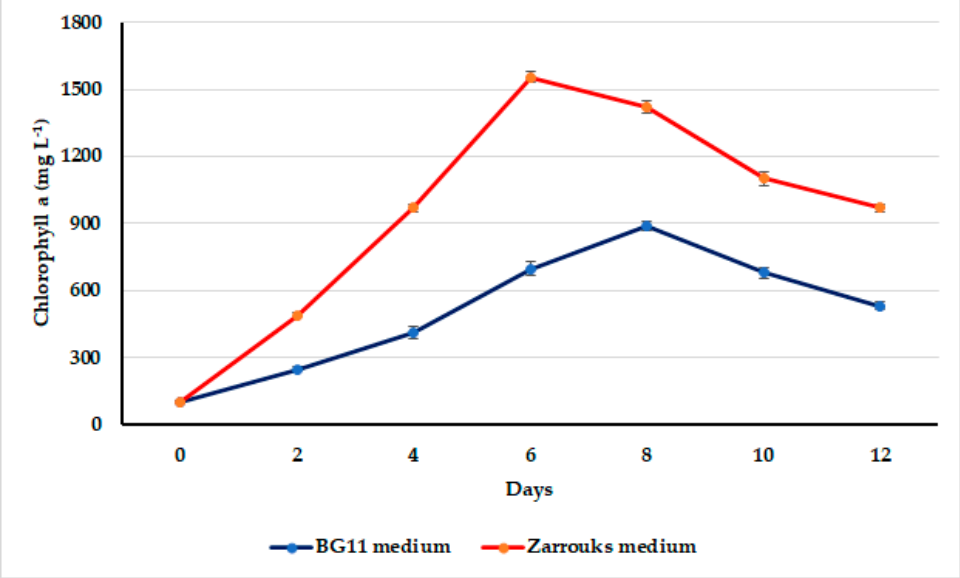

Figure 2. Mean changes in chlorophyll concentration (mg L^{-1}) of *O. sancta* grown on BG11 and Zarrouk's media.

3.3. Growth Rates of the Test Cyanobacterium Oscillatoria sancta

To perform an accurate growth comparison of *O. sancta* on both BG11 and Zarrouk's media, the specific growth rate (μ) and divisions per day (Dd^{-1}) were calculated. The results are shown in Figure 3. Zarrouk's medium exhibited a highly significant ($p \leq 0.05$) increment in specific growth rate (0.452 ± 0.029) and divisions per day (0.652 ± 0.034) of *O. sancta* by 1.68-fold compared with BG11 medium.

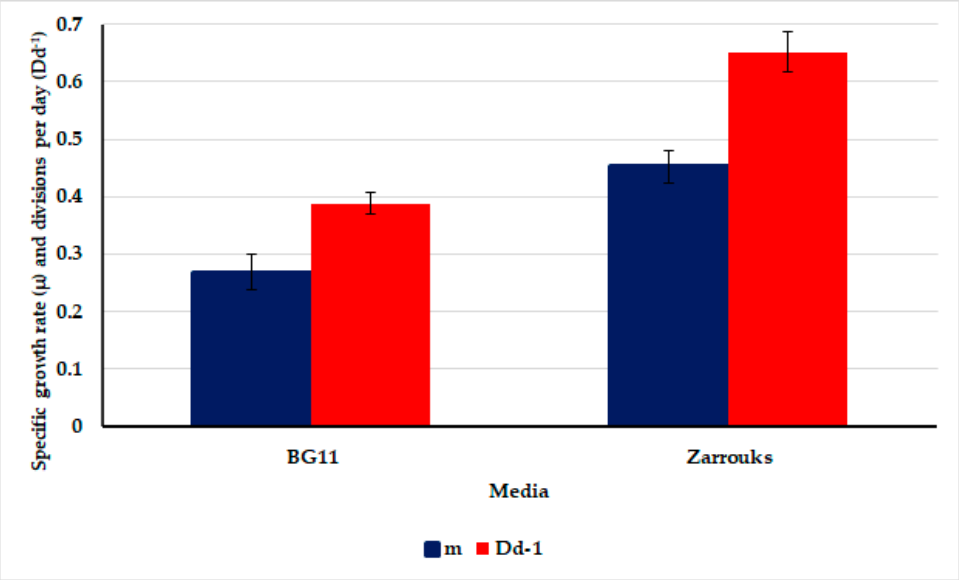

Figure 3. Specific growth rate (μ) and divisions per day (Dd^{-1}) of *O. sancta* grown on BG 11 and Zarrouk's media. Values are the averages of three replicates ± standard deviation.

3.4. Oscillatoria sancta Growth in Modified Zarrouk's Medium at Varying Nutrient Concentrations

Very slight and almost non-meaningful ($p \leq 0.05$) differences were observed in the dry weight (g L^{-1}) biomass of different *O. sancta* cultures grown at different modified Zarrouk's medium concentrations (T1 to T3) compared with control Zarrouk's medium (Table 2). The highest dry-weight biomass, 0.552 ± 0.002 g L^{-1}, was recorded on standard Zarrouk's medium and 0.509 ± 0.022 g L^{-1} was recorded at T3 (12.5% modified Zarrouk's medium). Accordingly, a 12.5% change in Zarrouk's medium concentration was selected for further growth test experiments.

Table 2. Dry weight biomass (g L^{-1}) of *Oscillatoria sancta* grown on different modified Zarrouk's medium concentrations (100%, 50%, 12.5%).

Treatments	Dry wt. (g L^{-1})
Control (standard Zarrouk's medium)	0.552 ^{a} ± 0.027 *
T1	0.539 ^{ab} ± 0.025
T2	0.53 ^{ab} ± 0.022
T3	0.509 ^{ab} ± 0.022

Note(s): * values shown are the averages of three replicates ± standard deviation. Different letters indicate significant differences at ($p > 0.05$).

3.5. Biochemical Composition of Oscillatoria sancta Grown on Both 12.5% Modified Zarrouk's Medium and Standard Zarrouk's Medium

The total carbohydrate, total protein, and total lipid contents of *O. sancta* dry biomass grown on both T3 (12.5% modified Zarrouk's medium) and standard Zarrouk's medium (control) are illustrated in Figure 4. There was no significant difference ($p > 0.05$) between *O. sancta* cultures cultured on T3 (47 ± 2.3%) and Zarrouk's medium (50.3 ± 2.5%) in terms of protein content.

Carbohydrate content was relatively high at T3 (26.2 ± 1.31%) compared with the control (15.4 ± 0.7%). Nutrient concentrations did not affect the lipid content. Wt.% of total lipids was 3 ± 0.14% and 2.8 ± 0.14% of biomass grown on T3 and Zarrouk's medium (control), respectively.

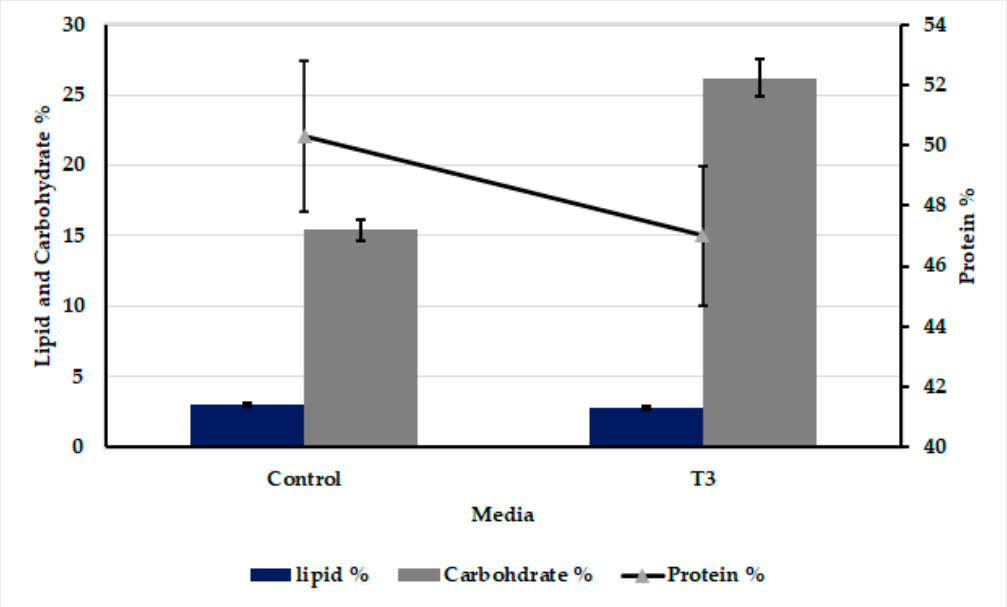

Figure 4. Biochemical composition of *O. sancta* grown on Zarrouk's medium (control) and 12.5% modified Zarrouk's medium concentration (T3). Error bars show the SD for three measurements.

3.6. Anticancer Activity of Oscillatoria sancta Methylene Chloride/Methanol Crude Extracts against MDA-MB-231 Cell Line

Cell viability in the MDA-MB-231 cell line was assessed using the MTT colorimetric test using crude methanol extracts of *O. sancta* cultures cultivated on T3 and Zarrouk's media. The extracts promoted MDA-MB-231 cell line anticancer activity in a dose-dependent manner (Figure 5). Compared with the conventional Zarrouk's medium (control, 195 ± 2.3 μg mL^{-1}), the *O. Sancta* crude extract cultivated in T3 demonstrated the strongest anticancer activity, with an IC_{50} value of 165.2 ± 0.54 μg mL^{-1}.

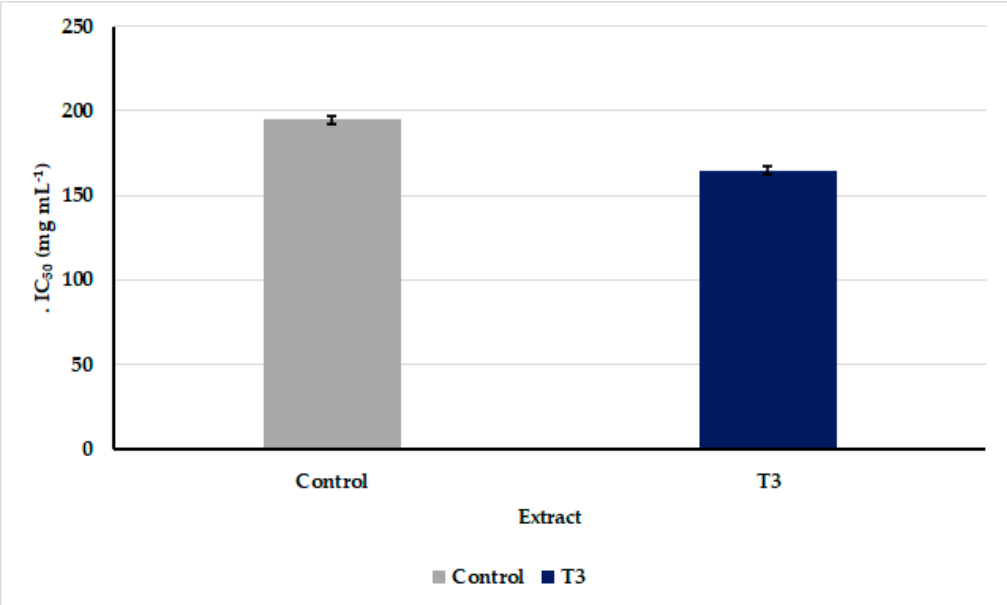

Figure 5. IC_{50} of *O. sancta* crude extracts grown on standard Zarrouk's medium (control) and modified Zarrouk's medium concentration (T3).

The *O. sancta* crude extracts exhibited antibacterial activity only against Gram + ve, *B. cereus*, and *S. aureus* (Table 3). With inhibition zones of 17.2 ± 0.44 mm and 15.3 ± 0.58 mm, *O. sancta* crude extracts prepared under nutrient scarcity (T3) exhibited the best antibacterial activity against Gram + ve, *B. cereus*, and *S. aureus*.

Table 3. The inhibition zone diameter (mm) measurement of different *O. sancta* crude extracts against pathogenic bacteria.

Bacteria	Diameter of Inhibition Zone (mm)		
	Zarrouk's Medium (Control)	T3 [a]	Solvent [b]
Pseudomonas aeruginosa	N	N	N [c]
Escherichia coli	N	N	N
Bacillus cereus	16.3 ± 0.5	17.2 ± 0.44	N
Staphylococcus aureus	12.3 ± 0.7	15.3 ± 0.58	N

Note(s): [a] 12.5% modified Zarrouk's medium concentrations, [b] methylene chloride/methanol in a ratio of 1:1 (*v*/*v*), [c] no inhibition.

3.7. Inhibition of Acetylcholinesterase Activity by Oscillatoria sancta Crude Extracts

Inhibition% of AChE activity calculated of the tested extracts are presented in Figure 6, where donepezil was used as the positive control. Moderate significant ($p \leq 0.05$) inhibition of AChE activity was shown in *O. sancta* crude extracts grown on both T3 and Zarrouk's medium, by 60.7 ± 3.1% and 58 ± 2.1%, respectively, compared with donepezil, which was recorded as 100 ± 0.0%.

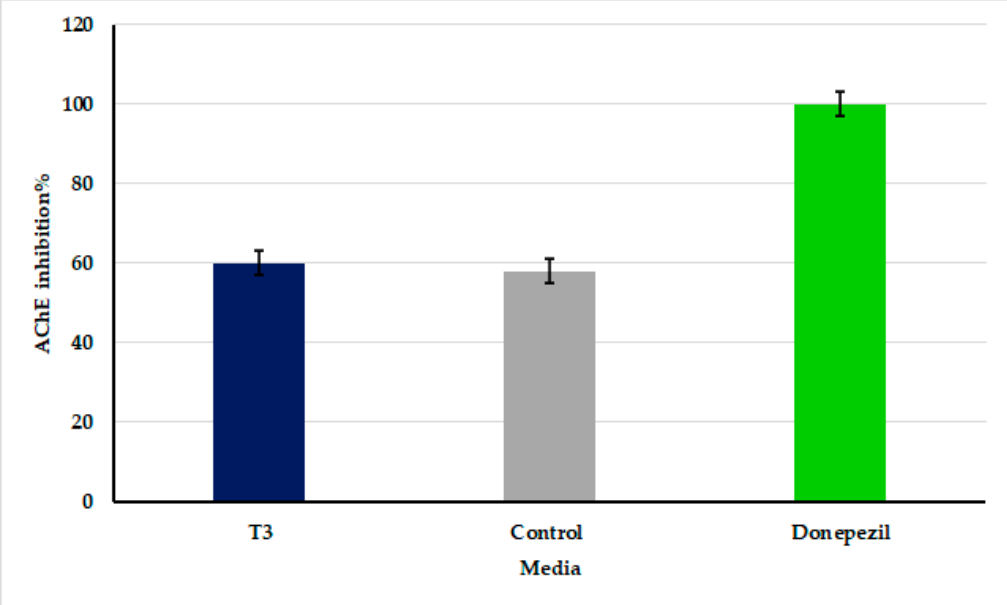

Figure 6. The AChE inhibitory activity of *O. sancta* crude extracts grown on Zarrouk's medium (control) and 12.5% modified Zarrouk's medium concentration (T3).

3.8. GC/MS

The gas chromatogram for *O. sancta* crude extract cultivated on T3 revealed ten peaks corresponding to ten compounds included (Figure 7). In Table 4, we recorded a complete list of chemical names, peak locations, retention times, and formulations. These substances comprise hydrocarbons, fatty acids, esters, and alkaloids.

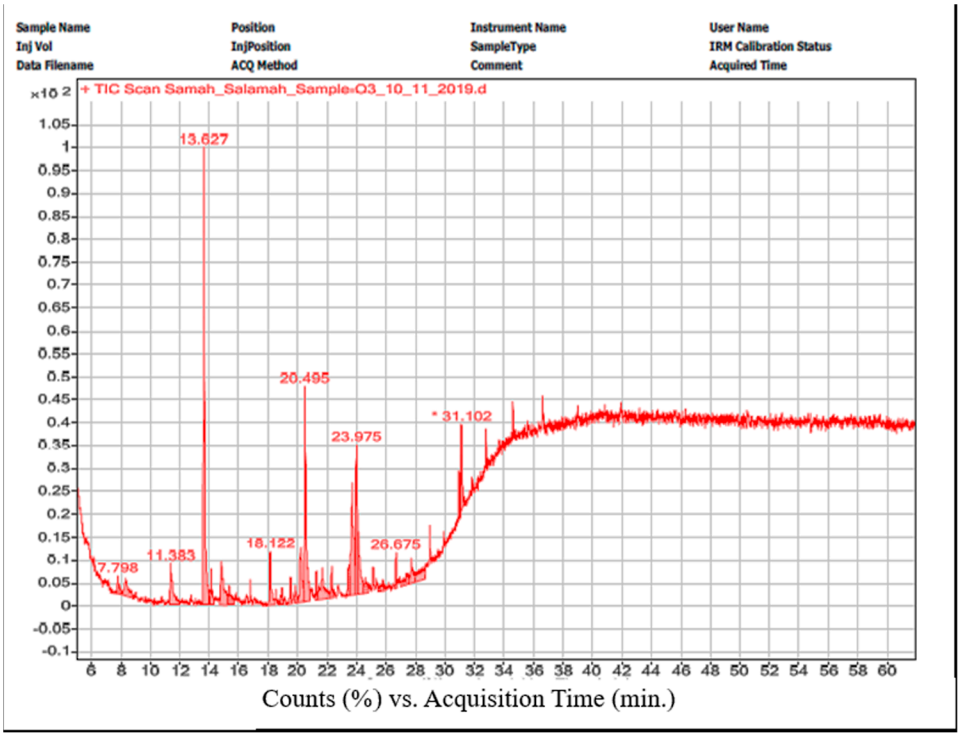

Figure 7. GC chromatogram of *O. sancta* crude extracts grown on T3.

Table 4. The data of GC-MS analysis of methylene chloride/methanol crude extract of *O. sancta* grown on 12.5% modified Zarrouk's medium concentration.

Peak No.	Compound	Chemical Group	Retention Time (min.)	Formula	Area%
1	Oleic acid, eicosyl ester	Oleic acid ester	11.383	$C_{38}H_{74}O_2$	5.5
2	Phenol, 2,4-bis(1,1-dimethylethyl)-	Phenol	13.652	$C_{14}H_{22}O$	25
3	Pentatriacont-17-ene	Alkene	14.808	$C_{35}H_{70}$	6.2
4	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-	Ester	18.134	$C_{34}H_{64}O_2$	3.8
5	7-Hexadecenoic acid, methyl ester, (Z)-	Methyl ester	20.175	$C_{17}H_{32}O_2$	5.7
6	Hexadecanoic acid, methyl ester	Methyl ester	20.532	$C_{17}H_{34}O_2$	16
7	Dasycarpidan-1-methanol, acetate (ester)	Indole alkaloid	23.477	$C_{20}H_{26}N_2O_2$	13.1
8	9,12-Octadecadienoyl chloride, (Z, Z)-	Linoleic acid chloride	23.668	$C_{18}H_{31}ClO$	10.5
9	Cholestan-3-ol, 2-methylene-, (3β,5α)-	Steroid	23.975	$C_{28}H_{48}O$	7.1
10	3′,8,8′-Trimethoxy-3-piperidyl-2,2′-binaphthalene-1,1′,4,4′-tetrone	Terpene	31.095	$C_{28}H_{25}NO_7$	7.15

4. Discussion

One of the major challenges influencing microalgae biomass production for high-value products or metabolites with applied purposes is the cost of nutrients. It is also possible for microalgae, particularly cyanobacteria, to develop high-value secondary compounds with a variety of medical applications in nutritional stress settings.

As seen in Figures 2 and 3, Zarrouk's medium exhibited the maximum biomass reproduction of *O. sancta*. Hong and others [43,44] have shown that the composition of nutritional media impacts both biomass production and the content and yield of a specific metabolic product in this situation. Owing to its increased growth rate, this cyanobacterium mainly depended on its culture medium's composition, making it a good candidate for future growth studies.

Since, the possible economic biomass production of microalgae is the key issue for microalgae-based applied purposes, it was decided to test the potential of *O. sancta* to grow

under nutrient deficiency using different nutrient concentrations of Zarrouk's medium after modification by substituting sodium nitrate with a cheaper nitrogen source (urea). According to Schwartz [45], cyanobacteria prefer inorganic nitrogen, particularly nitrates, for growth, but some strains can also thrive on organic nitrogen.

The experimental results (Table 2) may indicate relatively low nutritional demands of *O. sancta* to grow as dense as in standard Zarrouk's medium. They may highlight this cyanobacterium's feasible and economical biomass production in large-scale growth experiments. It is important to note that blue-green algae can develop under different nutrient conditions with flexible metabolic capabilities that enable them to grow under conditions of nutrient scarcity while synthesizing a variety of bioactive molecules [46,47].

Biochemical compositions of biomass grown on 12.5% modified Zarrouk's medium and standard Zarrouk's media should be analyzed because the culture medium composition influences not only the growth and metabolic activities of microalgae, but also their biochemical composition [44,48].

The protein analysis results (Figure 4) indicated that the biomass of this cyanobacterium is rich in protein content. This finding may trigger further research to investigate this cyanobacterium protein's composition and possible bioactivity. *Spirulina*, a type of cyanobacterium, is a high-protein food alternative to traditional plants like soybean, as it contains up to 70% of its dry-weight biomass as a protein [49].

Figure 4 reveals that the carbohydrate content of *O. sancta* is endorsed by some scientists [50,51]. They stated that minimal nitrate and phosphorus concentrations significantly increased the carbohydrate content in *Synechococcus* sp. and *Arthrospira platensis* biomass compared with the control (Figure 3).

These findings of lipid content (Figure 4) of *O. sancta* agree with the results stated by Dean and others [52,53]. It is relevant to highlight the almost comparable growth production, as well as protein, carbohydrate, and lipid contents, of *O. sancta* biomass grown in 12.5% modified Zarrouk's medium concentration. Standard Zarrouk's medium represents a concrete guideline to develop this cyanobacterium in 12.5% modified Zarrouk's medium and to use the produced biomass for further analysis and bioactivity assays.

The significant anticancer activity results of *O. sancta* methylene chloride/methanol crude extract grown under nutrient deficiency against the MDA-MB-231 cell line (Figure 5) may emphasize the potential role of cyanobacterium for breast cancer therapy. The MDA-MB-231 cell line underwent a thorough literature study and was a very invasive and aggressive triple-negative breast cancer cell line. Some scientists studied the cytotoxic effect of some cyanobacterial isolates *Chroococcus minutes* and *Anabaena oryzae*, against MCF-7 cells [54,55]. The MDA-MB-231 is a highly invasive, metastatic, and poorly differentiated human breast cancer, whereas MCF-7 is an invasive and well-differentiated human breast cancer [44]. The anticancer potential of *O. sancta* extract may be attributed to the GC-MS results (Table 4) that showed the presence of some compounds known previously with their antioxidant and anticancer activity, such as oleic acid, linoleic acid phenol, 2,4-bis (1,1-dimethylethyl)-, 3′,8,8′-trimethoxy-3-piperidyl-2,2′-binaphthalene-1,1′,4,4′-tetrone, hexadecanoic acid methyl ester, and cholestan-3-ol, 2-methylene-, (3β,5α) [56–60].

There is currently a lack of understanding of how cyanobacteria chemicals in tumor cell lines cause cytotoxicity, although multiple investigations have shown that apoptosis is activated [61]. It has been documented in many articles that several cyanobacteria compounds can stimulate an arrest in the cell cycle phase, particularly the G2/M phase, by disrupting microtubules and actin proteins [62,63] involved in mitosis and, thereby, apoptosis [63,64]. Furthermore, cyanobacteria secondary metabolites induce mitochondrial dysfunction, oxidative stress, and DNA damage to cancerous cells [65,66]. Apoptosis can be induced by cyanobacteria's ability to create various chemicals that boost the activity of caspase proteins, specifically caspase 1 and 3 [62,67,68]. Aside from this, they create chemicals that can start the phosphorylation of Bcl-2 protein and block human breast cancer cells' anti-apoptotic response [69]. Changes in tumor cell membrane sodium channel

kinetics in response to cyanobacteria chemical contact suggest its potential importance in inducing apoptosis [69–72].

The effectiveness of *O. sancta* crude extract against Gram + ve bacteria like *Staphylococcus aureas* and *Bacilli cereus* and Gram − ve bacteria like *Pseudomonas alginolyticus* and *E. coli* has been demonstrated in numerous studies [73–75]. The above result reveals that *O. sancta*, when grown in a nutrient-deficient media, shows the sharpest antibacterial activity against *S. aureus* and *B. cereus* [76]. Crude *O. sancta* extract has been connected to substances such as linoleic acid, hexadecanoic acid methyl ester, 9-decadienoic acid methyl ester, 2-bis(1,1-dimethylethyl), and dasycarpidan-1-methanol acetate, previously linked to antimicrobial action [77–79] (Table 4).

As shown in Figure 6, *O. sancta* extracts greatly reduced AChE activity, suggesting that they may be useful in treating dementias like Alzheimer's disease and dementia-related diseases like Parkinson's disease. As acetylcholinesterase inhibitors have been and will continue to be the primary treatment for Alzheimer's disease symptoms, there is little dispute (AD). Three studies [80–82] have shown that a person's genetic makeup can influence their risk of developing Alzheimer's disease. Owing to phytochemicals such as dasycarpidan-1-methanol, acetate (ester), cholestan-3-ol, 2-methylene-, (3,5), and 3′,8,8′ trimethoxy-3-piperidyl-2′,2′,4,4′-tetrone that have been demonstrated in GC-MS data, *O. sancta* extract may have anticholinesterase action (i.e., a possible role for AD treatment) [41,73,83].

5. Conclusions

It can be concluded that modifying Zarrouk's medium by excluding $CaCl_2 \cdot 2H_2O$, EDTA (Na), NaCl, micronutrients and substituting sodium nitrate with urea might be promising in reducing the medium cost. The marked biomass production of *Oscillatoria sancta* on 12.5% concentration of modified Zarrouk's medium indicates the possible economic mass cultivation of such algae under starvation conditions. The potency of *O. sancta* crude extract against the MDA-MB-231 cell line, pathogenic bacteria, and AChE activity showed the potential of *O. sancta* secondary metabolites as drug candidates for breast cancer, Alzheimer's disease, and bacterial diseases. More in-depth in vivo studies may be required to ascertain the bioactivity results of *O. sancta* extract against these diseases.

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