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Antioxidant and antibacterial activities of *Oscillatoria* sp. and *Chlorella* sp.

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Abstract

Chlorophyta and Cyanophyta are potential sources of physiologically active chemicals that are antibacterial, antifungal, anticancer, and antiviral. The total phenolic, flavonoid, antioxidant, and antibacterial properties of *Oscillatoria* sp. and *Chlorella* sp. were evaluated using methanol and acetone. The acetone extract of *Chlorella* sp. had the highest flavonoid (16.575±0.44 mg/g) and antioxidant activity (27.94±27.94 mg/g) while *Oscillatoria* sp. had the strongest antibacterial activity against the bacteria *Escherichia coli.* Both methanol and acetone extracts of *Oscillatoria* sp. and *Chlorella* sp. using Gas Chromatography Mass Spectroscopy showed the presence of twelve heterocyclic chemicals including Silanediol (37.121%) in the methanol extract and Oxime (28.587%). in both extracts. The antioxidant and antibacterial properties of the chemicals identified in the extracts appeared to be due to synergistic actions. Further research on the antibacterial prospect of *Oscillatoria* sp. and *Chlorella* sp. should be explored.

Keywords: GC-MS, antibacterial, antioxidant, *Eschericha coli*, phytochemicals

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Introduction

Secondary and primary metabolites produced by microalgae are sources of bioactive chemicals having antibacterial, antifungal, anticancer, and antiviral activities (Balaji *et al*., 2017; Patra *et al*., 2008; Tuney *et al*., 2006; Ely *et al*., 2004). Minerals, polysaccharides, amino acid derivatives, carotenoids, and phenolic compounds are common metabolites found in microalgae (Mimouni *et al*., 2012; Yusoff *et al*., 2019; Balaji *et al*., 2017; Marrez *et al*., 2019; El-Chaghaby *et al*., 2019). These metabolites are created for protective measures and chemical defense, which gives them adaptive flexibility, making them intriguing candidates for biotechnological applications in natural antioxidants for nutraceuticals and pharmaceuticals (Asif, 2015; Yusoff, 2019; Gnanakani, 2019).

Microalgae biomass can be used for a variety of purposes, including animal and fish feed, biofertilizers, and drug recovery extractions. Antioxidants have been investigated and are known to protect cells from free radical damage by stabilizing free radicals and preventing harm caused by them (Balaji *et al*., 2017; Haoujar *et al*., 2019). The principal contributors to antioxidant capacity are flavonoids, phenolic acids, and tannins, which have been studied for biological activities such as anti-inflammatory, anti-atherosclerotic, and anti-carcinogenic properties (Wang *et al*., 2018; Derong *et al*., 2016). Phenolic

substances have one (phenolic acids) or more (polyphenols) aromatic rings in their structures with connected hydroxyl groups. These hydroxyl groups and phenolic rings are linked to their antioxidant properties. Polyphenols are natural antioxidants that include thousands of molecules with a wide range of structures that can be classified into 10 different classes based on their basic chemical structure (Helenoa *et al*., 2015). Polyphenols work as antioxidants by transferring a single electron and a hydrogen atom.

According to Marinho *et al*. (2021), two basic processes produce plant phenolics: the shikimic acid pathway and the malonic acid system. Simple phenolic compounds and complex phenolic compounds are the two types of phenolic compounds. Trans-cinnamic acid (aromatic ring of C6 and a chain of C3) is a simple phenolic that participates in inter and intra-plant interactions. Coumarins and benzoic acid derivatives are two examples (an aromatic ring of C6 and a chain of C1).

Different microalgae screened for their radical scavenging activity against the stable radical 1,1-diphenyl-2-picrylhydrazyl by using aqueous, ethanol, acetone, and methanolic extracts and researchers have given reports of methanol as more efficient to extract selected group of compounds with a higher antioxidant activity (Belyagoubi *et al*., 2021). Although previous research has shown that phenolic compounds have antioxidant properties and that microalgae and cyanobacteria can be sources of these compounds, few studies have focused on their identification and quantification in microalgae, as well as the role of phenolics in microalgae defense mechanisms against high ROS levels (Anwera *et al*., 2021). The goal of this work was to quantify the phenolic and total antioxidant capacity content of locally isolated species of microalgae using DPPH-GCMS analysis and to evaluate their antioxidant activity.

Materials and Methods

Chemicals

Standard antibiotics, physiological saline, Mueller Hinton agar (Himedia Lab, India), antibacterial assay (amoxicillin and levofloxacin).

Algal Strain Collection and Growth

To examine the antibacterial and antioxidant activity against four bacteria species, *Chlorella* sp. and *Oscillatoria* sp., members of the Chlorophyta and Cyanobacteria families were selected. The algal species were separated from the phytoplankton community structure of Makoko Creek, which is located along the Lagos lagoon (N 06 29 659', E003 23.833'). Makoko Creek receives freshwater from Lekki Lagoon via Epe Lagoon in the north-east, as well as discharges from Magidun, Agboyi, and Ogudu creeks and Ogun River in the north-west (Lawal-Are and Nwankwo, 2011). *Chlorella* sp. *and Oscillatoria* sp. were cultured and maintained on Proteose Medium and MB3N medium respectively. To prevent algal cell clumping and adherence to the containers, the cultured media were incubated for 10 days and stirred every day.

Algal Extract Preparation

Total Phenolic Content Estimation

The spectrophotometric approach was used to determine the total phenolic (TP) content (Slinkard and Singleton, 1977). A 0.5 g extract sample was dissolved in 50 mL distilled water and weighed. 0.5 mL was collected and mixed with 0.1 mL Folin-Ciocalteu reagent (0.5 N), then incubated for 15 min at room temperature. After that, 2.5 mL sodium carbonate solution (7.5 percent w/v) was added and incubated at room temperature for another 30 min. The solution's absorbance was measured at 760 nm. Total phenol content was calculated as gallic acid equivalent (GAE) (mg/g dry mass).

Determination of Total Flavonoid Content

Zhishen *et al*. (1999) established a colorimetric method for determining total flavonoid (TF). 1 mL of sample solution (100 g/mL) was

combined with 3 mL methanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1 M potassium acetate, and 5.6 mL distilled water. The reaction mixture was incubated at room temperature for 30 min before being tested at 415 nm for absorbance. The calibration curve was created making quercetin solutions in methanol at various concentrations.

Total Antioxidant Capacity Determination

The sample extract solution (1 mL) was combined with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated for 90 min in a boiling water bath at 950 °C. The absorbance of each sample's aqueous solution was measured at 695 nm after it had cooled to room temperature. The overall antioxidant capacity was calculated as the ascorbic acid equivalent.

Assay for DPPH Radical Scavenging Activity

Blois approach was used to evaluate the radical scavenging activity of algal extracts quantitatively (1958). An aliquot of 0.5 mL extract in 95 % ethanol was combined with 2.0 mL reagent solution at various concentrations (25, 50, 75, 100 g/mL) and 0.004 g of DPPH in 100 mL methanol. The sample was replaced with a DPPH solution in the control, and methanol was used as the blank. The mixture was agitated vigorously and allowed to cool to room temperature. The decrease in absorbance of the test mixture (due to quenching of DPPH free radicals) was measured at 517 nm after 30 min.

Equation (1).

DPPH scavenging effect (% inhibition) = [A0-A1] x 100/A0

The absorption of the blank sample is A0, whereas the absorption of the extract is A1.

Assay for antibacterial resistance

Pure typed five bacteria cultures of ATCC29213 *Staphylococcus aureus,* ATCC11229 *Escherichia coli,* ATCC12022 *Shigella flexneri,* ATCC13311 *Salmonella typhimurium,* and *Bacillus* sp. were tested using modified Kirby-Bauer disc diffusion technique agar. Bacterial strains were grown in broth. The isolates were standardized in sterile physiological saline and compared with 0.5 McFarland standards. (Ochei and Kolhatkar, 2004; Ogah and Osundare, 2015). The agar plates were seeded by 100 µl bacterial suspensions approximately 10^6 - 10^8 CFU/mL. Sterile 8 mm filter paper discs were impregnated with an extract from the culture of *Chlorella* sp. and *Oscillatoria* sp. Standard antibiotics (Levofloxacin and Amoxicillin) were prepared in dimethyl sulfoxide (DMSO) to give a concentration of 5 mg/mL to serve as a positive control. The inoculated plates were placed for 1 h and incubated at 37 ˚C for 24 h and were examined for zones of inhibition. Each zone of inhibition was measured in millimeters with a ruler at 90˚ perpendicular to each other and the mean of the two readings was then calculated (Ochei and Kolhatkar, 2004; Ogah and Osundare, 2015).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The volatile constituents from cultures of *Chlorella* sp. and *Oscillatoria* sp. were analysed by GCMS using a 7820A gas chromatograph coupled to a 5975C inert mass spectrometer (with triple axis detector) and electron impact source (Agilent Technologies). The stationary phase of separation of the compounds was carried out on the HP-5 capillary column coated with 5% of Phenyl Methyl Siloxane (30 m length × 0.32 mm diameter × 0.25 µm film thickness) (Agilent Technologies). The carrier gas was helium used at a constant flow rate of 1.573 mL/min, an initial nominal pressure of 1.9514 psi, and an average velocity of 46 cm/s. One microliter of the samples was injected in splitless mode at an injection temperature of 260 °C. Purge flow was 21.5 mL/min at 0.50 min with a total gas flow rate of 23.355 mL/min; gas saver mode was switched on. The oven was initially programmed at 60 °C (1 min), then ramped at 4 °C/min to 110 °C (3 min), followed by

temperature program rates of 8 °C/min to 260 °C (5 min) and 10 °C/min to 300 °C (12 min). The run time was 56.25 min with a 3 min solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70 eV with an ion source temperature of 230 °C, quadrupole temperature of 150 °C, and transfer line temperature of 280 °C. Scanning of possible compounds was from 30 to 550 amu at a 2.62 s/scan scan rate and was identified by comparing measured mass spectral data with those in NIST 14 Mass Spectral Library.

Statistical Analysis

The data on changes in the antioxidant response of *Chlorella* sp. and *Oscillatoria* sp. was analyzed using a one-way Analysis of Variance. Shapiro Wilk and Levene's tests were used to checking for normality and homogeneity of the data before using ANOVA. The 'agricolae' R package's LSD test function was used to differentiate significantly different means between the treatments. The ggplot2 R software was used to create the plots. R version 4.1.0 GUI 1.76 High Sierra build for macOS was used for all statistical studies.

Results

Total Phenolic and Total Flavonoid Contents

The total phenolics and flavonoids of two different solvent extracts (methanol and acetone) of *Oscillatoria* sp. and *Chlorella* sp. are presented in Table 1.

Table 1: Total phenolic and flavonoid contents of *Oscillatoria* sp. and *Chlorella* sp. in two solvent extracts.

Algal speies	Solvents extract	
	Methanol	Acetone
	Total phenolic (mg/g)	
Chlorella sp.	7.03 ± 0.24	7.77 ± 0.2
Oscillatoria sp.	4.53 ± 0.31	6.99 ± 0.12
	Total flavonoid (mg/g)	
Chlorella sp.	16.22 ± 0.23	16.58 ± 0.32
Oscillatoria sp.	11.04 ± 0.23	11.90 ± 0.27
	Total antioxidant capacity (mg/g)	
Chlorella sp.	21.98 ± 0.33	27.94 ± 0.49
Oscillatoria sp.	18.02 ± 0.21	21.37 ± 0.21

DPPH Radical scavenging Activities

Results obtained from DPPH Radical scavenging evaluation of two microalgae strains extracts by different solvents using DPPH free-radical reduction method at four different concentrations (25 50, 75, and 100 µg/mL) are shown in Table 2.

Table 2: DPPH radical scavenging activities of *Chlorella* sp. and *Oscillatoria* sp.

	DPPH Scavenging Activity (%Inhibition)			
Methanol	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
Oscillatoria sp.	29.69	44.21	66.07	70.31
	28.71	44.86	65.58	71.78
Chlorella sp.	20.88	38.17	54.16	64.76
	19.90	37.68	54.98	63.95
Ascorbic acid	44.57	55.59	77.46	89.47
	45.53	57.51	76.38	90.18
	DPPH Scavenging Activity (%Inhibition)			
Acetone				
	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
Oscillatoria sp.	30.88	45.09	68.05	74.53
	29.86	45.76	69.51	76.09
Chlorella sp.	23.80	41.61	55.24	69.94
	22.09	39.19	57.18	71.62
Ascorbic acid	44.57	55.59	77.46	89.47
	45.53	57.51	76.38	90.18

Antioxidant capacity: Results obtained from antioxidant capacity of two microalgae strains extracts by different solvents are shown below in Table 3.

Table 3: Antioxidant capacity of *Chlorella* sp. and *Oscillatoria* sp.

Methanol	Total Antioxidant Capacity mg/100g	Total Flavonoid mg/100g	Total Phenol mg/100g
Oscillatoria sp.	22.30	15.99	6.79
	21.65	16.44	7.26
Chlorella sp.	17.81	11.26	4.22
	18.22	10.81	4.84
Acetone	Total Antioxidant Capacity mg/100g	Total Flavonoid mg/100g	Total Phenol mg/100g
Oscillatoria sp.	27.45	16.26	7.96
	28.43	16.89	7.57
Chlorella sp.	21.57	12.17	6.87
	21.16	11.63	7.10

Reducing power of *Oscillatoria* sp. and *Chlorella* sp. extracts

Results obtained from reducing power activity evaluation of two microalgae strains extracts

by different solvents using reducing power activity method at four different concentrations (25 µg/mL 50 µg/mL, 75 µg/mL, 100 µg/mL) are shown in Table 3.

Table 4: Reducing power of *Oscillatoria* sp. and *Chlorella* sp. extracts

	Reducing power Activity			
Methanol	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
Oscillatoria sp.	0.128	0.181	0.209	0.289
	0.132	0.187	0.213	0.291
Chlorella sp.	0.106	0.165	0.194	0.275
	0.104	0.163	0.192	0.272
Ascorbic acid	0.169	0.382	0.481	0.624
	0.163	0.379	0.485	0.626
Acetone	Reducing power Activity			
	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
Oscillatoria sp.	0.136	0.186	0.215	0.301
	0.133	0.183	0.209	0.297
Chlorella sp.	0.110	0.172	0.208	0.286
	0.111	0.166	0.198	0.280
Ascorbic acid	0.169	0.382	0.481	0.624
	0.163	0.379	0.485	0.626

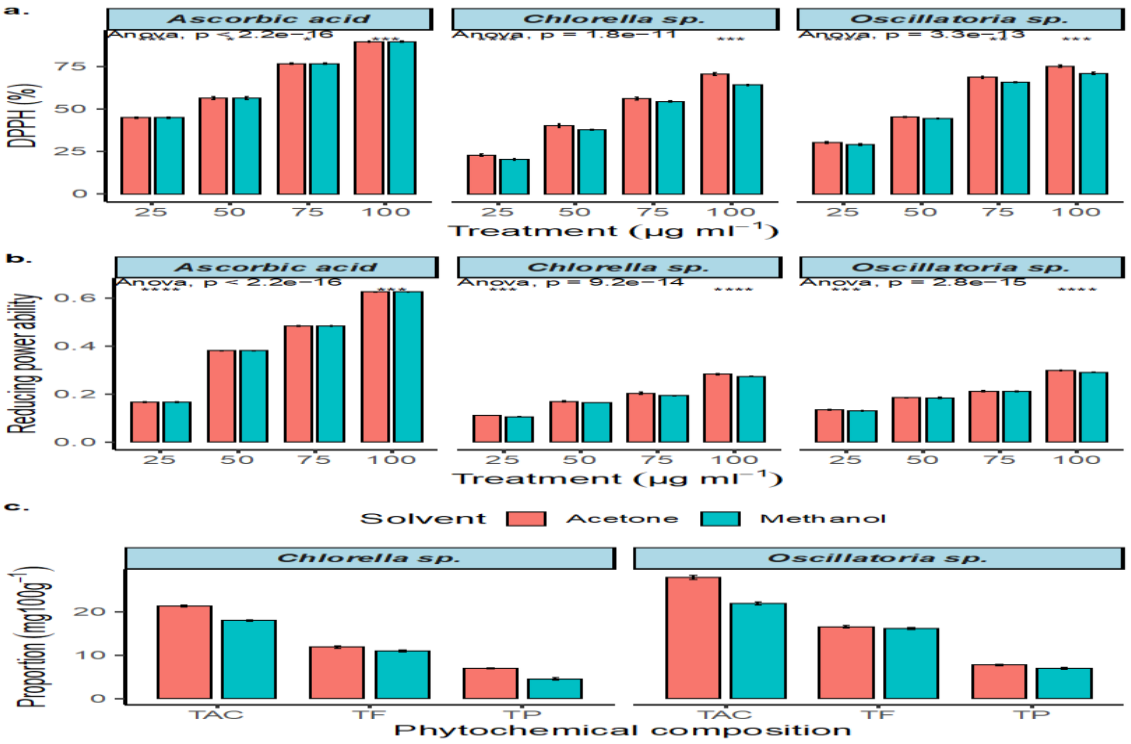

Figure 1: Antioxidant potential (a), reducing power ability (b), and phytochemical composition (TAC = total antioxidant capacity, TF = total flavonoid, and TP = total phenols) of *Chlorella* sp. and *Oscillatoria* sp. cultures.

Antibacterial Activity

The antibacterial activity of secondary metabolites produced by *Chlorella* sp. and *Oscillatoria* sp. against five species of bacteria was recorded in Table 4. Species of bacteria and algal species determine the degree of antimicrobial activity and intensity of inhibitory action. The antibacterial activity was compared to Amoxicillin and Levofloxacin. The experimental analysis indicated *Oscillatoria* sp. had the highest inhibition zone against *Escherichia coli* (20 mm inhibition zone) as shown in Table 4.

This was followed by *Salmonella typhimurium* (17 mm inhibition zone). Comparing antibacterial activity to Levofloxacin, the results showed that the antibiotics had stronger activity than tested algal strains as shown in Table (4). *Chlorella* sp. had one inhibition activity against *Salmonella typhimurium* (13 mm inhibition zone) contrary to *Oscillatoria* sp. (17 mm inhibition zone). *Chlorella* sp. showed no inhibition against *Staphylococcus aureus*.

The study revealed *Oscillatoria* sp. exhibited antibacterial activity on *Escherichia coli, Shigella flexneri*, and *Salmonella typhimurium.* In both studies, there was no antibacterial against *Staphylococcus aureus. N*o inhibition of growth was observed, using acetone, ethyl acetate, and methanol extracts of *Oscillatoria* sp. on *Bacillus subtillis*, and *S. aureus.*

Table 5: Antibacterial Activity of *Oscillatoria* sp. and *Chlorella* sp. against Standard bacterial strains

Bacterial strains	Diameter of inhibition zone (mm)			
	Chlorella sp.	*Oscillatoria* sp.	AMX (5 mg/mL)	LEV (5 mg/mL)
ATCC11229 *Escherichia coli*	N.D	20	19	36
ATCC12022 *Salmonella flexneri*		12	13	40
ATCC13311 *Salmonella typhimurium*	13	17	21	32
ATCC29213 *Salmonella aureus*	N.D	N.D	20	37
Bacillus sp.	N.D	N.D	21	35

Diameter of disc = 8 mm, AMX= Amoxicillin, LEV = Levofloxacin, N.D: Not detected

GC-MS Analysis

The GC-MS analysis of *Oscillatoria* sp. and *Chlorella* sp. using methanolic and acetone extracts resulted in the identification of 17 compounds; however, a few of them were predominant. The major compound was Silanediol which was presented in the methanolic extract at (37.121%) and Oxime (28.587%) in both methanol and acetone extracts. In Table (6) the GC/MS analysis of the methanol and acetone extracts of *Chlorella* sp. resulted in many compounds which have diverse use. Compounds having anti-inflammatory, antibacterial, and antifungal, properties have been identified. For methanolic extract, the highest concentrations were cyclopentaneundecanoic acid, methyl ester (11.06%), followed by methyl decadienoate (6.10%). Whereas the major compounds estimated in acetone extract were, NMethylyunaconitine-3-ol, (4.11%) followed by the 1-Pentanol (3.11%).

Table 6: Chemical composition of methanol and acetone extract of the *Chlorella* sp. extracts using GC/MS analysis.

	Composition (%)	
Compound name	Methanol (Retention time)	Acetone (Retention time)
Cyclotrisiloxane	1.048 (3.347)	2.188 (3.249)
Cyclotrisiloxane	-	1.431 (3.301)
Oxime	29.425(5.276)	28.587 (4.577)
9-phenanthrenamine	-	7.411 (4.993)
Cyclotrisiloxane	-	3.654 (5.466)
3-hydroxymandelic acid	2.788(6.991)	3.568(6.985)
Phosphonoacetic acid	1.071(8.654)	1.479(8.654)
Cyclohexane	-	1.314(10.231)
2-tetradecene	-	2.127(12.298)
1-docosene	-	2.983(15.833)
Silanediol	37.121(4.427)	-
Cyclotetrasiloxane	2.805(5.414)	-
Benzenepropanoic acid	1.057(15.473)	-

Table (7): Chemical composition of methanol and acetone extract of the *Oscillatoria* sp. extracts using GC/MS analysis.

	Composition (%)	
Compound name	Acetone (Retention time)	Methanol (Retention time)
Cyclotrisiloxane	3.324(5.366)	
Silanediol	3.439(45.422)	
Hydrazine	3.792(3.64)	
3-pentanone	3.907(3.349)	
Oxime	4.54 (8.493)	
1-anthracenamine	5.022(6.674)	
Cyclotetrasiloxane	5.507(2.756)	8.487 (18.945)
Cyclotrisiloxane	6.627(1.379)	3.208(1.249)
3-hydroxymandelic acid	6.991(2.965)	
Phosphonoacetic acid	8.654(1.15)	
Phthalic acid	15.654(1.679)	
Cyclopentasiloxane		10.162(9.465)
Cyclohexasiloxane		11.906(3.847)

Discussion

Flavonoids such as isoflavones, flavanones, flavonols, and dihydrochalcones are examples of phenolic chemicals. Microalgae have antioxidant activity that is comparable to, if not greater than, that of higher plants. Antioxidants are an important line of defense against free radical damage (Sen and Chakraborty, 2011). The highest phenolic content was found in the acetone extract (7.77±0.2 mg/g) of *Chlorella* sp. in comparison to the methanol extract (7.03±0.24 mg/g). However, *Oscillatoria* sp. gave 4.53±0.31 and 6.99±0.12 mg/g respectively. For flavonoids, the highest value of 16.58±0.32 mg/g was observed in the acetone extract of *Chlorella* sp. followed by methanol extract (16.22± 0.2mg/g) in *Chlorella* sp. The lowest flavonoid content was noticed from the methanol extract of *Oscillatoria* sp. (11.04±0.23 mg/g).

Antioxidants aid in the optimization of human physiological functions and play an important role in preventing oxidative damage caused by free radicals through scavenging activity, and/or play a key role in the prevention of degenerative neuropathies, diabetes, cardiovascular diseases, and cancers, as well as having anti-inflammatory, antiviral, and anti-aging properties (Gupta *et al.*, 2018; Galasso *et al*., 2017; Gomes *et al*., 2013; Scalbert *et al.*, 2005). In other applications, astaxanthin is a strong antioxidant that is 550 times more effective than vitamin E and has significant cosmetic and medicinal potential (Marino *et al*., 2020).

Variations in TP, TF, and TAC contents across species were observed using two solvents with different polarities, as shown in Table 1. These variations could be attributed to the polarity of solvents, flavonoids, and phenolic compounds present in each species, and genetic factors contributing to the antioxidant capacity of both algal species (Banskota *et al*., 2019). Acetone was considered to be the best solvent for the extraction of TP and TF. These findings agreed with the earlier investigation by Seal *et al*. (2014) and they clearly explain that the acetone extract to be having higher flavonoid content in *Scytonema ocellatum*. According to Kumar *et al*. (2018), the total phenolic content of the *Nannochloropsis salina* sample was measured in chloroform, methanol, and acetone extracts. The result showed higher content of phenolic compounds in acetone extract compared to methanol extract. This may be due to the difference in the polarity of the solvents used. Recent research by Anwer *et al*. (2021) observed that the highest total phenol recorded by *Spirulina* sp. in acetone extract compared to other solvent extracts used.

The color change of DPPH from purple to yellow is a measure of radical-scavenging activity; the more intense the color change, the higher the scavenging activity (Gontijo *et al.*, 2012). Antioxidants are substances that may protect cells from damage caused by free radicals, which are unstable molecules. Antioxidants interact with free radicals, stabilizing them and preventing part of the harm caused by them. As a result, antioxidants serve as cell defenders.

DPPH is characterized as a stable free radical under the delocalisation of the spare electron over the molecule as a whole (Fig. 1), so that the molecules do not dimerise, like most other free radicals. As shown in Figure (1), the extract of *Oscillatoria* sp. obtained by acetone solvent possessed the highest antioxidant activity, with (76.09%) inhibition of DPPH radical at 100 µg/mL followed by methanol. Ascorbic acid had the most favorable percentage inhibition (90.18%) of DPPH radical at the same concentration (100 µg/mL). These findings correlated with Khalili *et al*. (2018), who reported the lowest DPPH radical scavenging activity in the methanol extract of *Gracilaria gracili* compared to the acetone extract.

Increased amounts of all experimental materials – ascorbic acid, *Chlorella* sp., and *Oscillatoria* sp. – resulted in higher DPPH levels, as measured by DPPH levels (Figure 1a). There were substantial changes in DPPH levels between ascorbic acid, *Chlorella* sp. and *Oscillatoria* sp. extract concentrations. In biological samples, however, there was minimal difference between acetone and methanol extracts (microalga and cyanobacterium). Acetone extracts of *Chlorella* sp. and *Oscillatoria* sp. outperformed methanolic extracts in terms of antioxidant capacity. It is worth mentioning that ascorbic acid has a substantially higher antioxidant potential than the other two species investigated which is the same as observed by Simic *et al*. (2012) in which the tested extract revealed lower antioxidant activities than ascorbic acid. Arun *et al*. (2012), recorded the antimicrobial index for a methanolic extract of *Chlorella pyrenoidosa* showed a maximum percentage of inhibition (66.6%) against *Bacillus cereus*, followed by *Spirulina platensis* (55.5%) and *Nostoc muscorum* (44.4%). However, the effect of acetone extract on all selected algae revealed that *B. cereus* was the most sensitive strain. Rajendran *et al* (2014) reported a DPPH scavenging assay was used to study the antioxidant potential of four solvents extract. *Dunaliella* sp., *Chlorella* sp., and *Synechocystis* sp. showed maximum activity in acetone extracts, followed by methanol extracts of *Synechocystis* sp., *Oscillatoria* sp., *Tetraselmis* sp. and *Dunaliella* sp.

Phenolic compounds and flavonoids are electron-donor substances that play a vital role in exhibiting reduction capacity. Changes in reducing power followed the same pattern as DPPH alterations (Figure 2b). Ascorbic acid, in particular, had twice the reducing power of *Oscillatoria* sp. and *Chlorella* sp. The reducing power of both species' extracts rose with concentration, and these increases were significant ($p < 0.05$). At the 100 µg/mL treatment, the acetone extracts of *Chlorella* sp. and *Oscillatoria* sp. had slightly higher reducing power than their methanolic extracts. When acetone extracts of *Chlorella* sp. and *Oscillatoria* sp. were compared to methanolic extracts, the acetone extract showed the greatest total antioxidant capacity -TAC (Figure 1c). The total flavonoid (TF) content of the two extracts tested from the two organisms under examination did not differ appreciably ($p<0.05$). The acetone extract, on the other hand, had higher total levels than the methanolic extract. The total phenol (TP) content was significantly lower than the TAC and TF values. In addition, the amounts found in the methanolic extract were less than those found in the acetone extracts.

Increased amounts of all experimental materials – ascorbic acid, *Chlorella* sp., and *Oscillatoria* sp. – resulted in higher DPPH levels, as measured by DPPH levels (Figure 5a). There were substantial changes in DPPH levels between ascorbic acid, *Chlorella* sp., and *Oscillatoria* sp. extract concentrations. In biological samples, however, there was minimal difference between acetone and methanol extracts (microalga and cyanobacterium). The difference between the solvents, however, was significant ($p = 0.03$). Acetone extracts of *Chlorella* sp. and *Oscillatoria* sp. outperformed methanolic extracts in terms of antioxidant capacity. It is worth mentioning that ascorbic acid had a substantially ($p<0.01$) higher antioxidant potential than the other two species investigated, especially at 75% and 100% treatments. Also, for most of the concentrations tested, *Oscillatoria* sp. had considerably greater DPPH levels than *Chlorella* sp. ($p<0.05$). This was also reported by Pradhan *et al*. (2021). Dimova *et al*. (2019) reported a higher result using methanol in *Ulva rigida* and microalgae *Chlorella* sp. Abdel-Karim *et al*. (2020) compared acetone, methanol, and water in *Chlorella* sp., and acetone showed higher results.

When acetone extracts of *Chlorella* sp. and *Oscillatoria* sp. were compared to methanol extracts, the acetone extract showed the greatest total antioxidant capacity - TAC (Figure 5c). Differences in solvent type caused a significant variation in total antioxidant capacity ($p<0.05$). In this study, the effect of specific types on total antioxidant capacity was also significant ($p<0.01$). The overall antioxidant capacity of *Oscillatoria* sp. was found to be substantially higher than that of *Chlorella* sp. The total flavonoid (TF) content of the two extracts tested from the two organisms under examination did not differ appreciably ($p<0.05$). The acetone extract, on the other hand, had higher total flavonoid levels than the methanolic extract, but the difference was not significant ($p > 0.05$). The difference between the studied species per total flavonoid content was significant ($p < 0.01$). The total phenol (TP) content was significantly lower than the TAC and TF values. In addition, the amounts found in the methanolic extract were less than those found in the acetone extracts. The total phenol concentration of the methanolic and acetone extracts of both species, however, did not differ substantially ($p > 0.05$).

Chlorella sp. had one inhibition activity against *Salmonella typhimurium* (13 mm inhibition zone) contrary to *Oscillatoria* sp. (17 mm inhibition zone). *Chlorella* sp. showed no inhibition against *Staphylococcus aureus* as reported by Jayshreen *et al*. (2016). Ely *et al*. (2004) reported the ineffectiveness of aqueous extracts against tested bacterial species except for *Escherichia coli* in the case of aqueous extract of *Oscillatoria agardhii*. This is also shown in the present study which revealed *Oscillatoria* sp. exhibited antibacterial activity on *Escherichia coli, Shigella flexneri*, and *Salmonella typhimurium.* In both studies, there was no antibacterial against *Staphylococcus aureus.* Seddek *et al*. (2019) reported no inhibition of growth using acetone, ethyl acetate, and methanol extracts of *Oscillatoria* sp. on *Escherichia coli*, *Bacillus subtillis,* and *Staphylococcus aureus* which is also shown in the present study for *Bacillus subtillis* and *Staphylococcus aureus.*

The GC-MS analysis of *Oscillatoria* sp. and *Chlorella* sp. using methanolic and acetone extracts has result in agreement with a previous result of Castilho *et al*. (2012) who identified 1-hexacosanol, as one component of oregano essential oil using GC-MS. They also reported that non-esterified 1-hexacosanol could act as an antimicrobial and antioxidant compound. Moreover, the biological activity of some triterpenoid such as phytoene (PE) and phytofluene (PF) was reported by Engelmann *et al*. (2011)

Conclusion

In conclusion, the results distinctly showed the antioxidant activity of two algae (*Chlorella* sp. and *Oscillatoria* sp.) were screened for their total phenols and flavonoids, total antioxidant capacity, and antibacterial activities. Extraction was done with different solvents (acetone and methanol). The highest total flavonoid and highest antioxidant activity content were recorded by acetone extract of *Chlorella* sp. The highest antibacterial activity was detected in *Oscillatoria* sp. against *Escherichia coli*. Silanediol and Oxime are major compounds presented in both methanol extract, methanol and acetone extracts respectively. A total of twelve heterocyclic compounds were detected in both *Oscillatoria* sp. and *Chlorella* sp. Although, this was the first study on the antioxidant effects of algae from aquaculture wastewater of the University of Lagos, further research is needed to increase the lipid concentration for the enhancement and production of high nutritional value-added products for fish feed.

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Declaration of conflict of interest

The authors declare no conflict of interest.

Credit author statement

Adeniyi-Martins: Conceptualization and design of experiment; Primary Investigation; Data analysis and curation; Writing – original draft, review, and editing. Fasuba: Investigation; Supervision. Bolajoko: Investigation; Supervision. Nwankwo: Resources, Supervision. Adesalu: resources.

References

Anwer S. S., Sdiq, K. H., Muhammada, K. R. and Aladdin, L. M. (2022). Phenolic compound and fatty acid properties of some microalgae species isolated from Erbil City. *Brazilian Journal of Biology*, 82:256927. https://dx.doi.org/10.1590/1519-6984.256927

Arun, N., Gupta, G. and Singh, D.P. (2012). Antimicrobial and antioxidant property of commonly found microalgae *Spirulina platensis*, *Nostoc muscorum*, and *Chlorella pyrenoidosa* against some pathogenic bacteria and fungi. *International Journal of Pharmaceutical Sciences and Research*, 3(12): 4866-4875. https://doi.org/10.1016/j.biteb.2020.100477

Asif, M. (2015). Chemistry and antioxidant activity of plants containing some phenolic compounds. *Chemistry International*, 1:35–52. 10.12691/jfnr-8-12-3

Balaji, M., Thamilvanan, D., Vinayagam, S. C. and Balakumar, B. S. (2017). Anticancer, antioxidant activity and GC-MS analysis of selected micro algal members of Chlorophyceae. *International Journal of Pharmaceutical Sciences and Research*, 8(8): 3302-3314 10.13040/IJPSR.0975-8232.8(8).3302-14

Banskota, A.H., Sperker, S., Stefanova, R., McGinn, P.J. and O'Leary, S.J.B. (2019). Antioxidant properties and lipid composition of selected microalgae. *Journal of Applied Phycology*, 31:309–318. https://doi.org/10.1007/s10811-018-1523-1

Belyagoubi, L., Belyagoubi-Benhammou, N., Atik-Bekkara, F. and Abdelouahid, D.E. (2021). Influence of harvest season and different polarity solvents on biological activities, phenolic compounds and lipid-soluble pigment contents of *Spirogyra* sp. from Algeria. *Advances in Traditional Medicine*, 22: 359–369. https://doi.org/10.1007/s13596-021-00551-0

Castilho, P.C., Savluchinske-Feio, S., Weinhold, T. S. and Gouveia, S. C. (2012). Evaluation of the antimicrobial and antioxidant activities of essential oils, extracts and their main components from oregano from Madeira Island, Portugal. *Food Control*, 552-558. https://doi.org/10.1016/j.foodcont.2011.08.031

Derong, Lin., Mengshi, X., Jingjing, Z., Zhuohao, Li. Baoshan, X., Xindan, Li., Maozhu, K., Liangyu, L., Yaowen, Liu., Hong, C., Wen, Q., Hejun, W. and Saiyan, C. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules*, 21(10): 1374. https://doi.org/10.3390/molecules21101374

El-Chaghaby, G. A., Rashad, S., Abdel-Kader, S. F., Rawash, E. A. and Moneem, M. A. (2019). Assessment of phytochemical components, proximate composition and antioxidant properties of *Scenedesmus obliquus*, *Chlorella vulgaris*, and *Spirulina platensis* algae extracts. *Egyptian Journal of Aquatic*, 23(4): 521–526. https://dx.doi.org/10.21608/ejabf.2019.57884

Ely, R., Supriya, T. and Naaik, C.G. (2004). Antimicrobial activity of marine organisms collected of the coast of South East India. *Journal of Experimental Marine Biology and Ecology*, 309:121-127 https://doi.org/10.1016/j.jembe.2004.03.010

Engelmann, I., Griffon, A., Tichit, L., Montañana-Sanchis, F., Wang, G., Reinke, V., Waterston, R. H., Hillier, L. W. and Ewbank, J.J. (2011). A comprehensive analysis of gene expression changes provoked by bacterial and fungal infection in *C. elegans*. *PLoS ONE*, 6(5): e19055. https://doi.org/10.1371/journal.pone.0019055

Galasso, C., Corinaldesi, C. and Sansone, C. (2017). Carotenoids from marine organisms: biological functions and industrial applications. *Antioxidants*, 6, 96. https://doi.org/10.3390/antiox6040096

Gnanakani, P.E., Santhanam, P., Kumar, K.E. and Dhanaraju, M.D. (2019). Chemical composition, antioxidant, and cytotoxic potential of *Nannochloropsis* sp. extracts. *Journal of Natural Science, Biology and Medicine*,10:167-77. https://doi.org/10.4103/jnsbm.JNSBM_208_18

Gomes, F.S., Costa, P.A., Campos, M.B.D., Tonon, R.V., Couri, S. And Cabral, L.M.C. (2013). Watermelon juice pre-treatment with microfiltration process for obtaining lycopene. *International Journal of Food Science & Technology*, 48: 601–608. doi:10.1111/ijfs.12005

Gupta, V.K.M., Shrivastava, R.K. and Singh, N. (2018). Status of exogenous antioxidant, total antioxidant capacity and oxidative stress in SCA patients. *Indian Journal of Applied Research*, 8:112–118. 9. 10.36106/ijar

Helenoa, S.A., Martins, A., Queiroz, M.J.R.P. and Ferreira, I.C.F.R. (2015). Bioactivity of phenolic acids: metabolites versus parent compounds: A review. *Food Chemistry*, 173:501–513. https://doi.org/10.1016/j.foodchem.2014.10.057

Haoujar, I., Cacciola, F., Abrini, J., Mangraviti, D., Giuffrida, D., Oulad, Y., Majdoub, E., Kounnoun, A., Miceli, N., Taviano, M. F., Mondello, L., Rigano, F. and Senhaji, N. S. (2019). The contribution of carotenoids, phenolic compounds, and flavonoids to the antioxidative properties of marine microalgae isolated from mediterranean Morocco. *Molecules*, 24(22): 4037. https://doi.org/10.3390/molecules24224037

Jayshree, A., Jayashree, S. and Thangaraju, N. (2016). *Chlorella vulgaris* and *Chlamydomonas reinhardtii*: Effective antioxidant, antibacterial and anticancer mediators. *Indian Journal of Pharmaceutical Sciences*, 78(5):575-581. 10.4172/pharmaceutical-sciences.1000155

Khalili, H., Chan, S. S. M., Lochhead, P., Ananthakrishnan A. N., Hart, A. R. and Chan, A. T. (2018). The role of diet in the aetiopathogenesis of inflammatory bowel disease. *Nature Rev Gastroenterology Hepatology*, 15(9):525-535. https://doi.org/10.1038/s41575-018-0022-9

Kumar, S. S. and Saramma, A. V. (2018). In vitro antioxidant activity and total phenolic content of *Nannochloropsis salina*. *International Journal of Pharmacognosy and Phytochemical Research*, 10(4); 160-164. http://dx.doi.org/10.25258/phyto.10.4.7

Lawal-Are, A.O and Nwankwo, H. (2011). Biology of the hairy mangrove crab, *Sersema huzardii* (Decapoda: Graspidae) from a tropical estuarine lagon. *Jounal of American Science*, 7(7): 402-408.

https://ir.unilag.edu.ng/handle/123456789/6174

Marinho, T.A., Oliveira, M.G., Menezes-Filho, A.C.P., Castro, C.F.S., Oliveira, I.M.M., Borges, L.L., Melo-Reis, P.R. and Silva-Jr, N.J. (2021). Phytochemical characterization, and antioxidant and antibacterial activities of the hydroethanolic extract of *Anadenanthera peregrina* stem bark. Brazilian. *Journal of Biology*, 82:234476. https://doi.org/10.1016/j.biopha.2009.03.005

Marino, T., Lovinea, A., Casellab, P., Martinoc, M., Chianesea, S., Laroccac, V., Musmarraa, D. and Molinob, A. (2020). *Haematococcus pluvialis* microalgae, a powerful antioxidant for cosmetic applications, *Chemical Engineering Transactions*, 79: 2020. 10.3303/CET2079046

Marrez, D. A., Naguib, M.M., Sultan, Y. Y. and Higazy, A. M. (2019). Antimicrobial and anticancer activities of *Scenedesmus obliquus* metabolites. *Heliyon*, 5:1404. 10.1016/j.heliyon.2019.e01404

Mimouni, V., Ulmann, L., Pasquet, V., Mathieu, M., Picot, L., Bougaran, G., Cadoret, J., Morant-Manceau, A. and Schoefs, B. (2012). The potential of microalgae for the production of bioactive molecules of pharmaceutical interest. *Current Pharmaceutical Biotechnology*, 13(15):2733-50. 10.2174/138920112804724828

Ochei, J. and Kolhatkar, A. (2004). Medical Laboratory Science; Theory and Practice. Tata McGraw-Hill Publishing Company Limited, New Delhi. 10.12691/bb-1-2-3

Ogah, J. O. and Osundare, F. A. (2015). Evaluation of antibacterial activity and preliminary phytochemical screening of moringa oleifera against pathogenic bacteria. *International Journal of Pharmacological Research*, 5: 11-20. 10.7439/ijpr.v5i11.2658

Rajendran, P., Nandakumar, N., Rengarajan, T., Palaniswami, R., Gnanadhas, E.N., Lakshminarasaiah, U., Gopas, J. and Nishigaki, I. (2014). Antioxidants and human diseases. *Clinica Chimica Acta*, 25 (436):332-47. 10.1016/j.cca.2014.06.004

Sansone, C. and Brunet, C. (2019). Promises and challenges of microalgal antioxidant production *Antioxidants*, 8:199. https://doi.org/10.3390/antiox8070199

Scalbert, A., Manach, C., Morden, C., Remesy, C. and Jimenez, L. (2005). Dietary polyphenols and prevention of diseases. *Critical Reviews in Food Science and Nutrition*, 45, 287–306. 10.1080/1040869059096

Seddek, N.H., Fawzy M.A., El-Said, W.A. and Ragaey, M.M. (2019). Evaluation of antimicrobial, antioxidant and cytotoxic activities and characterization of bioactive substances from freshwater blue-green algae, *Global NEST Journal*, 21(3), 329-337. https://doi.org/10.30955/gnj.002949

Sen, S. and Chakraborty, R. (2011). The Role of antioxidants in human health. *ACS Symposium Series*,1083:1-37. 10.1021/bk-2011-1883.ch001

Simić, S., Kosanić, M. and Ranković, B. (2012). Evaluation of in vitro antioxidant and antimicrobial activities of green microalgae. *Trentepohlia umbrina*. *Notulae Botanicae Horti Agrobotanici*, 40(2):86-91. http://dx.doi.org/10.15835/nbha40279333

Slinkard, K. and. Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28: 49-55. 10.5344/ajev.1974.28.1.49

Tuney, I., Cadirci, B.H., Unal, D. and Sukatar, A. (2006). Antimicrobial activities of the

extracts of marine algae from the coast of Urla (Izmir, Turkey), *Turkish Journal of Biology*, 30:171-175. https://doi.org/10.4236/ns.2018.107025

Wang, T.Y., Li, Q. and Bi, K.S. (2018). Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. *Asian Journal of Pharmaceutical Sciences*, 13, 12–23. https://doi.org/10.1016/j.ajps.2017.08.004

Yusoff, F.M., Nagao, N., Imaizumi, Y. and Toda, T. (2019). Bioreactor for microalgal cultivation systems: strategy and development. In: Rastegari, A., Yadav, A., Gupta, A. (Eds.) Prospects of renewable bioprocessing in future energy systems. Springer, Cham, Switzerland. p. 10. https://doi.org/10.1007/978-3-030-14463-0_4

Zhishen, Z., Mengcheng, T. and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4):555-559. https://doi.org/10.1016/S0308-8146(98)00102-2