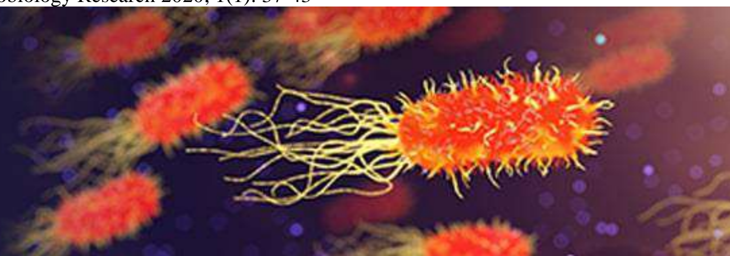

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Antimicrobial activities of green algae on microbial isolates

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Abstract
Algae are a group of organism that contain photosynthetic pigments but are not plants. They can appear in colonies that are very visible especially in the environment. They have been previously reported to have economical importance as a source of crude oil and as sources of food and a number of pharmaceutical and industrial products for humans. Several authors studied the antimicrobial activities of marine algae in different parts of the world. The aim of this study is to evaluate the antimicrobial activity and bioactive compounds of ethanol and n-hexane extracts of green algae from the environment on some microbial isolate. The Green algae was collected from the walls of the hostels and the active compounds were extracted using maceration in ethanol and nonpolar compounds were extracted using soxhlet extraction with n-hexane. The extracts were tested on certain microbial isolates which includes *Escheichia coli, Salmonella sp, Staphylococcus aureus, Bacillus sp* and *Candida sp*. From the results, on the *E. coli,* the highest activity of the extract was found at 400mg/ml. For the *Bacillus sp*, the highest activity of the extract was found at 50mg/ml with 9mm. The *Salmonella sp* inhibited at 50mg/ml. *S.aureus*, showed same mean zone of inhibition (MZI) for 400mg/ml, 200mg/ml and 100mg/ml at 1.50mm. Inhibition of the *Candida sp* was highest at 50mg/ml with 7.50mm. Compounds identified from the extracts includes Cyclotrisiloxane, hexamethyl- (22.71%), N-Methyl-1-adamantaneacetamide (9.06%), Pentasiloxane, dodecamethyl- (2.03%), Cyclohexasiloxane, dodecamethyl(4.37%), Caffeine (13.36%), Acridin-9-yl-(4-trifluoromethoxy-phenyl)-amine (11.04%), Glycine, N-[4-[(trimethylsilyl)oxybenzoyl]-, methyl ester (1.49%). The result shows that the extracts showed antimicrobial properties on the isolates tested due to certain compounds identified.

Keywords: Algae, antimicrobial, soxhlet, maceration, ethanol, n-hexane

Introduction
Algae, singular alga, members of a group of predominantly aquatic photosynthetic organisms of the kingdom Protista. Algae are microscopic organisms that carry out photosynthesis and possess photosynthetic pigments which help in their nutrition [1]. In addition to their ecological roles as oxygen producers and as the food base for almost all aquatic life, algae are economically important as a source of crude oil and as sources of food and a number of pharmaceutical and industrial products for humans.

Several authors studied the antimicrobial activities of marine algae in different parts of the world [2-6]. In recent year seaweeds are wildly used in several applications such as antimicrobial [7], antiviral [8-9], antifungal [10], anti-allergic activities. The antimicrobial activity of the two methanol extracts from selected marine (*Dunaliella salina*) and freshwater (*Pseudokirchneriella subcapitata*) microalgae have been reported to have antimicrobial properties on Staphylococcus *aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella* spp causing external otitis with MICs range of 1.4×10^9 to 2.2×10^{10} cells/mL [11]. Due to the emergence of drug resistance worldwide, there has been an emergency need to develop more therapeutic drugs from other sources which could include plants and even algae both microalgae and macroalgae.

Recently, their importance as a source of novel bioactive substances is growing rapidly and researchers have revealed that marine algal originated compounds exhibit various biological activities [12]. During the last years, many studies have been made on biological activities of the seaweed and identified as potential sources of natural antioxidants [13], anti-coagulant [14], anti-cancer [15], anti-fouling [16] and antioxidant activities [17].

The aim of this study is to evaluate the antimicrobial activity and bioactive compounds of ethanol and n-hexane extracts of green algae on some microbial isolate

Experimental Section

Specimen Collection and Identification

The algae used were collected from walls of St. Peters Hostel Madonna University Elele campus Nigeria. The algae were then dried using the oven and the air-drying method. The air-dried Green algae was used for the soxhlet extraction while the oven-dried Algae was used for the ethanol extraction.

Fig 1: Green algae on the wall of the wall of the hostel

Extraction of active ingredients

Ethanol Extraction

The Green algae was grounded to increase the surface area for proper mixing of the algae with the solvent. This process is done in a closed vessel (a covered bottle) where 750ml of ethanol which is the solvent used was added and allowed to stay 72hrs. After 72hrs, it was filtered and the filtrate was concentrated using a water bath at 50 °C for 24hrs so as to dry off the solvent left in the extract. Frequent agitation during maceration facilitates extraction.

Soxhlet Extraction method

It has been one of the most widely used extraction method which is still used extensively. Apparatus consists of an extraction chamber connected to a vapor duct and siphon tube which extends down to the joint where a round bottom flak can be attached. A cotton plug is placed in extraction chamber to prevent the blockage of siphon tube 200g of the powdered drug material is added in batches. The grounded algae are packed in the extraction chamber, and a condenser and a round bottom flask is attached to the Soxhlet apparatus at their respective positions. 600ml of the solvent which is n-hexane is measured out and poured into the round bottom flask which is placed in a heat source already. Heating takes place and the solvent finds its way into the extraction chamber, once the level of solvent reaches above the siphon bend, solvent flows into the flask through siphon tube back into the round bottom flask, the extraction chamber slowly gets filled with solvent (extract remains in contact with solvent in the chamber-maceration) till its level reaches above the siphon tube after which the solvent flows, through siphon tube, back to the round bottom flask and the cycle continues. Every time the extraction chamber receives fresh solvent, preventing saturation of solvent with solutes and therefore Soxhlet extraction is also known as continuous extraction and provides an exhaustive extraction of the plant material [1].

Preparation of the Serial dilution

The work bench was first disinfected with 75% ethanol before preparing the serial dilution. A serial dilution of the two extracts was prepared using 2-fold dilution method.

4g of the ethanol extracts were weighed out into beakers and dissolved in 10mls of Dimethylsulfoxide (DMSO) to get a stock solution of 400mg/ml. 6 test tubes labeled A-F with their corresponding concentrations that would be prepared in them (i.e A=200mg/ml, B=100mg/ml, C=50mg/ml, D=25mg/ml, E=12.5mg/ml and F=400mg/ml) and were arranged in a test tube rack and 5mls of DMSO was introduced into each test tube. 5mls was then transferred from the stock solution (test tube F) to test tube A giving the resulting concentration of 200mg/ml. The process repeated for the rest of test tubes until the final dilution in test tube E.

Confirmation of test isolates

The isolates were sub-cultured from preserved agar slants onto selective media including Nutrient agar, Salmonella-Shigella agar, Manitol salt agar, Eosine methylene blue agar for (media for bacteria) and Saboraud Dextrose agar (all-purpose media for fungi) 24 hours prior to use for the bacteris and 72 hours prior to use for the fungi. Biochemical tests were also used to confirm the isolates gotten from the lab. The biochemical tests used were catalase test, coagulase test, indole test, oxidase test and gram staining and lactophenol cotton blue staining for fungi.

Standardization of the Isolates

The required number of cells to be used as inoculum is 1.5 x 10^8 cfu/ml which is the 0.5 McFarland standard. This was achieved by using a spectrophotometer to dilute the cells to 0.08-0.1 OD at a wavelength of 600nm.

Antimicrobial Susceptibility Testing Isolates

The method used was agar well diffusion method. Widely used to evaluate antimicrobial activity of plants or microbial extracts. Agar plate is prepared according to the manufacturer's description and allowed to solidify. The agar plate surface is now inoculated by spreading 1mL of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 7mm is punched aseptically with a sterile cork-borer and a volume of 60ml of the extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The extract solution diffuses radially in the agar medium and inhibits the growth of the microbial strain tested. The plates were incubated at room temperature for 18-24 hrs, after which the inhibition zone diameters were measured to the nearest millimeters using a transparent plastic ruler. Two replicates were done for each microbial isolate and the average of the readings calculated.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. The minimum inhibitory concentration of the aqueous and ethanol extracts on the microbial isolates was obtained using the agar dilution technique which was plated out and observing for visibility after 24 hours. For the bacteria, 6 agar plates were prepared following the pour plate method. For this process, 6 bijou bottles containing 19mls of nutrient agar were autoclaved. Before pouring them into the petri dishes, 1ml of each

concentration for the ethanol extract was added to the corresponding bijou bottles to make up 20mls volume. The mixture was shaken slightly for proper mixing before being poured into the petri dishes. The petri dishes were divided into four equal quadrants, where each quadrant was swabbed with the corresponding bacterial organism. This same process was followed when using the aqueous extract.
For the fungi *C.albicans*, a total of 12 agar plates were prepared to check the MIC using both the ethanol and aqueous extracts. Following the same method used to check the MIC of the bacteria, 6 bijou bottles containing 19mls of Mueller-Hinton supplementary agar were autoclaved. After which 1ml of each concentration of the ethanol extract was added to a corresponding bottle to make up 20mls volume and they were slightly shaken to ensure proper mixing before being poured into the petri dishes and left to stand. The fungi organism was then swabbed properly unto the petri dishes and observed for 24 hours to check for growth [19].

Minimum Bactericidal Concentration (MBC)
The Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a bacterium over a fixed, somewhat extended period, such as 18 hours or 24 hours, under a specific set of conditions. The minimum bactericidal concentration of the aqueous and ethanol extracts on the microbial isolates was obtained from re-incubating the MIC for another 24hrs.

The GC-MS methodology
The GC-MS analysis was carried out using GC-MS-QP 2010 plus Shimadzu system and gas interfaced with a mass spectrophotometer system using the following conditions. Elite-1 column fused silica capillary column (30 m x 0.23 mm1 d x µL composed of 100% dimethyl polysiloxane). An electron ionization system with ionization energy of 70eV was used. 99.99% helium gas was used as the carrier gas with a flow rate of 1 ml/min with an injection volume of 2µL. injection temperature of 280 °C and ion source temperature of 280 °C was used. The oven temperature was programmed at110 °C. the relative percentage amount of each component was matched with the data available in the National Institute of Standard and Technology (NIST) library.

Data analysis
The experiments were carried out in duplicates and the results were expressed as mean and standard error of mean (x±SEM). The results were analyzed using the two-way ANOVA method with Bonferroni posttest were used to determine if there is any statistical significant difference between the mean zones of inhibitions of the extracts at different concentrations against the isolates with a significance value of $p<0.05$. Level of significance was set at 95% confidence interval, $p<0.05$ was considered statistically significant. The statistical package used was the Graphpad prisim 5 software.

Results
Standardization of extract
The extract of Algae was prepared and tested for its antimicrobial properties on microbial isolates *(staphylococcus aureus, salmonella sp., Escherischia coli, and bacillus sp).*
Weight of powered sample for Ethanol extraction = 300g
Weight of solvent =750ml
Weight of extract =4g
Concentration of extract =400mg/ml
Weight of powdered sample for n-hexane =200g
Weight of solvent =600ml
Weight of extract =4g
Concentration of extract =400mg/ml

Confirmation of the isolates
The isolates were confirmed using the selective media with their morphologies seen on the agar as one of the determinant of their confirmation. The biochemical characteristics also aided in their identifications as can be seen in table 1 below.

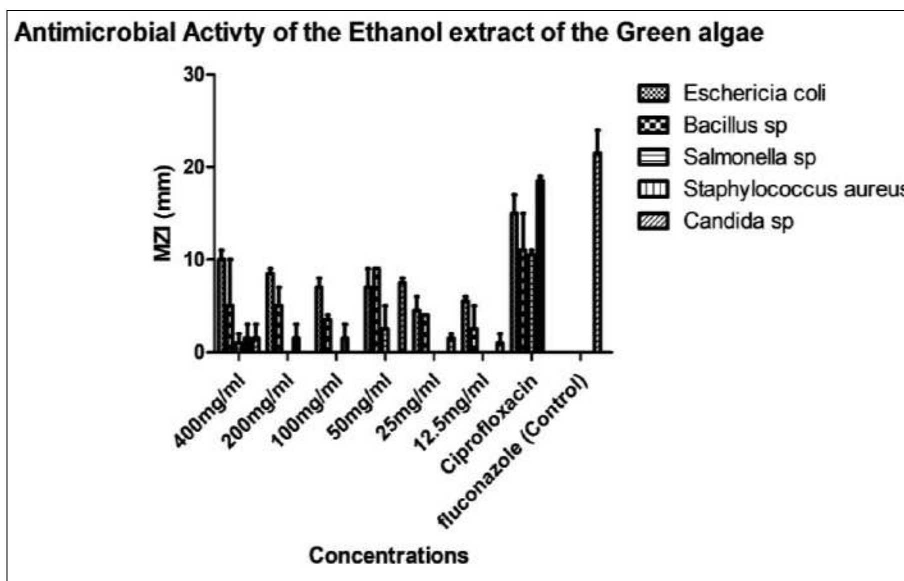
Table 1: Microbial and biochemical results of microbial isolates

Microbial Isolates	Cultural Characteristics	Gram Staining	Catalase	Coagulase	Indole	Oxidase	Lactophenol cotton blue staining	Organism
Escherichia coli	On Eosin methylene blue agar, colonies are 2-3mm in diameter and have a greenish metallic sheen in reflected light	-ve rod	+ve	-ve	+ve	-ve	ND	Affirmed
Salmonella sp.	On *Salmonella-Shigella* agar, Colonies are round, smooth and opaque with black cenred colonies.	-ve rod	+ve	-ve	-ve	-ve	ND	Affirmed
Staphylococcus aureus	On Mannitol Salt Agar, colonies are 2-3mm in diameter, with a smooth, shiny surface, opaque appearance and often pigmented golden yellow	+ve cocci	+ve	+ve	-ve	-ve	ND	Affirmed
Bacillus sp	On Nutrient agar, colonies appear rough, opaque, fuzzy white or slightly yellow with jagged edges.	+ve rod	+ve	-ve	-ve	-ve	ND	Affirmed
Candida sp	On Sabouraud Dextrose agar, *Candida albicans* shows white colored, smooth and yeast-like appearance.	ND	ND	ND	ND	ND	Circular bluish colonies appearing in clusters	Affirmed

Key: -ve= Negative; +ve=Positive; ND=not determined

Anti-susceptibility Activity of Ethanol Extract of green algae on Microbial Isolates
The graph in figure 2 shows the antimicrobial activities of the ethanol extracts of the green algae on the selected microbial isolates at the different concentrations. For the *E.coli,* the highest activity of the extract was found at

400mg/ml with 10mm followed by 200mg/ml having 8.50mm and the lowest found at 25mg/ml with 4.50mm. For the *Bacillus sp*, the highest activity of the extract was found at 50mg/ml with 9mm followed by 200mg/ml and 400mg/ml having 5mm and the lowest found at 12.5mg/ml with 2.50mm. The *Salmonella sp* inhibited at 50mg/ml with a mean of 2.50 followed by 400mg/ml which gave 1.0mm. The other concentrations showed resistance to the isolate. *S.aureus*, showed same mean zone of inhibition (MZI) for 400mg/ml, 200mg/ml and 100mg/ml at 1.50mm. Inhibition of the *Candida sp* was highest at 50mg/ml with 7.50mm followed by 400mg/ml and 200mg/ml at 1.50mm and the lowest was found 12.5mg/ml at 1.0mm. 100mg/ml and 25mg/ml showed resistance to the isolate**.**



Minimum inhibitory concentration (MIC) of the extracts on the isolates

From the results presented in table 2 below, the MIC of the extract on *Staphylococcus aureus* is 200mg/ml, 50mg/ml for *Bacillus sp, Salmonella sp* and *Candida sps*, 25mg/ml for *E. coli.*

Table 2: Minimum Inhibitory Concentration of the Extracts on the Isolates

Isolates	400 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Staphylococcus aureus	-	-	+	+	+	+
Bacillus sp.	-	-	-	-	+	+
Escherichia coli	-	-	-	-	-	+
Salmonella typhi	-	-	-	-	+	+
Candida sp	-	-	-	-	+	+

Key: + = Growth; - = No growth

Minimum bactericidal concentration of the extracts on the isolates

From the result presented in Table 3, the minimum bactericidal concentration of the extract on *Staphylococcus aureus* is 200mg/ml, 50mg/ml for *Salmonella sp* and *Bacillus sp* and 25mg/ml for *E.coli* and *Candida sp*

Table 3: Minimum Bactericidal Concentration (MBC) on Test Isolates

Isolates	400 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Staphylococcus aureus	-	-	+	+	+	+
Bacillus sp.	-	-	-	+	+	+
Escherichia coli	-	-	-	-	-	+
Salmonella typhi	-	-	-	-	+	+
Candida albican	-	-	-	-	-	+

Key: + = Growth; - = No growth

Bioactive compounds of the extract

The Gas chromatography-mass spectrometry is targeted and identifying, quantifying of bioactive principles of herbal extract.

From the result it can be deduced that the ethanol extract contains 38 peaks with compounds of 2H-1-Benzopyran-2-one-4-diethylphosphonate (30.72%), Cyclotrisiloxane, hexamethyl- (22.71%), Cyclopentasiloxane, decamethyl- (11.76%), N-Methyl-1-adamantaneacetamide (9.06%), Pentasiloxane, dodecamethyl- (2.03%), Cyclohexasiloxane, dodecamethyl (4.37%) with percentages ≥ 1%

The n-hexane extract contain 49 peaks with Cyclotetrasolixane (34.61%), Caffeine (13.36%), Cyclotrisiloxane hexamethyl (2.97%), Acridin-9-yl-(4-trifluoromethoxy-phenyl)-amine (11.04%), Glycine, N-[4-[(trimethylsilyl) oxybenzoyl]-, methyl ester (1.49%), Disiloxane, 1,3-diethoxy-1,1,3,3-tetramethyl (2.37%) and Bis(2-ethyl hexyl)phthalate (2.84%) and some others represented in Table 5 below, other compounds have percentage < 1%

Table 4: Bioactive compounds in the ethanol extract

Peak	Retention time	Area Percentage (%)	Chemical Name	Molecular Formula	Molecular Mass
1	5.173	1.51	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_5Si_4$	282.54g/mol
2	5.453	22.71	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_5Si_4$	282.54g/mol
6	7.725	9.06	N-Methyl-1-adamantaneacetamide	$C_{13}H_{21}NO$	207.31
7	7.834	1.83	2-Acetyl-1,3,3,4,4-pentamethylcyclopentene semicarbazone	$C_{13}H_{23}N_3O$	237.34
8	8.166	2.03	Pentasiloxane, dodecamethyl-	$C_{12}H_{36}O_4Si_5$	384.84
9	8.315	30.72	2H-1-Benzopyran-2-one-4-diethylphosphonate	$C_{11}H_{10}O_2$	174.20
16	10.786	11.76	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	370.77
20	13.235	4.37	Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	444.92
21	13.396	1.67	2,4'-Dimethoxy-2'-(trimethylsilyl)oxychalcone	$C_{17}H_{16}O_4$	284.31
25	15.433	1.78	Pentasiloxane, dodecamethyl-	$C_{12}H_{36}O_4Si_5$	384.84
26	15.599	1.12	Cycloheptasiloxane, tetradecamethyl	$C_{14}H_{42}O_7Si_7$	519.07

Table 5: Bioactive compounds in the n-hexane extract

Peak	Retention time	Area percentage (%)	Chemical name	Molecular formula	Molecular weight
1	5.156	11.04	Acridin-9-yl-(4-trifluoromethoxy-phenyl)-amine		
2	5.625	2.97	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.46
3	5.865	1.0	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.46
7	7.628	1.49	Glycine, N-[4-[(trimethylsilyl)oxybenzoyl]-, methyl ester	$C_{15}H_{25}NO_4Si_2$	339.53
8	7.708	2.37	Disiloxane, 1,3-diethoxy-1,1,3,3-tetramethyl	$C_8H_{22}O_3Si_2$	222.42
9	7.817	1.13	1-Methyl-4-azaphenanthrene-3-carboxylic acid	C_9H_{12}	120.19
11	8.166	1.55	Pentasiloxane, dodecamethyl-	$C_{12}H_{36}O_4Si_5$	384.84
12	8.303	34.61	Cyclotetrasiloxane, octamethyl	$C_8H_{24}O_4Si_4$	296.61
20	10.781	1.52	Cyclopentasiloxane, decamethyl	$C_{10}H_{30}O_5Si_5$	370.77
25	13.104	1.28	Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	444.92
36	17.967	1.45	1,2,4-Oxadiazol-5-amine, 3-(4-amino-1,2,5-oxadiazol-3-yl)-N-(2-thienylmethyl)-	C12H12N6O3	288.27
40	19.581	13.36	Caffeine	$C_8H_{10}N_4O_2$	194.19
47	25.578	2.84	Bis(2-ethylhexyl) phthalate	C 24 H 38 O 4	390.56

Discussion

Algae have been demonstrated to produce secondary metabolites other than those produced by terrestrial organisms [20]. Therefore, they have been indicated to be a source of compounds of biomedical interest [11, 21-22]. The antimicrobial potential of algae is highly dependent on: (i) type, brown algae being the most effective against foodborne bacteria; (ii) the solvent used in the extraction of bioactive compounds, ethanolic and methanolic extracts being highly effective against Gram-positive and Gram-negative bacteria; and (iii) the concentration of the extract [23]. The results of the study showed that the green algae extract using ethanol solvents possess antimicrobial activity by showing the inhibition zone around the holes created using a sterile cork borer. However, among all microorganisms tested *Bacillus subtilis* and *E. coli* showed inhibition zone at all the studied concentrations. Meanwhile, *S. aureus* showed little zone of inhibition around the hole and the lower concentrations did not even show activity at all (50mg/ml, 25mg/ml and 12.5mg/ml) *Salmonella typhi* showed inhibition zone at concentration 400 mg/ml and 50 mg/ml. There was no zone of inhibition at 400 mg/ml and 200mg/ml concentrations for *Candida sp* but the lower concentrations like 100 mg/ml, 50mg/ml 25 mg/ml and 12.5mg/ml showed high zone of inhibition round the hole. A study carried out on the green algae species by Gonzalez del Val *et al*. [24] showed that methanol extract of green algae possessed antimicrobial activity against *B. subtilis* and *S. aureus* which is in line with the current study. The study conducted by Indira *et al*. [25], claimed that the higher inhibition zone was recorded at concentration 1000 μg/ml than 500 μg/ml concentration of all extract. When the dosage level increases, the inhibitory effect also increased. Moreover, in this study ethanol extract of green algae showed equal effective against Gram-positive bacteria (*B. subtilis* and *S. aureus*) and Gram-negative bacteria (*S. typhi, E. coli*), *Escherichia coli* which is a gram-negative bacteria shows the highest reading for the zone of inhibition. These results parallel to other previous study conducted by Afifah *et al*. [26] that claimed the green algae extract were more effective against Gram-positive bacteria and less against Gram-negative bacteria. Basically, the differences in the antimicrobial activities may be due to the differences in cell walls of the bacteria. The cell wall of Gram-positive bacteria only consists of an outer peptidoglycan layer and that of the gram-negative bacteria is made up of lipopolysaccharides. it was observed that at 400g/ml which is the stock solution, there is high activity in *Eschericia coli* as compared to the other tested organism, the same thing is noticed in the 200mg/ml concentration as well, but It was observed that only 3 of the five organism showed noticeable activity at this concentration, the 3 organisms that are affected here are *Eschericia coli, Bacillus sp* and *staphylococcus aureus.* At 100mg/ml concentration same thing the was observed in the previous concentration happened here. At 50mg/ml concentration, it was observed that 4 organisms were significantly affected and here the activity shown in *Bacillus sp.* Is higher than that shown in the previous concentration, *Staphylococcus aureus* resisted the sample at this concentration as well. The extract at 25mg/ml concentration did not have activity on *Salmonella sp* and *Staphylococcus aureus.* Resistance was observed at 12.5mg/ml concentration from just 2 of the tested organisms, and the organism include *Eshericia coli* and

Bacillus sp

The MIC of the extract on *Staphylococcus aureus* is 200 mg/ml, 50mg/ml for *Bacillus sp, Salmonella sp* and *Candida sp*, 25mg/ml for *E. coli.*

The minimum bactericidal concentration of the extract on *Staphylococcus aureus* is 200mg/ml, 50mg/ml for *Salmonella sp* and *Bacillus sp* and 25mg/ml for *E.coli* and *Candida sp*

Following the statistical analysis of the result, there is a significant difference between the mean zone of inhibition of *E.coli* and *Salmonella sp* with $p<0.001$ at 400mg/ml, $p<0.01$ at 200mg/ml and $p<0.05$ at 100mg/ml. The mean zone of inhibition of *E.coli* and *Staphylococcus aureus* are significantly different with $p<0.01$ at 400mg/ml and $p<0.05$ at 200mg/ml. The mean zone of inhibition *E. coli* is significantly different from Candida with $p<0.01$ at 400mg/ml and 200mg/ml and $p<0.05$ at 100mg/ml. The mean zone of inhibition of *Bacillus sp* is significantly different from *Salmonella sp* with $p<0.05$ at 50mg/ml and different from *Staphylococcus aureus* with $p<0.001$ at 50mg/ml. *Staphylococcus aureus* is significantly different from *Candida sp* with $p<0.01$ at 50mg/ml.

For the GC-MS results cyclotrisiloxane hexamethyl a very common component in both the n-hexane and the ethanol extracts have said to constitute to the antimicrobial activity of the green algae extract as reported in antimicrobial activity and chemical constituents of west Anatolian olive (*Olea europea L.*) leaves by Keskin, *et al* [27]in which they tested antimicrobial activity of cyclotisiloxane hexamethyl showed similar antimicrobial activity in aqueous extract of olive leaves. In the study it was detected that cyclotrisiloxane hexamethyl, which happen to be the main constituents in olive extract caused the antimicrobial influence against the bacteria microorganisms used then. According to the work of Lekshmi *et al*., [28], N-Methyl-1-adamantaneacetamide in *Cissus quadrangularis* was attributed to the insulin regulation of the plant. This is because the compound has been reported to be an insulin secretagogues in nature [29]. Hence its present in the green algae ethanol extract can give it an antidiabetic effect. The presence of Acridin-9-yl-(4-trifluoromethoxy-phenyl)-amine is in line with the report of Goni *et al*. [30] which says that Acridine and its derivatives are found in natural plants and marine organisms, forming an important class of N-containing heterocycles [30]. Acridine derivatives posess anticancer, antitubercular, antiviral, antimalarial, antimicrobial, anti-inflammatory, antiparasitic, and fungicidal activities [31]. The presence of this compound in this extract could convey these properties to its biological activities. Glycine, N-[4-[(trimethylsilyl)oxybenzoyl]-, methyl ester is it is the hydrochloride of the methyl ester of the amino acid glycine. Glycine an amino acid, glycine contributes to cellular growth and health. Glycine is one of the amino acids essential to the body's synthesis of the antioxidant glutathione. Hence its presence in the extract can confer its function to the overall benefits of the extract. The presence of Caffeine in the extract could induce, increase in alertness and attentional performance [32-33].

Conclusion

Results gotten from the work as reported above can make one conclude that the demonstration of antimicrobial activity against both gram-negative and gram-positive bacteria is an indication that the green algae is a potential source for production of drugs with a broad spectrum of activity. The results of the study also support the traditional application of the plant and suggests that the plant extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel drugs. Some of the compounds identified from the extracts have been reported to have some biological effects in addition to antimicrobial activity which could include antidiabetic, anticancer, synthesis of important compounds in the body. Although more research geared towards other utilitarian activities of green algae can be carried out.

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Author's Contribution

Not available

Conflict of Interest

Not available

Financial Support

Not available

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