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Phytochemical Analysis, Antioxidant, Antibacterial Potentials and Chemical Composition of Methanol Extract of *Oscillatoria* sp.

A. M. SALAAM[1ACEF], B. C. ADEBAYO-TAYO[2*ACDEF], A. A. AJIBADE[2BCF]

[1] Department of Botany, Faculty of Science, University of Ibadan, Ibadan, Oyo state, Nigeria
[2]Department of Microbiology, Faculty of Science, University of Ibadan, Ibadan, Oyo state, Nigeria

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Algae contains some useful phytochemicals and *Oscillatoria* sp. is a green microalga which has various applications

Objectives: Phytochemicals, antioxidant, antibacterial potential and chemical composition of methanol extract of *Oscillatoria* sp. were evaluated.

Materials and Methods: Extraction of bioactive compound from *Oscillatoria* sp., determination of phytochemicals, antioxidant, antibacterial potential and chemical composition of the methanol extract.

Results: Saponin, alkaloids and flavonoids were detected in the methanol extract while glycosides and phenols were absent. The scavenging assay for the antioxidant increased in a dose dependent (100 -1000 µg/mL) manner. The DPPH scavenging activity, Total phenolic content, Ferric Reducing Antioxidant Power Assay (FRAP) and hydrogen peroxide scavenging activity ranged from 21.8 - 44.9%, 9.09 -21.46%, 0.43 - 42.49% and 19.4 - 74.4% respectively. The methanol extract had inhibitory activity against the test pathogens in which *Pseudomonas aeruginosa* ATCC 27853 (24.0 mm) had the highest susceptibility. GCMS chromatogram of the methanol extract of *Oscillatoria* sp. shows fifteen major peaks which indicated the presence of Oleic acid, stearic acid, aracidic acid, methyl laurate, methyl myristate, oxirane and palmitic acid.

Conclusion: *Oscillatoria* sp. methanol extract contain some phytochemicals and some heterocyclic compounds. The extract had good antioxidant capability and appreciable antibacterial activity against the test pathogens.

Keywords*: Oscillatoria sp.; methanol extract; Phytochemical; Antioxidant; GC-MS; Antibacterial*

INTRODUCTION

The emergence of microorganisms that have developed resistance to conventional chemotherapeutic agents is of great concern due to the threat it poses to public health. The infections caused by resistant microorganisms are usually hard to treat with chemotherapeutic agents hence the use of potent extracts of natural products which give better aid due to presence of diverse chemical compounds that are derived naturally (Mothana *et al*., 2010). Potent natural extracts could be of plant or algal source. The Green algae are photosynthetic algae that manufacture their food using energy from sunlight and carbon-dioxide as major carbon source (Rodea-Palomares *et al*., 2011). Microalgae are known to produce antibiotic compounds that have been tested against wide range of microorganisms including viruses, bacteria and fungi among others (Metting and Pyne, 1986; Herrero *et al*., 2006).

In humans, several metabolic reactions occur; amongst these chains of reactions are oxidation reactions that lead to formation of superoxide, hydroxyl, peroxyl radicals and other reactive oxygen species (ROS). These reactive oxygen species are formed in the presence of intermediates of free radicals in the course of human metabolic activities. Formation of reactive oxygen species can lead to the extensive oxidative damage causing related degenerative conditions and other diseases in humans (Aruoma, 1999; Finkel and Holbrook, 2008; Kannan *et al*., 2014). The oxidation reactions that lead to generation of reactive oxygen species (ROS) can be reduced by antioxidants which remove intermediates of free radicals (Kannan *et al*., 2014). Flavonoids such as catechins, anthocyanidins, flavones, proanthocyanidins, flavonols among others (Ndhlala *et al*., 2007) have wide range of biological and chemical potentials that include free radical scavenging and antioxidant potentials (Kahkonen *et al*., 1999). In this work, bioactive components of the microalgae *Oscillatoria* sp. were extracted using methanol. The phytochemicals, antioxidant potential and antibacterial activity of the methanol extract of *Oscillatoria* sp. against some clinical pathogens along with its chemical composition was evaluated.

METHODOLOGY

Sample collection and Identification

A thick mass of blue green algae sample identified as *Oscillatoria* sp. in the Department of Botany, University of Ibadan, Ibadan Nigeria was collected from the wall of the walkway of the Department of Theatre Arts, University of Ibadan, Ibadan, Nigeria (Latitude 03˚434' 43' E and longitude 07˚26' 41'' N) using sterile scapel and transported to the laboratory for further analysis. The algal sample was washed under running tap water and rinsed with sterile distilled water to remove extraneous materials from it. The washed samples were dried at room temperature for a week, powdered and stored at room temperature for further analysis.

Collection of indicator microorganisms

Pathogenic bacteria (*Staphylococcus aureus* ATCC29213, *Escherichia coli* ATCC 11775, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC27853, *Citrobacter* sp., *Salmonella typhi* ATCC 14028 and *Bacillus cereus*) were obtained from the Department of Microbiology, University of Ibadan, Ibadan Oyo state.

Extraction of the bioactive compound from the microalgae

Two hundred grams of the dried algae samples was ground and the bioactive compounds was extracted using Soxhlet extraction and methanol (300 mL) as solvent. The marc was filtered and solvents were removed under reduced pressure in a rotary evaporator. Dark brown pastes were obtained, weighed and stored in a refrigerator at 4˚C.

Phytochemical analysis

Qualitative determination of Phytochemicals present in the methanol extract of *Oscillatoria* sp.

Qualitative determination of the phytochemical in the *Oscillatoria* sp. methanol extracts was done to test for Saponins, Flavonoids, Alkaloids, Glycoside and Phenols (Harbourne, 1983; Sofowora, 1983; Meda *et al*., 2005).

Test for saponins in the methanol extract of *Oscillatoria* sp.

One gram of the methanol extract of *Oscillatoria* sp. was added to 9 mL of distilled water in a sterile test tube and well shaken. Formation of froth in the solution indicated the presence of saponin in the methanol extract of the microalga (Cuilei, 1982).

Test for the presence of flavonoid in the methanolic algal fraction

A mixture of 10 mL ethyl acetate and 2 g of the extract was placed in a steam bath for 3 minutes and filtered. 1 mL of ammonia solution was added to 4 mL of the filtrate and shaken. The observation of yellow colour in the reaction mixture showed the presence of flavonoids (Harbourne, 1983).

Test for Alkaloid in the methanolic algal fraction

Five milliliter (5 mL) of 1% hydrochloric acid was mixed with 0.2g of methanol extract of *Oscillatoria* sp., placed in steam bath, stirred gently and filtered. A few drops of Dragendorff's reagent was added to 1 mL of the filtrate. Formation of precipitate indicated the presence of alkaloid in the extract (Gundidza, 1985).

Quantitative determination of the phytochemicals

Determination of Total Alkaloids

The quantity of alkaloid was determined using the method of Harbourne (1983). To 2.5 g of the methanol extract (0.025g/mL), 100 mL of acetic acid in ethanol was added and covered for 4 hours. The mixture was filtered and concentrated in a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added to the mixture and allowed to settle before collecting the precipitate with filter paper.

The residue (alkaloid) was dried, weighed and the percentage alkaloid was expressed mathematically as:

$$\% \text{ alkaloids} = \frac{weight\ of\ alkaloids}{weight\ of\ sample} \times 100$$

Determination of Total Flavonoids

Total flavonoid contents in the methanol extract of *Oscillatoria* sp. was determined according to the method of Ejikeme *et al*., (2014); Boham and Kocipal (2002). 10 mL of 80 % aqueous methanol was added to 10 grams of the sample and incubated at room temperature for 24 hours. The supernatant was discarded and the residue was re-extracted (three times) with the same volume of ethanol. The solution was filtered using Whatman filter paper number 42 (125mm). The filtrate was dried using water bath, cooled in a desiccator and weighed. Percentage flavonoid was calculated ass:

$$\% \text{ flavonoids} = \frac{weight\ of\ alkaloids}{weight\ of\ sample} \times 100$$

Determination of Total Saponins

Total saponin content was determined using the method of Obadoni and Ochuko (2001). 20 mL of 20% aqueous ethanol was added to 2 grams of the methanol extract sample. The suspension was heated 4 hours with continuous stirring at 55˚C in a water bath. The mixture was filtered and the residue was re-extracted with another 20 mL of 20% ethanol. The combined extract was evaporated to 4 ml over water bath at 90˚C. 2 ml of diethyl ether was added to the concentrate in a separator funnel and vigorously agitated. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated twice and 6 ml of n-butanol was added aseptically, washed twice with 10 ml of 5% aqueous sodium chloride and the remaining solution was heated in a water bath. After the evaporation, sample was dried in the oven and saponin content was calculated as percentage:

$$\% \text{ saponin} = \frac{weight\ of\ alkaloids}{weight\ of\ sample} \times 100$$

Determination of antioxidant potential of the *Oscillatoria* sp. methanol extracts

DPPH Radical Scavenging Assay

The free radical scavenging activity of the *Oscillatoria* sp. methanol extract using stable free radical, DPPH (2, 2-Diphenyl-1-picrylhydrazyl) (Temraz and El-Tantawy, 2008; Rajaram and Kumar, 2011). 1 mL of 0.004 % DPPH-methanol solution was added to 2 mL of the methanol extract prepared at different concentration (50 – 100 µg/ mL). The absorbance changes were measured at 517 nm using spectrophotometer and ascorbic acid was used as standard. The percent inhibition was calculated using the formula:

Percentage Inhibition of DPPH activity (%) = (A – B / A) X 100

Where A = the absorbance of the control (DPPH) and B = the absorbance of test sample.

Total Antioxidant Capacity

Total antioxidant activity of methanol extract was done by mixing sulphuric acid of sodium sulfate and ammonium molybdate with distilled water and labeled as total antioxidant capacity (TAC). The extract was added to TAC and the absorbance was read at 695 nm after 15 minutes and ascorbic acid was used as standard, this was done according to method of Mitsuda *et al.* (1996).

Hydrogen Peroxide Scavenging Assay

Free radical scavenging activity of the extract was determined using a hydrogen peroxide assay according to method of Gulcin *et al*. (2004). A 10 mM hydrogen peroxide solution was prepared in phosphate buffer (0.1M, pH 7.4). Aliquot of 2 mL Hydrogen Peroxide (H_2O_2) was mixed with 1mL of methanol extract. The absorbance was taken at 230 nm after 10 minutes of incubation at 37˚C against a blank without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide was calculated:

Antibacterial activity of the methanol extract of *Oscillatoria* sp.

The antibacterial activity of methanol extract of *Oscillatoria* sp. against some pathogenic strains (*Escherichia coli* ATCC 11775, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Citrobacter* sp., *Salmonella typhi* ATCC 14028, *Staphylococcus aureus* ATCC 29213, and *Bacillus* sp.) was evaluated using agar well diffusion method (Abedin and Taha, 2008; Prakash, 2011). The test cultures were swabbed on Muller Hinton agar. Uniform wells were bored on the agar using a sterile cork borer of 7 mm diameter. 0.5 McFarland standard of the test microbes were prepared with 24 hours culture and were evenly swabbed on the freshly prepared agar plate. About 50 µL of the *Oscillatoria* sp. methanol extracts and positive control (10 mg / mL Streptomycin) was placed in the wells. The inoculated plates were incubated at 37ºC for 24 hours after which the diameter of the zone of inhibition was measured. The tests were done in triplicates.

Determination of chemical compounds in the samples using Gas Chromatography (GC)–Mass Spectrometer (MS*)*

The soluble constituents obtained from the organic fraction after dissolving the methanol extract in petroleum ether were re-dissolved in diethyl ether and subjected to GC/MS analysis. Gas Chromatography-Mass Spectrometer (model: GCMS-Qp2010 Plus Shimadzu, Japan) made up of an auto sampler (AOC-20i) and a gas chromatograph interfaced to a mass spectrometer was used. The interpretation of mass spectrum GC-MS for the identification of compounds was done by comparing the spectrum of the unknown component in the samples with the spectrum of the known components stored in the National Institute Standard and Technology (NIST) library. The name, molecular weight and structure of the components of the methanol extract of *Oscillatoria* sp. were ascertained.

RESULTS

Phytochemical analysis

The qualitative and quantitative determination of phytochemicals in the methanol extract of *Oscillatoria* sp. is shown in Table 1. Three secondary metabolites were detected in the sample. Alkaloids, flavonoids and saponins were present in the extract while glycosides and phenols were not detected. Quantitative analysis showed that saponins were abundantly present (7.0%) followed by Alkaloids (6.0 %) while Flavonoid had the lowest quantity (3.0 %).

Table 1: Bioactive components of methanol extract of *Oscillatoria* sp.

Phytochemical Compound	Qualitative determination of phytochemical	Quantitative determination of the phytochemicals (%)
Alkaloids	+	6.0
Flavonoids	+	3.0
Glycosides	-	-
Phenols	-	-
Saponins	+	7.0

Key: + present, - absent

Phytochemical screening was carried out in order to determine the biological compounds that are present in the extract. The presence of alkaloids, flavonoids and saponins in methanol extract of *Oscillatoria* sp. extract was in agreement with the report of Selvan *et al*. (2014), who also confirmed the presence of these three phytochemicals in *Tetraselmis* sp., *Dunaliella* sp., *Chlorella* sp., *Synechocystis* sp., and *Oscillatoria* sp. They equally reported the absence of glycosides from methanol extract of the *Oscillatoria* sp. The biological activity of flavonoids which include antioxidant and tumoristatic potentials have been reported (Li *et al*., 2007). The presence of these phytochemicals has confirmed their relevance and effectiveness in human physiological and cellular reactions (Guaadaoui *et al*., 2014). El-Din and Al-Ahwany, (2016) also reported the presence of alkaloid, flavonoid and saponin in three seaweeds. They reported that higher dielectric constant of methanol compared to dielectric constant of acetone, ethanol and chloroform is responsible for the most effectiveness of methanol in the extraction of extract from algae.

Flavonoid has been reported to have protective ability on different biotic and abiotic stresses and it has ability to act as unique UV filters (Takahashi and Ohnishi, 2004) . Flavonoids function as signal molecules, allopathic compounds, phytoalexins, detoxifying agents and antimicrobial defensive compounds (Panche *et al*., 2016). Flavonoids were reported by Ross and Kasum, (2002) and Williams *et al*. (2004) as inhibitors of lipid peroxidation, scavengers of reactive oxygen species, antioxidants and potential therapeutic agents against diseases. Saponin and steroid are related to sex hormones such as oxytocin which regulates the onset of labour in pregnant women and subsequent release of milk (Kalidass and Mahapatra, 2014).

Antioxidant analysis

DPPH scavenging activity of *Oscillatoria* sp. methanol extract

The antioxidant activity of the methanol extract of *Oscillatoria* sp. was determined based on the percentage of free radical scavenging activity. The DPPH activity increased in dose dependent manner. At concentration of 100 – 1000 µg/mL the activity ranged from 21.8 - 44.9 %. The maximum scavenging activity occurred at highest concentration (44.9% at 1000µg/mL) and the lowest scavenging activity was observed at the minimum concentration (21.8% at 100µg/mL). This implied that the DPPH scavenging activity of the methanol extract of *Oscillatoria* sp. increased with respect to the increase in concentration of the extract; the higher the quantity of the extract, the higher the inhibition of free radicals as shown in

Figure. 1. The DPPH activity of the extract was lower than that of the standard.

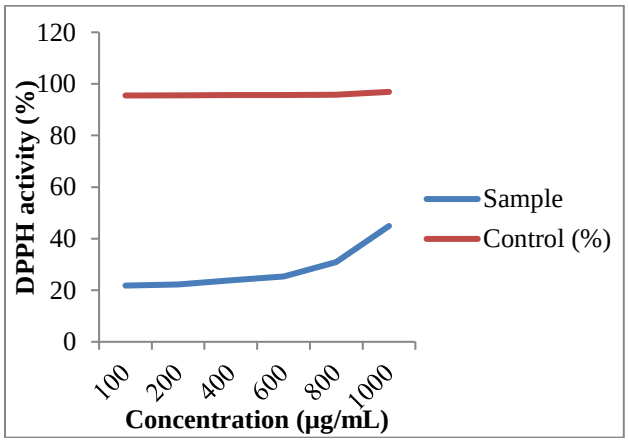


Figure 1: DPPH scavenging activity of methanol extract of *Oscillatoria* sp.

Total Phenolic Content (TPC)

The percentage of total phenolic content of the extract of *Oscillatoria* sp. ranged from 9.09 - 21.46%. The highest phenolic content was observed at the maximum concentration of the extract (1000 µg/mL) and the lowest phenolic content of the extract was observed at the minimum concentration (100 µg/mL). There was a significant difference in total phenolic content of the extract of *Oscillatoria* sp. as shown in Figure 2. The TPC of the extract was higher than that of the standard up to 800 µg/mL.

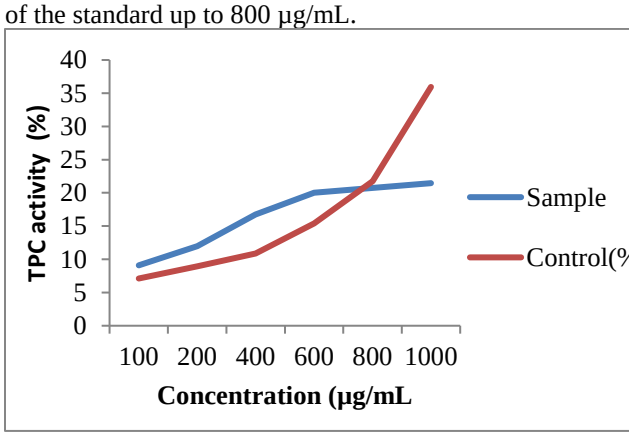


Figure 2: Total Phenolic Content of methanol extract of *Oscillatoria* sp.

Ferric Reducing Antioxidant Power Assay (FRAP)

The reducing power of Fe^{3+} to Fe^{2+} was analyzed and it was observed that the reduction ability increased in a dose dependent manner. The highest reducing power (42.49 %) was obtained at the maximum concentration of the extract (100µg/mL) as shown in Figure 3. *Oscillatoria* sp. methanol extract did not have FRAP capacity at a concentration below 10 µg / mL.

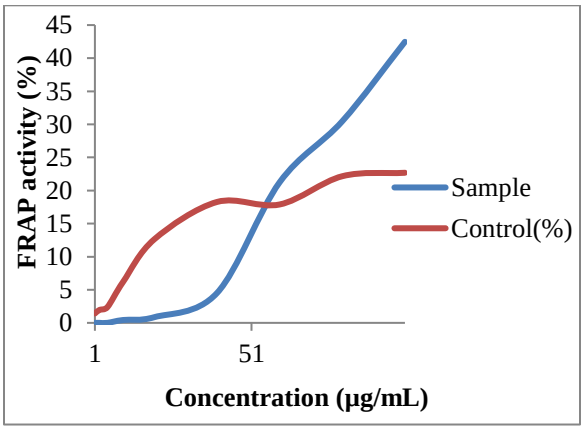


Figure 3: Ferric reducing antioxidant power (FRAP) of the extract

Hydrogen Peroxide Scavenging Activity

The antioxidant activity of the extract of *Oscillatoria* sp. was determined based on the percentage of free radical scavenging activity; assessing the ability to decrease the pro-oxidant H_2O_2. The maximum activity (74.4 %) occurred at the highest concentration (1000 µg/mL) and the lowest activity (19.4 %) was observed at the minimum concentration (100 µg/mL). This implied that hydrogen peroxide scavenging activity of the extract against the free radicals increased as the concentration of extract increased as shown in Figure. 4. The hydrogen peroxide scavenging activity of methanol extract of *Oscillatoria* sp. was higher than that of the standard.

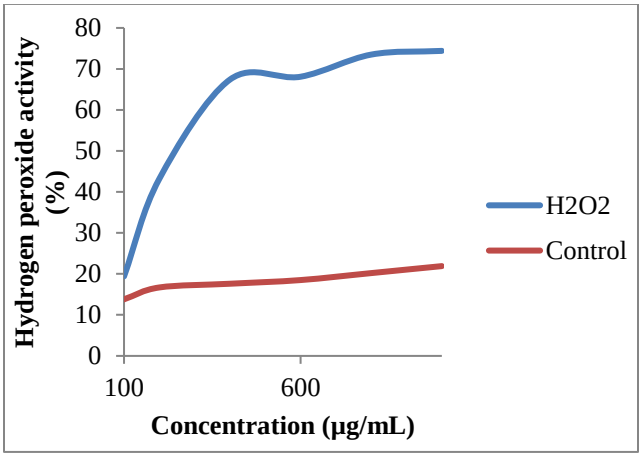


Figure 4: Hydrogen peroxide scavenging activity of the extract

The methanol extract showed inhibition of DPPH. DPPH scavenging activity of the methanol extract of *Oscillatoria* sp. increases with respect to the increase in concentration of the extract. Percentage scavenging activity of the extract on Iron as shown by the result increased in dose dependent manner and this reducing

power showed that the antioxidant compounds present in the extract are electron donors which possess the ability to reduce the oxidized reaction intermediates of lipid peroxidation process. This is in agreement with the work of El-Din and El-Ahwany (2016) on the bioactivity and phytochemical constituents of algae. He reported methanol as the most effective solvent for the extraction of bioactive compounds due to higher dielectric constant of methanol compare to other solvents.

The ability of the methanol extract of *Oscillatoria* sp. to scavenge hydrogen peroxide as shown by the result increased in dose dependent manner. The percentage inhibition of the extract was higher than that of the control (ascorbic acid).

Antibacterial Activity of the Extract

The antibacterial activity of the methanol extract of *Oscillatoria* sp. against some selected microorganisms is shown in Figure 5. The antibacterial activity of the extract ranged from 8.0 - 24.0 mm. The susceptibility patterns of test pathogens to methanol extract of *Oscillatoria* sp. was in ascending order of *Pseudomonas aeruginosa* ATCC 27853 > *Staphylococcus aureus* ATCC 29213 > *Escherichia coli* ATCC 35218 > *Salmonella typhi* ATCC 14028 > *Bacillus* sp. > *Escherichia coli* ATCC 11775 > *Citrobacter* sp.

Pseudomonas aeruginosa ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Salmonella typhi* ATCC 14028 were more susceptible to the extract than to commercial antibiotics used during this study. *Citrobacter* sp. had the lowest susceptibility to the extract.

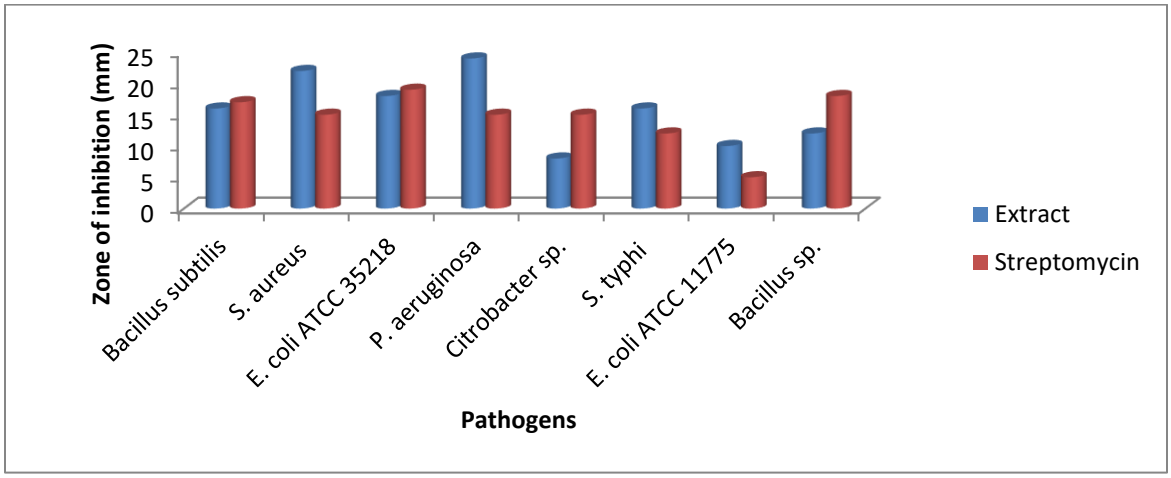


Figure 5: Antibacterial activity of the extract against some selected pathogens

The antimicrobial activity of the extract against the test pathogens may be as a result of the phytochemicals present in the methanol extract of *Oscillatoria* sp. Radhika *et al*. (2012) reported that the antimicrobial activity of algae extract was dependent on the algal species and solvent used for the extraction. This research is in agreement with the report of Osman *et al*. (2013) on the biological activities (antimicrobial, antiviral, neurotoxic, anti-inflammatory) of compounds derived from algae.

Methanol extract of *Oscillatoria* sp. showed more activity against most of the pathogens especially *Pseudomonas aeruginosa* ATCC 27853 (24.0). The antibacterial potential of methanol extract of *Sargassum polycystum* and *Sargassum tenerrimum* against some pathogens has been reported (Kausalya and Rao, 2015).

Gas Chromatography-Mass Spectrophotometry (GC-MS) of the methanolic extract of *Oscillatoria* sp. fraction

GC-MS chromatograph of methanol extract of *Oscillatoria* sp. showed fifteen (15) major peaks each of which signified a chemical compound (Figure 6, Table 2). Peak 4 showed the highest intensity while peak 15 had the lowest intensity in the chromatogram. Oleic acid, stearic acid, aracidic acid, methyl laurate, methyl myristate, oxirane and palmitic acid among others were detected in methanol extract of *Oscillatoria* sp.

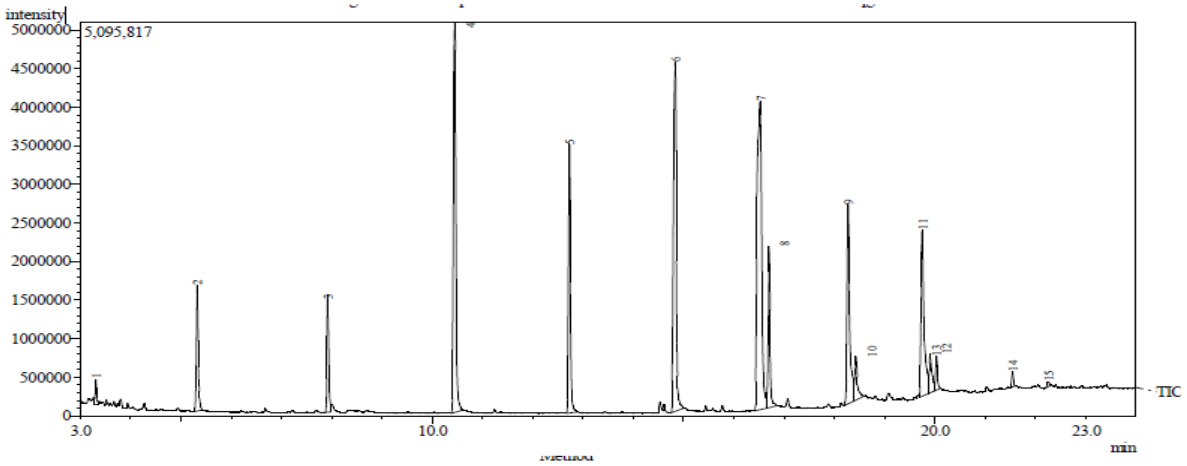
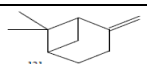
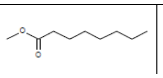
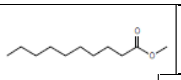
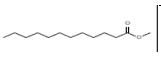
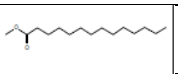
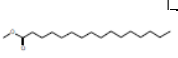
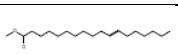
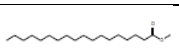
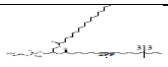
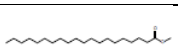
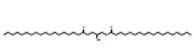
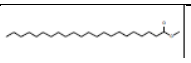
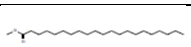
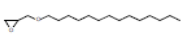


Figure 6: GC-MS Chromatograph of methanol extract of *Oscillatoria* sp.

Table 2: Bioactive components of methanol extract of *Oscillatoria* sp. by GC-MS

Peak No	Retention time	Peak area (%)	Structure of Compound	Name	Peak height (%)
1	3.306	0.58		6,6-Dimethyl-2-methylene-bicyclo heptanes	1.10
2	5.58	5.324		Methyl Octanoate	5.324
3	7.922	5.22		Methyl Decanoate	3.58
4	15.81	10.448		Methyl Laurate	17.35
5	8.8	12.729		Methyl Myristate	12.01
6	14.832	16.84		Methyl Hexadecanoate	15.51
7	16.522	23.28		Methyl-11-Octadecenoate	13.71
8	16.701	4.8		Methyl n-Octadecanoate	7.18
9	18.271	8.90		Palmitin, 1, 2-di-, 2-aminoethyl hydrogen phosphate	8.87
10	18.428	1.82		Methyl aracidate	1.94
11	19.747	8.28		Cis-Oleic acid	7.40
12	19.907	1.80		Glyceryl 1,3-distearate	1.71
13	20.029	1.52		n-docosanoic acid methyl ester	1.05
14	21.542	0.41		Methyl henicosanoate	0.67
15	22.256	0.20		Myristyloxymethyl oxirane	0.23

GC-MS analysis of the extract showed fifteen major peaks each of which represents a chemical compound. Oleic acid, stearic acid, aracidic acid, methyl laurate, methyl myristate, oxirane and palmitic acid among others were detected in methanol extract of *Oscillatoria* sp. Most compounds identified have previously been reported for antioxidant and anti-inflammatory among other health benefits of algae. Oleic acid and octadecanoic acid were also reported to contribute to bioactivity and phytochemical constituents of marine red seaweeds such as *Jania rubens Corallina mediterranea* and *Pterocladia capillacea* (El-Din and Al-Ahwany, (2016). Most of the chemicals such as palmitic, myristic, oleic, lauric and stearic acids obtained from this research are in agreement with the work of Agoramoorthy *et al*. (2007). He reported some chemicals such as palmitic acid, lauric acid, stearic acid, linoleic, oleic, myristic acid and linolenic acid from algae extract. Zhong *et al*. (2010) also reported greater inhibitory activity by octadecanoic acid obtained from neem oil extract against *S. aureus*, *Salmonella* sp. and *E. coli*. Octadecanoic acid was among the chemical compound found in the methanol extract of *Oscillatoria* sp.

CONCLUSION

The methanol extract of *Oscillatoria* sp. was found to contain vital phytochemicals which are valuable medicinal heterogeneous compounds. The presence of saponin, flavonoids and alkaloids and some other chemical compounds in the extract contribute to the antioxidant and antibacterial activity of the extract against the test pathogens.

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*Address for correspondence: Bukola C. Adebayo-Tayo
Department of Microbiology,
Faculty of Science,
University of Ibadan,
Ibadan, Oyo state,
Nigeria
Telephone: +234 8035522409
E-mails: bukola.tayo@gmail.com

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