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# Phytochemical, Chemical and Biomedical Characterization of Crude Extracts of *Macrosphyra longistyla* (DC.) Hiern

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**Abstract**

Phytochemical analysis of *Macrosphyra longistyla* leaf, stem bark, and root extracts revealed different constituents. The GC-MS analyses of their ethanolic extracts showed the presence of bioactive compounds: the stem bark yielded 9 compounds, such as squalene, vitamin E, and fatty acids; the root extract revealed 10 different compounds, especially morpholine, isophorone and fatty acids. The extracts demonstrated high proteinase inhibition potentials. The aqueous, ethanol and ethyl acetate extracts of the leaf, stem bark, and root were tested against *Methicillin-Resistant Staphylococcus aureus (MRSA) 144m, Escherichia coli ATCC11229, Salmonella typhimurium ATCC13311, Enterococcus faeciumATCC700221, Shigella flexneri ATCC12012* and laboratory strain of *Streptococcus mutans* with the diameter of zone of inhibitions ranging from 10 to 40 mm. The study revealed a marked susceptibility pattern of the test organisms to the ethanol and ethyl-acetate extracts showing varied diameter of zones of inhibitions. The aqueous extract was ineffective against the pathogens.. The minimum inhibitory concentrations (MIC) ranged from 25-100mg/ml. All the test organisms except *S. mutans* were susceptible to control antibiotic (streptomycin 10µg).. The presence of arrays of bioactive ingredients implicated in the treatment of specific ailments has provided a scientific justification for *Macrophyla longistyla* as an alternative remedy for the treatment of bacterial infections.

**Keywords**: *Macrosphyra longistyla*, DPPH scavenging, isophorone, plants alkyne, antibacterial agents.

## 1. Introduction

Numerous medicinal herbal plants have been associated with the treatment and the prevention of different diseases for thousands of years (Wang *et al.* 2012). This association, however, diminished with the discovery of antibiotics. As resistant pathogens develop and spread, the effectiveness of the antibiotics also diminished. In recent years, clinically relevant bacteria with multiple drug-resistant (MDR) strains had been reported globally (Andersson and Levin, 1999; Davies and Davies, 2010; Odumosu *et al.* 2017).

Bouyahaya *et al.* (2017) reported that numerous research works had demonstrated the potential of medicinal plants used in different traditional, complementary, and alternative disease treatments. Jaiswal and Sharma (2020) further added that in the last two decades, different parts of the plant such as leaves, stem, seeds, flowers, fruits, and roots of many medicinal plants and weeds had been documented to exert antibacterial potentials. They together with (Belakhdar *et al.* 2015; Maffo *et al.* 2015; Al-Jadidi and Hossain, 2016; Bouyahya *et al,* 2016; Lopez-Rubalava and Estrada-Camarena, 2016; Karthikeyan *et al.* 2009; Saranraj and Sivasakthi, 2014) had attributed this ability to various secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, glycosides, saponins, fatty acids, gums, resins, and steroids, present in these medicinal plants. Recently, interest in plant extracts exhibiting antimicrobials and pharmacological applications has been increasing (; Sacan 2018; Singh and Sharma, 2019; Jaiswal and Sharma, 2020; Mak *et al.* 2013; Yigit, 2018).

*Macrosphyra longistyla* (DC.) Hiern is a popular medicinal plant in West Africa (Burkill, 1995; Govaerts *et al*. 2003; Arbonnier, 2000). It belongs to the family Rubiaceae. The plant parts used include the fruit, leaf, and also flower. Generally, the flower is used for healing; the young leaves are boiled and eaten as vegetables. The leaves are excellent treatments for cutaneous, subcutaneous parasitic infections, abortifacients, embolic, and leprosy. The roots are diuretic and useful for treating kidney problems (Arbonnier, 2000).

Since the main antimalarial drug quinine is of Rubiaceae origin, researchers tend to infer that similar compounds with similar properties may occur in other genera of the family Rubiaceae (Karou *et al.* 2011). Singh and Sharma (2019) had reviewed the therapeutic potentials of plant-based natural compounds used for malaria treatment. From the available literature, reports on *M.*

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*longistyla* are scarce (Odugbemi, 2008; Elufioye *et al.* 2019). Hence, this study was set out to investigate the phytochemical components and antibacterial activities of ethanolic, aqueous and ethyl acetate of the leaf, stem bark, and root extracts of *M. longistyla* on selected bacteria.

## 2. Material and Methods

### *2.1. Collection of plant materials*

The fresh stem-bark, leaves, and roots of *Macrosphyra longistyla* were collected from within the premises of Redeemer's University Ede, Osun State, Nigeria, in March 2018. The plant specimen was identified by Dr. Ernest Durugbo of the Department of Biological Sciences Redeemer's University and later authenticated by Dr. George Nodza at the University of Lagos Herbarium and a voucher number LUH 8194 assigned. The plant materials were air-dried for two weeks at room temperature crushed and grounded into powder, using mortar and pestle ready for extraction.

### *2.2. Preparation of crude extracts*

The extraction of powdered plant material was carried out by maceration, as previously described (Ogah and Osundare, 2015). Thirty grams (30 g) of dry powdered materials percolated in 300 ml of absolute ethanol, ethyl-acetate, and distilled water in 500 ml conical flasks were stoppered and kept for 48 hours at room temperature (28±2ºC). The extracts were filtered with Whatman No. 1 filter paper, concentrated at 40$^{o}$C using a rotary evaporator (REL200, Bibby Sterlin, England, Agyare *et al.* 2014), and the concentrated extracts were used for the antibacterial assay and further analysis.

### *2.3. Selection of Bacteria culture*

The pure typed bacteria cultures of Methicillin-Resistant *Staphylococcus aureus* (MRSA)144m, *Escherichia coli* ATCC11229, *Salmonella typhimurium* ATCC13311, *Enterococcus faecium* ATCC700221, *Shigella flexneri* ATCC12012 and Laboratory strain of *Streptococcus mutans* used for the antibacterial assay were obtained from the pure culture laboratory of Microbiology Department of the University of Lagos, Akoka-Yaba, Lagos, Nigeria. They were maintained in glycerol-peptone water at 4$^{o}$C before use.

### *2.4. Antibacterial Study*

The bacterial cultures were inoculated in Mueller-Hinton Broth to obtain a fresh 18-h old culture for the assay. The leaf, stem bark and root extracts of *M. longistyla* were tested using the well-diffusion method described by CLSI. A stock solution of extract consists of 0.4g of plant material in 1ml of water. A loopful of bacterial colonies from overnight culture was inoculated into sterile Mueller-Hinton Broth (MHB). Broth inoculums were incubated at 37°C for 16-20 h. Broth inoculums were standardized to 0.5 McFarland standards using dilution with sterile MHB and measuring the optical density t at 625 nm wavelengths. Absorbance readings were fixed within the range of 0.08 - 0.13 (equivalent to approximately 1.5 x $10^{8}$ CFU/mL). Standardized broth inoculums were lawned onto sterile Muller Hinton Agar by swabbing with sterile cotton swabs and allowed to sit for 10 minutes. Wells (6 mm in diameter) were bored into the solidified and well-seeded agar equidistant to each other with a cork borer. Streptomycin, a standard antibiotic, was used as the positive control for the test organisms, while 1% DMSO was the negative control. Each plant extract (100 µl) was dispensed into the wells in triplicates. The agar plates were kept at room temperature for 10 min before incubation at 37°C for 18-24 h. The diameter zones of inhibition were measured using a ruler in millimeters, and the average zone was taken.

#### *2.4.1. Minimum Inhibitory Concentration of M. longistyla on the selected Bacterial Isolates*

The minimum inhibitory concentrations (MIC) of the extracts of *M. longistyla* were determined by the double dilution technique (Ochei and Kolhatkar, 2004). Known weights of plant extracts were diluted with physiological saline (0.85%) into four different concentrations (100, 50, 25 and 12.5 mg/ml). One hundred microliters (100 µl) of different dilutions were inoculated (Cheesbrough, 2013), and incubated at 37$^{o}$C for 24 h. After the overnight incubation, the lowest concentrations of the extract that inhibited the organisms' growth were taken as the minimum inhibitory concentration.

### *2.5. Qualitative phytochemical analysis*

Ethanol extracts of the various parts of *M. longistyla* were screened for the presence of bioactive chemicals such as alkaloids using Mayer’s reagents, flavonoids with sodium hydroxide test as described by Trease and Evans (2002). Tannins and saponins were detected using ferric salt and frothing tests, respectively, according to the standard procedures described by Parekh and Chanda (2007) while Steroids, terpenoids, and phlobatannins were screened as documented by Harborne (1998).

### *2.6. Quantitative phytochemicals analysis*

#### *2.6.1. Determination of total phenolic content*

The total phenolic compounds in the various extracts of the plant were determined with Folin-Ciocalteu reagent using the method of Ebrahimzadeh *et al.* (2008). To 0.5 mL of each sample (in triplicates) of plant extract in methanol solution (1 mg/mL), 2.5 mL of 10 % Folin-Ciocalteu reagent and 2 mL of $Na_2CO_3$ (2 % w/v) was added. The mixture was incubated at 50°C for 30 min, and the absorbance was measured at 765 nm using a U.V./visible spectrophotometer. Concentrations of the phenolic compounds in the extracts were extrapolated from a calibration curve of Gallic acid. Results were expressed as mg Gallic acid equivalent/ g of extract.

#### *2.6.2. Determination of total flavonoids content*

Total flavonoids content was determined using the colorimetric method of Singleton and Rossi (1965) to extract and estimate flavonoids with some modifications. Flavonoids react with vanillin to produce a colored product, which can be measured spectrophotometrically. According to the procedure, 250 µl of the extract was added to 1.25 ml of distilled water and 75 µl of 5% $NaNO_2$. After 5 min, 150 µl of 10% $AlCl_3.H_2O$ was added, followed by 500 µl of 1 M NaOH and 275 µl of distilled water after 6 min. The solution was adequately mixed, and the color intensity of the mixture read at 510 nm. The standard used was Gallic acid.

### *2.6.3. Estimation of antioxidant activity*

The antioxidant activity was measured using the DPPH assay. This spectrophotometric assay uses the stable radical 1, 1-diphenyl-1- picrylhydrazyl (DPPH) as a reagent (Amarowicz *et al.* 2004). The DPPH free radical is commercially available, and it was prepared at a 0.1 mM concentration (25 mg/L) in methanol (Sánchez-Moreno *et al.* 1998; Larrauri *et al.* 1999; Sasidharan *et al.* 2011). The absorbance at 518 nm was monitored in the presence of different concentrations of extracts. The blank experiment was carried out to determine the absorbance of DPPH before interacting.

### *2.6.4. Proteinase inhibition assay*

The method of Kunitz (1947) was used for the proteinase inhibition study. One mL aliquot of trypsin [EC 3.4.21.4, SRL, India (1000 units/mg)] (0.5 mg/mL prepared in 0.1 M phosphate buffer pH 7) was pre-incubated with 1 mL of varied concentration of the plant extracts and aspirin (standard) at 37° C for 15 min. To the mixture, 2 mL of 1% casein (S.R.L., India) (prepared in 0.1 M phosphate buffer) was added and incubated at 37° C for 30 min. The reaction was terminated with 2.5 mL of 0.44 M trichloroacetic acid (TCA) solution. The reaction mixture was centrifuged to remove the precipitated protein at 10,000 rpm for 15 min (Eppendorf, Germany). The absorbance of the clear supernatant was measured at 280 nm in a UV–Visible spectrophotometer (Shimadzu, Japan) against appropriate blanks. The formula below gave percentage inhibition:

$$\%\,Inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} X\ 100\%$$

### *2.7. GC-MS Analysis*

**Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Crude Extracts of *M. longistyla***

GC-MS analysis was carried out on the extracts according to the protocol previously described by Odumosu *et al.* (2017) using a 7890A Gas Chromatography system (Agilent Technology HP5MS), with a mass spectrometer (5975C VLMSD) as a detector. The column has a length of 30 m, the internal diameter of 0.32 mm, and thickness of 0.25 µm; volume injected was 1 µL, and the injector temperature was 250°C, Helium was the carrier gas, and oven temperature initially programmed at 80°C for 2 min, increased at 10°C per min to 240°C, and held for 6 min. Interpretation of the mass spectrum of GC-MS was conducted using the National Institute Standard and Technology (NIST) database. Unknown spectra were compared with those of the known ones stored in the NIST library.

## 3. Results and Discussions

Qualitative phytochemical screening of the ethanolic extract of *M. longistyla* revealed that the leaf extract contained almost all the tested phytochemicals except alkaloids. The stem bark had only alkaloids, flavonoids, saponins, steroids, and tannins. In contrast, the root contained flavonoids, saponins, steroids, and carbohydrates (Table 1). Some of these compounds are known to possess antimicrobial activities.

**Table 1.** Phytochemical content of ethanolic extracts of *Macrosphyra longistyla*

| Group | Plant parts | | |
|---|---|---|---|
| | Stem bark | Leaf | Root |
| Alkaloids | + | - | - |
| Flavonoids | + | + | + |
| Saponins | + | + | + |
| Steroids | + | + | + |
| Tannins | + | + | - |
| Terpenoids | - | + | - |
| Phobatannin | - | + | - |
| Carbohydrate | - | + | + |
| Amino acids and proteins | - | + | - |

The quantitative phytochemical contents (total polyphenols and total flavonoids) of the various parts of *Macrosphyra longistyla* are shown in Table 2. The leaves presented the highest content of total polyphenols (226.69 ± 4.53 mg/g gallic acid equivalents (GAE). Although the root extract has the lowest content of total polyphenols, it demonstrated high flavonoid content (254.25 ± 4.75).

**Table 2.** Total phenols, total flavonoids and DPPH scavenging activities of extracts

| Plant extract | Total polyphenols (G.A.E./g extract) | Total flavonoids (G.A.E./g extract) | DPPH Assay (IC50 (µg/mL) |
|---|---|---|---|
| GA.□ | - | - | 16.78± 1.14[a] |
| MLS | 226.60 ± 4.53 | 180.29 ± 5.61 | 43.33± 1.83[b] |
| MLL | 271.27 ± 19.88 | 287.10 ± 5.83 | 73.07± 2.86[c] |
| MLR | 115.13 ± 13.74 | 254.25 ± 4.75 | 114.87± 3.13[d] |

The DPPH assay is a spectrophotometric measurement of the color changes, from violet to yellow, when an antioxidant substance scavenges the radical, reducing it to hydrazine. The ML leaf extract showed relatively high antioxidant capacity with an IC50 of 43.33 ± 1.83µg/mL while the standard had an IC50 of 16.78 ±1.14 µg/mL (Figure 1 and Table 2). The ML root and stem fractions showed high IC50 values comparable to those of plants with low antioxidant activities. These results agreed with the content of polyphenols found in the fractions (MLL> MLR> MLS) and were significantly different from those of the total flavonoids; thus, it is impeccable to attribute the antioxidant capacity of the various ML parts to the polyphenol and/or flavonoid contents. This relationship has been described by many authors using various plants species (Schubert *et al.* 2007; Zadra *et al.* 2012) (Fig. 1).

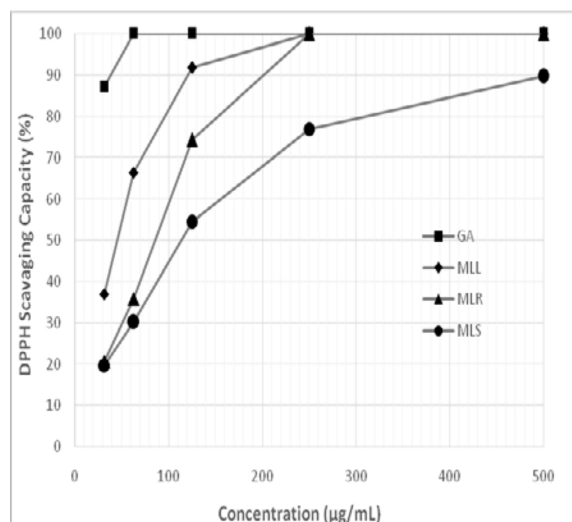


**Figure 1.** Antioxidant capacity of methanol extracts of various parts of M. longistyla using DPPH assay (n=3)

Figure 2 shows the reducing power potentials of the ethanol extracts of ML compared to the gallic acid standard at 700 nm. The reducing capacity of the extracts, a significant indicator of antioxidant activity, was found to be appreciable. The results showed that there were increases in the reductive capability of the extracts from ML. The leaf (MLL), and stem extracts (MLS) showed a comparable high reducing ability of 1.49 and 1.46 at 0.2 mg/mL when compared with the ascorbate control (1.67). This reducing activity is higher than that reported by Atolani *et al.* (2011), who worked on *Kigelia pinnata*, even at a high concentration of 0.4 mg/mL. However, the findings are in tandem with recent works of Kudumula *et al.* (2018), who did an extensive survey on selected medicinal plants used in the treatment of bacterial infections in South Africa (Fig. 2).

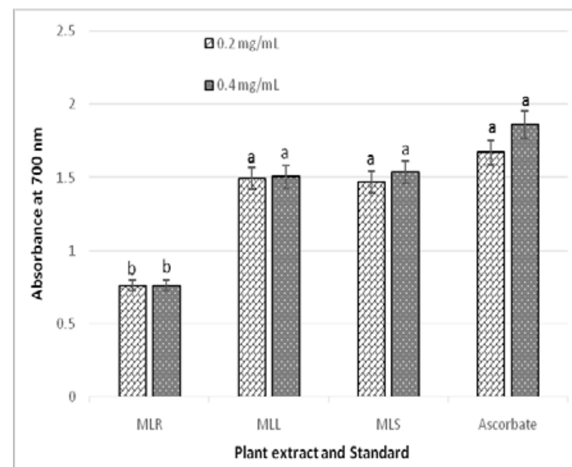


**Figure 2**. The reducing power potential of the selected plant extracts and ascorbic acid, with absorbance increasing with increasing concentration (values with different alphabets are significantly different at p =0.05, t-test).

Similarly, the extracts demonstrated high proteinase inhibition potential competing effectively with the Aspirin standard, as shown by their inhibition effect on trypsin (Figure 3). Trypsin inhibitors are serine based inhibitors and are of high pharmaceutical importance (Bejina *et al*. 2011). Proteinase inhibitors are generally good anti-inflammatory drugs, suggesting that ML will be a good candidate for drug development and direct consumption for locals as a pain-killer.

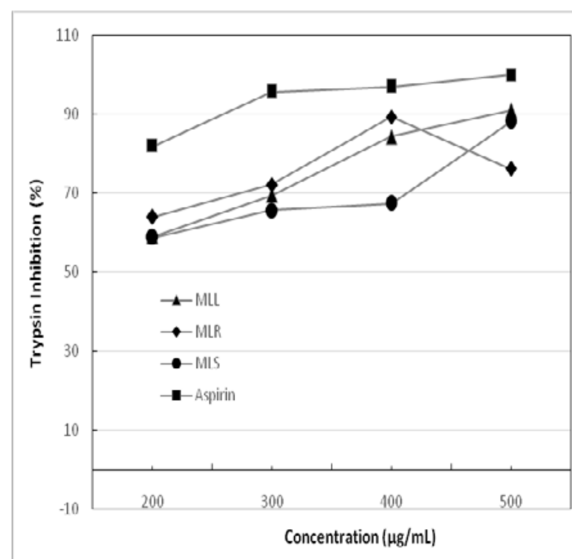


**Figure 3.** Trypsin inhibitory activities of extracts from various parts of Macrosphyra longistyla

The antibacterial activities of the crude extracts of stem-bark, leaves, and roots of *M. longistyla* showed zones of inhibition in millimetres against the clinical isolates ranging from 10 to 40 mm (Table 3) with minimum inhibitory concentration (MIC) ranging from 25 – 100 mg/ml (Table 4). GC- MS analyses showed a significant presence of bioactive compounds from ethanolic crude extracts and their characteristics (Tables 5 and 6). The chromatograms of the ethanolic extracts of the root and stem are shown in Figures 4 and 5, respectively.

**Table 3.** Antibacterial Activity of Extracts of *Macrosphyra longistyla* against Bacterial Isolates

| Bacterial Isolates | The diameter of the zone of inhibition in millimeter (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J |
| *MRSA (144m)* | - | - | - | 40 | 35 | 26 | - | 14 | 23 | 27 |
| *E. coli ATCC11229* | - | - | - | 27 | 23 | 33 | 22 | 15 | 24 | 11 |
| *S. typhimurium ATCC13311* | - | - | - | - | 39 | - | 32 | 31 | - | 15 |
| *E. faecium ATCC700221* | - | - | - | 24 | 36 | - | 19 | 26 | 20 | 12 |
| *S. flexneri ATCC12012* | - | - | - | 12 | 39 | - | - | 17 | - | 10 |
| *S. mutans* | - | - | - | 19 | 32 | 18 | - | 19 | 15 | - |

Zone of inhibition included 8mm cork-borer, - resistance, **A**=Aqueous stem extract, **B**=Aqueous leaf extract, **C**=Aqueous root extract, **D**=Ethanol stem extract, **E**=Ethanol leaf extract, **F**=Ethanol root extract, **G**=Ethyl-acetate stem extract, **H**=Ethyl-acetate leaf extract, **I**=Ethyl-acetate root extract, **J**=Streptomycin (10μg).

**Table 4.** Minimum Inhibitory Concentration of Extracts of *Macrosphyra longistyla* against Bacterial Isolates

| Bacterial Isolates | Minimum inhibitory concentration (MIC) in mg/ml | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I |
| *MRSA (144m)* | - | - | - | 25 | 25 | 50 | - | 50 | 50 |
| *E. coli ATCC11229* | - | - | - | 25 | 100 | 50 | 25 | 25 | 25 |
| *S. typhimurium ATCC13311* | - | - | - | - | 50 | - | 25 | 50 | - |
| *E. faecium ATCC700221* | - | - | - | 100 | 50 | - | 50 | 25 | 100 |
| *S. flexneri ATCC12012* | - | - | - | 100 | 50 | - | - | 100 | - |
| *S. mutans* | - | - | - | 25 | 25 | 50 | - | 100 | 100 |

Zone of inhibition included 8mm cork-borer, - resistance, **A**=Aqueous stem extract, **B**=Aqueous leaf extract, **C**=Aqueous root extract, **D**=Ethanol stem extract, **E**=Ethanol leaf extract, **F**=Ethanol root extract, **G**=Ethyl-acetate stem extract, **H**=Ethyl-acetate leaf extract, **I**=Ethyl-acetate root extract, **J**=Streptomycin (10μg).

The ability of the stem, leaf and root extracts of *M. longistyla* in this study to inhibit both Gram-positive and Gram-negative bacteria is an indication of its broad-spectrum activity; hence, a potential source of drugs for the treatment of dental caries, diarrhoea, skin and wound infections, typhoid and non-typhoid fever, gastroenteritis, urethritis, otitis media, septicemia, osteomyelitis and any other infections caused by these pathogenic bacterial strains. Aqueous extracts could not inhibit any of the isolates used, whereas ethanol extracts of stem-D inhibited *MRSA* (40), *E. coli* (27), *E. faecium* (24), *S. flexneri* (12) and *S. typhimurium* (R); leaf-E all tested organisms (23-39) and root-F *MRSA* (26), *E. coli* (33), *S. mutans* (15), *S. typhimurium* (R), *S. flexneri* (R) and *E. faecium* (R) millimetre zones of inhibitions. However, ethyl acetate extracts of stem-G inhibited *E. coli* (22), *S. typhimurium* (32), *E. faecium* (19), others resistance (R); Leaf-H all isolates (14-31) and root-I *MRSA* (23), *E. coli* (24), *E. faecium* (20), *S. mutans* (15), others resistance (R). Streptomycin-J inhibited all except *S. mutans* between 10-27 millimetre diameters. Results showed that the leaf extract of *M. longistyla* was more potent than extracts of other parts of the plant. Hence, *M. longistyla* leaf contains more active ingredients than the stem and root (Table 1). The inability of aqueous extracts to inhibit the growth of the bacterial strains may be due to its low extraction ability. The findings agreed with Ogah and Osundare's (2015) report, which observed that aqueous extract showed little or no activity against tested strains, suggesting that water may have a low penetration and extraction ability compared to organic solvents. Other researchers had also supported this view that organic solvent extracts exhibited higher antibacterial activity than aqueous extracts. The antibacterial principles may be either polar or non-polar since extraction can be with organic solvents or aqueous media (Britto, 2001). Odeleye *et al*. (2016) reported that aqueous extracts' poor inhibition ability might be due to the weak solubility nature of the plants' active components in water.

Minimum inhibitory concentration (MIC) of the plant extracts (Table 4) showed the following range against *MRSA* (25-50), *E. coli* (25-100), *S. typhimurium* (25-50), *S. flexneri* (25-100) and *S. mutans* (25-100) milligram per millimetre. The MICs implied that the extracts had some substantial effects on the test organisms.

The result of the GC-MS analysis revealed certain bioactive compounds such as fatty acids, amino acids, vitamin E, and their derivatives, which were shown to have medicinal properties. It showed that isophorone is prevalent in the three parts of the plant studied; it is the most prevalent in the MLL and MLR, and the second most prevalent in MLS (Table 5). Besides, the isophorone, which is common to the three parts, the leaf (MLL) has 1-methoxymethoxy-oct-2-yne, 7-chloro-3-heptyne, 11-dodecyn-1-ol acetate as prominent components. The root (MLR) also presented linoleic acid ethyl ester, 3-eicosene, octadecanoic, n-hexadecanoic acid, and their ester derivatives as prominent components. The stem (MLS), however, has 5H,6H,7H-cyclopenta[d]pyrimidin-2-amine as the most prominent compound alongside hexadecanoic ethyl ester, Vitamin E and Squalene. Other bioactive components identified include 3-ethyl-1-pyrroline and morpholines.

**Table 5.** Bioactive Compounds identified in the *Macrosphyra longistyla* ethanol extract of the leaf (MLL), root (MLR) and stem (MLS) and their characteristics

| Peak # | RT (Min) | Compound name | Molecular formulae | Molecular Weight ($gmol^{-1}$) | % Peak Area |
|---|---|---|---|---|---|
| MLL | | | | | |
| 1 | 4.191 | Aziridine, 1-(2-buten-1-yl)-, (Z)- | $C_6H_{11}N$ | 97.16 | 2.76 |
| 2 | 4.775 | 3-Methyl-3-hexene | $C_7H_{14}$ | 98.19 | 3.16 |
| 3 | 5.204 | Propanenitrile, 3-amino-2,3-dihydroxymino)- | $C_3H_4N_4O_2$ | 128.09 | 2.32 |
| 4 | 8.351 | Isophorone* | $C_9H_{14}0$ | 138.21 | 33.54 |
| 5 | 13.043 | 4-Cyclopentene-1,3-diol, trans- | $C_5H_8O_2$ | 100.12 | 1.06 |
| 6 | 13.861 | 2-Azatricyclo[4.3.1.1(4,8)]undecane | $C_{10}H_{17}N$ | 151.25 | 1.20 |
| 7 | 14.805 | Benzaldehyde, 2-nitroso- | $C_7H_5NO_2$ | 135.12 | 1.58 |
| 8 | 15.486 | Octadecane, 1-(ethenyloxy)- | $C_{20}H_{40}O$ | 296.50 | 1.21 |
| 9 | 16.127 | Oxirane, 2,2'-(1,4-butanediyl)bis- | $C_8H_{14}O_2$ | 142.20 | 1.15 |
| 10 | 17.684 | 2-Hexyn-1-ol | $C_6H_{10}O$ | 98.14 | 1.46 |
| 11 | 18.313 | 3-Heptyne, 7-chloro- | $C_7H_{11}CL$ | 130.61 | 9.10 |
| 12 | 21.672 | Paromomycin | $C_{23}H_{45}N_5O_{14}$ | 615.60 | 1.45 |
| 13 | 22.427 | 1-Pyrroline, 3-ethyl- | $C_{16}H_{15}N$ | 97.16 | 1.56 |
| 14 | 22.707 | 7-Oxabicyclo[4.1.0]heptane, 1,5-dimethyl-□ | $C_8H_{14}O$ | 126.20 | 2.48 |
| 15 | 22.851 | 11-(2-Cyclopenten-1-yl)undecanoic acid, (+)- | $C_{16}H_{28}O$ | 252.39 | 1.41 |
| 16 | 23.451 | 1-Methoxymethoxy-oct-2-yne** | $C_{10}H_{18}O_2$ | 170.25 | 27.02 |
| 17 | 23.583 | 11-Dodecyn-1-ol acetate | $C_{14}H_{24}O_2$ | 224.34 | 3.85 |
| 18 | 25.105 | 4-Acetoxy-3-methylbut-2-enoic acid, methyl ester □ | $C_8H_{12}O_4$ | 172.18 | 1.07 |
| 19 | 25.397 | 8-Nonynoic acid | $C_9H_{14}O_2$ | 154.21 | 1.08 |
| 20 | 26.318 | Trichloroacetic acid, 2-methyloct- 5-yn-4-yl ester | $C_{11}H_{15}Cl_3O_2$ | 285.60 | 1.54 |
| MLR | | | | | |
| 1 | 4.730 | 2(5H)-Furanone, 5-methyl- | C5H6O2 | 98.10 | 2.49 |
| 2 | 5.001 | Morpholine | C4H9NO | 87.12 | 4.05 |
| 3 | 8.268 | Isophorone* | C9H14O | 138.21 | 30.35 |
| 4 | 18.75 | (5-Carbamoyl-2,4-dioxo-3H-pyrimidin-1-yl) acetic acid | C7H7N3O5 | 213.15 | 0.40 |
| 5 | 19.562 | Pentadecanoic acid, 14-methyl | C16H32O2 | 256.42 | 2.29 |
| 6 | 20.493 | n-Hexadecanoic acid | C16H32O2 | 256.42 | 10.24 |
| 7 | 20.629 | Octadecanoic acid | C18H36O2 | 284.48 | 10.90 |
| 8 | 23.452 | Linoleic acid ethyl ester ** | C20H36O2 | 308.50 | 17.12 |
| 9 | 23.522 | 3-Eicosene, (E)- | C20H40 | 280.53 | 15.15 |
| 10 | 23.918 | Octadecanoic acid, ethyl ester | C20H40O2 | 312.53 | 3.729 |
| 11 | 28.651 | Benzene, 1-isothiocyanato-2-methyl | C8H7NS | 149.21 | 1.02 |
| 12 | 30.856 | 2-Buten-1-ol, (E)-, TBDMS derivative | C10H22OSi | 186.37 | 0.21 |
| 13 | 31.056 | 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl | C15H260 | 222.37 | 2.05 |
| MLS | | | | | |
| 1 | 5.078 | Propanoic acid, 2-methylpropyl ester | $C_7H_{14}O_2$ | 130.18 | 1.26 |
| 2 | 8.271 | Isophorone** | $C_9H_{14}O$ | 138.21 | 18.37 |
| 3 | 18.118 | Cyclohexanone, 2,2-dimethyl-5-(ethyloxiranyl) | $C_{12}H_{20}O_2$ | 196.29 | 1.24 |
| 4 | 18.49 | 5H,6H,7H-Cyclopenta[d]pyrimidin-2-amine* | $C_7H_9N3$ | 135.17 | 30.93 |
| 5 | 20.625 | Hexadecanoic acid, ethyl ester | $C_{18}H_{36}O_2$ | 284.48 | 16.46 |
| 6 | 23.451 | Linoleic acid ethyl ester | $C_{20}H_{36}O_2$ | 308.50 | 12.64 |
| 7 | 31.044 | Squalene | $C_{30}H_{50}$ | 410.72 | 4.90 |
| 8 | 33.585 | Vitamin E | $C_{29}H_{50}O_2$ | 430.71 | 14.20 |

***** most prevalent phytochemical in each plant's part

** the second most prevalent

Isophorone, the phytochemical, which is common to the three parts, is a well-known plant metabolite that has been used as an industrial solvent. It is used as a carrier of pesticide for plants, and when partly hydrogenated, it forms a derivative, trimethylcyclohexanone, a common raw material for polycarbonates production. Polycarbonate can further be reacted with phenol to give an analogue of bisphenol A with potent antimicrobial properties; the antimicrobial is usually used to disinfect skin or wound. The high level of this solvent may suggest exogenous sources from pesticide applications.

The long-chain fatty acids, which are the major components of the root extracts, linoleic acid ethyl ester and octadecanoic acid, are well known for their nutritional values. Besides, octadecanoate is a potent antifungal and antibacterial compound; the antioxidant, hypo-cholesterolemic, and nematicide, activities of n-hexadecanoic acid is also well known (Elaiyaraja and

Chandramohan, 2018). The stem, however, has 5H,6H,7H-cyclopenta[d]pyrimidin-2-amine as its prominent compound; pyrimidin-2-amine is presently being considered as an anticancer drugs and H3 receptor antagonist (Tadesse et al. 2018; Wagner et al. 2019). H3 receptor antagonists are principal in allergy prevention and autoimmune response suppression. The presence of vitamin E and squalene in stem bark collaborated the presence of flavonoid in the previous analysis, so did the lowest DPPH scavenging IC50 recorded by MLS extract in that study. Squalene has a role in topical skin lubrication and protection against pathogens (Pappas, 2009); in addition to the aforementioned, vitamin E has been reported to demonstrate antioxidant, anti-aging and anti-inflammatory activities (Saliha *et al.,* 2014). It was also observed that the plant extracts had particular alkyne groups such as 1-methoxymethoxy-oct-2-yne, 7-chloro-3-heptyne, and 11-dodecyn-1-ol acetate and some bioactive ring structure (Figure 4). These alkyne groups are known with rare bioactivities such as antiprotozoal and nematocidal activities (Jorgensen et al., 1996 ).

The presence of a 3-ethyl-1-pyrroline suggests that the compound could be responsible for the scent (aroma) of the plant leaf. A similar compound, 2-acetyl-1-pyrroline, has been implicated in aromatic rice and other plants (Routray and Rayaguru, 2018). Also, propanenitrile, 3-amino-2,3-di (hydroxymino)- has been reported in the volatile compounds identified from root exudates of chilli seedling primed with 6% *Bacillus amyloliquefaciens* (Sathya et al. 2016). Morpholines are widely used in organic synthesis. They serve as -building blocks in the preparation of the antibiotic linezolid, the anticancer agent gefitinib (Iressa), and the analgesic dextromoramide (Wikipedia, 2019). Recently, some studies have shown that morpholines derivative, 1-[4-(morpholin-4-yl) phenyl]-5-phenylpenta-2,4- dien-1- one, is a potent monoamine oxidase inhibitor (Maliyakkal et al. 2020). Monoamine oxidase inhibitors are effective antidepressant drugs that have found usage in social phobia and panic disorder.

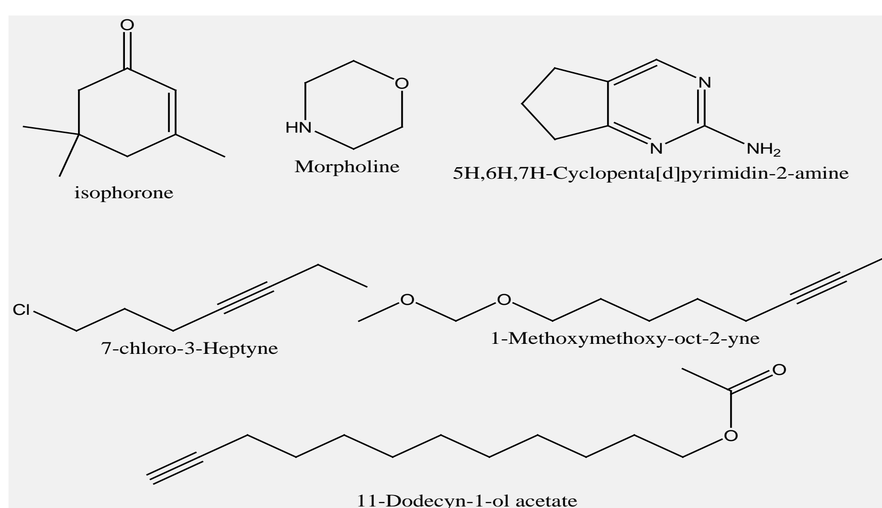


**Figure 4.** Prominent bioactive compounds and rare alkyne groups identified from the GCMS analysis of *Macrosphyra longistyla* extracts

## 4. Conclusion

The presence of arrays of phytochemicals or bioactive ingredients such as alkaloids, saponins, tannins, flavonoids, phlobatanins, isophorone, squalene, morpholine, vitamin E and host of others revealed in this study, which were implicated in the treatment of certain ailments, has provided a scientific justification for the use of *Macrophyla longistyla* as an alternative remedy for the treatment of bacterial infections, hence acting as a potential source of drugs with broad-spectrum activity. Furthermore, the GCMS identified plants alkynes, and pyrimidin-2-amine suggested the possible anticancer and antihistamine potentials of the plant. These, however, need to be verified through further studies. In the meantime, -studies are also ongoing on the toxicity, pharmacological evaluation, and structural elucidation of the plant's active principles since this will enhance the plant materials' potency at lower concentrations.

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