ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF SOME NIGERIAN GREEN LEAFY VEGETABLES

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Abstract
This study evaluated the antioxidant and antimicrobial properties of Piper guineense, Ocimum gratissimum, Murraya koenigii and Gongronema latifolium leave extracts using ethanol, petroleum-ether and aqueous media. The scavenging activities of the extracts was measured with free radical 2, 2-diphenyl-1-picrylhydrazyl, flavonoid and phenolic contents were assessed by spectrophotometry while, gas chromatographic flame ionization detector was used for phytochemical screening of the extracts. The micro-broth dilution and agar diffusion methods were used to assess Minimum inhibitory and bactericidal concentrations of the extracts respectively. The different leaves extracts differed significantly (P<0.05) at 25, 50, 75 and 100µg/ml of radical scavenging activities, with the lowest and highest values recorded at 25 and 100µg/ml respectively. The aqueous extract of G. latifolium had the highest value (67.60 ± 0.12mg GAE/100g DW) while, petroleum ether extract of P. guineense had the lowest value (30.65 ± 0.06mg GAE/100g DW) of total phenolic content. The highest and lowest flavonoid contents were found in petroleum ether extract of P. guineense (80.75 ± 0.37mg Rutin/g DW) and in aqueous extract of M. koenigii (36.27 ± 0.62mg Rutin/g DW). Phytochemicals screening indicated that the extracts were rich in steroids, terpenoids, tannins, saponins, phlobatannins, cardiac glycosides and alkaloids. The petroleum ether, ethanol and aqueous extracts were active against the growth of S. epidermidis, P. aeruginosa, K. pneumonia, E. coli, S. aureus, and S. typhimurium. This study proved that all crude extracts showed robust antioxidant and antimicrobial potentials which, could be harnessed for both their health and nutritional benefits in fisheries.

Key words: Antimicrobial, antioxidant; P. guineense, O. gratissimum, M. koenigii, G. latifolium

Introduction
Nigeria is richly endowed with indigenous plant species and over the years, several of these native herbaceous species have been consumed raw or cooked in many Nigerian homes as vegetables (Dhellot et al., 2006). They are rich, cheap and common sources of nutrients especially in rural communities where they add considerably to protein, minerals, vitamins, and fibres in addition to other nutrients that are deficient in

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daily diets (Mohammed and Sharif, 2011). Several of these vegetables, particularly the green leafy vegetables are mainly known for their nutritional content, without much attention channelled towards their medicinal importance. Apart from the variety which they add to the menu and their nutritional intake, they have the capacity to synthesize several ancillary metabolites of relatively complex structures possessing antioxidants and other health benefits (Yadav et al., 2013). The attention in the medicinal and nutritional properties of phytochemicals in foods and vegetables has increased over the years. This was as a result of the specific physiological roles they play on human health and other organisms (Wei and Shiow, 2001). Generally, antioxidants are substances that inhibit the oxidation course by reacting with free radicals, chelating catalytic metals and scavenge oxygen thereby shielding cells from oxidative impairment (Shahidi and Wanasundara, 1992). Escalation in formation on the free radicals has been linked with several diseases related to human and resulting in tissue damage (Bridges et al., 1993). Several reports have proven an inverse relationship between the dietary ingestion of antioxidant rich foods such as vegetables and fruits and the incidence of numerous chronic human diseases (Devasagayam et al., 2004; Kyro et al., 2013; Wang et al., 2013; Kruk, 2014).

Many vegetables such as Murraya koenigii, Gongronema latifolium, Piper guineense and Ocimum gratissimum were found to contain health protective constituents that avert diseases and sustain a state of well-being (Apple et al., 1997). Phyto-constituents present in plants are organic compounds of great structural variety that can act as chemotherapeutic, bacteriostatic, bactericidal and antimicrobial agent (Purohit and Mathur, 1999). Murraya koenigii (Rutaceae), commonly called curry leaf, represents more than 150 genera and 1600 species (Satyavati et al., 1987). It is widely distributed in both sub-tropical and tropical countries. The leaves are bi-pinnately compound, about 15-30cm long. The leaves are highly valued for their characteristic flavour and are used in folk medicine for the treatment of gastrointestinal disturbances such as vomiting, bites of poisonous animals, night blindness, bruises and eruption (Kirtikar and Basu, 1993).

Gongronema latifolium (Benth) is a tropical rainforest plant which belongs to the family Asclepiadaceae. It is commonly known as “utazi” and “arokeke” in South Eastern and South Western, Nigeria respectively (Ugochukwu and Babady, 2002; Chinedu et al., 2013). The plant leaf has a bitter taste and is consumed fresh, cooked or dried and also applied as a powdery spice in dishes. Apart from its nutritional value, it is locally use for the treatment of various ailments such as malaria, diabetes and hypertension, among others (Enemor et al., 2014).

Piper guineense belongs to the family Piperaceae, commonly referred to as black pepper or Ashanti pepper. It is widely distributed throughout the tropical and sub-tropical regions of the world. In Nigeria, the Igbo and Yoruba call it “uziza” and “Iyere” respectively (Isawumi, 1984). In a bid to contract the uterus, Piper guineense is used by the locals as a medicinal spice in the diet of
pregnant and lactating mothers and among post-partum women (Achinewhu et al., 1995). It is generally known to possess antioxidant and antibacterial properties (Iwu, 1993).

*Ocimum gratissimum* (Lamiaceae) is widely distributed in warm temperate and tropical parts of the world including West Africa. In Nigeria, it is called by different names among diverse ethnic groups, the Igbo (Ahuji), Yoruba (Effinrin-nla) and Hausa (Daidoya) (Dada et al., 2013). The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, ophthalmic, skin diseases, pneumonia, and also as a remedy for cough, fever, and conjunctivitis (Onajobi, 1986).

In order to provide additional information, on the health benefits of the utilization of these vegetables; the current study examines the antioxidant and antimicrobial properties of leaves extracts of the four Nigerian green leafy vegetables; *Ocimum gratissimum*, *Piper guineense*, *Murraya koenigii* and *Gongronema latifolium*.

**Materials and Methods**

**Collection of Plant Materials**

Fresh leaves of *Piper guineense*, *Ocimum gratissimum*, *Murraya koenigii* and *Gongronema latifolium*, were bought at Oyingbo local food market, Lagos, Nigeria. The samples were identified and authenticated at the Department of Botany Herbarium, University of Lagos, Nigeria.

**Preparation of Samples**

The vegetables were picked to remove debris, thoroughly rinsed with clean water and evenly spread on a mosquito net-size mesh to air dry at room temperature. After complete dryness, the leaves were grinded into uniform powder to increase their total surface area for the extraction of crude leaves extracts.

**Aqueous Extraction**

20g of air dried leaf was placed in a conical flask with 200ml hot distilled water, shaken on an orbital shaker (200rpm) for 72h and filtered (Ifesan et al., 2009). Residue was re-extracted twice, each filtrate was evaporated to dryness in a water bath at 50°C and extract was kept in a stoppered bottle at 4°C.

**Ethanol Extraction**

20g of air dried leaf was placed in a conical flask with 200ml ethanol (95%), shaken on an orbital shaker (200rpm) for 72h and filtered (Ifesan et al., 2009). Residue was re-extracted twice, each filtrate was evaporated to dryness in a water bath at 45°C and extract was kept in a stoppered bottle at 4°C.

**Petroleum Ether Extraction**

20g of air dried leaf was placed in a conical flask with 200ml Petroleum ether, shaken on an orbital shaker (200rpm) for 72h and filtered (Ifesan et al., 2009). Residue was re-extracted twice, each filtrate was evaporated to dryness in a water bath at 4°C and extract was kept in a stoppered bottle at 4°C.

**Measurement of Antioxidant Activity**

**Free Radical Scavenging Ability**

The solution was prepared by dissolving 24mg DPPH (2, 2-diphenyl-1-picrylhydrazyl) with 100ml methanol and refrigerated at 20°C. 3ml aliquot of the solution was mixed with 100μl of the sample at 25, 50, 75 and 100μg/ml. Discolouration was determined at 517nm after 30min incubation in the dark. Scavenging effect (%) = (control absorbance – sample absorbance)/ control absorbance.
absorbance x 100 (Brand-Williams et al., 1995; Bursal and Gulcin, 2011).

**Total Phenol Content**

1mg/ml was mixed with 1ml folin-ciocalteu’s phenol reagent. After 5min, 10ml 7% Na$_2$CO$_3$ solution was added, followed by 13ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90min at 23°C, after which the absorbance was read at 750nm. The result was expressed as mg of gallic acid equivalent/100g of dry extract (kim et al., 2003).

**Total Flavonoid Content**

0.3ml leaf extract, 3.4ml 30% methanol, 0.15ml NaNO$_2$ (0.5M) and 0.15ml AlCl$_3$.6H$_2$O (0.3M) were put in a 10ml test tube and mixed. After 5min, 1ml NaOH was added and mixed. The absorbance was determined against the reagent blank at 506nm. The total flavonoid content was calculated using a calibration curve of Rutin ($R^2 = 0.985$). The result was expressed as the Rutin equivalent/gm of dry weight of extract (Park et al. (2008)).

**Phytochemical Screening**

The screening of leaves extracts for Phytochemicals was investigated with the gas chromatographic flame ionization detector, for the investigation of Terpenoids, Steroids, Tannins, Saponins, Alkaloids, Cardiac glycosides, Anthraquinone, and Phlobatannin presence in different leaves extracts.

**Antimicrobial Assay**

**Microbial Strain and Growth Media**

The ethanol, aqueous and petroleum ether extracts of the leaves were individually tested against a range of six microorganisms. The microorganisms’ stock cultures which include *Pseudomonas aeruginosa* (ATCC 15442), *Klebsiella pneumonia* (ATCC 8309), *Staphylococcus aureus* (ATCC 700699), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 35218) and *Salmonella typhimurium* (ATCC 13311) were obtained from Department of Microbiology, University of Lagos. The microorganisms were maintained on nutrient agar slant (4°C) with sub-culturing done every two months. Prior to use for in vitro antimicrobial assay, a loop of each microbial culture on slant was placed in Mueller Hinton broth (5 ml) and grown at 37°C for 6h. The turbidity of the resulting broth culture was adjusted to 0.5 McFarland standards to give a cell density of $1.5 \times 10^8$cfu/mL and labelled as the standard inoculum.

**Screening of Leaves Extracts Using Disc Diffusion Technique**

The antibacterial screening of the extract was evaluated by establishing the zone of inhibition using disc diffusion method (Sahoo et al., 2006). The extracts were dissolved in sterile water to give a concentration of 0.5, 1.0, 1.5, 2.0 and 2.5mg/mL. The extracts were tested individually against six pathogenic bacteria strains of Gram-positive and Gram-negative bacteria using the pour-plate diffusion method. Each standard inoculum suspension of bacterial strain was plotted on the surface of the Mueller-Hinton agar (MHA) using a sterile cotton swab. 20μL of each extract was placed on the surface of sterile filter paper disc (6 mm) that was subsequently incubated at 4°C for 15min. Each extract disc was aseptically placed on MHA surface by pressing slightly. The plates were left at ambient temperature for 15min to allow excess diffusion of extracts prior to incubation at 37°C for 24h. The diameter of inhibition zone was measured and
expressed in millimetres. Each experiment was done in triplicate

**Estimation of Minimum Inhibitory Concentration (MIC)**

The MIC was the least concentration of the extract that produced no turbidity after 24h of incubation (Ezekwesili and Nwodo, 2013). The MIC of each leaf extract identified in the assay was estimated by micro-broth dilution method (Andrews, 2001). Serial dilutions of each extract at different concentrations, 0.5–32.0mg/mL in sterile Mueller-Hinton broth were dispensed into the wells of 96-well micro titre plate (50μL per well). This was followed by the addition of equal volume of each test microorganism at 10⁶ CFU/mL into the wells. The plate was covered with aluminium foil and incubated at 37°C for 24h. Results were compared with standard antibiotic ciprofloxacin (10μg/disc) from Oxford (UK).

**Estimation of Minimum Bactericidal Concentration (MBC)**

The MBC was regarded as the lowest concentration of the extract that produced no colonies of the test organisms after 24h of incubation (Ezekwesili and Nwodo, 2013). The MBC of each active or very active medicinal plant was investigated by the agar diffusion method. About 10μL aliquot of test bacteria culture was taken, from the micro titre plates used for the MIC assays. Each aliquot was used in the surface inoculation of Mueller- Hinton agar plate, which was incubated at 37°C for 24h.

**Statistical Analysis**

The data were analysed with statistical package SPSS version 22, using one way ANOVA and Duncan’s multiple range test was used to determine the significant differences between the means at 5% probability level and the results expressed as means ± standard deviation.

**Results and Discussion**

**DPPH Free Radical Scavenging Activity**

The radical scavenging capacity of each extract at different concentrations was measured using the DPPH radical assay (Table 1). The results were expressed as percentage of inhibition of DPPH free radicals in comparison to Ascorbic acid (standard control). The different leaves extracts recorded significant differences (P < 0.05) at 25, 50, 75 and 100μg/ml of radical scavenging activities. The ascorbic acid had the highest value (57.67 ± 0.44) while the aqueous extract of *O. gratissimum* recorded the lowest value (23.96 ± 1.86) at 25μg/ml. Petroleum ether extract of *G. latifolium* showed the highest scavenging effect (68.82 ± 1.10) while, petroleum ether extract of *M. koenigii* had the least scavenging effect (33.46 ± 0.55) at 50ug/ml. Also, at 75ug/ml, petroleum ether extract of *P. guineense* had the highest DPPH inhibition value (85.45 ± 0.64) whilst aqueous extract of *O. gratissimum* (48.54 ± 1.49) recorded the lowest value.

At 100μg/ml, the highest (96.96 ± 0.12) and lowest (60.86 ± 0.31) reducing power of plant extracts were recorded with ether and aqueous extracts of *O. gratissimum* respectively. In addition, the Petroleum ether extracts of *G. latifolium* (87.83 ± 0.37), *P. guineense* (90.48 ± 0.40) were more than the control (77.65 ± 0.22).

The above results showed that, the inhibition trend of DPPH radical by
extracts was concentration dependent, with the highest value at 100µg/ml concentration in all the plant extracts. These results were buttressed by the work of Alothman et al. (2009) who reported that the polarity of solvents can raise the solubility of antioxidant compounds. Although, all tested plant extracts possessed antioxidant activities, the petroleum ether extract of *O. gratissimum* recorded the highest reducing power than other plant extracts and ascorbic acid.

Table 1: Free radical scavenging activities of the various plants extracts (DPPH Inhibition (%))

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Solvents</th>
<th>Concentration (µg/ml)</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. gratissimum</em></td>
<td>P.E</td>
<td>34.33±1.33</td>
<td>55.11±0.89</td>
<td>81.24±1.07</td>
<td>96.96±0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>44.23±0.71</td>
<td>53.42±0.68</td>
<td>64.57±0.43</td>
<td>87.04±0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AQ</td>
<td>23.96±1.86</td>
<td>41.15±0.85</td>
<td>48.54±1.49</td>
<td>60.86±0.31</td>
<td></td>
</tr>
<tr>
<td><em>P. guineense</em></td>
<td>P.E</td>
<td>43.58±0.96</td>
<td>51.19±0.98</td>
<td>85.45±0.64</td>
<td>90.48±0.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>50.17±0.96</td>
<td>60.06±0.99</td>
<td>60.98±0.21</td>
<td>86.48±0.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AQ</td>
<td>44.97±1.02</td>
<td>56.19±1.19</td>
<td>76.13±0.46</td>
<td>81.11±0.73</td>
<td></td>
</tr>
<tr>
<td><em>M. koenigii</em></td>
<td>P.E</td>
<td>28.18±0.96</td>
<td>33.46±0.55</td>
<td>61.85±1.30</td>
<td>88.87±0.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>39.30±1.11</td>
<td>46.89±1.11</td>
<td>74.26±0.42</td>
<td>80.71±0.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AQ</td>
<td>43.33±0.77</td>
<td>54.36±1.62</td>
<td>66.67±1.06</td>
<td>89.67±0.01</td>
<td></td>
</tr>
<tr>
<td><em>G. latifolium</em></td>
<td>P.E</td>
<td>48.50±0.61</td>
<td>68.82±1.10</td>
<td>78.10±0.25</td>
<td>87.83±0.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>47.97±0.99</td>
<td>67.34±0.67</td>
<td>73.21±0.64</td>
<td>77.65±0.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AQ</td>
<td>40.43±1.34</td>
<td>63.05±0.18</td>
<td>63.85±0.30</td>
<td>74.26±0.42</td>
<td></td>
</tr>
<tr>
<td>Abscorbic acid</td>
<td></td>
<td>57.67±0.44</td>
<td>66.52±0.60</td>
<td>74.96±0.68</td>
<td>85.79±0.36</td>
<td></td>
</tr>
</tbody>
</table>

Different letters within the same row indicate significant differences (P < 0.05). Different letters within the same column indicate significant differences (P < 0.05). Data expressed as Mean±SD. P.E: Petroleum Ether; ET: Ethanol; AQ: Aqueous extract.

**Total Phenolic and Flavonoid Contents**

The results in Table 2 showed that the extracts of the four vegetables tested positive for the total phenolic and flavonoid contents which were significantly different (P < 0.05) across media. The aqueous extract of *G. latifolium* had the highest value (67.60 ± 0.12mg GAE/100g DW) of total phenolic content, while petroleum ether extract of *P. guineense* had the lowest value (30.65 ± 0.06mg GAE/100g DW). Also, the amount of flavonoids in the tested plants were significantly different (P < 0.05) and varied from 36.27 to 80.75mg/g Rutin equivalent of the crude extract. The highest flavonoid content was found in petroleum ether extract of *P. guineense* (80.75 ± 0.37mg Rutin/g DW), whilst the lowest amount was obtained in aqueous extract of *M. koenigii* (36.27 ± 0.62mg Rutin/g DW).

Results from the above indicated that different solvents demonstrated varying degree of extraction for different vegetables. Phenols present in medicinal plants have received considerable attention because of their potential antioxidant activity (Djeridane et al., 2006; Zheng et al., 2011). Besides, many studies have shown a direct relationship between antioxidant capacity and total phenolic content (TPC) in spices, medicinal herbs and other dietary plants.
(Al-Mamary et al., 2002). Though, plant extracts used in this study that contained high amounts of TPC were not of relatively high antioxidant activity and this is corroborated by several studies which reported a poor correlation between TPC and antioxidant activity (Capecka et al., 2005; Wong et al., 2006).

Flavonoids carry out biological activities such as extinguishing of active oxygen species, anti-inflammatory and being anti-carcinogenic in various animal models (Leake, 2001; Reddy et al., 2003; Kandaswami et al., 2005). However, the results from this study did not show that an increase in total flavonoids would bring about a corresponding increase in radical scavenging activity of plant extracts as reported by several authors (Heinonen et al., 1998; Anagnostopoulou et al., 2006; Nickavar et al., 2007; Olajire and Azeez, 2011) which indicated that flavonoids did not contribute to antioxidant activity of vegetables.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Solvents</th>
<th>Phenolic content (mg GAE/g DW)</th>
<th>Flavonoids content (mg Rutin/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. gratissimum</em></td>
<td>P.E</td>
<td>45.90±0.15f</td>
<td>46.51±0.46c</td>
</tr>
<tr>
<td></td>
<td>ETH</td>
<td>43.96±0.09e</td>
<td>41.64±0.13b</td>
</tr>
<tr>
<td></td>
<td>AQ</td>
<td>64.18±0.13j</td>
<td>57.12±0.26f</td>
</tr>
<tr>
<td><em>P. guineense</em></td>
<td>P.E</td>
<td>30.65±0.06a</td>
<td>80.75±0.37h</td>
</tr>
<tr>
<td></td>
<td>ETH</td>
<td>32.35±0.10b</td>
<td>48.33±0.44d</td>
</tr>
<tr>
<td></td>
<td>AQ</td>
<td>52.33±0.36i</td>
<td>57.01±0.43f</td>
</tr>
<tr>
<td><em>M. koenigii</em></td>
<td>P.E</td>
<td>38.53±0.34d</td>
<td>58.87±0.20g</td>
</tr>
<tr>
<td></td>
<td>ETH</td>
<td>50.91±0.13h</td>
<td>46.14±0.36c</td>
</tr>
<tr>
<td></td>
<td>AQ</td>
<td>34.94±0.71c</td>
<td>36.27±0.62a</td>
</tr>
<tr>
<td><em>G. latifolium</em></td>
<td>P.E</td>
<td>49.48±0.52g</td>
<td>67.83±0.11h</td>
</tr>
<tr>
<td></td>
<td>ETH</td>
<td>38.22±0.03d</td>
<td>52.53±0.21e</td>
</tr>
<tr>
<td></td>
<td>AQ</td>
<td>67.60±0.12k</td>
<td>71.92±0.08i</td>
</tr>
</tbody>
</table>

Mean values in the same column with different superscript differ significantly (P<0.05). DW: dry weight; mg GAE/g DW: milligram gallic acid equivalent per gram dry weight; mg Rutin/g DW: milligram Rutin equivalent per gram dry weight. P.E: petroleum ether; ETH: ethanol; AQ: aqueous.

**Phytochemical Screening**

The results in Table 3 showed the contents of the phytochemicals obtained from the extraction of the four vegetables which, showed that flavonoids and phenols were present in all the plant extracts while, anthraquinone was absent in all the crude extracts screened. Alkaloids were absent in petroleum ether and ethanolic extracts of *M. koenigii*, petroleum ether and ethanolic extract of *P. guineense*. All crude extracts of *O. gratissimum* were rich in steroid and alkaloid while, all crude extracts of *G. latifolium* were rich in terpenoids, saponin and cardiac glycoside.

Phytochemicals are secondary metabolites of plants known to exhibit diverse pharmacological and biochemical effects on living organisms (Trease and Evans, 1989). The qualitative phytochemical screening of the plants extracts demonstrated that most of the
plant extracts are quite rich in metabolites, containing steroids, terpenoids, tannins, saponins, phlobatannins, cardiac glycosides and alkaloids which support their uses in folk medicine. The extracts of *P. guineense* were found to contain alkaloids, flavonoids, phlobatannin, saponins, tannins and terpenoids as reported in the work of Okoye and Ebeledike (2013). Also, from this study the leaves extracts of *G. latifolium* contained flavonoids, terpenoids, steroids, saponins and cardiac glycosides. These compounds have been identified by previous workers (Morebise et al., 2002; Enemor et al., 2014; Ezekwe et al., 2014). The leaf extract of *O. gratissimum* was also found to have a good quality of phytochemicals which were similar to earlier study (Mann et al., 2012). Similarly, phytochemical screening of *M. koenigii* leaf extracts revealed the presence of alkaloids, flavonoids, saponins, steroids, phlobatanin and tannins which were also reported by Victoriya and Manimekalai (2016).

**Growth Inhibitory Activities of Plant Leaves Extracts Against the Test Microorganisms**

The results of the antimicrobial activity are presented in Table 4. Generally, plants extracts that had inhibition diameter zone between the ranges of 10 to 15mm were active against the tested bacteria while plant extracts with inhibition diameter zone greater than or equal to 16mm were assumed to be highly active against the pathogenic bacteria. The various leaves extracts of the vegetables used in this study presented variable inhibitory effects against the gram positive and negative pathogenic bacteria, the aqueous and petroleum ether extracts of *M. Koenigii* recorded the highest (36.00mm) and lowest (10.75mm) values for antimicrobial activities of the crude plants extracts. According to Bonjar et al. (2004) phytochemical constituents such as tannins, alkaloids, saponins, and some other aromatic compounds of plants serve as defense mechanism against numerous microorganisms and herbivores.

Also, flavonoids, alkaloids, terpenoids and other compounds of phenolic nature or free hydroxyl group are commonly found to have antimicrobial properties (Baskaran et al., 2011; Mehrangiz et al., 2011). Hence, the antimicrobial activities reported in this study for various leaves extracts may be due to the presence of their phytochemicals such as flavonoids, terpenoids, tannins, saponins, alkaloids, steroids, cardiac glycoside, anthraxquinone, phlobatanin and phenols.

**Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)**

The results of MIC and MBC of the plants extracts against the tested five microorganisms are shown in Table 5. The MIC of aqueous extract of *M. koenigii* showed a remarkable effect on all tested bacteria, with values ranging from 0.13 – 0.52g/ml. The petroleum ether extract of *M. Koenigii* had MIC values ranging between 0.22g/ml and 0.30g/ml against *E. coli* and *P. aeruginosa* respectively. Also, the aqueous extract of *M. Koenigii* recorded MIC values of 0.19g/ml against *E. coli*. The petroleum ether extract of *G. latifolium* recorded MIC of 0.15g/ml against *K. pneumonia* while, the ethanolic extract of *G. latifolium* showed
MIC value of 0.14g/ml against *E. coli* and 0.23g/ml against *K. pneumonia*.

The MBC value of ethanolic extract of *M. koenigii* was 0.77g/ml against *S. aureus* whilst, petroleum ether extract of *M. Koenigii* recorded 0.60 g/ml against *S. aureus* and *P. aeruginosa*. Also, the MBC value of aqueous extract of *M. Koenigii* was 0.71g/ml against *S. aureus* whilst, the ethanol extract value of *G. latifolium* ranged between 0.42 and 0.56g/ml against *E. coli* and *K. pneumonia*. Similarly, the petroleum ether extract of *G. latifolium* recorded MBC value of 0.37g/ml against *K. pneumonia*.

Furthermore, to quantify the expression of the antibacterial activity, the MICs and MBCs of *M. Koenigii* and *G. latifolium* were determined using standard method. The results exhibited that extracts of *M. koenigii* and *G. latifolium* were effective as antimicrobial agents with relatively low MIC and MBC values against tested Gram-positive and Gram-negative bacteria. In addition, the MBC values obtained from the five leaves extracts against the tested bacteria were generally higher than the values of MIC. This is an indication that the extracts were bacteriostatic at lower concentrations and bactericidal at higher concentrations.
<table>
<thead>
<tr>
<th>Plants</th>
<th>Solvents</th>
<th>Flavonoid</th>
<th>Terpenoids</th>
<th>Steroid</th>
<th>Tannin</th>
<th>Saponin</th>
<th>Alkaloid</th>
<th>Cardiac glycoside</th>
<th>Anthra-quinone</th>
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(-) = Absent; (+) = Present; P.E: Petroleum Ether; ETH: Ethanol; AQ: Aqueous
Table 4: Growth inhibitory activity of the plant extracts against the test microbes

<table>
<thead>
<tr>
<th>Plant Extracts</th>
<th>PEOG</th>
<th>ETOG</th>
<th>AQOG</th>
<th>PEPG</th>
<th>ETPG</th>
<th>AQPG</th>
<th>PEMK</th>
<th>ETMK</th>
<th>AQMK</th>
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<tr>
<td>S. aureus</td>
<td>12.25±0.35</td>
<td>11.5±0.71</td>
<td>14.25±0.35</td>
<td>14.0±0.71</td>
<td>17.25±0.35</td>
<td>15.5±0.71</td>
<td>36.0±1.41</td>
<td>16.5±0.71</td>
<td>21.0±0.71</td>
<td>12.0±0.71</td>
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<tr>
<td>S. epidermis</td>
<td>14.5±0.71</td>
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<td>15.0±0.71</td>
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<td>12.0±0.71</td>
<td>11.25±0.35</td>
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<td>11.25±0.35</td>
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<td>Gram negative bacteria</td>
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<td>E. coli</td>
<td>22.5±0.71</td>
<td>18.0±0.71</td>
<td>26.25±0.35</td>
<td>12.0±0.71</td>
<td>16.25±0.35</td>
<td>14.5±0.71</td>
<td>16.25±0.35</td>
<td>12.0±0.71</td>
<td>10.75±0.35</td>
<td>18.35±0.49</td>
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<td>P. aeruginosa</td>
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<td>16.5±0.71</td>
<td>14.0±0.71</td>
<td>13.25±0.35</td>
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<td>14.5±0.71</td>
<td>14.75±0.35</td>
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<td>S. typhimurium</td>
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<td>12.0±0.71</td>
<td>14.25±0.35</td>
<td>17.75±0.35</td>
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<td>13.5±0.35</td>
<td>12.5±0.35</td>
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<tr>
<td>K. pneumonia</td>
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Antimicrobial activities were expressed as inhibition diameter zones in millimetres (mm).
<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Plant Extracts</th>
<th>( M. ) koenigii (Ethanol)</th>
<th>( M. ) koenigii (Petroleum ether)</th>
<th>( M. ) koenigii (Aqueous)</th>
<th>G. latifolium (Ethanol)</th>
<th>G. latifolium (Petroleum Ether)</th>
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<tr>
<td></td>
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<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
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<td>S. aureus ATCC 700699</td>
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<td>0.46±0.02</td>
<td>0.77±0.10</td>
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<td>0.60±0.04</td>
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<td>P. aeruginosa ATCC 15442</td>
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<td>0.34±0.06</td>
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<td>S. typhi ATCC 13311</td>
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<td>K. pneumonia ATCC 8309</td>
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<td>0.22±0.06</td>
<td>N.D.</td>
<td>0.19±0.06</td>
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</table>

The table shows the Minimum Inhibitory Concentration (MIC, g/ml) and Minimum Bactericidal Concentration (MBC, g/ml) for various plant extracts and microorganisms.
Conclusion

In conclusion, the studies confirmed the antioxidant and antimicrobial potentials of some of the Nigerian vegetables, which could be harnessed for both their nutritional and health benefits in fish and fishery product industries.

References


