Antibacterial Activity of the Aqueous Extracts of the Leaves, Fruits and Bark of *Gmelina Arborea*

**ABSTRACT**

The antibacterial activity of the aqueous extracts of the leaves, fruits, and bark of *Gmelina arborea* against *Escherichia coli* was investigated in this study. Aqueous extraction was done, and three concentrations were prepared: 2.5 ml extract: 5 ml distilled H2O, 5.0 ml extract: 5 ml distilled H2O and 7.5 ml extract: 5 ml distilled H2O. Disk - diffusion method was used in the bioassay using E. coli after which, the filter paper discs were soaked separately in three concentrations of the *Gmelina arborea* extracts (bark, fruits, and leaves). The antibacterial activity was determined based on the zones of inhibition formed which was measured after 12, 24, 36, and 48 hours. The results showed that the highest average zone of inhibition (9.86 mm) was formed using the aqueous extracts of the fruits followed by the average zone of inhibition (8.45 mm) formed using the aqueous extracts of the bark and then that of the aqueous extracts of the leaves (7.44 mm). Thus, the aqueous extracts of the leaves, fruits, and bark of *Gmelina arborea* have antibacterial activity against *E. coli* antibacterial activity against *E. coli*.

*Keywords: Zone of inhibition, Gmelina arborea, Escherichia coli, disk - diffusion method.*

**INTRODUCTION**

Nature provides a complete storehouse of remedies to cure some human ailments. For several thousand years, plants have been used as traditional medicine (Akyala et al., 2013). However, scientific investigations and information on the therapeutic potential of medicinal plants are limited. This lack of scientific knowledge has restricted the use of traditional herbs as remedies to be used in conjunction with or as an alternative to orthodox medical treatment (El-Mahmood et al., 2014).

In recent decades, there has been an exponential growth in the practice and use of herbal medicine. Due to its natural origin and relative safeness, it has been popularized in developing and even in developed countries. Plant parts, such as leaves, roots, bark or fruit are known to contain chemicals that can inhibit or prevent the growth of microorganisms (Banu et al., 2013). They carry a wide range of antimicrobial agents that can kill or inhibit the growth of pathogenic or non-pathogenic microorganisms.

Plants are considered as promising sources of medicine in the traditional health care system. Nowadays, there is a revival of interest in herbal-based medicine as a source of new antibacterial drugs, because they have been used by some portions of the populace for a long time and...
have been known to be safe for humans and the environment (Banu et al., 2013). The World Health Organization has listed over 2,100 plant species used worldwide for medicinal purposes, but so far, only about 20% of the world medicinal plants have been screened for pharmacological and biological activities (El-Mahmood et al., 2014). One species of plant with a medicinal value which has not yet been thoroughly screened for pharmacological and biological activities is *Gmelina arborea* (white teak or “gamhar”), belonging to family Lamiaceae.

*Gmelina arborea*, a fast growing timber-yielding tree belonging to Family Lamiaceae, is one of the plants mentioned in ancient scriptures of Ayurveda (ancient Hindu system of healing) being used as folk remedies for jaundice, headache, hallucinations, fever and ‘Tridosha’ (the collective term for sustaining and regulating psychologic and physiologic functions). This species is found in Southern China, Bangladesh, Myanmar, Thailand, Vietnam, Indonesia and the Philippines (Farah et al., 2008). In Indian folk medicine, the root decoction was used to treat abdominal tumors (Chellappan & Pemiah, 2014), as lactagogue, galactagogue and as laxative (Baguet et al., 2013). The fruits were used to treat alopecia, anemia, and leprosy. The leaves were used to treat high blood pressure, malaria, scorpion and insect stings (Doughari et al., 2007). *Gmelina arborea* extracts have also been used by traditional practitioners in Nigeria to treat diarrhea (Moronkolaet al., 2012).

*Gmelina arborea* is a medium sized, deciduous (shedding the leaves annually) tree which grows up to a height of 20 m or more with a diameter of up to 0.5 meters. The bark is smooth, grayish-white and the flowers are brownish-yellow. The fruits are round, and the leaves are broad, heart-shaped and long-petioled (Figure 4). This species has antioxidant (Angamuthu, 2013), antibacterial (Akyala et al., 2013), antidiabetic (Bhabani et al., 2012), cardio protective (Palani et al., 2011), gastro-protective (Chellappan & Pemiah, 2014), antihelminthic (Ambujakshi et al., 2009), diuretic (Sravani et al., 2011), immunomodulatory (Shukla et al., 2010), anti-hyperlipidemic (David et al., 2012), antifungal (Kawamura et al., 2004), antipyretic and analgesic activity (Pravat et al., 2011). Thus, *G. arborea* has huge potential as a source of medicine.

Parekh and Chanda (2006) have shown the antibacterial activity of *Gmelina asiatica* against *Bacillus cereus* and *Klebsiella pneumoniae*. Rohit et al. (2012) reported antimicrobial activity of *G. arborea* crude leaves and stem bark extracts against gram negative and gram positive bacteria including *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Shigelladyenteria*. The ethanol extract of *G. arborea* showed significant antibacterial activity with zones of inhibition ranging from 13 to 17.75 mm, against both gram positive and gram negative bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* (Bhabani et al., 2012). The antibacterial activities shown by ethanol extract are somewhat comparable with Streptomycin, a standard brand of antibiotic (Bhabani et al., 2012).

Phytochemical screening of the bark, leaves, fruits and roots of *G. arborea* showed that they contain a large concentration of flavonoids (Chellappan & Pemiah, 2014). Quantitative estimation revealed flavonoid content of 15.2 % and 6.1 % saponins (Chugh et al., 2012), both secondary metabolites possessing antimicrobial activity. The aqueous plant extracts contain saponins, reducing sugar, steroids, flavonoids, glycosides, tannin and phenolics (Akyala et al., 2013; Chothani & Patel, 2012). The methanolic extracts contain flavonoids, saponins, steroids and glycosides while the hexane extract contains saponins and steroids (Rohit et al., 2012). In another study, Akyala et al. (2013) reported that
the aqueous fruit extract of G. arboreal showed higher antibacterial activity than methanol and hexane extracts.

The aqueous extract of the bark of G. arborea contains secondary metabolites such as tannins, anthraquinone, saponins, and carbohydrates but no phenolics (Rohit et al., 2012). The aqueous extract of the fruit contains tartaric acid, saponins, reducing sugar, steroids, flavonoids and glycosides (Ayala et al., 2013). The aqueous extract of the leaves contains carbohydrates, proteins, saponins, tannin, and phenolics but no flavonoids and cardiac glycoside (Chothani & Patel, 2012). Verbascoside was also isolated from the roots of G. Arborea (Bangou et al., 2012).

Notwithstanding previous research, a gap of a pool of knowledge of the antibacterial activity of Gmelina arborea against E. coli exists. There were similar studies done on the antibacterial activity of Gmelina arborea against gram positive and gram negative bacteria, however, in the study of Rohit et al. (2012), only leaves and stem bark extracts were used and fruit extracts in the report of Akyala et al. (2013). The concentrations used in this study were 2.5 ml plant extract: 5 ml distilled H2O; 5.0 ml plant extract: 5 ml distilled H2O; and 7.5 ml plant extract: 5 ml distilled H2O which were 1000 g/ml, 100 g/ml, 1 g/ml, 0.1 g/ml, 0.01 g/ml, 0.001 g/ml and 0.0001 g/ml in the study of Akyala et al. (2013). The concentrations were lower compared to the present study which resulted to lower inhibition zones of Gmelina arborea against gram positive and gram negative bacteria.

Thus, this study investigated and validated the antibacterial activity of the bark, fruit, and leaves crude extracts of Gmelina arborea against E. coli. The specific objectives include the following: 1) to measure and compare the diameter of the zones of inhibition formed using different concentrations of the aqueous extract of the bark, fruit, and leaves of G. arborea, and 2) to determine which part of the plant demonstrates the highest antibacterial activity against E. coli. The results and findings of this study will be used as basis for recommending the use of Gmelina arborea as alternative antibiotic. They will also serve as a significant contribution to the increasing knowledge of plants with antimicrobial values.

**MATERIALS AND METHODS**

**Plant collection and preparation of Gmelina arborea extract**

Using the methods of El-Mahmood et al. (2010), about 200 g each of fresh bark, fruit, and leaves of Gmelina arborea were randomly collected from Alangilan, Sta. Catalina on August 27, 2015. The samples were first washed thoroughly with tap water followed by distilled water, after which, they were air-dried at room temperature. The air-dried samples (bark, fruit, and leaves) were then sliced separately into small (fine) pieces using a sterile knife.

About 150 g of the finely-cut samples was placed into three 1000-ml beakers containing 300 ml distilled water. The mixtures were next heated for about 15 minutes at 60 °C. After heating, the mixtures were filtered using Muslin cloth first followed by the use of Whatman No. 1 filter paper (Figure 5). The filtrates were then heated again at 40 °C until only 15 ml of the extract was left in the beaker. Out of the remaining 15 ml extract, three test concentrations were prepared to be used for the later bioassay: 2.5 ml plant extract: 5 ml distilled H2O; 5.0 ml plant extract: 5 ml distilled H2O; and 7.5 ml plant extract: 5 ml distilled H2O (Figure 6). The positive control used was Amoxycillin trihydrate dissolved in 5 ml distilled H2O. The above three concentrations were designated as C-1, C-2, and C-3, respectively and C-4 was the control. This procedure was done separately for the bark, fruit, and leaves of
Culture media preparation

The amount (g) of nutrient agar needed in the preparation of culture medium was calculated first based on the formula:

\[ \frac{C1}{V1} = \frac{C2}{V2} \]

Whereas,

\[ \frac{C1}{V1} = \frac{23\, g}{1000\, mL} \] (specifications of Scharlau nutrient agar as indicated in the container)

\[ C2 \] – weight (g) of nutrient agar

\[ V2 \] – volume (mL) of distilled H2O (27 petri dish * 5 mL)

Thus,

\[ \left( \frac{C1}{V1} \right) V2 = C2 \]

\[ \left( \frac{23\, g}{1000\, mL} \right) \left( 27 \times 5\, mL \right) = C2 \]

\[ 0.023\, g/mL \times 135\, mL = C2 \]

\[ 3.10\, g = C2 \]

As computed, 3.10 g of Scharlau nutrient agar was dissolved in 135 ml distilled water using a sterilized Erlenmeyer flask. The mixture was heated over a hot plate for about 15 minutes with constant stirring until the agar became homogenized. The homogenized, melted agar was next sterilized for about 15 minutes in an autoclave, after which, 5 ml was poured into each of eleven petri dishes. The petri dishes or agar plates were allowed to solidify inside a wooden cabinet by placing them in upside-down position so that no drops of moisture remained on the agar surface.

Isolation of pure culture of \textit{Escherichia coli}

The test bacteria (Escherichia coli) were collected from a human stool sample using a sterile cotton swab. The cotton swab was dipped and swirled in a sterilized beaker containing 10 ml distilled water. Next, a sterile inoculating loop was dipped into the distilled water and then streaked onto one of the prepared agar plates. The agar plate was placed in an upside down position inside the cabinet and left overnight for the colonies of bacteria to grow.

The colonies of \textit{E. coli} that grew on the agar plate were rounded, white with the greyish periphery. Gram staining method was used to immobilize and stain bacteria for identification. The thin portion of the colony of bacteria was transferred to the slide using a sterilized inoculating loop. The slide was passed over a low flame. The smear was rinsed with crystal violet then with water after 1 minute. After which, iodine solution was used then smear was washed with water. For 5 seconds, the smear was rinsed with 95% ethanol and counterstained with safranin for 1 minute. The smear was air-dried. When viewed under the low power objective of the compound microscope, the bacteria appeared reddish and rod-shaped. To isolate pure culture of \textit{E. coli}, a sterile inoculating loop, was dipped on the isolated colony and was inoculated into another agar plate. The inoculum was spread and streaked over the surface of the agar following specific patterns (Figure 7). The agar plate was then placed in an upside-down position inside the cabinet and left overnight.

Serial dilution of \textit{Escherichia coli}

Using a sterile inoculating loop, a pure culture of \textit{E. coli} was obtained from the surface of the inoculated plate, out of which a four-fold dilution was made using test tubes. Test tube 1
contained 10 ml distilled water, and test tubes 2, 3 and 4 contained nine ml distilled water. Using a sterile inoculating loop, a loopfull of pure culture was dipped into Test tube 1, swirling the loop afterward. Next, one ml of the diluted bacteria was transferred from the Test tube 1 to Test tube 2, and from Test tube two, one ml of the diluted bacteria was transferred to Test tube 3, and finally, one ml of the diluted bacteria from Test tube three was transferred to Test tube 4. The diluted bacteria in Test tube four were used for subsequent inoculation.

Three drops of the *E. coli* dilute solution was transferred from Test tube 4 to nine agar plates. The plates were rotated, and a sterile inoculating loop was streaked on the agar surface to evenly spread the diluted bacteria to ensure complete coverage of the plate. The agar plates were then allowed to dry for about 5 minutes.

**Bioassay of Escherichia coli**

One by one, the sterile filter paper discs, each measuring 6 mm in diameter, were soaked separately in three concentrations of the *Gmelina arborea* extracts (bark, fruits and leaves) and the control solution. The discs were drained off of excess liquid by holding it against the side of the beaker using a sterile forceps. The discs were arranged in the four quadrants of the agar surface (Figure 8), gently pressing them on the agar surface to ensure good contact. Afterwards, the agar plates were deposited inside the cabinet in an upside-down position for the next 24 hours.

The zones of inhibition were measured following the guidelines set by the European Community on Antimicrobial Susceptibility Testing (EUCAST). In measuring the zone of inhibition, the agar plates were placed in an upside-down position against a dark background in a lighted room. The distance between the plates and the eyes was about 30 cm. The zones of inhibition were measured (in mm) after 12 hours, 24 hours, 36 hours and 48 hours (Figure 9) using a Vernier caliper that was positioned above the glass cover of the petri dishes.

Three trial experiments were performed for a total of nine agar plates per trial with three agar plates for every concentrations of extract dilution (2.5 ml plant extract: 5 ml distilled H2O; 5 ml plant extract: 5 ml distilled H2O; 7.5 ml plant extract: 5 ml distilled H2O) multiplied by the three parts of the plant (bark, fruit and leaves). All in all, a total of 27 agar plates were observed, and the zones of inhibition were measured plus the nine agar plates for the control.

**RESULT**

**Diameters of the zone of inhibition using the bark extracts of *Gmelina arborea***

Table 1 shows the data on zones of inhibition measured after treating Escherichia coli with the aqueous extract of the bark of Gmelina *arborea*. During the first trial, after 12 hours, the highest zone of inhibition was 9.04 mm in C-3 concentration while the lowest zone of inhibition was 7.0 mm in C-1 after 48 hours. The zones of inhibition decreased with increasing period of observation such that after 48 hours, the zone of inhibition was only 7.13 mm in C-3 and the lowest was also 7.0 mm in C-1. In Trial 2, the highest zone of inhibition was 8.17 mm after 12 hours in C-3 which decreased to 7.08 mm in 48 hours. The lowest zone of inhibition was 7.0 mm after 48 hours in C-1. In Trial 3, the area of inhibition was still highest in C-3(8.14 mm) after 12 hours which decreased to 7.05 mm after 48 hours while the lowest was 6.10 mm in C-1 after 12 hours and 6.03 mm after 48 hours.
Table 1. Data on zones of inhibition measured using the aqueous extracts of the bark of *G. arborea* to treat *E. coli* after 12, 24, 36 and 48 hours.

<table>
<thead>
<tr>
<th>Time (Hrs)</th>
<th>Trial 1</th>
<th></th>
<th>Trial 2</th>
<th></th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5 ml</td>
<td>5.0 ml</td>
<td>7.5 ml</td>
<td>2.5 ml</td>
<td>5.0 ml</td>
</tr>
<tr>
<td>12</td>
<td>7.07</td>
<td>7.10</td>
<td>9.04</td>
<td>7.08</td>
<td>8.16</td>
</tr>
<tr>
<td>24</td>
<td>7.06</td>
<td>7.08</td>
<td>8.09</td>
<td>7.12</td>
<td>8.07</td>
</tr>
<tr>
<td>36</td>
<td>7.03</td>
<td>7.04</td>
<td>7.65</td>
<td>7.05</td>
<td>8.06</td>
</tr>
<tr>
<td>48</td>
<td>7.00</td>
<td>7.02</td>
<td>7.13</td>
<td>7.00</td>
<td>7.08</td>
</tr>
</tbody>
</table>

Table 2 reveals that the diameters of the zone of inhibition were consistently high in the highest concentration of the fruit extract (C-3) from 12 to 48 hours, although in a decreasing trend. During the first trial, the highest diameter of the zone of inhibition was 10.40 mm in 12 hours which decreased to 9.03 mm in 48 hours. The lowest diameters of the zone of inhibition ranged from 8.15 mm after 12 hours to 8.03 mm after 48 hours. Likewise, the diameters of the zone of inhibition decreased with decreasing concentration of the fruit extract, that is, the diameters were highest in C-3 and lowest in C-1 at particular time points. The same pattern was observed during the second and third trials.

Table 3 also shows the same pattern of high and low diameters of zone of inhibition through the hours per concentration of the leaf extract. The highest diameter of zone of inhibition ranged from 7.08 mm to 6.14 mm in C-3 while the lowest ranged from 7.02 mm to 7.0 mm, from 12 hours to 48 hours of observation, respectively. The pattern was true in Trial 2 and Trial 3.

Note that in all cases, that is, using either the bark, fruit or leaves of *G. arborea*, C-2 showed diameters of zone of inhibition which are intermediate between C-1 and C-3. Moreover, the data revealed that of the three parts of the plant, it was the fruit extracts that yielded the highest diameters of zone of inhibition (Table 4).

Table 2. Data on zones of inhibition measured using the aqueous extracts of the fruit of *G. arborea* to treat *E. coli* after 12, 24, 36 and 48 hours.

<table>
<thead>
<tr>
<th>Time (Hrs)</th>
<th>Trial 1</th>
<th></th>
<th>Trial 2</th>
<th></th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5 ml</td>
<td>5.0 ml</td>
<td>7.5 ml</td>
<td>2.5 ml</td>
<td>5.0 ml</td>
</tr>
<tr>
<td>12</td>
<td>8.15</td>
<td>10.10</td>
<td>10.40</td>
<td>8.18</td>
<td>9.08</td>
</tr>
<tr>
<td>24</td>
<td>8.04</td>
<td>10.07</td>
<td>10.10</td>
<td>8.12</td>
<td>9.04</td>
</tr>
<tr>
<td>36</td>
<td>8.03</td>
<td>9.09</td>
<td>9.05</td>
<td>8.11</td>
<td>9.03</td>
</tr>
<tr>
<td>48</td>
<td>8.03</td>
<td>9.02</td>
<td>9.03</td>
<td>8.06</td>
<td>9.03</td>
</tr>
</tbody>
</table>
Table 3. Data on zones of inhibition measured using the aqueous extracts of the leaves of *G. arborea* to treat *E. coli* after 12, 24, 36 and 48 hours.

<table>
<thead>
<tr>
<th>Time (Hrs)</th>
<th>Bark</th>
<th>Fruit</th>
<th>Leaves</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 ml</td>
<td>7.02</td>
<td>7.04</td>
<td>7.08</td>
<td>7.11</td>
</tr>
<tr>
<td>5.0 ml</td>
<td>7.11</td>
<td>8.12</td>
<td>7.13</td>
<td>7.10</td>
</tr>
<tr>
<td>7.5 ml</td>
<td>7.10</td>
<td>8.08</td>
<td>8.11</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>6.75</td>
<td>7.46</td>
<td>8.45</td>
<td>7.08</td>
</tr>
<tr>
<td>24</td>
<td>6.76</td>
<td>7.41</td>
<td>8.11</td>
<td>7.05</td>
</tr>
<tr>
<td>36</td>
<td>6.71</td>
<td>7.37</td>
<td>7.28</td>
<td>6.74</td>
</tr>
<tr>
<td>48</td>
<td>6.68</td>
<td>7.03</td>
<td>7.09</td>
<td>6.71</td>
</tr>
</tbody>
</table>

Table 4 further shows that, regarding average diameters of zone of inhibition, the control group was far highest than any of the three kinds of extracts.

DISCUSSION

The present study was conducted to add to the pool of knowledge of the antibacterial activity of *Gmelina arborea* against *E. coli*. Previous research failed to examine plant parts of *Gmelina arborea*. In the study of Rohitet al. (2012), only leaves and stem bark extracts were used and fruit extracts in the report of Akyala et al. (2013). The results and findings of this study will be used as basis for recommending the use of *Gmelina arborea* as alternative antibiotic. They will also serve as an important contribution to the increasing knowledge of plants with antimicrobial values.

This study affirms previous reports on the antibacterial activity of *Gmelina arborea* against gram-positive and gram-negative bacteria done by El-Mahmood et al. (2014), Akyala et al. (2013) and Nayak et al. (2012). According to El-Mahmood et al. (2014), aqueous extracts of the bark and leaves of *G. arborea* showed antibacterial activity against Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, *Shigella dysenteriae* and *Salmonella typhi*. Reportedly, the fruit extracts showed antibacterial activity against gram-positive and gram-negative bacteria such as *Bacillus subtilis*, Staphylococcus aureus, and *Pseudomonas aeruginosa* (Nayak et al., 2012) and *Streptococcus pyogenes*, *Escherichia coli* and *Proteus morganis* (Akyala et al., 2013). Akyala et al. (2013) also indicated that aqueous extract showed higher activity on bacterial isolates than methanol and hexane extracts.

Table 4. Data on average zones of inhibition obtained using the aqueous extracts of the bark, fruit and leaves of *G. arborea* to treat *E. coli* after 12, 24, 36 and 48 hours. Also shown are the zones of inhibition obtained for the control group.

<table>
<thead>
<tr>
<th>Time (Hrs)</th>
<th>Bark</th>
<th>Fruit</th>
<th>Leaves</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 ml</td>
<td>6.75</td>
<td>7.46</td>
<td>8.45</td>
<td>22.49</td>
</tr>
<tr>
<td>5.0 ml</td>
<td>8.14</td>
<td>9.75</td>
<td>9.86</td>
<td>23.40</td>
</tr>
<tr>
<td>7.5 ml</td>
<td>7.08</td>
<td>7.75</td>
<td>7.44</td>
<td>24.54</td>
</tr>
<tr>
<td>12</td>
<td>18.74</td>
<td>18.73</td>
<td>18.68</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>16.08</td>
<td>15.21</td>
<td>15.40</td>
<td></td>
</tr>
</tbody>
</table>
Phytochemical screening of the bark, leaves and fruits of *G. arborea* for secondary metabolites showed that this species contains a large concentration of flavonoids (Chellappan & Pemiah, 2014) as well as saponins (Chugh et al., 2012). Flavonoids, which has a structural feature of 2-phenyl-benzo[α] pyrane consisting of two benzene rings (A and B), linked through a heterocyclic pyrane ring, inhibit nucleic acid synthesis and cytoplasmic membrane function of the cell. Nucleic acid synthesis is inhibited by intercalation or hydrogen bonding of the B ring of flavonoids with the stacking of nucleic acid bases, probably explaining the inhibitory action of flavonoids on DNA and RNA synthesis. Flavonoids also increase the polarization of the liposomes which reduced fluidity of the outer and inner layers of cell membranes. Flavonoids may perturb the lipid bilayers by directly penetrating them and disrupting the barrier function.

Saponins are high-molecular-weight glycosides, consisting of a sugar unit(s) linked to a triterpene or a steroid aglycone. They are known to possess gaming or detergent properties (Negi et al., 2013). They lower the surface tension of aqueous solutions and therefore form stable foams when in contact with water (Madland, 2013). Gunaseker et al. (2009) experimented on the antimicrobial activity of saponin fraction of *Solanum xanthocapum* and *Centella asiatica*. It inhibited the growth of *Staphylococcus aureus* with zones of inhibition ranging from 21 mm to 22 mm. The antimicrobial activity of *G. arborea* extracts might be might be explained by one of the mechanisms of action of flavonoids and saponins mentioned above.

The results revealed that the most potent extract is the aqueous extract of the fruit of *G. arborea* since it formed the highest zones of inhibition which ranged from 8.04 mm to 9.86 mm. Akyala et al. (2013) found out that only the aqueous extracts of the fruit of *G. arborea* possess both flavonoids and saponins while Chothani and Patel (2012) and Rohit et al. (2012) found out that the aqueous extracts of the leaves and bark of *G. arborea* has saponins only.

The higher concentration of the plant extracts showed higher diameters of zones of inhibition compared with the lower concentrations (Figure 1, 2, and 3). These findings agree with the study of Akyala et al. (2013), who used different concentrations of the extract: 1000 g/ml, 100 g/ml, 10 g/ml, 1 g/ml, 0.1 g/ml, 0.01 g/ml, 0.001 g/ml and 0.0001 g/ml. He found out that the highest concentration (1000 g/ml), formed the highest zone of inhibition while 0.0001 g/ml concentration formed the lowest zone of inhibition. Also, Beg et al. (2012) found out that Amoxycillin trihydrate is formed zones of inhibition ranging from 17.4 mm to 30.3 mm which compares with the present findings, 14.06 mm to 27.15 mm. Amoxycillin trihydrate is a semisynthetic penicillin belonging to the β-lactam family. It was found out to be highly effective against gram-positive and gram-negative bacteria by inhibiting cell wall synthesis. It binds with penicillin-binding proteins of the inner membrane of the bacterial cell wall which interferes with the process that produces peptidoglycan and recuperation of the cell (Beg et al., 2012).

Due to time constraint, this study focused only on the antibacterial activity of the crude extracts of the bark, fruit, and leaves of *G. arborea* against *Escherichia coli*. Also, Soxhlet apparatus for the extraction procedure was not used since it was not available at the time of the study.

**CONCLUSION**

The aqueous extracts of the bark, fruit, and leaves of *Gmelina arborea* have potent antibiotic properties. However, of the three kinds of extract, the aqueous extracts of the fruit showed the highest antibacterial activity. It was also found out
that the higher concentration of the plant extracts formed larger diameters of zone of inhibition compared with the lower concentrations which formed relatively smaller diameters of zone of inhibition. Presumably, the antibiotic properties of G. arborea are attributed to the two major groups of metabolites that they contain – flavonoids and saponins. It is recommended that aside from further antibacterial studies on this species, G. arborea may be subjected to clinical trials in order to determine the potency of the plant as an antimicrobial agent. Moreover, extraction solvents like chloroform, ethanol, methanol and hexane may be used. ASoxhlet apparatus for extraction is highly recommended, and if possible, phytochemical screening investigations and/or HPLC-finger print analyses would be conducted to identify the bioactive substances found in G. arborea.

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REFERENCES


**APPENDIX**

![Figure 1. Graph showing the average zones of inhibition (mm) measured using the aqueous extracts of the bark, fruit, and leaves of *G. arborea* after 12, 24, 36 and 48 hours in 2.5 ml plant extract: 5 ml distilled H2O concentration.](image1)

![Figure 2. Graph showing the average zones of inhibition (mm) measured using the aqueous extracts of the bark, fruit, and leaves of *G. arborea* after 12, 24, 36 and 48 hours in 5.0 ml plant extract: 5 ml distilled H2O concentration.](image2)
Figure 3. Graph showing the average zones of inhibition (mm) measured using the aqueous extracts of the bark, fruit, and leaves of *G. arborea* after 12, 24, 36 and 48 hours in 7.5 ml plant extract: 5 ml distilled H2O concentration.

Figure 4. The plant parts of *Gmelina arborea* used in the study: (A) bark, (B) fruit and (C) leaves.

Figure 5. Filtration of the Gmelina arborea mixture using (A) Muslin cloth followed by the use of (B) Whatman no. 1 filter paper.

Figure 6. Photographs of the three aqueous extract concentrations of (a) bark, (B) fruit, and (C) leaves of *Gmelina arborea*.

Figure 7. Streak patterns in spreading the inoculum over the agar plates to obtain pure culture of *Escherichia coli*. (A) Loop with *E. coli* was streaked over the agar surface repeatedly, (B) the plate was rotated so that next streaks will be perpendicular to the first, (C) the plate was rotated so that the third streak will be perpendicular to the second, and (D) the plate was rotated again and the final streak was made towards the center.
Figure 8. Distribution of the 6 mm filter paper discs impregnated with different concentrations of aqueous extracts of the bark, fruit and leaves of *Gmelina arborea*. Legend: B – Aqueous extract of the bark; F – Aqueous extract of the fruit; L – Aqueous extract of the leaves; and C+ – Positive control.

Figure 9. Photographs of the agar plates with zones of inhibition formed using the aqueous extracts of the bark, fruit and leaves of *G. arborea* after A) 12, B) 24, C) 36 and D) 48 hours.