PHYTOCHEMICALS AND ANTIMICROBIAL ACTIVITY OF *BOSWELLIA DALZIELII* STEM BARK AGAINST SOME CLINICAL ISOLATES

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**ABSTRACT**

Plants have been used in traditional medicine to treat common human illnesses in our local communities. Crude aqueous and methanol extracts of *Boswellia dalzielii* stem bark were screened to investigate the phytochemicals responsible for its antimicrobial activity. The crude extracts and methanol extract fractions were used to conduct antimicrobial activity against some clinical isolates (*Eschericia coli*, *Shigella dysenteriae* and *Salmonella typhi*). Results of the antimicrobial activity showed that both methanol and aqueous extracts had activity against the clinical isolates with higher activity observed in the methanol extract against *E. coli* (16.67±0.96 at 500 mg/ml) and *S. typhi* (16.17±0.75 at 500 mg/ml). Bioassay of the partitioned methanol extract showed n-butanol had the best activity against the organisms and the n-butanol fractions were more active against *E. coli* (16.00±0.00), then *S. dysenteriae* (14.00±0.00) and least active against *S. typhi* (11.00±0.00) all at 50 mg/ml. The MIC of all the organisms for both methanol and aqueous extracts is 7.81 mg/ml but 15.63 mg/ml in methanol for *S. dysenteriae* and the MBC range between 7.81 - 125 mg/ml in both extract for all the organisms. The results of this study therefore indicated that the plants’ stem bark contain potent phytochemicals which inhibited the growth of the tested clinical isolates which validate its continued use in traditional medicine to cure illnesses.

**Keywords:** Phytochemicals, extracts, *Boswellia dalzielii*, antimicrobial activity, fractions.

**INTRODUCTION**

*Boswellia dalzielii* is a very tall tree, more than 13 meters high (Mbiantcha et al., 2017) which is widely distributed in many African countries and in dry climates of tropical and subtropical regions, such as Northern Nigeria (Mamza et al., 2018). The plant is very popular among the local people as an important source (Nwude and Ibrahim, 1980) of cure used to treat a variety of ailments in Hausa traditional medicine. Different parts of *Boswellia dalzielii* have been employed in Ethnomedicine with various degree of success. Its parts have also been screened and found to contain numerous phytochemicals such as tannins, glycosides, flavonoids, alkaloids, anthracene, saponin and saponin glycosides as reported by Abdulazezez et al., (2013). Its’ aqueous stem bark extracts is used as anti-diarrhea (Etuk et al., 2006), antispasmodic (Hassan et al., 2009) and anti-ulcer (Nwinyi et al., 2004). In addition, it has been used in folk medicine as antiseptic, anti-arthritis, wound healing, antimalaria, antidiarhoea, anti-inflammatory, antibacteria, anti-fungal, anti-trypanosomal, anti-hepatitis, anti-HIV/AIDS, antitodes to arrow poison and for the treatment of rheumatism, leprosy, gastro-intestinal troubles (Hussain et al., 2013; Ohemu et al., 2014). The stem bark is used to treat rheumatism, septic sores, venereal diseases and gastrointestinal ailments (Burkhill, 1985a). The leaves are used in large quantity to make a wash of fever and rheumatism while it is also taken internally for gastro-intestinal troubles (Burkhill 1985b). Reports have also shown that the plant has been used for the treatment of dental problems, swellings, bronchitis, coughs, gastric disorder, asthmatic attack, pulmonary diseases and skin ailments, among others (Miller and Morris, 1988; Ben-Yehoshua et al., 2012). There is continuous investigation of the antimicrobial agents (Yamac and Bilgili 2006) and properties of plants by researchers across the globe due to the increase emergence of drug-resistant microorganisms (Tanaka et al., 2006). It is therefore very important that more research is carried out to ascertain that these medicinal plants are very effective and safe for use by man. This study was conducted to find out the effect of the crude extracts and different fractions of *Boswellia dalzielii* on some selected clinical isolates that cause some common infections.

**MATERIALS AND METHODS**

**Plant collection and identification**

*Boswellia dalzielii* stem bark was collected from Changwal forest Kibiya Local Government Area in Kano state on 29th September, 2016 and was taken to the herbarium section, Bayero University, Kano for identification and classification. Herbarium sample was prepared and deposited with voucher no. BUKHAN 0381.

**Extraction**

The stem bark of the plant was air-dried at room temperature and crushed with mortar and pestle were 500 g of each powdered plant material was weighed and extracted exhaustively with 5000 ml methanol and 5000 ml distilled water for two (2) weeks using maceration technique. The extracts obtained were transferred into clean sterile airtight containers, weighed and...
kept in desiccators until required for use (Vishnoi, 1979; Sofowora, 1993; Trease and Evans, 2002).

**Phytochemical screening**

Phytochemical screening of the methanol and aqueous extracts was carried out following standard methods described by Sofowora, (1993), Trease and Evans, (2002) and Yadav and Munin, (2011).

**Antimicrobial activity**

The clinical isolates used for the research were obtained from Microbiology Department of Murtala Muhammad Specialist Hospital, Kano. The organisms were maintained on nutrient agar, slope at 4 °C and sub-cultured before use. The organisms studied were those that are clinically important in causing several infections and it is essential to overcome them through some active therapeutic agents (Parekh and Chanda, 2007).

**Culture Media**

Mueller Hinton agar media was used for the growth of the clinical isolates.

**Standardisation of Inoculum**

A loopful of each of the confirmed test isolates were picked using a sterile wire loop and emulsified into 10 ml of sterile normal saline to match with the 0.5 McFarland Standard for sensitivity test as described by Perez et al. (1990) and Kirby (1996).

**Preparation of the Test Concentrations for Sensitivity**

Various test concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml were prepared from each of the methanol and aqueous plant extracts using serial doubling dilution method. Stock solutions of each of the extracts was prepared by dissolving two gram (2 g) of each extract in separate Bijou bottles containing 4 ml of Dimethyl Sulfoxide (DMSO) to give 1000 mg/ml solutions and labeled as the stock solution. The working solutions were prepared from the stock solution to obtain various concentrations (Esimone et al., 2012) of 500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml respectively. For the control, Ciprofloxacin tablet (500 mg) was dissolved in 1ml of DMSO to give a concentration of 500 mg/ml.

**Antibacterial assay**

This assay was conducted using agar well diffusion method where the plates were observed for zones of inhibition around the wells after incubation and the diameters of the zones were measured with a transparent ruler in millimeters (Esimone et al., 2012; Singh and Tafida, 2013). These tests were performed in triplicate.

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

The plant extracts that showed significant antimicrobial activity by the well diffusion method were subjected to minimum inhibitory concentration (MIC) assay by using tube doubling dilution technique with Dimethyl-sulfoxide (DMSO) to arrive at four different concentrations starting from the lowest concentration that indicated activity from the sensitivity test. The lowest concentrations that showed no evidence of growth (turbidity) after the tests were regarded as minimum inhibitory concentrations (MIC), (Baker et al., 1993, Vallekobia et al., 2001).

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

The Minimum Bactericidal Concentration (MBC) of methanol and aqueous extracts were determined by sub culturing from the test tubes from each of the MIC test tubes that showed no evidence of growth (turbidity). The plates were further incubated at 37 °C for 24 hours to determine the Minimum Bactericidal Concentration (MBC). The highest dilutions that yielded no single bacterial colony on the solid media were regarded as the minimum bactericidal concentrations (Baker et al., 1993; Vallekobia et al., 2001).

**Partitioning of methanol extract of B. dalzielii**

The methanol extract of the plant was subjected to fractionation by solubilization in distilled water then succeeding partitioning with n-hexane (Jamil et al., 2012) over and over for complete separation of n-hexane soluble materials. The remaining extracts were then partitioned with ethyl acetate and n-butanol following the same process leaving just the aqueous portion. All the fractions obtained were collected in beakers and evaporated to dryness (Javaid et al., 2017).

**Bioassay with Fractions of Methanol Extract**

The different partitioned fractions obtained were subjected to a second phase of antimicrobial test using the earlier described method. The fractions concentrations used were 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml. All tests were performed in duplicate. The fraction that showed the best activity on the organisms was selected for further purification using column chromatography.

**Column Chromatographic (CC) Separation of the different Plants Fractions.**

A glass tube of 60 cm high with a width of 3cm was used for the column chromatography. The adsorbent, silica gel (100, 60-120 μm) was carefully packed using wet slurry method to about 25 cm in the glass tube. The n-butanol fraction (6 g) of *Boswellia dalzielii* was loaded onto the packed adsorbent and allowed to stabilize for about 3 hours before elution started. The column was eluted with solvents of increasing polarity in the following stepwise gradients (Mudi and Dauda, 2013) using ethyl acetate (100 %), ethyl acetate: methanol (2:1), ethyl acetate: methanol (1:1), ethyl acetate: methanol (1:2) and methanol (100 %). Volumes of 50 ml and 100 ml were collected at a time until elution was completed per fraction and allowed to evaporate to dryness at room temperature. All the eluets were labeled accordingly.

**Bioassay with pooled Column Chromatographic (CC) fractions**

The different fractions obtained from the CC were subjected to another phase of antimicrobial test using the method described above to determine the effect of the different fractions. The extracts concentrations used were 50 mg/ml and 25 mg/ml where all tests were performed in duplicate.

**Statistical analysis**

Data on the mean zone of inhibition produced by the clinical isolates were analyzed using one-way analysis of variance (ANOVA) and presented as mean ± standard error mean (SEM), where significant difference was considered at p<0.05.

**RESULTS AND DISCUSSION**

The result of the phytochemical screening of the methanol and aqueous extracts of *B.dalzielii* as presented in Table 1 showed the presence of carbohydrates, monosaccharides, reducing sugars, combined reducing sugars, tannins, free anthraquinones, cardiac glycosides, glycosides, terpenoids, saponins, flavonoids, alkaloids and phenols in the extracts. This is in agreement with the screening conducted by Hassan et al. (2009) who also found carbohydrates, tannins, saponins, flavonoids and cardiac glycosides in the methanol extract of *B. dalzielii*. So also, flavonoids, sterols/terpenes, Saponins and tannins were...
reported in the stem bark of *B. dalzielii* (Nabèrè et al., 2013). Similarly, Mamza *et al.* (2018) found reducing sugars and combined reducing sugars in addition to other chemical compounds in the crude methanol stem bark extract of *B. dalzielii* and he reported that alkaloids, flavonoids, tannins, saponins and steroids are all associated with antibacterial properties. Flavonoids have also been attributed with antibacterial (Mamza *et al.*, 2015a; Mamza *et al.*, 2015b; Pingale *et al.*, 2017). In addition, Alkaloids have also been reported to possess antibacterial activities (Ogunsuyi and Ibitoye, 2003; Karthikeyan *et al.*, 2013). According to Cowan (1999) tannins inhibits the growth of microorganisms. Saponins are known to have antimicrobial properties. Steroids are equally known for their antibacterial activity. The antimicrobial property of plants is therefore associated with the presence of tannins, saponins, flavonoids and alkaloids (Adebajo *et al.*, 1983; Leven *et al.*, 1979; Ohadoma *et al.*, 2014) among others. Furthermore, incensole: a macroyclic diterpenoid; 1:12-epoxy-3,7-cembradien-11-ol was isolated from the ethanol stem bark extract of *Boswellia dalzielii* in a research conducted by Alemika *et al.* (2004). Terpenoids as well as glycosides have been shown to possess antibacterial activity (Mamza *et al.*, 2015b; Mamza *et al.*, 2017; Parekh *et al.*, 2006).

Table 1: Phytochemical Constituents of *B. dalzielii* Plant Extracts.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th><em>B. dalzielii</em> Extract</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Monosaccharide</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Combined reducing sugar</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Free anthraquinones</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**KEY:** + = present - = absent

The results of the antimicrobial activity for the stem bark of *B. dalzielii* against *Eschericia coli*, *Shigella dysenteriae* and *Salmonella typhi* is presented in Table 2. The results showed that at a concentration of 500 mg/ml, the extracts produced zones of inhibition between 17.67±1.47 mm and 15.00±1.00 mm in all the organisms. Higher zones were observed in *Eschericia coli* (17.67±1.46 mm) which is significantly the same with that observed in *S. typhi* (17.33±0.66 mm) in the methanol extract. The zones observed in *E. coli* are significantly the same with that of *S. dysenteriae* in the aqueous extract (15.67±1.20 mm and 15.33±0.34 respectively) and that observed in *S. dysenteriae*, 16.67±0.88 mm is significantly different from all other means observed. At 250 mg/ml zones of inhibition for *S. dysenteriae* in both extracts showed significant difference from all others (14.00±0.58 mm and 12.67±0.88 mm). At 125 mg/ml, higher zones were produced against *Salmonella typhi* (13.00±0.58 mm) but are significantly the same with 12.67±0.66 mm against *Escherisia coli*. Even at the least concentration of 62.5 mg/ml there were zones of inhibition of 10.33±0.34 mm against *Eschericia coli* and *Salmonella typhi* and 9.00±0.58 mm zone for *Shigella dysenteriae*. The result showed the methanol extract had higher activity on *E. coli* and *S. typhi* compared to *S. dysenteriae*. This is in line with the findings of Bahia’uddeen *et al.* (2017) whose result showed that the stem bark aqueous extract of *B. dalzielii* was active against *S. typhi* and *E. coli* with higher zones of inhibition of 19±0.00 mm for *S. typhi* at 50 mg/ml concentration where resistance was only observed in *E. coli* at 2.5 mg/ml concentration. Nwinyi *et al.* (2004) showed a contrary result where the aqueous extract of *Boswellia dalzielii* had no antimicrobial activity against the tested microorganisms. The methanol extract of the same plant was shown to possess antibacterial activity by Sylvester *et al.* (2016). A similar study conducted revealed that the methanol extract of the same plant had inhibitory effect on *E. coli*, *S. typhi* and *S. dysenteriae* at 200 mg/ml and 100 mg/ml with no significant difference but had no effect on *E. coli* and *S. typhi* at 50 mg/ml and 25 mg/ml where the mean zone of inhibition observed in *S. dysenteriae* was insignificant from those produced in the other organisms. In addition, aqueous stem bark of the same plant only presented antimicrobial activity on *E. coli* (19 mm at 80 mg/ml and 15.50 mm at 60 mg/ml) and *S. dysenteriae* (16.50 mm at 80 mg/ml) but *S. typhi* was resistant across all concentrations (Abdulazeez *et al.*, 2013). The positive control Ciprofloxacin 500 mg/ml produced 47 mm, 50 mm and 56 mm zones of inhibition respectively against *Eschericia coli*, *Shigella dysenteriae* and *Salmonella typhi* and 0mm for the negative control using 0.1 ml of DMSO.

In Table 3 the results for the minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of *B. dalzielii* stem bark extracts is shown. The MIC of methanol extract for *Eschericia coli* and *Salmonella typhi* is 7.81 mg/ml and 15.63 mg/ml for *Shigella dysenteriae*. This means that for *Eschericia coli* and *Salmonella typhi*, 7.81 mg/ml was the least concentration that inhibited the growth of the bacteria while for *Shigella dysenteriae*, the least concentration that inhibited their growth was 15.63 mg/ml for methanol extract. The MIC of the aqueous extract for all the three test organisms was 7.81 mg/ml as shown in Table 3. Positive control for all the organisms showed clear solution while turbidity was seen in all the negative controls.

The MBC of the methanol extract of *B. dalzielii* stem bark for *Eschericia coli* was 7.81 mg/ml. For *Shigella dysenteriae* the MBC for methanol extract was 125 mg/ml and for *Salmonella typhi* was 62.50 mg/ml. Results of the MBC of aqueous extract for *Eschericia coli* was 125 mg/ml and 31.25 mg/ml for both *Shigella dysenteriae* and *Salmonella typhi* as shown in Table 3. The MIC values of the two extracts for all the organisms is 7.81 mg/ml except for *S. dysenteriae* that had 15.63 mg/ml and their MBC values range from 7.81 mg/ml to 125 mg/ml. This is in contrast with the findings of Bahia’uddeen *et al.* (2017) who found out that lowest MIC value of 12.5 mg/ml was observed in *S. typhi* and 50 mg/ml in *E. coli* and MBC values did not exceed the corresponding MIC values by more than a factor of 2. A similar study conducted revealed that the MIC of both *S. typhi* and *E. coli* is 50 mg/ml while the MBC is 100 mg/ml and that of *S. dysenteriae* is 12.5 mg/ml (Mamza *et al.*, 2018) which does not also conform with the results of this research. Lower MIC was recorded in *Shigella dysenteriae* (12.5 mg/ml) in aqueous...
extract followed by *E. coli* then highest was recorded in *S. typhi* (50 mg/ml) and for the methanol extract, *S. dysenteriae* had lower MIC (12.5 mg/ml) and highest in the other organisms (25 mg/ml). In addition, Nas and Ali, (2017) studied the methanol and aqueous leaves extract of *B. dalzielii* and found that the extracts were active on the tested organisms with MBC range between 50 – 100 mg/ml.

Table 2: Effect of Different Solvents and Concentrations of Plant Extracts on the Growth of Some Clinical Isolates.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Extracts</th>
<th>Organisms</th>
<th>Concentration1 (62.5 mg/ml)</th>
<th>Concentration2 (125 mg/ml)</th>
<th>Concentration3 (250 mg/ml)</th>
<th>Concentration4 (500 mg/ml)</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. dalzielii</em></td>
<td>Methanol</td>
<td><em>E. coli</em></td>
<td>10.33±0.34^a</td>
<td>12.67±0.66^a</td>
<td>14.33±0.66^a</td>
<td>17.67±1.46^a</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td><em>S. typhi</em></td>
<td><em>S. typhi</em></td>
<td>10.33±0.34^a</td>
<td>13.00±0.58^a</td>
<td>14.67±0.34^a</td>
<td>17.33±0.66^a</td>
<td>Turbid</td>
</tr>
<tr>
<td></td>
<td><em>S. dysenteriae</em></td>
<td><em>S. dysenteriae</em></td>
<td>9.00±0.58a±cd</td>
<td>11.67±0.34bc</td>
<td>14.00±0.58b</td>
<td>16.67±0.98b</td>
<td>Clear</td>
</tr>
<tr>
<td>Aqueous</td>
<td><em>E. coli</em></td>
<td><em>E. coli</em></td>
<td>11.33±0.34^a</td>
<td>12.67±0.88^a</td>
<td>15.33±0.34^a</td>
<td>15.33±0.34^a</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td><em>S. typhi</em></td>
<td><em>S. typhi</em></td>
<td>9.33±0.66a±cd</td>
<td>12.00±0.58b</td>
<td>13.00±0.58bc</td>
<td>17.67±1.20c</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td><em>S. dysenteriae</em></td>
<td><em>S. dysenteriae</em></td>
<td>8.67±0.66b±cd</td>
<td>10.00±0.58^a±cd</td>
<td>13.00±0.58^a±cd</td>
<td>15.00±1.00^a±cd</td>
<td>Clear</td>
</tr>
</tbody>
</table>

NOTE: Means with different letters are significantly different from all other means and those sharing the same letters are significantly the same at p<0.05.

Table 3: Minimum Inhibitory (MIC) and Minimum Bactericidal Concentrations (MBC) of *B. dalzielii* Stem Bark extracts against Some Clinical Isolates.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Methanol extract</th>
<th>Aqueous extract</th>
<th>Positive Control</th>
<th>Negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>7.81±0.59</td>
<td>7.12±0.59</td>
<td>Clear</td>
<td>Turbid</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>15.63±0.59</td>
<td>125</td>
<td>Clear</td>
<td>Turbid</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>7.81±0.59</td>
<td>62.50</td>
<td>Clear</td>
<td>Turbid</td>
</tr>
</tbody>
</table>

The table (4) presents the result of antibacterial activity of partitioned fractions (mg/ml) of Boswellia dalzielii stem bark against *S. typhi*, *S. dysenteriae* and *E. coli*. The result shows that n-butanol fraction had higher activity against *E. coli* and *S. typhi* followed by ethyl acetate, aqueous and then the n-hexane fractions. In the case of *S. dysenteriae*, ethyl acetate fraction showed a higher activity, then n-butanol, aqueous and lastly the n-hexane fraction. The negative control (DMSO) showed no activity against all the organisms whereas the positive control (ciprofloxacin 200 mg/ml) recorded 41 mm, 42 mm and 30 mm respectively against *E. coli*, *S. typhi* and *S. dysenteriae*.

Table 4: Effect of *B. dalzielii* Stem bark Fractions and Concentrations on the Growth of the Clinical Isolates.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Solvent</th>
<th>Concentration (mg/ml)</th>
<th><em>E. coli</em></th>
<th><em>S. typhi</em></th>
<th><em>S. dysenteriae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. dalzielii</em></td>
<td>N Butanol</td>
<td>25</td>
<td>15.50±0.71ef</td>
<td>14.50±0.71ef</td>
<td>9.50±0.71hi</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>16.50±0.71bc</td>
<td>18.50±0.71cd</td>
<td>13.00±1.41f</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>19.00±0.00e</td>
<td>21.50±2.12h</td>
<td>16.50±0.71d</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>200</td>
<td>23.50±3.54a</td>
<td>23.50±2.83a</td>
<td>21.00±1.41b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>19.00±0.00e</td>
<td>21.50±2.12h</td>
<td>16.50±0.71d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>13.00±1.41bi</td>
<td>16.00±1.41e</td>
<td>19.00±1.41c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>13.00±1.41bi</td>
<td>16.00±1.41e</td>
<td>19.00±1.41c</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>200</td>
<td>22.00±2.83b</td>
<td>19.50±0.71c</td>
<td>23.00±2.83a</td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>25</td>
<td>8.50±0.71e</td>
<td>0.00±0.00k</td>
<td>0.00±0.00k</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>10.50±0.71j</td>
<td>0.00±0.00k</td>
<td>0.00±0.00j</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>13.00±1.41bi</td>
<td>8.00±0.00j</td>
<td>9.00±1.41g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>14.50±0.71g</td>
<td>8.50±0.71j</td>
<td>10.50±0.71h</td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>25</td>
<td>9.50±0.71el</td>
<td>10.50±0.71h</td>
<td>10.00±0.00h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>12.00±1.41f</td>
<td>12.00±2.83b</td>
<td>11.50±0.71f</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>13.50±0.71eb</td>
<td>13.50±2.12c</td>
<td>14.50±0.71e</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>15.50±0.71ef</td>
<td>15.00±2.83d</td>
<td>15.50±0.71d</td>
</tr>
</tbody>
</table>

NOTE: On each column, means with different letters are significantly different from all other means and those sharing the same letters are significantly the same (p<0.05).

This result is similar to Mohammed *et al.*, (2012) where the ethyl acetate and n-Butanol fractions of the leaves of the *B. dalzielii* was also active against *E. coli* but it was resistant at the lowest concentration of 50 mg/ml and for the aqueous extract it was only ac
ative at the highest concentration of 400 mg/ml which does not agree with the present finding. In addition the ethyl acetate fraction of the root of B. dalzielii showed significant activity on S. typhi (60.67 mm) with low activity on E. coli (10.33 mm) and the n- butanol fraction showed no activity on E. coli (00.00 mm) and good activity (26.33 mm) on S. typhi all at 1.5 mg/ml (Benjamin et al., 2019). Antimicrobial activity of n- butanol pooled fractions of B. dalzielii stem bark (mg/ml) against S. typhi, S. dysenteriae and E. coli in Table 5 showed that the fractions were more active against E. coli, then S. dysenteriae and least active against S. typhi. Th e positive control produced more zones of inhibition against S. dysenteriae (63 mm), then E. coli (57 mm) and lastly S.typhi (54 mm).

### Table 5: Effect of Butanol Sub-fractions of B. dalzielii Stem bark and Concentration on the Clinical Isolates.

<table>
<thead>
<tr>
<th>Plant/Fraction</th>
<th>Concentration (mg/ml)</th>
<th>E. coli</th>
<th>S. typhi</th>
<th>S. dysenteriae</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. dalzielii N Butanol</td>
<td>25</td>
<td>13.00±0.00</td>
<td>9.00±0.00</td>
<td>11.50±2.12</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>16.00±0.00</td>
<td>10.50±0.71</td>
<td>14.00±0.00</td>
</tr>
<tr>
<td>BAB</td>
<td>25</td>
<td>11.00±0.00</td>
<td>10.00±0.00</td>
<td>10.00±0.00</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>13.50±2.12</td>
<td>11.00±0.00</td>
<td>15.50±0.71</td>
</tr>
<tr>
<td>CAB</td>
<td>25</td>
<td>11.00±1.41</td>
<td>10.00±0.00</td>
<td>8.00±0.00</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>13.50±0.71</td>
<td>11.00±1.41</td>
<td>9.00±0.00</td>
</tr>
<tr>
<td>DAB</td>
<td>25</td>
<td>12.00±0.00</td>
<td>8.00±1.41</td>
<td>8.00±1.41</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>12.00±0.00</td>
<td>10.00±1.41</td>
<td>11.00±1.41</td>
</tr>
<tr>
<td>EAB</td>
<td>25</td>
<td>10.00±0.00</td>
<td>10.00±0.00</td>
<td>8.50±0.71</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>13.50±2.12</td>
<td>10.00±1.41</td>
<td>13.00±1.41</td>
</tr>
</tbody>
</table>

**NOTE:** On each column, means with different letters are significantly different from all other means and those sharing the same letters are significantly the same (p<0.05).

### CONCLUSION
Phytochemical screening of the crude extracts of B. dalzielii stem bark has revealed the presence of numerous phytochemicals that have been reported to possess antimicrobial activity. This shows that methanol and n-butanol are good solvents for extracting active compounds from medicinal plants. The results of the bioassay also showed that the crude extracts and fractions were active against all the clinical isolates which verify the continued use of the plant parts, especially the stem bark by local people for the treatment of microbial infection and other diseases.

### REFERENCES


