Phytochemical, Antibacterial and Toxicological Studies of Aqueous Stem Bark Extract of *Boswellia dalzielli*.

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**ABSTRACT**

The aqueous stem bark extract of *Boswellia dalzielli* was evaluated for its antibacterial activity against four bacterial strains by agar-well diffusion method and its phytochemical composition was determined. Its toxicity profile was also determined by studying its effect upon acute and sub-chronic administration in albino rats. The aqueous stem bark extract of *B. dalzielli* revealed the presence of tannins, glycosides, flavonoids, alkaloids, anthracene, saponin and saponin glycosides. The antimicrobial activity of the extract against *Staphylococcus aureus*, *Salmonella typhae*, *Esherichia coli* and *Shigella dysentrae* showed that it is effective against *Esherichia coli* and *Shigella dysentrae* at 60-80 mg/ml. Acute toxicity studies showed the LD₅₀ to be greater than 3000 mg/kg in rats. Sub-chronic toxicity studies showed that the extract caused significant (P < 0.05) changes in ALT, AST, ALP, total protein and total bilirubin level. There was also a significant (P < 0.05) change in haematological indices (WBC, PCV and Hb) at 1800 and 2700 mg/kg body weight. This result has shown that the consumption of the extract over a long duration and at higher dose level should be with caution as it seems to compromise liver function.

**KEYWORDS**: phytochemicals, antibacterial, LD₅₀, *Boswellia dalzielli*

**INTRODUCTION**

*Boswellia dalzielli* (Burseracea) is a tree plant of the savannah forest recognizable by its papery bark peeling off in a ragged manner. It is commonly known in Hausa as ‘hano’ or ‘harrabi’. This plant is very popular among the natives as a potent source of ethno medicine [1]. The extract from its leaves is used in the treatment of diarrhea in poultry. The root decoction of *B. dalzielli* and *Danielli oliverri* is taken to heal wounds. The fresh bark is eaten to induce vomiting and relieve symptoms of giddiness and palpitation. Oil from the leaves of *B. dalzielli* was reported to show anti-ulcer activity and reduced gastrointestinal motility [2]. The stem bark of *B. dalzielli* was also reported to be used as an antimalarial [3]. The aim of the present study was to evaluate the antibacterial activity, phytochemical composition and toxicity profile of aqueous stem bark extract of *B. dalzielli*.

**MATERIALS AND METHODS**

**Chemicals and reagents**: All the chemicals and reagents used were of analytical grade.

**Collection and Identification of Plant material**
The stem bark of *B. dalzielli* was obtained from a farmland in Sokoto State of Nigeria. The plant material was identified by a taxonomist in the Department of Botany, Usman Danfodiyo University, Sokoto. Voucher specimens were then deposited in the herbarium unit of the same Department.

**Preparation of plant material**
The stem bark of the plant was air-dried to a constant weight in an oven at a temperature of 45 °C. This was then pulverized using a mortar and pestle and sieved to a fine powder. The powdered stem bark (200 g) was soaked in 1.5 l of water for 24 h and filtered using a white muslin cloth. The filtrate was dried in vacuo at a reduced temperature of 45 °C. The percentage yield was 9.85 % w/w.
The extract was stored in sealed plastic container until required. A fresh 30% working standard of the extract was prepared by mixing 30g of the evaporated extract with 100ml of distilled water to obtain an aqueous extract as used by the locals when needed.

**Bacterial cultures**

Clinical isolates of Staphylococcus aureus, Salmonella typhae, Escherichia coli and Shigella dysenteriae obtained from the Department of Microbiology of Usmanu Danfodiyo University, Sokoto were used.

**Animals**

Wistar rats of both sexes weighting between (116-170 g) were obtained from the animal house of the Department of Microbiology in Ahmadu Bello University, Zaria and were housed in a well ventilated room under 12 h light / 12 h dark cycle and allowed to acclimatize for one week. The animals were fed with standard animals feed (Grower’s pelletized vital feed, Jos, Nigeria) and water was provided ad libitum.

**Phytochemical analysis**

The extract was evaluated for the presence of alkaloids, tannins, glycosides, saponins, volatile oil, anthraquinones, flavonoids and saponin glycosides using standard methods [4, 5, 6].

**Antibacterial studies**

This was done using the agar well diffusion method [7]. Standardized inoculum of each test organism was streaked by means of sterile wire loop onto the surface of the media so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 12.0 mm was used to bore wells into the agar plates. Subsequently, different concentrations of the extract were introduced into the well. The plates were allowed to set for an hour and finally inverted and incubated at 37 °C for 24 h. After this period, all the incubated plates were examined for any zone of inhibition following the sensitivity nature of the bacterium against the extract. The diameter of the zone of inhibition in millimeter were measured and recorded.

**Determination of LD₅₀**

This was determined using the limit test dose (Up and Down procedure) of Organization for Economic Co-operation and Development guidelines [8]. The test procedure is of value in minimizing the number of animals required to estimate the acute toxicity of a substance. Aqueous extract of B. dalzielli (3000 mg/kg body weight) was administered to five rats, one rat at a time orally using a syringe. The animal was observed for 48 h; during the first 30 min after dosing, periodically during 24 hours with special attention given during first 4 hours for any toxic symptoms and thereafter for a total of 14 days. LD₅₀ was expressed based on the survival rate of the animals.

**Oral Toxicity Studies**

Twenty five wistar rats weighing between (116 – 170 g) were randomly divided into five groups of 5 animals per group. Animals in each group were housed in standard metal animal cages under room temperature. Four of the groups were administered orally with aqueous extract of B. dalzielli (600, 900, 1800 and 2700 mg/kg body weight) per day for 28 days. The control (5th group) was given only drinking water through the same route. The animals were allowed free access to food and water and monitored for signs and symptoms of toxicity. The body weights of all the animals were monitored weekly. After the 28 days of treatment, animals were fasted overnight. On the 29th day, the animals were sacrificed and blood samples were collected. Anticoagulated blood was used for the analyses of haematological parameters while the coagulated blood was centrifuged at 3000 rpm for five minutes. The serum was collected using a micropipette and stored at -4°C for biochemical analysis.

**Assessment of Liver Function**

Serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by the method of Reitman and Frankel [9]; serum alkaline phosphatase (ALP) activity was determined using thymolphthalein monophosphate [10]; serum protein level was estimated by using biuret method [11]; serum albumin level by dye binding technique utilizing bromocresolgreen [12], and serum bilirubin level was estimated using method described by Jendrassik and Groff [13].

**Assessment of Kidney Function**

Serum creatinine level was measured by modified alkaline picrate method [14]; urea level by the method of urea berthelot colorimetric method [15]. Uric acid level was determined by utilizing its reaction property with Fe III [16]; sodium ion (Na⁺) and potassium ion (K⁺) were measured using flame emission spectrophotometer while bicarbonate (HCO₃⁻) was measured using titration method [17].
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Haematological indices
White Blood Cell (WBC) Count, Red Blood Cell (RBC) Count, Haemoglobin (Hb) and Packed Cell Volume (PCV) were also determined [18].

STATISTICAL ANALYSIS
The data are presented as mean ± SEM. The results obtained were statistically analysed by one-way ANOVA followed by Benforoni multiple comparisons, using Graphpad instat software. P value < 0.05 was considered significant.

RESULTS
Phytochemical analysis
Phytochemical screening of the aqueous stem bark extract of B. dalzielii revealed the presence of flavonoids, tannins, glycosides, alkaloids, anthracene, saponin and saponin glycosides as shown in Table 1

Table 1: Phytochemical constituents of B. dalzielii stem bark.

<table>
<thead>
<tr>
<th>PHYTOCHEMICAL GROUP</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanins</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
</tr>
<tr>
<td>Saponin glycosides</td>
<td>+++</td>
</tr>
<tr>
<td>Volatile oil</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>-</td>
</tr>
<tr>
<td>Terpenes</td>
<td>+</td>
</tr>
</tbody>
</table>
| ++: Present in large amount; ++: present in moderate amount; +: present in trace amount and -: Not detected

Antibacterial Studies
Table 2 presents the antibacterial activity of different concentration of the aqueous plant extract against Stapylococcus aureus, Salmonella typhae, Esherichia coli and Shigella dysentrae. The result shows that the extract had inhibitory effects at higher concentrations only on Esherichia coli and Shigella dysentrae.

Table 2: Inhibition of bacterial growth by aqueous stem bark extract of B. dalzielii

<table>
<thead>
<tr>
<th>Concentration (mgml⁻¹)</th>
<th>Stapylococcus aureus</th>
<th>Salmonella typhae</th>
<th>Esherichia coli</th>
<th>Shigella dysentrae</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>40</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>60</td>
<td>0.00</td>
<td>15.50</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>80</td>
<td>0.00</td>
<td>19.00</td>
<td>16.50</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Acute Toxicity Studies
All animals treated with the 3000 mg/kg limit dose of the extract of B. dalzielii were hyperactive to external stimuli such as touch in the first few hours after administration but later exhibited normal behavior throughout the 14 days observation period. The LD₅₀ of the extract is therefore greater than 3000 mg/kg.

Oral toxicity studies Body weight
The weights of rats after (28 days) administration of extract were compared with their initial weight before administration of extract. There was a significant (P<0.05) increase in the mean body weight of animals in the control group and those treated with lower doses of the extract (600-900 mgkg⁻¹) and a significant decrease in animals given the highest dose

Table 3: Mean body weights of rats before and after administration of aqueous stem bark extract of Boswellia dalzielii.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>119.10±1.22</td>
<td>177.40±9.42*</td>
</tr>
<tr>
<td>600</td>
<td>121.06±0.33</td>
<td>153.76±4.19*</td>
</tr>
<tr>
<td>900</td>
<td>137.56±0.98</td>
<td>146.05±2.80*</td>
</tr>
<tr>
<td>1800</td>
<td>143.40±1.32</td>
<td>139.95±7.08</td>
</tr>
<tr>
<td>2700</td>
<td>167.92±0.50</td>
<td>143.20±6.92*</td>
</tr>
</tbody>
</table>

*significant when compared with initial weight using unpaired student's t-test (P<0.05)

Liver function indices
The level of the hepatic enzymes, bilirubin, albumin and protein after the 28 days administration of extract are shown in Table 4. Alkaline phosphatase (ALP) and the transaminases concentration significantly (P<0.05) increased at 1800 mg/kg and 2700 mg/kg dose levels. This increase seemed to be dose dependent. Total bilirubin level in the
treated groups significantly (P<0.05) increased when compared to the control group while conjugated bilirubin level were unaffected. Total protein (P<0.05) concentration also increased significantly (P<0.05) in groups treated with 1800 mg/kg and 2700 mg/kg of extract while albumin level was only significantly (P<0.05) reduced at the highest dose level (2700 mg/kg).

### Table 4: Serum liver function indices in rats administered aqueous stem bark extract of *Boswellia dalzielii*

<table>
<thead>
<tr>
<th>Dose (mgkg⁻¹)</th>
<th>AST (UL⁻¹)</th>
<th>ALT (UL⁻¹)</th>
<th>ALP (UL⁻¹)</th>
<th>ALB (gdL⁻¹)</th>
<th>TP (gdL⁻¹)</th>
<th>TB (mgdL⁻¹)</th>
<th>CB (mgdL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.80±1.39</td>
<td>38.00±1.52</td>
<td>40.19±0.59</td>
<td>3.48±0.06</td>
<td>7.25±0.17</td>
<td>0.45±0.04</td>
<td>0.26±0.03</td>
</tr>
<tr>
<td>600</td>
<td>16.20±1.77</td>
<td>37.40±1.25</td>
<td>40.77±0.74</td>
<td>3.02±0.10</td>
<td>7.05±0.03</td>
<td>0.56±0.04</td>
<td>0.20±0.04</td>
</tr>
<tr>
<td>900</td>
<td>17.75±0.75</td>
<td>39.75±0.48</td>
<td>42.42±107</td>
<td>3.46±0.10</td>
<td>7.81±0.20</td>
<td>0.67±0.01*</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td>1800</td>
<td>22.75±0.75*</td>
<td>48.50±1.56*</td>
<td>51.28±1.11*</td>
<td>3.09±0.12</td>
<td>8.42±0.14*</td>
<td>0.92±0.04*</td>
<td>0.19±0.03</td>
</tr>
<tr>
<td>2700</td>
<td>24.60±0.81*</td>
<td>51.40±2.11*</td>
<td>61.25±1.70*</td>
<td>2.93±0.16*</td>
<td>8.52±0.08*</td>
<td>1.06±0.04*</td>
<td>0.18±0.03</td>
</tr>
</tbody>
</table>

Values are mean ± standard error of mean, n=5,*significantly different from control (P < 0.05).

### Table 5: Renal function indices in rats administered aqueous stem bark extract of *Boswellia dalzielii*

<table>
<thead>
<tr>
<th>Dose (mgkg⁻¹)</th>
<th>Urea (mgdL⁻¹)</th>
<th>Creatinine (mgdL⁻¹)</th>
<th>Uric acid (mgdL⁻¹)</th>
<th>Na⁺ (mmolL⁻¹)</th>
<th>K⁺ (mmolL⁻¹)</th>
<th>HCO₃⁻ (mmolL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36.36±3.20</td>
<td>1.40±0.20</td>
<td>3.73±0.63</td>
<td>133.56±1.96</td>
<td>4.68±0.30</td>
<td>23.80±0.97</td>
</tr>
<tr>
<td>600</td>
<td>34.55±1.00</td>
<td>1.06±0.15</td>
<td>4.19±0.62</td>
<td>132.72±1.03</td>
<td>4.22±0.12</td>
<td>24.60±0.87</td>
</tr>
<tr>
<td>900</td>
<td>35.91±2.81</td>
<td>1.26±0.11</td>
<td>3.64±0.37</td>
<td>136.05±2.27</td>
<td>4.38±0.35</td>
<td>26.75±0.75</td>
</tr>
<tr>
<td>1800</td>
<td>36.36±1.05</td>
<td>0.86±0.04*</td>
<td>4.09±0.19</td>
<td>135.38±1.60</td>
<td>4.15±0.06</td>
<td>23.25±0.75</td>
</tr>
<tr>
<td>2700</td>
<td>45.85±1.37*</td>
<td>1.14±0.10</td>
<td>4.20±0.43</td>
<td>134.76±0.49</td>
<td>3.70±0.14*</td>
<td>21.80±0.37</td>
</tr>
</tbody>
</table>

Values are mean ± standard error of mean. n=5,*significantly different from control (P < 0.05).

### Haematological indices

The Haematological indices of the animals following the 28 days oral administration of aqueous stem bark extract of *B. dalzielii* are presented in Table 6. Red blood cell (RBC), packed cell volume (PCV) and hemoglobin (Hb) level significantly (P < 0.05) reduced at 1800 mg/kg and 2700 mg/kg dose while the white blood cell (WBC) level significantly (P < 0.05) increased at the same dose levels.
DISCUSSION

Phytochemicals which possess many ecological and physiological roles are widely distributed as plant constituents. Plants can synthesize and accumulate in their cells a great variety of phytochemicals including alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins, lignins and lignans [19]. Phytochemical analysis of extract of stem bark of *B. dalzielii* revealed the presence of flavonoids, tannins, alkaloids and other phenolic compounds. The antibacterial effect of the extract observed may be indicative of the presence of some metabolic toxins for bacteria. There has been an earlier report on the antibacterial activity of *B. dalzielii* stem bark and oil from its leaves [2, 20]. The plant could be an important precursor for the development of an antimicrobial.

*B. dalzielii* at a single dose of 3000 mg/kg b.w did not show any toxicity signs. The result suggests that *B. dalzielii* is not toxic after an acute exposure in rats. In the sub-chronic toxicity study, a 28-day study was considered since it is well accepted for eliciting any toxicity on long term exposure [21].

The measurement of the activities of enzymes in tissues and body fluids plays a significant and well known role in investigation and diagnosis of diseases [22]. When a liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the bloodstream. Alkaline phosphatase (ALP), Alanine aminotransaminase (ALT) and Aspartate aminotransaminase (AST) are important markers for liver functions [23]. Results from the oral toxicity study showed a significant (p<0.05) increase in ALT, AST and ALP levels in animals administered 1800–2700 mg/kg. The observed dose-dependent increase in the activities of these enzymes can be traced to possible necrosis of the tissue where they are found. Serum ALT and AST levels are always found to increase in liver cell damage and the greater the degree of liver damage, the higher the activities of both enzymes [17]. Alkaline phosphatase is a marker enzyme for the plasma membrane and endoplasmic reticulum; it is often used to assess the integrity of plasma membrane [24]. The observed increased activity of this enzyme could be due to an injury of hepatocytes in plasma membrane thus leading to its leakage into the bloodstream.

Bilirubin is the major breakdown product that results from the destruction of red blood cells, it is removed by the liver; it is thus a good indicator of liver function [25]. Total bilirubin level increased significantly in groups that received the higher doses (900 – 2700 mg/kg). An increase in bilirubin level results from functional or mechanical impairment in biliary excretion and is found in most cases of acute hepatitis and cholestasis [26]. This dose dependent increment augments the fact that there may be evidence of liver dysfunction.

Albumin level significantly reduced at the highest dose level. This decrease could be as a result of decrease in the number of cells responsible for albumin synthesis in the liver through necrosis [27]. The study also showed that total protein level significantly (p<0.05) increased at the higher dose levels (1800 mg/kg and 2700 mg/kg). The enhancement in the level of serum protein has been reported to be an indication of tissue injury and reflection of hepatic injury [28]. This could also be due to exposure to any environmental stress which could lead to an increase in the production of stress proteins in the body [29].

The renal function indices of the treated groups were almost unaltered except for urea and potassium whose concentrations were significantly (p<0.05) different from control group only at the highest dose level (2700 mg/kg) and creatinine level that was significantly (p<0.05) reduced in group treated with 1800 mg/kg of the extract. This might indicate a near normal nephrotic function.

The decrease in PCV, Hb and RBC count observed may be an indication of anaemia which may occur as a result of inhibition of RBC production by bone marrow or haemolysis of blood cells by active component of the plant extract. The increased level of total bilirubin can thus be accounted for by the haem degradation that follows red blood cell destruction [30]. The increased in WBC count might
suggest a fight against an immunogen; which may be part of the constituents of the plant extract. The results of the study suggest that the administration of the extract at higher doses may be potentially toxic to the liver and red blood cells; this supports an earlier report [31].

CONCLUSION
The present study has shown that the extract could be a precursor for antibiotics and that its use for a long duration should be with caution as this could compromise the functioning of vital organs in the body.

REFERENCES