Phytochemical Screening and Haemopoetic Study of the Ethanolic Root Extract of *Baphia nitida* Lodd on Albino Rats

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Abstract: The potential haematological effects associated with the oral administration of ethanolic root extract of *Baphia nitida* was investigated in rats. Phytochemical screening of *Baphia nitida* root extract revealed the presence of alkaloids, saponins, terpenes, tannins and flavonoids. The median lethal dose (LD$_{50}$) studies revealed that the mice treated intraperitoneally (i.p.) with *Baphia nitida* root extract tolerated a considerable high dose of 5000 mg/kg of body weight without any manifestation. Twenty five Albino rats were randomly divided into five groups. The first 3 groups were the test groups. The fourth group served as negative control, the fifth group served as the positive control with 5 rats each. Groups I, II and III were gavaged with the extract of *Baphia nitida* in concentrations of 500 mg/kg, 1000 mg/kg and 1500 mg/kg respectively for 21 days. The negative control group received normal saline while the positive control received 10 mg/kg of folic acid (standard drug). The extract at the doses administered was found to increase in a dose-related fashion PCV and Hb ($p < 0.05$ for 500 mg/kg and $p < 0.05$ for 1000 mg/kg and 1500 mg/kg), RBC ($p < 0.05$ for 100 mg/Kg and 1500 mg/kg) and marginal increases that were not significant ($p < 0.05$ for 500 mg/kg); MCH and MCV ($p < 0.05$ and $p < 0.01$ for 1000 mg/kg and 1500 mg/kg respectively) 500 mg/kg was not significant. MCHC recorded no significant change. WBC recorded marginal increases that were not significant ($p < 0.05$), similarly, the differential white blood cell recorded marginal increases that were not significant, except lymphocytes that recorded significant increase in group III ($p < 0.05$). The result of this study thus indicates haematopoietic potentials of the extract and could possibly remedy anaemia.

Keywords: *Baphia nitida*; haemopoiesis; anaemia; gavage; phytochemical screening.
1. Introduction

*Baphia nitida* Lodd is belonging to Fabaceae family commonly known as camwood. *Baphia nitida* is very abundant in under wood of the African dense forests. Camwood is a fast-growing smallish tree that can be as tall as 5 meters. Leaves are simple, alternately arranged, oblong-elliptic, 10-15 cm long and strongly tipped. Flowers are bisexual, white, solitary or up to 4 grouped together on the main branch, faintly fragrant and inconspicuous (Wee, 2003). Fruits are straight pods, 10-15 cm long and 12-16 mm wide, sharply pointed at both ends and with 2-4 brown, flat seeds. Many-stemmed erect shrub or small tree up to 9 m tall with glabrous to densely pubescent branchlets. Leaves and entire; stipules quickly caducous; petiole 1–4 cm long, prominently thickened at base and at top; obovate or lanceolate, 5–21 cm × 3–9 cm, base rounded to cuneate, apex acuminate, slightly leathery, leaves almost glabrous, pinnately veined. Fruit a compressed pod 8–16.5 cm × 1–1.5 cm, pointed at both ends (Soladoye, 1985; Wee, 2003).

The young leaves are used as vegetables, especially in Ikono and Ini Local Government Areas of Akwa Ibom State and the Igbo-speaking areas of eastern Nigeria, as well as folder for goats. The twigs and small branches are popularly used as chewing-sticks. The pulp is sweet and silky (Etukudo, 2003). Leaves of *Baphia nitida* have been shown to possess diverse pharmacological properties, acting as a sprain, nosebleed, arthritis and asthma (Poorter et al., 2004). In essence, healers and patients in many communities still rely on locally available phytomedicines. As part of a broad based search for plants with anaemic activity, this study was conducted to investigate the possible usefulness of the ethanolic root extract of *Baphia nitida* in the treatment of anaemia.

2. Materials and Methods

2.1. Plant Collection and Identification

The fresh roots of *Baphia nitida* used in this research were obtained from Ibiaku Offot in Uyo Local Government Area in Akwa Ibom State, Nigeria in April 6th, 2012. The plant samples were identified and authenticated by Dr. (Mrs.) M. E. Bassey, a plant taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. The voucher specimen were prepared and deposited in the herbarium (*Baphia nitida*: Etim, UUH 3017 Uyo) of the Department.

2.2. Preparation of Plant Extracts

*Baphia nitida* roots sample were shed-dried and pulverised by graded using a manual blender. Five hundred grams (500 g) of the coarse powdered sample of *Baphia nitida* roots was macerated with 1000 mL of 70% ethanol and allowed to stand for 72 hours. The solution was filtered using glass
funnel packed with cotton wool. The filtrate was evaporated to dryness by heating in a water bath at 40 °C which give a yield of 56.8 g of semi-dry extract with black colour. This was reconstituted in distilled water to an appropriate concentration for administration and phytochemical screening.

2.3. Collection and Maintenance of Animals

Healthy male and female albino rats weighing 150 – 250 g were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo and maintained according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals.

2.4. Phytochemical Screening

The experiment was carried out in the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo. The phytochemical screening involves the simple chemical test to detect the presence of secondary metabolites. The methods of Trease and Evans (2009) were used for phytochemical screening.

2.5. Determination of Median Lethal Dose (LD50)

Swiss albino mice weighing 25-32 g were dose by the intraperitoneal (i.p.) route using the method of Lorke (1983). The animals were administered with 5000 mg/kg, 4000 mg/kg, 3000 mg/kg, 2000 mg/kg, 1000 mg/kg and 500 mg/kg of Baphia nitida extract in 5 groups of 5 mice each. The animals were observed for manifestation of physical signs of toxicity and the number of death within 24 hours was recorded. The LD50 was calculated as the geometric mean of the maximum dose producing 0% mortality and the minimum dose producing 100% mortality. Food was withdrawn for 18 h before the onset of the experiment according to methods of Amresh et al. (2008).

\[ LD_{50} = \sqrt{D_0 \times D_{100}} \]

Where, \( D_0 \) = Maximum dose producing 0% mortality, \( D_{100} \) = Minimum dose producing 100% mortality.

2.6. Experimental Design

Twenty five (25) Albino rats weighing 150 – 250 g were randomly assigned into 5 sets: 5 animals per group for haematological and haemopoietic activities. The 10%, 20% and 30% of LD50 were used as working doses (low, middle and high dose respectively).
Groups:
Group I received 10 mL/kg of normal saline;
Group II received 10 mg/kg of haematinic (folic acid);
Group III received 500 mg/kg of Baphia nitida extract;
Group IV received 1000 mg/kg of Baphia nitida extract;
Group V received 1500 mg/kg of Baphia nitida extract.
The daily administration (orally) was for twenty one days (21-days) and during these period animals were allowed for free access to feed and water ad libitum.

2.7. Blood Samples Collection

After twenty one days of oral administration of the soluble fraction of the two extracts, forty eight hours after the last dose were administered to each of the groups, the animals were anesthetised with chloroform, dissected to exposed the cardiac cavity of the heart, blood was obtained using a sterile syringe (5 mL) by cardiac puncture and carefully discharged into ethylene diamine tetraacetic acid (EDTA) bottle by running it down the side of the bottle, covered and rolled gently to mix with EDTA so as to avoid clotting. The sample bottles were labelled accordingly for all the 5 groups.

2.8. Haematological Analysis

The methods of Lewis et al. (2001) were adopted to determine the packed cell volume (PCV), haemoglobin concentration (Hb conc.), red blood cell count (RBC count), white blood cell count (WBC count), platelet count (PLT count), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), absolute count of lymphocytes (Lym.#), absolute count of neutrophils (Neut.#), platelet distribution width (PDW), mean platelet volume (MPV), reticulocyte count (Retics. count) and mean corpuscular haemoglobin (MCH). All the haematological parameters was analysed using Mindary 5 Path Differential BC 5300 Autoanalyser at University of Uyo Teaching Hospital (UUTH), Uyo.

2.9. Statistical Analysis

Data were expressed as mean ± S.E.M (standard error of the mean) of three replicates and were subjected to statistical analysis using the student’s t-test using SPSS by comparing the control with the treated groups. Probability limit was set at ninety five percent (95%) level of significance (P < 0.05).

3. Results

The results of phytochemical screening of Baphia nitida are summarized in Table 1. Baphia
**Baphia nitida** root extract revealed the presence of alkaloids, saponins, terpenes, tannins and flavonoids and cardiac glycosides. The median lethal dose (LD$_{50}$) studies revealed that the mice treated intraperitoneally (i.p.) with *Baphia nitida* root extract tolerated a considerable high dose of 5000 mg/kg of body weight without any manifestation.

Tables 2 and 3 show oral administration of *Baphia nitida* extract for 21 days produced various effects on the haematological parameters of Wistar Albino rats. The results of haematological indices showed a marginal dose related declined in body weight of the animals in test groups on the final day, this changes were however significant (P < 0.05), except group III (1500 mg/kg) that was significantly higher than group 1 (P < 0.05). The mean values of the PCV, haemoglobin concentration, red blood cell count, MCH, MCHC, MCV, white blood cell count and the differential white blood cell count in control and rats gavaged with ethanolic root extract of *Baphia nitida* are shown in Table 1. The test groups I, II and III showed significant increases in PCV, total haemoglobin concentration and RBC count that were dose related.

The PCV was significantly lower in the control group, and was compared to the test groups, (P < 0.01) when compared to group I (37.6 ± 1.55), (P < 0.001) when compared to groups II and III animals (37.63 ± 1.58 and 38.87 ± 4.52 respectively). The increase in PCV of rats gavaged with 1500 mg/kg body weight (38.87 ± 4.52), was significantly higher (P < 0.05) than those gavaged with 1000 mg/kg and 500 mg/kg body weight respectively (Table 1). Increase in haemoglobin (Hb) concentration followed the same trend as in PCV, again the increases in test groups were dose dependent. Red blood cell count was not significantly different from control except in groups II and III gavaged with 1000 mg/Kg and 1500 mg/kg body weight respectively that the increases were significantly higher (P < 0.05) than the control group, increases in test groups were dose dependent and were significant.

The mean corpuscular haemoglobin (MCH) rises in test groups in a dose related fashion. The increase in MCH in group I was not significantly different from group IV (control). But groups II (19.13 ± 0.71) and III (18.2 ± 0.36) were significantly (P < 0.05 respectively) higher than group 1 (control). Mean corpuscular haemoglobin concentration (MCHC) in both the control and test groups were not statistically significant (P < 0.05). Similarly, the mean corpuscular volume (MCV) was marginally higher in group II (58.07 ± 0.68) than group IV (control) (51.45 ± 1.06), but the increase in group I and II were significantly higher than group IV (P < 0.05) (Table 2).

There were marginal increases of WBC that were dose-related in the test groups. These increases were however not significantly different from group IV (P < 0.05). There were marginal increases in the groups gavaged with the extract of *Baphia nitida* that were dose dependent, again these increases were not significantly different from the control group, except lymphocytes (LYM#) in group III that was significantly different from group IV (P < 0.05). In similar manner platelet showed a
marked increase that was significant (P < 0.05) in groups I, II and III when compared with group IV (control) Table 3.

**Table 1. Phytochemical analysis of Baphia nitida root extract**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
</tr>
<tr>
<td>Terpenes</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td></td>
</tr>
<tr>
<td>Salkowski</td>
<td>++</td>
</tr>
<tr>
<td>Keller Killiani’s test</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
</tbody>
</table>

Note: +++ = High concentration, ++ = Moderate concentration, + = Trace concentration, - = Absent.

**Table 2. Hematological indices of rat treated with ethanolic extract of Baphia nitida roots**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>RBC (10^6/µL)</th>
<th>HGB (g/dL)</th>
<th>HCT (PCV) (%)</th>
<th>MCV (fL)</th>
<th>MCH (Pg)</th>
<th>MCHC (dL)</th>
<th>MPV (fL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Low dose</td>
<td>6.59±0.35</td>
<td>12.03±0.12*</td>
<td>37.6±1.55</td>
<td>57.07±2.06*</td>
<td>15.3±0.82</td>
<td>32.03±1.10</td>
<td>6.73±0.25</td>
</tr>
<tr>
<td>II</td>
<td>Middle dose</td>
<td>6.31±0.38</td>
<td>12.07±0.29*</td>
<td>37.63±1.85</td>
<td>58.07±0.68*</td>
<td>19.13±0.71*</td>
<td>32.97±1.08</td>
<td>6.67±0.15</td>
</tr>
<tr>
<td>III</td>
<td>High dose</td>
<td>6.74±0.68*</td>
<td>12.27±1.46*</td>
<td>38.87±4.52*</td>
<td>56.07±1.45</td>
<td>18.2±0.36*</td>
<td>32.4±0.89</td>
<td>6.33±0.23</td>
</tr>
<tr>
<td>IV</td>
<td>-ve Control</td>
<td>6.41±0.22</td>
<td>11.7±0.14</td>
<td>35.5±0.57</td>
<td>51.45±1.06</td>
<td>14.3±0.85</td>
<td>32.95±0.92</td>
<td>6.4±0.14</td>
</tr>
<tr>
<td>V</td>
<td>+ve Control</td>
<td>5.7±0.7</td>
<td>15.6±0.5</td>
<td>48.5±0.3</td>
<td>82.5±0.4</td>
<td>30.6±0.3</td>
<td>34.8±0.3</td>
<td>7.2±0.4</td>
</tr>
</tbody>
</table>

Note: Results are expressed as mean values ± standard errors of mean. * Significant (P < 0.05).

4. Discussion

The results of this work showed that Baphia nitida root are moderate in cardiac glycosides. Similar results were obtained by Farine et al. (1996) and Trease and Evans (2009) who worked on Morinde citrofolia and Digitalis pupurea respectively. They reported the presence of cardiac glycosides in these plants. Cardiac glycosides can be used in the treatment of diseases associated with the heart and they are currently used by herbalist to treat tumour in Akwa Ibom state (Piett, 2000). The cardiac glycosides found in Baphia nitida root could be used for treatment of heart disease problems. In Baphia nitida root, saponins are present in trace amount. This result is in line with the study of Okoko (2011) who reported that the presence of saponins in Mucuna pruriens. The work indicated that

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saponins have the properties of precipitating proteins, cholesterol-binding and haemolysis. Flavonoids were present in *Baphia nitida* root in moderate amount. Trease and Evans (2009) stated that some of the biological functions of flavonoids include protection against allergies, free radicals, platelet aggregation microorganisms, ulcers, hepatotoxins and tumors (Okwu, 2004).

Table 3. Platelet and white blood cell and its differential count of Wistar Albino rats treated with ethanolic extract of *Baphia nitida* root

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>PLT (10³ µL)</th>
<th>PDW (fL)</th>
<th>WBC (10³/µL)</th>
<th>LYM # (10³/µL)</th>
<th>NEUT # (10³/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Low dose</td>
<td>796.67±125.89*</td>
<td>7.73±0.68</td>
<td>14.87±5.92</td>
<td>13.17±5.00</td>
<td>1.43±0.91</td>
</tr>
<tr>
<td>II</td>
<td>Middle dose</td>
<td>791±64.95*</td>
<td>7.5±0.2</td>
<td>15.37±4.36</td>
<td>13.2±3.42</td>
<td>1.8±0.85</td>
</tr>
<tr>
<td>III</td>
<td>High dose</td>
<td>797.33±143.55*</td>
<td>7.5±0.17</td>
<td>15.77±1.80</td>
<td>13.8±1.44*</td>
<td>1.6±0.44</td>
</tr>
<tr>
<td>IV</td>
<td>-ve Control</td>
<td>805±0.01</td>
<td>7.25±0.07</td>
<td>13.2±2.69</td>
<td>13.4±2.26</td>
<td>1.45±0.35</td>
</tr>
<tr>
<td>V</td>
<td>+ve Control</td>
<td>738±52.7</td>
<td>16.4±2.2</td>
<td>12.8±4.6</td>
<td>8.5±1.6</td>
<td>8.5±1.6</td>
</tr>
</tbody>
</table>

Note: Results are expressed as mean values ± standard errors of mean. * Significant (P < 0.05).

In some parts of the World and indeed Nigeria, local medicinal herbs are employed in the management of various diseases. For instance, the erythropoietic values of extracts of pumpkin leaf and *Sorghum bicolor* have been previously reported (Ajayi et al., 2000; Ogwu, 2002). The haematopoietic activities of *Baphia nitida* have been investigated in this study. The determination of haematological indices provides physiological information on a proper blood assessment in the body. In this study, rats gavaged with ethanolic root extract of *Baphia nitida* recorded significant increases in packed cell volume, haemoglobin concentration, and red blood cell counts. White blood cells were also estimated. The mean values of the packed cell volume, haemoglobin concentration, red blood cell count, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume, white blood cell count and the differential white cell in control and rats gavaged with ethanolic root extracts of *Baphia nitida* are shown in Tables 2 and 3. The test groups I, II and III showed significant increases in PCV, total haemoglobin concentration and RBC count as compared to the control. The increases in PCV of test groups were significantly different from the control group (i.e. group IV) (P < 0.05). Increases that were dose-related were observed in the groups gavaged with *Baphia nitida* extract. Group III (1500 mg/kg) was significantly higher than groups II (1000 mg/kg) and III (500 mg/kg) (P < 0.05). There were increases in haemoglobin concentration that followed the same trend as in PCV. There were increases in red blood cell count that were dose-related and were not significant except in groups gavaged with higher doses (1500 mg/Kg and 1000 mg/kg body weight) of
the extract that the increases were significantly higher (P < 0.05) than group IV (Control). From the foregoing the extract showed an enhanced erythropoietic activity. The MCH and MCV also recorded increases in a dose-related fashion. Although, group I was not significantly different from group IV (control), higher dosage showed significant change (1500 mg/kg and P < 0.05 respectively). MCHC recorded no significant change. It is interesting however to note that high dosage of the extract (1500 mg/kg and 1000 mg/kg) significantly increase blood parameters such as PCV, Hb concentration, RBC counts, MCH and MCV. Anaemia by definition is a state of lower than normal concentration of haemoglobin that falls below the mean for a normal population to two standard deviations (Ibu, 1999). The least PCV value was recorded in group 2 with a lowest dose of 200 mg/kg to be 59.50 ± 1.50% and thereafter increase in a dose-related fashion. The ingestion of this extract may possibly be an acceptable blood booster in an anaemic condition targeted at the haematopoietic system. The mechanism of action is however not ascertain, but three possibilities have been postulated, it is possible that Baphia nitida components stimulate the kidney directly to cause formation and secretion of erythropoietin to stimulate haematopoiesis, or could be due to the high iron content of the plant, and if this be the case, it will then lend credence to the earlier report that erythropoietic value of pumpkin leaf extract is the function of the high level of protein, iron and vitamins found in the plant (Ajayi et al., 2000).

In this work, a marked increase in packed cell volume, haemoglobin concentration were observed coupled with raised red blood cell count that could be as a result of a direct effect of the extract on the haematopoietic systems. This increase was most pronounced at higher dosage of the extract (1500 mg/kg and 1000 mg/kg).

5. Conclusions

*Baphia nitida* root extracts contains bioactive components and are good potentials for clinical applications as they significantly increased blood parameters with good haematopoietic potentials. The results lead to the conclusion that *Baphia nitida* root might possess haematopoietic activity that could possibly remedy anaemia. Based on the findings from this research work, further investigation is therefore necessary: quantify the bioactive component responsible for haemopoietic activities and further experimental investigations are also needed to exploit its relevant therapeutic effect to substantiate its ethnomedicinal usage of this plant.

References


