Comparative Study of The Phytochemical Analysis And Mineral Constituent of The Powdered Telfairiaocidentalis, Vernomiaamygdalina, And Hydrophyllumvirginianum: An Index of Erythropoiesis

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Abstract: The comparative study on Telfairiaocidentalis, Vernomiaamygdalina, Hydrophyllumvirginianum was carried out to determine the major element associated with erythropoiesis such as iron, copper and cobalt and also phytochemical that plays the role of antioxidant such as polyphenol and flavonoid; An index of erythropoiesis. Telfairiaocidentalis, Vernomiaamygdalina, Hydrophyllumvirginianum were collected, rinsed and dried and Chemical tests were carried out on the powdered specimens using standard procedures to identify the constituents as described by Trease and Evans and also Mineral analysis using Atomic absorption Spectrophotometer. The qualitative analysis result of Phytochemical showed the presence of Polyphenol and Flavonoid the present of which have antioxidant activity and also the three elements Fe, Cu, Co which are related to erythropoiesis. The three plants show the presence of antioxidant defense and mineral constituent associated with erythropoiesis with fluted pumpkin showing the highest value when compared to bitter leaf and water leaf. It can be concluded that fluted pumpkin has a better erythropoietic potential when compared to bitter leaf and water leaf.

Keywords: Erythropoiesis, Hydrophyllumvirginianum, Phytochemicals, Telfairiaocidentalis, Vernomiaamygdalina.

1. Introduction

Hemolytic anemia is a condition in which there are not enough red blood cells in the blood, due to the premature of red blood cells. It is also a form of anemia due to hemolysis, the abnormal breakdown of red blood cells (RBCs), either in the blood vessels (intravascular hemolysis) which does occur in severe acute exacerbations and in some profoundly hemolytic patient(1) or elsewhere in the human body (extravascular). Hemolytic anemia involves the abnormal and accelerated destruction of red cells and in some anemias, their precursors. Increased indirect breakdown of hemoglobin, which may result in: increased bilirubin level (mainly- indirect-reacting) with jaundice increased fecal and urinary uroblinogen and hemoglobinemia, hemoglobinuria and hemosiderinuria (where there is significant intravascular hemolysis).(2)

In general hemolytic anemia occurs as a modification of the RBC life cycle. That is, instead of being collected at the end of its useful life and disposed of normally, the RBC disintegrated in a manner allowing free iron-containing molecules to reach the blood. Sickle Cell disease is a type of hemolytic genetic disorder with high morbidity and mortality (3) It is perhaps then helpful to understand the physiology of the RBC and things that can go wrong to cause it to “die” prematurely, with their complete lack of mitochondria, RBC rely on glycolysis for the materials needed to reduce oxidation damage. Any limitations of glycolysis can result in more susceptibility to oxidative damage and a short or abnormal lifecycle. If the cell is unable to signal to the reticuloendothelial phagocytes by externalizing phosphatidylserine, it is likely to lyse through uncontrolled means.(4)

The cause of hemolytic anemia could be of congenital hemolytic anemia or acquired hemolytic anemia. They may be classified according to the means of hemolysis, being either intrinsic in cases where the cause is related to the red blood cell RBC itself, or extrinsic in cases where factors external to the RBC dominate intrinsic effects may include problems with RBC proteins or oxidative stress handling, whereas external factors include immune attack and microvascular angiopathies (RBCs are mechanically damaged in circulation) Maellaro et al 2013.(5)
Acquired hemolytic anemia may be caused by caused by immune-mediated (e.g. mycoplasma pneumonia infection or systemic lupus erythematosus) drugs and other miscellaneous cause such as burns and lead poisoning from the environment. Some of the symptoms of hemolytic anemia includes: Dark urine, Enlarged spleen, fatigue, fever, pale skin color, rapid rate, shortness of breath, yellow skin color (jaundice).

Erythropoiesis is the process by which red blood cells (erythrocytes) are produced. It is stimulated by decreased O₂ in circulation, which is detected by the kidneys, which then secrete the hormone erythropoietin. This hormone stimulates proliferation and differentiation of red cell precursors, which activates increased erythropoiesis in the hemopoietic tissues, ultimately producing blood cells. In postnatal birds and mammals (including humans), this usually occurs within the red bone marrow. In the early fetus, erythropoiesis takes place in the mesodermal cells of the yolk sac. By the third or fourth month, erythropoiesis moves to the spleen and liver. After seven months, erythropoiesis occurs in the bone marrow. Decreased level of physical activity can cause a increase in erythropoiesis. However, in humans with certain diseases and in some animals, erythropoiesis also occurs outside the bone marrow, within the spleen or liver. This is termed extramedullary erythropoiesis.

Recently, some plant such as fluted pumpkin (telfairiaocidentalis) better leaf (vernoniaamygdalina), water leaf (hydrophyllumvirginianum). Was demonstrated to have effect on erythropoiessis (the pumpkin patch 2007). The result of this finding provokes interest in determining whether same plants will have effects on other anemia such as hemolytic anemia.

II. Materials And Methods

Collection of plant samples
Fresh leaf samples were collected from the Institute of Agricultural Research (IAR) Ahmadu Bello University farm, Identified and authenticated at the herbarium Section of Biological science Ahmadu Bello University, Zaria

Processing of plant samples
The fresh leaf of Telfairiaocidentalis, Vernoniaamygdalina, and Hydrophyllumvirginianum were rinsed and destalked after collection, this was followed by air-drying at room temperature (235°C). The dried samples were homogenized using an automated electric blender and stored in an airtight container prior to analysis.

Phytochemical screening
Chemical tests were carried out on the powdered specimens using standard procedures to identify the constituents as described by Trease and Evans (1989) and Harborne (1973). Alkaloids:
70ml of 10% HCl will be added to 4g of each sample in appropriately labeled conical flasks and boiled for 10 mins. Each boiled sample will be filtered and allowed to cool. The filtrates will then be poured into four labeled test tubes. Few drops of Dragendoff's, Mayer's, Wagner's reagents will be added to each test tube separately. Alkaloids will be recorded as present in the sample if turbidity or a brownish precipitate is observed

Saponins:
4g of each sample will be dissolved in distilled water and heated for 2–5 mins. The mixtures will be filtered, allowed to cool and shaken continuously for 2 mins to induce the production of froth. They will then be left to stand for 15 mins. The observation of frothing will be an indicative of presence of saponin.

Test for tannins:
1g of each sample will be heated with 20ml of water for 5 mins in appropriately labeled test-tubes. Each solution will be cooled and then filtered. 1ml of each filtrate will be diluted with 5ml distilled water in a test tube; few drops of 0.1% ferric chloride solution will be added. A characteristic blue, blue-black, green or blue-green colour and precipitate indicate the presence of tannin.

Anthraquinones:
1g of each sample will be shaken with 10ml of ferric chloride solution mixed with 5ml of HCL. Each mixture will be heated in a water bath for 10–15 mins, filtered and allowed to cool. The filtrate was extracted with chloroform and shaken gently. The clear layers at the base were pipette into test tubes and 2ml each of ammonia solution was added. An observation of a delicate pink rose colour indicated the presence of anthraquinones.

Test For Flavonoids
Weigh about 0.2 gm plant extract in separate test tubes and dissolved diluted Sodium hydroxide and add diluted Hydrochloride. And observe for yellow solutions that turn colorless. This indicates the presence of flavonoids.

**Test For Polyphenol**

2cm of the extract was diluted with distilled water in the ratio of 1:4 and few drops of 10% Iron (III) chloride solution was added. A greenish colour is observed which indicates the presence of polyphenols.

**Mineral Analysis**

9ml of concentrated HCl was added into 1.5g of the sample, followed by 3ml of Conc HNO₃ and heated on a hot plate slowly at first, until frothing ceases. Heating was continued until HNO₃ evaporated and a while fumes observed. It will be allowed to cool and filtered. This was diluted and made up to 100ml with distilled water. The following elements were determined using atomic absorption spectrophotometer. The elements are calcium, chromium, manganese copper, zinc, iron, magnesium and nickel.

### III. Result

Mineral composition of *Telfairia occidentalis*, *vernonia aryngdalina*, and *hydrophyllum virginianum* in mg/100g

<table>
<thead>
<tr>
<th>ELEMENT</th>
<th>PUMPKIN LEAF</th>
<th>BITTER LEAF</th>
<th>WATER LEAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>04.82±0.09</td>
<td>2.55±0.01</td>
<td>03.14±0.03</td>
</tr>
<tr>
<td>Nickel</td>
<td>05.00±0.05</td>
<td>09.13±0.11</td>
<td>04.92±0.02</td>
</tr>
<tr>
<td>Zinc</td>
<td>05.52±0.00</td>
<td>03.00±0.01</td>
<td>05.82±0.06</td>
</tr>
<tr>
<td>Chromium</td>
<td>10.97±0.00</td>
<td>71.33±0.07</td>
<td>73.91±0.02</td>
</tr>
<tr>
<td>Lead</td>
<td>22.91±0.05</td>
<td>03.74±0.02</td>
<td>44.55±0.08</td>
</tr>
<tr>
<td>Manganese</td>
<td>06.71±0.10</td>
<td>07.6±0.10</td>
<td>06.10±0.00</td>
</tr>
<tr>
<td>Iron</td>
<td>15.64±0.09</td>
<td>10.03±0.05</td>
<td>07.48±0.07</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>76.33±0.58</td>
<td>61.5±0.30</td>
<td>65.3±1.78</td>
</tr>
<tr>
<td>Cobalt</td>
<td>0.99±0.06</td>
<td>0.48±0.52</td>
<td>0.35±0.17</td>
</tr>
<tr>
<td>Potassium</td>
<td>85.25±0.76</td>
<td>121.19±0.51</td>
<td>54.49±0.04</td>
</tr>
<tr>
<td>Magnesium</td>
<td>101.92±0.07</td>
<td>62.53±0.01</td>
<td>122.12±0.04</td>
</tr>
<tr>
<td>Calcium</td>
<td>67.19±0.06</td>
<td>54.72±0.76</td>
<td>76.38±0.63</td>
</tr>
</tbody>
</table>

Values are means and standard deviation of duplicate determination. (mean±SD)

Three mineral element Iron, copper, and cobalt have been shown to be essential for normal erythropoiesis in at least one the species (10,11).

The result of the minerals analysed as shown above in the table 1, pumpkin leaf popularly known as Ugu leaf has a iron estimation of (15.64±0.09) which when compared to bitter leaf (10.03±0.05) and water leaf (7.48±0.07) shows a relatively high difference with water leaf (7.48±0.07). Also comparing another element associated with erythropoises such as cobalt which is essential for normal bone marrow function which as dietary supplements is proclaimed to increase erythropoietin., pumpkin has a cobalt estimation of (0.99±0.06) which is relatively higher when compared with bitter leaf (0.48±0.52) and water leaf (0.35±0.17) but show relatively similar estimation. and finally the third element Copper which is the cofactor required by the enzymes Hephaestin and seroplasmin which is needed to iron absorption increasing erythropoiesis. The copper has an estimation of (4.82±0.09) which is moderately higher than that seen in bitter leaf (2.55±0.01) and water leaf (3.14±0.03).

### Qualitative Phytochemical Analysis

Phytochemicals present in the plants were tested but few were absent.

<table>
<thead>
<tr>
<th>Phytochemical component</th>
<th>Pumpkin leaves (telfairia occidentalis)</th>
<th>Bitter leaves (vernonia aryngdalina)</th>
<th>Water leaves (hydrophyllum virginianum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Phytochemical detected  
- = Phytochemical not detected

### IV. Discussion
Three mineral elements, iron, copper, and cobalt, have been shown to be essential for normal erythropoiesis in at least one species each. Iron is probably required for erythropoiesis in all mammals. A deficiency result, at least in chronic stages in a microcytic hypochromic anaemia and is accompanied by a normoblastic, hyperplastic bone marrow and a low serum iron level, and increased amount of protoporphyrin in the erythrocytes, and an elevated serum copper level. Nucleated red blood cells are occasionally seen in the peripheral blood and the reticulocytes are increased. The fundamental concepts of iron metabolism have changed greatly in recent years. These may be summarized. Iron is absorbed chiefly in the duodenum in man it is absorbed principally as ferrous iron. Dogs absorb both valence forms well although some animals absorb the ferrous form more readily than the ferric form. Rats absorb both forms equally well. The absorption of iron is dependent upon the concentration of the iron in the intestine, upon the solubility of the iron salt, and in the human being at least upon the presence of reducing substances in the diet as well as the reducing action of the gastric hydrochloric acid. In addition to these factors the need of the body for iron may determine, to a certain degree, the amount absorbed. This is known as the “selective absorption” theory. Recently it has been suggested that apoferritin acts as a receptor compound in the mucosal cell. As the concentration of the plasma iron falls, ferrous iron is removed from the mucosal cell resulting in a diminution of ferritin in the mucosa. When the ferritin has diminished to a point where the cell is no longer saturated with respect to ferrous iron, more iron is absorbed into the mucosal cell. Once absorbed the iron is transported in the plasma to the tissues where it is stored to a great extent as ferritin, a protein-iron complex. The iron is then used over and over again for hemoglobin synthesis. Iron is excreted from the body in only insignificant quantities. This theory requires substantiation.

Copper has been shown to be essential for normal erythropoiesis in chickens, mice, rats, rabbits, dogs, pigs, sheep, cattle, and infants. A deficiency of this mineral in rats is manifested by a microcytic hypochromic anemia and a moderate reticulocytosis. A condition due to deficiency of copper, known as “enzoctic ataxia,” occurs in sheep in western Australia. Anemia may be severe. In young lambs it is microcytic and hypochromic and is accompanied by demyelinization of the nervous system and hemosiderosis of the tissues. In adult sheep the anemia is slightly macrocytic and hydrochromic. Blood smears reveal anisocytosis, poikilocytosis, Howell-Jolly bodies, normoblasts, normoerycrites, stippling and polychromatophilia. Similar blood changes have been reported in copper-deficient cattle in western Australia. In nutritional anemia in infants the rate of erythropoiesis is accelerated when copper is given in addition to iron. In adults supplemental copper therapy may be of value in few cases. Such cases, if they occur are rare. Most cases will respond if adequate doses of iron are given. This does not necessarily indicate that copper is not needed for erythropoiesis or that it is not a dietary essential but rather that the quantities needed are so small that sufficient copper is present in the body stores in adult life, in the diet or as a contaminant in the iron used therapeutically to supply the needs. No case of uncomplicated copper deficiency has been reported in man. The manner in which copper is related to the formation of red cells is not understood.

The role of cobalt in erythropoiesis is unique. A deficiency results in anemia. The administration of small amounts to normal animals produces a polycythemia, whereas the administration of large amounts depresses erythropoiesis. The enzoztic occurrence of cobalt deficiency in sheep and cattle has been reported from various regions of the world. Anemia is present and oftentimes severe. The anemia is either normocytic or microcytic and usually hypochromic. Blood smears reveal anisocytosis, poikilocytosis. There is a hypoplasia of erythrogenic tissue in the bone marrow, hemosiderosis of the tissues and a reduction in reticulocytes in the blood. An experimental anemia due to cobalt deficiency has not been produced in either rats or dogs. There is no substantial or convincing evidence that cobalt is needed by human beings for normal erythropoiesis. The administration of small amounts of cobalt to normal rats, dogs, guinea pigs, frogs, mice, rabbits, chickens, pigs, and ducks produces a marked polycythemia which is accompanied by reticulocytosis, hyperplasia of the bone marrow, and an increased erythropoiesis activity in the spleen and liver.

In conclusion, certain vitamins, namely, riboflavin, nicotinic acid, pyridoxine, “folic acid,” and the intrinsic factor, have been shown to be essential for normal erythropoiesis in at least one species each.

V. Conclusion

It can be concluded that fluted pumpkin has a better erythropoietic potential when compared to bitter leaf and water leaf.

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References
[15].