Mineral and phytochemical composition of baobab (Adansonia digitata L.) root tubers from selected natural populations of Malawi

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Abstract

Background
Most studies on baobab have focused mainly on the nutritional value of baobab fruit pulp. Information on nutritional value and medicinal properties of the baobab root tuber has not been fully investigated and is scarce. This study was conducted to assess mineral and phytochemical composition of baobab root tubers from selected natural populations of Malawi.

Methods
Baobab seeds from Chikhwawa, Mwanza, Salima, Karonga and Likoma were sown at Mzuzu University. At the age of five months after sowing, mineral content of the resulting root tubers was determined using atomic absorption spectrophotometer whereas phytochemical composition was determined qualitatively.

Results
Magnesium (44.16mg/100g) and calcium (69.39mg/100g) levels were highest in baobab root tubers from Chikhwawa and Karonga, respectively. Mwanza and Karonga root tubers contained highest amount of lead (0.2100mg/100g) while iron content was highest (8.89mg/100g) in root tubers from Karonga. Salima and Mwanza root tubers showed strong concentration of terpenoids. Moderate concentrations of saponins were detected in Mwanza, Salima, Karonga and Chikhwawa root tubers. Alkaloids and flavonoids were absent in all families from the five sampled provenances.

Conclusion
Minerals and phytochemicals present in baobab root tubers suggest their nutritional and medicinal potential. However, further research is required to establish the causes of high levels of lead in baobab root tubers.

Key words: baobab, calcium, iron, lead, magnesium, saponins, terpenoids

Introduction
Baobab (Adansonia digitata L.) is a deciduous indigenous fruit tree that belongs to the family Malvaceae and is native to Africa¹. The tree is found in most of sub-Saharan Africa’s semi-arid and sub-humid regions as well as in western Madagascar³. In southern Africa, Adansonia digitata is commonly found in Malawi, Mozambique, South Africa and Zimbabwe⁴. In Malawi, Adansonia digitata is mostly found in dry woodlands⁵. It usually grows as a solitary individual though sometimes it can be found in small groups depending on soil type⁶. A baobab tree can grow up to 25 meters in height, 28 meters in girth and can live for several hundred years⁷. It has a shallow root system that rarely extends beyond 2 meters in depth for mature trees⁸. The tree excels in a wide range of well drained soils but not in deep unconsolidated sands³. Fluviosols, which are not subjected to flooding, have been reported to favour the growth of baobab⁹. The species does not occur in water-logged and frost areas³. It requires an annual temperature of range 20-30°C but can also tolerate high temperatures of 40-42°C. Lower altitudes with annual rainfall in the range of 100-1000ml have been reported to be ideal for the growth of baobab tree⁹, ¹⁰.

A baobab is a multi-purpose tree mostly valued for food and traditional medicine. The root tubers, twigs, fruits, seeds, leaves and flowers are all edible and have been found to possess various minerals (calcium, iron, copper, magnesium, zinc, potassium, magnesium) and phytochemicals⁸. In addition, a variety of phytochemicals (terpenoids, flavonoids and steroids) which are responsible for medicinal purposes have been chemically isolated from various parts of the baobab tree¹⁰. Even without such knowledge, rural communities have developed unique indigenous knowledge related to use of traditional medicine¹¹. The use of baobab root tubers has for a long time been used to treat sore throats¹². The wide ecological adaption of baobab tree suggests the species has evolved a wide genetic diversity across its geographical range¹². In this regard, it is possible that variations in mineral and phytochemical composition of baobab root tubers are influenced by geographical or genetic differences, hence their use cannot be generalized. This study was carried out to determine the variation in mineral and phytochemical composition of baobab root tubers from selected natural populations of Malawi. This information would be useful for baobab populations whose root tubers are exploited for food and medicinal purposes. In addition, findings of this study will be essential for future selection programmes aimed at producing baobab populations of specific mineral and phytochemical content.
Methods

Study area and experimental material

Baobab seeds representing a total of 59 half-sib families were collected in Malawi from five provenances (Karonga, Salima, Mwanza, Chikhwawa and Likoma). For easy data collection, these provenances were abbreviated as K, S, M, C and L, respectively. Mature baobab fruits were harvested from trees by plucking and/or collection on the ground. The fruits were collected at the peak of fruit season between April and May, 2008. The fruits were collected from randomly selected parents at a distance of at least 100 m apart. Fruits were kept in plastic bags and were transferred from provenances to the nursery by car. The fruits were then crushed using stones to obtain seeds that were covered with pulp. Seeds were washed with tap water to remove pulp and then dried. Before sowing, baobab seeds (Orthodox) were stored in plastic bags at room temperature. In October 2015, the seeds were sown at Mzuzu University Forestry department nursery. Table 1 shows site characteristics for the five sampled baobab provenances. The seeds were pretreated through nicking to allow water penetration. Mzuzu University lies in silvicultural zone M and falls at an altitude of 1270 m above sea level with mean annual temperature range of 13.5°C to 24°C and mean annual rainfall of 1150 mm.

Table 1: Site characteristics for five sampled baobab provenances

| Provence | Silvicultural zone | Altitude (m) | MAR (mm) | T (°C) | Soil type               |
|----------|-------------------|--------------|----------|--------|-------------------------|
| Karonga  | L                 | 750-1000     | >1800    | 23-25  | Ferrisols, regosols     |
|          |                   |              |          |        | Aluvial calcimorphic soils |
| Salima   | Ba                | 200-1000     | 710-850  | 20-25  | Sandy ferrallitic        |
| Mwanza   | J                 | 900-1500     | >1200    | 19-21  | Vertisols               |
| Chikhwawa| A                 | <200         | 710-840  | <25    | Ferrisols, regosols, lithosols |
| Likoma   | L                 | 475-1000     | >1600    | 23-25  |                        |

Adapted from Hardcastle.

Experimental design

The trial was laid out as a complete randomized design with four replicates. From 59 baobab families, 25 families were selected for sowing on the basis of seed quality. Seeds which were relatively small and damaged were left. For each treatment, two seeds for each of 25 families were sown in ten black polythene tubes (30 cm x 15 cm) at 4 cm depth. Sand and dark-grey miombo soils mixed in the ratio 1:2, respectively, were used as rooting medium. Watering was carried out twice a day to keep the rooting medium moist. After germination, the seedlings were thinned to remain with one seedling per tube.

Collection and processing of baobab root tubers

At the age of five months after sowing (October 2015 – March, 2016), baobab root tubers (Figure 1) were collected from the nursery by uprooting the entire plant. The root tubers were then cleaned to remove mud. Thereafter, the root tubers were sliced into small pieces by sterilized blades. The samples were then pounded using a mortar and pestle. The wet samples were then weighed (50 g) and transferred into dry beakers and then dried in an electric oven (Series 9000) at 105°C overnight to constant mass.

Figure 1: Baobab root tubers

Determination of minerals

Mineral content of baobab root tubers was determined using atomic absorption spectrophotometer. For each treatment, the dried powdered sample (5 g) of baobab root tuber was placed in a 50 mL porcelain crucible in triplicate. The samples were then ashed by heating at a temperature of 650°C for 6 hours in an electric muffle furnace (CWF 1200). After cooling, hydrochloric acid solution (6M) was then added (7 mL) to the ash and boiled on a hot plate with the aid of four anti-bumping granules until the solution had just dried. The crucible containing the sample was removed from the hot plate and hydrochloric acid solution (3M) added (10 mL) to it, and boiled for 10 seconds on a hot plate. After cooling, each sample was filtered into 100 mL volumetric flask. Blank sample which contained all reagents except the sample was treated in a similar manner as the samples. The filtered samples were finally diluted with distilled water up to the 100 mL mark. The sample solutions were then taken to Agricultural Research and Extension Trust (ARET) in Lilongwe for determination of minerals using atomic absorption spectrophotometer (AAS). From each metal calibration curve, the mineral (metal ion) content was calculated using the following formula:

Metal content (mg/100 g) = [(a-b) x V] / 10W

Where W is the weight (g) of the sample, V is the volume (mL) of extract (filtered sample); a and b are concentrations (mg/L) of a sample solution and blank determined from a calibration curve, respectively.

Phytochemical screening of baobab root tuber

Phytochemical screening of baobab root tubers was carried out using qualitative methods. The analyses were carried out on dry and pounded samples. All the analyses were carried out at Mzuzu University Chemistry laboratory.

Test for alkaloids

A test for the presence of alkaloids was done using Dragendorff and Mayer’s reagents. A dried powdered sample (5 g) was macerated in 5% (v/v) hydrochloric acid solution for 24 hours. Two portions of the filtrate (1 mL each) were treated with 10 drops of Dragendorff and Mayer’s reagents separately. Absence of the red precipitate and cream white precipitates were taken as an indication for the absence of alkaloids.
**Test for terpenoids**

A dried powdered sample (1g) was macerated in 20mL of diethyl ether in a stoppered conical flask for 48 hours. A portion of the filtrate (1mL) in a porcelain crucible was dried on a hot plate followed by the addition of 10 drops of concentrated sulphuric acid. The colour produced was recorded. Another portion (1mL) was treated the same but starting with the addition of acetic anhydride (1mL) followed by concentrated sulphuric acid, 1mL). The appearance of green, blue and pink to purple colors indicate the presence of terpenoids\(^{16}\).

**Test for saponins**

An infusion (5%, w/v) was prepared by macerating 1g of dried powdered sample in 20 mL of distilled water. The mixture was left to stand for 24 hours and the extract was filtered using Whatman filter paper No.1. A portion of the filtrate (10mL) was transferred into a test tube and was shaken vigorously for 10 seconds. The foam that persisted for 10 minutes was measured using a ruler and used as an indication for the presence of saponins\(^{17}\).

**Test for flavonoids**

A dry powdered sample (5g) was macerated in 50mL of distilled water and the mixture was left to stand for 24 hours. The mixture was then filtered and a solution (0.5mL) containing hydrochloric acid, methanol and water (1:1:1) was added to the filtrate followed by some few magnesium turnings. Absence of the pink or red colour was used to indicate absence of flavonoids\(^{18}\).

**Data analysis**

Data for mineral composition of baobab root tubers from 25 families were tested for normality and homogeneity with Komolgorov-Smirnov using Minitab 16. After meeting the two criteria, data were subjected to one-way ANOVA at (P=0.05) using the same statistical package. Means were separated using Fisher's test. The concentration of phytochemicals was assessed using qualitative scores (+++, ++, +, -) where +++ denoted strong concentration, ++ representing moderate concentration, + indicating weak concentration and – indicating absence of a particular phytochemical.

**Results**

**Mineral composition of baobab root tubers**

Variations of mineral elements in all the treatments are summarized in Table 2. There were great variations in the mineral content of baobab root tubers among the study populations.

### Table 2: Variation in mineral content of baobab root tubers

| Element | Mean (mg/100g) | S.E. Mean | Interval | Variance | Variance % |
|---------|----------------|-----------|----------|----------|------------|
| Mg      | 22.72          | 1.89      | 8.41-56.01 | 47.90    | 565.99     |
| Ca      | 42.91          | 2.60      | 15.89-123.39 | 107.50  | 676.53     |
| K       | 6.606          | 0.267     | 3.180-10.300 | 7.12    | 223.89     |
| Fe      | 5.141          | 0.22      | 2.10-10.59 | 8.49    | 404.29     |
| Cu      | 0.0694         | 0.0052    | 0.0200-0.1900 | 0.17   | 850        |
| Zn      | 0.2212         | 0.0131    | 0.1100-0.6400 | 0.53   | 481.81     |
| Mn      | 0.4910         | 0.0181    | 0.2600-1.0400 | 0.93   | 357.69     |
| Cd      | 0.01475        | 0.0015    | 0.0014-0.0364 | 0.035  | 2500       |

**Table 3: Mineral content of baobab root tubers for 25 families in five provenances**

| Code | Mg (mg/100g) | Ca (mg/100g) | K (mg/100g) | Fe (mg/100g) | Cu (mg/100g) | Zn (mg/100g) | Mn (mg/100g) | Cd (mg/100g) |
|------|--------------|--------------|-------------|--------------|--------------|--------------|--------------|--------------|
| S1   | 22.72 | 42.91 | 6.606 | 5.141 | 0.0694 | 0.2212 | 0.4910 | 0.01475 |
| S2   | 9.15  | 26.07 | 4.050 | 3.783 | 0.0304 | 0.1417 | 0.3510 | 0.0074  |
| S3   | 16.64 | 38.42 | 3.820 | 4.050 | 0.0304 | 0.1417 | 0.3510 | 0.0074  |
| S4   | 22.72 | 42.91 | 6.606 | 5.141 | 0.0694 | 0.2212 | 0.4910 | 0.01475 |
| S5   | 9.15  | 26.07 | 4.050 | 3.783 | 0.0304 | 0.1417 | 0.3510 | 0.0074  |

Generally, calcium was shown to be the highest mineral (42.91±2.60mg/100g) present in the baobab families under study whilst the least mineral was cadmium (0.01475±0.0015mg/100g). The variance percentage ranged from 223.89% for K to 2500% for Pb and Cd. Variations of mineral elements in all the treatments are summarized in Table 3. There were significant differences (P<0.05) in the amount of magnesium (Mg), calcium (Ca), iron (Fe), copper (Cu), manganese (Mn), cadmium (Cd) and lead (Pb) among the families. No significant differences (P>0.05) were observed in the levels of potassium (K) and zinc (Zn) among the families. Highest amount of magnesium (44.160mg/100g) was recorded in a family from Chikhwawa (C2) while the lowest amount of magnesium (9.84mg/100g) was also detected in a family from Chikhwawa (C5). Cadmium levels were highest (69.39mg/100g) in Karonga family (K12) and lowest (25.02mg/100g) in a family from Likoma (L7). Iron content was highest (8.89mg/100g) in Karonga family (K8) and lowest (3.13mg/100g) in a family from Chikhwawa (C6). Levels of copper were highest (0.155mg/100g) in Salima

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family (S15) and lowest (0.0250mg/100g) in three families from Karonga (K11, K5 and K8). The highest amount of manganese (0.815mg/100g) was found in a family from Karonga (K8) while the lowest amount (0.335mg/100g) was detected in two families from Chikhwawa (C2 and C5). Cadmium levels were highest (0.0343mg/100g) in a family from Likoma (L7) and lowest (0.0024mg/100g) in a family from Mwanza (M7). The highest amount of lead (0.2100mg/100g) was recorded in families from Mwanza (M15) and Karonga (K8) while lowest levels (0.025mg/100g) of lead were found in families from Chikhwawa (C2), Salima (S13) and Mwanza (M9).

**Phytochemical variation of baobab root tubers**

Table 4 shows phytochemical composition of *A. digitata* root tubers from the five study areas. Root tubers from Salima and Mwanza showed strong concentration of terpenoids. Moderate concentrations of terpenoids were observed in root tubers from Karonga and Chikhwawa while weak concentrations were recorded in root tubers from Likoma. Moderate concentrations of saponins were detected in root tubers from Mwanza, Salima, Karonga and Chikhwawa while weak concentrations were recorded in root tubers from Likoma. Alkaloids and flavonoids were absent in root tubers from all the five provenances.

**Table 4: Phytochemical variation of baobab root tubers from five provenances**

| Provenance | Terpenoids | Saponins | Alkaloids | Flavonoids |
|------------|------------|----------|-----------|------------|
| Mwanza     | +++        | +        | -         | -          |
| Salima     | +++        | ++       | -         | -          |
| Karonga    | +          | ++       | -         | -          |
| Chikhwawa  | +          | ++       | -         | -          |
| Likoma     | +          | +        | -         | -          |

**Discussion**

This study has shown that the mineral content and phytochemical composition of baobab root tubers from different geographical localities of Malawi differ significantly when raised in the same environment. Variations have been reported to arise from genetic and environmental differences. Differences in the mineral content and phytochemical composition of baobab root tubers in this study could, therefore, be attributed to genetics as all the families were raised in the same environment. This conclusion supports earlier hypothesis that baobab has evolved a wide genetic diversity across its geographical range. It could be possible that the species utilizes its long life span in successfully adapting to various climatic and environmental effects in different geographical localities. The high variance percentages (Table 2) in the important minerals (Mg, Ca, K, Fe, Cu, Zn) present in baobab root tubers clearly show the possibility of selection at family level. The effect of various quantitative trait loci generally explains the genetic variance in the mineral content of specific organisms.

Magnesium is important in protein synthesis, release of energy from muscle storage and is essential in regulating body temperature. High levels of magnesium (44.16mg/100g) in Chikhwawa family (C2) suggest baobab root tubers are an important source of magnesium for nutritional purposes. Moreover magnesium levels (44.16mg/100g) in this study are higher than reported values in raw sweet potatoes (30mg/100g), cassava (16mg/100g) and yams (17mg/100g). Calcium is very important in tooth formation and reduces the risk of osteoporosis, a condition in which decreased bone mass weakens the bone. In all the families, calcium levels (25.02mg/100g-60.39mg/100g) were much higher compared to lower levels (18.20mg/100g) recorded in baobab leaves. The high content of calcium (69.39mg/100g) in baobab root tubers indicates that they may be used to improve tooth and bone strength. Potassium plays an important role in lowering blood pressure and release of energy from fats, proteins and carbohydrates.

Statistical insignificant levels of potassium among the study populations indicates that its content is the same in different geographical localities of Malawi. Iron is a major component of hemoglobin and has been reported to be very important in the oxidation of carbohydrate, protein and fats. High iron levels found in this study (8.89mg/100g) in Karonga (K8) are greater than the value (3.95mg/100g) recorded in dry baobab fruit pulp by Phytotrade Africa. Iron content could therefore support use of baobab roots in improving levels of hemoglobin as well as the general human nutrition.

Manganese supports brain functioning and is required for blood sugar regulation. High levels (Table 3) of manganese (0.185mg/100g) in Karonga (K8) indicate potential use in treating diabetes. Furthermore, Mn levels recorded in this study (0.185mg/100g) are higher compared to Mn content (0.035mg/100g) in raw apple and avocado (0.095mg/100g). Zinc is involved in digestion, metabolism and is an important antioxidant. Zinc levels (0.2212mg/100g) among the study populations indicate that its content in baobab root tuber is also the same across geographical localities of Malawi. However, the amount of Zn (0.221mg/100g) recorded in this study is lower compared to levels (0.680mg/100g) found in avocado. Copper is a redox active metal necessary for the formation of hemoglobin and is required for the function of over 30 proteins. Presence of copper in baobab root tubers in this study indicates potential use in prevention of anemia and malnutrition deficiencies common in Malawi. However, the levels of copper (0.025-0.155mg/100g) found in this study are very low compared to copper levels (13.00mg/100g) reported in baobab leaves. Lead is an element that is not needed in the body. Levels of lead (>0.03mg/100g) may cause impairment of the central nervous system in children. High levels of lead reported in this study could be due to the soil where the baobab tubers were growing was contaminated by lead. However, the validity of this hypothesis would be true only if the soil was analysed before and after the experiment for the presence of lead. Cadmium is an inorganic metal that causes anemia and heart diseases if ingested in high concentrations. High levels of Cadmium (0.0343mg/100g) in population from Likoma (L7) indicate a health concern in the utilization of baobab root tuber either for food or medicinal purposes. A study in Nigeria failed to detect lead but found cadmium levels (0.85mg/100g) in baobab leaves and reported that lead and cadmium are naturally present in the environment. As mentioned earlier, in the current study, soil samples and water used in the experiment were not tested for the presence of lead and cadmium. It could be possible that high levels of lead and cadmium were attributed to the soil and water used in the experiment. Still more, presence of lead and cadmium in baobab root tubers could be genetically influenced. Further research is required to establish the sources of high levels.
of lead and cadmium in baobab roots. In southern Malawi, children consume baobab root tuber to treat sore throat. However, no oral knowledge has so far been reported on the harmful side effects relating to the use of baobab root tubers.

The presence of terpenoids and saponins in baobab root tubers agree with the previous work in Nigeria which also reported the occurrence of saponins and terpenoids in baobab root tubers. The antimicrobial activity of baobab root has been reported to be influenced by the presence of saponins and terpenoids. The availability of terpenoids and saponins in baobab root tubers therefore justifies their traditional application in the treatment of microbial infections. From the results of this study, baobab root tubers from all the provenances could hence be utilized to treat microbial infections. However, the varying concentration of terpenoids and saponins in baobab root tubers still needs to be considered in order to optimize their efficacy in the treatment of microbial infections. Terpenoids are precursors in the human body which help to produce steroids like sex hormones such as testosterone. The traditional use of *Adansonia digitata* root tubers either for food or medicinal purposes could hence be helpful in increasing testosterone levels in males with fertility problems. Because of strong concentrations of terpenoids, baobab root tubers could be utilized to optimize male testosterone levels. It has been reported that saponins regulate blood sugar levels in the human body.

Presence of saponins in baobab root tubers from all the provenances could therefore defend their traditional use in treating diabetes. Flavonoids are chemical compounds with antidiarrheal activity. Absence of flavonoids points out that baobab root tubers from all the five provenances may not be a practical remedy for treating diarrhea. Alkaloids are chemical compounds mostly containing basic nitrogen atoms and are used as a remedy for gout with analgesic and anti-malarial activity. The absence of alkaloids in baobab root tubers from all the provenances therefore shows that they are not an ideal remedy for treating gout, malaria and for eliminating body pain. Absence of alkaloids and flavonoids differs with other findings which have proved the availability of alkaloids and flavonoids in baobab root and attributed their variations to environmental differences and maturity of the plant part used. In the current study, seedlings were used perhaps before the plants started reserving or storing alkaloids and flavonoids in the roots.

**Conclusion and Recommendations**

This study has revealed that baobab root tubers are an important source of magnesium, calcium, potassium, iron, copper, zinc and manganese which are required for the proper functioning of the human body. But baobab root tubers have also shown to contain heavy metals (Pb and Cd), which are not required in considerable doses by the human body. Baobab root tubers have demonstrated to possess important phytochemicals such as terpenoids and saponins which are crucial in the treatment of ailments. However, alkaloids and flavonoids were absent. The mineral and phytochemical composition of baobab root tubers has proved to be distinct across geographical localities of Malawi when raised in a similar environment. Therefore, because of these differences, the use of baobab root tubers for food and medicinal purposes should not be generalized.

Further studies are required to establish the causes of high lead and cadmium levels in baobab root tubers. In addition, domestication efforts must consider variation in mineral and phytochemical composition of baobab root tubers if target ideotypes of baobab are to be produced.

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