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Phytochemical and Elemental Determination of *Balanites aegyptiaca* Leaves and Stem bark in Hong Local Government Area of Adamawa State, Nigeria

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Abstract The leaves and stem bark of *Balanites aegyptiaca* are popularly consumed as vegetables or herbs in Adamawa state of Nigeria. In view of its importance in Hong local government area, the leaves and stem bark were collected from 10 locations for elemental and phytochemical determination. Atomic absorption spectrophotometer (AAS) was employed for the elemental determination. The results obtained contained some essential elements (Na, K, Mg and Ca) in the leaves and stem bark. For heavy metals, Cd was recorded in the entire samples analyzed, Cu was found in four locations (Gaya, Mugili, Pubba and Uba), and Zn was only recorded in the leaves and stem bark from two locations (Pubba and Mugili respectively). Pb was not observed in the entire samples by the instrument used. Quantitative methods were used for phytochemical screening. Tannin was recorded in the samples investigated while flavonoid and saponin were recorded in some areas. The essential elements and phytochemicals revealed could be good source of minerals and herbs.

Keyword: Phytochemical, Element, *Balanites aegyptiaca*, herbs, mineral

Introduction

Balanites aegyptiaca is a member of Zygophyllaceae family. The Hausa name is aduwa. It is mainly distributed in semi-arid and arid zones in tropical Africa. The species are found wild in Adamawa and Borno state [1]. It is used for various needs such as fodder, medicines, charcoal and pesticides. The almond is rich in saturated fatty acid that is used as cooking oil. It contains steroids (saponins, sapogenins, diosgenins) used as raw materials for industrial production of contraceptive pills, corticoids, anabolisants and other sexual hormones [2]. The leaves are eaten by humans. It has considerable amount of vitamins and minerals [3]. The fruits and the kennels are rich in vitamin A and B respectively [4].

Wild and semi-wild plant leaves are frequently consumed as dominant leafy vegetables especially in the rural communities [5, 6]. They constitute indispensible constituents of human diet in Africa most especially West Africa. Vegetables are important protection foods and highly beneficial for the maintenance of health and prevention of diseases and also reinforce the resisting ability of the cells to counter bacterial infections due to the fact that they contain valuable food ingredient which is utilized to build and repair body [7]. Green leafy vegetables are best cooked when freshly harvested because some of their vitamin contents deteriorate on storage. Most leafy vegetables particularly the dark green variety contain pro-vitamin A as do carrots, sweet peppers and some tomatoes. They also supply vitamin C and in many cases, iron and calcium to the diet. The search for nutrient-rich food like vegetable is therefore essential [8].

Vegetable accumulate heavy elements in their edible and non-edible parts. Although some of the heavy elements such as Zn, Mn, Ni and Cu act as micro-nutrients at lower concentrations, they become toxic at higher concentrations. Health risk due to heavy elements contamination of soil has been widely reported [9,10,11]. Phytochemicals are rich sources of organic substances which are used for production of many drugs. Investigation into therapeutic efficacy of plants is therefore urgently needed because of widely usage, acceptability and so many claimed activities for treatment of ailments and diseases [12]. The phytochemicals that have medicinal importance are the secondary metabolites. These secondary metabolites include phenolic compounds such as tannins, alkaloids, glycosides, saponins, terpens, volatile oils, gums and mucilage. Epidemiological studies suggest that consumption of a diet high in fruits and vegetables is associated with a reduce risk of chronic disease [13]. Therefore, consuming a variety of plant-based foods helps to ensure the individuals receive the optimum benefits from the fruits and vegetable consumed [14]. This study therefore, presents data on levels of some elements (Na, K, Ca, Mg, Cu, Zn, Pb and Cd) and phytochemicals (alkaloids, tannin, saponins, flavonoids and glycosides) in *Balanites aegyptiaca* leaves and stem bark in Hong Local Government area of Adamawa State.

Materials and Methods

Sampling

Fresh leaves and stem bark of *Balanites aegyptiaca* were collected from 10 locations (Pella, Hong, Hildi, Uba, Gashala, Gaya, Garaha, Mugili, Fa'a and Pubba) in Hong Local Government area of Adamawa State. Each plant part was dried under shade.

Sample Preparation

The dried samples were ground to powder and sieved. Representative samples were obtained from each sample by coning and quartering techniques. This was repeated until the sample was reduced to the size required for final analysis and stored in an air tight container.

Sample Digestion

3.0g of each of the powdered sample was weighed and pre-treated with 20 mL nitric acid and allowed to stay overnight. 10 mL perchloric acid was added and heated gently, then vigorously until clear solutions were obtained. The solutions were allowed to cool and then transferred to 100 ml volumetric flask and made up to mark with distilled water. The solutions were filtered and stored in plastic bottles for the analysis of Na, K, Ca, Mg, Cu, Zn, Pb and Cd using Atomic Absorption Spectrophotometer (AAS) GBC Avanta GF300 model, equipped with digital read-out.

Phytochemical Determination

10 g each of the powered plant leaves and stem bark were soaked in ethanol for 24 h. The extracts were filtered through a Whatman filter paper. The filtrates were then concentrated using a rotary evaporator with the water bath set at 40^{0}C [15]. The phytochemical analyses of the extracts were carried out according to the methods described by Trease and Evans [16].

Statistical Analysis

Data generated was subjected to Analysis Of Variance (ANOVA) to determine the level of significance using statistical software (CROPSTAT 7.2.3 International Rice Research Institute IRRI, Philippine). Significant means were separated using least significant difference (LSD) technique. Differences were considered significant if probability is less than 5% ($p \leq 0.05$) for all the data.

Results and Discussion

Phytochemical Screening

Table 1 presents the phytochemical composition of *Balanites aegyptiaca*. The leaves and the stem bark demonstrated the presence of tannin in all the locations. Alkaloids were present in the leaves around Hildi and Fa'a. Glycosides were not observed in the entire samples from all the locations. The leaves demonstrated the presence of flavonoid. Saponin was recorded in the leaves and the stem bark around Hong, Fa'a and Garaha areas. However, saponin was observed in the leaves from all the locations.

Table 1: Phytochemical screening *of Balanites aegyptiaca* extracts

Location	Plant parts	Tannin	Alkaloid	Glycoside	Flavonoid	Saponin
Pella	Leaves	+	-	-	+	+
	Stem bark	+	-	-	-	-
Hong	Leaves	+	-	-	+	+
	Stem bark	+	-	-	-	+
Hildi	Leaves	+	+	-	+	+
	Stem bark	+	-	-	-	-
Uba	Leaves	+	-	-	-	+
	Stem bark	+	-	-	-	-
Gaya	Leaves	+	-	-	+	+
	Stem bark	+	-	-	-	-
Fa'a	Leaves	+	+	-	+	+
	Stem bark	+	-	-	-	+
Garaha	Leaves	+	-	-	+	+
	Stem bark	+	-	-	-	+
Gashala	Leaves	+	-	-	+	+
	Stem bark	+	-	-	-	-
Mugili	Leaves	+	-	-	+	+
	Stem bark	+	-	-	-	-
Pubba	Leaves	+	-	-	+	+
	Stem bark	+	-	-	-	-

+ = present, - = absent

Elemental Determination

The mean distribution for essential elements and heavy metals in *Balanites aegyptiaca* leaves among ten locations in Hong Local Government is presented in Table 2. Na was present in the entire samples analyzed with a total mean value of 0.504 µg/g. K had a total mean value of 0.703µg/g. For Mg and Ca, highest mean value was obtained in samples around Gashala (0.420 µg/g) and Uba (0.410 µg/g) respectively. There was no significant difference observed among the locations in terms of Na, Mg and Ca contents at $p \leq 0.05$. Cu was recorded in Gaya (0.115 µg/g), Mugili (0.120 µg/g) and Pubba (0.160 µg/g) areas. Significant difference ($p \leq 0.05$) was observed among the locations with respect to Cu content. Zn was observed in the samples around Pubba (0.210 µg/g) only. Pb was below the detectable limit (<0.06 µg/g) of the machine used. Cd was recorded in all the samples with an overall mean of 0.266 µg/g. there was no significant difference ($p \leq 0.05$) revealed in terms of Cd levels among the plants.

Table 3 presents mean levels of essential elements in *Balanites aegyptiaca* stem bark in ten locations around Hong Local Government Area. The results indicate that Na ranged between 0.540µg/g to 0.600µg/g with a mean value of 0.566µg/g. for K, the value was below the detectable limit (<0.008µg/g) of the instrument used in all the samples investigated. In terms of Mg, The mean value ranged between 0.240µg/g to 0.280µg/g with an overall mean of 0.259µg/g. Ca was recorded highest in the sample around Pella (0.380µg/g). There was no significant difference ($p \leq 0.05$) observed in terms of Na, K, Mg and Ca contents among the locations. The results showed the presence of Cu in samples around Uba (0.145µg/g), Mugili (0.1000µg/g) and Pubba (0.098µg/g) areas while Zn was recorded in Uba (0.056µg/g) and Mugili (0.110µg/g) with a total mean value of 0.023µg/g. Pb was below the detectable limit (<0.06µg/g) of the instrument used in all the samples. Cd was recorded in all the samples with highest amount around Hildi (0.233µg/g). There was no significant difference ($p \leq 0.05$) among the locations in terms of heavy metal compositions.

Table 2: Mean levels of Elements in *Balanites aegyptiaca* leaves (µg/g dry weight)

Locations	Elements							
	Na	**K**	**Mg**	**Ca**	**Cu**	**Zn**	**Pb**	**Cd**
Pella	0.510	0.770	0.380	0.400	<0.025	<0.008	<0.060	0.260
Hong	0.500	0.780	0.410	0.380	<0.025	<0.008	<0.060	0.280
Hildi	0.520	0.760	0.400	0.40	<0.025	<0.008	<0.060	0.270
Uba	0.480	0.800	0.390	0.410	<0.025	<0.008	<0.060	0.260
Gaya	0.520	0.780	0.400	0.380	0.115	<0.008	<0.060	0.266
Fa'a	0.510	0.770	0.390	0.390	<0.025	<0.008	<0.060	0.270
Garaha	0.510	0.780	0.400	0.410	<0.025	<0.008	<0.060	0.280
Gashala	0.480	0.800	0.420	0.380	<0.025	<0.008	<0.060	0.280
Mugili	0.490	0.780	0.370	0.400	0.120	<0.008	<0.060	0.230
Pubba	0.520	0.008	0.370	0.350	0.160	0.210	<0.060	0.260
Mean	0.504	0.703	0.393	0.390	0.057	0.0280	0.06	0.266
SE	0.026	0.046	0.026	0.030	0.032	0.0020	0.011	0.023
LSD(5%)	0.079	0.137	0.078	0.088	0.097	0.006	0.034	0.068

Any two mean having difference greater than LSD are significantly different at 5% level of probability.

Table 3: Mean levels of Elements in *Balanites aegyptiaca* Stem bark (µg/g dry weight)

Locations	Elements							
	Na	**K**	**Mg**	**Ca**	**Cu**	**Zn**	**Pb**	**Cd**
Pella	0.580	<0.008	0.250	0.380	<0.025	<0.008	<0.060	0.220
Hong	0.550	<0.008	0.260	0.120	<0.025	<0.008	<0.060	0.227
Hildi	0.540	<0.008	0.260	0.100	<0.025	<0.008	<0.060	0.233
Uba	0.546	<0.008	0.250	0.100	0.145	0.056	<0.060	0.210
Gaya	0.540	<0.008	0.250	0.340	<0.025	<0.008	<0.060	0.210
Fa'a	0.550	<0.008	0.270	0.100	<0.025	<0.008	<0.060	0.200
Garaha	0.560	<0.008	0.260	0.100	<0.025	<0.008	<0.060	0.200
Gashala	0.580	<0.008	0.240	0.110	<0.025	<0.008	<0.060	0.210
Mugili	0.580	<0.008	0.280	0.080	0.100	0.110	<0.060	0.190
Pubba	0.600	<0.008	0.276	0.320	0.090	<0.008	<0.060	0.100
Mean	0.566	0.562	0.259	0.175	0.051	0.023	0.057	0.200
SE	0.024	0.003	0.030	0.115	0.041	0.015	0.013	0.025
LSD (5%)	0.072	0.010	0.091	0.343	0.012	0.044	0.039	0.074

Any two mean having difference greater than LSD are significantly different at 5% level of probability.

Phytochemical composition in the Plant

Tannin: The samples investigated showed the presence of tannin in the leaves and stem bark. Tannins are widely distributed in many plants species. They have astringent properties which are important anti-oxidant and in wound healing [17].

Alkaloid: The presence of alkaloid was only recorded in the leaves around Hildi and Fa'a. Alkaloids are produced by large variety of organisms, plants and animals. They almost uniformly invoke bitter taste [18]. They have pharmacological effects and often used as medications and recreational drugs.

Flavonoid: The results obtained showed the presence of flavonoid in the leaves of the entire samples investigated. Flavonoids are the most common group of polyphenolic compounds in the human diet and are found in plants [19]. The widespread distribution of flavonoids and their low toxicity compared to other active plant compounds means that many animals, including humans, ingest significance quantities in their diet. Flavonoids have anti-allergic, anti-inflammatory, anti-bacterial, anti-cancer, anti-diarrheal and anti-oxidant properties [20, 21].

Saponin: The samples investigated demonstrated the presence of saponin in most of the locations, while Kubmarawa *et al*. [22] reported saponin in the roots. The difference in the composition could be due to geographical location. Saponins have the potential to lower cholesterol levels in humans due to their hypocholesterlemetric effect. They form complexes with cholesterol to reduce plasma cholesterol [23].

Glycoside: The presence of glycoside was not recorded in the entire samples screened. Glycosides play important roles in living organisms. They are used as medications for treatment of congestive heart failure and cardiac arrhymia [24].

Elemental Composition in the Plant

Sodium: The amount for sodium recorded in the entire samples investigated was highest in the stem bark around Pubba area (0.600 µg/g). The values observed for sodium in the samples were lower than the World Health Organization (WHO) permissible limits range of 400 to 500 µg/g in plants as reported by Mustapha *et al*. [12]. Sodium is one of the chief extracellular ions in the body. It involves in the production of energy, transport of amino acids and glucose into the body cells and its deficiency results in hyponatremia [25].

Potassium: the total mean values for potassium observed in the leaves of samples from the entire locations were 0.703 µg/g. potassium was below the detectable limit (<0.008 µg/g) of the instrument used in the stem bark of the samples investigated. The amounts of potassium in the samples investigated are within the safety limits of 10 to 100 µg/g recommended by WHO [26]. Potassium is the principal intracellular cation. It helps to regulate osmotic pressure and pH equilibrium. The recommended daily intake is 4700 mg [27]. Its deficiency causes muscle weakness, decrease reflex responses and respiratory paralysis.

Magnesium: Magnesium was obtained in the leaves and the stem bark of samples investigated with a mean value of 0.393 µg/g and 0.259 µg/g respectively. The amounts of magnesium obtained in the plant parts investigated are lower than the safety limits of 100 to 200 µg/g in plants as reported by WHO [26]. Magnesium plays important role in maintaining electrical potential in nerves and membranes. It improves insulin sensitivity, protect against diabetes and its complications and also reduce blood pressure [28].

Calcium: The average values for calcium recorded in the leaves and stem bark of the entire samples were 0.390 µg/g and 0.175 µg/g respectively which is lower than the amount (3.78 µg/g) reported by Kubmarawa et al. [6] in the roots. The difference in the results reported may be due to geographical location.

Copper: The values for copper was only recorded in the leaves and stem bark around Mugili and Pubba areas with a total mean values of 0.057 µg/g and 0.051 µg/g respectively. The values obtained in the samples were lower than the range of 100 to 300 µg/g recommended as normal copper concentration in plants by WHO [26]. Copper plays important role in treatment of chest wound and prevent inflammation arthritis and similar diseases. It is also essential for the formation haemoglobin of the red blood cells. It is required by trace quantity by humans [28].

Zinc: The presence of zinc was observed in the leaves around Pubba (0.210 µg/g). It was also found in the stem bark around Uba and Mugili with an average concentration value of 0.023 µg/g. The concentration of zinc in the plant parts investigated is lower than the permissible limits range of 150 to 2000 µg/g in plants as reported by WHO [26]. Zinc is essential constituents of enzymes that involve in carbohydrate and protein metabolism and nucleic acid synthesis. Its deficiency results in impaired growth and development, skin lesion and loss of appetite [27].

Cadmium: Cadmium was found in the entire samples investigated with a mean value 0.266 µg/g and 0.200 µg/g in the leaves and stem respectively. The concentration of cadmium in the leaves (0.266 µg/g) investigated is slightly higher than 0.20 µg/g fresh weight recommended as the maximum permissible level of cadmium in vegetables as reported by FAO/WHO [29]. The high level of cadmium obtained in the plant leaves may be due to agricultural activities carried out in the studied area.

Lead: The value for lead was below the detectable limit (<0.06 µg/g) of the instrument used in the entire samples. Pb has low geochemical mobility and bioavailability. Its transportation to above ground tissues in plants is minimal due to its retention in roots and precipitation [30]. An ultra-structural study using transmission electron spectroscopy revealed the retention of Pb in the cell wall of roots, particularly around intercellular spaces [31]. This explains why Pb was not observed in the leaves and stem bark of the plants investigated by the analytical instrument used. Lead is toxic metal and non-essential element for human body as it causes a rise in blood pressure, kidney damage and miscarriage [28].

Conclusion

The results obtained from this study revealed the presence of some essential elements (Na, K, Mg, and Ca) and heavy metals (Cu, Zn and Cd) in the plant parts investigated. The concentrations of elements in the plant is within the range of safety limits as reported by WHO, except for Cd in the leaves which is slightly above the recommended safety limit (0.2 µg/g), but lower than the results reported in cabbage (41-55 µg/g) and amaranths (6.0 µg/g). Pb was not detected in the entire samples analyzed by the method used. The essential elements and phytochemicals revealed by this leafy plant could be rich sources of minerals and also serve as basis for medicinal plant researchers.

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