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EVALUATION OF NUTRITIONAL, PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF AQUEOUS ETHANOL LEAF EXTRACT OF *BALANITES AEGYPTIACA* (L.) DELILE

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ABSTRACT

Purpose: *Balanites aegyptiaca* have been used in Northern-Nigeria for nutritional and medicinal purposes. Malnutrition due to food insufficiency and poverty are major burden of the third world countries. The research was aimed at evaluating the nutritional value of *B. aegyptiaca* leaves and to determine the phytochemical and antioxidant activity of the aqueous ethanol leaf extract.

Principal methodologies: The air-dried powder leaf was subjected to proximate analyses using standard methods. The leaf powder was analyzed for its mineral elements and heavy metal contents using Atomic Absorption Spectrophotometry (AAS) and Flame Photometry (FP). The leaf was extracted by maceration using 70% ethanol for the phytochemical and antioxidant studies. The extract was screened for its phytochemical constituents both qualitatively and quantitatively. The antioxidant activity of the extract was determined using DPPH (2,2-diphenyl-1-picryl hydrazyl) and ascorbic acid was used as standard.

Key findings: The proximate analyses of the leaves revealed crude fat (0.99%), crude fiber (7.80%), crude protein (14.25%) and nitrogen free extract (62.4%). The mineral element analyses revealed phosphorous (1524.60 mg/kg), nitrogen (4.20%), calcium (2126.10 mg/kg), potassium (11270.30 mg/kg), manganese (0.08%), copper (0.01%), selenium (0.02%), organic carbon (51.59%) and organic matter (88.94%). The heavy metal content revealed lead (0.01%) and cadmium (0.03%). The percentage yield of the aqueous ethanol extract was 41.02 %. The qualitative and quantitative phytochemical analyses revealed saponin (7%), tannins (1.35%), flavonoids (14%), steroids (3.15%), cardiac glycosides (0.75%). The total phenolic and total flavonoid contents were 24.92 mg GAE/g and 12.70 mg CTE/g respectively. The extract and the ascorbic acid scavenged the DPPH free radical with IC_{50} of 368.1 µg/mL and 46.10 µg/mL respectively.

Conclusion: The outcome of the research revealed that *B. aegyptiaca* leaf is a source of nutrients and phytochemicals with antioxidant property. The results have provided scientific basis for the use of the plant in traditional medicine for treatment of diseases associated with free radicals in the human body.

Keywords: Antioxidant, *Balanites aegyptiaca*, DPPH, malnutrition, nutritional, phytochemical
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INTRODUCTION

Malnutrition refers to a deficiency, excess, or imbalance of energy and other macro and micro-nutrients [1]. It is an urgent global health issue resulting in the death and disability of millions of children yearly [2, 3, 4]. Furthermore, malnutrition is a public health concern and it is one of the risk factors responsible for non-communicable diseases [5]. In recent past, it has been estimated that about 925 million people suffer from hunger and about 180 million preschool children are victims of chronic undernutrition [2, 6]. Poverty is defined as the scarcity or the lack of a certain amount of material possessions or money [7]. It is important to note that lower levels of education lead to decrease in economic productivity and the resultant effect is poverty [8]. Poverty is one of the challenges the world is currently facing [7]. Reports have shown that in the year 2019, there were about 1.3 billion people, in 101 countries living in poverty. This necessitated the 2030 Global Agenda for Sustainable Development Goals with the aim of eliminating all forms of poverty worldwide [9]. Poverty is the condition of being unable to meet the basic needs of life due to inadequate income or material goods [9]. Poverty is multifaceted and may include social, economic and political elements [10,

11]. Extreme poverty, is the complete lack of the means necessary to meet up with the basic needs such as food, clothing and shelter [10].

Plants have been the main source of nutrition for humans since time immemorial with the wild edible plants serving as sources of calories [11]. There is a strong interaction between human survival and plants [12]. The nutrient derived from edible plants could be beneficial or harmful to humans and phytochemicals derived from plants have played a key role in the maintenance of human health [12].

B. aegyptiaca (L.) Delile of the family Zygophyllaceae is an African plant with high socioeconomic interest especially, for the rural dwellers due its high demand during food scarcity [13, 14]. Previous researches on the fruits, seeds, roots and barks have revealed the presence of flavonoids, tannins, anthraquinones, terpenoids, steroids, cardiac glycosides and saponins [15, 16, 17]. *B. aegyptiaca* has been used for its medicinal and nutritional values in the Northern-Nigeria [18]. Rural women harvest the young fresh leaves from the wild to prepare a delicious soup while it is fresh and sometimes after air-drying in a dark room. Although, there are existing data on the chemical, nutritional and antioxidant properties on the other parts of the plant, little is available on the leaves. Therefore, this research presents data on the nutritional contents of the leaves, phytochemical and antioxidant activity of the leaf extract.

MATERIALS AND METHODS

Plant collection, identification and authentication of *B. aegyptiaca*

The fresh leaves, flower and fruits were collected at the Botanical Garden, Department of Plant Biology, Bayero University Kano, Nigeria with the help of the Curator (Buha'uddeen Said Adam). The plant was identified and then authenticated by scanning, using a computer programme. The specimen voucher with number: BUKHAN0359 was prepared and then kept in the herbarium of the Department for future reference.

Proximate analysis of *B. aegyptiaca* leaves

The moisture, ash, protein, fat, fibre, total carbohydrate and free nitrogen extract contents were estimated as outlined by the Association of Official Analytical Chemist [19]. The moisture content was determined by loss of weight on drying method. The ash content was obtained by burning 1 g of the powdered plant sample in a muffle furnace at 550°C for 3-4 hours. The alcohol and water-soluble extractive values were determined by maceration. The crude protein was determined by Kjeldahl method. The crude fat was estimated by the ether extract method with the aid of the Soxhlet apparatus where 1 g of moisture free sample of the leaf was extracted using petroleum ether. The crude fibre was determined using the moisture free and ether extracted samples. The samples were digested with dilute H_2SO_4 and then with dilute KOH solution. The undigested residue collected after digestion was ignited in a muffle furnace at 550°C for 3-4 hours and loss in weight after ignition was recorded as crude fiber. The Nitrogen Free Extract (NFE) was calculated as follows:

NFE = (100 - % moisture + % crude protein + % crude fat + % crude fiber + % ash)

Mineral content determination of *B. aegyptiaca* leaves

The mineral content of the leaf was determined according to AOAC [19]. Chromium (Cr), Cadmium (Cd), Lead (Pb), Manganese (Mn), Calcium (Ca) and Magnesium (Mg**)** were analyzed with atomic absorption spectrometry (AAS) while Potassium (K) and sodium (Na) were analyzed using flame photometry. The wet digestion method was used for the sample preparation and analyses.

Preparation and extraction of *B. aegyptiaca* leaves

The procedure described by Ncube *et al*. [20] was followed. The leaves were washed with distilled water and then air dried under the shade for seven days. The sample was size reduced to fine powder using wooden pestle and mortar. The powdered leaf (100 g) was macerated for 24 hours using 70% ethanol (1 L). The solution was decanted, filtered through Whatman No.1 filter paper and then concentrated using a rotary evaporator. The residue was allowed to dry and the percentage yield calculated.

Phytochemical screening of *B. aegyptiaca* leaf extract

The methods outlined by Sofowora and Harbone [21, 22] were used for the qualitative and quantitative analyses of saponins, tannins, flavonoids, alkaloids, anthraquinones, steroids, anthraquinones and cardiac glycosides.

Determination of total phenolic and total flavonoid contents of *B. aegyptiaca* leaf extract

The total phenolic content was determined using the Folin-Ciocalteu's reagent [23] and the $AlCl_3$ method outlined by Sushant *et al*. [24] was adopted for the determination of total flavonoid content.

Determination of antioxidant activity of *B. aegyptiaca* leaf extract using DPPH free radical

The method of Zhu *et al*. [25] was used for the determination of antioxidant activity using DPPH free radical scavenging assay. The samples were mixed with 2 mL of DPPH solution (0.1 mM, in ethanol) at various concentrations (7.8125, 15.625 31.25, 62.5,

125, 250, 500, 1000 µg/mL). The reaction mixture was allowed to incubate in the dark at room temperature (27ºC) for 30 minutes before the absorbance was measured at 517 nm. The percentage inhibition was calculated and the IC_{50} was determined using the Graph Pad prism 8.0.

RESULTS

Proximate parameters of *B. aegyptiaca* leaves

The proximate parameters (%) of the dried powdered leaf are presented in Table 1, with the nitrogen free extract having the highest percentage.

Table 1: Proximate parameters of *B. aegyptiaca*

Proximate parameters	Content (%)
Crude fat	0.99
Crude fibre	7.80
Crude protein	14.25
Nitrogen free extract	62.40
Total carbohydrate	62.40
Moisture content	4.70
Ash content	9.86
Alcohol soluble extractive	8.13
Water soluble extractive	15.3

Mineral content of *B. aegyptiaca* leaves

The mineral content of the dried powdered leaves revealed a high amount of calcium (Table 2).

Table 2: Mineral content of *B. aegyptiaca* leaves

Mineral	Content
Nitrogen (%)	4.20
Phosphorous (mg/kg)	1524.60
Potassium (mg/kg)	11270.30
Calcium (mg/kg)	2126.10
Manganese (%)	0.08
Copper (%)	0.01
Selenium (%)	0.02

Heavy metals content of *B. aegyptiaca* leaves

The percentage concentration of the heavy metals analyzed were found to be low in the leaves (Table 3).

Table 3: Heavy metals contents of the dried leaves of *B. aegyptiaca*

Heavy metal	Concentration (%)
Cadmium	0.03
Lead	0.01

Percentage yield and phytochemical constituents of *B. aegyptiaca* leaf extract

The mass and percentage yield of the crude hydro-ethanol extract obtained were 20.51g and 41.02% respectively. The phytochemical screening revealed the presence of some important classes of secondary metabolites (Table 4).

Table 4: Qualitative and quantitative phytochemical screening *B. aegyptiaca* leaf extract

Phytochemical	Qualitative	Quantity (%)
Flavonoids	+	14.00
Tannins	+	1.35
Steroids	+	3.15
Saponins	+	7.00
Cardiac glycosides	+	0.75

Total phenolic and total flavonoid contents of *B. aegyptiaca* leaf extract

The total phenolic and total flavonoid contents were found to be 24.92 mg GAE/g (Gallic acid equivalence) and 12.70 mg RE/g (Rutin equivalence) respectively.

DPPH Free radical scavenging activity of *B. aegyptiaca* leaf extract

The antioxidant activity demonstrated by the crude extract was expressed in terms of IC_{50} as determined from the percentage inhibition and the concentration (Figure 1). The IC_{50} of the crude extract was 368.1 µg/mL and that of the ascorbic acid was 46.10 µg/mL.

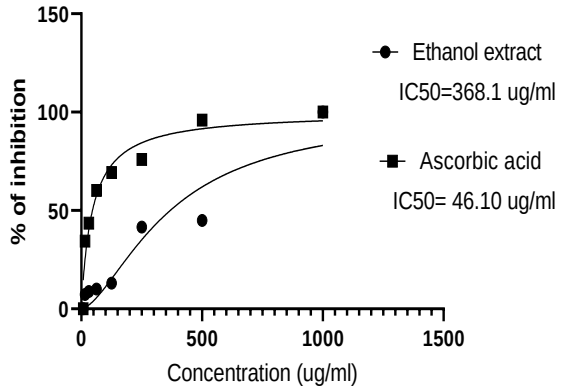

Figure 1: Inhibition (%) of extract and ascorbic against concentration (µg/mL)

DISCUSSION

The moisture content of the dried leaf was found to be 4.70% w/w. When compare with digitalis (5 % w/w) and ergot (8% w/w) [26], it was lower. This result indicated that the leaf was properly dried and stored. The presence of high amount of moisture in crude drugs encourages fungal growth and this leads to deterioration of crude drugs. On the other hand, low moisture may prevent deterioration of the crude drug [27]. The total ash content of 9.86% w/w obtained from this study was higher than that obtained from the seeds of *Balanites aegyptiaca* with total ash of 3.55%. This difference may be due to the difference in the morphological parts of the plant [28]. High ash content may be due to adulteration of the crude drugs with siliceous materials or due high mineral contents [26]. Extractive values are helpful in determining the amounts of plant constituents that could be extracted using a given solvent. The alcohol soluble extractive (8.13%) was found to be lower than the water-soluble extractive (15.3%). This may be due to high polarity of water which tends to extract polar plant constituents [26].

The nutritional potential of *B. aegyptiaca* leaves could be expressed in term of the crude protein. The crude protein of 14.25% obtained from this study was higher than in the flower which had 10.8% [29]. This variation may be due to the fact that the leaves are directly involved in food synthesis for the plant and most of the plant proteins (70%) are stored in the chloroplast [30]. As it is known that certain phytochemicals accumulate in specific part of the plant [31]. Fat is a key nutrient in plants required by humans especially the essential fatty acids. The crude fat obtained from leaf was low when compared with those of the flowers (4.5%) [29]. Furthermore, the crude fibre (7.80%) obtained from this study was higher when compared with the flowers which had crude fibre of 3.8%. Plants with high fibre are of nutritional interest as the fibers play a role in relieving constipation [32]. The nitrogen-free extract (62.40%) obtained in this study is higher when compared with the study of [33] who reported nitrogen free extract (44.50%) from *B. aegyptiaca* leaves. Nitrogen free extract is a component in rations of animal feeds and also source of energy for body processes and for the deposition of fat. The *B. aegyptiaca* leaf contains high carbohydrate as shown by the total carbohydrate (62.40%). Although the flower was reported to have higher carbohydrate content (74.2%) [29].

The mineral composition of the *B. aegyptiaca* leaf (Table 2) showed the presence of some important elements. The amount of nitrogen (4.20%) in the leaf obtained in the present study was found to be higher than in the flower [29]. These differences may be due to due to the fact that many plant constituents are found concentrated in the leaves [31]. Nitrogen is needed for the synthesis of amino acids, DNA, RNA, and ATP in the body [34]. The amount of calcium (2126.10 mg/kg) obtained from this study was higher than the earlier reported value from the leaf (416.40 mg/100g). Calcium is an important mineral especially in children as it is required for bone and teeth formation [35]. Also, the amount of potassium (11270.30 mg/kg) was found to be greater in previous research (1164.8 mg/100g) by (Idris *et al*., 2010)**.** Potassium plays an important role in maintaining the bioelectrical potential of the body [36]. In the current study, the concentration of manganese obtained was 0.08 % and this differs from the previous study (15.35 mg/100g) [33]**.** Manganese activates enzymes transfer ATP to ADP. The amount of copper obtained was 0.01% and copper helps in melanin pigment formation. The amount of selenium was 0.02 % and it is an important component of many enzymes and proteins, called seleno-proteins, which helps in the production of DNA [37].

Heavy metal analysis revealed the presence of cadmium (0.03%) and lead (0.01 %) in the leaf. These results are found to be lower in the leaf when compared with the limits set by the WHO and FAO for the various elements in vegetables for human consumption [38]. This suggest that the leaf may be safe for consumption. Appreciable percentage yield (41.02%) was obtained from the aqueous ethanol extract of the dried leaves. The polarity of the extracting solvent may be responsible for the high yield [39]. The qualitative and quantitative phytochemical analysis revealed the presence of saponins (7%), flavonoids (14%), cardiac glycosides (0.75%), steroids (3.15%) and tannins (1.35%). The presence of these secondary metabolites may be responsible for the various biological activities of the extract.

The antioxidant activity of plant extracts has been attributed to the flavonoids, tannins and other phenolic compounds [40]. The total phenolic content of *B. aegyptiaca* leaves is equivalent to 24.92 mg GAE/g. This result is lower, when compared with previous study by Khorasani *et al*. [41] who reported 46.88 mg GAE/g total phenolic content of methanolic extract of *B. aegyptiaca* leaves. This observation may be due to the fact that phenolic compounds are more easily extracted from methanol than other solvents [41]. The total flavonoid content of 12.70mg RE/g was obtained from this study. This result is lower than the total flavonoid content of methanolic extract of *B. aegyptiaca* leaves reported by Khorasani *et al*. [41] which was found to be 26.61mg CTE/g. Flavonoids have the potential to protect humans from lipid peroxidation by scavenging free radicals [42]. The aqueous ethanol extract of the leaf showed concentration dependent activity. It had the highest percentage inhibition at 1000 g/mL and the lowest at 7.8125 g/ml. The ascorbic acid exhibited greater antioxidant activity when compared with the aqueous ethanol leaf extract. The antioxidant activity was further expressed on the basis of the IC_{50}. The lower the IC_{50} value the more potent is the test sample [43]. The IC_{50} value of the plant extract was 368.1 µg/mL while that of the ascorbic acid was 46.10 µg/mL.

CONCLUSION

B. aegyptiaca leaves is an excellent source of dietary nutrients. The high proportions of carbohydrate and protein give it a good nutritional value. It is also a good source of dietary minerals containing nitrogen, phosphorus, potassium, calcium, copper, manganese and selenium. The presence of flavonoid, tannins and other secondary metabolites, indicated the leaves to be a potential source of natural antioxidant with health promoting properties as supported by the ethnomedical claims on the plant.

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