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Phytochemical and Proximate Analyses of Methanol Leaf Extract of Neem *Azadirachta indica*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FMM, AYK and JE designed the study, wrote the protocol and supervised the work. Authors FMM, SCM and JE carried out all laboratories work and performed the statistical analysis. Authors FMM and MTBO managed the analyses of the study. Author FMM wrote the first draft of the manuscript. Authors FMM, JE and RUH managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: To determine the phytochemical content, proximate and mineral analysis of neem leaves.
Place and Duration of Study: The experiment was carried out in the Department of Biochemistry, Federal University of Technology, Minna, Bosso Campus, Niger State. from March 2013 to July, 2013.
Methodology: Reflux extraction was carried out using 70% methanol as extraction solvent standard analytical methods were used to determined the phytochemical content and AOAC official method was used to determined the proximate and mineral contents.
Results: The result of the phytochemical screening of methanol leaf extract indicated the presence of cardiac glycosides, saponins, steroids, flavonoid, alkaloids, tannin, phenol, terpene, and reducing sugar while athraquinone and carbohydrate were absent.

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The proximate analysis of Neem leaves showed the following result; moisture% 9.50±0.24, ash content 2.81±0.21' protein% 1.58±0.34' fat% 2.07±0.35, fibre% 5.92±0.47' carbohydrate% 78.12±0.35, Vitamin A (mg/100 g) 180±0.10 and Vitamin C (mg/100 g) 287±6.22.
The result of mineral analysis shows that neem leaves contains potassium 235.65±5.05, calcium 170.05±10.12, sodium 180.65±8.83 and phosphorus 39.34±3.25.
Conclusion: It can be deduced from these results of this study that neem leaves contained appreciable amount of phytochemicals, nutrients and minerals that aid it medicinal properties.

Keywords: Phytochemical; proximate analysis; mineral analysis and Azadirachta indica (Neem).

1. INTRODUCTION

The medical value of the plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [1]. Presently, many scientist and organizations are in search of traditional remedies as alternative medicine. It has been estimated that about 25% of all prescribed medicines today are substances derived from plants [2,3]. The African continent is one of the continents endowed with the richest biodiversity in the world with avalanche of many plants used as herbs, health and food for therapeutic purpose [4]. It is however observed that these purposes vary from one country to another. Numerous plants and herbs are used all over Nigeria by traditional medicine practitioner [5].

Plants have contributed more than 7,000 different compounds in use today antibiotics, laxative, anticancer agents, contraceptives, diuretics, decongestants and analgesics compounds. The world Health Organization (WHO) estimates that up 80% of the world's people rely on plants for their primary health care, since western pharmaceuticals are often expensive, inaccessible or unsuitable and are always accompanied with various side effects [2].

Azadirachta indica (Neem) is an evergreen tree, cultivated in various parts of subcontinent. Every part of the tree has been used as traditional medicine for household remedy against various ailments from antiquity. Neem has been extensively used as Ayuveda, Urani and Hemeophatic medicine. The Sanskrit name of Neem tree is Arishtha, meaning reliever of sickness [6]. Chemical investigation on the products of Neem tree was extensively undertaken in the middle of the 20th century. The Neem Tree is an incredible plant that has been declared the Tree of the 21st century by the United Nations [2]. In India, it is commonly known as 'Divine Tree', 'Life giving tree', 'Nature's Drugstore', 'Village Pharmacy' and 'Panacea for all diseases' [5] (Neem Foundation, 1997). Extracts from the Neem tree (*A. indica* A Juss) also called 'Dogonyaro' in Nigeria are most consistently recommended in ancient medical texts for gastrointestinal upsets, diarrhoea and intestinal infections, skin ulcers and malaria [7]. Its leave can be used as drug for diabetes, eczema and reduce fever. Barks of Neem can be used to make toothbrush and the roots has an ability to heal diseases and against insects. The seed of Neem tree has a high concentration of oil. Neem oil is widely used as insecticides, lubricant, drugs for variety of diseases such as diabetes and tuberculosis [2,8,9]. In Africa, extracts from Neem leaves have provided various medicinal preparations. In Nigeria Neem leaves is mostly used and therefore it is imperative to evaluate the chemical constituents and proximate content of this part of neem plant. Which will further enhance the knowledge of how Neem plant (*A. indica*) exerts its biochemical, pharmacological, and medicinal properties.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection of plant materials

The leaf was collected as part of the plant for phytochemical screening and proximate analysis. The fresh plant sample (*A. indica*) was collected around the staff quarters of The Federal University of Technology, Minna, Bosso Campus, Niger State. They were found growing freely as wild plant collected in March, 2013 and identified by a Mr Adamu; a botanist from the department of Biological Science Federal university of Technology, Minna Niger State.

2.1.2 Preparation of plant material

The leaves of the identified plant were collected and air dried, the dried sample was milled into

fine powder by pounding manually with a clean sterile mortar and pestle to increase surface area, and the powder was weighed and collected into clean cellophane bags and labeled to prevent mix up. The sample was kept in a cool dry place till further use, while fresh leaves were use immediately after collection from the tree.

2.1.3 Extraction of plant materials

Fifty grams (50 g) of the pounded dried plant materials (leaves powder) was weighed and extracted with 400 ml of aqueous(distilled water) and 400 ml of methanol using reflux extraction method. The processes was run for 2 hours each after which the samples was evaporated to dryness using water bath. The dried extract was weighed and kept in a well labeled sterile specimen bottles and stored in a refrigerator at 4 degree celsius until is required.

2.2 Phytochemical Screening

The methanol extracts of the plant was subjected to phytochemical test to determine their chemica1 constituents using standard method of [10-12].

2.3 Proximate Analysis

The proximate analysis of the samples for moisture, total ash, crude fibre, fat, protein were carried out in duplicate using methods described by Onwuka [13]. The nitrogen was determined by micro Kjeldahl method described by Onwuka [13] and the nitrogen content was converted to protein by multiplying by a factor of 6.25. Total carbohydrates content was estimated by 'difference'. All the proximate values were crude fiber, percentage crude protein, carbohydrate content.

2.4 Determination of Vitamin A

One gram of sample was weighed.10ml of distilled water was added and homogenized using ceramic mortar, then filtered with filter paper. Two mls of filtrate, then it was shaken for 1 minute, heat under water bath at 60°C for 20 minute, after this 2 mls of xylene was added and mixed thoroughly using cyclo mixer, and then it was centrifuged at 1500 rpm for 10 minutes. The whole of separated upper layer was collected and transfer into a test tube. Absorbance was measured at wavelength of 335 nm against xylene, the extract was irradiated in a test tube for 30 minutes.

Calculation:

$C_{x =(}A_1 – A_2) \times 22.23$
C_X = concentration of vitamin A
A_1 = Absorption before irradiation
A_2 = Absorbance after irradiation measured in mg/100 ml

2.5 Determination of Vitamin C

0.5 g of the sample was homogenized with 5mls of 2% HCL acid using ceramic mortar and pestles, then filtered with filter paper, the filtrate was made up to 100 ml, 5 ml of the diluted solution was measured into 2 conical flasks and titrated with iodole phenol dye and the titre value was determined.

Calculation:

0.5 / 13.1 x titre value
Divide answer by 5 then multiply by 100
Measured in mg/ 100 ml

2.6 Mineral Analysis

The mineral composition of the sample was analyzed on aliquots of dry-ashing. 1 g of the test portion, was weighed into glazed, high-form porcelain crucible and was ashed for 2 hrs at 500°C, and allowed to cool. The ash was wet with 10 drops of water, and carefully 3-4 ml of HNO_3 (1+1) was added. Excess HNO_3 was evaporated on hot plate set to 100-120°C. The crucible was returned to the furnace for reashing for additional 1 hr at 500°C after which the crucible was cooled and the ash dissolved in 10 ml HCl (1+1), and transferred quantitatively into 50 ml volumetric flask. The mineral elements were determined by Atomic Absorption Spectrophotometer (Model Accusy 211 Bulk Scientific USA), sodium and potassium by flame photometer (Model FP6410 Harris Medical Essex, England), phosphorus was determined by colorimetric means using the vanadomolybdate (yellow) method [14] (AOAC official method 975.03; AOAC).

3. RESULTS

The results of phytochemical component of 70% methanol extracts of *Azadirachta indica* leave are summarize in Table 1.

The result of mineral analysis of fresh leaves of *azadirachta indica* is shown in Table 2.

The result of mineral analysis of fresh leaves of *Azadirachta indica* is shown in Table 3.

Table 1. Phytochemical constituent of and methanol extracts of *Azadirachta indica*

Phytochemical	Result
Cardiac glycosides	+
Saponins	+
Steroids	+
Flavonoids	+
Alkaloids	+
Tannins	+
Phenols	+
Terpenes	+
Anthraquinones	–
Carbohydrates	–
Reducing sugar	+

Absent - , present +

Table 2. Proximate composition of fresh leaves of *Azadirachta indica*

Parameters	Result %
Moisture%	9.50±0.24
Ash content%	2.81±0.21
Protein%	1.58±0.34
Fat%	2.07±0.35
Fibre%	5.92±0.47
Carbohydrate%	78.12±0.35
Vitamin A (mg/100 g)	180±0.10
Vitamin C (mg/100 g)	287±6.22

Values are means of duplicate results ± SEM

Table 3. Mineral constituents of fresh leaves of *Azadirachta indica*

Parameters	Result (mg/100 g)
Potasium	235.65±5.05
Calcium	170.05±10.12
Sodium	180.65±8.83
Phosphorus	39.34±3.25

Values are means of two different determination ± SEM

4. DISCUSSION

The results of the phytochemical screening of methanol extract reveals the presence of terpenes, cardiac glycosides, flavonoids, alkaloids and saponin, steriods, tannins, phenols, reducing sugar, while carbohydrate and anthraquinones were absent. It showed from the results that the methanol extract exhibited more phytochemical constituent, compared to the result reported by Tiwari and Rao [15]. This could be due to variation in geographical location. However, some of these components have been shown to have antinutritional effect due to their ability to decrease palatability and digestibility of food stuffs [16] as shown by the bitter nature of neem leaves.

The results of the proximate analysis of the fresh neem leaves as shown in Table (2), reveals that the fresh - leaves of *A. indica* contain more moisture and carbohydrates. These value indicate that fresh - leaves of *A. indica* can serve as good source of energy because carbohydrate and fat are known to be the main source of energy for organism which include human, animal, or microorganism. The protein content of *A. indica* in this study is lower than the percentage recommended by the Food and Agriculture Organization [17] which is in the range of 12-15%. This suggests the need to supplement a diet base on *A. indica* with a complementary protein source to make it more nutritious. The ash content indicates the degree of the inorganic matter content of the samples. The percentage ash content is (-2.81±0.2-), this can be attributed to the mineral composition of the leaves. When ash content is abnormally high, there is a very good chance the forage is contaminated with soil which is not desirable. The normal content of legume grass forage is near 9.0%. Those with more than 10-18% ash are likely contaminated with increasing amount of soil, excess ash content can have negative effect on lactation for example in cattle, hence the amount of non- fermentable inorganic matter in some dairy cattle diets get high [18,19] on this basis *A. indica* can be considered suitable for animal feeds. The percentage crude fibre was (5.92±0.47) obtained for fresh leaves. These indicate that *A. indica* leaves contained fibre which can aid in paristalysis. The moisture content was (9.50±0.24) which is a good attribute for storage [20].

The result of vitamin analysis revealed that fresh leaves contain high concentration of vitamin A and C. The recommended dietary allowance for vitamin C are based on its known physiological and antioxidant functions in white blood cells and are much higher than the amount required for protection from deficiency. Recommended dietary allowance for vitamin C for female is 75 mg while for male is 90 mg. Vitamin C also known as L- ascorbic acid, is a water soluble vitamin that is naturally present in some food which is required for the biosynthesis of collagen and also involved in protein metabolism [21,22]. The Recommended dietary allowance for vitamin A is 900 mg, vitamin A is fat soluble vitamin that

is naturally present in some food which is essential for healthy skin, eyesight, growth, and reproduction the biosynthesis of collagen and also involved in protein metabolism, these vitamins and mineral elements have variously been shown to posses' antioxidant activities.

The result shown of mineral analysis of fresh neem leaves shows low concentration of minerals such as Na, P, K, and Ca compared to the result to the result reported by Afolabi and Tunde [18]. The Na^+/k^+ ration of *A. indica* leaves is less than one, this on the basis of the daily recommendation which suggest that *A. indica* could be suitable for reducing high blood pressure. Conversely the ca^{2+}/ p ration of *A. indica* leaves is far less than one, thus its consumption is likely to reduce the intestinal absorption of calcium. An excess of phosphorus (Ca^{2+}/p ratio lower than 1 to 2) has shown in several species to cause secondary hyperparathyroidism with loss of bone loss and increase intestinal calcium absorption in rats. The mineral contents of *A. indica* in this study is lower than the percentage recommended by the Food and Agriculture Organization [17] (FAO, 2003) sodium is 2400 mg, phosphorus (700 mg), potassium (4700 mg), calcium (1000 mg). This suggests the need to supplement a diet base on *A. indica* leaves with a complementary mineral element source to make it more nutritious.

5. CONCLUSION

This studies reveals that neem leaves contains phytochemical constituents that enables it elicit medicinal properties. *A. indica* leaves could also serve as a good source nutritional supplement in the diet of animal.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical clearance was given by Federal University of Technology Minna/Nigeria Ethical Review Board (CUERB) in accordance with internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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