**Research Article**

**Proximate, elemental, phytochemical and anti-fungal analysis of *Acacia nilotica* fruit**

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**ABSTRACT**

Objective: *Acacia nilotica* fruit has been used to treat different diseases. The significance of the plant in traditional medicine and the importance of the distribution of these chemical constituents were discussed with respect to the role of this plant in ethno-medicinal usage in Nigeria. In the present study, the fruit of *A. nilotica* was subjected to elemental, phytochemical and anti-fungal analysis.

Methods: The extraction was done with ethanol and hexane using soxhlet apparatus. The elemental analysis was done using an Atomic Absorption Spectrophotometer. The phytochemical investigation of the ethanol and hexane extract of *A. nilotica* fruit was carried out. The extracts were evaluated for their antifungal activity.

Results: The proximate analysis showed that moisture content was 12.6±0.02 %, crude fibre 11.1±0.03 %, crude lipid 15.8±0.01 %, ash content 5.0±0.01 %, crude protein 1.3±0.02 % and carbohydrate 54.2±0.02 %. The elemental analysis showed various concentrations of Ca, Zn, Mg, Mn, Ni, Cr, Fe, Cd while Co was absent. The phytochemical investigation revealed the presence of tannins, steroids, saponins, phenols, alkaloids, cardenolides, terpenoids, carbohydrates, cardiac glycosides, resins and balsams. The antifungal analysis of ethanol extract on *Aspergillus niger*, *Aspergillus flavon*, *Fusarium oxyfurum* and *Penicillium Spp* showed an increase in the zone of inhibition and an increase in the concentration of the *A. nilotica* fruit extracts when measured in mm.

Conclusion: Acacia nilotica has both nutritional and medicinal values based on the presence of numerous secondary metabolites and essential metals. The plant studied here can be seen as a potential source of useful drugs and further studies are going on in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds.

**Keywords:** *Acacia nilotica*, Phytochemical, Antifungal, Elemental, Inhibition

**Introduction**

Plants have great significance due to their nutritive value and are also a major source of medicines. Plants have served mankind throughout the history of human civilization⁴. 30 to 40% available drugs are based on the medicinal and curative properties of various plants and are in use as herbal supplements, botanicals and nutraceuticals²,³. Wild edible
plants, many of which are potentially valuable as an alternative food for humans can play an important role in striking a balance between population explosion and limited agriculture productivity, especially in developing countries\(^4\). The important values of some plants have long been published but a large number of them remain unexplored as yet. So there is a necessity to explore their uses and conduct pharmacological studies to ascertain their therapeutic properties\(^5\).

*Acacia nilotica* also known as gum Arabic, Egyptian thorn in Egypt, scanted tree in English, thorn in mimosa in Australia and commonly known as “Bagaruwa” among the Hausas, is a species of vachellia and a genus of Acacia. It is a thorny Acacia found in different parts of the world (West Asia, Africa, Eastwards India, Australia)\(^6\).

*A. nilotica* has been designated and used as a medicinal plant in Northern Nigeria, West Africa, and other parts of the world. It is known as a multipurpose medicine, used to treat illnesses like diarrhea, leprosy, male sterility, diabetes, cancer, ulcer, inflammation, anti-microbial and some others. It is also used as a local vegetable and tanning material by tanning industries in Nigeria. The pods are greatly utilized for their higher tannins because chemical analysis has shown that it contains both hydrolysable and condensable tannins\(^7\). The fresh parts of these plants have been reported to be most active against Hepatitis C varius\(^8\). The leaves of *A. nilotica* inhibit and active against *A. fumigates*, *P. expansum* and *C. albicans* while its stem and bark does not show activity against these fungal\(^9,10\). The herbal product derived from acacia species are sold in the market either pure or mixed from like Babool tooth paste, Ajur shampoo and Nye shampoo etc\(^9\). Phytochemical screening was done on the plant extracts to determine the bioactive constituents of the plant, as some plants are highly medicinal. Proximate analysis of the plant was done to reveal the relative amount of macro-nutrient and non-nutrients in the plant. Elemental analysis was carried out to determine the amount of known metals in the sample while the anti-fungal analysis was done to determine the anti-fungal activity of the fruits of the plant.

**Materials and Methods**

**Collection of plant materials**

The fruit of *A. nilotica* was collected from Gwagwalada market, Federal Capital Territory (FCT), Abuja, Nigeria. It was identified at the Biotechnology Laboratory of Sheda Science and Technology complex, (SHESTCO) Federal Capital Territory (FCT), Abuja, Nigeria. The fruit of *A. nilotica* was washed under running tap water and dried in an oven at 40°C for 24 h. It was grounded into coarse powder with an electronic blender and then stored in air-tight containers for further analysis.

**Chemicals and reagents**

All the chemicals and reagents used in this study were of analytical grade and were products of British Drug House Laboratory, England.

Sample analysis: The proximate analyses were carried out in triplicates and the results obtained were the average values. The moisture content was determined by drying in an oven at 100-107°C to constant weight\(^11\). The crude fat was determined by continuous extraction in a soxhlet apparatus for 8 h using hexane as a solvent, ash by incinerating in a furnace at 550°C for 5 h, crude fibre by sequential hot digestion of the defatted samples with dilute acid and alkaline solutions\(^11\). The crude protein content was evaluated by digestion of the samples using Kjeldahl’s method\(^12\), nitrogen determination by a spectrophotometric method describe by Devani\(^12\). The total carbohydrate was determined by subtracting the % sum of protein, crude lipid, crude fibre, moisture and ash from 100\(^11\).

Mineral analysis was carried out after 1 g of the fruit sample was ash and 10 ml of concentrated HNO\(_3\) was added to it and digested until a clear solution was obtained. The digestion was allowed to cool and then transferred into a 100 ml standard flask and made up to mark with de-ionized water. The mineral elements were analyzed with an atomic absorption

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spectrophotometer (GBC AvantaVer 2.02 Model, Australia) equipped with air-acetylene flame. Sodium and potassium were determined using a flame photometer (Gallenkamp flame analyzer, UK).

**Hexane and ethanol extraction of fruit**

The hexane and ethanol extracts were extracted from the resulting powder by adopting the method described by Association of Official Analytical Chemist (AOAC)\(^{[13]}\), which entailed using soxhlet apparatus with hexane and then ethanol. 200 g of the ground fruit were packed in muslin cloth and inserted into the soxhlet extractor and hexane was used as the extracting solvent for a period of eight hours. At the end of this period, the solvent was recovered by rotary evaporator and residual oil was oven-dried at 75°C for one hour. Ethanol was also used for extracting solvent for another period of eight hours. At the end of this period, the solvent was recovered by a rotary evaporator and the residual extract dried at 85°C for one hour. The two extracts were then transferred to a desiccator and allowed to cool before being kept for analytical tests.

**Phytochemical screening**

Chemical tests were carried out on the hexane and ethanolic extracts for the qualitative determination of phytochemical constituents as describe before\(^{[14-16]}\).

**Anti-fungal Analysis**

Anti-fungal analysis was carried out using the well diffusion method described by Aliyu and Sani\(^{[17]}\) to determine the anti-fungal activity of the plant extract. The media was first prepared by dissolving 14 g of Potato Dextrose Agar (PDA) in 50 ml diluents (DMSO and distilled water in the ratio of 1:1) according to Akinpelu and slide modification\(^{[18]}\). The mixture was put in a water bath for complete dissolution. It was then put in an autoclave for sterilization. Pour plating of the media was done and a thick gel was allowed to form. Different concentrations of the extract were prepared by dissolving 0.5 g in 2 ml of the diluents; it was then double diluted giving concentrations 500 mg/ml, 250 mg/ml and 125 mg/ml. The Fungi species *Aspergillus niger*, *Penicillium spp.*, *Aspergillus flavus* and *Fusarium oxysporum* were then inoculated in each plate using the streaking method, 5 holes were bored on the media and 2 ml of each concentration of the extracts were put in 3 holes, a positive control (Nystatin) and negative control (Diluents) where put in remaining 2 holes. This was repeated for each plate. It was then incubated at room temperature for 58 h and the zone of inhibition was measured.

**Statistical analysis**

For the fractions, samples were analyzed and assays were carried out in triplicate. The results were analyzed using one way analysis of various (ANOVA).

**Results and Discussion**

The results obtained from the proximate and mineral analysis were documented in Table 1 and 2 respectively. The preliminary phytochemical analysis results were reported in Table 3. The anti-fungal activity study results were given in Table 4.

<table>
<thead>
<tr>
<th>Table 1: Chemical Composition of A. nilotica.</th>
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<tbody>
<tr>
<td><strong>Component</strong></td>
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<tr>
<td>Moisture</td>
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<tr>
<td>Crude fibre</td>
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<tr>
<td>Crude fat</td>
</tr>
<tr>
<td>Ash</td>
</tr>
<tr>
<td>Crude Protein</td>
</tr>
<tr>
<td>Carbohydrates</td>
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</tbody>
</table>

<table>
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<tr>
<th>Table 2: Mineral Composition of A. nilotica.</th>
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<tbody>
<tr>
<td><strong>Minerals</strong></td>
</tr>
<tr>
<td>Na</td>
</tr>
<tr>
<td>K</td>
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<tr>
<td>Ca</td>
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<tr>
<td>Fe</td>
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<tr>
<td>Mn</td>
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<tr>
<td>Cu</td>
</tr>
</tbody>
</table>
The proximate analysis profile of *Acacia nilotica* showed that the ash content, which is a measure of mineral content, was obtained as 5.0±0.01%. The moisture content is known to affect the processing, preservation and storage of food and herbal products.[19] High moisture content renders plant products susceptible to microbial attack and thus leads to spoilage and a lowered shelf life.[20] The moisture content 12.6±0.02% of *A. nilotica* showed that it needed to be dried further before storage. The crude fibre represents the non-digestible carbohydrates as lignin. The contents of crude fibre are known to enhance digestibility but a high level can lead to intestinal irritation, lowered digestibility and decreased nutrient absorption.[21] The content of crude fibre in *A. nilotica* is high and may be considered inappropriate for consumption.[20] Lipids are a rich source of energy and aid in the transport of total fat soluble vitamins insulate and protect internal tissues and contribute to important cell processes.[22,23] The total lipid content of *A. nilotica* was found to be 15.84±0.01%. According to Pearson (1976), a plant-based food that provides more than 12% of its caloric value from protein is considered as a good source of proteins.[24] In the case of *A. nilotica*, total protein is contributed to approximately 3% of the total calorific value, making it a bad source of proteins. The plant is a good source of carbohydrates (54.19±0.02%) when compared with the Recommended Dietary Allowance (RDA) values for children, adults, pregnant and lactating mothers.[25]

The result of mineral element composition of *A. nilotica*, in mg/g dry matter is shown in Table 2. The zinc content of *Acacia nilotica* was 0.08±0.01mg/g. The Recommended Dietary Allowance (RDA) for zinc is 13mg/kg.[26] Zinc is essential in the activation of certain enzymes such as dehydrogenase, alkaline phosphatase and carboxypeptidase. Zinc containing organic compounds is employed as astringent and antifungal agents. It aids wound healing and the metabolism of nucleic acids and insulin. Excess of zinc causes anaemia and can lead to dermatitis if deficient in the body. The manganese content of *Acacia nilotica* was 0.04±0.02 mg/g. The Recommended Dietary Allowance (RDA) for manganese varies between 2mg/kg to 8mg/kg.[26] Certain trace elements such as copper, iron, and manganese constitute an essential part of any balanced diet. Some of them are micronutrient to the plants and if not presented in the right proportion, they may have adverse effect on human and plants. The content of copper was 0.25±0.01 mg/g. The Recommended Dietary Allowance of copper according to Jones et al., (1985)[26] is 3.5 mg. Copper is very vital in a diet because it is involved in the proper usage of iron (Fe) and especially for the synthesis of cytochrome oxidase, which contains both iron (Fe) and copper (Cu). Excess of copper can lead to jaundice (Wilson’s disease).[27]

The potassium content was 4.19±0.03 mg/g. According to National Research Council (1974)[28], the Recommended Dietary Allowance of potassium is 1875-5625 mg/kg for adults. Potassium is very vital in the regulation of water, electrolyte balance and acid-base balance in the body, as well as responsible for nerve actions and the functioning of the muscles.

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**Table 3: Phytochemical analysis of *A. nilotica***.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Hexane extract</th>
<th>Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tri-terpenoid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycoside</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Balsams</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Volatile oil</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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Deficiency of potassium leads to muscle paralysis. The sodium content was 6.86±0.02 mg/g. Sodium is a very important mineral element that aids the transmission of nerve impulses as well as maintenance of osmotic balance of the cells. According to National Research Council (1974)[28], the Recommended Daily Allowance for sodium is 1100-3300 mg/100 g for adults. Deficiency of sodium may lead to dehydration or muscle cramp[29].

The iron content of A. nilotica was 0.09±0.01 mg/g. According to Bolt (1978)[30], the recommended daily requirement of iron for man is 6 – 40 mg/kg. Iron is an essential nutrient required by every human cell[31]. Its atomic structure gives rise to a number of biochemically useful properties, including the unusual capacity to both donate and accept electrons, and to reversibly bind to ligands such as oxygen and nitrogen. As such, iron plays a vital role in the transport and storage of oxygen, in oxidative metabolism and in cellular growth and proliferation[32]. The value obtained for calcium was 1.25±0.01 mg/g. The Recommended Dietary Allowance for calcium is 600-1400 mg[30]. Calcium is essential for bone and teeth formation and development, blood clotting and for normal functioning of the heart, nervous system and muscles. Calcium deficiency can lead to rickets, osteomalacia and tooth decay[31]. Excess of calcium in the soil interfere with phosphorus and boron nutrients and may encourage chlorosis because of a reduction of soil manganese, iron and zinc[33].

The two extracts of A. nilotica showed the presence of most of the secondary metabolites: Tannins, steroids, saponins, phenol, alkaloids, terpenoids, carbohydrates, cardiac glycosides, resins and balsams (Table 3). Alkaloids contribute to a plant species fitness of survival. They often have pharmacological effects and are used as medication and recreational drugs[34]. Phenolic compounds are reported to exert a wide spectrum of biological effects such as antioxidant and free radical scavenging activity and antimicrobial activity[35,36]. Similarly, tannins are well known for their antioxidant and antimicrobial properties as well as skin regeneration, anti-inflammatory and diuretic properties[36,37]. Flavonoids are widely recognized for exerting antioxidant, antimicrobial, anti-carcinogenic and antitumor properties[36,37]. Many pharmacological activities such as antibiotic, antifungal, antiviral, hepatoprotective, and anti-inflammatory and ant- ulcer activities have been reported for saponins[38]. Steroids have been reported to exert analgesic properties[39] while cardiac glycosides are reported to have antibacterial and antifungal activity[40,41].

The antifungal activities of A. nilotica fruit Table 4 showed that the extracts possessed antifungal activity at various concentrations, ranging from 500 mg/ml to 125 mg/ml. The best results were obtained on Aspergillus niger and A. flavus, which showed a zone of inhibition ranging from 9.00 mm to 17.00 mm, while the ethanolic extracts showed activity on F.
oxysforum at 500 mg/ml and 250 mg/ml (11.00 mm and 9.00 mm) respectively. Nevertheless, the extracts showed activity on Penicillium spp at 500 mg/ml (9.00 mm) only. Many literatures provided the information concerning the antifungal activities of A. nilotica from different parts of the plant such as root leaves, stem and stem back. A. nilotica species can be regarded as a promising resource for antibacterial and antifungal drugs due to its highly active nature. Researchers have showed antifungal activity of methanolic extracts and aqueous extract of A. nilotica with percentage inhibition ranging from 34.27± 1.45 to 93.35±1.99[42]. Dried fruits of Acacia nilotica are active against C. albicans and are used to treat oral candidasis [43]. Another result reported on ethanolic extracts of A. nilotica against two pathogenic fungi, namely A. niger and A. flavus, using an agar tube dilution method which showed 4.91 % and 116 mm growth inhibition against A. niger and 4.61 % and 124 growth inhibition against A. flavus [44].

Conclusions

Acacia nilotica has both nutritional and medicinal values based on the presence of numerous secondary metabolites and essential metals. The plant studied here can be seen as a potential source of useful drugs and further studies are, in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds.

Acknowledgements

The authors offer their appreciation to Sheda Science and Technology Complex (SHESTCO), Abuja, Nigeria for granting laboratory facilities to the authors and for the antimicrobial screening and atomic absorption spectrophotometer (AAS) analysis of the samples.

Funding: No funding sources
Conflict of interest: None declared

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