

Assessment of the Dietary Minerals, Phytochemical Composition and Antimicrobial Potential of Acacia Nilotica Seeds Extract Obtained in Sagamu, Ogun State

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Abstract

Acacia nilotica (*A. nilotica*) is a medicinal plant widely used in Nigeria due to its nutritional and bioactive potential. Understanding its mineral composition and phytochemical constituents is vital for determining how the components translate into nutritional and biological activity. This research, therefore, aimed to screen the methanolic crude extract of *A. nilotica* seeds obtained in Sagamu, Ogun State, Nigeria for phytochemicals, dietary minerals, and antimicrobial activities. Standard methods were employed for phytochemical analysis of the seeds, the dietary minerals were determined using Atomic Absorption Spectrophotometer (AAS) and antimicrobial screening was accomplished using agar well diffusion method. Alkaloids, steroids, flavonoids, tannins, phenol, terpenoids, and cardiac glycosides were the phytochemicals found in the seed extract. Fe (822.0 mg/kg) was the most abundant element found in *A. nilotica* seeds followed by Ca (104.2 mg/kg) > Mg (48.6 mg/kg) and Mn (10.2 mg/kg) while Zn (6.7 mg/kg) was the least abundant. The seeds contain flavonoids and phenolics with a % mean \pm standard deviation value of 2.40 ± 0.20 and 1.81 ± 0.09 , respectively. The crude extract displayed broad-spectrum antimicrobial activity against five bacterial strains. For the antibacterial screening, the optimal activity was observed against *Bacillus subtilis* with the highest inhibition zone (27 mm) at 200 mg/mL followed by *Pseudomonas aeruginosa* = *Staphylococcus aureus* (26 mm) > *Escherichia coli* = *Salmonellae typhi* (24 mm). The findings of this work justifies the application of *A. nilotica* in folk medicine and a source of dietary supplement for people with diseases relating to mineral deficiency amongst others.

Keywords: *Acacia nilotica*, Phytochemicals, Minerals, Antimicrobial, Microorganism

1. Introduction

Dietary minerals, also known as macro and micronutrients, are essential parts of the human diet that the body needs for many vital processes, including blood clotting, disease prevention and bodily development among others. Examples of these minerals are sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), iron (Fe), and zinc (Zn). When these nutrients are absent or insufficiently absorbed in the right amounts from food, a nutritional deficiency occurs leading to different health problems (Ali, 2023). For example, Mg, Ca, and K have been reported in some studies to reduce blood pressure in patients diagnosed with hypertension (Houston and Harper, 2008). Ca in particular is known to promote stronger bones and healthy teeth, hence reducing the risk of dental caries and fractures. Furthermore, Fe exhibits a variety of biological activities, including binding and transporting oxygen, electron transfer processes, gene regulation, immune system function, and cell

development and differentiation (Siddiqui, et al., 2014; Ajayi et al., 2018).

One of the main issues facing world health today is the insufficient or lack of micronutrients in humans; it has been estimated that over 2 billion people lack essential minerals like I, Fe, and Zn (WHO, 2007). These minerals are obtained from diet, which can come from either plants or animals. Particularly, plants usually possess abundant minerals and have garnered interest because they can provide humans with numerous medicinal remedies. Plants continue to be valuable sources of income and make the earth habitable to man and animals. Many of these plants are used by humans as medicines, and it may be claimed that these plants' bioactive chemicals are essentially what give them those abilities. Among these compounds are terpenes, flavonoids, alkaloids, and tannins. Typically, the plant ingredients are either refined into synthetic pharmaceuticals or used as medicines in their unrefined state as decoctions (Osinubi et al., 2020, Osibote, et al., 2021). *A. nilotica* can be considered as one of those plants with both nutritional and therapeutic properties because of the various secondary metabolites as well as essential minerals it present in its different parts (Ajayi et al., 2018, Bwai, et al., 2015). Many practitioners of traditional medicine have asserted that *A. nilotica* possesses a variety of biological activities that can treat conditions like

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diarrhea, eye infections, toothaches, and wound healing.

Numerous studies on the various applications of *A. nilotica* plant parts have also been published in the literature, demonstrating the plant's adaptability as a medicinal plant. For instance, a group of researchers examined the phytochemical composition, the antioxidant as well as the antibacterial properties of *A. nilotica*'s leaves, stem bark, and pods. They found that every plant part they screened had inhibitory effect against isolates of *S. typhi* and *E. coli* that were resistant to other known antibiotics (Sadiq et al., 2015). Another group also assessed the antibacterial activity of *A. nilotica* seed extract in dermatological forms and found that the extract demonstrated strong antibacterial activity against the tested microorganisms when it was incorporated into cream formulations (Aremu et al., 2020). According to a recent study, bioactive lipid compounds from the bark of *A. nilotica* trees may be used as sustainable feed additives in the diets of growing rabbits to boost growth rate, improve carcass standard, antioxidant status as well as antibacterial qualities in hot climates (Abdel-Wareth et al., 2023). Despite the abundance of reports on the different parts of *A. nilotica* plant parts in the literature, research on the dietary minerals of *A. nilotica* seed as it relates to its broad spectrum antimicrobial potential is rare. Furthermore, it is impossible to overstate the danger of a little kid developing a delayed physical or mental development due to a vitamin shortage, or even the chance of losing one to infectious diseases. Consequently, this study aims to assess the various minerals content, phytochemicals composition, as well as assess the antimicrobial potential of *A. nilotica* seed crude extract against selected drug resistant microorganisms.

2. Materials and Methods

2.1. Plant collection and preparation

The pod containing *A. nilotica* seed was collected locally from Awolowo market, Sagamu, and authenticated at the herbarium of the Botany Department, University of Lagos, Nigeria with voucher No. LUH 6613. The pod containing the seed was air-dried, and the seeds were separated from the pod. The seeds were then chopped in a mortar with a pestle to smaller sizes, then ground into fine powder in a mechanical blender. Extraction was then carried out on the fine sample using previously reported maceration procedures with minor modifications (Osinubi et al., 2020). 500 g of the fine sample was macerated (soaked) with 1 liter of methanol in a glass jar for 72 hours. After 72 hours the solution was filtered and filtrate was allowed to evaporate naturally leaving behind the paste-like methanolic extract in the glass jar. Qualitative and Quantitative phytochemical screening was then carried out on the methanolic crude extract of the seed sample.

2.2. Test Microorganisms

Five clinical isolates were assayed for the *in vitro*

antimicrobial study, the bacterial strains screened include *S.aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa*, and *S.typhi*. The positive control for the antibacterial screening was gentamycin.

2.3. Methods

Qualitative tests were carried out to determine the different plant chemicals present in the seed of the plant and quantitative analysis was conducted to ascertain the total phenolic content, total flavonoid content, levels of some dietary mineral composition. The antimicrobial screening was achieved using the agar well diffusion method.

2.3.1. Qualitative phytochemical analysis

Phytochemical screening was carried out for Alkaloids, Saponins, Flavonoids, Steroids, Tannins, Cardiac glycoside, Phenols and Terpenoids according to standard procedures (Sofowora, 1993; Trease and Evans, 2002).

2.3.1. Quantitative analysis

2.3.1.1. Determination of Total Phenolic content

The total phenolic content of the extract was determined by the Folin–Ciocalteu method with mild modification (Annisworth and Gillespie, 2007; Osinubi et al., 2024). Simultaneously, the crude extract and a reference sample (blank) were prepared. 0.04 g of the crude extract of *A. nilotica* seed was dissolved in 100 mL distilled water (400 µg/mL) and mixed thoroughly with 0.5 mL of Folin–Ciocalteu reagent for 3 min (light green color observed), thereafter, 2 mL of 20 % Na₂CO₃ was added to the mixture and the mixture was kept in a dark cupboard undisturbed for 2 hours and a dark blue colour was developed. The same procedure was performed on the blank sample (distilled water) (the light green color remains unchanged). The absorbance of both solutions were then read on the Ultraviolet-Visible spectrophotometer (UV-Vis spectrophotometer). The absorbance of the sample was measured at 765 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent (GAE) per g dry weight.

$$Y = 0.0981x - 0.0099(GAE), \quad (1)$$

where Y = absorbance

$$Total\ phenolic\ content = \frac{x \times Total\ volume}{Mass\ of\ crude\ sample} \quad (2)$$

2.3.1.2. Determination of total flavonoid content

The total flavonoid content was determined gravimetrically by adopting the method of Harborne (1973). 5 g of the methanolic crude extract of *A. nilotica* seed was dissolved in 50 mL of HCl and the resulting solution was boiled for 30 mins. After this, the solution was allowed to cool and then filtered to obtain a brownish-yellow. 5 mL of the filtrate was treated with 5 mL of ethyl acetate, and immediately precipitate of the flavonoid begins to grow. The precipitate was filtered with a pre-weighed filter paper

(w_1). The filter paper containing the precipitated flavonoid was then oven-dried and reweighed (w_2). % flavonoid composition is obtained according to equation 3.

$$\text{Flavonoid}(\%) = \frac{W_2 - W_1 \times 100}{W_0}, \quad (3)$$

where:

W_0 is the weight of the crude sample.

W_1 = Weight of empty filter paper

W_2 = Weight of Filter paper and precipitate after oven drying

2.4. Antimicrobial activity

The antibacterial activity of the methanolic crude extract of *A. nilotica* seed were achieved using the agar well diffusion technique with mild modification (Osinubi et al., 2020; Russell and Furr, 1977). Sensitivity test: nutrient agar plates were seeded with 0.1 mL of an overnight culture of each microorganism strain (equivalent to $10^7 - 10^8$ CFU mL) Mueller-Hinton agar plates (for bacteria). The seeded plates were allowed to set and uniform wells were cut on the surface of the agar with a standard cork borer of 7 mm diameter. 0.3 mL of the dissolved crude extract (dissolved in distilled water which served as the negative control and had no activity against the organisms) was then introduced into the wells and set in the incubator at 30 °C for 24 h. The diameter of the zone of inhibition (ZI) (clear area around each well) was measured and recorded for each bacteria screened.

2.4.1. Dose-response diffusion assay (DrD)

Using the agar well diffusion method, the DrD assay of the crude extract of *A. nilotica* seed was determined. Different concentrations (100, 50, 25, 12.50, 6.25 mg/mL) of the extract were prepared from the stock solution. 0.3 mL of each concentration was then poured into the inoculated wells and set in the incubator for 24 h at 30 °C. After 24 h, the wells were examined for the lowest concentration at which the growth of the microorganisms was inhibited to establish the dose response.

2.5. Dietary minerals screening

Nitric acid (5 mL) was added to a representative sample of *A. nilotica* extract (1g) in a beaker. The filled beaker was placed in a digestion tube at a temperature of 135 °C for about 2 hours to enable digestion to take place. The digested sample was then filtered into a 100 mL volumetric flask and made up to the mark with distilled water. The resulting solution was shaken thoroughly. Sample bottles were filled with the digested sample and subjected to AAS analysis on a RAYLEIGH WFX – 130B Atomic Absorption Spectrophotometer to determine the quantity of the elements in the plant extract.

3. Results

3.1. Qualitative Phytochemical analysis result

The result of the qualitative phytochemical screening

of *A. nilotica* seed revealed that the seeds were rich in alkaloids, steroids, flavonoids, tannins, phenol, terpenoids and glycosides while saponin was absent as shown in Table 1.

3.2. Quantitative Phytochemical Analysis Result

Table 2 shows the result of total flavonoid and phenolic content in % Mean \pm Standard deviation. The test was done in duplicates. The total phenolic content obtained for the methanolic crude extract of the seed was 1.81 % gallic acid equivalent, and the total flavonoid content determined was 2.40%.

This result corroborated the result of the qualitative phytochemical screening where the flavonoids were observed to be predominantly present compared to the other phytochemicals screened (Table 1) besides alkaloids. The presence of the phenolic and flavonoids can be said to be contributory to the bioactivity of *A. nilotica* seed's crude extract.

3.3. Dietary minerals composition.

The composition (mg/kg) of five dietary minerals (micronutrients) present in the seed of *A. nilotica* as obtained on the AAS is presented in Figure 1.

The result indicates that the *acacia* seed is rich in iron with the element having a concentration of 822 mg/kg, making it the most abundant followed by Calcium (104.2 mg/kg), Magnesium (48.6 mg/kg), Manganese (10.2 mg/kg), and Zinc (6.7 mg/kg).

3.4. Antibacterial activity of *A. nilotica* seed

Data from the result of antibacterial screening indicates that the extract displayed the best activity against *B. subtilis* followed by *P. aeruginosa* and *S. aureus* at a concentration of 200 mg/mL. While its activity against *E. coli* and *S. typhi* were comparable at the different concentrations (200, 100, 50, 25, 12.5, 6.25 mg/mL) at which the experiment was carried out. Though the crude extract of the plant displayed antibacterial activity against the bacteria screened, the positive control (10 mg/ mL gentamycin), however, had better activity as displayed in Figure 2.

4. Discussion

The result of the qualitative phytochemical screening in this study revealed that the seeds of *A. nilotica* were rich in alkaloids, steroids, flavonoids, tannins, and phenol, while terpenoid, glycosides were found in traces but saponin was absent, a similar observation was reported by Sharma *et al.* 2014 however, with minute difference because contrary to the present finding, saponins were found present in the sample they analysed. This disparity can be attributed to the difference in sample location or chemical transformations that may have taken place during storage/drying (Osinubi et al 2020). The quantitative analysis revealed the presence of flavonoids and phenolic, even though in little quantities, their presence play a pivotal role contributing to the bioactivity of *A. nilotica* seed's crude extract.

Table 1: Phytochemical components of *Acacia nilotica* seed

Natural product	Observation	Result
Alkaloid	Brick red precipitate	+++
Saponin	No froth or emulsion observed	---
Steroid	A red coloration	++
Flavonoid	Yellow coloration	+++
Tannin	Bluish-green coloration	++
Phenol	Green precipitate	++
Terpenoid	Reddish-brown color	++
Cardiac glycoside	Formation of brown ring	+

--- Indicates absence, + (low concentration), ++ (moderately present), +++ (highly present).

Table 2: Table of value for total phenols and flavonoid

Total Flavonoid content (%)	2.40 ± 0.20
Total Phenolic content (%)	1.81 ± 0.09

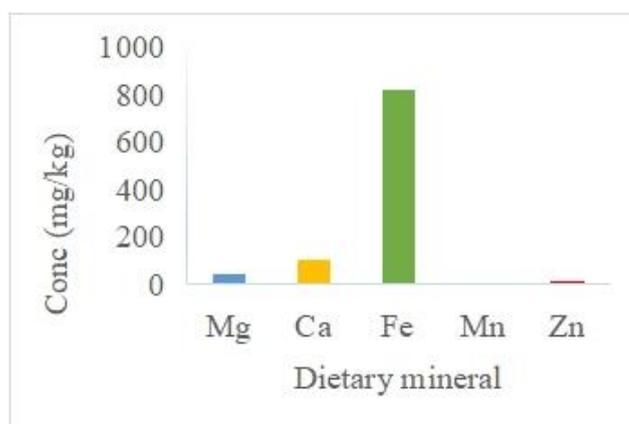


Figure 1: Concentration of some dietary minerals in the seed of *A. nilotica* extract.

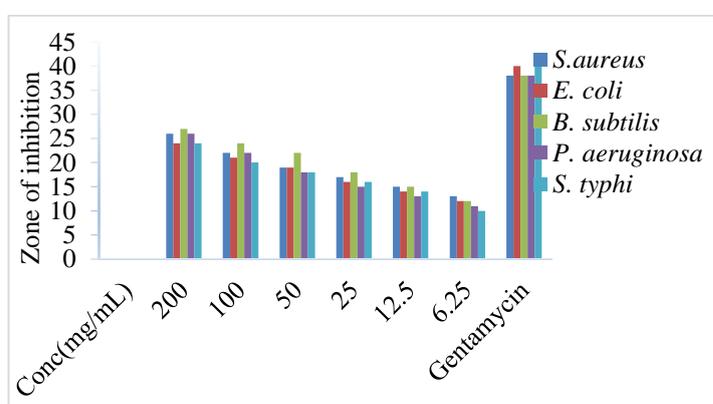


Figure 2: Antibacterial activity of *Acacia nilotica* seed

The methanolic extract of the seed of *A. nilotica* was tested against five bacterial strains (*S. aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa*, and *S. typhi*). Data obtained from the inhibition zones (mm) indicates that the antibacterial activity of the methanolic extract of *A. nilotica* seed against all test organisms required a quantitative amount of the test extract because a decrease in the concentration (200 > 100 > 50 > 25 > 12.5 > 6.25 mg/mL) of the *A. nilotica* seed extract resulted in decreased activity. *B. subtilis* was the most susceptible bacterial strain and the highest zone of inhibition (ZI: 27 mm at 200 mg/ml) was recorded against it. The plant extract had similar zones of inhibition (26 mm) against *S. aureus* and *P. aeruginosa*, and ZI of 24 mm against *E. coli* and *S. typhi* respectively. At the least concentration (6.25 mg/ml), the zone of inhibition of the organisms was observed to fall within a range of 10 mm – 13 mm. *S. aureus* (13 mm), *E. coli* and *B. subtilis* (12 mm), *P. aeruginosa* (11 mm), and *S. typhi* (10 mm). Gentamycin (10 mg/ml) which was the positive control against the test organisms for the bacterial strain was most active on *E. coli* and *S. typhi* with a ZI at 40 mm, while it had a ZI of 38 mm against other bacterial strains screened. This observation corroborates the work of Saini *et al.*, (Saini *et al.*, 2008) who compared antimicrobial activity among *Acacia* species. Their research revealed that the *Acacia* species had high antimicrobial potential against *S. aureus*, *E. coli*, and *S. typhi*. A similar observation was reported against *E. coli* and *Salmonella* by Jabaka *et al.* (Jakata *et al.*, 2019) who made use of different fractions of *A. nilotica* stem bark.

Phytochemical presence in the different plant parts have been broadly reported to be responsible for the therapeutic roles that different medicinal plants play when consumed. Based on the foregoing, the biological activity of the seed extract of *A. nilotica* can be associated with the presence of the plant chemicals (phytochemicals) found in the plant, coupled with the effect of the dietary mineral contents which can be said to play contributory role in the bioactivity of the plant hence the reason for the observed activity (Altemimi *et al.*, 2017). Alkaloids found present for example have been shown to possess anticonvulsant and anxiolytic properties, terpenes as well also have muscle relaxant ability (Sama, *et al.*, 2022). While steroid which was also detected in the plant sample is known to display analgesic and free radical scavenging properties (Sama, *et al.*, 2022; Nacoulma *et al.*, 1996).

Dietary minerals on the other hand are well known to serve functions like improving bone health such as is well known with calcium, balancing water levels as well as maintaining healthy skin, hair and nails. All of these functions are vital for the wellbeing and proper development of an individual.

The present finding indicates that *A. nilotica* obtained in Shagamu contains a higher level of Fe compared to other minerals this however, negates the findings of Wati and Khabiruddin, (2018) who recorded higher

concentration in Ca in the samples obtained in Palwal and Histar regions of India, This disparity may be associated with the difference in geographical location (Osinubi *et al.*, 2024) which may be a factor in the mineral uptake of plants from the soil. The presence of these elements in the present plant of study supports claims of its use in traditional medicine for the treatment of ailments such as fever, haemorrhages, diarrhoea, and toothache, besides other infections (Ohouko *et al.*, 2020), many of which require the influence of the nutrients, Ca and Mg, especially, which are known to support bone health and antioxidant defenses while Zn supports physiological and metabolic process (Osinubi *et al.*, 2025). In addition, these minerals have been reported to possess the ability to improve the health and well-being of patients with heart failure by reducing the severity of symptoms, increasing work capacity, and improving left ventricular ejection fraction. (Pan *et al.*, 2019). Summarily, going by the variety of phytochemicals and minerals found present in its seed, coupled with its potential to inhibit microbial activity, the use of *A. nilotica* could be said to be beneficial as a natural source of medicine, which can facilitate the well-being of the heart as well as inhibit microbial activity of organisms which pose threats to humans due to drug resistance. The sample could be a valuable dietary supplement for essential minerals, which are required for different biological processes, such as oxygen transport by Fe (Osinubi *et al.*, 2025) as well as bone health support by Ca and Mg.

5. Conclusion

Conclusively, the findings of this investigation reveals the methanolic crude extract of the *A. nilotica* seed as a rich source of phytochemicals and nutritional minerals with Fe being the most abundant mineral. The crude extract also exhibited broad-spectrum antimicrobial activity as it inhibited the growth of five different bacterial strains. The potential of the extract of *A. nilotica* seed to inhibit microbial growth can be ascribed to the plant chemicals as well as the nutritional minerals found in it. These two - phytochemical and dietary minerals can be said to work synergistically in facilitating the therapeutic activity of the plant.

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