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Correspondence:

Email: *ammadrufai@gmail.com*

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ANTINUTRITIONAL AND PHYTOCHEMICAL PROPERTIES OF AQUEOUS EXTRACT OF Newbouldia laevis (BOUNDARY TREE) LEAVES

Ahmed, K.R* ., `Anyekema, M ., Abdulhakeem, Z and Abubakar, U

Department of Science Technology, College of Science Technology, Waziri Umaru Federal Polytechnic, Birnin Kebbi, Kebbi State,Nigeria.

Abstract

Newbouldia laevis, which is commonly called the Boundary tree is a tropical plant whose medicinal values have been in existence for quite a long time. Plants are known to contain active metabolites such as cardiac glycoside, flavonoids, alkaloid and tannins which possess medical properties and are therefore harnessed for the treatment of different diseases. This present study was aimed at investigating the phytochemical and antinutritional properties of aqueous extract of N. laevis leaves. Qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, tannins and cardiac glycosides. The *N. laevis* leave extract showed the presence of antinutritional compounds which include, Phytate (4.224 ± 7.182mg%), Flavonoid (4.000±6.240mg%), Oxalate (0.081±0.105mg%), alkaloids (0.0032±0.0008mg%) Tannin (0.008 ± 0.0032mg%). This result suggests that N. laevis leave extract contains bioactive compounds which have potential health benefit inherent in them, additionally, there is also the presence of antinutritional factors which has a significant impact on nutritional value of this plant. However, further research is needed to explore the therapeutic values as well as safe use of this plant material.

Keywords: *Newbouldia laevis*, Phytochemicals, Antinutritional Properties, Metabolites

INTRODUCTION

A vast area of Nigeria geographical zones is known to contain many plant species that have been found to be useful to human either directly or indirectly (Oliver, 2021). The medicinal values of many of these plants cannot be over emphasized. *Newboudial. laevis* (Bignoniaceae) is commonly known as African Border tree or boundary tree, it is called "Aduruku" in Hausa; "Ogirisi" in Igbo; "Ikhimi" in Edo and "Akoko" in Yoruba languages (Ogunlana and Ogunlana, 2018). It grows to a height of about 7.8 (up to 15 m), more usually a shrub of 2 to 3 m, many - stemmed forming clumps of gnarled branches. It is easily recognized by its short branches, coarsely toothed leaflets and purple and white flowers. *N. laevis* is native to tropical Africa and grows from Guinea Savannahs to dense forests, or moist and well drained soils (Burkill, 2022).

Plants contained active components such as anthraquinones, flavonoids, glycosides, saponins, tannins, etc which possess medical properties that are harnessed for the treatment of different diseases (Feher and Schmidt, 2013). Phytochemicals are naturally occurring and are believed to be effective in combating or preventing disease due to their antioxidant effect (Ejele *et al.*, 2020).

Anti-nutritional factors are those substances or chemical compounds found in leaves, fruits and food substances in general. Anti-nutritional factors are present in different food substances in varying amounts depending on the kind of food, mode of its propagation, chemicals used in growing the crop as well as those chemicals used in storage and preservation of the food substances. These anti-nutritional factors are known to interfere with metabolic processes such that growth and bioavailability of nutrients are negatively influenced (Halliwell and Gutteridge, 2020). Examples of antinutritional factors present in most leaves and fruits are alkaloids, tannins, phytate, trypsin inhibitor, cyanide, saponins, and oxalates. Antinutritional factors reduce the nutrient utilization in human or animal feeds (Gbile and Adessina, 2021). In most parts of the developing world, food poisoning arising from anti-nutritional factors has been poorly addressed, and death arising from these antinutritional factors have been attributed to ignorance, poverty and inadequate nutritional information and education, especially within sub-saharan African region. (Gbile and Adessina,2021).In addition, it is known that some fruits possess "anti-nutritional" factors such as phytate, tannins, and oxalate that can diminish the nutrient bioavailability, especially if present at high level (Enye, 2018). Although, it has been reported that these anti-nutritional factors could help to prevent and treat several diseases; remarkably, the anti-carcinogenic activity of phytate, and the antidiarrhe activity of tannins. Plant foods are the only sources of dietary fiber which plays an important role in decreasing the risk of many disorders such as constipation, diabetes, cardiovascular diseases, and obesity (Enye, 2018) Certain harmful effects might also be due to the breakdown products of these active metabolite in plant tissues. However, some anti-nutritional factors as well as their break down products may possess beneficial health effects even if present in small amounts. The mechanism through which the

beneficial and harmful effects of anti-nutritional factors are elicited are the same (Hassan, et al., 2010) Thus, manipulating processing conditions, in addition to removing certain unwanted compounds in foods, may be required to eliminate the deleterious effects of anti-nutritional factors to harness their health benefits (Hassan *et al.,* 2010). Therefore, the aim of this present study is to determine the phytoconstituents and nutritional properties of *N. laevis* leaves.

MATERIALS AND METHODS

Sample Collection, Identification

The leaf samples of *Newbouldia laevis* was obtained from Ministry of Environment and Solid Mineral Resources Department of Forestry Birnin Kebbi, Kebbi State and subsequently transported to the Biology Laboratory, Waziri Umaru Federal Polytechnic Birnin Kebbi, Kebbi State for onward processing.

Sample processing

The leaves were chopped into bits and shade dried for four weeks at room temperature. The leaves were then grinded in to powder with micro plant grinding machine the grinded leaves were then sieved through a 0.25mm pore size to obtain a uniform fine uniform powdered particle. The resulting powder was stored in separate clean containers with screw cap at room temperature prior to use.

Aqueous Extraction

Dried fine powder of each plant material (10g) was poured in the glass beaker and filled with 100ml of distilled water at room temperature and the mixture was shaken continuously and allowed to stand for 24hh After which the mixture was filter through the use of Whatman no. 1 filter paper and subsequently concentrated to dryness by using water bath at 70^{O} C. Yield of the extract was then weighed on the weighing balance. The extract was then transferred into glass vial and refrigerated before use.

Determination of Antinutritional Factors

Determination of Tannins

Folin-Denis spectrophotometric method

A measured weight of each sample (1.0 g) was dispersed in 10 mL distilled water and agitated. This was allowed to stand for 30 mins at room temperature while continuously stirring every 5 mins. At the end of 30 mins, it was centrifuged and the extract obtained. 2.5 mL of the extract was dispersed into a 50 mL volumetric flask. Similarly, 2.5 mL of standard tannic acid was dispersed into a separate 50-ml flask. A 1.0 mL Folin-Denis reagent was measured into each flask followed by the addition of 2.5 mL of saturated Na_2CO_3 solution. The mixture was diluted and made up to the 50 mL mark of the flask and was incubated for 90 mins at room temperature. The absorbance was measured at 250 nm in a UV spectrophotometer; readings were taken with the blank sample at zero.

Calculation

% Tannin = An/As x C x 100/w x VF/VA

An= absorbance of the test sample

As = absorbance of standard solution

C = concentration of standard solution

W = weight of sample used

VF = total volume of extract

VA = volume of extract analyzed

Determination of Oxalate

Procedure

One gram (1g) of the sample was added to 75 mL of 15% H_2SO_4 the solution was carefully stirred intermittently with a magnetic stirred for 1h and filtered using What man No. 1 filter paper, the filtrate (25 mL) was then collect and titrated against 0.1N $KMNO_4$ solution till a faint pink colour appeared that persisted for 30 seconds. $1cm^3$ of 0.1N $KMNO_4$ = 0.0045g of oxalic acid.

Calculation

(%Oxalate g%) = Titre value x 0.0045

Determination phytate

Phyatate was determine using the procedure described by Lucas and Marakaka (2015

Procedure

The phytic acid was determined using the procedure described by Lucas and Markaka (1975). 2 g of sample was weighed and poured into 250 mL conical flask. 100mL of 2% concentrated hydrochloric acid was used to soak the sample in the conical flask for 3hrs. This was then filtered through a double layer of hardened filter paper. 50m of the filterate was placed in 250mls beaker and 100mL of distilled water was added to give proper acidity. Ten mL of 0.3% Ammonium thiocyanate solution was added into the solution as indicator. This was titrated with standard iron (iii) chloride solution, which contained 0.00195-g iron per ml. the end-point was slightly brownish-yellow which persisted for 5mins. The percentage phytic acid was calculated using the formula:

% phytic acid = $\frac{X\ x\ 1.19x100}{2}$

Where X = Titre value x 0.00195

Qualitative Analysis of Phytochemical Constituents:

Preliminary phytochemical screening was carried out on the leaf extract of *N. leaves* using standard procedures as described by Trease and Evans (1989), Sofowora (1993), Ushie *et al.*,2016)

Detection of Alkaloids

Extract was dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

Mayer's Test

Filtrates was treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Detection of Flavonoids

Lead Acetate Test

Extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Detection of Steroids

2 mL of acetic anhydride was added to 0.5 mL of each extract in a test tube, followed by the addition of 2 mL of sulfuric acid. A color change from violet to blue or green indicated the presence of ste-roids.

2.8. Detection of Phlobatannins

2 mL of the extract of the plant sample was boiled with 1% aqueous hydrochloric acid. Disposition of red precipitate indicated the presence of phlobatannins.

Detection of Anthraquinones

Borntrager s Test

0.5 mL of the extract was boiled with 2 mL of 10% HCl for few minutes in a water bath. The resulting solution was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few

drops of 10% NH3solution was added to the mixture and heated. Formation of rose
pink color indicated the presence of anthraquinones in the extracts.

Detection of Terpenoids

Salkowski s Test

0.5 mL of the extract was mixed with 2 mL of chloroform, and 3mL of concentrated H_2SO_4 was carefully added to form a layer. An appearance of a reddish brown color interface indicated the presence of terpenoids.

Detection of Phenol

10 mL of the extract was treated with few drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

Detection of Glycosides

0.5 mL of the extract was dissolved in 1 mL of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

Detection of Tannins

1 mL of the extracts was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. Formation of a dark green color indicated the presence of tannins. **Detection of Saponins**

Foam Test

1 mL of each of the extracts was shaken with 5 mL of distilled water. Formation of stable persistent foam indicated the presence of saponins.

Quantitative Phytochemical Analysis

The phytochemicals detected in the leaf extract on *N. laevis* were quantified using standard procedures as described by Harborne (1973).

Determination of Alkaloids

To 5 g of the sample, 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. It was filtered and the filtrate was concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added drop wise to the filtrate until precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue is the alkaloid, which was dried and weighed.

Determination of Flavonoids

10 g of plant sample was repeatedly extracted with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through a Whatman No.42 filter paper into a pre weighed 250 mL beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed (Krishnaiah *et al.*, 2009*).*

Determination of tannin

5g of the sample was weighed into 100 ml conical flask and 50 ml of distilled water was then added and was shaken continuoslyfor one hour in a mechanical shaker. The resultant solution was then filtered into a 50 ml volumetric flask using whatman's No 1 filter paper and made up to the mark. Then 5 ml of the filtrate was pipette out into a test tube and mixed with 3 ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was then measured in a spectrophotometer at 120 nm wavelengths, within 10 minutes. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured.

RESULTS

Table :1 Antinutritional composition of *Newbouldia laevis*

Parameters	Concentration mg/100g
Phytate	4.224 ± 7.182
Oxalate	0.081 ± 0.105
Tannin	0.008 ± 0.0032

Table 2: Qualitative Analysis of *Newbouldia laevis*

Parameters	level
Quinone	-
Cardiac Glycoside	+ ++
Tannin	+
Phenolic Compound	-
Anthocyanin	-
Alkaloid	++
Saponin	-
Flavanoid	+

Keys: + Slightly present, +++ Highly presen

t, ++ Moderately present, - Absent

Table 3: Quantitative Analysis of *Newbouldia laevis*

Parameters	Concentration mg%
Alkaloid	4.000 ± 6.000
Flavonoid	4.000 ± 6.240
Tannin	0.008 ±0.0032

DISCUSSION

The result of this study revealed that the phytate concentration of *Newbouldia laevis* was 4.224mg/100g This results in line with the value reported by Popoola and Tee, (2019) and the Alanda (2022a) who reported the range of value from 5.224mg/100g. (Zaid *et al.,* (2021).The phytate in food can bind some essential mineral elements such as Ca, Mg, Zn and Fe in the digestive tract and render them not bioavailable. Protein and starch solubility digestion was also reported to have been affected by phytate (Bello, 2008a, Hassan et al., 2018). The result also shows that the oxalate concentration of *Newbouldia laevis* was 0.081mg. The value is higher than the range of 0.061mg reported by Popoola and Tee, (2019). High Oxalate content causes irritation of the mouth and interferes with the absorption of divalent minerals particularly Calcium by forming insoluble salt leading to kidney stone which may eventually lead to death (J.Imam *et al*., 2013) The Tannin concentration of *Newbouldia laevis* is 0.008 This resultis in line with the value reported by Komlaga *et al.,* (2018.who reported the range of value from 0.008 (Zaid *et al.,* 2021).The variation may be due to the types of materials used during the experimental processes or due to the environmental condition as reported by Jackson (2019).

The preliminary phytochemical analysis revealed the presence of cardiac glycosides present, Alkaloid, Flavonoids and Tannin. Quinone, Phenolic Compound, Anthocyanin and saponin were not detected in the leaves extract.

Alkaloids: the alkaloid concentration is 4.000 g% in *newbouldia laevis* is lower in contrast to the findings of Usman and Osuji (2017) and Azando *et al.* (2019) who reported a higher concentration of 41.74g% . alkaloids are nitrogen-containing compounds with a wide range of biological activities. Alkaloids have been shown to have pharmacological effects, including analgesic, anti-inflammatory, and antisplasmodic properties Ejele *et al.* (2020).

Tannin has the concentration of 0.008mg% in *Newbouldia laevi* which is lower than the result observed by *Akerele et al*., (2019) who reported higher concentration of 1.74. Tannins are polyphenolic compounds with astringent properties. They can bind to proteins, leading to the formation of precipitates. Tannins have also been found to posess antioxidant properties and may have antiviral, antibacterial, and anti-inflammatory effects. They are often found in fruits, tea, and some nuts (Kittakoop *et al.,* 2022).

Flavonoids: the flavonoid concentration is 4.000 g% in *newbouldia laevis* is lower compared with the result obtained by Dandjesso *et al.,* (2020) who reported the concentration of 0.100g%. Flavonoids are a diverse group of polyphenolic compounds found in plants. They are known for their antioxidant properties and can have various health benefits, including anti-inflammatory and anticancer effects (Jackson, 2022). These variations may be due to the types of materials used during the experimental processes or due to the environmental condition Jackson (2019.The presence of those metabolites no doubt is indication of the potential medicinal usefulness of the plant extracts.

CONCLUSION

This result suggests that N. laevis leave extract contains bioactive compounds which have potential health benefit inherent in them, additionally, there is also the presence of antinutritional factors which has a significant impact on nutritional value of this plant. However, further research is needed to explore the therapeutic values as well as safe use of this plant material.

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