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Pharmacognostic Evaluation of leaf of *Newbouldia laevis* (P.Beauv.) Seem. Ex Bureau (BIGNONIACEAE)

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ABSTRACT

***Background*:** *Newbouldia laevis*, commonly known as the boundary tree, belongs to the Bignoniaceae family and is the sole species in its genus. It is widely used in traditional medicine for its antiulcer, antimicrobial, anticancer, and antidiabetic properties. Despite its broad use, there is limited pharmacognostic data available, necessitating this study to support its safe and effective use.

***Methods*:** Standard pharmacognostic techniques were employed, including microscopy, micrometry, chemomicroscopy, moisture content, ash and extractive values, fluorescence analysis, and micromeritic assessments.

***Results*:** Microscopy revealed a hypostomatic leaf with stomata (anomocytic, anisocytic, and anomalous types) only on the abaxial surface, alongside glandular trichomes. The adaxial surface lacked both stomata and trichomes. The stomatal index for the abaxial surface was 27.31%. Micromeritic properties included bulk and tapped densities of 0.22 ± 0.01 g/mL and 0.32 ± 0.004 g/mL, respectively, with a Hausner ratio of 1.47 ± 0.09 and Carr's index of 31.83 ± 4.46%, indicating poor flow. Angle of repose was 43.5°. Moisture content was 12.7% w/w. Chemomicroscopy identified mucilage, lignin, starch, oil, cellulose, and proteins. Fluorescence analysis showed solvent-dependent color changes. Extractive values were 11.00% (water), 8.33% (ethanol), and 9.33% (methanol). Ash values were 6.58% (total), 1.0% (acid-insoluble), and 1.5% (water-soluble).

Conclusion: This study provides essential pharmacognostic parameters for *N. laevis*, aiding its identification, authentication, and the establishment of quality standards for its safe and effective use in herbal medicine

Keywords: Bignoniaceae, Hausner's ratio, Hypostomatic, Micromeritics, *Newbouldia laevis*.

1. INTRODUCTION

Newbouldia laevis also known as boundary tree is a small tree of about 7-20 m tall depending on the region where it is found and the stem grows vertically with few branches. It is a medium sized angiosperm in the Bignoniaceae family. The leaves are large, glossy and deep green and the tree is propagated by cutting [1]. It is native to Africa and it is a monotypic genus. Some species are edible and have economic value. Ghanaians, Cameroonians and Nigerians use the bark, roots and leaves for the treatment of toothache, stomachache, diarrhoea, dysentery, malaria, fever, breast cancer, sexually transmitted diseases (STDs), anaemia, ulcer, arthritis, rheumatism, hemorrhoids, constipation, cardiovascular diseases, diabetes, cough, elephantiasis and urinogenital tract infection [2]. *N. laevis* extract contains a large amount of pyrazole alkaloids. Withasomnine and newbouldine derivatives were the main molecules [3].

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Classification of *N. laevis* according to Angiosperm Phylogeny Group System (APG, 2016) [4]

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Lamiales
Family:	Bignoniaceae
Genus:	*Newbouldia* seem ex bureau
Species:	*N. laevis*
Common name:	Boundary tree, Tree of life

Fig 1a

Fig 1b

Figure.1a: leaves of *Newbouldia laevis* seem ex bureau (Source: Field data (2024). University of Uyo Town Campus, Ikpa Road behind the Medical Centre.
Figure.1b:leaves and flowers of *Newbouldia laevis* seem ex bureau (Source: Field data (2025).A location in Nung Udoe Itak Junction, Ikono Local Government Area of Akwa Ibom State.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological Materials

The leaves of the plant *Newbouldia laevis*

2.1.2 Chemicals and reagents

The chemicals and reagents used include; distilled water, glycerol, sodium hydroxide, 10% Concentrated Hydrochloric acid, ferric chloride, concentrated sulphuric acid, dichloromethane, ethylacetate, methanol, ethanol, n-hexane, dragendorff's reagent, ferric chloride, phloroglucinol, ruthenium red, millon's reagent, N/50 iodine, sodium hypochloride.

2.1.3 Equipment and Apparatus

Materials used include: Beakers, electronic weighing balance, test tubes, filter paper, oven, water bath, pen, pencil, funnel, glass stirrer, measuring cylinders, conical flask, sieves, spatula, marker, masking tape, foil paper, tongs, evaporating dish, silica gel, knife, mortar and pestle, desiccator, muffle furnace, ashless filter paper, Olympus CX21 electronic microscope, microscope slides, cover-slips, foolscap sheets, meter rule, Amscope MD 500.

2.2 Methods

2.2.1 Collection and Identification of Plant Material

The plant *N. laevis* from the family Bignoniaceae was collected from the University of Uyo town Campus, Ikpa Road. It was identified by Dr. I.I. Johnny of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Akwa Ibom State, Nigeria. The Herbarium Specimen with the Voucher Number UUPH1C(i) was deposited in the Herbarium, Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo.

2.2.2 Microscopic Evaluation of Leaf

The standard median portion of the well-expanded matured leaf was obtained. Microscopical examinations of the transverse section were made, the Epidermis of both adaxial and abaxial surfaces were also made by placing the leaf on a glass slide, scraping gently with a sharp razor blade, irrigated with water until loose cells from the epidermis were washed away and the desired epidermis was reached. The epidermal peels were further cleared with sodium hypochlorite rinsed gently with water and stained with an aqueous solution of safranin-O for (five) 5 minutes and mounted with 10% glycerol. The stained samples were mounted on a binocular microscope. Photomicrographs were taken from good preparations using the Olympus CX21 binocular microscope fitted with an MD500 amscope microscope eyepiece camera. Measurements were done at ×10 while ×40 for photomicrographs [5].

2.2.3 Quantitative Microscopy of the Leaf

Quantitative microscopy parameters such as leaf constant studies namely stomatal length and width, guard cell length and width, stomatal number, stomatal index, epidermal cell length and width, epidermal cell number, epidermal cell thickness were carried out using standard procedures. All measurements were made using a calibrated ocular micrometer and 10 microscopic fields chosen at random were used and data were presented as mean ± Standard Error of Mean (SEM).

2.2.4 Stomatal Index Determination

The stomatal index (S.I) was determined according to Metcalfe and Chalk [6,7, 8] The sample (quantitative microscopy) was placed under the microscope and the stomatal index was determined using the formula.

$$S.I. = \frac{S}{E+S} x100$$

Where S = Number of stomata per unit area, E = Number of epidermal cells in the same area

2.2.5 Evaluation of Powders

2.2.5.1 Micromeritic Analysis

The flow property was determined using standard methods [9]. Which consists of: Bulk Density and Tapped Density. The weight of 10 g of dried powdered leaf was weighed into a 100 ml measuring cylinder and the volume occupied was noted as the bulk volume (Vb). The cylinder was gently tapped repeatedly to obtain a constant volume noted as the tapped volume (Vt). Bulk density was calculated using the formula below.

$$B\rho = \frac{M}{Vp}$$

$$T\rho = \frac{M}{Vt}$$

Where Bρ = Bulk density, M=Mass of powder, Vb = Bulk volume of powder, Tρ = Tapped density Vt = Tapped volume

2.2.5.2 Hausner's Ratio and Carr's index

Hausner's ratio -a function of interparticle friction was calculated using the formula.

$$\text{Hausner's ratio} = \frac{Tp}{Bp}$$

$$\text{While Carr's index} = 1 - \frac{Bp}{Tp} x\ 100$$

Where; Tp = Tapped density, Bp = Bulk density,

$$\text{Angle of repose } (\theta) = \text{Tan}^{-1}\left(\frac{\text{Heap height of powder}}{\text{Radius of heap base}}\right)$$

2.2.5.3 Chemomicroscopic Analysis of Leaf Powder
Powdered leaf was examined for its chemomicroscopic properties namely mucilage, lignin, starch, oils, calcium carbonate and calcium oxalate crystals using standard procedures [10].

2.2.5.4 Fluorescence Analysis of Leaf Powders
The fluorescent analysis of dried leaf powder was carried out using the standard method [11].

2.2.5.5 Physico-chemical Evaluation of Leaf Powders
The physicochemical parameters such as moisture content, ash values (total ash, acid-insoluble ash and water-soluble ash values), soluble extractive values such as ethanol, methanol and water-soluble extractive values were performed according to the official method prescribed by the WHO guidelines on quality control methods for medicinal plant materials [6,7,11].

2.3 Statistical Analysis
Data obtained were expressed in Mean ± SEM using (Statistical Package for Social Sciences) SPSS 17.0 and the terminology used in describing epidermal features is that of Metcalfe and Chalk (1979).

3. RESULTS
Table 1: Quantitative micro-morphological characters for the adaxial and abaxial surface of leaves of *N. laevis*

Leaf surface	Abaxial	Adaxial
Stomata morphology type	Anomocytic, Anisocytic, Anomalous	-
Stomata distribution	Hypostomatic	-
Stomatal Length (µm)	20.46 ± 0.49	-
Stomatal Width (µm)	13.94 ± 0.39	-
Stomatal Pore Length (µm)	15.27 ± 0.70	-
Stomatal Pore Width (µm)	3.01± 0.24	-
Stomatal Number	86 ± 2.39	-
Guard Cell Length (µm)	11.09 ± 0.26	-
Guard Cell Width (µm)	4.15 ± 0.32	-
Stomatal Index (%)	27.31	-
Length of Trichome (µm)	-	-
Width of Trichome (µm)	-	-
Epidermal Layer Number	348.33 ± 9.196	392.86±2.11
Epidermal Cell Length (µm)	45.68± 1.926	38.75±1.92
Epidermal Cell Width (µm)	24.90 ± 1.95	20.22±0.75
Epidermal Cell Wall Thickness	2.99 ± 0.097	3.06±0.26
Vein Termination Number	5.8± 0.34	2.90±0.29
Vein Islet Number	2.9± 0.25	1.50±0.18

Mean ± SEM of 15 determinations

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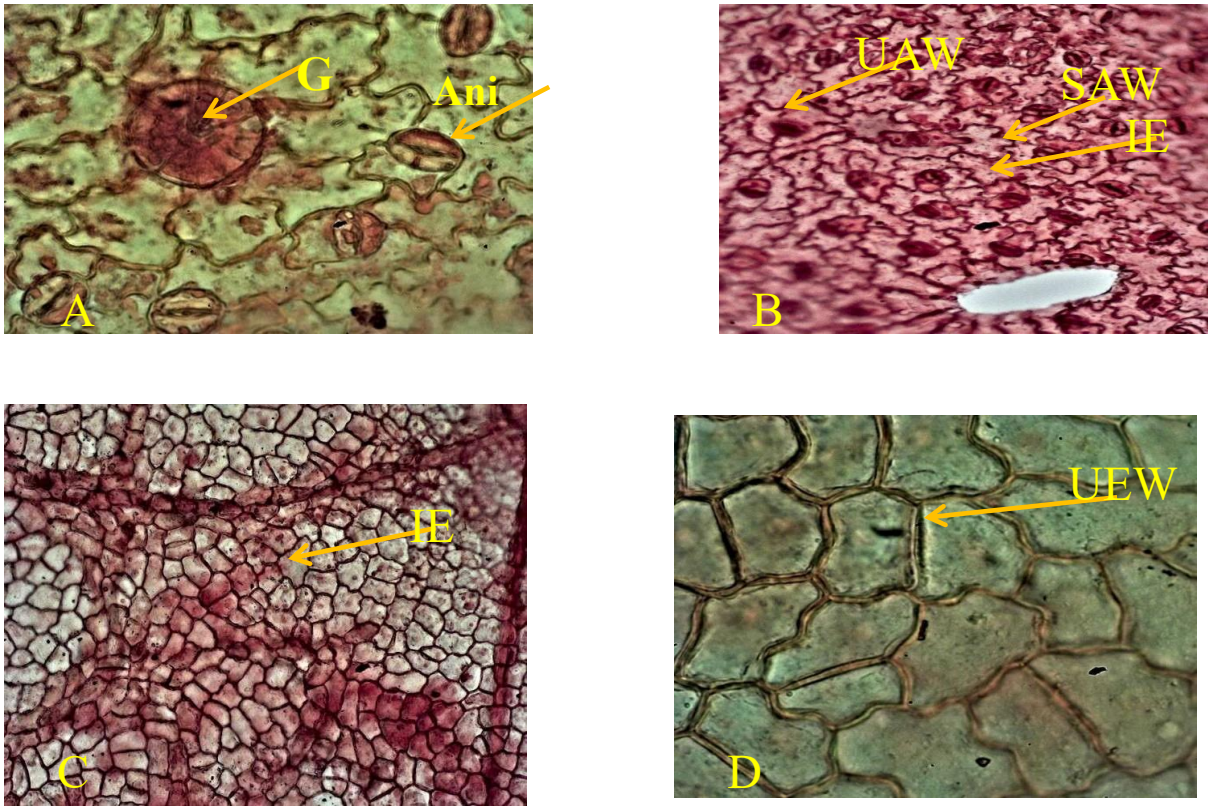

Figure 2: Abaxial (Lower) Surface of *N. laevis* leaf showing A. (×40) Glandular Trichome and Anisocytic stomata, B. (×10) Irregular epidermal Cell, Sinous anticlinal wall pattern, Undulate Anticlinal wall pattern. Adaxial (upper) Surface (×10) C. (×10) Showing Irregular epidermal cell, D. (×40) showing Undulate epidermal wall pattern.

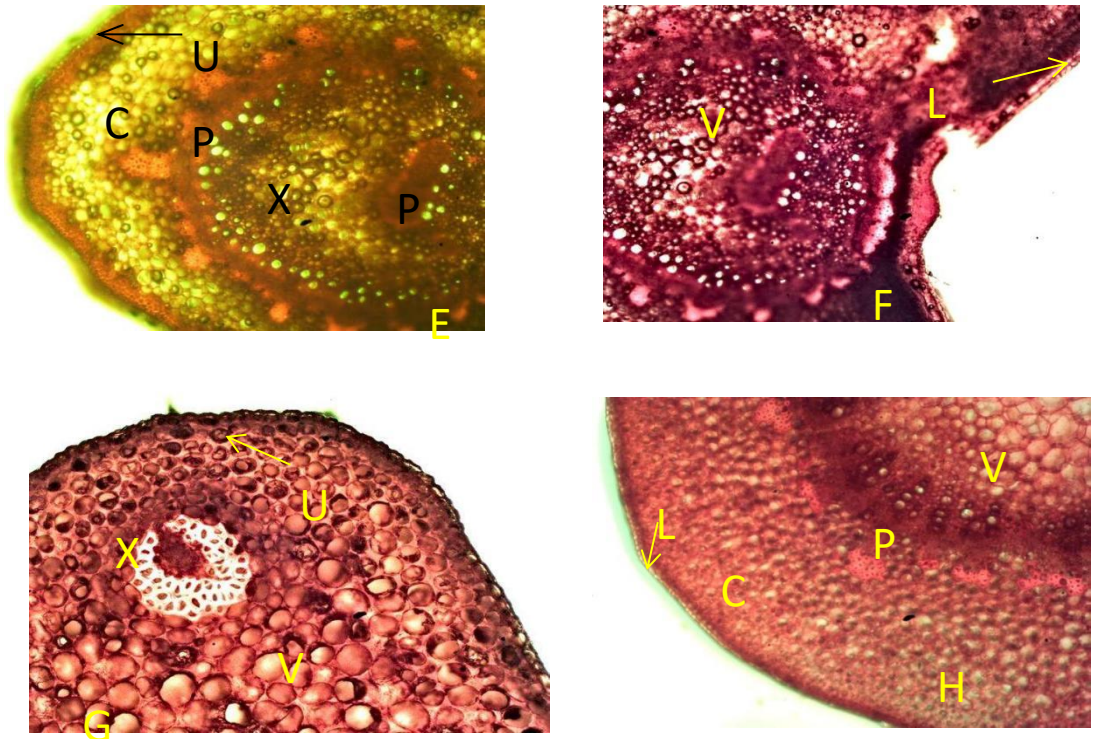

Figure 3: *N. laevis* leaf Transverse section showing (E) (×40) UE; Upper epidermis, Co; Collenchyma, P; Parenchyma, Xy; Xylem, Ph; Phloem. (F) (×40) LE; Lower epidermis, Vb; Vascular bundle. *N. laevis* Transverse section of petiole showing (G) (×10) UE; Upper epidermal cell, Vb; Vascular bundles, Xy; Xylem. (H) (×40) LE; Lower epidermis, Co; Collenchyma, P; Parenchyma, Vb; Vascular bundle.

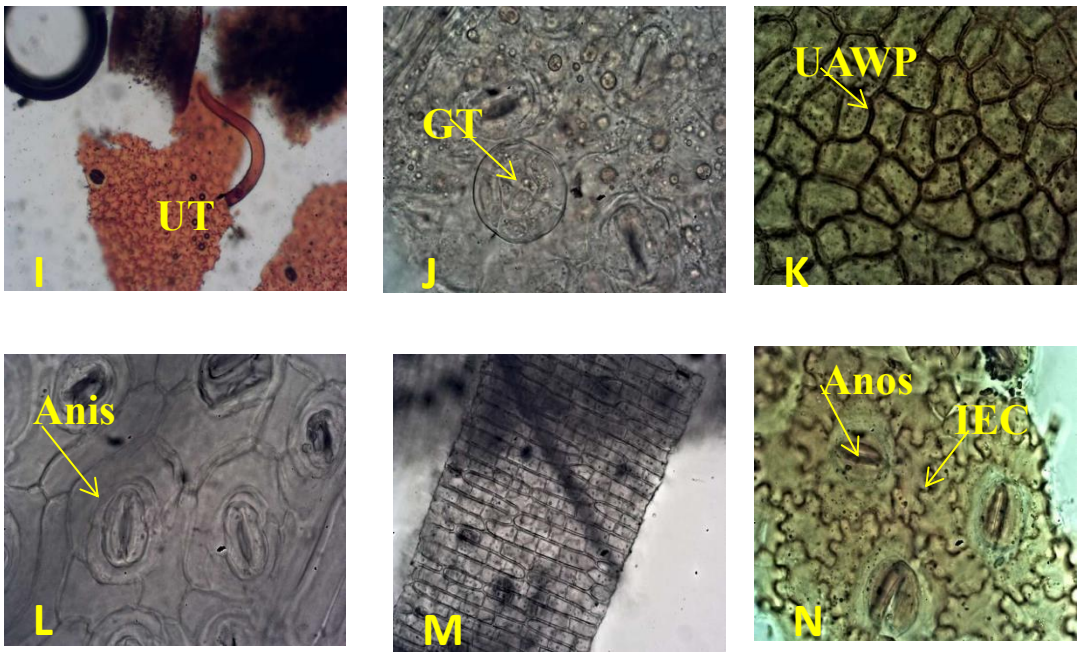

Figure 4: *N. laevis* leaf powder showing (I). (×10) UT; Unicellular Trichome, (J) (×40) GT; Glandular Trichome (K). (×40) UAWP; Undulate Anticlinal wall pattern, (L) (×40) Anis; Anisocytic stomata, (M) (×10) (N). (×40) Anos; Anomocytic stomata, IEC; Irregular epidermal Cell

Table 2: Micromeritic evaluation of powdered leaf of *N.laevis*

Micromeritic Parameters	Leaf Powder
Bulk Volume (mL)	44.66 ± 2.27
Tapped Volume (mL)	30.05 ± 0.38
Bulk Density (g/mL)	0.22 ± 0.01
Tapped Density (g/mL)	0.32 ± 0.004
Hausner Ratio	1.47± 0.09
Carr's Index (%)	31.83 ± 4.46
Diameter of Heap (cm)	7.43 ± 0.14
Height of Heap (cm)	3.53 ± 0.20
Flow Time (sec)	15.14 ± 1.70
Flow Rate (g/sec)	1.25
Angle of Repose (0)	43.50

Mean±SEM (Standard Error of Mean) of Three (3) Replicate

Table 3. Fluorescence analysis of *N. laevis* leaf powder

Extract	Sample	Visible Light	UV Light Long wavelength (365.0 nm)
n-Hexane	Leaf	Brown	Grey
Ethyl acetate	Leaf	Green	Pink
Chloroform	Leaf	Green	Pinkish yellow
Ethanol	Leaf	Green	Light grey
Methanol	Leaf	Green	Light pink
Water	Leaf	Brown	Blue

Table 4: Results for Water soluble extractive value, ethanol soluble extractive value and methanol soluble extractive value and standard error of mean for leaf powders of *N. laevis*

Parameters	Weight	Percentage(% w/w)
Water- soluble extractive value	0.11± 0.007	11
Ethanol- soluble extractive value	0.833± 0.008	8.33
Methanol- soluble extractive value	0.0933± 0.004	9.33

Table 5: Results of Moisture content, total ash, Acid-insoluble ash, water-soluble ash and standard error of mean for leaf powders of *Newbouldia laevis*

Parameters	Weight	Percentage (% w/w)
Moisture content	0.2533± 0.004	12.7
Total ash	0.1316± 0.003	6.58
Acid-insoluble	0.02± 0.00	1
Water-soluble	0.03± 0.007	1.5

Values are represented as mean of six (6) replicates ± SEM for moisture content and Total ash
Values are represented as mean of three (3) replicates ± SEM for Acid insoluble ash and Water-soluble ash

Table 6: Results for Chemomicroscopy of *Newbouldia laevis*

Constituents	Qualitative test	Observation/Inference
Mucilage	Sample + Ruthenium red	Pink/Mucilage present
Lignin	Sample + phloroglucinol + Conc. HCl	Red/Lignin present
Starch	Sample + N/50 iodine	Blue-black/Starch present
Cellulose	Sample + N/50 iodine + Sulphuric acid	Blue colouration/Cellulose present
Protein	Sample + Picric acid (1%)	yellow stains strand/ Protein present

4.0 DISCUSSION

Newbouldia laevis a member of Bignoniaceae family, have various bioactive secondary metabolites with diverse pharmacological activities. They are widely used in traditional medicinal systems of a number of countries for the treatment of ailments like cancer, snake bite, skin disorders, gastrointestinal disorders, respiratory tract disorders, hepatic disorders, epilepsy, cholera, pain, urinary problems, malaria, heart problems and sexually transmitted diseases [12]. These studies will offer this plant like any other medicinal plants that could be confused with other species due to their relative similarities and so provide some basis for its proper identification. The quality control of vegetable crude drugs is paramount importance under current European Union (EU) regulations: herbal products can only be manufactured under license in uniformity with the rules and guidance for pharmaceutical manufacturers and distributors [13]. According to WHO, all drugs synthetic or plant-based must meet safety and efficacy standards. Hence, quality control of herbal drugs is critical for ensuring authenticity and detecting adulteration. Microscopic analysis of *N. laevis* revealed anisocytic and anomocytic stomata on the abaxial surface while the adaxial surface had no stomata, irregular epidermal cell shapes, and undulate anticlinal walls. Stomatal distribution was Hypostomatic, with a stomatal index of 27.31% on abaxial surface and absent for (adaxial). Stomatal number was 86 ± 2.39 (abaxial) and absent for (adaxial). The transverse section of the midrib revealed vascular bundles (Fig. 3). Micromeritics indicated poor flow properties, including an angle of repose of 43.50°, Hausner's ratio of 1.47± 0.09 and Carr's Index of 31.83 ± 4.46 (Table 2). Chemomicroscopy confirmed cellulose, mucilage, starch, lignin, and oils (Table 6). Water extractive value was higher than methanol and ethanol aligning with Umoh et al. (2022) [14]. Moisture contentis foundto be within range at 12.7% w/w of the African Pharmacopoeia limit. [8] Total ash value was 6.58%w/w, acid-insoluble ash value was 1%w/w and water-soluble ash value was1.5 % w/w, with total ash and acid-insoluble ash being within European Pharmacopoeial limits [15][16].

5. CONCLUSION

The results obtained from the pharmacognostic studies have provided critical insights into ensuring proper identification and standardization for medicinal use of the plant *Newbouldia laevis*.

Declarations

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Conflict of Interest
The authors have declared that no conflict of interest exists.

Contribution of the Authors
This work was carried out in collaboration with all other authors. CIA and RAU designed the study, performed the experimental procedures, and author IIJ prepared statistical analysis, and CIA wrote the first draft of the manuscript. Authors RAU and IIJ supervised lab experiments. Authors CIA, IIJ, RAU, NAA and GEC organized data, managed the literature searches and assisted in plant material preparation. All authors read and approved the final manuscript.

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