# Pharmacognostic Profiling and Standardisation of *Praxelis clematidea* (Griseb): Morphological, Physicochemical, and Phytochemical Insights

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| Article Info | A B S T R A C T |
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| *Article type:*<br>Original Article | **Objective:** *Praxelis clematidea* (Asteraceae) has attracted medicinal interest but remains poorly characterized pharmacognostically. The lack of standardized data limits its therapeutic application and broader acceptance. Its variable phytochemical profile across regions underscores the need for a comprehensive pharmacognostic framework. This study provides foundational data for standardization, supporting reliable herbal preparation development, guiding pharmacological research, and differentiating it from morphologically similar Asteraceae species. |
| | **Methods:** Macroscopic and microscopic analyses were performed to characterize tissues and cellular inclusions, and leaf constants were quantified. Physicochemical parameters, including ash values, extractive values, and moisture content, were assessed. Phytochemical screening and thin-layer chromatography (TLC) were used to detect secondary metabolites. Acute toxicity was evaluated in animal models to determine $LD_{50}$. |
| ✉ *Correspondence to:*<br>Tunde Owolabi<br><br>**Email:**<br>owolabitunde1@gmail.com | **Results:** *P. clematidea* displayed distinctive morphological and anatomical features. Quantitative microscopy recorded vein islets ($20 \pm 1.45$), vein terminations ($52 \pm 5.27$), and stomatal indices of $20 \pm 1.25$ (upper surface) and $18 \pm 0.94$ (lower surface). Physicochemical analysis revealed total ash $12.667 \pm 0.219\%$, loss on drying $7.433 \pm 0.636\%$, water-soluble extractive $8.967 \pm 0.393\%$, and alcohol-soluble extractive $15.733 \pm 0.561\%$. Phytochemical screening identified flavonoids, alkaloids, and terpenoids, with five TLC spots. Acute toxicity indicated moderate toxicity ($LD_{50} = 565.69$ mg/kg). |
| | **Conclusion:** This study establishes standardized diagnostic parameters for *P. clematidea*, ensuring accurate identification and quality control. The characterized bioactive constituents highlight its medicinal potential, and toxicity assessment indicates preliminary safety for therapeutic use. These findings support its inclusion in pharmacopoeial monographs and safe application in herbal medicine. |
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## Introduction

*P. clematidea* (commonly known as praxelis), a member of the Asteraceae family, is globally acknowledged as a highly invasive plant with far-reaching ecological and economic consequences. Originally native to some part of America, such as Brazil, Argentina, Bolivia, Paraguay, and Peru, this species has rapidly spread beyond its native range, primarily due to anthropogenic activities [1]. It is now naturalised in several regions such as China, Taiwan, Australia, Florida, and across tropical and subtropical zones like Nigeria, where it forms dense, and outcompetes other plants and alters natural ecosystem functions [2-4].

Despite these invasive and suppressive reputations, phytochemical investigations have repeatedly identified flavonoids and other phenolic compounds as major constituents of *P. clematidea* [5], including isolation and structural elucidation of multiple flavonoids and other known natural products from the leaf and flower materials, with methoxylated flavones, phenylpropanoids, lignans/benzofurans as the major bioactive constituents [6].

In traditional medicine, *P. clematidea* is used for the management of wounds, fever, inflammation, gastrointestinal disorders, and microbial infections [7]. Decoctions or poultices prepared from its leaves and aerial parts are applied topically for wound healing and skin ailments, while aqueous or ethanolic extracts are used internally as antipyretic, analgesic, and antimicrobial remedies [8]. These traditional uses are supported by the presence of several bioactive secondary metabolites such as flavonoids, terpenoids, alkaloids, and phenolic compounds [5,6] that confer multiple pharmacological activities.

Phytochemical and pharmacological investigations have attributed antioxidant, anti-inflammatory, antimicrobial, and cytoprotective effects [6,9,10] to *P. clematidea*, aligning with its ethnopharmacological applications. The flavonoid constituents, including methoxylated flavones and flavonols [5], exhibit strong radical scavenging and anti-inflammatory properties [11,12], while terpenoids and alkaloids contribute antimicrobial, wound-healing, and analgesic effects [13-15]. These multi-target bioactivities suggest that *P. clematidea* could serve as a valuable source of novel phytotherapeutic agents.

Despite its therapeutic potential, the species remains underexplored in terms of pharmacognostic characterization, standardization, and quality control measures.

Standardization of medicinal plants is essential to ensure their identity, purity, safety, and efficacy [16]. Variations in environmental conditions, harvesting time, processing methods, and geographical origin can significantly influence the phytochemical profile and pharmacological potency of herbal materials [17]. The lack of standardised diagnostic and physicochemical parameters often leads to adulteration and misidentification, undermining the reliability of herbal preparations [17-19].

For *P. clematidea*, establishing detailed pharmacognostic characteristics, including macroscopic and microscopic features, physicochemical constants, and chromatographic fingerprints, is crucial for its inclusion in pharmacopoeial monographs and for developing evidence-based phytomedicines. This motivates the current standardisation work.

*P. clematidea*, despite been a plant with promising therapeutic applications, the absence of standardised pharmacognostic data significantly limits its therapeutic potential and broader acceptance. Its phytochemical composition, which is notably diverse and subject to variation across different geographic regions revealed the pressing need for a comprehensive pharmacognostic framework. This includes detailed assessments of its macroscopic, microscopic, physicochemical, and phytochemical properties to ensure precise identification, quality control, and safe medicinal application. The current study is the first as far as we know presenting foundational data essential for the standardization of *P. clematidea*, these data will support the development of reliable herbal preparations and guide future pharmacological research, while also facilitating

differentiation from other morphologically similar species within the Asteraceae family.

## Materials

The fresh whole plant of *P. clematidea* (figure 1a) was obtained within the University of Benin, Ugbowo campus, Ovia South, LGA, Edo State, Nigeria (6°20'1"N 5°36'1"E) in June, 2023, identified and authenticated at the pharmacognosy department of Dora Akunyili college of pharmacy, Igbinedion University, Okada, Edo State, Nigeria and voucher specimen (figure 1b) deposited in the herbarium with herbarium number IUO/23/356.

The plant samples were sorted, and prepared accordingly base on the experimental need, fresh plant samples were used for the macroscopy, quantitative microscopy, microscopy except for the powder microscopy, physico-chemical evaluations where powder sample are required and prepared as follows; after sorting, the whole plants were rinsed and air-dried at room temperature for 7 days and transferred into an oven maintained at 40°C for an additional 4 hrs before pulverization into powder form using an electrical miller (Chris Norris, England). The powder was stored in an air-tight container and used for the experiment. A portion of the powder material was subjected to Soxhlet extraction using methanol to obtain a crude extract, which was used for phytochemical and toxicological evaluations.



**Figure 1:** *P. clematidae*: a) in its natural habitat b) Deposited voucher specimen

## Chemical, Reagents, and Tools

The chemical and reagents used include; N/50 iodine, sulphuric acid, phloroglucinol, concentrated hydrochloric, ferric chloride, Ruthenium red, Sudan iv, picric acid, chloral hydrate, glycerol, Drangedroff reagent, Mayer's reagent, Wagner's reagent. All other reagents were of analytical grade and the solvents were redistilled before use. The study utilized various apparatus and equipment, including a digital microscope (AXL, LABO DIGICAM 1300), digital venial caliper, glass slide heating mantle, hot air oven (DHG-9053A), muffle furnace, water bath, grinding machine (DE-DAMAK; GX160, Japan), centrifuge, Soxhlet apparatus, water circulator.

## Methods
## Macroscopical and Morphology Examination

Sensory characteristics such as odor, taste, color, and texture were assessed using the unaided sense organs, while measurements of shape and size were performed using a digital vernier caliper and a hand lens, in line with standard procedures [19,20].

## Microscopic Examination

Fresh leaf, stem, and root samples were fixed using a solution composed of 5 ml formalin, 5 ml glacial acetic acid, and 90 ml of 70% ethanol. After 24 hours of fixation, the specimens were dehydrated through a graded series of tertiary butyl alcohol (TBA). Paraffin wax was gradually introduced for infiltration until the TBA was fully saturated. The embedded tissues were then cast into paraffin blocks [20,21], and photomicrographs were captured using a LABO DIGICAM 1300 at varying magnifications.

## Powder Microscopy

A rough powder of the plant was prepared and observed microscopically to identify diagnostic plant tissues, using standard microscopy protocols [18,19].

## Chemo-Microscopic Analysis

Chemical microanalysis followed methods outlined standard procedures [19,22].

## Quantitative Microscopy

Leaf anatomical constants, including stomatal index, vein islet number, and vein termination number, were determined using established protocols [22,23].

## Physicochemical Analysis

The Physicochemical evaluations were conducted following standard procedures [24]. The parameters evaluated include; Total Ash, Acid-Insoluble Ash, Moisture Content, Water-Soluble Extractive Value, and Alcohol-Soluble Extractive Values.

## Qualitative Phytochemical Screening

Preliminary phytochemical analysis [25] was performed to detect the presence of major secondary metabolites.

## Thin Layer Chromatography (TLC) Analysis

The crude extract was redissolved in methanol, diluted with water, and partitioned with chloroform using a separatory funnel until a clear chloroform layer was obtained. This process produced aqueous and chloroform fractions. These, along with the crude extract, were spotted onto pre-coated silica gel TLC plates (Silica gel 60 F254, Sigma Aldrich, Germany) using microcapillary tubes. The plates were developed in a TLC tank (Shandon Southern T.L.C Chromatank, Unikit) using two mobile phase systems: n-hexane:methanol (4:6) and n-hexane:ethyl acetate (9:1). After development, the plates were dried in an oven at 105°C for five minutes and visualized under UV light (356 nm). The Rf values for each spot were recorded [25].

## Biological Evaluation: Acute Toxicity

The acute oral toxicity of *P. clematidea* was assessed following Lorke's method, with modifications according to OECD Guideline 425 (Up-and-Down Procedure [26,27].

Briefly, Phase One: Nine mice were divided randomly into three groups (3 mice per group) and given 10, 100, and 1000 mg/kg of extract orally. Observations were made over 24 hours.

Phase Two: Four individual mice received doses of 200, 400, 800, and 1600 mg/kg respectively, based on the outcomes of Phase One. The animals were monitored for signs of toxicity, behavioral changes, weight loss, or mortality during the initial 24 hours and daily for seven days. The $LD_{50}$ was then calculated by the formula:

$$LD_{50} = \sqrt{(D_0 X\ D_{100})}$$

where $D_0$ = Highest dose that gave no mortality, $D_{100}$ = Lowest dose that produced mortality.

## Statistical Analysis

## Results
## Macroscopy
## Seed Morphology

The seeds (achenes) of *P. clematidea* are small, black, and equipped with about 40 bristles (pappi) measuring 3 - 4 mm in length. Samples collected

## Flower Morphology

The flower heads (capitula) are densely clustered at the ends of terminal cymes. Each capitulum measures approximately 8 - 12 mm in length and 5.1 mm in width, borne on hairy stalks 2.5 - 11 mm long. Each head contains 40 - 80 purple florets, encircled by around 30 green bracts. The florets feature 4 - 6 petals, which are fused to form a corolla tube of 3.5 - 4.8 mm in length (Figure 2).

All experiments were performed in triplicate, and results are presented as mean ± standard error of the mean (SEM) for n = 3. Statistical analyses were conducted using SigmaPlot version 15.0, and differences among treatments were evaluated by one-way ANOVA. Values of $p < 0.05$ were considered statistically significant.

from three populations across Edo State (Ewu - Esan Central, Benin - Egor, and Okada - Ovia North-East) revealed seed lengths ranging from 2.9 to 3.6 mm, widths from 0.83 to 1.1 mm, and a thickness of about 0.6 mm. Seed weight ranged between 0.14 and 0.33 mg, with each capitulum yielding approximately 44 to 58 seeds.

## Stem and leaves Morphology

The branched stems are rounded or angular and covered in long hairs. The leaves are oppositely arranged along the stems and borne on stalks (petioles) 3.5-20 mm long. The leaf blades (2.5-6 cm long and 1- 6 cm wide) are ovate to rhomboid and have acute apices. They are hairy, particularly underneath, and have deeply toothed margins (Figures 3a, b & c).



**Figure 2:** *P. clematidea* flowers

### Root characters

The root is fibrous with a few adventitious roots developing from the base of the stem. they are thin, cylindrical, and moderately branched. They vary in length depending on the plant's maturity, but are slender and elongated. The outer surface is slightly creamy and appears off-white when cut. It is slightly aromatic in odour, characteristic of essential oils present in the plant and taste bitter, slightly acrid, but not strongly pungent (Figure 3d below).
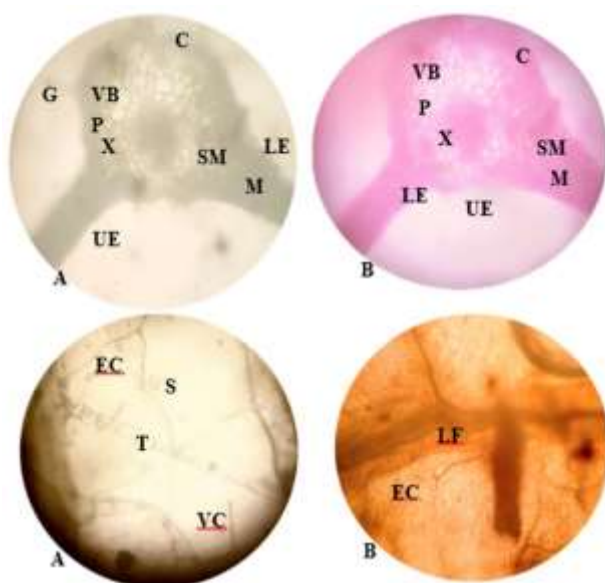
### Microscopic characteristics
### Leaf microscopy
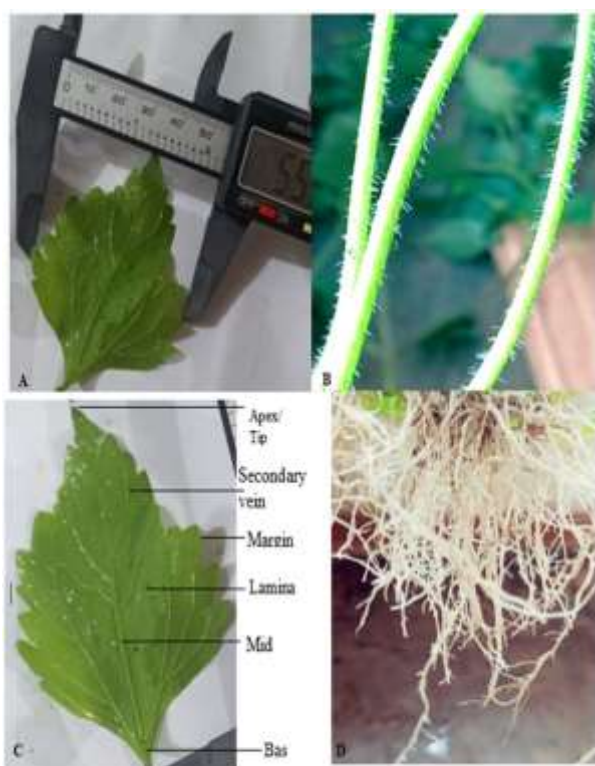


**Figure 3**: *P. clametidea* (a) Heavily toothed leaf with caliper b) Hairy



Figure 4. Microscopy of P. Clematidea leaf; a) Transverse section without stains b) Transverse section after staining with phloroglucinol and HCL, c) cross section without stains d) cross section after staining with phloroglucinol and HCL, UE=Upper Epidermis, LE=Lower Epidermis, GT=Glandular Trichome, M= Mesophyll, SM=-Spongy Mesophyll, C=Collenchyma, VB=Vascular Bundles, X=Xylem, P=Phloem, EC= Epidermal Cell, T=Trichome, VC= Vacuole,

The transverse section of the leaf (Figures 4a & b above) exhibits a single-layered upper and lower epidermis composed of polygonal cells with slightly undulating margins. Some epidermal cells are modified into biseriate, multicellular, glandular trichomes that serve as covering hairs. Beneath the upper epidermis lies a well-organized palisade layer, while the lower mesophyll region consists of loosely arranged spongy parenchyma with conspicuous intercellular spaces. The midrib region contains two to three layers of collenchymatous cells on both adaxial and abaxial sides. The vascular bundle is encased by parenchymatous tissue, with distinct radiating xylem and phloem elements. Diacytic stomata occur on both leaf surfaces.
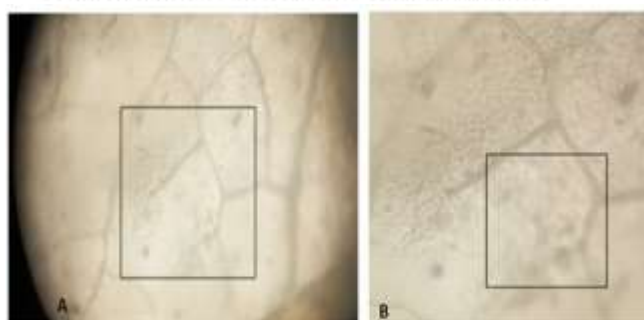
### Stem Microscopy

The transverse section of the stem (Figure 5 below) reveals an epidermis made up of quadrangular, parenchymatous cells covered by a thin cuticle and interrupted by stomata. The cortical region is differentiated into an outer chlorenchymatous zone composed of radially elongated cells and an inner zone of spongy parenchyma. The pericycle consists of lignified fibers located beneath the ridges, while mesocortical fibers occur in compact groups. Calcium oxalate crystals are distributed within the cortical tissues. The vascular bundles are collateral, and the secondary xylem forms a continuous ring.
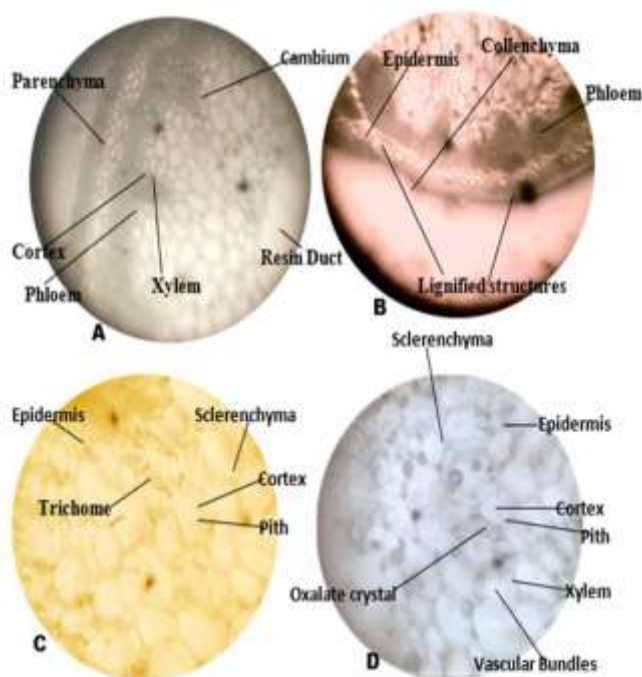


**Figure 5:** Cross section of P. clematidea stem; a) X10 without staining, b) X10 after staining with phloroglucinol + HCL, c) X40 after staining with phloroglucinol + HCL, d) X40 without
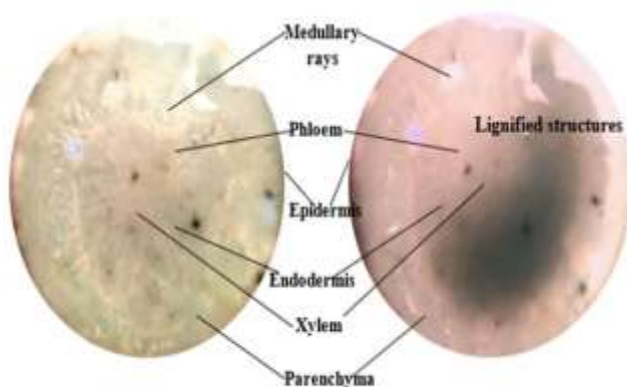


**Figure 6:** Microscopic features of *P. clematidea* root



**Figure 7:** Leaf Constant a) Vein islet and termination number b)

## Root Microscopy

The transverse section of the root (Figure 6 above) demonstrates a stratified cork layer comprising one to three rows of small outer cells, followed by two layers of larger, suberized and lignified cells. The phelloderm is composed of one to two rows of thin-walled, parenchymatous cells. The phloem consists of sieve tubes and parenchyma alternating with two to three-celled medullary rays. The xylem contains small vessels, parenchyma, and fibers, with broad medullary rays separating the xylem patches.

## Powder Microscopy

Microscopic examination of the powdered material from the whole plant revealed characteristic fragments such as epidermal cells with diacytic stomata, multicellular glandular trichomes, lignified fibers, phloem elements, calcium oxalate crystals, cork cells, parenchyma containing oil globules, and xylem vessels as shown in Table 1 below.

**Table 1**. Chemo-microscopic characters of powder *P. clematidea*

| Reagents | Observation | Results |
|---|---|---|
| Phloroglucinol + HCL | Cherry red colouration of the walls of cork cells, sclereids and fibres | Lignin present |
| Ferric chloride solution | Greenish-black colouration observed in cork cells | Tannins (Condensed) present |
| N/50 iodine solution | No bluish colouration in cortical parenchyma and cork cells | Starch grains abssent |
| Ruthenium red solution | Nom reddish colouration in the cortex | Mucilage abssent |
| N/50 iodine + 66% $H_2SO_4$ | Blue black colouration | Cellulose present |
| Sudan III | Orange-red colouration | Fat and fatty oil present |
| Picric acid solution | No yellow colouration | Protein absent |
| 80% $H_2SO_4$ | The bright prismatic crystals disappeared on the addition of the reagent. Prismatic crystals found in parenchyma cells | Calcium oxalate crystals present |
| Glycerol + acetic acid solution | No evolution of gas | Calcium carbonate absent |

## Quantitative microscopy of leaf constants

The stomatal number was determined by calculating the ratio of the number of stomata to the total number of epidermal cells, with each stoma considered as a single epidermal cell. Vein islet number was quantified as the total number of vein islets per square millimeter of the leaf surface, measured midway between the midrib and the leaf margin. Similarly, the veinlet termination number was evaluated as the number of veinlet terminations per square millimeter of the same region as depicted in Figure 7 above. The obtained results are presented in Table 2 below.

**Table 2:** Leaf Constants of *P. clematidea*

| Vein Islet Number | Termination Number | Upper Stomatal Index | Lower Stomatal Index |
|---|---|---|---|
| 20 ± 1.45 | 52 ± 5.27 | 20 ± 1.25 | 18 ± 0.94 |

Data represent Mean ± SEM, n = 8

## Physicochemical parameter

The physicochemical evaluation of the powdered whole plant included the assessment of key parameters such as moisture content, total ash, sulphated ash, acid-insoluble ash, alcohol- and water-soluble extractives, and crude fiber content. The detailed results are provided in Table 3.

**Table 3:** Physico-chemical parameters of the whole plant

| Physicochemical Parameters | (%) |
|---|---|
| Total Ash | 12.667 ± 0.219 |
| Water Soluble Ash | 8.667 ± 0.219 |
| Acid In Soluble Ash | 3.267 ± 0.120 |
| Sulphated Ash | 10.743 ± 0.406 |
| Moisture content | 7.433 ± 0.636 |
| Water Soluble Extractive | 8.967 ± 0.393 |
| Alcohol Soluble Extractive | 15.733 ± 0.561 |
| Crude fibre | 8.733 ± 0.120 |

Data represent Mean ± SEM, n = 3

## Phytochemical screening

Preliminary screening of the plant extract revealed the presence of key bioactive constituents such as flavonoids, alkaloids, reducing sugars, terpenoids, cardiac glycosides, and polysaccharides. Conversely, saponins, tannins, phlobatannins, and steroids were not detected (Table 4).

**Table 4:** Results of the qualitative phytochemical screening of *P. clematidea*

| Phytoconstituents | Results |
|---|---|
| Cardiac Glycoside | - |
| Saponin | - |
| Tannin | - |
| Phlobatannin | + |
| Flavonoid | + |
| Steroid | + |
| Alkaloid | + |
| Reducing sugar | + |

| | |
|---|---|
| Terpenoid | + |
| Polysaccharide/Starch | - |

Note: (+) means present, and (-) means

absent.

## Thin Layer Chromatography (TLC) Analysis

The successful solvent-solvent partition yielded a highly polar fraction (aqueous), and a non-polar fraction (chloroform layer). After the development of the TLC plates, and viewed for photo-reactive components under UV light at 365 nm, the results revealed that the chemical components of *P. clematidea* are relatively non-polar as five fluorescent constituents were conspicuously visible for plate developed in n-Hexane: Ethylacetate (9:1) for chloroform fraction, four for crude which were initially unseparated with combination of n-Hexane: Methanol (4:6) as shown in figure 8 below. The breakdown of the rf values is presented in Tables 5 & 6 below.
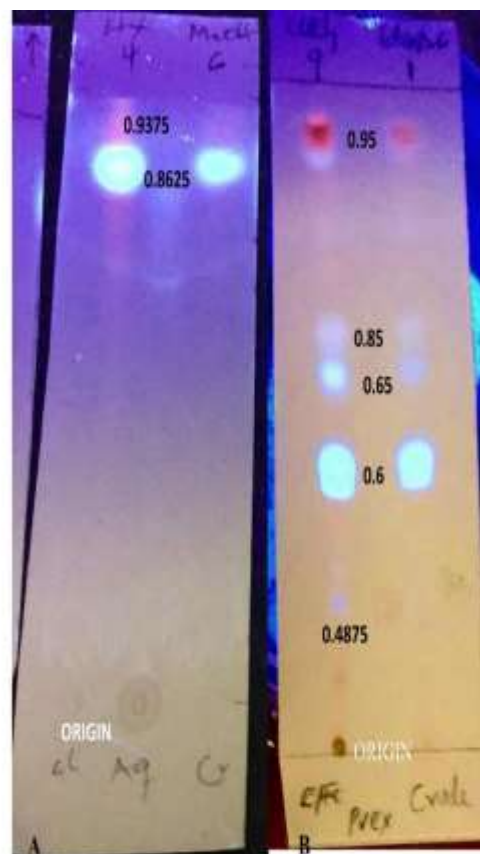


**Figure 8.** Chromatograms of *P.clematidea* on silica gel GF254 with A) n-hexane:Methanol (4:6) and B) n-hexane:ethyl acetate (9:1) solvent system. Viewed under ultraviolet light at 365 nm.

**Table 5:** TLC developed in n-Hexane:Methanol (4:6)

| Components | Color | $R_f$ (cm) | Aq | CF |
|---|---|---|---|---|
| 1 | Red | 0.9375 | 0.85 | 0.9375 |
| 2 | Sky Blue | 0.8625 | | 0.8625 |

CF = Chloroform fraction, AF = Aqueous fraction, Rf = Retention Factor

**Table 6:** TLC developed in n-hexane - ethyl acetate (9:1)

| Components | Color | Rf (cm) | CF |
|---|---|---|---|
| 1 | Pink | 0.95 | 0.95 |
| 2 | Sky Blue | 0.85 | 0.85 |
| 3 | Sky Blue | 0.65 | 0.65 |
| 4 | Sky Blue | 0.6 | 0.6 |
| 5 | Sky Blue | 0.4875 | 0.4875 |

CF = Chloroform fraction, Rf = Retention Factor

## Biological Evaluation
## Acute toxicity study of methanol extract in Mice

In the initial phase of toxicity testing, mice treated with 10 and 100 mg/kg orally exhibited no mortality, while 66.67% mortality was observed at 1000 mg/kg. In the second phase, animals receiving 200 and 400 mg/kg survived, whereas those administered 800 and 1600 mg/kg all died. Based on these observations, the $LD_{50}$ for the methanol extract was calculated to be 565.69 mg/kg (*p.o.*) (Table 7). Symptoms included reduced activity, though the mice resumed feeding and normal behavior within three hours post-treatment. All surviving animals fully recovered within 24 hours.

**Table 7:** Determination of median lethal dose ($LD_{50}$) of the methanol extract in mice

| Treatment (mg/kg) group/dose | Number of animals used | Number of Deaths | Survival (%) |
|---|---|---|---|
| PHASE 1 | | | |
| Extract (10) | 3 | 0 | 100 |
| Extract (100) | 3 | 0 | 100 |
| Extract (1000) | 3 | 2 | 33.3 |
| PHASE 2 | | | |
| Extract (200) | 1 | 0 | 100 |
| Extract (400) | 1 | 0 | 100 |
| Extract (800) | 1 | 1 | 0 |
| Extract (1,600) | 1 | 1 | 0 |

$LD_{50}$ = 565.69 mg/kg body weight *P.O*

## Discussion

This study presents a holistic pharmacognostic profile of *P. clematidea*, a member of the Asteraceae family, combining macroscopic, microscopic, physicochemical, and phytochemical analyses. Given the plant's dual reputation as an invasive species and a source of medicinal bioactives [1-3,5-10].

The plant exhibits hallmark traits typical of Asteraceae, particularly in its reproductive structures. Its small, black achenes with 25 - 40 pappus bristles are well-adapted for wind dispersal; an attribute common among invasive weeds [4]. Seed size variations (2.9 - 3.6 mm in length; 0.14 - 0.33 mg in weight) across different locations suggest possible chemotypic diversity, which is in line with previous reports [28] and may reflect underlying phytochemical variability [29]. The floral architecture, consisting of capitula with 40–80 florets surrounded by about 30 green bracts, aligns with the Astereaceae classification [30].

The anatomical investigations revealed distinguishing features, including diacytic stomata and glandular trichomes on the leaf surface, both key diagnostic markers in Asteraceae as earlier reported in literatures [20,30,31]. The well-organized palisade and spongy mesophyll layers are potential sites for metabolite storage [32], justifying the plant as a reservoir of bioactive constituents. The stem and root sections displayed lignified pericyclic fibers and layered cork structures, indicative of structural resilience and useful in raw material verification [16-18]. The detection of calcium oxalate crystals and distinct medullary rays further supports species-level identification [31]. These traits also help differentiate *P. clematidea* from visually similar or adulterant species [30,31]. Powder microscopy additionally revealed diagnostic elements such as lignin, condensed tannins, cellulose, and calcium oxalate crystals, while the absence of starch and mucilage provides distinguishing criteria during quality control [18,33].

Quantitative evaluation yielded a vein-islet number of $20 \pm 1.45$, vein termination number of $52 \pm 5.27$, and stomatal indices of $20 \pm 1.25$ (upper epidermis) and $18 \pm 0.94$ (lower epidermis), aligning with the expected range for Asteraceae members [30-31]. These anatomical constants are critical for establishing pharmacognostic benchmarks; the values obtained fall within acceptable limits outlined by standard guidelines [17,33]. The total ash (12.667 $\pm$ 0.219%) and acid-insoluble ash (3.267 $\pm$ 0.120%) levels confirm minimal contamination by inorganic impurities [18].

The high alcohol-soluble extractive value (15.733 $\pm$ 0.561%) compared to the water-soluble fraction (8.967 $\pm$ 0.393%) indicates a richness in moderately non-polar compounds, such as terpenoids and flavonoids, which were confirmed by phytochemical analysis. The moisture level (loss on drying: 7.433 $\pm$ 0.636%) is within safety limits, reducing the likelihood of microbial growth or degradation [17-18,33].

Phytochemicals are the determinant of any biological activities of medicinal plants [34]. The presence of flavonoids, alkaloids, reducing sugars, terpenoids, cardiac glycosides, and polysaccharides supports the plant's ethnomedicinal uses, these constituents have well documented bioactivities, for instance, alkaloids are associated with analgesic, antimalarial, antitumor, and antimicrobial effects [35,36]. Their mechanisms often involve interactions with neurotransmitter receptors, ion channels, or enzymes, thereby modulating physiological functions [36], indole and isoquinoline alkaloids influence central nervous system activity through dopaminergic and serotonergic pathways [37,38], while quinoline and pyrrolizidine alkaloids exhibit antimicrobial and cytotoxic properties by intercalating DNA or inhibiting nucleic acid synthesis [39,40]. The presence of alkaloids in *P. clematidea*, may contribute to its antimicrobial and analgesic potential.

Flavonoids are widely recognized for their antioxidant, anti-inflammatory, antimicrobial, and cytoprotective activities [41–43]. Their primary mechanism involves free radical

scavenging and metal ion chelation, which protect biological membranes and macromolecules from oxidative stress [41]. Flavonoids also modulate key signaling pathways such as NF-κB, MAPK, and Nrf2, leading to the downregulation of pro-inflammatory cytokines (IL-1β, TNF-α) and upregulation of antioxidant enzymes (superoxide dismutase, catalase) [44]. Certain flavones and flavonols demonstrate vasoprotective, hepatoprotective, and neuroprotective activities by stabilizing cellular redox balance and maintaining mitochondrial integrity [45,46]. The presence of methoxylated flavonoids may therefore underlie the plant's reported anti-inflammatory and gastroprotective effects [6,9].

Terpenoids represent one of the largest classes of natural compounds, exhibiting anti-inflammatory, antioxidant, antimicrobial, and anticancer properties [47,48]. Their mechanisms are primarily associated with the modulation of inflammatory enzymes such as COX-2 and iNOS, inhibition of lipid peroxidation, and suppression of ROS-mediated signaling [49]. Monoterpenes and sesquiterpenes, common in aromatic plants, exert membrane-stabilizing and antimicrobial effects by altering cell membrane permeability and interfering with bacterial quorum sensing [50]. In *P. clematidea*, terpenoid constituents may explain its mild aromatic property and potential anti-inflammatory activities [6], consistent with findings in related Asteraceae species [20].

The coexistence of flavonoids, alkaloids, and terpenoids in *P. clematidea* suggests multitarget pharmacological synergy, where the antioxidant and anti-inflammatory effects of flavonoids complement the antimicrobial potency of alkaloids and the membrane-stabilizing action of terpenoids [51,52]. Such combined bioactivity supports its traditional use in treating inflammatory conditions, infections, and gastrointestinal disorders, positioning the plant as a promising candidate for standardised phytomedicine development.

Thin-layer chromatography of the chloroform fraction yielded five distinct bands with Rf values ranging from 0.4875 to 0.95, indicative of multiple mid-polar constituents. These chemical signatures are invaluable for preliminary compound identification and could serve as markers in future standardization and chemotaxonomic efforts. Fractionation, a purification process that groups similar constituents, has been associated with improved purity and efficacy [53]. In this study, fractionation enhanced the purity observed with the crude methanol extract, with sub-fractions showing significantly higher resolution and more chromatograms than the crude extract.

According to the OECD and Globally Harmonized System (GHS) of classification, substances with an $LD_{50}$ value between 300 and 2000 mg/kg fall under Category 4 [26-27], signifying that the material is harmful if swallowed, with an $LD_{50}$ of 565.69 mg/kg (p.o.), the methanol extract of *P. clematidea* falls under OECD Category 4, indicating moderate toxicity [27]. Though it holds promise as a source of therapeutic agents, its use should be approached cautiously, with attention to dosage and the need for further toxicological evaluation, particularly for chronic exposure.

## Conclusion

The findings provide baseline diagnostic, physicochemical, and phytochemical parameters essential for the inclusion of *P. clematidea* in pharmacopoeial monographs. The detailed macroscopic and microscopic descriptions serve as reliable identification criteria to prevent adulteration and misidentification. Quantitative microscopy and validated physicochemical values establish measurable quality benchmarks for purity and consistency, while the phytochemical and TLC profiles define chemical fingerprints for authentication. Furthermore, the toxicity assessment supports preliminary safety classification. For the first time, *P. clematidea* has been provided with standardised data that form the foundation for developing an official pharmacopoeial monograph to ensure the plant's quality, efficacy, and safety in herbal formulations. Having established pharmacognostic standards, future research should focus on chronic toxicity studies, bioactivity-guided isolation, mechanism-of-

action studies linking isolated compounds to observed in vivo effects, and well-designed preclinical models to support translation.

## Statements and Declarations
## Competing interests

## Sources of funding

## Ethics Approval

All animal procedures were conducted in compliance with the Research and Ethical Review Committee of Igbinedion University, Okada, and approval was subsequently granted with the number IUO/Ethics/62/25

## Consent to Participate

All authors have approved the submission of this manuscript for publication in *Plant Biotechnology Persa.*

## Author Contributions

Conceptualization, B.A.A.; Methodology, B.A.A.; Software, T.A.O.; Validation, B.A.A.; Formal Analysis, T.A.O, B.A.A.; Investigation, T.A.O; Resources, T.A.O., B.A.A.; Data Curration; B.A.A.; Writing - Original Draft, T.A.O.; Writing - Review & Editing, B.A.A.; Visualization, T.A.O.; Supervision, B.A.A.; Project Administration, B.A.A, T.A.O. All authors have read and agreed to the published version of the manuscript.

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