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

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A B S T R A C T

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}.

Results: *P. clematidea* displayed distinctive morphological and anatomical features. Quantitative microscopy recorded vein islets (20 ± 1.45), vein terminations (52 ± 5.27), and stomatal indices of 20 ± 1.25 (upper surface) and 18 ± 0.94 (lower surface). Physicochemical analysis revealed total ash 12.667 ± 0.219%, loss on drying 7.433 ± 0.636%, water-soluble extractive 8.967 ± 0.393%, and alcohol-soluble extractive 15.733 ± 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.

Keywords: *Praxelis clematidea*; Phytochemistry; Standardization; Quality Control; Microscopy; Thin Layer Chromatography; Secondary Metabolites

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Introduction

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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

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.

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 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.

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).

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

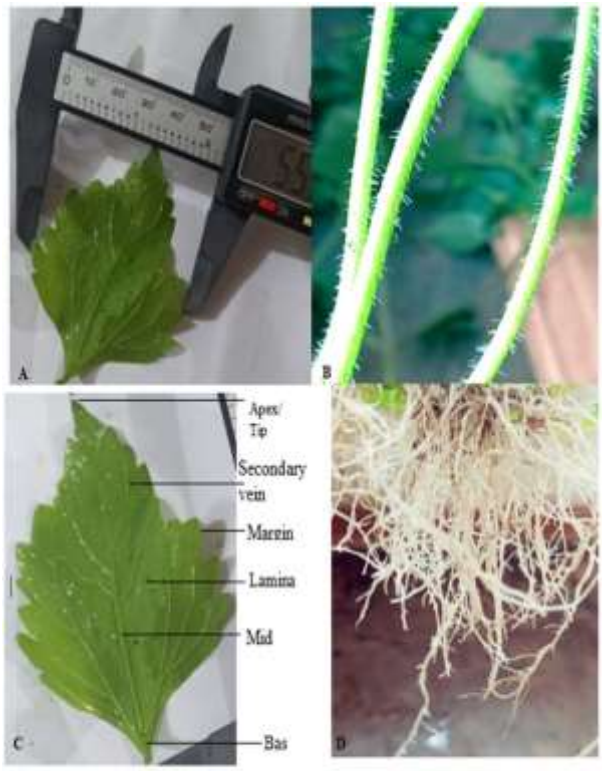

Figure 3: *P. clametidea* (a) Heavily toothed leaf with caliper b) Hairy

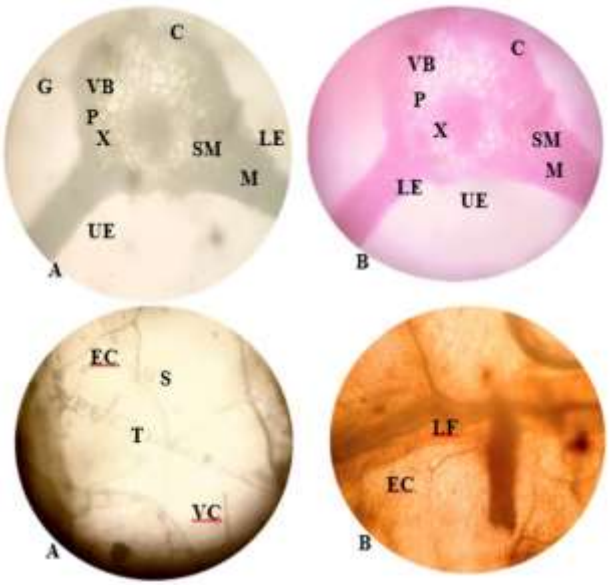

Figure 4. Microscopy of P. Clematidea leaf; a) Transverse section without stains b) Transverse section after staining with phloroglucinol and HCL, c) cross section without stains d) cross section after staining with phloroglucinol and HCL, UE=Upper Epidermis, LE=Lower Epidermis, GT=Glandular Trichome, 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.

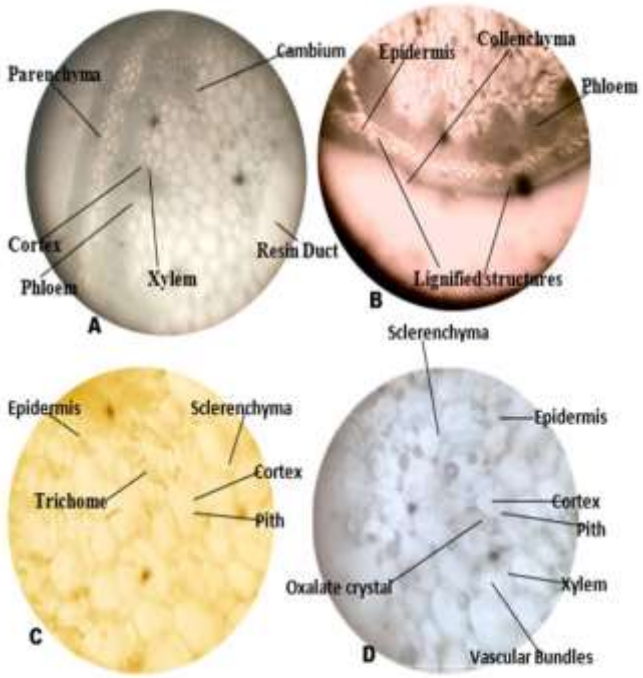

Figure 5: Cross section of P. clematidea stem; a) X10 without staining, b) X10 after staining with phloroglucinol + HCL, c) X40 after staining with phloroglucinol + HCL, d) X40 without

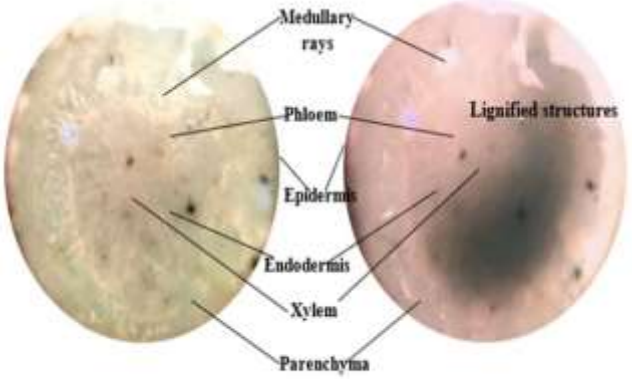

Figure 6: Microscopic features of *P. clematidea* root

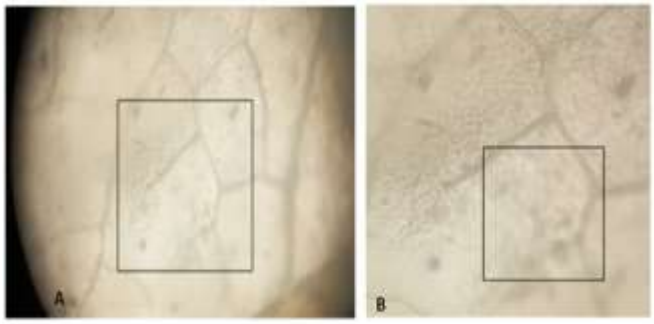

Figure 7: Leaf Constant a) Vein islet and termination number b)

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 absent
Ruthenium red solution	Nom reddish colouration in the cortex	Mucilage absent
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.

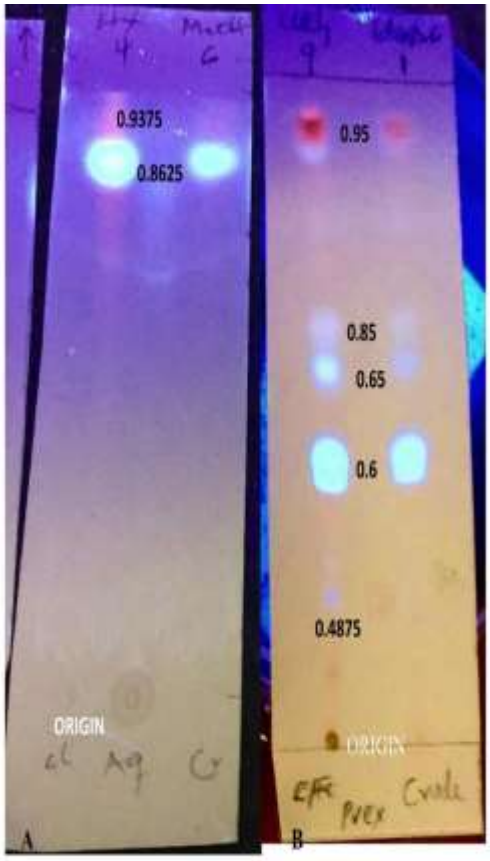

Figure 8. Chromatograms of *P.clematidea* on silica gel GF254 with A) n-hexane:Methanol (4:6) and B) n-hexane:ethyl acetate (9:1) solvent system. Viewed under ultraviolet light at 365 nm.

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	R_f (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 group/dose (mg/kg)	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 ± 1.45, vein termination number of 52 ± 5.27, and stomatal indices of 20 ± 1.25 (upper epidermis) and 18 ± 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 ± 0.219%) and acid-insoluble ash (3.267 ± 0.120%) levels confirm minimal contamination by inorganic impurities [18].

The high alcohol-soluble extractive value (15.733 ± 0.561%) compared to the water-soluble fraction (8.967 ± 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 ± 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

The authors declare no competing interest.

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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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References

1. Salgado VG, de Viera Barreto JN, Rodríguez Cravero JF, Grossi MA, Gutiérrez DG. Factors influencing the global invasion of the South American weedy species Praxelis clematidea (Asteraceae): a niche shift and modelling based approach. Bot J Linn Soc. 2024. https://doi.org/10.1093/botlinnean/boae079
2. Yu SS, Song CS, Song WW. Occurrence of Praxelis clematidea witches' broom disease associated with 16SrI group 'Candidatus Phytoplasma asteris' in Hainan Island of China. Plant Dis. 2024. Published online April 8. https://doi.org/10.1094/PDIS-01-24-0063-PDN
3. Wardini TH, Afifa IN, Esyanti RR, Astutiningsih NT, Pujisiswanto HA. The potential of invasive species Praxelis clematidea extract as a bioherbicide for Asystasia gangetica. Biodiversitas. 2023;24:4738–46. https://doi.org/10.13057/biodiv/d240914
4. Wang J, Zhao M, Zhang J, Zhao B, Lu X, Wei H. Characterization and utilization of biochars derived from five invasive plant species Bidens pilosa L., Praxelis clematidea, Ipomoea cairica, Mikania micrantha and Lantana camara L. for Cd^{2+} and Cu^{2+} removal. J Environ Manage. 2021;280:111746. https://doi.org/10.1016/j.jenvman.2020.111746
5. Maia GL, Falcão-Silva VS, Aquino PG, de Araújo-Júnior JX, Tavares JF, da Silva MS, Rodrigues LC, de Siqueira-Júnior JP, Barbosa-Filho JM. Flavonoids from Praxelis clematidea R.M. King and Robinson modulate bacterial drug resistance. Molecules. 2011;16(6):4828–35. https://doi.org/10.3390/molecules16064828
6. Liang S, Wang L, Xiong Z, Zeng J, Xiao L, Xu J, et al. Anti-inflammatory phenolics and phenylpropanoids from Praxelis clematidea. Fitoterapia. 2023;167:105476. https://doi.org/10.1016/j.fitote.2023.105476
7. Shelly. Praxelis clematidea: The hidden herb that supports your health in surprising ways. 2025 Feb 13. Available from: https://1millionideas.com/health-benefits-of-praxelis-clematidea?utm_source=organic&utm_medium=bing&utm_campaign=Shelly&utm_content=health-wellness&utm_term=2-2025
8. Wawstock. Praxelis clematidea: Benefits and uses of this medicinal plant. 2025 Feb 10. Available from: https://wawstock.com/2025/02/10/praxelis-clematidea-benefits-and-uses-of-this-medicinal-plant/
9. Xiao L, Huang Y, Wang Y, Xu J, He X. Anti-neuroinflammatory benzofurans and lignans

from Praxelis clematidea. Fitoterapia. 2020;140:104440. https://doi.org/10.1016/j.fitote.2019.104440
10. Silva DF, Albuquerque ACL, Lima EO, Baeder FM, Luna ABH, Ribeiro ED, et al. Antimicrobial and anti-adherent potential of the ethanolic extract of Praxelis clematidea (Griseb.) R.M. King & Robinson on pathogens found in the oral cavity. Res Soc Dev. 2020;9(10). http://dx.doi.org/10.33448/rsd-v9i10.9237
11. Shen N, Wang T, Gan Q, Liu S, Wang L, Jin B. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. Food Chem. 2022;383:132531. https://doi.org/10.1016/j.foodchem.2022.132531
12. Intharuksa A, Kuljarusnont S, Sasaki Y, Tungmunnithum D. Flavonoids and other polyphenols: bioactive molecules from traditional medicine recipes/medicinal plants and their potential for phytopharmaceutical and medical application. Molecules. 2024;29(23):5760. https://doi.org/10.3390/molecules29235760
13. Adamski Z, Blythe LL, Milella L, Bufo SA. Biological activities of alkaloids: from toxicology to pharmacology. Toxins. 2020;12(4):210. https://doi.org/10.3390/toxins12040210
14. Kumar Dash D, Kishore Tyagi C, Kumar Sahu A, Tripathi V. Revisiting the medicinal value of terpenes and terpenoids. In: IntechOpen. 2022. https://doi.org/10.5772/intechopen.102612
15. Kaushik B, Sharma J, Kumar P, Shourie A. Phytochemical properties and pharmacological role of plants: secondary metabolites. Biosci Biotechnol Res Asia. 2021;18(1):23. https://doi.org/10.13005/bbra/2894
16. Umamaheswari D, Muthuraja R, Kumar M, Venkateswarlu BS. Standardization of herbal drugs–an overview. Int J Pharm Sci Rev Res. 2021;68(1):213–9. http://dx.doi.org/10.47583/ijpsrr.2021.v68i01.033
17. European Pharmacopoeia (Ph. Eur.) — 11th Edition. Strasbourg: European Directorate for the Quality of Medicines & HealthCare (EDQM); 2023. Contains general chapters and monographs on herbal drugs and herbal preparations; Ph. Eur. is legally binding in member states. Available from: https://www.edqm.eu/en/european-pharmacopoeia-ph.-eur.-11th-edition
18. European Directorate for the Quality of Medicines & HealthCare (EDQM). Guide for the elaboration of monographs on herbal drugs and herbal drug preparations. Strasbourg: EDQM; 2023. Guidance for authors and laboratories preparing Ph. Eur. herbal monographs. Available from: https://www.edqm.eu/en/d/1766460
19. Zaman W. Morphology, Palynology and Phytochemicals of Medicinal Plants. Horticulturae. 2024; 10(3):202. https://doi.org/10.3390/horticulturae10030202
20. Giacò A, De Giorgi P, Astuti G, Caputo P, Serrano M, Carballal R, Sáez L, Bacchetta G, Peruzzi L. A morphometric analysis of the Santolina chamaecyparissus complex (Asteraceae). Plants. 2022;11(24):3458. https://doi.org/10.3390/plants11243458
21. Zubairova US, Fomin IN, Koloshina KA, Barchuk AI, Erst TV, Chalaya NA, Gerasimova SV, Doroshkov AV. Image-based quantitative analysis of epidermal morphology in wild potato leaves. Plants. 2024;13(21):3084. Available from: https://doi.org/10.3390/plants13213084
22. Deepak Rajput. Quantitative microscopy of crude drugs: leaf constant method. Pharma Academias. 2024 May 10. Available from: https://www.pharmaacademias.com/quantitative-microscopy-of-crude-drugs-leaf-constant-method/
23. Das Sarkar R, Roy GS. Micro-qualification by estimation of leaf constants of the ethnobotanical plant Costus igneus N.E. Br by optical microscopical analysis. World Journal of Biology Pharmacy and Health Sciences. 2023;13(3):120–127. Available from: https://doi.org/10.30574/wjbphs.2023.13.3.0130
24. Singla C, editor. Recent advances in pharmaceutical sciences. Vol. 6. New Delhi: AkiNik Publications; 2021. 125 p. ISBN: 978-93-91538-19-4. https://doi.org/10.22271/ed.book.1318
25. Owolabi T, Osaretin D, Eyinayan B. Bioactive composition and TLC profile data on Pax Herbal Malatreat Tea. Drug Anal Res. 2022;6(1):35–9. https://doi.org/10.22456/2527-2616.125038
26. OECD guidelines for testing of chemicals No. 423 (acute oral toxicity – acute toxic class method). Paris: OECD; 2001. p.2–5 [cited 2022 Dec 16]. Available from: https://www.oecd-ilibrary.org/environment/test-no-423-acute-

oral-toxicityacute-toxic-class-method_9789264071001-en
27. Gothe SR, Pawade UV, Nikam AV, Anjankar MP. OECD guidelines for acute oral toxicity studies: an overview. Int J Res Ayurveda Pharm. 2023;14(4):137–40. http://dx.doi.org/10.7897/2277-4343.1404130
28. Intanon S, Wiengmoon B, Mallory Smith CA. Seed morphology and allelopathy of invasive Praxelis clematidea. Not Bot Horti Agrobo. 2020;48(1). https://doi.org/10.15835/nbha48111831
29. Wang JG, Wu JW, Li WJ. Ecological niche changes and risk regionalization of the invasive plant Praxelis clematidea. Ecol Evol. 2025;15(6):e71546. https://doi.org/10.1002/ece3.71546
30. Barone G, Domina G, Bartolucci F, Galasso G, Peruzzi L. A nomenclatural and taxonomic revision of the Senecio squalidus group (Asteraceae). Plants. 2022;11(19):2597. https://doi.org/10.3390/plants11192597
31. Ekeke C, Ogazie CA. Systematic significance of petiole anatomical characteristics in some members of Asteraceae from some parts of Nigeria. Singapore J Sci Res. 2020;10:387–399. https://scialert.net/abstract/?doi=sjsres.2020.387.399
32. Borsuk AM, Roddy AB, Théroux-Rancourt G, Brodersen CR. Structural organization of the spongy mesophyll. New Phytol. 2022;234(3):946–960. Available from: https://doi.org/10.1111/nph.17971
33. World Health Organization. Draft WHO traditional medicine strategy: 2025–2034. Geneva: World Health Organization; 2025. https://cdn.who.int/media/docs/default-source/tci/draft-traditional-medicine-strategy-2025-2034.pdf
34. Ansari P, Reberio AD, Ansari NJ, Kumar S, Khan JT, Chowdhury S, Abd El-Mordy FM, Hannan JMA, Flatt PR, Abdel-Wahab YHA, Seidel V. Therapeutic potential of medicinal plants and their phytoconstituents in diabetes, cancer, infections, cardiovascular diseases, inflammation and gastrointestinal disorders. Biomedicines. 2025;13(2):454. Available from: https://doi.org/10.3390/biomedicines13020454
35. Bhambhani S, Kondhare KR, Giri AP. Diversity in chemical structures and biological properties of plant alkaloids. Molecules. 2021;26(11):3374. https://doi.org/10.3390/molecules26113374
36. Prajapati AK, Shah G. Alkaloids and their mechanisms of action in cardiovascular diseases. Int J Drug Discov Pharmacol. 2025;4(3):100017. https://doi.org/10.53941/ijddp.2025.100017
37. Zhou Y, Chen Y, He H, Peng M, Zeng M, Sun H. The role of indoles in the microbiota–gut–brain axis and potential therapeutic targets: A focus on human neurological and neuropsychiatric diseases. Neuropharmacology. 2023;239:109690. https://doi.org/10.1016/j.neuropharm.2023.109690
38. Basavarajaiah SM, Pattanashettar R, Yernale NG. A comprehensive review on the biological interest of quinoline and its derivatives. Bioorg Med Chem. 2021;32:115973. https://doi.org/10.1016/j.bmc.2020.115973
39. Letchuman S, Madhuranga HDT, Madhurangi BLNK, Premarathna AD, Saravanan M. Alkaloids unveiled: A comprehensive analysis of novel therapeutic properties, mechanisms, and plant-based innovations. Intell Pharm. 2025;3(4):268–276. https://doi.org/10.1016/j.ipha.2024.09.007
40. Jayawickreme K, Świstak D, Ozimek E, Reszczyńska E, Rysiak A, Makuch-Kocka A, Hanaka A. Pyrrolizidine alkaloids—Pros and cons for pharmaceutical and medical applications. Int J Mol Sci. 2023;24(23):16972. https://doi.org/10.3390/ijms242316972
41. Safe S, Jayaraman A, Chapkin RS, Howard M, Mohankumar K, Shrestha R. Flavonoids: structure–function and mechanisms of action and opportunities for drug development. Toxicol Res. 2021;37(2):147–162. https://doi.org/10.1007/s43188-020-00080-z
42. Hamsalakshmi A, Alex AM, Arehally Marappa M, et al. Therapeutic benefits of flavonoids against neuroinflammation: a systematic review. Inflammopharmacol. 2022;30:111–136. https://doi.org/10.1007/s10787-021-00895-8
43. Gajender, Mazumder A, Sharma A, Azad MAK. A comprehensive review of the pharmacological importance of dietary flavonoids as hepatoprotective agents. Evid Based Complement Altern Med. 2023;2023:4139117. https://doi.org/10.1155/2023/4139117
44. Barreca MM, Alessandro R, Corrado C. Effects of flavonoids on cancer,

cardiovascular and neurodegenerative diseases: Role of NF-κB signaling pathway. Int J Mol Sci. 2023;24(11):9236. https://doi.org/10.3390/ijms24119236
45. Hamsalakshmi A, Alex AM, Arehally Marappa M, et al. Therapeutic benefits of flavonoids against neuroinflammation: a systematic review. Inflammopharmacol. 2022;30:111–136. https://doi.org/10.1007/s10787-021-00895-8
46. Gajender, Mazumder A, Sharma A, Azad MAK. A comprehensive review of the pharmacological importance of dietary flavonoids as hepatoprotective agents. Evid Based Complement Altern Med. 2023;2023:4139117. https://doi.org/10.1155/2023/4139117
47. Boncan DAT, Tsang SSK, Li C, Lee IHT, Lam HM, Chan TF, Hui JHL. Terpenes and terpenoids in plants: interactions with environment and insects. Int J Mol Sci. 2020;21:7382. https://doi.org/10.3390/ijms21197382
48. Naji EF, Abdulfatah HF, Hashim KS. Plant secondary metabolites, their classification and biological roles: a review. J Univ Anbar Pure Sci. 2024;18(1). https://doi.org/10.37652/juaps.2023.144549.1164
49. Bergman ME, Davis B, Phillips MA. Medically useful plant terpenoids: biosynthesis, occurrence, and mechanism of action. Molecules. 2019;24(21):3961. https://doi.org/10.3390/molecules24213961
50. Lanier ER, Andersen TB, Hamberger B. Plant terpene specialized metabolism: complex networks or simple linear pathways? Plant J. 2023;114:1178–1201. https://doi.org/10.1111/tpj.16177
51. Mukherjee PK, Banerjee S, Kar A, Chaudhary SK, Bhardwaj PK, Haldar PK, Sharma N. Synergy and network pharmacology—Establishing the efficacy of herbal medicine. In: Mukherjee PK, ed. Evidence-Based Validation of Herbal Medicine. 2nd ed. Amsterdam: Elsevier; 2022:501–510. https://doi.org/10.1016/B978-0-323-85542-6.00017-2
52. Chaachouay N. Synergy, additive effects, and antagonism of drugs with plant bioactive compounds. Drugs Drug Candidates. 2025;4(1):4. https://doi.org/10.3390/ddc4010004
53. Smith RJ, Brown LM. Evaluating the biological efficacy of fractionated natural extracts. Phytother Res. 2024;38(5):1821–34. https://doi.org/10.1002/ptr.7840