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Pharmacognostic, Antidiabetic, and Antioxidant Properties of *Mitracarpus scaber* Zucc. (Rubiaceae) on Alloxan-Induced Diabetic Rats

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Abstract
Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and oxidative stress. *Mitracarpus scaber* Zucc. (Rubiaceae), a plant used in African ethnomedicine, was evaluated for its pharmacognostic features, antidiabetic, and antioxidant potential.This study aims at assessing the pharmacognostic parameters, antidiabetic activity, and antioxidant effects of dichloromethane and methanolic extracts of *Mitracarpus scaber* in alloxan-induced diabetic rats. Plant materials were subjected to macroscopic, microscopic, and chemomicroscopic analyses. Extracts were prepared via cold maceration, and phytochemical screening (qualitative and quantitative) was performed. Antidiabetic activity was evaluated in albino rats (n=30) divided into six groups (n=5/group), with doses of 100, 200, and 400 mg/kg extract compared to glibenclamide (1 mg/kg) and controls. Blood glucose was monitored weekly for 3 weeks. Antioxidant parameters (SOD, CAT, GPx, MDA) were assayed in serum. Macroscopic analysis revealed an erect annual herb (10-50 cm) with scabrous leaves and quadrangular stems. Microscopic features included amphistomatic leaves with higher abaxial stomatal density (45.38±1.47 mm^{-2}). Chemomicroscopy confirmed tannins, oils, starch, lignin, and calcium oxalates. Extraction yield was 4.45%. Phytochemicals results were dominated by tannins (975.63±2.23 mg/100g), phenols (925.70±2.09 mg/100g), and alkaloids (665.68±2.45 mg/100g). The 200 mg/kg extract showed the highest glucose inhibition (21.931%), approaching glibenclamide (27.929%). Antioxidant assays at 200 mg/kg revealed elevated SOD (36.692±1.627 U/mg) and GPx (23.951±1.029 U/mg), with reduced MDA (10.256±0.294 mmol/mg). Secondary metabolites, particularly tannins and phenols, underpin *Mitracarpus scaber*'s antidiabetic and antioxidant properties, validating its ethnomedicinal use.

Keywords: *Mitracarpus scaber*, antidiabetic, antioxidant, alloxan, Rubiaceae

Introduction
Medicinal plants have been integral to healthcare for millennia, with secondary metabolites like flavonoids, alkaloids, and tannins conferring therapeutic effects such as antidiabetic and antioxidant activities (Gurib-Fakim, 2006; Petrovska, 2012) [5, 11]. Diabetes mellitus, affecting over 463 million globally ((International Diabetes Federation. (2023). IDF Diabetes Atlas (11th ed.)., n.d.) involves hyperglycemia and oxidative stress from reactive oxygen species (ROS), leading to complications like neuropathy and cardiovascular disease (Onugwu et al., 2024). Synthetic drugs like glibenclamide are effective but pose risks of hypoglycemia and resistance (Grover et al., 2002) [4]. *Mitracarpus scaber* Zucc. (Rubiaceae), known as blackweed or tropical soapwort, is a 10-50 cm annual herb native to West Africa and South America. Traditionally used for skin disorders, infections, and diabetes management (e.g., leaf decoctions for hyperglycemia in Nigeria and Ghana; Table 1), it contains iriDOIds, flavonoids, and phenolics with reported antimicrobial and anti-inflammatory effects (Lahlou et al., 2024; Manuel et al., 2020) [8, 9]. Despite ethnopharmacological promise, pharmacognostic standardization and preclinical antidiabetic/antioxidant data are limited (So et al., 2022) [12]. This study aimed to: (1) characterize *Mitracarpus scaber* via macroscopic, microscopic, and chemomicroscopic analyses; (2) quantify phytochemicals; and (3) evaluate antidiabetic and antioxidant activities in alloxan-induced diabetic rats. These findings bridge traditional

knowledge with evidence-based validation, supporting sustainable herbal integration into diabetes therapy.

Table 1: Historical Use of *Mitracarpus scaber* in Diabetes Management

Region	Traditional Preparation	Reported Effect	Reference
Nigeria	Leaf decoction (oral)	Lowers blood glucose	Abere *et al.* (2012)
Ghana	Crushed leaves (topical/oral)	Improves glycemic control	Agyare *et al.* (2015)
West Africa	Infusion with honey	Reduces oxidative stress symptoms	Ogunmoye *et al.* (2018)

Methods

Fresh *Mitracarpus scaber* plants were collected in June 2025 from Enugu State, Nigeria (rainy season optimal growth). Voucher specimens (ESUT/Pharmacog/2025/024) were authenticated at the Herbarium, Department of Pharmacognosy, Enugu State University of Science and Technology. Leaves and stems were air-dried at 25-30 °C, pulverized (mesh size 40), and stored. Macroscopic assessment of shape, size, color, texture, odor, and taste according standard procedures (WHO, 2017). Microscopic Analysis of fresh leaf sections were done according to standard procedures (Sofowora, 1993) [13]. Chemomicroscopy of the powdered samples were done (Evert et al., 2006) [3]. Pulverized material (944 g) was cold macerated with methanol and dichloromethane (1:1) filtered, and concentrated using rotary evaporator. Yield was calculated as % w/w. Qualitative phytochemical tests were done according standard procedures (Harborne, J.B., 1973). Quantitative phytochemical tests were done using spectrophotometric assays for tannins (Evans, 2009), Male albino rats (150-250 g, n=30) acclimatized (22±3 °C, 12-h light/dark, ad libitum feed/water) per CPCSEA guidelies and Ethical approval of ESUT/IACUC/2025/002 gotten. Diabetes was induced by alloxan monohydrate (130 mg/kg i.p., Sigma-Aldrich) after 12-h fast; confirmed if fasting blood glucose >200 mg/dL. The animals were randomly assigned to six groups (n=5): Normal control (saline), diabetic control (saline), standard (glibenclamide 1 mg/kg p.o. in 12% Tween 80), and extracts (100, 200, 400 mg/kg p.o.) daily for 3 weeks. Blood glucose monitored at baseline, post-induction, and weekly. Antioxidant Assays was analysed using serum analyzed for Sodium Oxidase Dehydrogenas (SOD, Catalase enzyme (CAT), Malondehyde (MDA) and Gluthathione peroxidase (GPx) via spectrophotometry (Evans, 2009; Harborne, J.B., 1973). The data were analysed using one way ANOVA.

Results

Macroscopic result of *Mitracarpus scaber* revealed an erect annual herb (10-50 cm) with quadrangular stems (1-3 mm diameter, green-brown), opposite elliptical-lanceolate leaves (5-25 mm, scabrous, serrated, piquant odor), white tetramerous flowers (2-4.2 mm, axillary cymes), schizocarp fruits (1-2 mm, hairy), and fibrous taproots (5-15 cm, whitish-brown). Microscopy results showed amphistomatic leaves with undulated epidermal cells, paracytic stomata (adaxial density: 13.24±1.47 mm^{-2}; abaxial: 45.38±1.47 mm^{-2}), and unicellular trichomes. Transverse sections revealed single-layered epidermis and concentric vascular bundles. Chemomicroscopy (powder) confirmed unicellular trichomes, lignified vessels, prism-shaped calcium oxalates, pitted/unwinding vessels, and fiber strands (Figures 6-12; Table 3).

Table 2: Summary of Microscopic Study of Fresh Leaf

Parameter	Adaxial Surface	Abaxial Surface
Stomatal Density (mm^{-2})	13.24±1.47	45.38±1.47
Stomatal Index (%)	15.2±0.8	18.5±1.2
Trichome Type	Unicellular	Unicellular

Table 3: Result of Powder Chemomicroscopy of the Leaf

Compound/Feature	Test/Reagent	Observation
Tannins	$FeCl_3$	Greenish precipitate
Oils/Lipids	Sudan III	Red staining
Starch	Iodine	Blue-black
Lignin	Phloroglucinol	Red-violet
Calcium Oxalate	Polarized light	Prisms

The extraction yield was 4.45% (42 g from 944 g). Qualitative screening showed high (+++) tannins, phenols, alkaloids; moderate (++) saponins, glycosides; low (+) terpenoids, steroids, cyanide (Table 5). Quantitative: Tannins (975.63±2.23 mg/100g), phenols (925.70±2.09 mg/100g), alkaloids (665.68±2.45 mg/100g), glycosides (431.20±1.89 mg/100g), terpenoids (187.68±1.34 mg/100g), saponins (0.682±0.045 mg/g) (Table 6).

Table 5: Qualitative Phytochemical Screening

Phytochemical	Presence (+++ High, ++ Moderate, + Low)
Tannins	+++
Phenols	+++
Alkaloids	+++
Flavonoids	++

Table 6: Quantitative Phytochemical Analysis (mg/100g, mean ± SD)

Compound	Concentration
Tannins	975.63±2.23
Phenols	925.70±2.09
Alkaloids	665.68±2.45

Post-induction glucose: 239-266 mg/dL (diabetic groups). After 3 weeks, 200 mg/kg extract reduced glucose to 208.25±15.777 mg/dL (21.931% inhibition), outperforming 100 mg/kg (12.463%) and 400 mg/kg (15.455%), nearing glibenclamide (27.929%). Toxic group worsened (-14.286%) (Table 7; Figure 13, raw data in Table 10).

Table 7: Mean Blood Glucose (mg/dL, mean ± SD) and % Inhibition

Group	Baseline	Post-Induction	Week 3	% Inhibition
Control	108.25±3.304	104.25±6.752	88±2.160	15.588
Standard	112.50±4.030	239±12.437	172.25±34.053	27.929
Toxic	110.75±5.123	245±8.367	280±3.000	-14.286
100 mg/kg	109.00±3.606	250.75±9.465	219.5±29.354	12.463
200 mg/kg	111.25±4.787	266.75±15.692	208.25±15.777	21.931
400 mg/kg	108.50±2.645	247.5±6.028	209.25±14.546	15.455

Antioxidant Activity

200 mg/kg extract elevated SOD (36.692±1.627 U/mg) and GPx (23.951±1.029 U/mg), reduced MDA (10.256±0.294 mmol/mg) vs. toxic group (Table 8). Standard group showed highest SOD/CAT.

Table 8: Antioxidant Parameters (mean ± SD)

Group	SOD (U/mg)	CAT (U/mg)	MDA (mmol/mg)	GPx (U/mg)
Control	37.219±2.731	12.384±1.086	8.498±0.550	24.677±0.950
Standard	38.266±1.187	11.760±0.978	9.696±0.651	24.481±0.309
Toxic	34.204±2.486	10.875±0.528	11.390±0.865	23.600±0.941
100 mg/kg	36.062±0.999	11.398±1.356	10.467±1.233	23.511±0.623
200 mg/kg	36.692±1.627	11.145±1.091	10.256±0.294	23.951±1.029
400 mg/kg	35.910±3.201	11.749±0.565	9.935±1.324	23.782±0.660

Discussion

Pharmacognostic profiling authenticated *Mitracarpus scaber*, with scabrous leaves and trichomes aligning with Rubiaceae traits. High stomatal density on abaxial surfaces of *M scaber* enhances CO_2 uptake, supporting metabolite synthesis Chemomicroscopic detection of tannins and oxalates correlates with bioactivity of the plant. The 4.45% yield indicates efficient extraction, though scalability needs optimization. Phytochemical dominance of tannins/phenols (975-926 mg/100g) explains efficacy; these inhibit α-glucosidase/amylase, enhance insulin sensitivity, and scavenge ROS. Alkaloids (666 mg/100g) may stimulate insulin secretion (Ekalu, 2021). Antidiabetic results show dose-dependent efficacy peaking at 200 mg/kg (21.931% inhibition), comparable to prior studies on the plant family (Sunday et al., 2024). Non-linear response (400 mg/kg < 200 mg/kg) suggests saturation or toxicity at higher doses. Antioxidant results indicate ROS mitigation via upregulated SOD/GPx and reduced MDA, protecting against diabetic complications. Standard glibenclamide outperformed extracts, but *Mitracarpus scaber*'s multi-target profile offers adjunctive potential in diabetes management.

Conclusion

Mitracarpus scaber exhibits robust pharmacognostic, antidiabetic, and antioxidant properties driven by polyphenolics which validates its antidiabetic ethnomedicinal use. The 200 mg/kg dose optimizes glucose reduction and oxidative stress alleviation, positioning it as a good herbal drug

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