Nigerian Research Journal of Chemical Sciences (ISSN: 2682-6054)
Volume 15, Issue 2, 2026

Phytochemical Analysis, Antimicrobial Susceptibility and Antioxidant Activity of Leaf Extract of *Leptadenia hastata* (Pers.) Decne, Grown in Biu Local Government Area, Borno State, Nigeria

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Accepted: *November 16, 2026* **Published Online***: November 25, 2026*

ABSTRACT

Phytochemical analysis, antimicrobial susceptibility tests and antioxidant activity of methanolic and aqueous leaf extracts of *Leptadenia hastata* were carried out using standard procedures. The phytochemical tests revealed the presence of alkaloids, flavonoids, tannins, saponins, cardiac glycosides, terpenoids and phenols in the methanolic extract. The aqueous extract showed the presence of tannins, saponins, cardiac glycosides and phenol. The two extracts exhibited antifungal activity against *Candida albicans*, at 100 mg/ml. Methanolic and the aqueous extracts exhibited a growth inhibition zone of 16 mm and 10 mm respectively. The antibacterial sensitivity test of the two extracts against four tested bacteria showed inhibition zones of 19, 21, 15 and 16 mm against *E. coli, S. aureus, S. epidermis* and *S. typhi* respectively at 100 mg/ml for methanolic extract. At concentration of 40 mg/ml, the extract was not sensitive against all tested microbes. The aqueous extract gave zones of 20 and 22 mm against *E. coli, and S. aureus* respectively at 100 mg/ml but not sensitive against *S. typhi* and *S. epidermis*. The antioxidant assay revealed that aqueous extract has slightly higher antioxidant activity (IC_{50} = 187.21 µg/mL) than methanolic extract (IC_{50} = 197.34 µg/ml), whereas the standard ascorbic acid has IC_{50} = 40.9462 µg/ml.

Keywords: Antimicrobial susceptibility, antioxidant, 2,2-diphenyl-1-Picrylhydrazyl, *Leptadenia hastata*, phytochemicals.

INTRODUCTION

Medicinal plants serve humanity as primary source of natural therapeutics across the globe. According World Health Organization (WHO), larger percentage of rural populace relies on traditional medicines for their ill-health needs in all developing countries [1]. Several factors such as accessibility, affordability, knowledge of the medicinal herbs and foremost the societal values

devoted to cultural practices explained the association of man and plant-based remedies. Recently, traditional medicines were the most patronized healthcare for treating various ailments in Sub-Saharan Africa**.** This could be due to promising impacts demonstrated by the plants [2,3].

The presence of natural biological agents in medicinal plants was cited as responsible for antimicrobial activity such as tannins [4], alkaloids [5] and several molecules of natural origin demonstrated antifungal [6] as well as antidiabetic properties [7]. Antimicrobial resistances expressed by the pathogenic organisms to current drugs use in the treatment of infectious diseases were reported by Prestinaci et al. [8] and Vaou et al. [9]. This has necessitated for continuous research for potent antimicrobial agents particularly from natural origin like medicinal plants.

The current investigation of antioxidant properties of medicinal plants facilitates in the identification and development of natural antioxidants that could provide health advantages both for young and old age [10]. Notable health benefits of antioxidants include the ability to delay or prevent the effects of free radicals in the human body. They can also maintain and intensify the functioning of immune system mechanism especially during old age or in some certain disease conditions [11]. Free radical scavenging activity of plants could mean the ability of extracts of plants to deactivate harmful free radicals release in the body, which might produce oxidative damage prompting other various diseases such as cancer. Several foods like fruits, vegetables, nuts and seeds and many plants were reported to contained antioxidants [11,12].

Global attention has been focused on plants antioxidants since synthetic antioxidants have proven serious adverse side effects especially their carcinogenic potentials and complications in some chronic diseases as such they are now restricted in their uses in foods [11]. Therefore, the search for the safe and effective natural antioxidants is on increase worldwide.

Leptadenia hastata is a perennial climbing shrub that belong to the family *Apocynaceae* and subfamily *Asclepiadoideae* [13]. It is widely distributed across arid and semi-arid regions of tropical Africa particularly, Nigeria, Niger, Sudan, Mali, Chad, Burkina Faso, Senegal and Burkina Faso [14,15]. The plant typically grows in sandy soils, often along riverbanks, roadsides and cultivated fields. It produces flexible stems that become woody at the base, with characteristic hastate (spear-shaped) leaves that give the plant its specific epithet “hastata.” The plant yields a milky latex when cut and produces small, greenish-yellow flowers followed by paired follicles

containing numerous seeds with silky pappus for wind dispersal [14]**.** Its uses as medicine and food especially in rural communities with shortage of food highlighted the significant of the plant. *Leptadenia hastata* has been widely used in traditional medicine to treat various ailments such as infections, wounds, gastrointestinal disorders and diabetes in several parts of West Africa [16,17]. It was reported to have use in the management of high blood pressure, sugar level and relief pain for pregnant women during labour [7]. The leaves are also used as medicine and food in some rural communities including Biu Local Government Area, however, the scientific validation of these traditional claims remains inadequate.

Therefore, scientific evaluation of phytochemical composition, antimicrobial susceptibility and antioxidant properties of the leaf extracts of *Leptadenia hastata* was carried out. The research findings could not only validate its applications in traditional medicine but also will contribute to the effort being made in the search for new alternative therapeutic agents from natural plant-based products.

MATERIALS AND METHODS

Sample collection and identification of the plant

Fresh leaves of *Leptadenia hastata* were collected in clean and dried polythene bag from Filin-Jirgi along Army Barracks Road in Biu Town in May 2026. Leaves were carefully handpicked in the morning hours to ensure freshness and sample uniformity and transported to the Department of Chemistry, Nigerian Army University Biu. The plant sample was identified and authenticated by a plant taxonomist and a voucher specimen no. 26/00412 was deposited at the Herbarium Unit of Biology Department, Faculty of Natural and Applied Sciences (FNAS), Nigerian Army University, Biu, for future reference.

Sample preparation

Freshly collected leaves were washed under running tap water followed by rinsing with distilled water to remove dust and surface contaminants. The washed leaves were air-dried under shade at room temperature. The dried plant material was pulverized into a fine homogeneous powder using blender, mortar and pestle, sieved through a 2 mm mesh and stored in an air-tight container at room temperature until analysis [18].

Extraction

Cold maceration was used as the extraction method. Five hundred grams (500 g) of the powdered leaf material was macerated in 500 mL of 80% methanol (v/v) for 72 hours at room temperature with intermittent shaking. The mixture was then filtered through Whatman No. 1 filter paper, and the filtrate concentrated using a rotary evaporator at 40 °C to yield the crude methanolic extract. An aqueous extract was similarly prepared by maceration in distilled water for 72 hours, filtered, and evaporated to dryness. Both extracts were stored at 4 °C [19].

Qualitative phytochemical analysis

Qualitative test was carried out on all extracts for the following eight (8) important secondary metabolites using standard tests as described by standard phytochemical procedures consistent with those reported by Zhang, et al., [10], Chukwuma et al., [19] and Adetunji et al., [23].

(a) Dragendorff's Test: About 2 mL extract was treated with Dragendorff's reagent. An orange-red precipitate indicates the presence of alkaloids.

(b) Shinoda (Lead Acetate) Test: About 2 mL extract was treated with magnesium ribbon fragments and concentrated HCl. A pink, red, or orange colouration indicates flavonoids. Additionally, 2 mL of extract was treated with 2 mL of 1% lead acetate; a yellow precipitate confirms flavonoids.

(c) Ferric Chloride Test: A few drops of 1% ferric chloride were added to 2 mL of extract. A blue-black or greenish-black colouration was confirmed the presence of tannins.

(d) Froth Test: About 2 mL of extract was vigorously shaken with 5 mL of distilled water. Persistence of stable froth for $\geq$ 10 minutes indicated the presence of saponins.

(e) Liebermann-Burchard Test: About 2 g of extract dissolved in acetic anhydride was treated with concentrated H_2SO_4 acid. A colour change to blue-green confirmed the presence of steroids.

(f) Keller-Killiani Test: A mixture of 2 mL of extract in glacial acetic acid and a drop of ferric chloride was carefully underlaid with concentrated H_2SO_4 acid. A reddish-brown ring at the interface indicated the presence of cardiac glycosides.

(g) Terpenoids and Phenols: About 5 mL of extract was mixed with chloroform and concentrated H_2SO_4 acid, a reddish-brown interface confirmed the presence of terpenoids. The presence of Phenols was confirmed by a green black colouration with ferric chloride.

Antimicrobial susceptibility test

Test organisms are *Staphylococcus aureus, Escherichia coli, staphylococcus epidermis* and *Salmonella typhi*. The organisms were obtained as stock culture from the General Hospital Microbiology Laboratory Biu, Borno State.

Agar disc diffusion method

Standard agar disc diffusion method was used to evaluate the antimicrobial activity of the leaf extracts [16**,** 20]. Bacterial suspensions were standardized to 0.5 McFarland turbidity standard of approximately 1.5 × 10 CFU/mL. Sterile Mueller-Hinton agar plates were surface-inoculated with each suspension. Wells (6 mm diameter) were bored with a sterile Cork borer, and 0.1 ml of extracts at different concentrations (40, 60, 80, and 100 mg/mL prepared in 10% DMSO) were placed in each well. The Plates were incubated at 37 °C for 24 hours, and the zones of inhibition were measured in millimetres (mm). Standard antibiotic, Ciprofloxacin (10 µg/disc) was used as the positive control, while Ketoconazole (2.5 mg/ml) served as standard antifungal drug and dimethyl sulphoxide (DMSO) served as the negative control.

2,2-Diphenyl-1-PicrylHydrazyl (DPPH) radical scavenging assay

The DPPH assay was carried out as described by Souratie et al., [12]. A 0.1 mM DPPH solution in methanol was prepared. Serial dilution of concentrations of each extract was prepared as 12.5, 25, 50, 100, and 200 µg/mL and mixed with equal volumes of DPPH solution, incubated in the dark for 30 minutes at room temperature and measured the absorbance at 517 nm. Ascorbic acid was prepared to serve as the standard antioxidant and compare with the crude leaf extracts. The Percentage inhibition was then calculated as:

$$Percentage\ inhibition = \frac{Absorbance\ Control\ -\ Absorbance\ Sample}{Absorbance\ Control} \times 100$$

Statistical analysis

The DPPH assay was carried out in triplicate, with statistical analysis performed using a one-way ANOVA. Data are presented as the mean ± standard deviation (SD) and IC_{50} values were determined via linear regression of the dose-response curves

RESULTS AND DISCUSSION

Table 1 presents the extraction yields and physical characteristics of the methanolic and aqueous leaf extracts of *Leptadenia hastata*. The results show that distilled water produced a higher extraction yield (13.0%) than methanol (6.2%). Specifically, 10.4 g of crude extract was obtained from 80 g of powdered leaf sample using distilled water, whereas 6.2 g of extract was obtained from 100 g of leaf sample using methanol. Both extracts were carried out for 72 hours. The methanolic extract appeared dark green and viscous, while the aqueous extract was light brown and solid. The higher yield obtained with distilled water suggests that a greater quantity of water-soluble constituents was extracted under the experimental conditions.

Table 1. Extraction yield of leaf sample of *Leptadania hastata*

SN	Solvent used	Polarity	weigh(g) of sample	Volume of solvent (ml)	Weight of extract (g)	Duration (Hour)	% Yield	Color	Texture
1	Methanol	Polar	100	400	6.2	72	6.2	Dark green	Viscous
2	Distilled water	Highly Polar	80	500	10.4	72	13	Light brown	Solid

Table 2 shows the results of the qualitative phytochemical screening of the methanolic and aqueous leaf extracts of *L. hastata*. The results revealed the presence of tannins, saponins, cardiac glycosides, and phenols in both extracts. Alkaloids, flavonoids, and terpenoids were detected in the methanolic extract but were absent in the aqueous extract. Steroids were not detected in either extract. These findings indicate that the leaf extracts contain several classes of secondary metabolites, with the methanolic extract showing a broader range of detected phytochemicals than the aqueous extract. The differences in phytochemical composition may be associated with the solvent polarity and the solubility of individual plant constituents. Previous research studies have also reported phytochemicals such as flavonoids, alkaloids, terpenoids, tannins, and nutritional values of the leaves [24, 25].

Table 3 presents the antifungal activity of the methanolic and aqueous leaf extracts of *Leptadenia hastata* against *Candida albicans*, with ketoconazole serving as the standard antifungal drug. The results indicate that antifungal activity varied with the type of extract and concentration

used. The methanolic extract exhibited the highest activity, producing a zone of inhibition of 16 mm at 100 mg/mL, while the aqueous extract produced a zone of 10 mm at the same concentration. At lower concentrations (80, 60, and 40 mg/mL), both extracts recorded inhibition zones of 6 mm. In comparison, ketoconazole produced a zone of inhibition of 26 mm at 2.5 mg/mL. These findings suggest that the methanolic extract demonstrated greater antifungal activity than the aqueous extract at the highest concentration tested. However, both extracts showed lower activity than the standard drug.

Table 2: Qualitative phytochemical analysis of leaf of *Leptadania hastata*

S/N	Phytochemicals	Type of Analysis	Methanol	Distilled water
1.	Alkaloids	Mayer's test	+	-
2.	Flavonoids	Shinoda (Lead Acetate) Test	+	-
3.	Tannins	Ferric Chloride Test	+	+
4.	Saponins	Froth Test	+	+
5.	Steroids	Liebermann-Burchard Test	-	-
6.	Cardiac glycosides	Keller-Killiani Test	+	+
7.	Terpenoids	Chloroform & ferric chloride test	+	-
8.	Phenols	Chloroform & ferric chloride test	+	+

Table 3. Antifungal activity of leaf extract of *L. hastata* against *Candida albicans*

Extract/Drug	Concentration (mg/ml)	Zone of inhibition (mm)
Methanolic		
	100	16
	80	6
	60	6
	40	6
Aqueous		
	100	10
	80	6
	60	6
	40	6
*Control	2.5	26

***Standard drug = Ketoconazole**

Table 4 presents the antibacterial activities of the methanolic and aqueous leaf extracts against four bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Salmonella typhi*. The results reveal variations in antibacterial activity depending on the extract type, concentration, and bacterial strain. The methanolic extract exhibited its highest activity against *S. aureus*, with a zone of inhibition of 21 mm at 100 mg/mL, followed by *E. coli* (19 mm), *S. typhi* (16 mm), and *S. epidermidis* (15 mm). At lower concentrations, the inhibitory activity generally decreased, although some variation was observed among the bacterial strains. The aqueous extract also showed its highest activity against *S. aureus* (22 mm) and *E. coli* (20 mm) at 100 mg/mL. Notably, the aqueous extract produced an inhibition zone of 21 mm against *E. coli* at 80 mg/mL. However, no measurable inhibition beyond the recorded 6 mm was observed against *S. epidermidis* and *S. typhi* at the tested concentrations. The standard drug, ciprofloxacin, produced inhibition zones of 22, 36, 22, and 23 mm against *E. coli*, *S. aureus*, *S. epidermidis*, and *S. typhi*, respectively.

Table 4: Antibacterial activity of leaf extract of *L. hastata*

Extract/Conc.	Zone	Of	Inhibition	(mm)
Methanolic	*E. coli*	*S. aureus*	*S. epidermis*	*S. typhi*
100	19	21	15	16
80	16	17	15	6
60	6	15	14	6
40	6	6	6	6
Aqueous				
100	20	22	6	6
80	21	19	6	6
60	17	18	6	6
40	15	6	6	6
*Control	22	36	22	23

***Standard drug = Ciprofloxacin**

Table 5 and Figure 1 present the DPPH radical scavenging activities of the methanolic and aqueous leaf extracts of *L. hastata*, using ascorbic acid as the standard antioxidant. The results show that the methanolic extract exhibited a progressive increase in percentage inhibition with increasing concentration, rising from 13.6% at 12.5 µg to 49.7% at 200 µg. This indicates an increase in radical scavenging activity as the concentration of the methanolic extract increased. The aqueous

extract also demonstrated radical scavenging activity, although the percentage inhibition fluctuated across the tested concentrations. The reported values increased from 35.2% at 12.5 µg to 44.3% at 25 µg, decreased to 25.9% at 50 µg, and subsequently increased to 45.2% at 100 µg. The mean value of 0.713 at 200 µg corresponds to 71.3% inhibition, rather than the reported 17.3%. The calculated IC_{50} values were 197.34 for the methanolic extract, 187.21 for the aqueous extract, and 40.95 for ascorbic acid. The lower IC_{50} value of the aqueous extract suggests slightly stronger radical scavenging activity than the methanolic extract. However, ascorbic acid exhibited substantially greater antioxidant activity than both plant extracts, as indicated by its lower IC_{50} value.

Table 5. DPPH radical scavenging activity of the leaf extract of *Leptadenia hastata*

Extracts	Concentration (µg/mL)	Mean ±SD	% Inhibition	IC_{50}
Methanolic	12.5	0.136 ± 0.001	13.6	197.3407
	25	0.176 ± 0.001	17.6	
	50	0.222 ± 0.001	22.2	
	100	0.331 ± 0.001	33.1	
	200	0.497 ± 0.001	49.7	
Aqueous	12.5	0.352 ± 0.001	35.2	187.211
	25	0.443 ± 0.001	44.3	
	50	0.259 ± 0.001	25.9	
	100	0.452 ± 0.001	45.2	
	200	0.713 ± 0.001	17.3	
*Ascorbic acid	12.5	0.066 ± 0.001	6.6	40.9462
	25	0.065 ± 0.001	6.5	
	50	0.065 ± 0.001	6.5	
	100	0.070 ± 0.001	7.0	
	200	0.085 ± 0.001	8.5	

*Standard antioxidant

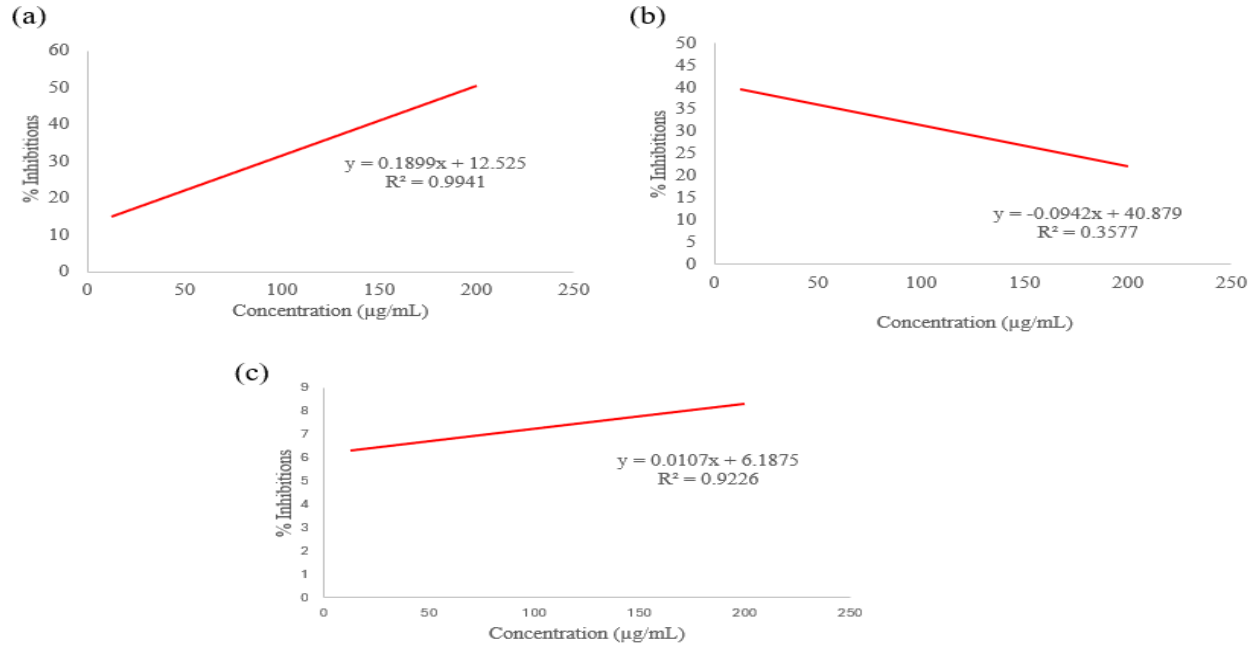

Fig.1. DPPH Radical Scavenging Activity of (a) Methanolic Extract, (b) Aqueous Extract and (c) Standard antioxidant (Ascorbic acid)

CONCLUSION

Preliminary *in vitro* investigation of medicinal plants for biological activities still remains an important step towards attaining the goal of discovering and developing safe natural therapeutics for management of multifaceted diseases ravaging humans and animals alike. This research paper reports the results of preliminary evaluation on the phytochemicals constituents and bioactivity of the leaves of *L. hastata* and it revealed fundamental information supporting the claims of local uses of the plants in herbal remedies for management of numerous human ailments because the leaves contained bioactive metabolites and exhibited bacterial sensitivity as well as free radical scavenging potentials. Further studies on the quantitative analysis, isolation and characterization of bioactive agents to identify the specific phytochemical compounds present in the leaves is recommended.

Acknowledgement: The authors wish to thank and acknowledged the Department of Biology, Nigerian Army University Biu. for identification of the plant and the assistance rendered by Technologists of Biology Laboratory in conducting the antimicrobial sensitivity studies. We also

extend our profound appreciation to the Chief Medical Officer of the General Hospital, Biu, for providing us with the needed microorganisms in this study.

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