Science Forum (Journal of Pure and Applied Sciences)

Journal Homepage: https://atbuscienceforum.com.ng/index.php/jpas

Assessment of Phytochemicals and Antifungal Potency of *Leptadenia hastata* Ethanolic Extract Against Some Selected Fungal Pathogens

Auwal Magaji[1], Zuhairu Ibrahim[1], Zinat Mahmud[1], Adamu Yakubu[1], Nathaniel Luka Kwarau[1], Zakariyya Abubakar Muhammad[2], Ibrahim Mustapha[3], Badamasi Abdullahi[4], Wasila Muktar Adam[1], Abdullahi Aliyu Jibo[1], Umar Bilyaminu[1], Zailani Hafiz Liman[6] Haladu Ali Gagman[5] and Ibrahim Musa Moi[1]

[1]Department of Microbiology, Sa'adu Zungur University, Bauchi State Nigeria
[2]Department of Microbiology, Federal University of Dutsin Ma Katsina State, Nigeria
[3]Department of Medical Laboratory Science College of Health Sciences and Technology Nguru Yobe State, Nigeria
[4]Department of Human Nutrition and Dietetic, Bill and Melinda Gates College of Health Sciences and Technology Ningi, Bauchi State Nigeria
[5]Department of Bilogical Sciences, Sa'adu Zungur University, Bauchi State Nigeria
[6]Department of Science Laboratory Technology, Abubakar Tatari Ali Polytechnic, Bauchi, Bauchi State Nigeria

Abstract

Leptadenia hastata *is a perennial plant widely recognized for its diverse ethnomedicinal applications, including notable antifungal properties. This study aimed to evaluate the phytochemical composition and antifungal efficacy of L. hastata leaf extract prepared using ethanol as the solvent. Air-dried leaves of the plant were subjected to ethanolic extraction and subsequently screened for key phytochemical constituents such as tannins, saponins, alkaloids, flavonoids, phenols, and steroids. The antifungal activity of the extract was assessed using both disc diffusion and broth dilution techniques. These methods were employed to determine the zones of inhibition, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) against selected fungal pathogens. Fluconazole was used as a standard antifungal drug for comparison. Phytochemical screening confirmed the presence of multiple bioactive compounds, suggesting a potential synergistic role in the antifungal mechanism. The ethanolic extract exhibited dose-dependent antifungal activity, with zones of inhibition ranging from 13.0 ± 0.8 mm to 50.3 ± 1.3 mm at concentrations between 25 and 100 mg/mL. The MIC for all tested fungal isolates was determined to be 25 mg/mL. MFC values varied, with Aspergillus fumigatus showing the lowest MFC at 25 mg/mL, while Candida albicans, Cryptococcus neoformans, and Aspergillus flavus required 50 mg/mL. These findings demonstrate that the ethanolic extract of L. hastata possesses significant antifungal activity against a range of pathogenic fungi. The results support its potential development as a natural antifungal agent and warrant further investigation into its active compounds, mechanisms of action, and therapeutic applications.*

Keywords
Phytochemical, Analysis, Antifungal, Efficacy, *Leptadenia hasata*

Corresponding Author: Auwal Magaji, [1]Department of Microbiology, Sa'adu Zungur University Gadau, Bauchi State Nigeria, Email: auwalmagajiaza@gmail.com

INTRODUCTION

Medicinal plants have long served as a vital source of inspiration for novel therapeutic drugs, significantly contributing to human health and well-being. They have been utilized for centuries as curative agents against various infections, with their efficacy well-documented in traditional medicine systems (Dubey *et al.*, 2004; Oche *et al.*, 2022). Among these, certain plants, particularly those common in Nigeria and West Africa, hold significant value due to their traditional medicinal applications (Haruna *et al.*, 2017; Galani *et al.*, 2020). These plants, often used in treating health issues, represent an integral part of the global cultural heritage (Sarkiyayi *et al.*, 2015; Chukwuma *et al.*, 2022). Although research has validated the medicinal properties of numerous tropical plants, a substantial number remain underexplored, lacking empirical evidence to substantiate their therapeutic claims (Obidah *et al.*, 2010; Abdallah and Ali, 2022).

The growing challenges associated with synthetic drugs, including high costs, toxic side effects, and limited efficacy in disease treatment, especially in developing countries, have heightened interest in alternative remedies (Makinde *et al.*, 2015). Concurrently, the rise of multi-drug-resistant microbial strains and reduced susceptibility to antibiotics—primarily due to the indiscriminate use of broad-spectrum antibiotics and immunosuppressive agents—has exacerbated global health threats. Consequently, there has been a surge in research on plant extracts and biologically active compounds for natural therapies (Rios and Recio, 2005; Chukwuma *et al.*, 2022).

Leptadenia hastata, a perennial plant belonging to the family *Asclepiadaceae*, exemplifies the medicinal potential of African flora. Taxonomically classified as part of the Dicotyledon group, this plant is widely distributed across West Africa and thrives under harsh environmental conditions (Umaru *et al.*, 2017; Oche *et al.*, 2022). It is valued both as a vegetable and a medicinal resource, offering a range of benefits, including anti-snake venom, analgesic, anti-inflammatory, anti-tumour, antihypertensive, and anti-diabetic properties (Dambatta and Aliyu, 2011; Betti *et al.*, 2011; Galani *et al.*, 2020). Additionally, its adaptability to tropical dry lands and sandy soils supports food security during seasonal changes.

The diverse applications of *L. hastata* further underscore its importance. Its extracts are commonly used in traditional medicine for treating hypertension, skin diseases, prostate cancer, rheumatism, and placental retention (Bayala *et al.*, 2011; Chukwuma *et al.*, 2022). Studies also reveal its antifertility, antiandrogenic, and antibacterial effects, alongside its potential in treating wounds and stomach disorders (Aliero and Wara, 2009; Oche *et al.*, 2022). Despite its extensive traditional use, further scientific investigation is warranted to substantiate and expand upon its therapeutic claims. This study aimed to evaluate the phytochemical properties of *L. hastata* ethanolic extract and to assess its antifungal efficacy.

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MATERIALS AND METHODS

Procurement, identification and preparation of Plant sample

The leaves of *L. hastata* were purchased from Tatari Ali market Azare, Bauchi State Nigeria, and authenticated by Dr Umar Aminu Muhammad at the Department of Biological Sciences, Sa'adu Zungur University Gadau, Bauchi State Nigeria. The Voucher number SZ1243A was assigned to the plant to allow for future reference. The plants leaves were air-dried at room temperature and grinded in to powder using motor and pistil.

Fungal Isolates

The fungal isolates were obtained from the stocked isolates of the Microbiology laboratory, Sa'adu Zungur University Gadau.

Extraction of the Plant

The method reported by Magaji *et al.*, (2023) was employed. About 100 g of the powdered leaf sample

was weighed and soaked in 1000 mL of ethanol (99%) for 72 hours at room temperature with occasional shaking. The extract was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure, and the crude extract was stored at 4°C for further analysis.

Phytochemical Screening

The chemical tests were carried out according to the method described by Debiyi & Sofowora, (1978), Trease & Evans, (1989).

Alkaloids (Mayer's Test): about 2 mL of extract was mixed with 2 mL of 1% HCl and heated. Mayer's reagent was then added dropwise. A creamy white precipitate indicates the presence of alkaloids.

Flavonoids (Shinoda Test): about 2 mL of extract was treated with a few drops of concentrated HCl and magnesium ribbon. A pink or reddish coloration confirmed the presence of flavonoids.

Saponins (Frothing Test): about 2 mL of extract was vigorously shaken with 5 mL of distilled water. Persistent frothing indicates the presence of saponins.

Tannins (Ferric Chloride Test): exactly 2 mL of extract was mixed with a few drops of 1% ferric chloride solution. A blue-black or green coloration indicates tannins.

Phenols (Ferric Chloride Test): about 2 mL of extract was mixed with 2 mL of 5% ferric chloride solution. A deep blue or green coloration confirmed the presence of phenols.

Steroids (Liebermann-Burchard Test): about 2 mL of extract was dissolved in chloroform, followed by the addition of acetic anhydride and concentrated H_2SO_4. A greenish color indicates the presence of steroids.

Glycosides (Keller-Kiliani Test): about 2 mL of extract was treated with glacial acetic acid and ferric chloride, followed by sulfuric acid. A brown ring at the interface indicated the presence of glycosides.

Preparation of the Crude Discs

Different concentrations (25, 50 and 100g/ml) of the ethanolic extracts of the plant's leaves were prepared. Five-millimeter diameter discs were made from Whatsman's No1 filter paper and soaked separately in the different concentrations of the extracts and allowed to stay for 24hours for proper absorption. The discs were then air dried. Fluconazole (25 µg) was used as reference antifungal agent (Trease and Evans, 1989, Magaji *et al.*, 2023).

Determination of the Antifungal Activity of the Crude Discs

Sabouraud Dextrose Agar (SDA) was prepared in accordance with specified guidelines, then autoclaved at 121°C for 15 minutes and subsequently cooled. The sterile petri dishes were filled with the media and allowed to solidify. Following this, the media underwent drying in an oven at 45°C before use. A standardized spore suspension, containing $1x10^6$ spores/mL, was evenly spread onto the surface of the SDA plates and allowed to dry. The crude extract discs of different concentrations were placed onto each of the appropriately labeled plates. The negative control consisted of the distilled water, while the positive control was represented by the standard disk impregnated with fluconazole (0.025mg). The plates were incubated at ambient temperature for 1-14 days. Growth was observed, and all tests were conducted in triplicate. The resulting zones of inhibition around the disks were measured and recorded as mean diameters of inhibition zones (CLSI, 2009; Magaji *et al*, 2023).

Determination of Minimum Inhibitory Concentration and Minimum Fungicidal Concentration

The broth dilution method, as cited by Magaji *et al.*, (2023), was employed to determine the minimum inhibitory concentration of the *L hastata* extracts. Sabouraud Dextrose Broth was prepared following the manufacturer's instructions, with 1 mL dispensed into individual test tubes. These tubes were then sterilized at 121°C for 15 minutes and allowed to cool. Different concentrations of the extracts, ranging from the stock concentration (200mg/mL) to concentrations of 100 mg/mL, 50 mg/mL and 25 mg/mL were prepared. 0,1 mL of the standardized inoculum ($1x10^6$ spores/mL) was introduced into each concentration in the broth. Incubation took place at 30°C for 1-7 days, and visible growth was monitored. The minimum inhibitory concentration was identified as the lowest concentration without observable growth in the test tube.

To determine the Minimum Fungicidal Concentration (MFC) of the extracts, fresh Sabouraud Dextrose Agar media were prepared, sterilized at 121°C for 15 minutes, and poured into sterile petri dishes to solidify. The contents of the tube containing the minimum inhibitory concentrations was subcultured onto the agar media. All plates were then incubated at 30°C for 1-7 days and observed for growth. The Minimum Fungicidal Concentration was identified as the lowest concentration of the extracts without any growth on the agar plate (CLSI., 2009; Magaji *et al.*, 2023).

Statistical Analysis

The experiments in the study were carried out in triplicates, and the results were presented as mean ± standard deviation (SD). The data were analyzed using SPSS version 23.0. To compare variations in antifungal activity among the agents one-way ANOVA, and statistical significance level of $p < 0.05$ were used.

RESULTS

Phytochemical analysis

The phytochemical analysis revealed the presence of Tannins, Saponins, Alkaloids, Flavonoids, Phenols and Steroids in the plants extract, and the result is as presented in Table 1.

Table 1: Phytochemical compositions of the ethanolic extract of *L hastata*

S/N	Secondary metabolites	Status
1.	Tannins	+
2.	Saponins	+
3.	Alkaloids	+
4.	Flavonoid	+
5.	Glycosides	-
6.	Phenol	+
7.	Steroids	+

Key: "+" = present "- "= absent

Antifungal evaluation

The antifungal studies revealed that, at 25mg/ml of the ethanolic extracts zones of 13.0±0.8mm, 19.0±0.0mm, 15.3±0.5mm and 15.3±0.5mm were produced against *C albicans, A fumigatus, C neofomans* and *A flavus* respectively. The zones of inhibition recorded at 50mg/ml were 26.7±2.1mm, 29.0±2.2mm, 30.7±1.6mm, and 25.2±0.5mm against *C albicans, A fumigatus, C neofomans* and *A flavus* respectively. Similarly, the study showed that, at 100mg/ml zones of 48.0 ± 2.2mm, 42.7±1.3mm, 50.3±1.3mm, and 46.3±2.1mm against *C albicans, A*

fumigatus, *C neofomans* and *A flavus* respectively. The control (fluconazole) produced zones of 23.3± 0.5mm, 48.0± 0.0mm, 48.0±0.0mm and 35.6±1.3mm against *C albicans*, *A fumigatus*, *C neofomans* and *A flavus* respectively, and the result is as presented in Table 2.

Table 2: Zones of inhibition (mm) produced by different concentrations of the *L hastata* ethanolic extracts

Isolates	Control	L (25mg/mL)	L(50mg/mL)	L(100mg/mL)	Fluconazole (0.025mg)
C albicans	0.00	13.0±0.8	26.7±2.1	48.0 ± 2.2	23.3± 0.5
A fumigates	0.00	19.0±0.0	29.0±2.2	42.7±1.3	48.0± 0.0
C neofomans	0.00	15.3±0.5	30.7±1.6	50.3±1.3	48.0±0.0
A flavus	0.00	15.3±0.5	25.2±0.5	46.3±2.1	35.6±1.3

Table 3: One way ANOVA for the efficacy of the plants extract and fluconazole against the pathogens

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	131.220	1	131.220	1.748	0.234
Within Groups	450.495	6	75.082		
Total	581.715	7			

"df": degree of freedom

"Sig": significance level

"*": significant at p< 0.005

Minimum inhibitory and Minimum Fungicidal concentration

The study indicated that, the minimum inhibitory concentration of *L hastata* ethanolic extract was found to be 25mg/mL against all the four isolates as presented in Table 4. The study also showed that, the minimum fungicidal concentration of the *L hastata* was 25mg/mL against *A fumigatus,* and 50mg/mL against the remaining isolates (*C albicans, C neofomans* and *A flavus*), and the result is as presented in Table 5.

Table 4: Minimum inhibitory concentration of *L hastata* ethanolic extract

Pathogens	MIC (mg/mL)
C albicans	25
A fumigates	25
C neofomans	25
A flavus	25

Table 5: Minimum fungicidal concentration of *L hastata* ethanolic extract

Pathogens	25 mg/mL	50 mg/mL	100 mg/mL
C albicans	-	+*	+
A fumigatus	+*	+	+
C neofomans	-	+*	+
A flavus	-	+*	+

Key: (*) minimum fungicidal concentration

DISCUSSION

Phytochemical analysis

The findings of this study confirm the presence of several bioactive compounds in *Leptadenia hastata* leaf extract, including tannins, saponins, alkaloids, flavonoids, phenols, and steroids. These results align with prior studies by Zulaiha *et al*. (2019), and Doughari & Obidah (2008), which similarly reported the presence of these phytochemicals in various plant extracts. The consisten**cy** of these findings underscored the reliability of the methods used in phytochemical screening and highlighted the therapeutic potential of *Leptadenia hastata*.

These results reinforce the importance of *Leptadenia hastata* as a valuable medicinal plant and support its traditional use in natural therapies. However, further studies are recommended to isolate and characterize these compounds, assess their pharmacological properties, and explore their potential applications in drug development.

Antifungal evaluation

Several plant parts are widely utilized for medicinal purposes and form a significant element of global cultural heritage, serving as essential resources for addressing health problems (Sarkiyayi *et al*., 2015). The findings revealed that at 25 mg/mL of the ethanolic extract, zones of inhibition measuring 13.0 ± 0.8 mm, 19.0 ± 0.0 mm, 15.3 ± 0.5 mm, and 15.3 ± 0.5 mm were recorded for *Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, and *Aspergillus flavus*, respectively. At 50 mg/ml, inhibition zones increased to 26.7 ± 2.1 mm, 29.0 ± 2.2 mm, 30.7 ± 1.6 mm, and 25.2 ± 0.5 mm, respectively. Similarly, at 100 mg/ml, the zones of inhibition were 48.0 ± 2.2 mm, 42.7 ± 1.3 mm, 50.3 ± 1.3 mm, and 46.3 ± 2.1 mm against the same fungal isolates.

According to the Clinical and Laboratory Standards Institute (CLSI), the standard inhibition zone for fluconazole is 16.0 mm. At 25 mg/mL, all fungal isolates were resistant to the ethanolic extract, except *A. fumigatus*, which was found to be sensitive. However, all fungal isolates were sensitive to the extract at 50 mg/mL and 100 mg/mL.

These results are consistent with previous studies by Umaru *et al*. (2017), and Doughari & Obidah (2008), which documented the antifungal activity of *Leptadenia hastata* extracts against similar fungal isolates. Furthermore, Aliero & Wara (2009) reported the efficacy of the plant against *Aspergillus niger* and *Fusarium oxysporum*. These findings underscore the potential of *L. hastata* as a natural antifungal agent, warranting further investigation into its active compounds and mechanisms of action.

The results of one-way ANOVA showed no significant difference in efficacy between fluconazole and the *L hastata* at 100mg/mL ($p = 0.234$) suggesting that *L hastata* extract has comparable antifungal activity to fluconazole. This indicated that the *L hastata* extract can serve as an alternative to fluconazole, especially in settings where access to synthetic antifungal agents is limited, or when considering natural, cost-effective, and sustainable treatment options.

Minimum inhibitory and Minimum Fungicidal concentration

The study demonstrated that the minimum inhibitory concentration (MIC) of the ethanolic extract of *Leptadenia hastata* was 25 mg/ml against all four fungal isolates (*Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, and *Aspergillus flavus*). Additionally, the minimum fungicidal concentration (MFC) was determined to be 25 mg/ml for *A. fumigatus* and 50 mg/ml for the other isolates (*C. albicans*, *C. neoformans*, and *A. flavus*).

The findings corroborate the earlier observation that the ethanolic extract exhibited dose-dependent antifungal activity, with increasing concentrations yielding larger zones of inhibition. The MIC results indicate that *L. hastata* ethanolic extract is effective in inhibiting fungal growth at relatively low concentrations, particularly against *A. fumigatus*. The MFC findings further highlighted

the extract's fungicidal potential, requiring slightly higher concentrations for complete fungal eradication, particularly for *C. albicans*, *C. neoformans*, and *A. flavus*.

These results align with previous studies that documented the antifungal efficacy of *L. hastata* and its potential as a natural therapeutic agent (Umaru *et al.*, 2017; Doughari and Obidah, 2008). The observed MIC and MFC values also support the findings of Aliero & Wara (2009), who highlighted the effectiveness of the plant against other fungal pathogens such as *Aspergillus niger* and *Fusarium oxysporum*. The consistent activity at low concentrations underscores the promising antifungal properties of *L. hastata* and its potential to address resistance associated with conventional antifungal agents.

CONCLUSION

This study highlighted the antifungal efficacy of *Leptadenia hastata* ethanolic leaf extract, underpinned by its rich phytochemical compositions. The plant extract showed promising inhibitory and fungicidal activities, with results comparable to fluconazole. These findings validate the traditional use of *L. hastata* for treating fungal infections and advocate further research to optimize its application in herbal medicine.

Conflict of Interest:

The authors declare that, there is no conflict of interest

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