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Evaluation of the Antimycotic Activity of *Lantana camara* Extracts against Aspergillus Species

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Abstract

Introduction: Aspergillus species are common filamentous fungi that primarily affect immunocompromised hosts, causing various respiratory conditions. The rising resistance to antimicrobial agents has spurred the search for novel therapeutic molecules from natural products, such as medicinal plants. Lantana camara is known for its diverse bioactivities, including antimicrobial properties. This study aimed to evaluate the Aqueous and ethanolic extracts of Lantana camara leaves have in vitro antimycotic efficacy against clinical isolates of Aspergillus species.

Methods: One hundred clinical sputum specimens were collected from tuberculosis patients at the Public Health Department of Chest and Respiratory Disease Clinic in Wasit Province, Iraq. Pathogenic fungi were identified using direct microscopic inspection (KOH test) and cultural traits on Sabouraud Dextrose Agar. L. camara's ethanolic and aqueous extracts' antifungal qualities leaves (at 6.25, 12.5, and 25 mg/mL concentrations were examined utilising disc diffusion and poisoned food techniques.

Results: Out of 62 positive samples, A. fumigatus was the most frequently isolated fungus (45.16%), followed by A. niger (25.80%). Both extracts inhibited the growth of Aspergillus species, with the ethanolic extract demonstrating a significantly greater effect than the aqueous extract. The maximum growth inhibition was observed with the ethanolic leaf extract against A. niger at a concentration of 25 mg/mL (inhibition zone 35.50 mm; 62.46% inhibition), whereas the minimum inhibition was noted with the aqueous extract against A. fumigatus at 6.25 mg/mL (inhibition zone 5.50 mm; 1.15% inhibition). A. niger was the most susceptible species to the extracts.

Conclusion: L. camara leaves' ethanolic extract exhibits potent antifungal activity against clinical isolates of Aspergillus species, particularly A. niger. The results of this research recommend that L. camara extracts could be used as a promising source of natural antifungal agents for biocontrol and therapeutic applications.

Keywords: Lantana camara, Crude extract, Biocontrol, Antimycotic activity, Aspergillus

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Introduction

Aspergillus is a prevalent filamentous fungus that predominantly impacts immunocompromised individuals and those with preexisting conditions. Lung problems. *Aspergillus* species in the environment proliferate asexually via conidia and exploit dead organic material as a nutritional source (1, 2). *Aspergillus fumigatus* is the most commonly identified pathogen, followed by *A. terreus* and *A. flavus*, despite this fact that over 24 *Aspergillus* species can infect humans. It is important to think of aspergillosis as a range of processes that differ significantly based about the host's immune status (3, 4).

Antimicrobial agent resistance is a major worldwide health concern, and the quantity of recently discovered microbial strains resistant to to multiple drugs is constantly rising. This circumstance has spurred the development of effective new antimicrobial drugs, leading to an ongoing quest for novel therapeutic molecules in natural products (5, 6). The majority of medicinal plants could serve as potential replacement sources for currently used antibiotics (against which microorganisms have acquired resistance) because they are generally safe, have few or no side effects, are inexpensive, and may affect a variety of antibiotic-resistant germs (7).

The chemical composition and bioactivities of *Lantana camara* flowers, leaves, roots, fruits, and essential oils have been extensively investigated. It has been valued for its medicinal properties, showcasing antimicrobial, anti-inflammatory, and antioxidant effects harnessed in traditional medicine (8, 9). Various studies have indicated that *L. camara* exhibits microbial, nematicidal, and insecticidal activities. For instance, a study on the nematicidal effects of *L. camara* leaf extract showed the highest mortalities in second-stage juveniles (J2s) of root-knot nematodes (10). The bioactive components of the extract that seem to contribute to its antimicrobial activity include alkaloids, flavonoids, phenols, tannins, and terpenoids. (11). Studies on the phytochemical composition and antimicrobial properties of *L. camara* leaves and stems have revealed that they contain bioactive substances that have antibacterial properties against harmful bacteria and fungi. (12). Thus, the purpose of this study was to assess how *Lantana camara* extracts affected *Aspergillus* species.

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Materials and Methods

Plant Material

The plant *Lantana camara* leaves were obtained out of the local market in Wasit Province, and seeds were sourced from the market in Basrah Province, Iraq. The plant material was identified by Prof. Dr. Majid Hannon Sharhan at the Wasit University College of Science, Department of Biology.

Collection of Fungal Isolates

One hundred clinical sputum specimens were collected from tuberculosis (TB) patients at the Public Health Department of Chest and Respiratory Disease Clinic in Wasit Province. The specimens were carefully transferred to sterilised Sabouraud Dextrose Agar (SDA) added with chloramphenicol in Petri dishes (0.05 g/L) together with cycloheximide (0.5 mL/L).to inhibit bacterial and saprophytic fungal growth, respectively (13). In addition to morphological traits, colony size, and pigmentation generated on the culture's front and back sides on SDA, the fungal isolates were identified morphologically through direct microscopic examination (KOH test). Pure subcultures were created for each isolate for subsequent applications.

Preparation of Plant Materials

Leaves were shade-dried at room temperature for eight to ten days before being processed utilising a mechanical grinder to turn it into a fine powder.After sieving through a standard mesh, the powder was utilised for extraction. Specifically, *L. camara* leaves were dried for two hours at 60 °C in an oven after being cleaned with distilled water. This dried material was then blended and converted into form of powder. The powders were kept in plastic bins until required (14).

Preparation of Aqueous Extracts

A flask holding 500 mL of distilled water was filled with 50 g of the dry powder to produce an aqueous extract. For twenty minutes, the mixture was boiled. Turning into a cream-coloured liquid. After passing through 0.45 µm Whatman filter paper, the supernatant was dried in a rotary evaporator at 50 °C. The resulting extract was weighed and stored for later use, Figure 1 (14).

Preparation of Ethanolic Extracts

The ethanolic extract was made by mixing 500 mL of 70% ethanol with 50 g of the dry powder.The mixture was kept at laboratory temperature for 24 hours and stirred with a glass rod every few hours. After passing the extract through 0.45 µm Whatman filter paper, it was dried at room temperature, weighed, and saved for future use (14).

Disc Diffusion Method

The effects of L. camara ethanolic and aqueous extracts on the development of Aspergillus species were assessed using the disc diffusion technique. 2 g of the dry extract were dissolved in 80 mL of distilled water to create a stock solution of 25 mg/mL. The concentrations of 12.5 mg/mL and 6.25 mg/mL were then obtained by diluting this stock solution. Paper discs (5 mm in diameter) were autoclave-sterilised and submerged in the respective extract solutions. The discs were placed in Petri dishes containing SDA previously inoculated with the target fungi. The agar to encourage fluid diffusion, plates were left at room temperature for two hours before being incubated at 25 °C for a set amount of time. The inhibitory zones were quantified. In millimetres by calculating the entire diameter and subtracting the disc diameter (15-21).

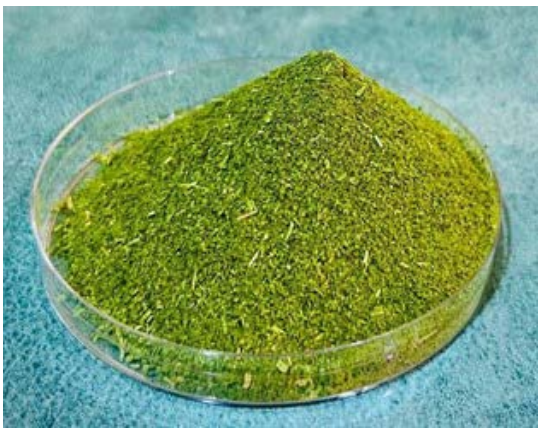

Figure 1. dried powder of plant material

Poisoned Food Technique

To assess the effects of the leaf extracts on fungal development using the poisoned food technique, 10 mL of each extract concentration (25, 12.5, and 6.25 mg/mL) was combined with 90 mL of previously autoclaved PDA medium (potato dextrose agar) was added to 9 cm Petri plates.Control dishes contained media devoid of the extract. Mycelial discs (10 mm in diameter) of the tested fungi were put in the middle of every plate and kept at 25 °C for incubation. It was determined how big the colony was, and the inhibition percentage was calculated using the following equation (15): Proportion of inhibition (%) = [(Control growth - Test growth) / Control growth] × 100

Statistical Analysis

Data were statistically interpreted use the software known as the Statistical Package for the Social Sciences (SPSS). The Least Significant Difference (LSD) test was used to assess treatment differences at the $p < 0.05$ probability level using analysis of variance (ANOVA).

Results

The findings demonstrated that morphological and microscopic characteristics were essential for isolating and identifying fungal isolates from patient sputum. Out of 100 clinical samples, 62 (62%) were positive for fungal growth, while 38 (38%) were negative. As displayed in Table 1, *Aspergillus fumigatus* was the most common

fungus, detected in 28 samples (45.16%) (Figure 2). This was followed by *Aspergillus niger* in 16 samples (25.80%) (Figure 3), *Aspergillus flavus* in 10 samples (16.12%) (Figure 4), and *Aspergillus nidulans* in 3 samples (4.83%) (Figure 5). Other opportunistic fungi, such as *Candida albicans* (3 samples, 4.83%) (Figure 6) and *Penicillium* sp. (2 samples, 3.22%) (Figure 7), were also identified.

The impacts of the aqueous and ethanolic extracts on *Aspergillus* species growth using the disc diffusion method are presented in Table 2. The findings showed that the ethanolic extracts were more effective than the aqueous extracts. At all concentrations. The inhibitory effect increased depending on the concentration.The largest inhibition zone was recorded with the ethanolic extract at 25 mg/mL against *A. niger* (35.50 mm), whereas the smallest zone was observed with the aqueous extract at 6.25 mg/mL against *A. fumigatus* (5.50 mm). For A. fumigatus, A. flavus, and A. niger, the average inhibition zones were 16.90 mm. 17.48 mm, and 18.52 mm, respectively, indicating that *A. niger* was the most sensitive Species (Figure 8). Furthermore, the overall mean inhibition zone was highest for the ethanolic extract (34.52 mm at 25 mg/mL) and lowest for the aqueous

Table 1. percentages of fungus identified from patients' sputum

Fungi	No. of samples	Percentage (%)
Aspergillus fumigatus	28	45.16%
Aspergillus niger	16	25.80%
Aspergillus flavus	10	16.12%
Aspergillus nidulans	3	4.83%
Candida albicans	3	4.83%
penicillium sp	2	3.22
Total	62	100

Figure 2. A. *fumigatus*

Figure 4. A.Flavus

Table 2. Effect of *Lantaba camara* extracts leaves on growth of Disc diffusion technique for Aspergillus spp

Treatment		Inhibition zones (mm)			Means
Extract	Concentration (%)	A.fumigatus	A.flavus	A. niger	
Ethanolic 70%	25mg/ml	33.66	34.42	35.50	34.52
	12.5mg/ml	16.74	17.02	18.66	17.47
	6.25mg/ml	14.00	14.66	15.33	14.66
Aqueous	25mg/ml	21.50	22.33	23.33	22.38
	12.5mg/ml	10.33	10.66	11.67	10.88
	6.25mg/ml	5.50	5.80	6.66	5.98
LSD T*F			0.718		LSD T 0.414
Means		16.9	17.48	18.52	
LSD F (0.05)			0.293		

The mean of three replicates is used for each value.

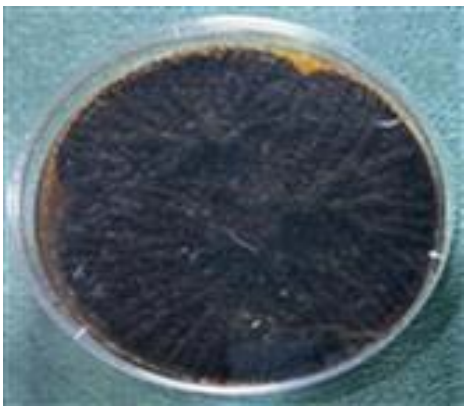

Figure 3. *A. niger*

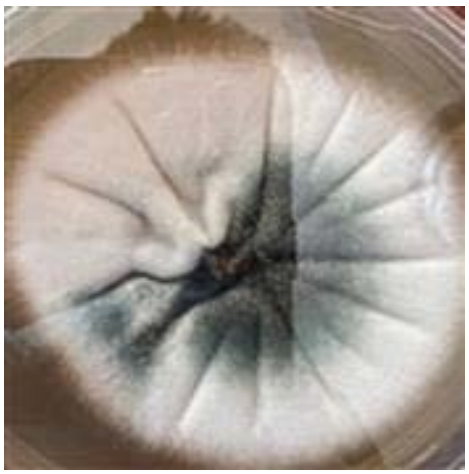

Figure 5. A. nidulans

extract (5.98 mm at 6.25 mg/mL).

Using the poisoned food technique (Table 3), the ethanolic extract consistently outperformed the aqueous extract, with efficacy rising alongside concentration. Compared to the control radial growth (80.6 mm), *A. niger* treated with 25 mg/mL ethanolic extract exhibited the lowest radial growth (30.25 mm), corresponding to the highest inhibition percentage (62.46%). Conversely, *A. fumigatus* treated with 6.25 mg/mL aqueous extract showed the highest radial growth (79.67 mm) and the lowest inhibition percentage (1.15%). The mean inhibition percentages were 30.01%, 33.55%, and 36.38% A. flavus and A. fumigatus, and *A. niger*, respectively, reaffirming that *A. niger* is the most susceptible Species (Figure 9).

Discussion

The current study demonstrated that Lantana camara leaf extracts, both ethanolic and aqueous, had a substantial impact on Aspergillus species growth. This antimycotic activity may be attributed to the existence of active phytochemical constituents including alkaloids and flavonoids triterpenes (22-26). Previous phytochemical examinations of *L. camara* leaf extracts have revealed modest quantities of alkaloids and cardiac glycosides, alongside significant yields of tannins, flavonoids, and anthraquinones (27). The superior efficacy of the ethanolic extract over the aqueous extract observed in our study aligns with the findings of other researchers who reported that ethanolic extracts demonstrated notable antifungal effects against Aspergillus niger and Rhizopus oryzae (28). Similarly, a separate investigation demonstrated that the ethanolic extract of *L. camara* leaves was superior to both aqueous and methanolic extracts against *A. fumigatus* and *A. flavus*, exhibiting 71% and 66% inhibition, respectively (29).

Furthermore, in vitro evaluations of extracts from *L. camara* using different solvents (acetone, chloroform, ethanol, and methanol) confirmed the presence of diverse phytoconstituents, including carbohydrates, proteins, amino acids, phenolic compounds, phytosterols, glycosides, and saponins. Ethanol and methanol extracts generally performed better than other solvents (30). Comparable antibacterial efficacy was also noted for methanolic and ethanolic extracts at 100 µg/mL, highlighting the broad-spectrum potential of these solvent extractions (31). The strong antibacterial and antifungal characteristics of the ethanolic extract against microorganisms such as *Staphylococcus*

Table 3. Effect of *Lantaba camara* leaves extracts on *Aspergillus* spp by poisoned food technique

Treatment		Radial growth(mm)						Means of inhibition%
Extract	Concentrations (%)	A. fumigatus	Inhibition %	A. flavus	Inhibition %	A. niger	Inhibition %	
	25mg/ml	34.50	57.1	32.33	59.88	30.25	62.46	59.81
Ethanolic 70%	12.5mg/ml	48.33	40.03	45.80	43.17	42.67	47.05	43.41
	6.25mg/ml	61.00	24,31	59.50	26.17	57.66	28.46	26.25
	25mg/ml	48.50	39.82	44.66	44.59	40.00	50.37	44.93
Aqueous	12.5mg/ml	66.33	17.7	61.33	23.9	60.33	25.14	22.24
	6.25mg/ml	79.67	1.15	77.67	3.63	76.71	4.82	3.2
Control				80.6				LSD T 0.74
LSD T*F				1.28				
Means		56.38	30.01	53,54	33.55	51.27	36.38	
LSD F (0.05)				0.52				

The mean of three replicates is used for each value. LSD value were calculated based on inhibition percentage

Figure 6. C. *albicans*

Figure 7. Penicillium sp

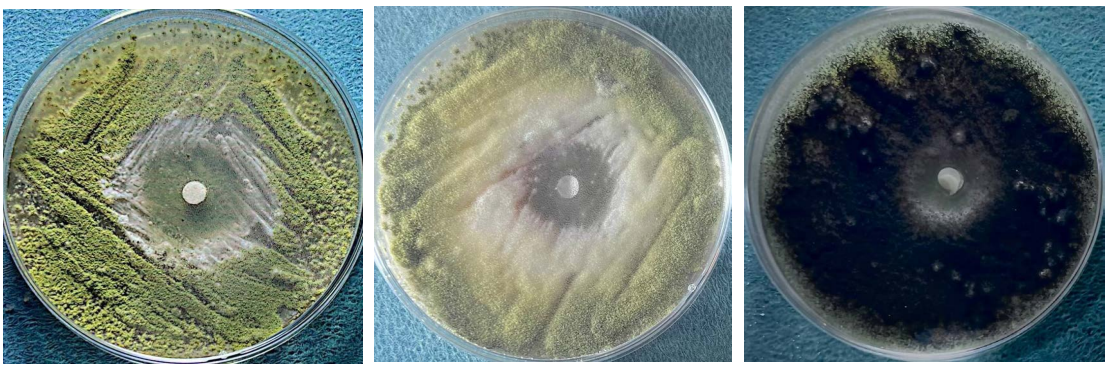

Figure 8. The impact of ethanolic leaf extracts (12.5mg/ml) on Aspergillus spp utilising the disc diffusion method (a- A. fumigatus, b- A. flavus, c- A. niger)

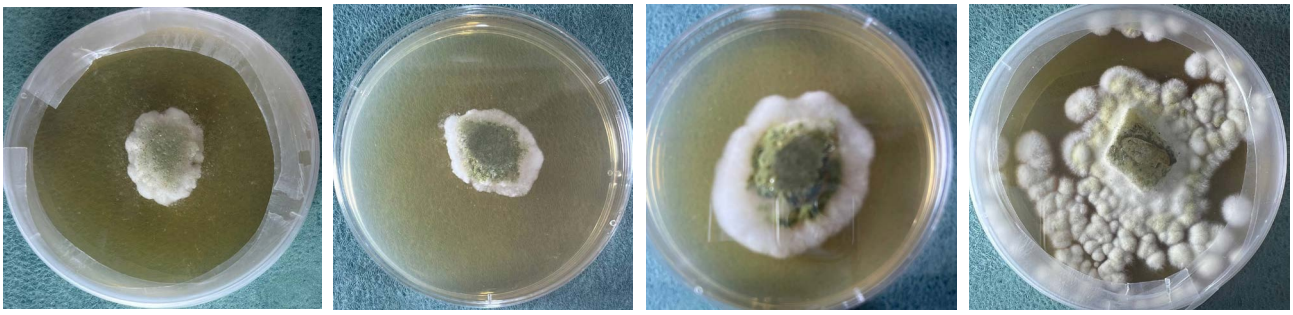

Figure 9. Impact of Ethanolic Extracts (mg/ml) on *Aspergillus fumigatus* by poisoned food technique (a-25mg/ml , b-, 12.5mg/ml c-6.25mg/ml, d- control)

aureus,*Pseudomonas aeruginosa*, *A. niger*, and *Candida albicans* have been shown to be concentration-dependent (32). The specific anti-*Aspergillus* activity of the aqueous extract may be linked to flavonoids, triterpenes, and terpenoids, warranting further targeted investigation (33). Acetone extracts derived from different *L. camara* sections have also shown strong to moderate inhibition against multiple phytopathogenic fungi, with foliage extracts displaying higher activity levels than those from seeds or flowers (34).

Regarding the prevalence of fungal isolates, *Aspergillus* species are ubiquitous in nature, producing airborne conidia that humans inhale daily. The clinical outcome of this exposure heavily depends on the host's immunological state (16). These fungi primarily infect the lungs, leading to conditions such as allergic, chronic, subacute, and invasive pulmonary aspergillosis. *A. fumigatus* is frequently isolated from the respiratory tracts of asthmatic patients and occasionally from healthy individuals (17). In our study, *A. fumigatus* was the most prevalent isolate (45.16%), it is compatible with epidemiological surveys identifying it as the predominant species among clinical isolates (18). A. fumigatus is categorised as a critical priority fungal pathogen by the World Health Organization (WHO). Especially given the recent rise in invasive aspergillosis cases among patients with influenza and COVID-19 (19, 20).

While some studies have found *A. flavus* to be the most prevalent isolate overall, the most frequent species found in *A. fumigatus* is still respiratory collections and blood (21). Other research corroborates our findings, reporting the *A. fumigatus* complex as the overwhelmingly dominant species (88.8%) in respiratory samples, followed by *A. niger* and *A. flavus* complexes (22). Variations in the distribution of *Aspergillus* species across different studies may be linked to the prevalence of underlying host illnesses, the geographical and ecological variety, and the extensive use of broad-spectrum antifungal drugs. (23, 24).

The enhanced antifungal efficacy of the ethanolic extract observed in this study is well-supported by literature. High antifungal properties against *Alternaria alternata*, *A. flavus*, and *A. niger* have been reported for ethanolic leaf extracts (35). Comparative studies have consistently found alcoholic extracts too much outperform hot water extracts in lowering *Aspergillus* species' radial growth rate. (36). Beyond antifungal action, ethanolic extracts of *L. camara* also exhibit insecticidal properties (37) and can completely inhibit the growth of phytopathogenic fungi like *A. alternata* (38). Specific secondary metabolites, particularly alkaloids, have shown potent antifungal action at very low quantities, successfully inhibiting the radial growth of *A. niger*, *Nigrospora oryzae*, and *Fusarium oxysporum*, whereas saponins were found to be less potent (39-42).

Conclusion

This study concludes that leaf extracts of *Lantana camara* possess significant in vitro antimycotic activity against clinical isolates of *Aspergillus* species, with Compared to the aqueous extract, the ethanolic extract is noticeably more effective. *Aspergillus niger* showed the greatest

sensitivity to the plant extracts, although *Aspergillus fumigatus* was the most common species identified from patient sputum. These results demonstrate the possibility of ethanolic extracts from *L. camara* as a practical, natural substitute for the biocontrol of *Aspergillus* species and the creation of innovative antifungal treatments.

Authors' Contribution

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Validation: Abdul hussein M. Ali, Mohammed J. HANAWI
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Writing – original draft: Abdul hussein M. Ali, Mohammed J. HANAWI
Writing – review & editing: Abdul hussein M. Ali, Mohammed J. HANAWI

Competing Interests

The writers affirm No conflicting interests, either monetary or not.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

Before sample collection began, the appropriate institutional ethics committee granted ethical permission for the study.

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