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Research Article
Antifungal Activity of *Lantana camara* L. Leaf Extracts in Different Solvents Against Some Pathogenic Fungal Strains

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Abstract

Background and Objective: Out of a large number of plant species used for curing various ailments, there are certain species which are not used widely but occur immensely in major parts of the state. Folklore claims also support the uses of such species for curing various diseases. In the present study one of such species, *Lantana camara* (*L. camara*) have been selected to investigate the *in vitro* antifungal activities of the leaf extract in different solvents viz., acetone, chloroform, ethanol and methanol extracts of the selected plant *Lantana camara*. **Methodology:** Poisoned food technique was carried out to perform the antifungal activity of acetone, chloroform, ethanol and methanol extracts. **Results:** All the extracts of *L. camara* gave positive for all the phytoconstituents viz. alkaloids, carbohydrates, flavonoids, glycosides, phytosterols, phenols, proteins and amino acids, saponins and tannins. Among all the extracts ethanol and methanol extracts showed better results. Methanol leaf extract of *L. camara* showed broad antifungal activity against both the fungal strains. Terbinafine which was used as standard completely inhibited fungal growth of both *Aspergillus flavus* and *Aspergillus niger*. **Conclusion:** The phytochemical screening and efficient antifungal activity of *Lantana camara* from the present investigation revealed that the methanol leaf extracts of the selected plant have significant potential to inhibit the growth of pathogenic fungal strains than other leaf extracts.

Key words: Antifungal activity, leaf extract, *Lantana camara*, pathogenic, terbinafine

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nature has been a source of therapeutic agents for thousands of years and a good number of modern drugs have been isolated from natural resources[1]. The presence of different phytochemicals in different parts of plants validates the ability of the plant to provide a source of natural medicines[2]. Plant extracts and their active phytochemical compounds have been used for antimicrobial activities and have significant remedial properties. Medicinal plants represent a rich source of secondary metabolites, many of which possess antimicrobial properties [3]. A good perceptive of plants offers the potential of developing effective broad spectrum antibiotics. Medicinal plants represent an alternative cure in non-severe cases of infectious diseases[4]. Some Indian plants have been reported to show antimicrobial, antioxidant and anti-inflammatory properties[5-7]. Plants can act as a source for new effective antibiotics.

Lantana camara L. is a species of flowering plant of the family Verbenaceae. It is a small perennial shrub which can grow to around 2 m tall and form dense thickets in varied environments. The plant is native to American tropics[8]. It has been widely used in traditional medicine for the treatment of various disorders like cancer, tumors, malaria, tetanus, ulcers, high blood pressure, eczema, wound healing, eye infections, chicken pox, measles, asthma, rheumatism and fever[9]. It is an excellent source of various classes of bioactive natural products like steroids, flavonoids, oligosaccharides, triterpenoids, glycosides and naphthoquinones[10-12].Various important phytomolecules such as oleanolic acid, lantanoside, linaroside, verbascoside, ursolic acid, camarinic acid, umuhengerin and phytol have been isolated from *L. camara* and their biological actions such as anticancer, antioxidant, hepatoprotective, leishmanicidal, antibacterial, nematicidal and antiulcer have been reported[9,10].

Different plants are being used for medicinal purposes in many countries as a source of potent drugs[13]. The interest in present scientific research of *L. camara* was based on different evidences of its effective use for the curing many diseases. Present study revealed the presence of phytochemicals and *in vitro* anti-fungal properties of acetone, chloroform, ethanol and methanol extracts of the leaves of *L. camara* against *Aspergillus flavus* and *Aspergillus niger* fungal strains.

MATERIALS AND METHODS

Plant material: Fresh leaves of *L. camara* were collected from the Medicinal Plant Garden, School of Studies in Botany, Jiwaji University, Gwalior during 2015-2016. The plant was identified by the Institute of Ethnobiology Herbarium, Jiwaji University, Gwalior (India).

Processing of the plant material: To avoid contamination, leaves were washed with running tap water and then rinsed properly with distilled water. The shade dried leaves of *L. camara* were finely grinded using electric grinder and stored in air tight containers for future use. The powdered material (approx. 100 g) was extracted in soxhlet apparatus for 6 h in acetone, chloroform, ethanol and methanol (Merck, India). The extracts were then filtered through Whatman's No. 1 filter paper. The excess solvent in the extracts was removed by distillation and the concentrated extracts so obtained were further dried at a temperature not exceeding 100°C in water bath. The extracts were collected in air tight containers and stored at 4°C for further analysis.

Test microorganisms: The test microorganisms used in the investigation were *Aspergillus flavus* and *Aspergillus niger.* These fungal isolates were sub-cultured on a potato dextrose agar (PDA) for 72 h at 25°C.

Positive and negative control: Fluconazole (1mg mL^{-1}) and terbinafine (1 mg mL^{-1}) were used as positive control for the test of fungal strains. Petri plates without plant extract were used as negative control.

Assay for anti-fungal activity: The poisoned food technique was used for the determination of anti-fungal activity of *L. camara* leaf extracts in different solvents[14]. One petri plate containing potato dextrose agar (PDA) and standard anti-fungal was used as positive control. Petri plates without plant extract were used as negative control. The petri plates were incubated at 37°C for 7 days. Cultures were examined thrice weekly during the incubation period. Readings were taken by measuring mycelial growth zone of fungus in petri plates and percentage inhibition of fungal growth for each concentration of all extracts was determined as:

$$\text{Mycelial inhibition (\%)} = \frac{\text{Mycelial growth (control)} - \text{Mycelial growth (treatment)}}{\text{Mycelial growth(control)}} \times 100$$

Statistical analysis: The data were analyzed by using Statistical Ezanova version 2. The data is presented as mean±standard error of two replicates.

RESULTS

Phytochemical screening: All the extracts of *L. camara* gave positive test when different reagents were used to test viz. alkaloids, carbohydrates, flavonoids, glycosides, phytosterols, phenols, proteins and amino acids, saponins and tannins (Table 1). Although some of the phytoconstituents were found in trace amount but there was presence of these phytoconstituents. Among all the extracts ethanol and methanol extracts showed better results.

Antifungal activity: The methanol leaf extract of *L. camara* showed broad antifungal activity against the tested microorganism at concentration of 3 mL (15%). Methanol leaf extract inhibited the fungal growth of *Aspergillus flavus* by 80.74 at 15% concentration (3 mL) while as acetone, chloroform and ethanol showed almost similar inhibition results against *A. flavus* i.e., 77.78% at the concentration of 15% (Table 2). Fluconazole inhibited fungal growth of *A. flavus* by 75.56% (Fig. 1 and 2).

Table 1: Qualitative phytochemical analysis of the leaf extracts of *Lantana camara* L.

		Solvents used			
Phytochemicals	Tests	Acetone	Chloroform	Ethanol	Methanol
Alkaloids	Dragendorff's test	++	++	++	+
	Mayer's test	++	++	++	+
	Wagner's test	++	++	++	+
Carbohydrates	Benedict's test	+	+	+	+
	Camnelisation	+	+	++	++
	Fehling's test	+	+	++	+
	Molisch's test	++	+	++	++
Glycosides	Legal's test	++	+	+	+
	Modified Borntrager's test	+	+	+	+
	Raymond test	++	+	++	++
Saponins	Foam test	++	++	++	++
	Froth test	++	++	++	++
Phytosterols	Libermann-Buckhard's test	+	+	+	+
	Salkowski's test	+	+	+	+
Phenols	Ferric chloride test	++	++	+++	++
Tannins	Gelatin test	+	+	+	++
Flavonoids	Alkaline reagent test	++	++	+++	++
	Lead acetate test	++	++	+++	++
Proteins and amino acids	Millon's test	++	+	+++	++
	Ninhydrin test	+	+	++	++
	Xanthoproteic test	+	+	++	+++

+: Trace amount, ++: Moderate amount, +++: Good amount

Table 2: Antifungal activity of different extracts of *Lantana camara* L. Leaf against *Aspergillus flavus*

Extract/standard	Concentration (%)	Diameter of fungal growth zone (mm) (Mean±SD)	Mycelial growth inhibition (%)
Acetone	1	76.67±5.77	14.81
	5	52.00±7.21	42.22
	10	24.67±3.06	72.59
	15	20.00±3.46	77.78
	20	*	*
	25	*	*
Chloroform	1	42.67±3.06	52.59
	5	30.67±3.06	65.92
	10	25.33±2.31	71.86
	15	20.00±2.00	77.78
	20	*	*
	25	*	*
Ethanol	1	47.33±6.43	47.41
	5	33.33±3.06	62.97
	10	26.00±2.00	71.11
	15	20.00±2.00	77.78
	20	*	*
	25	*	*
Methanol	1	34.67±3.06	61.48
	5	28.00±2.00	68.89
	10	21.33±1.15	76.30

Table 2: Continue

Extract/standard	Concentration (%)	Diameter of fungal growth zone (mm) (Mean±SD)	Mycelial growth inhibition (%)
	15	17.33±1.15	80.74
	20	*	*
	25	*	*
Fluconazole	1	37.33±4.16	58.52
	5	30.67±7.02	65.92
	10	22.00±3.46	75.56
	15	*	*
	20	*	*
	25	*	*
Terbinafine	1	*	*
	5	*	*
	10	*	*
	15	*	*
	20	*	*
	25	*	*

*No fungal growth

Table 3: Antifungal activity of different extracts of *Lantana camara* L. Leaf against *Aspergillus niger*

Extract/standard	Concentration (%)	Diameter of fungal growth zone (mm)(Mean±SD)	Mycelial growth inhibition (%)
Acetone	1	45.33±6.11	47.29
	5	43.33±3.06	49.62
	10	36.00±9.17	58.14
	15	33.33±8.33	61.24
	20	20.00±2.00	76.74
	25	*	*
Chloroform	1	62.67±2.31	27.13
	5	52.67±3.06	38.76
	10	46.00±4.00	46.51
	15	44.67±5.03	48.06
	20	*	*
	25	*	*
Ethanol	1	75.33±5.03	12.41
	5	62.67±6.43	27.91
	10	48.67±2.31	43.41
	15	35.33 ±3.06	58.92
	20	26.67±6.11	68.99
	25	*	*
Methanol	1	32.67±3.06	62.01
	5	29.33±7.57	65.90
	10	22.67±2.31	73.64
	15	18.00±2.00	79.07
	20	*	*
	25	*	*
Fluconazole	1	65.33±5.03	24.03
	5	40.67±1.15	52.71
	10	*	*
	15	*	*
	20	*	*
	25	*	*
Terbinafine	1	*	*
	5	*	*
	10	*	*
	15	*	*
	20	*	*
	25	*	0

*No fungal growth

Highest inhibition in fungal growth of *A. niger* was exhibited by methanol (79.07%) followed by acetone (76.74%) at 20% concentration. Chloroform and ethanol leaf extracts inhibited the fungal growth of *A. niger* by 48.06% (at 15% conc.) and 68.99 at 20% conc. (Fig. 2). Fluconazole inhibited fungal growth of *A. niger* by 52.71% (Table 3).

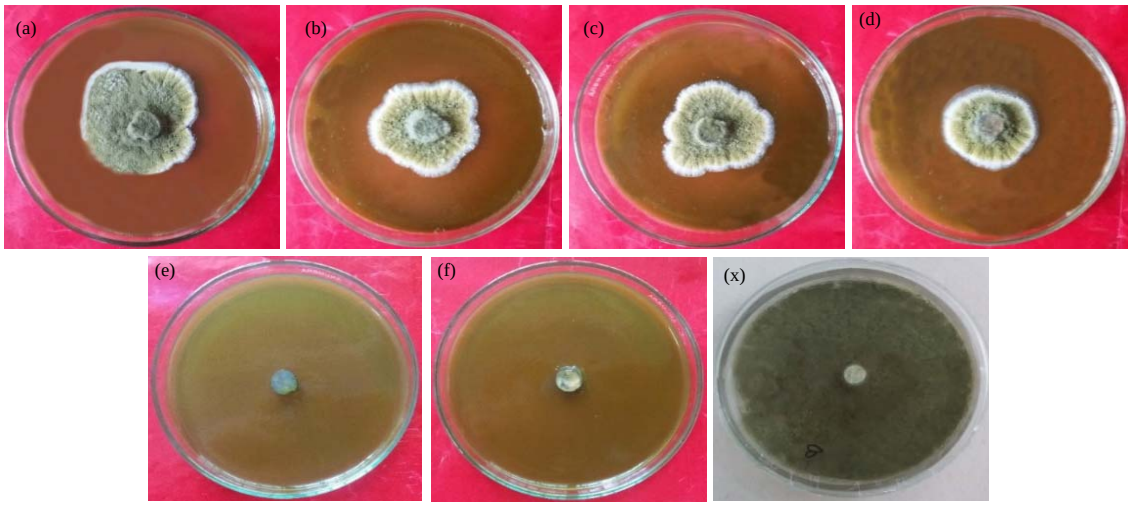

Fig. 1(a-f, x): Antifungal activity of different extracts of *Lantana camara* stem against *A. flavus*, (x) Control (PDA+tested organism), (a-d) 1, 5, 10 and 15% concentrations of methanol extract of *L. camara* leaf showing inhibition in the fungal growth and (e-f) 20 and 25% concentrations of methanol extract of *Lantana camara* leaf showing no fungal growth

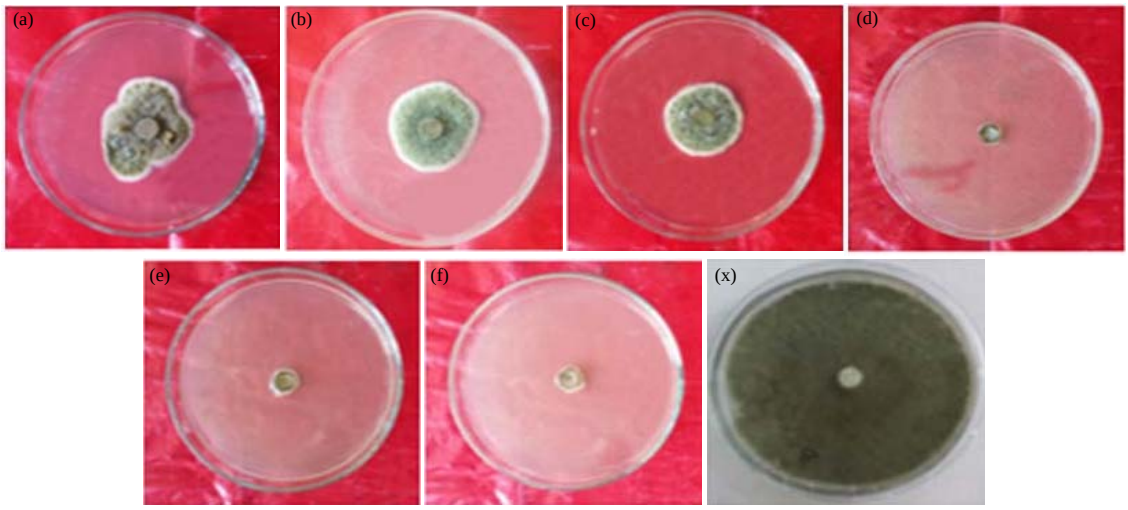

Fig. 2(a-f, x): Antifungal activity of fluconazole against *A. flavus*, (x) Control (PDA+tested organism), (a-c) 1, 5 and 10% concentrations of antifungal standard (fluconazole) showing inhibition of fungal growth and (d-f) 15, 20 and 25% concentrations of fluconazole showing no fungal growth

Terbinafine completely inhibited fungal growth of both *A. flavus* and *A. Niger* (Fig. 3 and 4).

DISCUSSION

Preliminary phytochemical screening, antifungal activity and the percentage inhibition of the acetone, chloroform, ethanol and methanol extract of the leaves of *L. camara* L. against *Aspergillus flavus* and *Aspergillus niger* fungal strains have been evaluated in the present research work. The *in vitro* antimicrobial activity evaluation of different plant extracts was a first step towards the development of new potential drugs. The acetone, chloroform, ethanol and methanol leaf extracts of *Lantana camara* L. tested on *Aspergillus flavus* and *Aspergillus niger* fungal strains showed a dose dependent antifungal activity. This agrees with several reports in which similar observations were made[15]. The antifungal activity of the leaf extracts at higher doses on *Aspergillus flavus* and

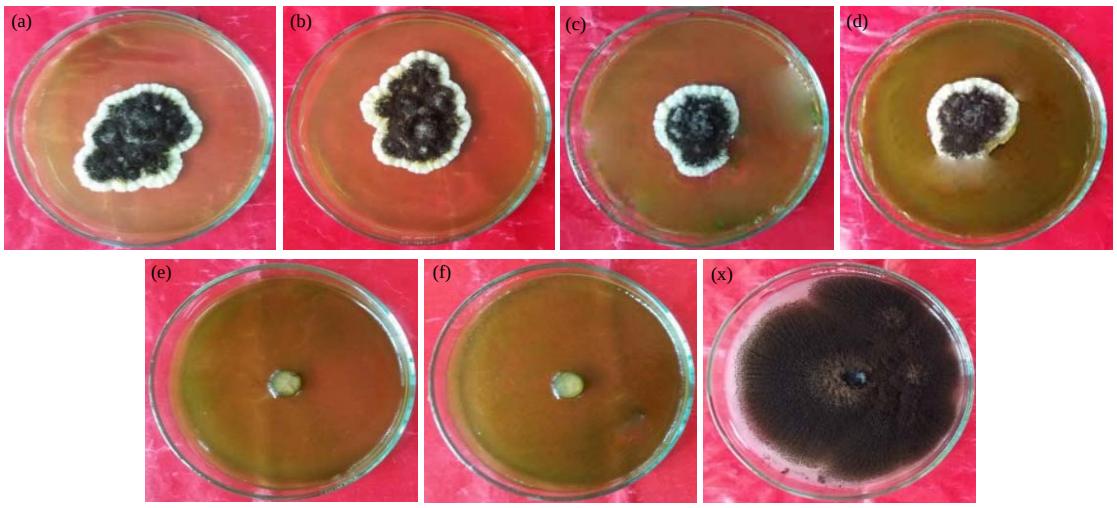

Fig. 3(a-f, x): Antifungal activity of different extracts of *Lantana camara* leaf against *A. niger*, (x) Control (PDA+tested organism), (a-d) 1, 5, 10 and 15% concentrations of methanol extract of *L. camara* leaf showing inhibition in the fungal growth and (e-f) 20 and 25% concentrations of methanol extract of *Lantana camara* leaf showing no fungal growth

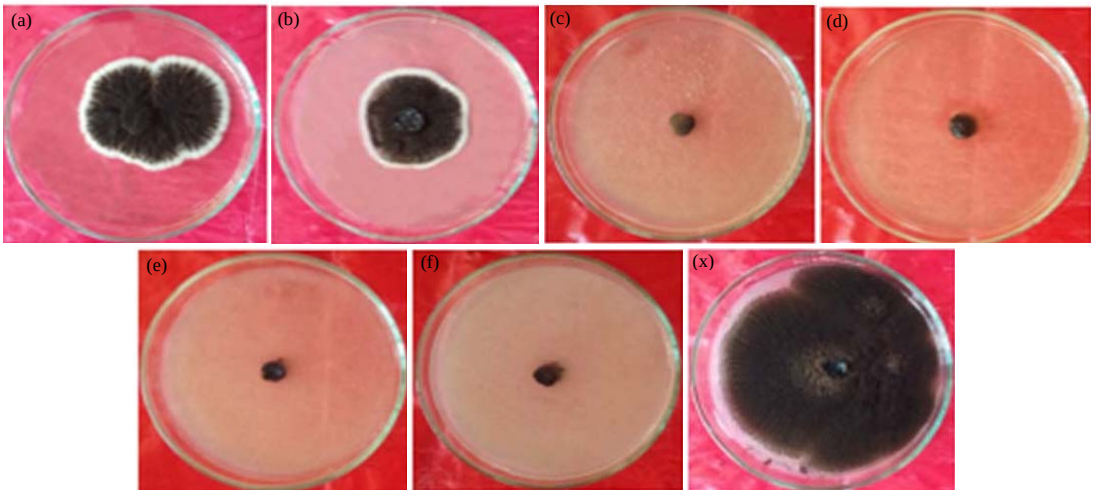

Fig. 4(a-f, x): Antifungal activity of fluconazole against *A. niger*, (x) Control (PDA+tested organism), (a, b) 1 and 5% concentrations of antifungal standard (fluconazole) showing inhibition of fungal growth and (c-f) 10, 15, 20 and 20% concentrations of antifungal standard (fluconazole) showing no fungal growth

Aspergillus niger could be attributed to the presence of some bioactive components in the extracts. Generally the methanol leaf extract showed a higher growth inhibition on all the organisms used in this study. The effect of methanolic leaf extract is significantly higher on *Aspergillus flavus* and *Aspergillus niger* as compared to other solvents used. This finding agrees with the report of Pranoothi and co workers[16] in which the whole plant extract of *Leucas indica* was found to significantly inhibit the growth of *Candida rogasa*, *Rhizopus oryzae*, *Aspergillus niger* and *Candida albicans*. The report of Satish *et al.*[17] revealed that methanol plant extract was found to exhibit marked antifungal activity than ethanol, chloroform, benzene and petroleum ether. This study also agrees with the report of Sailaja[18] in which methanolic extracts of *Lantana camara* L. and *Oscimum basilicum* L., showed highest MIC value of 0.7 mg mL^{-1} against *Aspergillus niger*. The results of the present study did not agree with the report of Pandithurai *et al.*[19] in which the maximum activity was recorded from chloroform extract against the non dermatophytic fungi *Aspergillus flavus* (98.83%)[19]. The study of Ashraf *et al.*[20] on antifungal activity of methanol, chloroform and aqueous extracts of *Origanum vulgare*

against *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus pterus* showed high susceptibility of fungal strains to chloroform extract which is in disagreement with the present study. This study showed methanol extract had higher antifungal activity against *Aspergillus flavus* and *Aspergillus niger* as compared to the other extracts of the same plant. The MIC showing *Aspergillus flavus* and *Aspergillus niger* being more susceptible to the methanolic leaf extract when compared with did not agree with Linthoingambi and Singh[21], who reported that the petroleum ether leaf extract had highest antifungal activity followed by methanol and chloroform extracts.

With the spread of antibiotic resistance and noticeable challenges with medicinal systems in the treatment of many infectious diseases, such plants should be considered to obtain their all possible antimicrobial benefits. The biologically active compounds having antimicrobial potential must be extracted and then identified. The tolerable level and toxic effects of such compounds should be properly investigated. This study provided a scientific validation to this medicinal plant to be used as antimicrobial drug. This study further suggested the isolation and identification of the phytochemicals and active compounds in the plant.

CONCLUSION

The extracts of the plant part used showed prominent antifungal activity against *Aspergillus niger* and *Aspergillus flavus* which are severe pathogens. Thus, the use of this plant in the treatment of pathogenic diseases associated with the infection of these pathogens is validated, scientifically supported by the results obtained in this study. Therefore, further efficacy and safety studies are encouraged onthis medicinal weed as alternative and effective in clinical practice.

SIGNIFICANCE STATEMENT

The present finding shows that different solvent extracts of a plant species may have different spectra of antifungal activity that can be explained by the solubility or insolubility of the active compound(s) in the solvent used for extraction. This study adequately justifies the ethno-medical use of this plant in the management of some fungal diseases. This study further suggests the researchers to isolate and identify the phytochemicals and active compounds in the plant.

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