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## ANTI-ASPERGILLUS ACTIVITY OF *LANTANA CAMARA* LINN.

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**ABSTRACT:** Herbal fungicides are gaining great interest because of their cost-effective and eco-friendly attributes. If this antifungal potency resides in weeds, it will be a great advantage because of their large population and to effectively develop a novel drug from weeds being considered as waste material. The present study aims to assess the anti-aspergillus activity of a common weed *Lantana camara*. The crude extract of leaves was obtained using polar and non-polar solvents and tested against *Aspergillus niger* ATCC 16888. Results reveal, the aqueous extract showed maximum growth inhibition of  $14 \pm 0.142$  mm zone at 30 mg/ml, and it was  $37 \pm 0.124$  mm for the standard antifungal agent voriconazole at 30 mg/disc. Further, the minimum inhibitory concentration of the extract was calculated as 18 mg/ml. Experiments on the mode of action suggested that the extract lyse the cells and alter the membrane integrity by depleting the ergosterol content, which avoids the reoccurrence.

**INTRODUCTION:** For many centuries, it is a known fact that humankind depends on plants as an indirect source of energy and shelter. It has been found that near about 80% of all established natural products originate from plants<sup>1</sup>. Natural products continue to play an important role in the discovery and development of new pharmaceuticals as clinically useful drugs as starting materials to produce synthetic drugs. If this antifungal potential resides in weeds, it will be a great advantage because of their large population and to effectively develop novel drugs against *Aspergillus sp.*, which is responsible for Aspergillosis in human and different plant diseases especially in vegetable plants such as onion, tomato, maize, grapes, etc.

Although, the synthetic fungicides are effective in control diseases, indiscriminate application caused severe health hazards in animal and humans due to their residual toxicity<sup>2,3</sup>. Furthermore, the danger alarm on resistance development in pathogenic microorganisms restricts the use of synthetic fungicides, which seriously hinders the management of diseases of crops and agricultural products<sup>3</sup>. These undesirable attributes of synthetic fungicides demand alternative treatments that are less hazardous to humans and animals and less impact on the environment. Extracts isolated from several plants have been reported to have a biological activity such as antimicrobial, antifungal, anti-inflammatory and anti-oxidant activities<sup>4</sup>.

*Lantana camara* also is known as Spanish flag, or West Indian Lantana is a species of flowering plant in the Verbenaceae family. It has been introduced into other parts of the world as an ornamental plant and is considered as an invasive species in many tropical and subtropical areas.

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Its various parts are used in the conventional and traditional system of medicine for the cure of several problems, which include itching, inflammation, and tumors <sup>5</sup>. **Table 1** depicts brief details on the constituents and application *Lantana camara* and suggested the immense application of the extracts of *Lantana camara*.

Since, only a few studies were on the medicinal properties of the extracts of *Lantana camara*; the present study has been taken up to assess the anti-aspergillus activity of the extracts of *Lantana camara* and identify the mode of action.

**TABLE 1: ACTIVE CONSTITUENTS AND APPLICATIONS OF THE EXTRACTS OF LANTANA CAMARA**

S. no.	Active constituents and Applications	References
1	Alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, amino acids, protein, sterols and triterpenoids, carbohydrates, oils and fats, organic and inorganic acids	Mamata and Jyoti <sup>12</sup>
2	Camaryolic acid, methylcamaralate, triterpenoids and other known compounds such as octadecanoic acid, camaric acid, lantnolic acid, Lantanone, and lantanoside	Begum et al., <sup>13</sup>
3	Oleanolic acid	Misra and Laatsch <sup>14</sup>
4	Flavonoids, triterpenoids and a mixture of stigma sterol, campsterol	Lai et al., <sup>15</sup>
5	Cardioactive glycosides	Qaisar et al., <sup>7</sup>
6	The whole plant antibacterial activity against <i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> , and <i>S. saprophiticus</i>	Basu et al., <sup>16</sup>
7	Antifungal activity of essential oil of <i>L. camara</i>	Deena et al., <sup>17</sup> Srivastava et al., <sup>11</sup>
8	Larvicidal activity of Leaves of <i>L. camara</i>	Kumar and Maneemegalai <sup>18</sup>
9	Anticancer activity of Leaves of <i>L. camara</i>	Fourie et al., <sup>19</sup>
10	The antihelmintic activity of Leaves of <i>L. camara</i>	Begum et al., <sup>20</sup>
11	Antiseptic for wounds and leprosy and scabies	Sharma et al., <sup>21</sup>
12	The wound healing potential of leaves of <i>L. camara</i>	Pan et al., <sup>22</sup>

## MATERIALS AND METHODS:

**Collection of Plant:** Leaves of *Lantana camara* **Fig. 1** collected from different parts of Tamil Nadu, India, and authenticated by Siddha Hospital and Research Institute, Chennai. The leaves were washed with tap water once, subsequently with distilled water (X2) and shade dried under room temperature. The dried leaves were powdered well and stored in a plastic airtight container and used for the experimental studies.



**FIG. 1: THE IMAGE OF THE LANTANA CAMARA PLANT USED IN THE PRESENT STUDY**

**Preparation of the Extract:** The air-dried and coarsely powdered leaves extracted using Soxhlet apparatus. About 10g of the dried powder extracted

with water and methanol individually. The resulting extract was concentrated using roto evaporator and stored in a sterile container at -4 °C until use.

**Aspergillus niger:** *Aspergillus niger* strain ATCC 16888 obtained through HiMedia, India, used as a test strain for the study.

**The anti-aspergillus activity of leaf crude extract of *Lantana camara*:** The anti-aspergillus activity of crude extract of *Lantana camara* leaves assessed using the protocol of CLSI (M 44-A and M 38-A) by microdilution method <sup>6</sup> and disc diffusion method (Andrews). In brief, for the disc diffusion method, a lawn of inoculum containing  $1 \times 10^7$  CFU/ml of conidial spores of *A. niger* was made in the Petri dish containing pre-sterilized Czapek dox medium. The plain sterile disc with (a) sterile water; (b) methanol and (c) with aqueous extract at different concentration was placed in the center of the lawn and incubated at 25 °C for 4 days. Voriconazole was used as a standard antifungal agent at 30 mg/disc (Hi-Media, India) concentration. Antifungal activity was determined by measuring the zone of inhibition in mm.

The anti-aspergillus activity index of the extract was calculated by dividing the zone of inhibition exhibited by the extract with the zone of inhibition exhibited by the standard drug. Duplicate experiments were performed and repeated three times for concurrence.

About broth microdilution method, step 1 is the preparation of the stock inoculum suspension, and it was prepared from a 7-day old culture grown in potato dextrose agar slants using 1% Tween 80. In step 2, RPMI 1640 with L-glutamine, buffered at pH 7.0 with 0.165M Morpholine propane sulphonic acid was used as the dilution medium. The crude extract was diluted with the medium for 100 times, and 100  $\mu$ l of the diluted sample was dispensed into 96 Well plates. The conidia obtained from step 1 was also diluted in RPMI stock solution and dispensed and incubated at 35 °C for 24, 48, and 76 hours. Triplicate experiments were conducted and the mean value was recorded. Minimum fungicidal concentration (MFC) was also determined by subculturing method, and the least concentration showing no visible growth was considered as MFC.

**Mode of Action:** To determine the exact target of extracts of *L. camara* concerning *A. niger*, followed by exposure to the extract, determinations on the lesion of the plasma membrane and variation in ergosterol content was determined according to the procedure summarized below;

In brief, for the determination of lesion of the plasma membrane, the spore suspension of *A. niger* containing  $4 \times 10^6$  spores/ml was taken in a glass test tube and to that extract was added in the increasing concentrations. The mixtures were then incubated for 12 h at  $28 \pm 2$  °C in an incubator shaker. After 12 h of incubation, the mixture was suspended with 15ml of Czapek dox Agar medium and allowed to solidify. The plates were incubated at 28 °C for 4 days and observed the growth of the organism. Water and methanol were used as controls.

Concerning ergosterol content determination, an amount of 100 $\mu$ l containing  $10^7$  spores/ml of *A. niger* inoculated in a Czapek dox broth medium. The extract was added at increasing concentrations. Samples without any plant extracts were considered as controls. The flasks were incubated at 28 °C for

4 days for the generation of mycelia in the medium. After incubation, the mycelia were harvested and washed twice with distilled water. The net weight of the cell pellet was determined. Five milliliters of 25% alcoholic Potassium hydroxide solution was added to each sample and vortex for 2 min, followed by incubation at 85 °C for 4 h. Sterols were extracted from each sample by adding a mixture of 2 ml sterile distilled water and 5 ml n-heptane. The mixture was then sufficiently mixed by vortex for 2 minutes allowing the layers to separate for 1 hour at room temperature. The n-heptane layer was analyzed using Jasco J715 spectropolarimeter at room temperature, using a circular quartz cell with a path-length of 0.1 cm.

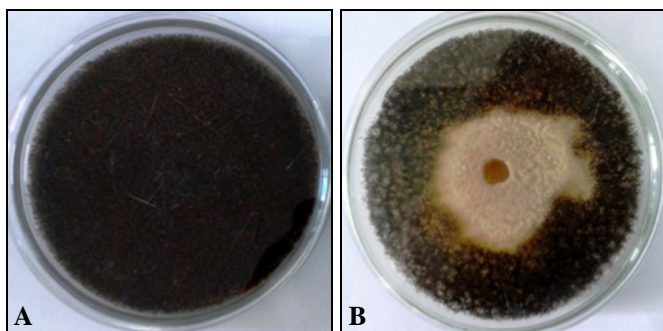
**RESULTS AND DISCUSSION:** Combating fungal contamination in materials of food, leather, cotton, books, etc., is a challenging task. The existing antifungal agents though restrict the growth of the fungus at the time of exposure, the reoccurrence is a major drawback realized. In most of the antifungal agents of azole family exhibit reoccurrence considerably. Moreover, the side effects shown by these synthetic antifungal agents restrict its application at a greater level. Hence, a suitable non-toxic antifungal agent from natural sources is badly required. In traditional medicines, most of the plant extracts showed potential antifungal activity. However, antifungal activity concerning *A. niger* is meager. However, some of the weeds and ornamental plants showed anti-aspergillus activity at an appreciable level. Hence, the present study has been taken up to assess the anti-aspergillus activity of a plant, namely *Lantana camara*.

The anti-aspergillus activity of both aqueous extract and methanolic extract of *L. camara* was tested using the diffusion method and the zone of inhibition of growth was measured. **Fig. 2** depicts the zone of inhibition of growth of *A.niger* observed with aqueous extract in comparison with control.

The inhibition was measured as  $40 \pm 2.4$  mm. With methanolic extract, no inhibition was observed.

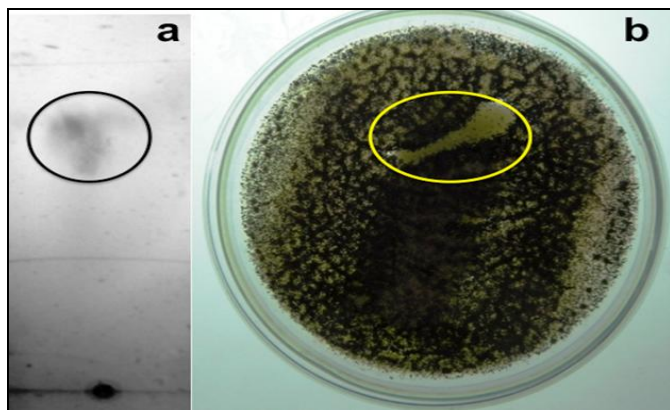
Thin layer chromatographic analysis was done for *L. camara* but with differences in the solvent system due to the solubility of different

constituents in different solvents having antifungal activity. The spot found at the  $R_f$  value of 0.511 in *L. camara* might be the presence of Flavonoids as depicted by Qaisar *et al.*<sup>7</sup>



**FIG. 2: ANTI-ASPERGILLUS ACTIVITY OF AQUEOUS EXTRACT OF *L. CAMARA*** (a) Control plate of *A. niger* (b) The zone of inhibition of *A. niger* towards *L. camara* extract.

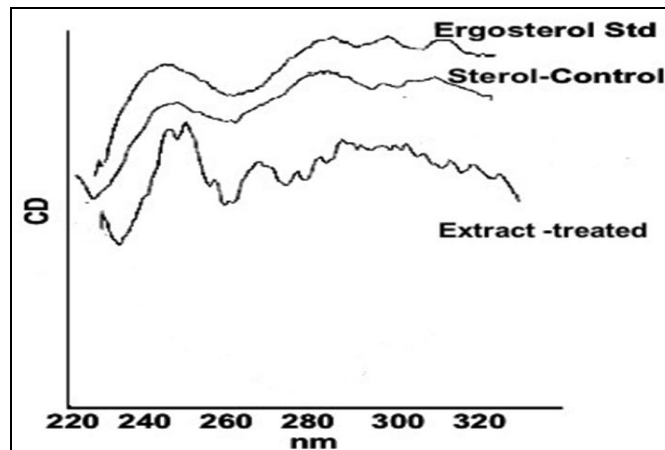
The bioautography analysis was performed as described by Qaisar *et al.*<sup>7</sup> and zone of clearance near the spot was observed, which indicates the presence of active antifungal components **Fig. 3**. The flavonoid component in *L. camara* contains pharmacological activities and is commonly present in medicinal herbal weeds. Concerning the minimum inhibitory concentration determination by microbroth dilution method, we observed MIC<sub>50</sub> as  $18 \pm 0.2$  mg/ml and MIC<sub>90</sub> as  $25 \pm 0.6$  mg/ml.



**FIG. 3: (A) TLC ANALYSIS OF *L. CAMARA* EXTRACT. (B) BIOAUTOGRAPHY ANALYSIS OF *L. CAMARA* EXTRACT AGAINST *A. NIGER***

Further, the absence of growth of *A. niger* at the concentration of 18 mg/ml and 25 mg/ml observed from the lesion of plasma membrane studies suggested that lysis of plasma membrane after exposure to the water extract of *L. camara*. According to Rodriguez *et al.*,<sup>8</sup> ergosterol, the major sterol component of the cell membrane is responsible for cell function and integrity.

Kelly *et al.*,<sup>9</sup> suggested that growth inhibition by antifungal agents reduces the ergosterol biosynthesis. In the present study, an about more than 75% reduction in ergosterol content was observed with the aqueous extract of *L. camara* **Fig. 4**.



**FIG. 4: CD SPECTRUM OF ERGOSTEROL CONTENT OF *A. NIGER* AFTER TREATMENT WITH THE WATER EXTRACT OF *LANTANA CAMARA***

Kalita *et al.*,<sup>10</sup> reported that *L. camara* has a wide spectrum of medicinal properties. Srivastava and Singh<sup>11</sup> reported the antifungal activity of *L. camara* against *Alternaria* sps. The reason for the anti-aspergillus activity of water extract of *L. camara* could be due to the presence of flavonoids, triterpenes, and terpenoids. Further research exploration is required for these active constituents.

**CONCLUSION:** Use of traditional medicinal plants for human health is well known. However, to combat fungal growth, special attention is needed, because of high nuisance and health impact.

A number of synthetic agents shown to reduce fungal growth but fails in controlling the reoccurrence and thus necessitates the need for potential and safe antifungal agents. In the present, a weed plant has been chosen to study the anti-aspergillus activity of its extract. *L. camara* was the plant chosen, and its water extract was studied for anti-aspergillus activity by measuring the zone of inhibition, minimum inhibitory concentration, lesion of the plasma membrane, and quantification of ergosterol content.

The results revealed that the aqueous extract of *L. camara* shown appreciable zone of growth

inhibition, and MIC 90 was calculated as 25 mg/ml. At this MIC concentration, a lesion in the plasma membrane was encountered, and more than 75% reduction in ergosterol content was observed. It has been understood from the literature that the presence of triterpenes and terpenoids could be the reason for the anti-aspergillus activity. Thus, these results suggested that aqueous extract could be a suitable anti-aspergillus agent. However, much more scientific explorations are required before going for clinical applications.

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**CONFLICT OF INTEREST:** Nil

## REFERENCES:

- Philipson JD: Plants as a source of valuable products. In: Charwood BV, Rhodes MJC, Editors. Secondary product from plant tissue culture. Oxford: Clarendon Press 1990: 1-21.
- Strange RN: Plant disease control towards environmentally acceptable methods, London: Chapman and Hall 1993.
- Raghavendra MP, Satish S and Raveesha KA: Phytochemical analysis and antibacterial activity of *Oxalis corniculata*; a known medicinal plant. mySCIENCE 2006; 1(1): 72-78.
- Yusuf NA, Ibrahim H and Khalid N: Studies on antibacterial evaluation and tissue culture studies of *Curcuma rubescens Roxb.* and *Curcuma* species. Seminar on medicinal and aromatic plants: Towards modernization of research and technology in the herbal industry; 2001: 24-25; FRIM Kuala Lumpur. Programme abstracts 24.
- Kiritker KR and Basu BD: Indian Medicinal plants 1918.
- Balajee SA and Marr KA: Conidial viability assay for rapid susceptibility testing of *Aspergillus* species. Journal of Clinical Microbiology 2002; 40: 2741-45.
- Qaisar N, Chaudhary BA, Dasti A, Malik A and Zafar R: Phytochemical study of aerial parts of *L. camara* for the pharmacologically active compounds. Applied Pharmacy 2009; 1: 19-26.
- Rodriguez RJ, Low C, Bottema CD and Parks LW: Multiple functions for sterols in *Saccharomyces cerevisiae*. Biochim Biophys Acta 1985; 837: 336-43.
- Kelly SL, Lamb DC, Corran AJ, Baldwin BC and Kelly DE: Mode of action and resistance to azole antifungals associated with the formation of 14 alpha-methylergosta-8, 24(28)-dien-3 beta, 6 alpha-diol. Biochemical Biophysical Research Communications 1995; 207: 910-15.
- Kalita S, Kumar G, Karthik L and Roa KVB: A review on medicinal properties of *Lantana camara* Linn. Research Journal of Pharmacy and Technology 2012; 5(6): 711-15.
- Srivastava D and Singh P: Antifungal potential of two common weeds against plant pathogenic fungi- *Alternaria* sps. Asian Journal of Experimental Biological Science 2011; 2 (3): 525-28.
- Mamata S and Jyoti S: Phytochemical screening of *Acorus calamus* and *Lantana camara*. International Research Journal of Pharmacy 2012; 3(5): 324-26.
- Begum S, Wahab A and Siddiqui BS: Pentacyclic triterpenoids from the aerial parts of *Lantana camara*. Chemical and Pharmaceutical Bulletin 2000; 51: 134-37
- Misra L and Laatsch H: Triterpenoids, essential oil and photo-oxidative 28 → 13-lactonization of oleanolic acid from *Lantana camara*. Phytochemistry 2000; 54(8): 969-74,
- Lai JS, Chan YF and Huang KF: Constituents from the stems of *Lantana camara* (II). Chinese Pharmaceutical Journal 1998; 50: 385-92.
- Basu S, Ghosh A and Hazra B: Evaluation of the antibacterial activity of *Ventilago madraspatana Gaertn.* *Rubia cordifolia* Linn. and *Lantana camara* Linn.: Isolation of emodin and physcion as active antibacterial agents. Phytotherapy Research 2005; 19: 888-94.
- Deena MJ and Thoppil JE: Antimicrobial activity of the essential oil of *Lantana camara*. Fitoterapia 2000; 71(4): 453-55.
- Kumar MS and Maneemegalai S: Evaluation of the larvicidal effect of *Lantana camara* Linn against mosquito species *Aedes aegypti* and *Culex quinquefasciatus*. Advance Biological Research 2008; 2(3-4): 39-43.
- Fourie N, Van der Lugt JJ, Newsholme SJ and Nel PW: Acute *Lantana camara* toxicity in cattle. Journal of South African Veterinary Association 1987; 58: 173-178.
- Begum S, Wahab A, Siddiqui BS and Qamar F: Nematicidal constituents of the aerial parts of *Lantana camara*. Journal of Natural Products 2000; 63: 765-67.
- Sharma OP, Makkar HPS and Dawra RK: A review of the noxious plant *Lantana camara*. Toxicon 1988; 26 (11): 975-87.
- Pan WD, Mai LT, Li YJ, Xu XL and Yu DQ: Chemical constituents of the leaves of *Lantana camara*. Yaoxue Xuebao.1993; 28: 35-39.

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