



PHARMACOGNOSTIC STUDY, PHYTOCHEMICAL EVALUATION AND ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS OF LANTANA CAMARA LINN.

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ABSTRACT

Purpose: *Lantana camara* (Verbenaceae) is one of the most popular medicinal plant in the traditional medicine. Different parts of *Lanata camara* have been used for the treatment of various human ailments such as itches, cuts, ulcers, swellings, bilious fever, eczema, tetanus, malaria, tumor, rheumatism and headache. To evaluate the scientific basis for the use of the plant, the antimicrobial activity of leaves extracts were evaluated against some Gram negative and Gram positive bacteria.

Pharmacognostic characterization: Fresh leaf of *Lantana camara* was studied for microscopical characterization. Pulverized dried *Lantana camara* leaves were investigated for its physical constant (LOD, ash value, extractive values, fluorescence analysis) and extracted with ethanol using soxhlet apparatus and macerated with chloroform water.

Method: Antimicrobial activity of *Lanata camara* leaf extracts were studied by determination of the diameter of zone of inhibition against both Gram negative and Gram positive bacteria using agar disc diffusion and minimum inhibitory concentration (MIC).

Result: Phytochemical studies revealed the presence of alkaloids, glycosides, steroids, flavonoids, tannins and the extracts were active against both Gram positive and Gram negative bacteria. Ethanolic extract at dose 500mg/ml showed potent activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* when compared with aqueous extracts.

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INTRODUCTION

Lantana camara (Verbenaceae) is the plant well known as medicinal plant in traditional medicine¹. *Lantana camara* whole plant and plant parts viz. leaves, flowers, and essential oils have been thoroughly studied for their chemical compositions^{2, 3}. The bioactive compounds mainly forms triterpenoids, flavonoids, tannins, alkaloids and glycosides isolated from *Lantana camara* plant gives biological activities^{4,5}. The ethanolic extract of *Lantana camara* leaves showed healing of gastric ulcers and also prevents development of duodenal ulcers in rats. Extracts of the fresh leaves are antibacterial and are traditionally used in Brazil as an antipyretic, carminative and in the treatment of respiratory system infection, antidiabetic, antispasmodic, diaphoretic, tonic and useful in the treatment of titanus. Concentrates of fresh roots is a good gargle for odontalgia and it is used by hill tribes for all types of dysentery.

Powdered leaves are useful for cuts, wounds, ulcers, swelling and infusion of the leaves is good for bilious fever, eczema and eruption. The fruits are useful in fistula, pastules, tumours and rheumatism^{6,7,8}. Other medicinal activity reported it's antitumor, analgesic, antifungal, and antihepatotoxic activities⁹. It is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections.¹⁰

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs¹¹. Therefore, discovery of new antibiotics is an exclusively important objective. Natural products are still one of the major sources of new drug molecules today.¹² Hence an attempt has been made to investigate antimicrobial activity of *Lantana camara*.

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Figure No. 1 Leaf and Whole Plant of *Lantana camara linn.*

MATERIAL AND METHOD

Collection of Plant material

The leaves of *Lantana camara* were collected from the Satara district, Maharashtra, during the month of July and authenticated by Dept. of Botany, Y. C. I. S, Satara, Maharashtra, India. Specimen voucher was deposited in the college herbarium for future reference. Fresh drug obtained were dried and coarsely powdered and passed through sieve 100 mesh sizes and stored in air - tight containers for further use.

Preparation of Extract^{13,14, 15, 16.}

The pulverized dried *Lantana camara* leaves were extracted with ethanol using soxhlet apparatus. The powder of *Lantana camara* leaves were also macerated with chloroform water. Ethanolic and water extracts were filtered & evaporated to dryness.

Macroscopic Characteristic^{17,18, 19.}

The macroscopy of fresh leaves were studied according to standard methods.

Microscopic characteristics²⁰

For microscopy hand section of leaf was taken, stained & mounted following usual micro-techniques.

Physical Evaluation^{21,22, 23}

The ash values, extractive values and Loss on drying were performed according to the official methods prescribed in Indian Pharmacopeia.

Fluorescence Analysis

Many drugs shows fluorescence when their powder is exposed to ultraviolet radiation. It is important to observe all materials on reaction with different chemical reagents under U.V. light. The fluorescence characteristics of powdered drug were studied under U.V. light after treating with different chemical reagents is reported. Fluorescence analysis was carried out according to the method of Chase and Pratt²⁴ and Kokoski.²⁵

Phytochemical Screening

The dried leaves were extracted with ethanol and water and preliminary chemical tests for ethanolic and aqueous extracts were carried out according to the standard procedures described by Kokate²⁶ and Horborne.²⁷

Antimicrobial study^{28,29 30, 31}

Collection of microbes

Bacterial strains such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* were used for the study. The collected microbes were maintained in Nutrient agar broth and cultured in Nutrient Agar media. (Hi Media (P) Ltd Mumbai).

Preparation of the medium

Nutrient agar medium was prepared by dissolving 2gm of nutrient agar in 100 ml of distilled water. The solution was sterilized in an autoclave at 121°C for 15 min. It was cooled and poured into sterile petri dishes to solidify. The agar depth of the medium was measured (1.3 cm).

Determination of antimicrobial activity

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) plates were swabbed (sterile cotton plug) with 8 hour old-broth culture of respective bacteria. Three wells (10mm diameter) were made in each of the plates using sterile cork borer. About 0.3 ml of different concentration of plant solvent extracts were added using sterilized dropping pipettes in to the wells and allowed to diffuse at room temperature for two hours. The plates were incubated at 37°C for 18-24 hr for bacterial pathogen. Respective proper controls of solvent plant extracts were also maintained. Diameter of the inhibition zones and the values were recorded.

Chromatographic Studies^{32,33, 34}

Thin Layer Chromatography studies were carried out for extracts to confirm the presence of different phytoconstituents in these extracts. TLC is a mode of liquid chromatography, in which the extract is applied as a small spot or band at the origin of thin sorbent layer supported on a glass. The mobile phase migrates through the stationary phase by capillary action. The separation of solutes takes place due to their differential absorption/ partition coefficient with respect to both mobile and stationary phases. Each separated component has same migration time but different migration distance. The mobile phase consists of a single solvent or a mixture of solvents. Although, a number of sorbent like silica gel, cellulose, polyamide, alumina, chemically modified silica gel etc. are used, silica gel (type 60) is most commonly used sorbent. Handmade plates are prepared by using techniques like pouring, dipping or spraying. The retardation factor (R_f) is calculated using following formula,

$$R_f = \frac{\text{Distance traveled by sample from base line}}{\text{Distance traveled by solvent from base line}}$$

Thin Layer Chromatography^{35,36, 37}

The extracts were subjected to thin layer chromatography for the presence of phyto-constituents. In this technique, the Silica gel-GF254 (for TLC) was used as an adsorbent and plates were prepared by spreading technique, then air dried for an overnight and activated for one hour at 110°C and used.

Preparative Thin Layer Chromatography

A thick layer of silica gel GF-254 was coated on the plate and activated at 110°C for one hour. The broad band (2 mm width) of extracted sample was applied on the plate.

Characterization of Isolated Compound

From the separated bands, the substance of interest was scrapped from the plate and it dissolved in methanol. The mixture was filtered and the filtrate was evaporated to dryness. The isolated compound was then subjected for further studies. (1 mg/ml concentrations of the extracts were used).

IR of isolated compound^{38,39, 40, 41}

IR spectrum was recorded in IR- spectrometer in 400-4000 frequency in cm^{-1} for isolated moiety. IR spectrum of compound was carried in KBR pellet and the important absorption can be correlated.

RESULT

Macroscopic Characteristic of *Lantana camara* Leaf

Fresh leaf of *Lantana camara* is green in colour. It is simple type of leaf having ovate shape with hairy surface and reticulate venation. Leaf possesses acute apex and symmetric base with crenate margin.

Microscopic Characteristics of *Lantana camara* Leaf

The Transverse Section of leaf is dorsiventral consists of Midrib and Lamina.

Midrib

It consist of single layered closely arranged elongated cells externally covered with striated cuticle on either sides of leaf named as upper and lower epidermis. Leaf surface contains simple, unicellular covering trichomes, glandular trichomes and paracytic type of stomata. Below the upper epidermis 3-4 layers of well developed more or less isodiametric collenchymatous tissues were observed.

Midrib contains centrally located vascular bundle which is collateral surrounded by some parenchymatous cells. Xylem is well developed and the phloem consists of strands of sieve tubes and small celled parenchyma.

Lower epidermis consist of single layer of elongated cells with cuticle and just above the lower epidermis 2-3 layers of parenchymatous cells followed by the layers of collenchymatous cells were present. Calcium-oxalate crystals were found in spongy parenchyma.

Covering trichomes are more in number as compared to glandular trichomes.

Lamina

It consists of Dorsi-ventral structure with single layered upper and lower epidermis. Below upper epidermis single layered palisade cells followed by 5-7 layers of mesophyll parenchyma which are rounded in shape and are devoid of intracellular spaces.

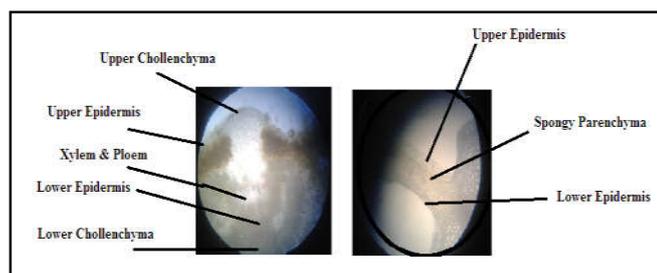


Figure No 2 Microscopy of *Lantana camara* leaf

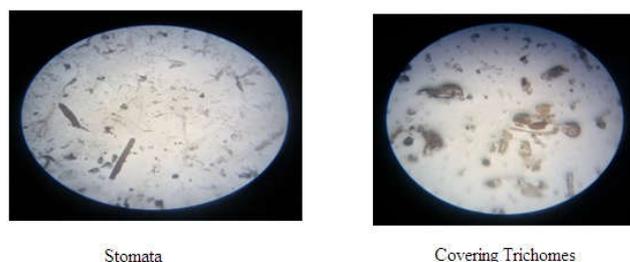


Figure No 3 Powder microscopy of *Lantana camara* leaf

Physical evaluation

The Loss on Drying, Ash Values (Total Ash, Acid insoluble ash, and Water soluble ash), ethanol soluble extractive, Water soluble extractive of leaf powder are given in table-1.

Table No. 1 Physical Constants of *Lantana camara* Leaves

Sr. No.	Physical Constants	Result
1.	Ash Value (% w/w)	
	• Total Ash	8.3
	• Acid Insoluble Ash	2.15
	• Water Soluble Ash	0.45
2.	Loss on Drying (% w/w)	87.3
3.	Extractive Values (% w/w)	
	➤ Ethanol soluble extractive.	25.12
	➤ Aqueous soluble extractive	29.3

Fluorescence Analysis

The fluorescence characteristics of powdered drug were studied under U.V. light after treating with different chemical reagents is reported.

Table No.2 Result of Fluorescence Analysis of *Lantana camara* Leaves.

Reagents	Fluorescence Observed		Reagents	Fluorescence Observed	
	At 254nm	At 366nm		At 254nm	At 366nm
Powder + 1N NaOH In Methanol	Green	Light Green	Powder + Chloroform	Dark Green	Faint Green
Powder + 1N NaOH In Water	Dark brown	Light Green	Powder + Picric Acid	Yellow	Dark Green
Powder + 50% HCl	Yellowish Green	Faint Green	Powder + 5% FeCl ₃ Iodine	Dark brown	Faint Green
Powder + 50% H ₂ SO ₄	Dark Green	Light Green	Powder + 5% Iodine	Dark brown	Dark Green
Powder + 50% HNO ₃	Dark Yellowish	Yellowish	Powder + Methanol	Black	Dark Green
Powder + Petroleum Ether	Dark Yellow	Faint Green	Powder + (HNO ₃ +NH ₃)	Brown	Light Green

Table No. 3 Phytochemical investigation of Ethanolic and Aqueous extracts of *Lantana camara*.

Sr No.	Name of the test	Leaf Extracts	
		Ethanolic extract	Aqueous extract
1.	Test for sterols	+	+
2.	Test for Triterpenoids	+	-
3.	Test for glycosides	+	+
4.	Test for carbohydrates	+	+
5.	Test for alkaloids	-	+
6.	Test for flavonoids	+	+
7.	Test for tannins	+	+
8.	Tests for proteins	-	-
9.	Test for amino acids	-	-
10.	Test for fats	+	-
11.	Test for Volatile oils	-	-

Table No.4 Antimicrobial activity of Ethanolic extracts of *Lantana camara* leaf.

Organism	Diameter of inhibition zone in cm		
	Ethanolic extract		Streptomycin 100(µ/ml)
	300(µ/ml)	500(µ/ml)	
<i>Staphylococcus aureus</i>	2.3	4	4
<i>Escherichia coli</i>	2.4	3.2	3.4
<i>Salmonella typhi</i>	2	3	3.5
<i>Pseudomonas aeruginosa</i>	2.1	2.6	3.2

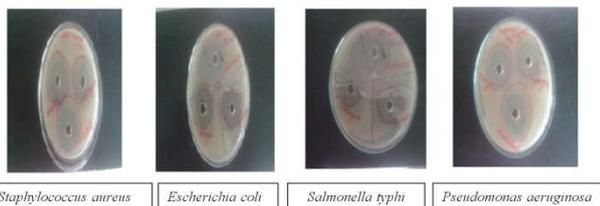


Figure No 4 Antimicrobial activity of Ethanolic extracts of *Lantana camara* leaf.

Table No.5 Antimicrobial activity of Aqueous extracts of *Lantana camara* leaf.

Organism	Diameter of inhibition zone in cm		
	Aqueous Extract		Streptomycin 100(µ/ml)
	300(µ/ml)	500(µ/ml)	
<i>Staphylococcus aureus</i>	2.3	2.9	3
<i>Escherichia coli</i>	1.9	3	3.2
<i>Pseudomonas aeruginosa</i>	2.2	3.1	3.4
<i>Salmonella typhi</i>	2.3	3.2	3.5

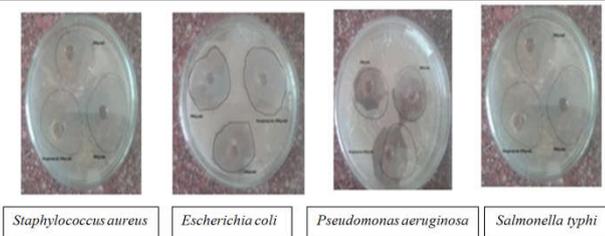


Figure No 5 Antimicrobial activity of Aqueous extracts of *Lantana camara* leaf.

Thin layer chromatography:

Thin layer chromatography of Aqueous extract

Stationary phase: Silica gel GF-254
 Mobile Phase: Toulene: ethyl acetate: Formic Acid (8.5:1:0.5).
 Detection: UV-366
 Solvent front: 4.9
 Spot detection: 2.3

Thin layer chromatography of Ethanolic extract

Stationary phase: Silica gel GF-254
 Mobile Phase: Toulene: ethyl acetate: Formic Acid (8.5:1:0.5).
 Detection: UV-366
 Solvent front: 4.8
 Spot detection: 2.2



TLC of Ethanolic extracts TLC of Aqueous extract

Figure No 6 Thin layer chromatography of Ethanolic and Aqueous extracts

Table No 6 TLC Profile of steroids

Extracts	Observation		R _f values
	No. of spots	Colour of spots	
Ethanolic	1	Yellow	0.45
Aqueous	1	Yellow	0.46

IR Spectroscopy

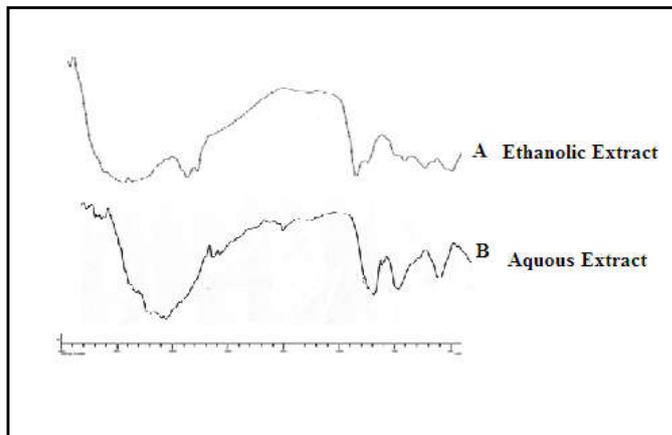


Figure No. 7 IR Spectra of Ethanolic and aqueous extracts of *Lantana camara*.

Table No.7 IR Spectral peaks of Ethanolic extract of *Lantana camara*.

Peak Observed	Assignment	Absorption Expected (cm ⁻¹)
719.45	Alkanes	600-1500
1035.77	Alcohol, Ether, Esters	1000-1300
1373.32	Nitro compound	1330-1540
1635.34	Alkenes	1620-1680
1722.47	Aldehydes, Ketones	1680-1760
2924.09	Hydrogen bonded Acid	2500-3000
3061.09	Aromatic Ring	3000-3100
3224.98	Amines	3300-3500

Table No.8 IR Spectral peaks of Aqueous extract of *Lantana camara*.

Peak Observed	Assignment	Absorption Expected (cm ⁻¹)
792.84	Alkanes	600-1500
1188.15	Alcohol, Ether, Esters	1000-1300
1531.63	-C=C-	1400-1600
2918.30	Hydrogen bonded Acid	2500-3000
3140.11	-CH	3050-3150
3219.19	-OH	3200-3400

DISCUSSION

- The leaves were collected from Satara district, Maharashtra region and authenticated. The leaves were subjected for Pharmacognostic investigation which includes determination of physical constants such as ash value, extractive values, loss on drying and fluorescence analysis. The powder of leaves shows fluorescence at 254nm and 366nm.
- Macroscopic and microscopic characteristics of the leaf were studied. The microscopic study shows that it contains midrib and lamina portion. The lamina shows upper and lower epidermis, spongy parenchyma, palisade cell layer while midrib portion shows upper

and lower epidermis, collenchyma, vascular bundles, etc., Powder characteristics shows presence of paracytic stomata and covering trichomes.

3. The leaves of plant were subjected to extraction by using ethanol and water and these extracts were subjected to phytochemical investigation.
4. Phytochemical investigation of extracts of *Lantana camara* shows that aqueous extract contains sterols, glycosides, carbohydrates, alkaloids. While ethanolic extract shows presence of sterols, flavonoids, glycosides, carbohydrate, alkaloids and tannins.
5. Chromatographic study of the extracts was carried out. Where Thin layer chromatography were carried out by using mobile phase Toulene: Ethyl acetate: Formic Acid (8.5:1:0.5) which shows R_f value 0.45 and 0.46 for steroids for ethanolic and aqueous extract respectively.
6. For these isolated compounds infra red spectroscopy was carried out which shows that ethanolic extract contains aromatic ring, hydroxyl group, amines, esters, alkenes, aldehydes, nitro group, etc. While aqueous extract shows alkenes, aldehyde, acidic group, esters, etc.

CONCLUSION

Lantana camara is widely found in India during any season. As there is less information available on pharmacognostical work on leaves hence morphological study, microscopical studies, physico-chemical parameters, fluorescence analysis and chemical tests performed will guide in the proper identification of the plant species as well as help in authentication of the purity of the plant. All these parameters also help to build up a suitable plant profile.

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