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Pharmacognostical, phytochemical and antimicrobial studies on the leaves of *Lantana camara* Linn.

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ABSTRACT

Lantana Camara. is a common weed that grow abundantly in our surround, however the weed used traditionally for many ailments like Malaria, Epilepsy, Dysentery, Eczema etc. During our experiment the pharmacognostical study was done for the identification from the adulterants where as the phytochemical study reveals it contains many phytoconstituents like alkaloids, glycosides, flavanoids, tannins etc. The anti-microbial activity of the petroleum ether, methanolic extract and water extract of Lantana Camara were investigated.. During the study the MIC value and zone of inhibition of bacteria and fungi were compared to standard drug ciprofloxacin and Fluconazole respectively. Petroleum ether and Methanolic extract of Lantana camara only when compared with Standard drug the activities were less potent but can be useful if explored either alone or in combination with other antimicrobial agents.

Key Words: *Lantana camara*,, Microscopy, Physicochemical property, Antimicrobial activity, Anti fungal property, cup-plate method.

INTRODUCTION

Life on the earth is a gift from nature and plants which are inherent part of nature are a great boon to mankind. The very existence of mankind is threatened by various diseases. As life is precious, it has to be protected. So in order to survive, man had to learn to identify plants that were useful as food or as drug. Due to toxicity and harmful effect associated with long use of synthetic drugs the use of plants as source of medicine has been considerable increased. The use of plant based medical therapeutics by primitive man is as old as the history of man himself. As civilization advances, these particles tend to disappear from our sight. A great deal of awareness in therefore generated amongst scholar to focus on plant based crude drugs and validates their folk claims for their pharmacy-chemical properties. The wider acceptability of herbal therapeutics by the society in relation to modern drugs in another driving force for rapidly ongoing research in the field of alternate medicinal systems. As a step in this direction we focused our attention on one of the selected medicinal plants *Lantana camara* belonging to family Verbenaeceae. Literature survey revealed that traditionally it is used as Carminative, Antispasmodic, Tonic, Tetanus, Malaria, Epilepsy etc. So the collected information prompted us to take up Pharmacognostical, Phytochemical and antimicrobial studies on the leaf of the plant in our laboratory.

MATERIALS AND METHODS

Collection of plant material

The leaves of *lantana camara* were collected from the periphery of R.C.P.H.S campus in the month of august and September.

Date of collection: 12-11-2011 Time of collection: 10 A: M
Date of collection: 14-11-2011 Time of collection: 10 A: M

Authentication: The collected plant was authenticated by Dr.P.Lakshminarasimhan, scientist, Central National Herbarium, Botanical survey of India, Howrah (Authenticated no. NH/27/2011/Tech.II/483).

PREPARATION OF EXTRACT: The collected part of the plant such as leaves were separated and cut into small pieces to accelerate shade drying, after drying the prepared course powder was subject to Soxhelation. Same procedure was followed for extraction of *lantana camara* with petroleum ether and water.

MICROSCOPIC STUDY

Quantitative microscopy: Quantitative microscopy like Stomata number, Stomatal index, palisade ratio, Vein islet number and Vein termination number were determined.

Qualitative microscopy: Qualitative microscopic evaluation was carried out by taking transverse sections of fresh Leaf of *lantana camara* . The thinnest section was selected and cleared by boiling with chloral hydrate solution for 20 minutes and then observed under microscope.

Powder microscopy: A judicious amount of Powder was taken in a glass slide; it was added with few drops of chloral hydrate and observed under microscope.

Physical evaluation: The determination of various physicochemical parameters such as Extractive values, Moisture content, total ash, Acid insoluble ash were calculated

Phytochemical screening: Phytochemical screening, powder was subjected to various chemical test to determine the presence of various phytoconstituents like alkaloids, glycosides, flavonoids etc.

Anti-bacterial studies:

Nutrient Broth (single strength): Nutrient broth of following composition was used for revival, culturing and sub culturing of the bacteria.

Nutrient Agar Media:
Peptone : 10 gm
Sodium chloride : 5 gm
Beef extract : 10 gm
Nutrient Agar : 30 gm
Distilled water : 1.0 L
p^H adjusted 7.2 to 7.4

Bacterial strains: The required strains like *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus faecalis* were procured from Institute of Microbial Technology, Chandigarh.

ANTI-FUNGAL STUDIES:

Media for *Candida albicans*

Sobouraud-Dextrose agar media:
Peptone (meat and casein) :10gm
Dextrose monohydrate : 40gm
Nutrient agar : 30gm
Distilled water : 1000 ml.
pH adjusted 5.4 to 5.8

Media for *Malassezia furfur*

Emmons's Modification of Sabouraud's Agar medium:
Special peptone : 10gm
Agar : 20gm
Dextrose monohydrate : 20gm
Sterile corn oil : few drops
Distilled water : 1000 ml.
pH adjusted to 6.8 to 7.0

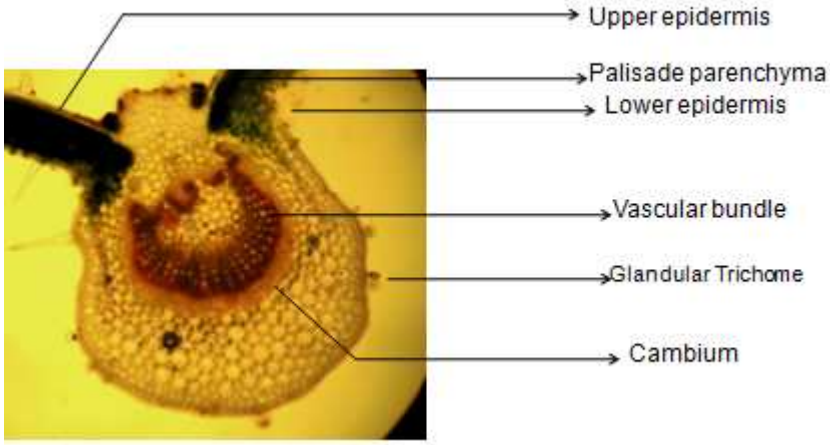
Fungal stains: *Candida albicans* MTCC 227, *Malassezia furfur* MTCC 1374

Methods: (Cup-Plate method)
Stock solutions of all extracts were prepared in the concentrations of 5mg/ml with sterile DMSO (Petroleum ether extract), sterile 15% DMSO (Methanolic extract) and sterile water (water extract) and measured quantities of stock solutions were diluted to prepare concentration of 2000, 1000, 500, 250, 125, 62.5, 31.25 mcg/ml of the extracts. Twenty four hours cultures of required bacterial strains were maintained. Half ml (for 100 ml of media) of 24 hrs culture of bacteria was inoculated into the nutrient agar at 40-50°C. Inoculated media was poured into the Petri plate up to 4-5 mm thickness (30ml) and allowed to solidify. Cylinder/cups were prepared by using sterile cork boarer of 5 mm internal diameter. About 0.1 ml of the different concentrations of the extract was added to the cups. The well on Petri plates were loaded with solvent (100% DMSO, 15% DMSO and water) & Ciprofloxacin, Fluconazole simultaneously. Care was taken to prevent the overflow of extract from cup. The extract was allowed to diffuse uniformly, by keeping it in refrigerator for 1 hr. The Petri plates were incubated for 24hrs at 37°C and the zone of inhibition was measured. for the fungi the plate were incubated for 5 days at 37°C and zone of inhibition was measured.

RESULT AND DISCUSSION

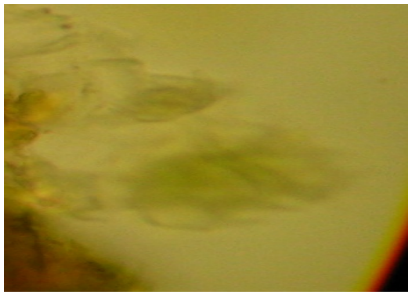
MICROSCOPY: T.S of Leaf:
A transverse section of the leaf of Lantana camara through midrib showed the presence of following characters.

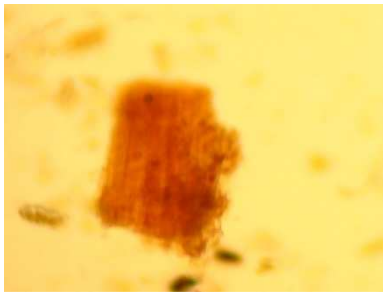
- Upper epidermis
- Palisade parenchyma
- Lower epidermis
- Vascular bundle
- Glandular trichome
- Cambium
- Spongy tissue



Powder Microscopic Observations:
During the powder microscopic study of leaf of lantana camara following observations were made like trichomes, stomata, vessels etc.


Unicellular Trichome


Paracytic Stomata


Spiral vessels

(a) Stomatal number and Stomatal index:
Stomatal number is defined as the average number stomata per square mm of epidermis of leaf. Stomatal index is the percentage proportion of the ultimate divisions which has been converted into stomata.

Table no.-I: Determination of stomatal number and stomatal index of *Lanatana camara*

Parts used	Stomatal number/mm^2	Epidermal cell/mm^2	Stomatal index
Lower Epidermis	22	55	28.27
	19	62	23.45
	24	61	28.23
	23	57	28.75
	17	48	26.15
	20	56	23.31
Upper Epidermis	15	105	12.5
	18	110	14.06
	14	107	11.5
	16	102	13.5
	16	104	13.3
	13	104	11.0
	14	106	11.6
	15	104	12.6
	16	106	13.1
	14	105	11.7

Lower Epidermis-
Average stomatal number 20.8
Average stomatal index 26.4
Upper Epidermis-
Average stomatal number 14.0
Average stomatal index 24.1

(b) Palisade ratio: The average number of palisade cells beneath each upper epidermal cell is termed as palisade ratio.

Table no.-II: Palisade ratio determination of *Lantana camara* Linn

Sl. No.	Epidermal cells	Palisade cells			Palisade ratio		
		Base	Middle	Apex	Base	Middle	Apex
1	4	40	42	39	10	10.5	9.75
2	4	39	38	45	9.75	9.5	11.25
3	4	46	44	43	11.5	11	10.75
4	4	41	42	37	10.25	10.5	9.25
5	4	43	40	42	10.75	10	10.5
6	4	42	37	42	10.5	9.25	10.5
7	4	44	44	43	11	11	10.75
8	4	40	40	41	10	10	10.25
9	4	43	43	44	10.75	10.75	11
10	4	38	48	42	9.5	10	10.5

Average palisade ratio:
Base 10.40
Middle 10.30
Apex 10.45

(c) Vein-islet number: Vein-islet number is a term used to indicate the minute areas of photosynthetic tissues and encircled by the ultimate divisions of the vascular strands. Vein-islet number is defined as the number of vein-islet per square mm.

Table no.-III: Determination of vein-islet number sq.mm

Sl. No.	No. of vein islet per mm^2
1	12
2	10
3	13
4	14
5	13
6	11
7	13
8	12
9	10
10	11

Average Vein-islet number 11.9

(d) Vein termination number: It is defined as the number of veinlet termination per mm^2 of the leaf surface, mid rib and margin.

Table no.-IV: Determination of vein termination no. of *Lantana camara*

Sl. No.	No. of vein termination per mm^2
1	8
2	8
3	10
4	8
5	9
6	8
7	10
8	7
9	9
10	10

Average Vein termination number 8.7

Physical evaluation:

Ash value: The residue left after incineration of a drug is called ash. Ash values are helpful in determining the quality and purity of a crude drug. Crude drug normally leave an ash usually contain carbonates, phosphates and silicates of potassium, sodium, calcium and magnesium.

Table no.-V: Determination of ash value *Lantana camara* Linn. Leaf

Sl.No	Parameter	Average Value in Percent
1	Total ash	8.06
2	Water soluble ash	0.95
3	Acid insoluble	1.96

Extractive value:

An extractive value gives the nature of the constituents present in the crude drug material.

Table no.-VI: Determination of water soluble and alcohol soluble extractive value of *Lantana camara* Linn. Leaf

Sl.No	Parameter	Average Value(%) w/w
1	Water soluble extractive value	27.5
2	Alcohol soluble extractive value	25.1

Phytochemical studies:

Different phytoconstituents and their amount were observed from different extracts.

Table no.-VII: Phytoconstituents present in different extracts of *Lantana camara* Linn. Leaf

Phytoconstituents	Extract			
	Water	Methanol	Petroleum ether	Toluene
Alkaloids	++	+	-	-
Carbohydrates	++	++	-	-
Flavanoids	+	+	-	-
Glycosides	+	+	-	-
Saponins	+	-	-	-
Sterols	-	-	-	-
Steroids	-	-	+	+
Triterpinoids	-	++	++	-
Tannins	+++	++	-	-
Proteins	-	-	-	-

++=Positive test, +=Positive test but in small amount, - =Not found

Anti-bacterial activity:

Table no. VIII-: Zone of inhibition (ZOI) Petroleum ether extract, methanolic extract, water extract and Ciprofloxacin against *Escherichia coli*.

Sl. No.	Conc. of extracts (µg/ml)	Conc. Of Ciprofloxacin (µg/ml)	Average zone of inhibition (diameter in mm)			
			Ciprofloxacin	Petroleum ether extract	Methanolic extract	Water extract
1	2000	-	-	16	14.5	-
2	1000	-	-	15	13	-
3	500	500	47	14.5	12	-
4	250	250	45	14	10.5	-
5	125	125	44	11.5	-	-
6	62.5	62.5	42	9	-	-
7	31.25	31.25	35	-	-	-

Table no.IX-: Zone of inhibition (ZOI) of petroleum ether extract, methanolic extract, water extract and Ciprofloxacin against *Bacillus subtilis*

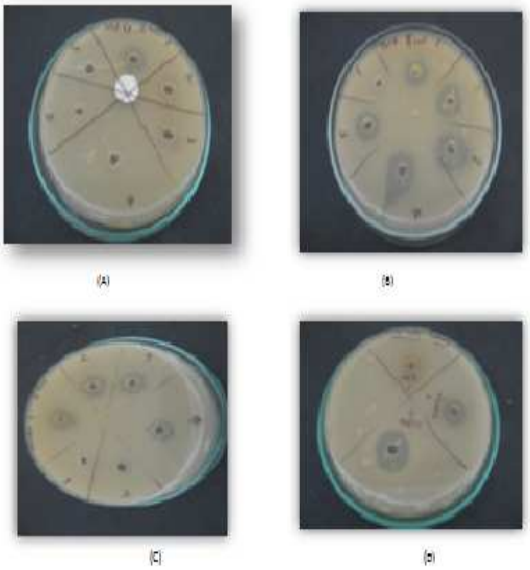
Sl. No.	Conc. of extracts (µg/ml)	Conc. of Ciprofloxacin (µg/ml)	Avg zone of inhibition (Diameter in mm)			
			Ciprofloxacin	Petroleum ether extract	Methanolic extract	Water extract
1	2000	-	-	16	12.5	-
2	1000	-	-	15	12	-
3	500	500	46	14	11	-
4	250	250	44	13	10	-
5	125	125	40	12	-	-
6	62.5	62.5	37	10	-	-
7	31.25	31.25	28	-	-	-

Table no .X-: Zone of inhibition (ZOI) of Petroleum ether extract, methanolic extract, water extract and Ciprofloxacin against *Pseudomonas aeruginosa*

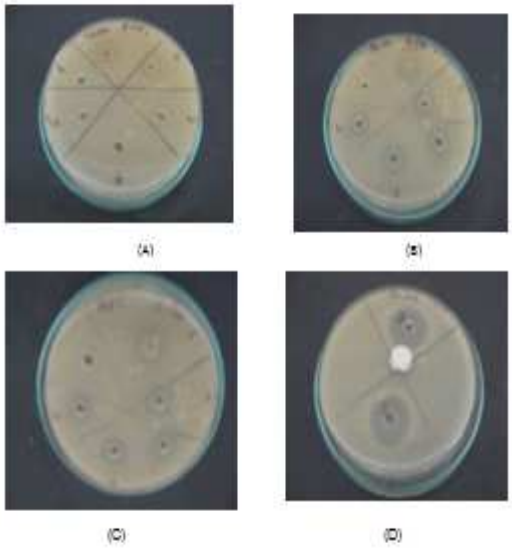
Sl. No.	Conc. Of extract (µg/ml)	Conc. Of Ciprofloxacin (µgm/ml)	Avg. zone of inhibition (Diameter in mm)			
			Ciprofloxacin	Petroleum ether extract	Methanolic extract	Water extract
1	2000	-	-	14.5	14	-
2	1000	-	-	13.5	13.5	-
3	500	500	46	12.5	12	-
4	250	250	44	12	10.5	-
5	125	125	42	10	8	-
6	62.5	62.5	35	8	-	-
7	31.25	31.25	27	-	-	-

Table no.XI-: Zone of inhibition (ZOI) of petroleum ether extract, methanolic extract, water extract and Ciprofloxacin against *Staphylococcus faecalis*

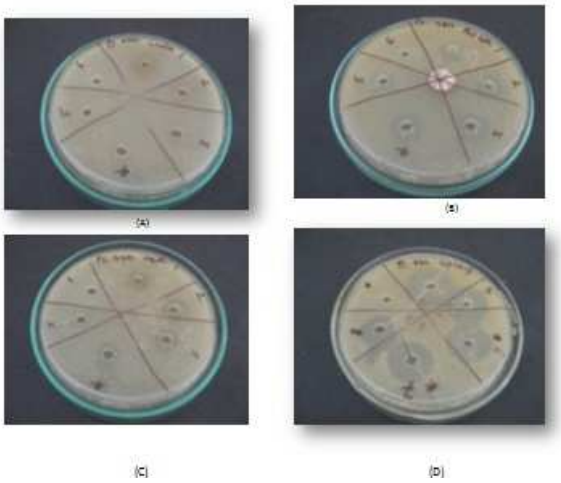
Sl. No.	Conc. Of extract (µg/ml)	Conc. of Ciprofloxacin (µg/ml)	Avg. zone of inhibition (Diameter in mm)			
			Ciprofloxacin	Petroleum ether extract	Methanolic extract	Water extract
1	2000	-	-	15.5	13.5	-
2	1000	-	-	14	12.5	-
3	500	500	43	13	11	-
4	250	250	42	12	10.5	-
5	125	125	40	10.5	9	-
6	62.5	62.5	32	-	-	-
7	31.25	31.25	27	-	-	-



Pic.-1
Zone of Inhibition against *E.Coli*

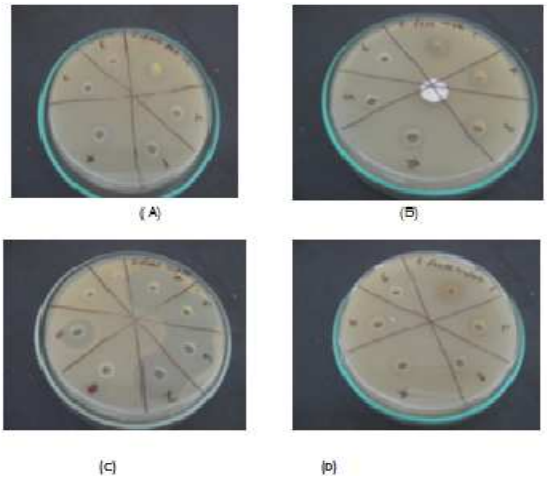


pic.-II
Zone of Inhibition against *Bacillus subtilis*



Pic-III

Zone of Inhibition against *Pseudomonas aeruginosa*



Pic-IV

Zone of Inhibition against *Staphylococcus faecalis*

A) Water extract (B) Petroleum ether extract (C) Methanolic extract (D) Ciprofloxacin standard

Anti-fungal activity:

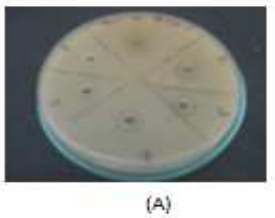
Fluconazole was used as standard drug for the comparison antifungal activity.

Table no. XII-: Zone of inhibition (ZOI) of petroleum ether extract, methanolic extract, water extract and Fluconazole against *Candida albicans*

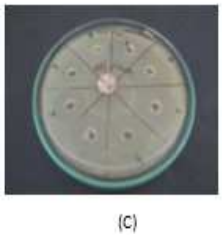
Sl.No.	Conc. Of extracts (µg/ml)	Conc. Of Fluconazole (µg/ml)	Avg. zone of inhibition (Diameter in mm)			
			Fluconazole	Petroleum ether extract	Methanolic extract	Water extract
1	2000	-	-	11.5	9	-
2	1000	1000	42	10	7.5	-
3	500	500	39	8	-	-
4	250	250	37	6.5	-	-
5	125	125	25	-	-	-
6	62.5	62.5	22	-	-	-
7	31.25	31.25	18	-	-	-

Table no.XIII-: Zone of inhibition (ZOI) of Petroleum ether extract, Methanolic extract, Water extract and Fluconazole against *Malassezia furfur.*

Sl.No.	Conc. of extract (µg/ml)	Conc. of Fluconazole (µg/ml)	Avg. zone of inhibition (Diameter in mm)			
			Fluconazole	Petroleum ether extract	Methanolic extract	Water extract
1	2000	-	-	13.5	10.5	-
2	1000	1000	39	12	10	-
3	500	500	35	11	9	-
4	250	250	28	10	-	-
5	125	125	25	9.5	-	-
6	62.5	62.5	22	-	-	-
7	31.25	31.25	20	-	-	-



Pic-V
Zone of Inhibition against *Candida albicans*



Pic-VI
Zone of Inhibition against *Malassezia furfur*

(A) Petroleum ether extract (B) Methanolic extract (C) Fluconazole standard

CONCLUSION

From the phytochemical investigation it was found that in Lanata leaf alkaloid, volatile oil,tannins. sugars and saponin glycosides are present. After performing the antimicrobial activity by using late method we found that petroleum ether extract has excellent antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa* , *Bacillus subtilis* and *Staphylococcus faecalis* at the various concentration but the results obtained are not much comparable with standard drug Ciprofloxacin. Similarly Petroleum ether extract and methanolic extract showed good and significant antifungal **activity** respectively against *Malassezia furfur* at the various concentrations. But these extracts are not much comparable with standard drug fluconazole. can be further studied for developing Herbal antibiotic Preparation, Herbal antidandruff hair oil and shampoo.

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