PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION AND ANTIPYRETIC ACTIVITY OF LEAVES OF LANTANA CAMARA LINN.

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Abstract

*Lantana camara* is a heavily branched shrub that can grow in compact clumps, dense thickets or as a climbing vine. The plant is Antidiabetic, Antiseptic, Anti-inflammatory, Antipyretic, Carminative, Anti-spasmodic, Diphoretic and are useful for cuts, wounds, ulcers, swelling, tumours and rheumatism. Evaluation of fresh, powdered and anatomical sections of the leaves was carried out to determine the morphological, microscopical and phytochemical profiles. Leaves of *L. camara* are dark green with characteristic odour, opposite, blade ovate, 4-10cm long, with coarse surfaces and toothed margins etc. Microscopy shows covering trichomes, Sclerenchyma, collenchyma, xylem, phloem etc. Phytochemical evaluation revealed the presence of alkaloids, triterpenoid, essential oil, flavonoids, carbohydrates, tannins and saponins. The investigations also included evaluation of physical parameter such as the moisture content, ash values, and extractive value. Concerning on the Antipyretic activity, Lantana camara ethanolic and ethyl acetate extract start lowering the body temperature from 1.5th hour, while between 2nd and 3rd hour both the extract showed significant (P<0.01) Antipyretic activity when compared with the negative control group by one way ANOVA followed by dunnett’s t-test.

Keywords: *Lantana camara* Linn, Phytochemical screening, TLC, Antipyretic activity

1. Introduction

Any plant and its parts containing any substance which can be used therapeutically, or can be used as raw material for Chemical/Pharmaceutical synthesis is categorized as herbal medicine.1,2. *Lantana camara* L. (Verbanaceae), universally known as wild or red sage is the most widespread species of this genus and regarded both as a notorious weed and a popular ornamental garden plant. However, it is listed as one of the important medicinal plants of the world. *L. camara* whole plant and plant parts viz., leaves, flowers, and essential oils have been thoroughly studied for their chemical compositions.3,5,6,16,17. The methanolic extract of Lantana camara leaves shown 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 infection2 Anti Diabetic, Anti spasmodic, Diaphoretic, Carminative, Tonic and useful in the treatment of tinitus, vitiated 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 and swelling and infusion of the leaves is good for bilious fever, eczema and eruption. the fruits are useful in fistula, pastules, tumours and rheumatism18,19,20,21. Antidiabetic, Antiseptic, Anti-inflammatory, Carminative, Anti-
spasmodic, Diphoretic and wound healing activity have been previously done on this plant.

There are no data available regarding its antipyretic activity of *Lantana camara* leaf there for, the present study was undertaken to evaluate the antipyretic activity of ethanolic leaf extract in rabbits.

2. Materials and Methods:

2.1. Plant material: Leaves of *Lantana camara* plant were collected from the campus of the TIT College of Pharmacy campus Bhopal, Madhya pradesh India during the period from September to December 2010. The identification of the plant was done at Safia College of science, Peergate, Bhopal, Madhya Pradesh. The voucher specimen no. 236/Bot/Safia/Lantana camara Linn was submitted in Department of Pharmacognosy TIT college of Pharmacy, Bhopal, Madhya Pradesh.

2.2. Pharmacognostical study

2.2.1. Macroscopical evaluation:

Organoleptic evaluation of drug refers to the evaluation of drugs by color, odour, size, shape, taste and special features including touch and texture etc. Organoleptic evaluation can be done by means of organs of special sense which includes the above parameters and thereby define some specific characteristics of the material which can be considered as a first step towards establishment of identity and degree of purity.\(^\text{12}\)

2.2.2. Microscopical evaluation:

Microscopical parameters observed were, Unicellular covering trichomes with pointed head, Phloem, glandular trichomes with unicellular head & stalk, collenchyma, palisade cells at upper side, vascular bundles, selerenchymatus layers etc. All determination was carried out by using Almicro compound microscope (10x, 40 xs) attached with a camera.\(^\text{12}\)

2.2.2.1. Powder microscopy. The dried Leaves of plant *Lantana camara* Linn were powderd and sieved to obtained fine powder. It was taken up for powder microscopy evaluation as follows:

A small quantity of powder was kept on a slide and after mounting on glycerine, 10min were provided as spared out time. Finally, it was observed for powder microscopical characters.\(^\text{12}\)

2.3. Physicochemical studies

Physicochemical studies include ash value and extractive value to determine the quality and purity of the powder of plant of *Lantana camara* Linn.

2.3.1. Determination of Ash-value

2.3.1.1. Total ash: Weigh accurately previously weighed and tarred crucible. Add 2-4gm of ground material. Ignite the material by increasing the heat to 500-600°C. Cool in desiccators and weigh. If carbon free ash cannot be obtained in this manner, cool the crucible and moisten the residue with 2ml. of water or a saturated solution of ammonium nitrate R. Dry on a water bath or hot plate and ignite. Allow the residue to cool for 30min. and weigh. Calculate content of total.\(^\text{10}\)

2.3.1.2. Acid-insoluble ash: To the crucible containing total ash, add 25 ml of hydrochloric acid, cover with a watch glass and boil gently for 5 minutes. Rinse the watch glass with 5ml of hot water and add this liquid to crucible. Collect the insoluble matter on an ash less filter paper and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, and then ignite at 450-500°C to constant weight. Cool in dedicator for 30 min and weigh without delay. Calculate
the content of acid-insoluble ash in mg per gram of air-dried material.\textsuperscript{10}

\textbf{2.3.1.3. Water-soluble ash:} To crucible containing total ash, add 25ml. of water and boil for 5min. collect mater on ash less filter paper. Wash with hot water and ignite for 15min. at temperature not exceeding 450$^\circ$C. Subtract the weight of this residue in mg obtained from total weight of total ash.\textsuperscript{10}

\textbf{2.3.1.4. Loss on drying:} This test determines both water and volatile matter. Drying can be carried out either by heating to 100-150$^\circ$C or by drying in a desiccators over phosphorus pentaoxide R under atmospheric or reduced pressure at room temperature for a specified period of time.\textsuperscript{11}

\textbf{2.3.2. Determination of solvent Extractive value:} Weigh accurately, about 4g of coarsely powdered air-dried material to a glass stoppered conical flask. Macerate with 100ml of the required solvent for the given plant material for 6 hour, shaking frequently then allow to stand for 18 hour. Filter rapidly taking care not to lose any solvent, transfer 25 ml of the filtrate to a treated flat-bottomed dish and evaporate to dryness an a water bath. Dry at 10$^\circ$ Celsius for 6 hour, cool in a desiccators for 30 minutes and weigh without delay. Calculate the contents of extractable matter in mg per g of air-dried material.\textsuperscript{15}

\textbf{2.4. Extraction of plant leaf material:} The powdered plant leaf material was subjected to successive solvent extraction taking from polar to nonpolar solvents like water, ethanol, methanol, acetone, chloroform, benzene and petroleum ether. 20gms of powdered plant material was subjected to soxhlet extraction for 8 hrs with 250ml of the various solvents. The extracts obtained were later kept for evaporation to remove the excessive solvents. These extracts were stored in a cool dry place for the analysis for the presence of preliminary pytochemicals.\textsuperscript{15}

\textbf{2.5. Phytochemical screening}

The aqueous and alcoholic extract of the powdered drug were subjected to various qualitative tests for the identification of various plants constituents present in that species.

\textbf{2.5.1. Tests for Alkaloid:}

- Extract was treated with 1ml of Dragondroff’s reagent. An orange-red precipitate indicates the presence of alkaloid.
- Extract was treated with 1ml of Mayer’s reagent. Whitish yellow or cream-colored precipitate indicates the presence of alkaloids.\textsuperscript{11}

\textbf{2.5.2. Tests for Carbohydrates}

- To 1ml of the extract, add equal quantities of Fehling A and B, upon heating formation of brick red precipitate indicate the presence of sugar.
- To 1ml of Benedict reagent, add 1ml of extract solution and boil.\textsuperscript{11}

\textbf{2.5.3. Test for Tannin:} To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins.\textsuperscript{11}

\textbf{2.5.4. Test for Flavanoids:} Extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow color solution formed, disappear on addition of an avid indicate the presence of flavanoids.\textsuperscript{11}

\textbf{2.5.5. Test for Saponins:} Take small quantity of alcoholic and aqueous extract
separately and add 20ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1 cm. layer of foam indicates the presence of saponins.11

2.5.6. Test for Triterpenoids: Extract (300 mg) was mixed with 5 ml chloroform and warmed for 30 minutes. Few drops of concentrated sulphuric acid was added and mixed well. The appearance of red color indicates the presence of triterpenes.11

2.6. Thin layer chromatography:
TLC is most widely used technique in biological, chemical and pharmaceutical laboratories. In TLC methods, the separation of the components in the material is based principally on adsorption, or partition, or combination of both, depending on the adsorbent and the solvent used. TLC is prepared on Glass plates of good quality, slurry is prepared which is a mixture of silicagel G (stationary phase) and water and spread on the plates, normally a thickness of 0.25 mm is used for analytical purpose, after setting plates are activated by keeping in an oven at 100 degree for 1 hour. After activation spot of the sample is placed above 2 cm on the base of the plate, and the plate is kept in the development tank containing mobile phase of different solvents. After the development of TLC plates the spots are visualized. for detecting, colourless spots are observed by keeping the plates in Iodine chamber.7,8

The ethanolic extract is referred to produce TLC because these extract only having more active constituents. The ethanolic extract showed resolution of spot with following solvent system.7

Ethyl acetate: Methanol = (80:20)

2.7. Antipyretic activity
2.7.1. Animal care and handling: The experiment was carried out on albino rabbits of 5-8 months, of both sexes, weighing between 1.5 to 1.6 kg. They were provided from Sapience Bio-analytical Research Lab, Bhopal, (M.P.) and were kept in mesh bottom iron cages to avoid caprophagy. The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 44 –56% and light and dark cycles of 11 and 13 hours respectively fed with cauliflower, cabbage, carrot and tap water for 10 days before the experiment. Food and water were withdrawn 14 hours prior to the experiment. The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no. 1413/a/11/CPCSEA).

2.7.2. Grouping and treatments of experimental animals: Four animals in each group were taken and total four groups were grouped. Group I (-ve control, vehicle treated), Group II (standard, paracetamol 100mg/kg treated), Group III (ethanolic extract 300mg/kg treated), Group IV (ethyl acetate extract 300mg/kg treated).

2.7.3. Preparation of extract dose: 2% acacia suspension was prepared by suspending 2 gram of accurately weighed acacia powder in 100 ml of 0.9% saline. 20 ml of vehicle was taken separately to which 2 gram of dried extract was added and sonicated, this produce suspension of 100 mg/ml strength. Both ethanolic extract and ethyl acetate extract suspension were prepared in such manner.19

2.7.4. Toxicity study: Both the extract of Lantana camara were tested for acute toxicity test. No toxicity was observed at the doses of 100, 200, 300 mg/kg of body weight. Thus for the screening of antipyretic activity, the dose selected was
300 mg/kg of body weight (maximum safe dose) as per the OECD guidelines (OECD 2000).20

2.7.5. Hyperpyrexia Induced in Rabbits by Typhoid-Vaccine:

- The room temperature was maintained at 22–24°C.
- Only animals with a body temperature of at least 38 °C were taken into the test.
- Typhoid-Paratyphoid A, B vaccine (0.1 ml) was injected into the marginal ear-vein of rabbits of each group.
- Standard and test groups were treated with paracetamol (I.V.) 15 minute and ethanolic extract, ethyl acetate extract (P.O.) 1 hour after the administration of Typhoid-Paratyphoid A, B vaccine respectively while control group was treated with vehicle.
- The rectal temperature each animal was recorded 15 minute before and at the interval of 30 minute after treatment using telethermometer upto 4 hours.19

2.7.6. Statistical analysis

All the values of Body temperature were expressed as mean±standard error of mean (S.E.M.) and analyzed by ANOVA followed by Dunnett's test. Differences between groups were considered significant at $P < 0.01$ and $P < 0.05$ levels.

3. Result and Discussion

Morphology of leaf of Lantana camara Linn is shown in Fig no.1 and Table no.1. Transverse section of Lantana camara Linn shows presence of unicellular covering trichomes with pointed head, xylem and phloem vessels, glandular trichomes with unicellular head and stalk, collenchyma, selerenchyma, palisade cells at upper side and oil glands are shown in Figure No.2. Lantana camara Linn leaves powder shows the presence of fibres, xylem vessel, covering trichomes, calcium oxalate crystal, collenchymas, and parasitic stomata shown in Figure No.3.

The leaves of Lantana camara Linn was collected and analysed the various standardization parameters. Physiochemical parameters of the leaves of Lantana camara Linn are tabulated in Table No.2. Preliminary phytochemical screening was performed in the leaves of Lantana camara Linn.

The leaves are extracted with different solvent and the percentage yield are tabulated in the Table No.3. Quantitative phytochemical analysis is performed in all extracts and the results showed the presence and absence of certain phytochemicals in the drugs. Phytochemical tests revealed the presence of alkaloids, tannins, saponins, flavanoids, carbohydrates, steroids and triterpinoids and results are given in Table No.4.

Thin layer chromatography technique was used to separate the chemical compounds present in the drugs. Various solvent systems were checked to separate the maximum number of chemical compound in the drugs. TLC of ethanolic extract develop in the mobile phase of Ethyl acetate: methanol (80:20) and observed 4 spots shown in fig.no.4. The Rf values were correspondingly calculated and showed in the Table No.5.

Concerning on the Antipyretic activity which was carried out on albino rabbits of either sex, Typhoid vaccine induce “pyrexia” in rabbits due to presence of lipopolysaccharides in it which acts as pyrogens and elevate the body temperature which was treated by paracetamol 100mg/kg, Lantana camara ethanolic and ethyl acetate extracts 300mg/kg. LC
ethanolic and ethyl acetate extract start lowering the body temperature from 1.5th hour, while between 2nd and 3rd hour both the extract showed significant (P<0.01) Antipyretic activity when compared with the negative control group by one way ANOVA followed by dunnett’s t-test.

**Conclusion and Future Prospects**

Preliminary phytochemical as well as various aspects of the leaves sample were studied and described along with physicochemical and TLC studies in authentification adultration for quality control of raw drugs. The leaves of *Lantana camara Linn* exhibits a set of diagnosistic characters which will help to identify the drug in dried condition. It has been concluded from this study that estimation is highly essential for raw drugs or plant parts used for the preparation of compound formulation drug. The periodic esessment is essential for quality assurance and safer use of herbal drugs.

The antipyretic activity of *Lantana camara* could be at least in part due to COX-1, COX-2 enzyme inhibition and free radical-scavenging activities which may be attributed to the presence of flavonoids and other polyphenols in the extracts. The results of this study provided a scientific support for the use of *Lantana camara* in the treatment of Pyrexia.

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Table No.01: Morphology of leaf of lantana camara linn leaves.

| S. No | Criterion     | character   |
|-------|---------------|-------------|
| 1     | Base          | Symmetric   |
| 2     | Shape of Lamina | Ovate       |
| 3     | Leaf Margin   | Crenate     |
| 4     | Leaf Apex     | Acute       |
| 5     | Type of Leaf  | Simple      |
| 6     | Leaf Surface  | Hairy       |
| 7     | Vention       | Reticulate  |
| 8     | Colour        | Dark green  |

Table No.02: Physiochemical parameters of leaves of lantana camara linn leaves

| S.NO. | Ash value              | % W/W |
|-------|------------------------|-------|
| 1     | Total ash              | 10    |
| 2     | Acid insoluble ash     | 2.5   |
| 3     | Water soluble ash      | 5     |
| 4     | Loss of Drying         | 2.5   |
Table No.03: Extractive values of leaves of Lantana camara linn leaves

| S.NO. | Physicochemical parameters           | % w/w |
|-------|-------------------------------------|-------|
| 1     | Chloroform soluble extractive       | 5.9   |
| 2     | Pet.ether soluble extractive        | 5.5   |
| 3     | Acetone soluble extractive          | 5.2   |
| 4     | Alcohol soluble extractive          | 11.2  |
| 5     | Water soluble extractive            | 10.3  |

Table No.04: Phytochemical screening

| S.No. | Experiment | Aqueous Extract | Alcoholic Extract | Pet Ether Extract | Chloroform Extract | Acetone Extract |
|-------|------------|----------------|-------------------|------------------|-------------------|----------------|
| 1     | Alkaloids  | +              | +                 | +                | +                 | +              |
| 2     | Glycosides | -              | -                 | -                | -                 | -              |
| 3     | Tannins    | +              | +                 | -                | +                 | +              |
| 4     | Saponins   | +              | +                 | -                | +                 | -              |
| 5     | Flavonoids | +              | +                 | -                | -                 | -              |
| 6     | Carbohydrates | +       | +                 | +                | +                 | -              |
| 7     | Steroids   | -              | +                 | +                | -                 | -              |
| 8     | Triterpenoids | -        | +                 | +                | +                 | -              |

(+) Present  (–) Absent

Table No.05: TLC analysis of Ethanol extract of Lantana camara linn leaves.

| S.No | Extract          | Solvent system                  | No. of spot | Rf value          |
|------|------------------|---------------------------------|-------------|-------------------|
| 1    | Ethanol extract  | Ethylacetate:Methanol (80: 20)  | 4           | 0.48,0.68,0.85,0.91 |
**Table No. 06: Hyperpyrexia induced in rabbits by typhoid-vaccine**

0.1 ml Typhoid-Paratyphoid A, B vaccine was injected into the marginal ear-vein of rabbits of each group before treatment.

| Group No. | I     | II    | III               | IV    |
|-----------|-------|-------|-------------------|-------|
| Drug      | Vehicle | Paracetamol | LC Ethanolic extract | LC Ethyl acetate extract |
| Dose      | 1 ml/100gm | 100 mg/kg | 300 mg/kg | 300 mg/kg |

**Body temperature at a regular time interval in °C (Mean ±SEM) after treatment**

| Time (hrs) | 0 min | 30 min | 1 hr | 1.5hr | 2hr | 2.5hr | 3hr | 3.5hr | 4hr |
|------------|-------|--------|------|-------|-----|-------|-----|-------|-----|
|            | 37.5±0.21 | 37.2±0.62 | 37.4±0.91 | 37.2±0.51 | 41.17±0.32 | 39.45±0.56* | 40.47±0.21 | 40.15±0.51 | 41.37±0.11 | 39.52±0.43* | 39.87±0.47* | 40.33±0.42 | 41.52±0.18 | 39.15±0.52* | 39.57±0.67 | 39.62±0.58 | 41.65±0.32 | 38.63±0.19** | 39.11±0.34** | 38.71±0.33** | 40.82±0.32 | 38.26±0.21** | 38.61±0.62** | 38.82±0.30** | 40.43±0.22 | 38.23±0.37** | 38.44±0.69* | 38.48±0.28* | 39.82±0.32 | 38.25±0.33 | 38.27±0.25 | 38.21±0.91 |
|            | 38.82±0.21 | 38.11±0.14 | 38.23±0.21 | 38.43±0.19 |

- LC- *Lantana camara*,
- No. of animals in each group = 4
- Data expressed as mean ± S.E.M One way ANOVA followed by Dunnett's t-test
- Group I negative control, II positive control, III and IV test
- All test and standard group were compared with group I (negative control)
- ** P<0.01, * P<0.05

| Time (hrs) | 0   | .5  | 1   | 1.5 | 2   | 2.5 | 3   | 3.5 | 4   |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| F value(3,12) | 0.097 | 4.81 | 4.34 | 4.14 | 22.6 | 8.55 | 5.73 | 2.26 | 2.69 |

**Figure No.01: Morphology of *Lantana camara linn* leaf.**
Figure No. 02 Microscopy of *Lantana camara linn* leaf.

*T.S. OF LEAF OF LANTANA CAMARA*

- Upper epidermis
- Palisade layer
- Lower epidermis
- Covering trichomes
- Xylem vessels
- Phloem vessels
- Glandular trichomes
- Sclerenchyma
- Chollenchyma
- Oil glands

Figure no. 03 Powder characteristics of *Lantana camara linn* leaves.

- Calcium oxalate crystal
- Oil glands
- Xylem vessels
- Covering trichomes
- Fibres
- Paracytic stomata
Figure No.4: TLC of Ethanolic extract of *Lantana camara linn* leaves