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Phytochemical analysis and evaluation of *in vitro* anti-Inflammatory activity of *Lantana camara* L

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ABSTRACT: Background: The plant *Lantana camara* L are widely used in folk medicine in India for the management of various diseases that are antiulcer, anticancer, anti-hypertensive, antiseptic, antimalarial and against asthma. **Aim:** The main aim of the present study is to perform preliminary phytochemical screening and *in vitro* anti-inflammatory activity of the leaves extract of *L. camera* L. **Methods**: The plant *L. camara* leaves was extracted in Soxhlet apparatus. The preliminary phytochemicals was performed as per the standard procedures and *in vitro* anti-inflammatory activity was performed by Fresh egg albumin denaturation assay**. Results:** The plant extract prominently contains phytochemicals that are Flavonoids, Sterols and Tannins. The ethanolic extracts fractions of the leaves of *L. camara* L have significant anti-inflammatory activity in concentration dependent inhibition of protein (albumin) denaturation by all the test fractions throughout the concentration range of 100 to 500 µg/ml. The Diclofenac sodium (at the concentration range of 10 to 50 µg/ml) was used as the standard drug, which also exhibited concentration- dependent inhibition of protein denaturation. **Conclusion**: The present study establishes the anti-inflammatory activity of *L. camera* L leaves.

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INTRODUCTION:

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants [1]. It is a protective attempt by the organism to remove the injurious stimuli as well as to initiate the healing process for the tissue. Inflammation is not a synonym for infection. Even in cases where inflammation is caused by infection, the two are not synonymous: infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen. In the absence of inflammation, wounds and infections would never heal and the progressive destruction of the tissue would

compromise the survival of the organism. However, an inflammation that runs unchecked can also lead to a host of diseases, such as hay fever, atherosclerosis, and rheumatoid arthritis [2,3].

L. camara is an important medicinal plant belonging to family *Verbenaceae*. This plant possess simple leaf with opposite and decussate arrangement. The shape of leaves are ovate and oblong with cuneate, rounded and cordate apex, having crenulate or crenate margin (Fig 1).

Fig 1. The flowering *Lantana camara* plant.

The leaves possess several therapeutic properties that are used to treat cuts, rheumatisms, ulcers, catarrhal infection, tetanus, rheumatism, malaria, cancer, chickenpox, asthma, ulcer, swelling, eczema, tumor, high blood pressure, bilious fever, ataxy of abdominal viscera, sores, measles, fevers, cold and high blood pressure. In Ghana, infusion of the whole plant is used to cure bronchitis and the powdered root in milk was given to children for stomach-ache and as a vermifuge. Lantana oil is used in the treatment of skin, itches, as an antiseptic for wounds. In leprosy and scabies, decoctions were applied externally [1-5]. The phytochemical composition of the *L. camara* has been extensively studied in the last few decades. Different parts of *L. camara* are reported to possess essential Oils, Phenolic compounds, Flavonoids, Carbohydrates, Proteins, Alkaloids, Glycosides, Iridoid glycosides, Phenylethanoid, Oligosaccharides, Quinine, Saponins, Steroids, Triterpenes, Sesquiterpenoids and Tannin as major phytochemical groups. The Sesquiterpene hydrocarbons bicyclogermacrene (19.4 %), Isocaryophyllenes (16.7 %), Valecene (12.9 %), Germacrene D (12.3 %) are main components of essential oil [6-10]. The literature survey reveals that there is a lack of scientific reports regarding the *in vitro* anti-inflammatory activity of *L. camara*. Based on traditional medicinal uses, the present study was designed to investigate the *in vitro* anti-inflammatory activity of *L. camara* by egg albumin methods.

METHODS AND MATERIALS:

The Diclofenac sodium was procured from Jagsanpol Pharmaceutical, India. Ethanol was purchased from Changu and Hu, China. The Petroleum ether, Chloroform, ethyl acetate and all other chemicals and reagents were of analytical grade and procured from Hi-media laboratory, India.

Collection, authentication and processing of plant material:

The leaves of the *L. camera* were collected during November-December month of 2017, from Ahalia Campus, Palakkad District, Kerala, India. The plant *L. camera* was identified and authenticated by Dr. Prabhu Kumar, Scientist, Plant Systematics and Genetic Resources Division, Centre for Medicinal Plant Research (CMPR), Department of AYUSH, Government of India, Kottakal, Malappuram District, Kerala and a voucher specimen has been deposited in our laboratory for further reference. The leaves of the plant were washed with distilled water to remove foreign matter, shade dried at room temperature for 15 days and powdered with a mechanical grinder. The powdered plant material was then passed through sieve No.60 and stored in an airtight container for future use.

Preparation of the crude plant extract:

The shade dried coarse powdered Plant material (100 g) was packed in the Soxhlet apparatus (Borosil, India) and extracted with 1 L of 95 % ethanol at a temperature of 40 to 50 °C for 72 h. The extract was filtered and the filtered extract was then concentrated to semisolid mass using an electrical water batch at 40 to 60 °C. The resultant green color residue was stored in a desiccator for use in subsequent experiments and considered as the crude ethanol extract.

Fractionation of crude extract:

The prepared crude ethanolic extract about (10 g) was dissolved in water, poured into a separating funnel and then distilled petroleum ether was added into it. The separating funnel was shaken for 1 h to separate the two phases as petroleum ether being immiscible with water. Compounds soluble in the upper petroleum ether phase (petroleum ether being lighter than water) were collected and the lower aqueous phase was extracted with petroleum ether. The fractions of petroleum ether poured

into China dish and evaporated in a water bath at about 50 to 55 °C and were stored in the desiccator until further use. The same process of fractionation was carried out for chloroform (heavier than water) and ethyl acetate (lighter than water) respectively resulting in chloroform and ethyl acetate fractions [11,12].

Preliminary Phytochemical Screening:

All the extracts were analyzed by using the qualitative method for the identification of various phytoconstituents like Alkaloids, Glycoside, Phenolics, Flavonoids, Tannins, Steroids, Carbohydrates, Terpenes, Terpenoids and Saponins according to the standard protocol [13].

Anti-inflammatory (Fresh egg albumin denaturation assay) Test:

Denaturation of tissue proteins may be the cause behind the production of auto-antigens in certain arthritic diseases. So it may be said that tissue protein denaturation is a marker for inflammatory and arthritic diseases. Agents that can prevent protein denaturation, therefore, would be a possible candidate for anti-inflammatory drug development [14].

The present study was carried out in fractions. As per the literature, 5 ml of the reaction mixture was prepared which comprised of 0.2 ml of eggs albumin, 2.8 ml of phosphate-buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations (100, 200, 300, 400 and 500 µg/ml) of fractions of the crude extracts. A similar volume of double distilled water served as a control. Then the mixture was incubated at 37 °C in an incubator for about 15 min and then the mixture was heated at 70 °C for 5 min. The mixture was cooled and the solution absorbance was measured at 660 nm by using UV-Vis Spectrophotometer (UV-1900i, Shimadzu, Japan) against pure blank. Diclofenac sodium (standard drug) of varying concentrations of 10 to 50 µg/ml was used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation was calculated by the formula mentioned below [14].

Inhibition (%) = [Ac - At)/Ac] × 100 ….. (1)

Where Ac and At are Absorbance of control and test. The percentage inhibition of denaturation of fresh egg albumin is considered as anti-inflammatory activity.

RESULTS AND DISCUSSION:

The results of the preliminary phytochemical analysis were shown in the Table 1. The Petroleum ether fractions showed the presence of Flavonoids and steroids. The Chloroform factions showed the presence of Alkaloids, Flavonoids, Steroids and Tannins. The ethyl acetate fractions showed the Flavonoids, Steroids and tannins. These results correlated with the previously reported phytochemicals.

Table 1. Preliminary phytochemical analysis.

Phyto-chemicals	PEF	CF	EAF
Alkaloids	**(-)**	**(+)**	**(-)**
Carbohydrate	(-)	**(-)**	**(-)**
Flavonoids	(+)	**(+)**	**(+)**
Glycosides	(-)	**(-)**	**(-)**
amino acids	(-)	**(-)**	**(-)**
Steroids	(+)	**(+)**	**(+)**
Tannins	(-)	**(+)**	**(+)**
Saponins	(-)	**(-)**	**(-)**

(+) indicate the presence (-) indicate the absence. PEF - Petroleum ether fractions, CF - Chloroform fractions and EAF - Ethyl acetate fractions.

The *in vitro* anti-inflammatory effect of *L. camara* fractions was evaluated against the denaturation of egg albumin. The present findings revealed a concentration-dependent inhibition of protein (albumin) denaturation by *L. camara* (100 to 500 µg/ml) (Table 2).

Table 2. Effect of various fractions on albumin denaturation.

Conc. (µg/ml)	Inhibition (%)		
	PEF	CF	EAF
100	44.99	31.59	39.95
200	45.22	36.09	42.45
300	49.95	39.57	47.20
400	55.93	49.79	60.96
500	66.53	51.20	69.96

PEF - Petroleum ether fractions, CF - Chloroform fractions and EAF - Ethyl acetate fractions.

Diclofenac sodium (at the concentration range of 10 to 50 µg/ml) was used as a reference drug which also exhibited concentration-dependent inhibition of protein denaturation; nonetheless, the effect of Diclofenac sodium was found to be high compared to *L. camara* fractions as shown in Table 3 [7-10]. The increased absorbance in the fractions and the standard drug concerning control indicates the protein stabilizing activity (denaturation is inhibited) with increased dose.

The petroleum ether fractions inhibited the albumin denaturation in a dose-dependent manner 44.99 % at a low dose to 66.53 % inhibition at a high dose. Similarly with Chloroform fractions inhibited 31.59 % at a low

dose and 51.20 % at a high dose. The ethyl acetate fractions inhibited 39.95 % at a low dose and 69.96 % at a high dose. With the results, the ethyl acetate fractions showed the highest percentage of inhibition of about 69.96 % compare to other fractions at a high dose and compare to the standard drug Diclofenac sodium.

Table 3. Effect of standard drug on albumin denaturation.

Sl. No.	Concentration (µg/ml)	Inhibition (%) by Diclofenac Na
1	10	37.68
2	20	49.91
3	30	56.55
4	40	66.91
5	50	77.97

CONCLUSION:

Hence, it may be concluded from the present findings that the fractions of the ethanolic extract of the leaves of *Lantana camara* L have significant anti-inflammatory activity *in vitro*. Further research on isolating the responsible components and *in vivo* studies may be undertaken and they may be incorporated into existing anti-inflammatory herbal compositions to improve their efficacy.

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