Phytochemical Composition and *In Vitro* Hemolytic Activity of *Lantana Camara* L. (Verbenaceae) Leaves

Sanjeeb Kalita, Gaurav Kumar, Loganathan Karthik, Kokati Venkata Bhaskara Rao*

Molecular and Microbiology Research Laboratory, Environmental Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore, Tamil Nadu - 632 014, India

***Corresponding author**
Dr. Kokati Venkata Bhaskara Rao, Assistant Professor (Senior), Environmental Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore, TN - 632 014, India
Telephone: 09894350824
E-mail: kokatibhaskar@yahoo.co.in

Summary

L. camera L. is an important medicinal plant with several ethnomedicinal properties. In this study leaves of *L. camera* were screened for the presence of major phytochemical groups and haemolytic activity towards human erythrocytes. The hemolytic activity of *L. camera* aqueous extract and its solvent fractions was performed by modified spectroscopic method at four different concentrations (125, 250, 500, 1000 µg/ml). The aqueous extract and its solvent fractions exhibited very low hemolytic activity towards the human erythrocytes. The hemolytic activity of the different extracts was found in the following order: **chloroform fraction > hexane and ethyl acetate fraction (50:50) > aqueous extract > ethanol fraction > methanol fraction.** Phytochemical screening of the leaves of *L. camera* showed the presence of phytosterols, glycosides, carbohydrates, phenolic compounds, saponins, alkaloids, flavonoids and tannins as major phytochemical groups.

Keywords: *L. camera* L., hemolytic activity, erythrocytes, phytochemical analysis

Introduction

Since ancient time human kind depends on herbs and plants as direct or indirect source of food and shelter. Furthermore, plants are widely used in traditional medicinal system to cure a variety of diseases such as fever, cough, cold, pain, headache etc. In the last century, plants are reported scientifically to possess various medicinal properties viz., antibacterial [1, 2], antifungal [3], anticancer [4], anti-inflammatory [5], anti-helminthic [6], antioxidant [7], larvicidal activity [8] etc. These reports indicate the possible use of plants for the development of new therapeutic compounds.

L. camara is a flowering plant belonging to family Verbenaceae. *L. camara* is a popular ornamental garden plant and commonly known as wild sage or Lantana. [9] *L. camara* is of tropical origin plant and native to Central and Northern South America and Caribbean. *L. camara* is reported in Mexico, Florida, Trinidad, Jamaica and Brazil [10]. *L. camara* is now naturalized in approximately 60 countries or island groups between 35° N and 35° S. It is reported in many African countries including Kenya, Uganda, Tanzania and South Africa. [11] *L. camara* was probably introduced in to India before 19th century, currently *L. camara* is found throughout India [11, 12] and known by different names viz, Raimuniya (Hindi), Chaturangi and Vanacehedi (Sanskrit), Arippu and Unnichedi (Tamil), Aripoov, Poochedi, Konginipoo and Nattachedi (Malayalam), Thirei, Samballei and Nongballei (Manipuri), Tantani and Ghaneri (Marathi), Pulikampa (Telegu), Kakke and Natahu (Kanada).

L. camara is an important ethno medicinal plant with several medicinal properties and used widely in traditional medicinal system to cure a varieties of diseases [13] viz, influenza, cough, mumps, incessant high fever, malaria, cervical lymph node tuberculosis, dermatitis, eczema, pruritus, rheumatism, sprains, wounds, contusions, tetanus, toothaches, ulcers and swellings. In the last decade, *L. camara* has been extensively studied for its medicinal properties by scientific methods. The plant is reported to possess antioxidant activity, anti-proliferative activity [14], antimotily activity [15], antipyretic activity [16], antifilarial activity [17], anti-inflammatory activity [18], anti ulcerogenic activity [19], anti bacterial activity [20], anti-cancer activity [21], antifertility activity, anti-mutagenic activity [22], pharmacognostic activity, anti hyperglycaemic activity [23], hepato protectective activity [24], anti tumour activity [25], larvicidal activity [26], termiticidal activity [27]. Above cited literature represents *L. camara* as an important source of novel pharmaceutically important compounds and a future candidate for the drug discovery.

For discovery and development of novel drugs, scientists are looking forward to the alternative sources and in last few decades, medicinal plants have been extensively studied for their bioactive principles to develop new lead molecules for pharmaceutical use. Toxicity of the active molecule is a key factor during drug designing, and hemolytic activity represents a useful starting point in this regard, it provides the primary information on the nitration between molecules and biological entities at cellular level. [28]

The aim of this study was to investigate the *L. camara* leaves for its phytochemical composition and haemolytic activity *L. camara* leaves against human erythrocytes. As per our literature survey this is the first report of haemolytic activity of *L. camara.*

Material and Methods

Chemicals

Acetic anhydride, Ammonium hydroxide solution, Ammonium solution, Chloroform, Concentrated hydrochloric acid, Copper sulphate, Iodine, Sodium chloride, Disodium hydrogen phosphate, Ferric chloride, Nynhydrin solution, Potassium chloride, Potassium dihydrogen phosphate, Potassium iodide, Sodium carbonate and Sulphuric acid

Plant material

L. camera was collected from the natural population growing in the industrial area Ranipet (12.9275°N 79.3302°E), Vellore, Tamil Nadu, India, during August 2010. The plants were carried to the Molecular and Microbiology Research Laboratory, VIT University, Vellore. Plant was identified in Herbal Garden of VIT University, Vellore. A voucher specimen was maintained in our laboratory for the future reference (LC/VIT/MMRL/17.08.2010-6).

Processing of plant

The leaves of *L. camera* was collected and washed thoroughly in tap water followed by distilled water. The leaved were shade dried at room temperature. Dried leaves were uniformly grinded using mechanical grinder to make fine powder. The leave powder (50 gm) was soaked in sterilized distilled water (10% w/v) loaded on a shaker at a speed of 120 rpm for 48 hour at room temperature. Mixtures were filtered by using filter cloth followed by whatman number 1 filter paper. The filtrate was concentrated at 40˚C under reduced pressure (72 mbar) with a Rotary evaporator and dried using lyophilizer. Dried extract was collect in air tight container and stored at 4°C up for further use. [29]

Phytochemical screening

Phytochemical screening of the leaves of *L. camera* was carried out by using the standard protocols. Plants were screened for carbohydrates, phenolic compounds, saponins, alkaloids, proteins, oil and fats, flavonoids, glycosides, phytosterols and tannins. [30]

Test for tannins

A pinch of the powdered plant sample was mixed with 5 ml of water. The mixture was boiled in a water bath and then filtered. A few drops of 0.1% ferric chloride was added to the filtrate. Brownish green or a blue-black coloration indicates the presence of tannins.

Test for proteins and amino acids (Ninhydrin test)

The plant powder was extracted in different solvent and solvent free plant extract was mixed with few ml of diluted HCl and filtered. Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of Acetone) was added to the filtrate. The mixture was mixed properly. Purple color indicates the presence of Proteins and Amino acids.

Test for flavonoids

To a portion of filtrate, 10 ml of ethyl acetate was added and heated in water bath and filtered. To the filtrate 1 ml of dilute ammonia solution was added to the filtrate and shaken well. A yellow coloration indicates the presence of flavonoids.

Test for saponins

A pinch of the dried powdered plant was added to 2-3 ml of distilled water. The mixture was shaken vigorously. Formation of foam indicates the presence of saponin.

Test for oils and fats

A small quantity of the extract was pressed in between the two filter papers. Oil stain on the filter papers indicates the presence of oils and fats.

Test for phenolic compounds
Test extract (50 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds.

Test for carbohydrates
100 mg of the plant extract was dissolved in 5 ml of water and filtered. To 0.5 ml of the filtrate, 0.5 ml of Benedict's reagent was added and heated on a boiling water bath for 3 minutes. A characteristic color indicates the presence of carbohydrates.

Test for alkaloids
20 ml of distilled water was added to 2 gm of the powdered plant sample and was boiled in a water bath and filtered. About 10 ml of this filtrate was mixed with 5 ml of Wagner's reagent and shaken vigorously for a stable persistent froth. The Reddish Brown precipitate indicates the presence of alkaloids.

Glycosides (Brontrager's Test)
50 mg of extract was Hydrolyse with concentrated HCl for two hours on water bath. The hydrolyzed mixture was filtered and 3 ml of chloroform was added to the 1 ml of the filtrate. The mixture was shaked and the chloroform layer was separated out. 10% ammonium solution was added to the chloroform layer, pink colour indicates the presence of glycosides.

Phytosterols
50 mg of extract was dissolved in 2 ml of acetic anhydride, to this few drops of concentrated sulphuric acid was added slowly along the sides of the test tubes. An array of colour changes showed the presence of phytosterols.

Hemolytic activity
In vitro hemolytic activity was performed by spectrophotometer method. [31] Five milliliters of blood was collected from a healthy individual. The blood was centrifuged at 1500 rpm for three minutes. The pellet was washed three times with sterile phosphate buffer saline solution (pH 7.2±0.2) by centrifugation at 1500 rpm for 5 min. The cells were resuspended in normal saline to 0.5%. A volume of 0.5 ml of the cell suspension was mixed with 0.5 ml of the plant extracts (125, 250, 500 and 1000 µg/ml concentrations in saline). The mixtures were incubated for 30 min at 37˚C and centrifuged at 1500 rpm for 10 min. The free hemoglobin in the supernatants was measured in UV-Vis spectrophotometer at 540 nm. Phosphate buffer saline and distilled water were used as minimal and maximal hemolytic controls. Each experiment was performed in triplicates at each concentration.

The level of percentage hemolysis by the extracts was calculated according to the following formula:

$$\% \text{ Hemolysis} = \frac{A_t - A_n}{A_c - A_n} \times 100$$

Here: A_t is the absorbance of test sample.
A_n is absorbance of the control (saline control)
A_c is the absorbance of the control (water control)

Statistical Analysis
All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD). Results were analyzed statically by using Microsoft Excel 2007 (Roselle, IL, USA).

Results and Discussion

L. camara is a gregarious, erect, half climbing and hairy aromatic shrub. It grows up 1.2 meter height and branches are growing all four sided with recurved prickles. Leaves elliptic, about 3 inches long and 1.5 inches wide, pointed at the tip and rounded in the base and toothed in the margins. Flowers are pink, orange, yellow, white, lilac in colour and colour usually changes with the age. Seeds germinate very easily throughout the year. Selection of *L. camara* for this study was based on its medicinal use in traditional medicinal system and easy availability in nearby areas.

Percentage yield
50 gm of dried leaves powder of *L. camera* was extracted in sterilized distilled water to obtain the test extract. After drying the filtrate yielded 3.05 gm of extract that is 6.10% of the initial plant powder (50 gm).

Phytochemical screening
Aqueous extract of *L. camera* leaves showed the presence of phytosterols, glycosides, carbohydrates, phenolic compounds, saponins, alkaloids, flavonoids, and tannins as major phytochemical groups (Table 1), earlier different organic extracts of *L. camera* leaves were reported to possess triterpenoids, steroids, carbohydrates, lactones, proteins, flavonoids, resins, tannins and fixed oils. [27] These phytochemical compounds are the key candidates in the medicinal value of the plant.

Table 1: Phytochemical analysis of *Lantana camara* leaves

Phytochemicals	***Lantana camara***
Phenoic compounds	++++
Flavonoids	+++
Alkaloids	+++
Tannins	++
Saponins	++
Phytosterols	++
Charbohydrades	+
Proteins	-
Oil and fats	-
Glycosides	-

Here, +: present, -: not present

Hemolytic activity

Hemolytic activity of the leaves of *L. camera* aqueous extract and its hexane and ethyl acetate fraction (50:50), chloroform fraction, methanol fraction and ethanol fraction was screened against normal human erythrocytes. Extracts exhibited low to mild hemolytic effect toward human erythrocytes. Hemolytic activity of the plant is expressed in % hemolysis and reported as mean ± standard deviation of three replicates. Result indicated that the methanolic fraction of the aqueous extract (at dose 1000 µg/ml) possess minimum hemolytic activity (4.62±0.23%) with an IC_{50} value12332.0 µg/ml, where as chloroform fraction (at dose 1000 µg/ml) possess highest hemolytic activity (20.51±0.98) with an IC_{50} value 2739.8 µg/ml. Hemolytic percentage was found to be increasing with increase in dose (Table 2 and Figure 1). This is the first report on the hemolytic activity of *L. camera* leave.

Table 2: Hemolytic activity of *Lantana camara*

Test extracts	% Haemolysis				IC_{50} value (µg/ml)
	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	
AE	2.40±0.2	3.66±0.11	4.53±0.64	7.93±0.23	8035.9
H:EA	1.69±0.35	4.29±0.44	7.12±0.29	12.51±0.82	4470.4
CF	5.16±0.24	7.79±0.22	12.86±0.93	20.51±0.98	2739.8
MF	0.87±0.16	1.95±0.18	2.81±0.27	4.62±0.23	12332.0
EF	2.80±0.33	4.27±0.18	5.23±0.13	7.79±0.28	9496.4

Here: AE = aqueous extract, H:EA = hexane and ethyl acetate fraction (50:50), CF = chloroform fraction, MF = methanol fraction, EF = ethanol fraction. All values represent the mean±standard deviation (*n* = 3 test).

2739.812332

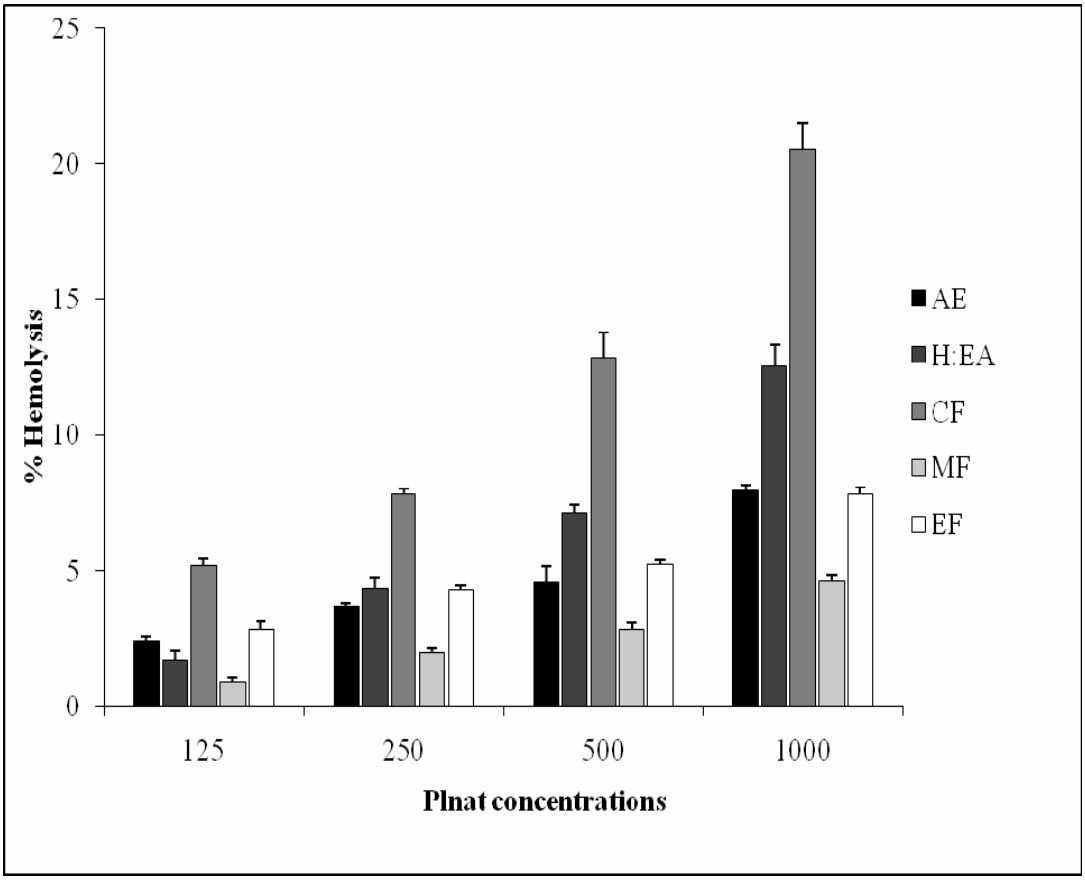

Figure 1: Hemolytic activity of *Lantana camara*
Here: AE = aqueous extract, H:EA = hexane and ethyl acetate fraction (50:50), CF = chloroform fraction, MF = methanol fraction, EF = ethanol fraction. All values represent the mean±standard deviation (n = 3 test).

Conclusion

L. camera is an important medicinal plant with several medicinal uses in folk and traditional medicinal system. In this study we have reported the hemolytic activity of *L. camera* leave aqueous extract and its various solvent fractions. The results reveled that the leaves possess very less hemolytic activity and can further use for the isolation of bioactive compounds.

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