

Phytochemical And Antidiabetic Evaluation Of *Lantana Camara* L. Roots (Family: Verbenaceae)

B. Udayabhanu^{*1}, Sumanta Mondal², L. Siddhartha³, Y. Praharsha⁴, Subhash Sahoo⁵

¹Assistant Professor, Department Of Pharmaceutical Chemistry, Pullareddy Institute Of Pharmacy, Hyderabad.

²Professor, Department Of Pharmaceutical Chemistry, Gitam University, Visakhapatnam

³Associate Professor, Head Of The Department, Department Of Pharmacy Practice, Arya College Of Pharmacy, Hyderabad.

⁴Assistant Professor, Department Of Pharmaceutical Chemistry, Mlr Institute Of Pharmacy, Hyderabad.

⁵Professor, Department Of Pharmaceutical Chemistry, Pullareddy Institute Of Pharmacy, Hyderabad.

ABSTRACT

Herbal drugs have a great significance in the field of therapeutics. Ethno Pharmacological surveys indicate that more than 1200 plants were used in traditional medicine for their alleged hypoglycaemic activity. *Lantana camara* is a low, erect or sub scandent, vigorous shrub and its root system is very strong with a main taproot. Leaf extracts of *Lantana* exhibit antimicrobial, fungicidal, insecticidal and nematocidal properties. The methanolic extract of *L. camara* leaves shown healing of gastric ulcers and also prevents development of duodenal ulcers in rats. Two major components have been reported from *L. camara* leaves are Lantadene A and Lantadene B. The hepatotoxins are pentacyclic triterpenoids called “lantadenes”. Roots of *Lantana* plant are rich in oleanolic acid, a hepatoprotective triterpenoid. A novel triterpenoid Lantadene D and Lantadene C, were isolated from the leaves. The present study focuses on the hydroalcoholic extract of *L. camara* roots which was subjected to phytochemical studies by using different chromatographic techniques. The isolated compounds were characterized using physical, chemical and spectral data. Screening for hypoglycaemic activity was performed on Wistar rats by the Streptozotocin induced hyperglycaemic study and it has shown promising Anti-diabetic activity when compared with standard drug Glibenclamide.

Keywords: *Lantana camara*, Phytochemical studies, Streptozotocin, Anti-diabetic activity.

INTRODUCTION

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity because of their natural origin and minimal side effects. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. India is the largest producer of medicinal herbs and is called as “Botanical Garden of the world”. Herbal medicines are the synthesis of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine for over hundreds of years. Herbal medicines are now in great demand in the developing world for primary health care not because

they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. Herbal medicines are generally considered comparably safer than synthetic drugs. While this may be probably correct, case reports show that severe side effects and relevant interactions with other drugs can occur.

PLANT PROFILE

Name of the plant: *Lantana camara* L.

Family: Verbenaceae

Vernacular Names: Lantana weed (English),

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Chaturang (Hindi),

Vanacchedi (Sanskrit),

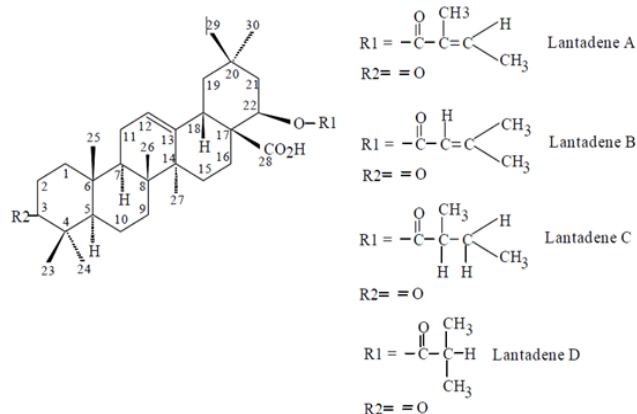
Pulikampa (Telugu),

Aripoo (Malayalam)



Phytochemical Information

- The hepatotoxins are pentacyclic triterpenoids called “lantadenes”. Two major components have been reported from *L.camara* leaves are lantadene A and lantadene B.
- Roots of lantana plant are rich in oleanolic acid, a hepatoprotective triterpenoid (Sharma, O.P et al., 1988.).
- Several studies had shown that phytoconstituents like sesquiterpenes, saponins and triterpenes were beneficial in the treatment of diabetes.
- A novel triterpenoid Lantadene D and Lantadene C was isolated from the leaves (Sharma, O.P. and Dawra, R.K., 1991).



MATERIALS AND METHODS

Plant Materials: *Lantana camara*.

Standard Drugs used for activity comparison:

Glibenclamide (2.5mg/kg, p.o.) - antidiabetic study.

Animals used:

- Adult Wistar albino rats (70-200 g) of either sex - sub-acute toxicity study, antidiabetic activity on normal and Streptozotocin induced diabetic rats.
- The test extracts were separately suspended in 1% tween 80 and 1% tween 20
- Animals used: Swiss albino mice
- (18–20g) for Gross behavioral and acute toxicity studies.
- No. of animals in each group: 02 (Two)
- Route of administration: Oral
- Doses selected: 100, 200, 300, 600, 800, 1000 and 2000 mg/kg

The Results of Gross behavioral and acute toxicity studies has shown the following:

The hydroalcoholic extract produced sedation, analgesia, diuresis and purgation at all tested doses.

No mortality was seen with the extract at the tested doses till the end of two weeks of observation.

EXTRACTION:

Plants Material

The fresh plant materials of *L. camara* roots were collected from young matured plants and authenticated. After authentication, the plant materials were collected in bulk, washed under running tap water to remove adhering dirt followed by rinsing with distilled water. The plant materials were then shade dried and separately pulverized in a mechanical grinder to obtain coarse powder.

Preparation of Extracts

The dried powdered plants materials (500 g each) were separately defatted with petroleum ether (600–800⁰ C) and successively extracted with Diethyl ether, Ethyl acetate and Hydro alcoholic using a soxhlet extractor. The period of extraction was fixed at 48 h for every solvent at every stage of the extraction process. The solvents were purified by distillation prior to extraction. After completion of extraction, the

extractive value was determined with respect to the dried plant material. The above extracts were studied for their colour, consistency and extractive values and reported in Table 1.

Fluorescence characteristics of *L. camara* root powder in different reagents was observed under daylight and ultraviolet light separately at short and long wavelengths (Dash G.K et al., 2003) and the results are depicted in Table 2

TABLE 1:

Solvent extract	Colour	Consistency	%w/w Extractive value
Petroleum Ether	Yellowish brown	Sticky with light brown stain	2.0
Diethyl ether	Dark yellow	Coarse powder	1.4
Ethyl acetate	Brownish red	Amber coloured waxy residue	3.8
Hydro alcoholic	Dark brown	Sticky	3.1

TABLE 2: Fluorescence Characteristics of *L. Camara* Root Powder in Different Reagents

Powdered drug	Visible/Day light	UV 254 nm (short)	UV 365 nm(long)
Powder as such	Husk	Brown	Yellowish brown
Powder + 1M NaOH	Brown	Blackish brown	Yellowish green
Powder + CH ₃ COOH	Brown	Dark brown	Light greenish yellow
Powder + 1M HCl	Light brown	Brown	Greenish yellow
Powder + dil. HNO ₃	Light brown	Reddish brown	Light green
Powder + 5% I ₂	Dark reddish brown	Black	Yellowish brown
Powder + 5% FeCl ₃	Light brown	Brown	Yellowish green
Powder + HNO ₃ + 25% NH ₃	Light brown	Black	Very light green
Powder + CH ₃ OH	Brown	Reddish brown	Light greenish yellow
Powder + 50% HNO ₃	Light brown	Brown	Light yellow
Powder + 1 M H ₂ SO ₄	Light brown	Dark brown	Light green
Powder + dil. NH ₃	Light brown	Black	Light yellowish green

TABLE 3: Preliminary Phytochemical Screening of *L. Camara* Roots

Sr. No.	Chemical constituent	Diethyl ether extract
1	Carbohydrates	+ve
2	Saponins	-ve
3	Cardiac Glycosides	+ve
4	Alkaloids	-ve
5	Tannins and phenolic substances	-ve
6	Proteins	-ve
7	Flavonoids	+ve
8	Steroids	+ve
(+) = positive; (-) = negative		



Preliminary Phytochemical Studies:

A plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man, but also for a multitude of compounds like glycosides, alkaloids, volatile oils, saponins, etc., that exert a physiological effect. The compounds that are responsible for therapeutic effects are usually the secondary metabolites. A systematic study of a crude drug embraces the consideration of both primary and secondary metabolites derived as a result of plant metabolism. The plant material may be subjected to preliminary phytochemical screening for the detection of various plant constituents. (Harborne J.B., 1984 and Wagner H., Bladt S., 1996).

1. Tests for alkaloids:

a) Mayer's reagent: - Dissolve 1.36 g of mercuric chloride in 60 ml. Distilled water (a) Dissolve 5 g potassium iodide in 60 ml. distilled water (b) Mix (a) & (b) and adjust the volume to 100 ml with distilled water. With alkaloids, it produces white to buff coloured precipitate.

b) Wagner's reagent: - Dissolve 1.27 g of iodine and 2 g of potassium iodide in 5 ml of water and make up the volume 200 ml with distilled water. With alkaloids, it produces reddish brown precipitate.

c) Dragendorff's reagent: - Boil 14 g of sodium iodide with 5.2 gm of bismuth carbonate in 50 ml glacial acetic acid for a few minutes. Allow it to stand over night and filter of the precipitate of sodium acetate crystals. Preserve the stock solution in amber coloured bottle. When needed, add 20 ml of acetic acid to 10 ml of this stock solution and make up to 100 ml with water. With alkaloids, it produces orange brown precipitate.

d) Hager's reagent: - A saturated aqueous solution of picric acid used for detection of alkaloids. It gives characteristics crystalline precipitate with many alkaloids.

2. Test for glycosides:

Test for cardiac glycosides:

a) Keller-Kiliani test:- To an extract of the drug in glacial acetic acid, few drops of ferric chloride and conc. Sulphuric acid are added. A reddish-brown colour is formed at the junction of two layers and the upper layer turns bluish green. The test confirms presence of cardiac glycosides with presence of digitoxose as the glycone moiety.

Test for anthraquinone glycosides:

a) Borntrager's test:- Boil 0.1 g of the powdered drug with 5 ml. 10% sulphuric acid for 2 min. Filter while hot, cool the filtrate and shake gently with equal volume of benzene. Allow the benzene layer to separate completely from the lower layer. Pipette out and transfer the benzene layer to a clean test tube. Add about half its volume of aqueous solution of ammonia (10%). Shake gently and allow the layer to separate. The lower ammoniacal layer will acquire pink to red colour due to the presence of free anthraquinones.

b) Modified Borntrager's test:- The C-glycosides of anthraquinones requires more drastic conditions for hydrolysis and thus a modification of the above test is used. Ferric chloride and hydrochloric acid are used to effect oxidative hydrolysis. 0.1 gm of the drug is boiled with 5ml of dil. HCl and 5 ml of 5% solution of ferric chloride for five minutes cool the solution and filter. This filtrate is shaken with benzene. Separate the benzene layer and add an equal volume of dilute solution of ammonia. This ammonical layer shows pink to red colour.

3. Test for carbohydrates:

a) Molisch's test:- To aqueous or alcoholic solution of the substance in a test tube add 10% alcoholic solution of α -naphthol. Shake well and add a few drops of Conc. Sulphuric acid along the side of the test tube. A violet ring at the junction of two liquids confirms the presence of carbohydrates.

b) Fehling's test:- Add 2ml of Fehling's solution A and 2 ml of Fehling's solution B to 2 ml of liquid extract in a test tube and boil. If yellow or bricked precipitate appears, then reducing sugars are present.

c) Benedict's test:- Add 5 ml of Benedict's reagent to 3 ml of test solution in a test tube and boil on a water bath. The appearance of brick red precipitate at the



bottom of the test tube shows the presence of monosaccharides.

4. Test for gums and mucilages:

a) Precipitation with 95% alcohol:- Gums and mucilages precipitate with addition of 95% alcohol, being insoluble in alcohol.

b) Molisch's test:- To aqueous or alcoholic solution of the substance in a test tube add 10% alcoholic solution of α -naphthol. Shake well and add a few drops of Conc. Sulphuric acid along the side of the test tube. A violet ring at the junction of two liquids confirms the presence of carbohydrates, gums and mucilages.

c) Ruthenium red test:- Dissolve 0.8gm of ruthenium red in 10 ml of 10% solution of lead acetate. It stains mucilage to red colour.

5. Test for proteins and aminoacids:

a) Biuret test:- To 2 ml of extract, 2 ml of 10% NaOH solution and 2 to 3 drops of 1% CuSO₄ solution is added and mixed. Appearance of violet or purple colour confirms presence of proteins.

b) Ninhydrin test:- To 2 ml. of extract add 0.5 ml of ninhydrin solution. Boil for 2 minutes and cool. If blue colour appears then proteins are present.

c) Xanthoproteic test:- To 2 ml of extract add 1 ml of Conc. HNO₃, boil, cool and add 40% NaOH drop by drop. Appearance of coloured solution indicates presence of proteins.

d) Millon's test:- To 2 ml of extract add 2 ml of Millon's reagent, boil, cool and add few drops of NaNO₂ solution. Appearance of red precipitate or colouration indicates presence of proteins.

6. Test for tannins and phenolic compounds:

a) Ferric chloride test: - A 5% W/V solution of ferric chloride in 90% alcohol are used for detection of phenols.

b) Lead acetate test: - Tannins are precipitated with lead acetate.

c) Gelatin solution test:- To a solution of tannins (0.5-1%) aqueous solution of gelatins (1%) and sodium chloride (10%) are added. A white to buff coloured precipitate is formed.

7. Test for steroids and sterols:

a) Lieberman Burchard reagent:- To about 2 ml of a solution extract in chloroform in a dry test tube, add 2 ml of acetic anhydride and 2-3 drops of Conc. Sulphuric acid Mix and stand for a few minutes. An emerald green colour develops if steroids or sterols are present.

b) Salkowski's test:- To 5 ml of a solution of extract in chloroform in a dry test tube add gently along the sides, on equal volume of conc. Sulphuric acid. Observe the upper chloroform layer and the lower acid layer. The acid layer develops a yellow colour with a green fluorescence. The chloroform layer will give a play of colours first from bluish red to gradually violet red.

8. Test for triterpenoids:

a) Noller's test:- To the extract dissolved in chloroform, add piece of metallic tin. Add 1 drop of thionyl chloride. If pink coloured develops then triterpenoids are present.

9. Test for saponins:

a) Foam test (1ml of extract + 9 ml of water):- About 1 ml of alcoholic or aqueous extract is diluted separately with distilled water to 10 ml and shaken in a graduated cylinder for 15 minutes and kept aside. About one cm layer of foam after standing for 30 minutes indicates the presence of saponins.

b) Haemolysis Test (3 drops of blood + 1 drop of extract):- Haemolysis occurs if saponins are presents.

10. Test for flavonoids:

a) Sodium hydroxide test:- The extract dissolved in water, filtrate treated with sodium hydroxide a yellow colour is observed if flavonoids are present.



b) Sulphuric acid test:- A drop of Conc. Sulphuric acid when added to the above, the yellow colour disappears.

c) Shinoda's test:- To the aqueous or alcoholic solution of the extract, add a piece of magnesium ribbon and few drops of Conc. HCl. A pink colour develops which indicates presence of flavonoids.

The results of the preliminary phytochemical studies are tabulated (TABLE 3).

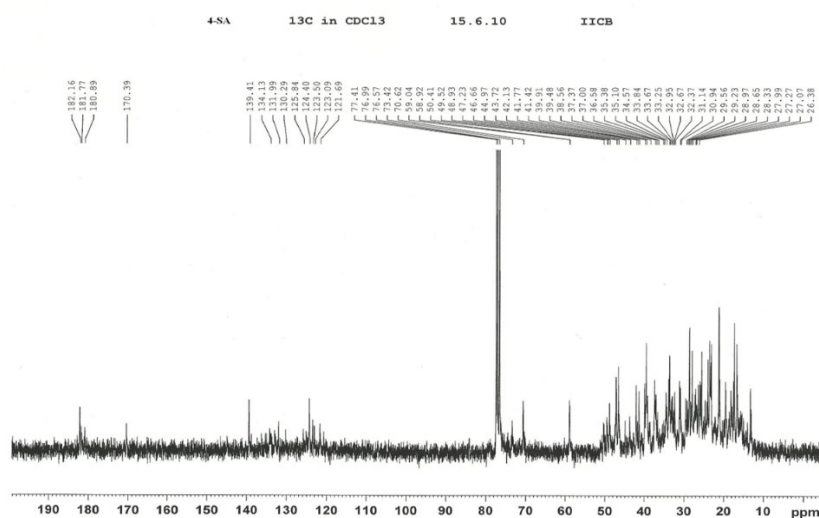
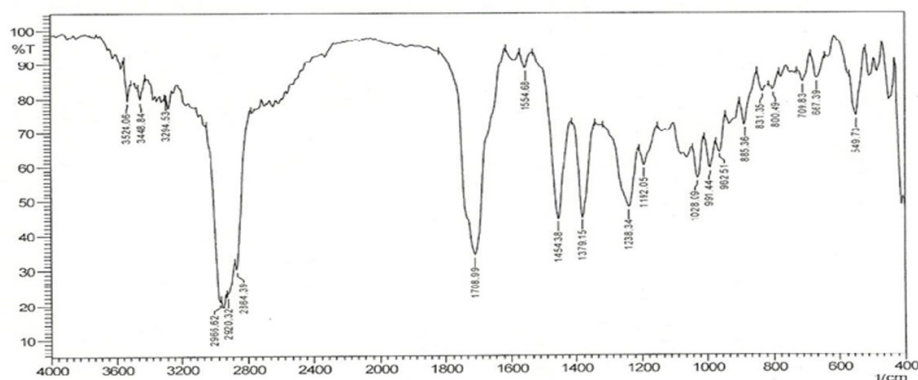
SPECTRAL ANALYSIS:

➤ Yellow powder, m.p. 274-276 degree Celsius (104 mg). On TLC, a purple spot was observed under UV light and darkened when exposed to ammonia. It gave dark green colour with ferric

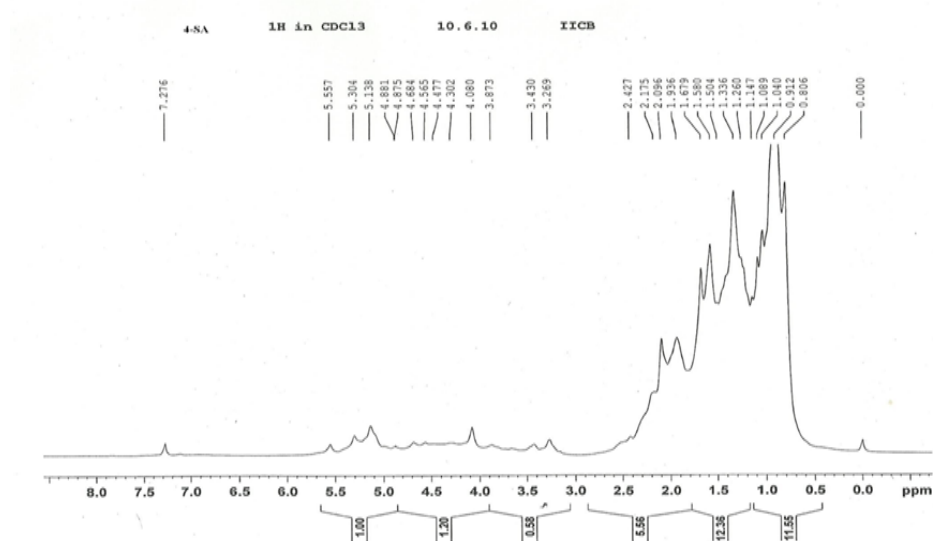
chloride, orange colour in Shinoda's test suggesting that it was a flavone. The compound had UV absorption maxima at 269 nm.

- Rf value 0.62 (ethyl acetate: methanol-1: 0.25).
- The IR (ν cm⁻¹) spectrum of compound showed absorption bands at 3524.0 to 3294.53 (O-H, free hydroxyl group), 2966.6 (Cyclic C-H, str), 2864.3 (Allyl C-H, str), 1708.9 (C=O stretch), 1554.6 (C-C ring stretch), 1454.3 (CH₃ of CH₃CO), 1238.6 to 1192.0 (C-C stretching), 1028.0 to 962.0 (O-H, out of plane bend), 709.8 to 667.3 (monosubstituted in aromatic ring).

IR Spectrum of compound: 3'-methoxy-4'-hydroxy-flavan-3-ol.



13C NMR Spectrum of compound: 3'-methoxy-4'-hydroxy-flavan-3-ol.

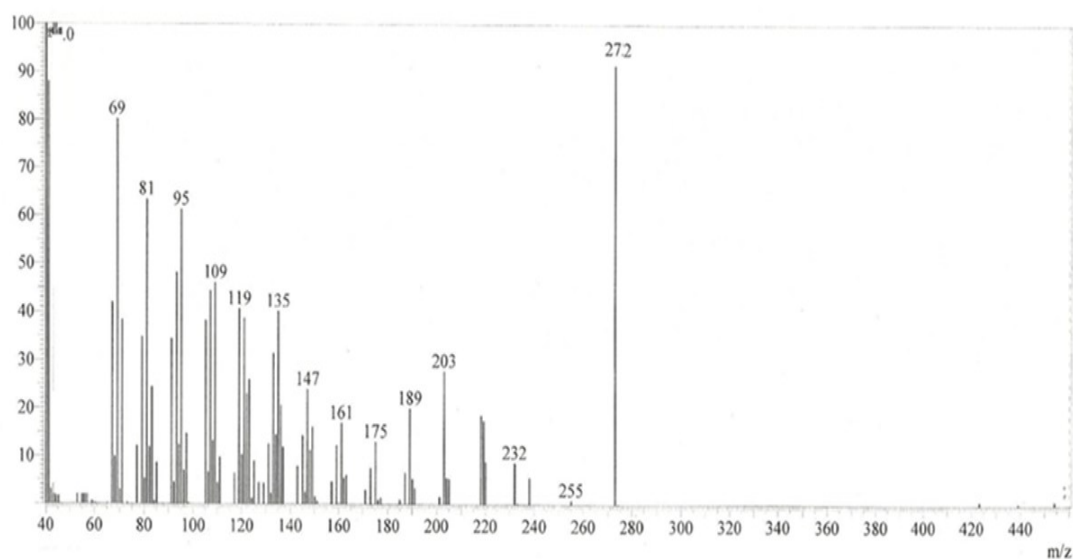
^1H NMR Spectrum: 3'-methoxy-4'-hydroxy-flavan-3-ol.

- The ^1H -NMR spectrum: δH 7.27(OH-3, s), 5.55 (OH- 3', s), 5.138 (H- 5',s), 4.881 to 3.430 (H- 5, 6, 7 & 8, s), 3.269 (OMe- 3', s), 2.175 (H- 4, d), 1.67 to 1.33 (H- 5', 6', & 2',s)
- ^{13}C -NMR: δH 2-77.41, 3-130.29, 4-50.41, 5-76.99, 6-73.42, 7-76.57, 8-70.62, 3'-139.41, 4'-131.99, 1'-170.39, 2'-121.69, 5'-123.09, 6'-123.50.

The ^{13}C NMR spectrum and ^1H - NMR of compound displayed the characteristic signals as mentioned above.

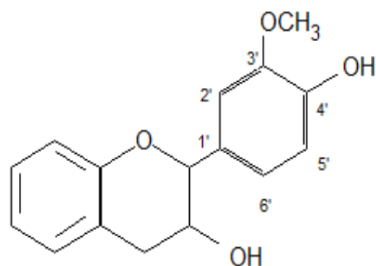
Mass spectrum of 3'-methoxy-4'-hydroxy-flavan-3-ol

The mass data which showed $m/z = 272$ (100) $[\text{M}^+]$ indicative of $\text{C}_{16}\text{H}_{16}\text{O}_4$.



RESULTS AND DISCUSSION

The chemical examination of the diethyl ether extractive from the root parts of *Lantana camara* yielded one compound 3'-methoxy-4'-hydroxy-flavan-3-ol on column chromatography and purification. The compound was characterized by spectral data and chemical test.



3'-methoxy-4'-hydroxy-flavan-3-ol.

Screening for hypoglycaemic activity of *L. camara* roots

Extracts of the selected plant studied: Hydro alcoholic extract

Animals used: Adult Wistar rats (150 -200g)

Doses studied: 250 and 500 mg/kg

Reference standard: Glibenclamide (2.5 mg/kg)

Route of administration: Oral

Method used: Normoglycaemic study (Ponnachan et al., 1993)

Streptozotocin induced hyperglycaemic study

Screening for hypoglycaemic activity of hydro alcoholic extract of *L. camara* roots. Screening for hypoglycaemic activity of the methanol extract was performed on wistar rats and glibenclamide (2.5 mg/kg, p.o.) was used as reference standard for activity comparison. All the test samples were administered through oral route.

Using normoglycaemic rats:

The method of Ponnachan et al., 1993 was followed (C.H. Jithendra et al., 2007). The acclimatized animals were fasted for 18 h, but were allowed free access to

water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), blood collection was done by tail vein method of each rat under mild anaesthesia. The blood glucose level was measured with Senso card blood glucose meter supplied by M/s Avecon Health Care Pvt. Ltd., Himachal Pradesh. The normal rats were then divided into four groups of four animals in each. Group-I served as negative control and received vehicle (2 ml/kg) through oral route. Group-II received glibenclamide (2.5 mg/kg). Group-III and IV received the extract at doses of 250 and 500 mg/kg, p.o., in a similar manner. Blood glucose levels were measured after 0, 30, 60, 90 and 120 mins of administration of single dose of test samples (Table-4).

Oral glucose tolerance test (OGTT) in rats:

The method of Dash et al., 2008 was followed (E. Edwin et al., 2007). Fasted rats were divided into four groups of four rats each. Group I served as a control and received only vehicle

(2 ml/kg) through oral route. Group-II received glibenclamide (2.5 mg/kg). Group-III and IV received the test extract at doses of 250 and 500 mg/kg, p.o. respectively in a similar manner. After 30 min of treatment, rats of all groups were loaded orally with glucose (2 g/kg, p.o). Blood samples were collected before and at 0, 60, 90 and 120 min after glucose administration as per the method described earlier.

Using hyperglycaemic rats:

The method of Dash et al., 2008 was followed (E. Edwin et al., 2007). The acclimatized animals were kept fasting for 24 hours with water ad libitum and injected intraperitoneally a dose of 55 mg/kg of Streptozotocin in normal saline. After one hour, the animals were provided standard laboratory diet ad libitum. The blood glucose level was checked before administration of Streptozotocin and 24 h after administration of Streptozotocin by withdrawing blood from the tip of the tail of each rat under mild anaesthesia. The blood glucose level was measured as above. Rats having the blood glucose level above 225 mg/dl Vidyarthi RD. (1977) were selected and grouped into four groups consisting of four animals each. This condition was observed at the end of 48 h



after administration. The first group (Group-I) served as diabetic control and received orally 1% Tween 80 solution (2 ml/kg p.o), second group (Group-II) received glibenclamide (2.5 mg/kg), third and fourth groups (Group-III and IV) received the hydro

alcoholic extract at doses of 250 mg/kg and 500 mg/kg (in 1% Tween 80) respectively in a similar manner. Blood glucose levels were measured after 1, 2, 4 and 6 hrs of administration of single dose of test samples. (Table-5).

TABLE 4: Effect of Hydroalcoholic extract of *L. camara* roots on the blood glucose level in normal rats.

Group	Treatment	Dose	Blood glucose concentration (mg / dl) (normoglycaemic study)				
			Fasting	Time (min) after treatment			
				30	60	90	120
I	Control	2 ml/kg	104.57±1.17	99.66±5.92	104.16±1.37	102.5±2.01	104.66±2.24
II	Glibenclamide	2.5 mg/kg	103.5±2.34	83.36±1.93**	81.47±1.08**	74.89±2.27**	71.16±2.5**
III	Hydro alcoholic extract	250 mg/kg	101.83±1.7	90.66±1.38	93.83±3.33*	89.66±2.51**	88.5±4.06**
		500 mg/kg	102.66±1.92	86±1.26*	85.5±2.39**	84.16±3.49**	81.83±3.18**

Results expressed as Mean ±SEM from six observations (n=6). *P<0.05, **P<0.01 as compared with control group (One way, ANOVA followed by Dunnet's t-test).

TABLE 5: Effect of Hydroalcoholic extract of *L. camara* roots on the blood glucose level in Streptozotocin induced diabetic rats.

Group	Treatment	Dose	Blood glucose concentration (mg / dl)				
			0 hour	1 hour	2 hour	4 hour	6 hour
I	Control	2 ml/kg	234.00±8.21	239.19±6.61	246.55±6.17	252.5±5.65	255.66±4.33
II	Glibenclamide	2.5 mg/kg	232.33±4.06	205.66±7.84* (11.47 %)	178.5±3.79** (23.16 %)	122.83±6.27** (47.13 %)	83.00±7.30** (64.27 %)
III	Hydro alcoholic extract	250 mg/kg	236.67±8.90	232.16±8.98 (1.90 %)	222.66±7.91 (5.91 %)	172.83±14.5** (26.97 %)	135.33±8.51** (42.81 %)
		500 mg/kg	241.33±7.75	230.16±6.21 (4.62 %)	204.00±5.06** (15.13 %)	161.00±6.92** (33.28 %)	89.00±6.39** (63.12 %)

Results expressed as Mean ±SEM from six observations (n=6). *P<0.05, **P<0.01 as compared with control group (One way, ANOVA followed by Dunnet's t-test). Figures in parenthesis denote percentage reduction of blood glucose.



RESULTS AND DISCUSSION

Reports of the normoglycaemic study (Table 4) reveals that the hydro alcoholic extract of *L. camara* roots exhibited significant reduction in blood glucose concentration in dose dependant manner as compare to control. It was observed that hydro alcoholic extracts reduced 85.66 ± 1.92 and 77.16 ± 3.15 mg/dl blood glucose levels respectively where as glibenclamide showed 71.16 ± 2.5 mg/dl blood glucose levels in rats after 120 mins. The effect of test extracts on glucose tolerance test in normal rats is shown in Table – 4. At 30 min after glucose administration the peak of blood glucose level increased rapidly from the fasting value and then subsequently decreased. The tested extract at 250 and 500 mg/kg, p.o., exhibited significant hypoglycaemic effect when compare with control group. In antihyperglycaemic study (Table – 5, the rise in the blood glucose level was observed after 24 h of administration of Streptozotocin to the animals. Single administration of hydro alcoholic extracts of *L.camara* roots (250 and 500 mg/kg) in diabetic rats showed significant reduction in blood glucose level in dose dependent manner, The results of the hydro alcoholic extract are comparable to that of the reference standard glibenclamide.

Traditional medicinal plants with various active principals and properties have been used from ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes, cancer and coronary heart diseases. Beneficial multiple activities like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring the integrity and function of beta-cells, releasing insulin activity, improving glucose uptake and utilization, and the antioxidant properties present in medicinal plants, offer an exciting opportunity to develop them into novel therapeutics Chau T. (1989).The anti-hyperglycaemic activity of hydro alcoholic extract may be due to the presence of several bioactive antidiabetic principles. The active ingredient in the extract that reduces the blood sugar is not known at present. There is ongoing research to isolate and characterize the bioactive compound(s) responsible for the antidiabetic activity of *L. camara* roots. From the present study it is apparent that the roots of *L. camara* possess hypoglycaemic activity and it justify

the use of the roots of the plant for treating diabetes as suggested in the folklore remedies.

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