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RESEARCH ARTICLE

Pharmacognostical and pharmacological evaluation of the leaf of Gmelina Arborea Roxb.

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ABSTRACT

Gmelina Arborea Roxb. (Verbenaceae) is commonly known as gambhari. Gambhari is one of the herbs mentioned in all ancient scriptures of Ayurveda, as one of the member of brhat pancamulas. Acarya Vagbhata has cited gambhari, one of the members from the group dasamulas, used in vata dosa (vata samsamana). It is traditionally used as diuretic, tonic, aphrodisiac, alternative astringent to the bowels, promote growth of hairs, useful in treatment of anaemia, leprosy, ulcers and vaginal discharge, alopecia, anemia, etc. The plant *Gmelina arborea* Roxb. traditionally reported for the treatment of diabetes. So in the present study the unexplored plant *Gmelina arborea.Roxb* has been investigated for its pharmacognostical and phytochemical and medicinal values.

Key Words: Gmelina arborea Roxb., diabetes, pharmacognostical and phytochemical properties, medicinal values.

INTRODUCTION:

Medicines in india are used by about 60 per cent of the world's population. These are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used. While the traditional medicines are derived from medicinal plants, minerals, and organic matter, the herbal drugs are prepared from medicinal plants only.

Gmelina arborea Roxb. (Family: Verbenaceae) is locally known as 'Gambhari'. In English it is known as the 'Candahar tree' or 'White teak'. It is a moderate sized, deciduous tree, found distributed in deciduous*Gmelina arborea* is a fast growing tree, which grows on different localities and prefers moist fertile valleys with 750–4500 mm rainfall. It does not thrive on ill-drained soils and remains stunted on dry, sandy or poor soils; drought also reduces it to a shrubby form. The root and bark of *Gmelina arborea* are claimed to be stomachic, galactagogue laxative and anthelmintic; improve appetite, useful in hallucination, piles, abdominal pains, burning sensations, fevers, 'tridosha' and urinary discharge. Leaf paste is applied to relieve headache and juice is used as wash for ulcers [12].

Flowers are sweet, cooling, bitter, acrid and astringent. They are useful in leprosy and blood diseases.

In Ayurveda, it has been observed that Gamhar fruit is acrid, sour, bitter, sweet, cooling, diuretic tonic, aphrodisiac, alternative astringent to the bowels, promote growth of hairs, useful in 'vata', thirst, anaemia, leprosy, ulcers and vaginal discharge.

For this experiment we have chosen a steroidal drug which is having diabetic induing property i.e.Dexamethasone, a very potent and highly selective glucocorticoid. People are taken as steroidal supplement but Corticosteroids profoundly affect carbohydrate and protein metabolism. Teleologically, these effects of glucocorticoids on intermediary metabolism can be viewed as protecting glucose-dependent tissues (*e.g.,* the brain and heart) from starvation. They stimulate the liver to form glucose from amino acids and glycerol and to store glucose as liver glycogen. In the periphery, glucocorticoids diminish glucose utilization, increase protein breakdown and the synthesis of glutamine, and activate lipolysis, thereby providing amino acids and glycerol for gluconeogenesis. The net result is to increase blood glucose levels. Because of their effects on glucose metabolism, glucocorticoids can worsen glycemic control in patients with over diabetes and can precipitate the onset of hyperglycemia in patients who are otherwise predisposed [6].To overcome this problem patient should take an antidiabetic drug with glucocorticoid.

MATERIALS AND METHOD:

Collection and Identification of the Plant:

The plant *Gmelina arborea* collected in the month of Nov -Dec from locally, guwahati region. The plant was identified from the standard literature (wealth of India,

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2001) then confirmed and authenticated by Department of Botany, Gauhati University, Guwahati, Assam. The collected plant material (leaf) were washed thoroughly with the help of water to remove the earthy matter or adherent impurity and then shade dried .The dried material were powdered by means of mechanical grinder. The resulting powdered material was stored in air tight glass container for further studies

Chemicals:

Phloroglucinol , conc. HCl (1:1), iodine solution, Mayer's reagent, Wagner's reagent, Hager's reagent, Dragendorff's reagent, Fehling solution, Benedict's reagent, Barfoed's reagent, $CHCl_3$,
Dexamethasone (Himedia Laboratories).

PHARMACOGNOSTICAL EXAMINATION:

MACROSCOPIC EXAMINATION:

The macroscopical observation were carried out as per performed by the standard methods to determine the shape, size, taste, colour, odour of powdered drug.

MICROSCOPICAL EXAMINATION:

Powder Microscopy:

The plant material were properly cleaned and dried first in the shade and then artificially in an oven at 60^0 C for approximately 50 hours. The dried plant material of the leaf was then subjected to size reduction to coarse powder using mixture grinder. The powder was passed through sieve no 60. This was further subjected with different reagents like chloral hydrate, Phloroglucinol and conc. HCl (1:1), iodine solution for the presence of the constituents like lignin, starch and calcium oxalate crystals.

Transverse section of midrib of leaf:

Midrib of the leaf of the plant was section by using blade, a clean glass slide taken and placed a drop of glycerine water in the centre of slide into this the section of midrib was placed then into this one drop of phluroglucinol and HCL (1:1) given. Placed the cover slip by using the finger and thumb of the left hand and let the edge of the cover slip rest on the slide at the left hand edge of the drop. Insert a dissecting needle under the right hand edge of the cover slip and let the latter rest on the needle. Lower the cover slip slowly on to the drop of the liquid exactly fills the space between the slide and the cover slip without any air bubbles being trapped inside. Placed the slide in position on the stage of microscope. Observed the T.S of the leaf by using 10X and 45X lens [1-5].

PROXIMATE ANALYSIS:

Extractive value:

Water soluble extractive:

Macerated about 5gm of the coarse powdered air dried leaf of *Gmelina arborea* with 100ml of chloroform water (2.5ml in 1000ml water) in a stoppered flask for 24 hrs, shaking frequently during first six hrs. filter through filter paper taking precaution against excessive loss of solvent. Evaporate 25ml of water extract to dryness in a tarred flat bottomed shallow dish. Dried at 105^0C and weighed. The percentage w/w of water soluble extractive value was calculated with reference to the air dried drug.

Alcohol soluble extractive:

Macerated about 5gm of the coarse powdered air dried leaf of *Gmelina arborea* with 100ml of alcohol (95% ethanol) in a stopper flask for 24 hrs, shaking frequently during first six hrs. Filter through filter paper taking precaution against excessive loss of alcohol. Evaporate 25ml of water extract to dryness in a tarred flat bottomed shallow dish. Dried at 105^0C and weighed.

The percentage w/w of alcohol soluble extractive value was calculated with reference to the air dried drug [1.]

DETERMINATION OF MOISTURE CONTENT:

About 5 gm of the air dried crude drug was accurately weighed in a tarred watch glass. The drug was kept in hot air oven at 105^0 c and dried for a period until constant weight obtained. The difference in weight gives the moisture content of the drug [1.]

DETERMINATION OF ASH VALUE:

Total Ash:

Method A:

Weigh accurately 2 to 3 gm of air dried drug in a tared platinum or silica dish and incinerate at a temperature not exceeding 450^0 until free from carbon, cool and weigh. If a carbon free ash is not obtained, wash the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper until the ash is white or nearly white, add the filtrate to the dish, evaporate to dryness and ignite at a temperature not exceeding 450^0. Calculate the percentage of ash on the dried drug basis.

Acid-Insoluble ash:

Method B:

Boil the ash (method A) with 25ml of *2 M* HCL acid for 5 minutes, collect the insoluble matter in a Gooch crucible or on an ashless filter paper, wash with hot water, ignite, cool in a desiccator and weigh. Calculate the percentage of acid- insoluble ash on the dried drug basis.

Water soluble ash:

Method C:

Boil the ash (method A) with 25ml water for 5 minutes, collect the insoluble matter in a Gooch crucible or on an ashless filter paper, wash with hot water, ignite for 15 minutes at a temperature not exceeding 450^0c. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water – soluble ash. Calculate the percentage of water soluble ash on the dried basis.

QUALITATIVE CHEMICAL ANALYSIS:

Extraction of Crude Drugs:

About 800gm of leaf of *Gmelina arborea* was taken in a 5000ml of round bottom flask and extracted for 72 hrs by maceration process by using alcoholic water. The extract was filtered through whatmann filter paper to remove impurities present. The extract was then concentrated by vacuum distillation, cooled and placed in desiccators to remove the excessive moisture [2].

PHYTOCHEMICAL ANALYSIS:

The concentrated extracts were subjected to chemical test as per the methods mentioned below for the identification of the various constituents.

Detection of Alkaloid:

Solvent free extract, 50mg is stirred with few ml of dilute hydrochloric acid & filtered. The filtrate is tested carefully with various alkaloidal reagents as follows:

- **Mayer's test:** To a few ml of filtrate, a drop or two of Mayer's reagent are added by the side of the test tube. A white or creamy ppt indicates test as positive.
- **Wagner's test:** To a few ml of filtrate, few drops of Wagner's reagent are added by the side of the test tube. A reddish-brown ppt indicates test as positive.
- **Hager's test:** To a few ml of filtrate, 1 or 2ml of Hager's reagent are added by the side of the test tube. A prominent yellow ppt indicates test as positive.
- **Dragendorff's test:** To a few ml of filtrate, 1 or 2ml of Dragendorff's reagent are added by the side of the test tube. A prominent yellow ppt indicates test as positive.

Detection of Carbohydrates

The extract (100mg) is dissolved in 5ml of water & filtered. The filtrate is subjected to the following test.

- **Molish's test:** To 2 ml of filtrate, 2 drops of alcoholic sol^n of alpha-naphthol are added, the mixture is shaken well & 1ml of conc. H_2SO_4 is added slowly along the side of the test tube & allowed to stand. A violet ring indicates the presence of carbohydrate.
- **Fehling's test:** 1ml of filtrate is boiled on water bath with 1ml each of Fehling solution A & Fehling solution B; a red ppt indicates the presence of sugar.
- **Benedict's test:** To 0.5ml of filtrate, 1ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 min. a characteristic colored ppt indicates the presence of sugar.
- **Barfoed's test:** To 1 ml of filtrate, 1 ml of Barfoed's reagent is added & heated on a water bath for 2 min. Red ppt. indicates presence of sugar.

Detection of Saponins

The extract (50 mg) is diluted with distilled water & made upto 20ml. The suspension is shaken for 15 min. A layer of 2 cm of foam indicates the presence of saponins.

Detection of Phenolic compounds

- **Ferric Chloride Test:** The extract (50mg) is dissolved in 5ml of distilled water. To this few drops of natural 5% ferric chloride solution is added. A dark green color indicates the presence of Phenolic compounds.
- **Gelatin Test:** The extract (50mg) is dissolved in 5ml of distilled water & 2ml of 10% sodium chloride solution is added. White ppt. indicates the presence of Phenolic compounds.

Detection of Glycoside

50gm of extract is hydrolyzed with concentrated hydrochloric acid for 2hr on a water bath, filtered & the hydrolysate is subjected to the following test:

- **Borntrager's test:** To 2ml of filtered hydrolysate, 3ml of $CHCl_3$ is added & shaken, $CHCl_3$ layer is separated & 10% NH_3 solution is added to it; pink color indicates the presence of glycosides.
- **Lead Acetate Test:** The extract (50mg) is dissolved in distilled water & to this; 3ml of 10% lead acetate solution is added. A bulky white ppt. indicates the presence of Phenolic compounds.
- **Magnesium & Hydrochloric acid reduction:** The extract (50mg) is dissolved in 5ml of alcohol & few fragment of magnesium ribbon & conc. HCl acid (drop wise) is added. If any pink to crimson color develops, presence of flavanol glycosides is inferred.
- **Alkaline reagent test:** An aqueous solution of the extract is treated with 10% NH_4OH solution. Yellow fluorescence indicates the presence of flavonoids.

Detection of Proteins & Amino acids:

The extract (100mg) is dissolved in 10ml of distilled water & filter through Whatman filter paper no-1& the filtrate is subjected to tests for proteins & amino acids.

- **Millon's test:** To 2ml of filtrate, few drops of Millon's reagent are added. A white ppt. indicates the presence of proteins.
- **Biuret test:** An aliquot of filtrate is treated with one drop of 2% copper sulphate solution. To this 1ml of ethanol (95%) is added, followed by excess of potassium

hydroxide palate. Pink color in the ethanolic layer indicates the presence of proteins.

- **Ninhydrin test:** Two drops of ninhydrin solution (10mg of ninhydrin in 200 ml of acetone) are added to 2ml of aqueous filtrate. A characteristic purple color indicates
- **Legal's test:** 50mg of extract is dissolved in pyridine, sodium nitroprusside solution is added & made alkaline using 10% NaOH & presence of glycoside is indicated by pink color.
- **Keller-Killiani test:** To an extract of drug in glacial acetic acid, few drops of ferric chloride & conc. Sulfuric acid are added. A reddish brown color is formed at the junction of two layers & the upper layer turns bluish green.

Detection of Phytosterols:

Liebermann- Burchard's test: Extract (50 mg) + 2 ml acetic anhydride. To this solution 1-2 drops of conc. Sulfuric acid is added, along the sides of the test tube. An array of color changes shows the presence of phytosterols.

Detection of Fixed Oils & Fats:

- **Spot test:** Press a small quantity of extract separately between two filter papers. Oil stains on the paper indicate the presence of fixed oil.
- **Saponification test:** Add a few drops of 0.5 n alc. KOH to a small quantity of extract along with a drop of phenolphthalein. Heat the mixture on water bath for 1-2 hr. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils & fats.

Detection of Gums & Mucilage

Extract (100 mg) is dissolved in 10 ml of distilled water & to this 25 ml of absolute alc.is added with constant stirring. White or cloudy ppt. indicates the presence of gums & mucilage.

Detection of coumarin

Extract (50mg) is dissolved in 10ml absolute alcohol and to this few drops of ferric chloride is added. Greenish fluorescence indicates the presence of coumarin.

Detection of lignin

Extract (50mg) is treated with conc.hydrochloric acid and phloroglucinol solution. Pink colour indicates presence of Lignin [4] .

PHARMACOLOGICAL ACTIVITY:

Acute toxicity study:

Healthy Wister albino rats of either sex weighing 200 ± 20 g were divided into 5 groups of 6 animals each. The animals were housed under standard conditions and room temperature (25 ± 2° C) was controlled. All animals were fed with standard rat pelleted diet and had free access to tab water *ad libitum.* Hydro alcoholic extract of leaf was administered orally through gastric intubation in water at doses of 100, 200, 500, 1000, 2000 and 3000 mg/kg bw and control group received water. The animals were observed continuously for 72 hr for any signs of behavioral changes, toxicity and mortality.

Dexamethasone-induced insulin resistance in rats:

Animals were divided in to 5 groups, each consisting of six rats. Rats in the first group received vehicle and served as control group, while the second group of rats received vehicle plus dexamethasone (10 mg/kg s.c.) and served as positive control group. Rats in experimental groups 4 and 5 were treated with extract of leaf of *Gmelina arborea* (100 & 200 mg/kg) plus dexamethasone, whereas rats in the 3rd group were treated with standard drug (500 µg/mg). All the animals received their respective assigned treatment daily for a period of 12 days. Rats of group 2-5 were daily fasted over night before dexamethasone treatment. On day 4th, 8th blood were collected from tail and measure the blood sugar level and on 12th day the animals were anesthetized with ether and blood was collected from retero-orbital plexus. Serum was then separated for the estimation of glucose by using respective kits [3] .

RESULT AND DISCUSSION:

Pharmacognostical examination:

Macroscopic Examination:

Gamhar is a tree that can grow to 30 m high, with smooth, whitish to greyish reddish-brown bark and a straight trunk. Its leaves are 8 to 20 cm long, 4.5 to 15 cm wide, and covered with star-shaped hairs. Two large glands are paired at the base of each leaf. The outer surface of the calyx (sepals) is scattered with flat, round glands. The flowers are reddish-yellow, hairy and five-lobed. The hairless fruits are 10 to 15 mm in diameter and glossy yellow when mature. They are recorded as having a bittersweet taste.

Leaf of *Gmelina arborea Roxb:*

Upper surface: Light green		**Lower surface**: Light green	
Lenght:	8 - 20 cm	**Width**:	4.5 - 15 cm
Taste:	Slight bitter	**Odour**:	Characteristic
Apex:	Acuminata	**Shape**:	Ovate
Margin:	Entire	**Leaf arrangement**:	Opposit

MICROSCOPICAL EXAMINATION:

Powder microscopy of leaf:

The powdered leaf of *Gmelina arborea* is dark green in colour. When powder was mounted with chloralhydrate,

phloroglucinol and HCl and stained with saffranin following elements were observed:

Trichomes, starch grain, ca-oxalate crystals, stomata, palisade cell, spongy tissue.

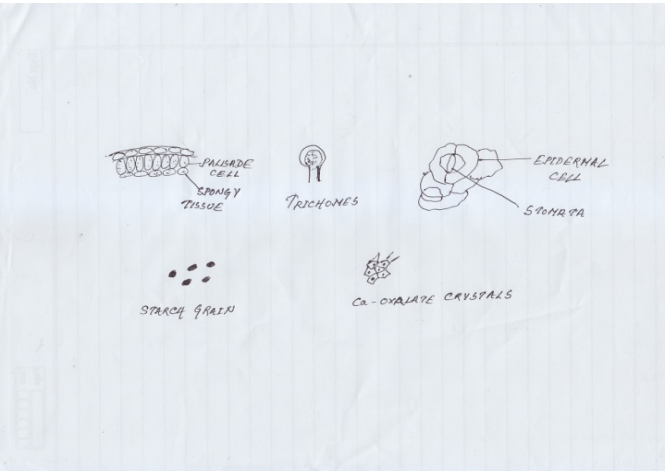

Figure 1: Powder microscopy of leaf of *Gmelina arborea Roxb.*

T.S *OF GMELINA ARBOREA.ROXB LEAF* (MIDRIB):

T.S of midrib shown that the leaf consist of upper and lower epidermis. Upper epidermis single layered, rectangular cells. Lower epidermis also single layered, having glandular trichorme and stomatal pores. Under upper epidermis palisadeparenchyma were seen with some spongy parenchyma. Vascular bundles were also seen under the microscope which is consisting of xylem and phloem.

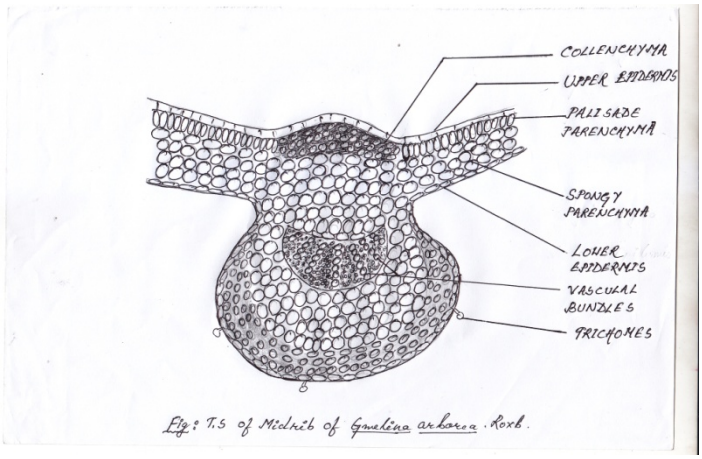

Figure 2: Transverse section of midrib of leaf of *Gmelina Arborea. Roxb.*

PROXIMATE ANALYSIS:

The result of extractive values and moisture content Fluorescence analysis are shown in table below:

Extractive value:

Table 1: Extractive value of leaf of *Gmelina Arborea. Roxb*

Sr. No.	Type of extract	Percentage(w/w)
1	Alcohol	7.4%
2	Water	24%

Moisture Content:

Table 2: Moisture content of leaf of *Gmelina Arborea Roxb.*

Wt. of drug	Initial wt of drug+pet dish (gm)	Constant wt. after dring (gm)	Loss on dring (gm)	Moisture content
5 gm	39.97	39.72	0.25	5.0%

Ash Value:

Table 3: Ash value of leaf of *Gmelina Arborea Roxb.*

Plant parts	Total ash	Acid insoluble ash	Water soluble ash
Leaf	5.5%	1.5%	0.5%

PHYTOCHEMICAL ANALYSIS:

The extracts were subjected to preliminary phytochemical analysis and the results are represented in the table below.

Table 4. Preliminary photochemical analysis

Test	Hydroalcoholic Extract
Alkaloids	+
Carbohydrates	+
Glycosides	+
Lignin	+
Fixed oil and fats	_
Phenolic compound and Tannins	+
Saponins	_
Proteins and Aminoacids	_
Gums and Mucilage	_
Flavonoid	+
Coumarin	+

(+) = Present, (-) = Absent

PHARMACOLOGICAL ACTIVITY:

Acute toxicity study:

There was no mortality or any signs of behavioral changes or toxicity observed after oral administration of hydroalcoholic extract of leaf upto the dose level of 3000 mg/kg bw in rats.

DEXAMETHASONE INDUCED INSULIN RESISTANCE IN RAT:

Effect on biochemical parameters:

The entire group significantly (P<0.001) decreased dexamethasone induced elevation of serum glucose. Hydroalcoholic extract of leaf at a dose level of 200 mg/kg produced lower effect in serum glucose level when compared with lower dose (100mg/kg). The standard and high dose of extract treated group significantly (P<0.001) decrease the level of serum glucose when compared with positive control group (Diabetic control).

Table 5: Effect on biochemical parameters

Group	Serum Glucose mg/dl		
	4th day	8th day	12th day
Control	71.5 ± 2.11	74.3 ± 3.12	75.2 ± 2.05
Diabetic control	165.5 ± 1.91	170.5 ± 2.11	172.5 ± 3.02
Standard	82.5± 2.05	78.5 ± 3.75	77.4 ± 2.33
Extract 100mg/kg	115.3 ± 4.02	110.5 ± 2.13	102.5 ± 3.05
Extract 200mg/kg	103.5 ± 3.75	96.3 ± 4.12	92.5 ± 4.72

All value expressed in Mean ± SEM, Oneway ANOVA followed by StudentNewmanKeuls Method
P<0.001 (diabetic control vs std.), P<0.001 (Diabetic control vs low dose)
P<0.001 (Diabetic control vs high)

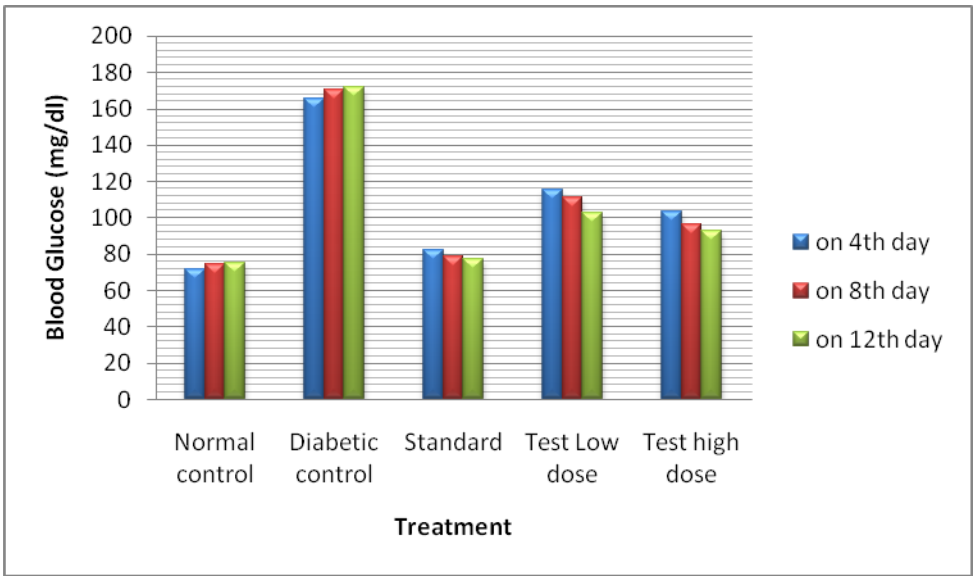

Figure 3: Treatment graph

CONCLUSION:

In conclusion, oral administration of hydroalcoholic extract of leaf lowers serum glucose level in dexamethasone-administered rats. Extract showed significant anti-diabetic effect in rats after oral administration. Thus the claim made by the traditional Indian systems of medicine regarding the use of this plant in the treatment of diabetes stands confirms. The results suggest the presence of biologically active principle flavonoids which may be worth further investigation, elucidation.

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