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Phytochemical and pharmacological evaluation of *Cordia gharaf* bark isolated compounds

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Abstract
Objective: The present study includes the preliminary screening of phytochemicals and evaluations of an *in vivo* antidiabetic activity of *Cordia gharaf* bark extract isolated compound.
Materials and Methods: Plant material was subjected to the extract preparation by soxhlet apparatus using ethanol. The various kinds of phytochemicals were studied and *in vivo* antidiabetic activity of *Cordia gharaf* bark were detected by using Dexamethasone induced insulin resistance in mice
Results: The study reveals the presence of phytochemicals such as carbohydrates, glycosides, flavonoids, alkaloids, steroids, tannins. Column chromatography using pet ether: acetone (90:10) and methanol: ethyl acetate (50:50) as mobile phase and all the collected fractions were subjected for TLC. The ethanol isolated compound of *C.gharaf* (300 mg/kg b.w) significantly reduces elevated blood glucose levels (P<0.0001). Treatment with the antidiabetic drug Glibenclamide (600 µg/ kg b.w). Blood samples were drawn from tail tip of mice at week interval till the end of study. Blood sugar level estimated by using one touch electronic glucometer. All the values of fasting blood sugar level and biochemical estimations were analyzed for one way ANOVA and post hoc Dunnett's t test using graph pad prism 6.0 version computer software.
Conclusion: The present study establishes that the drug was found safe up to 2000mg/kg/po. 200mg/kg was found as lethal dose and 100,200, and 300 mg/kg was used for evaluation of anti-diabetic activity. 300 mg/kg dose was found to be effective for anti-diabetic activity. *Cordia gharaf* bark may contain the active constituent amongst the alkaloid, sterol, glycosides, and carbohydrate. It has significant anti-diabetic activity as compared to standard drug (Glibenclamide).

Keywords: *Cordia gharaf*, dexamethasone, glibenclamide. antidiabetic activity

Introduction
Herbal products have gained increasing popularity in the last decade, and are now used by approximately 20% of the population. Herbal products are complex mixtures of organic chemicals that may come from any raw or processed part of a plant, including leaves, stems, flowers, roots, and seeds. Under the current law, herbs are defined as dietary supplements.[1] Herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients [2].
Diabetes is the most common endocrine disorder and by the year 2010. It is estimated that more than 200 million people worldwide will have DM and 300 million will subsequently have the disease by 2025 [3]. Oral hypoglycemic agents are also useful in the treatment of type 2 DM. Oral hypoglycemic agents include sulphonylureas, biguanides, alpha glycosidase inhibitors and thiazolidinediones. The main objective of these drugs is to correct the underlying metabolic disorder, such as insulin resistance and inadequate insulin secretion. They should be prescribed in combination with an appropriate diet and lifestyle changes. Diet and lifestyle strategies are to reduce weight, improve glycaemic control and reduce the risk of cardiovascular complications, which account for 70% to 80% of deaths among those with diabetes. Diabetes is best controlled either by diet alone and exercise (non-pharmacological), or diet with herbal or oral hypoglycemic agents or insulin (pharmacological) [4].
Cordia gharaf is a small tree growing mostly in tropical and temperate India, Sri Lanka and Abyssinia. In India, it is mostly found in Gujarat, Maharashtra, Rajasthan and Punjab. The bark of plant possess antidote activity, fruit pulp has astringent, antidiarrhoeal and antiseptic activities and reduces burning sensation of urinary tract, root has abortifacient and anti-inflammatory activities, while the whole plant has antidiabetic & antileprotic activities. In Ayurveda, the plant is considered as a source of Laghusleshmataka, a substitute of Sleshmataka (*Cordia dichotoma* Forst.), the bark of which is used in dyspepsia and fevers [5].
The bark is used in heart ailments, and its efficacy was found to be better than other Ayurvedic cardiotonic drugs, like Arjuna (Terminalia Arjuna W. & A.) Bark and Javsar (*Pterocarpus marsupium* Roxb.) wood. β-sitosterol (from stem and leaf), D-galactose,

D-fructose, D-xylose, L-rhamnose and D-galactouronic acid (from fruit mucilage), hydrocarbon and n-hexacosanol (from leaf) have been reported from the plant bark of *Cordia gharaf* [6] contains alkaloids, glycoalkaloids, coumarins and salts of potassium chloride. The aim of this study was to find out the scientific basis of the use *Cordia gharaf* in the management of diabetes used by traditional practitioners using ethanol extracts on Dexamethasone induced insulin resistance in mice.

Material and Methods

Plant material

The bark of *Cordia gharaf* bark were collected from local area of Otur (Pune, Maharashtra).These plant parts were authentified by P.G. Diwakar Joint Director of Botanical Survey Of India Western regional Centre, Koregaon Road, Pune, Maharashtra.. (Voucher no. COGBIJ3).

Plant profile

Fig 1: *Cordia gharaf* plant

General information

Scientific Name: *Cordia gharaf* (Forsskal) Ehrenb. ex Asch.

Synonyms [7, 8]

Common name	Cordia rothi, Gondani, Gundi
Botanical name	Cordia rothi
English	Grey Leaved Saucerberry, Narrow Leaved Sepistan
Hindi	Lasora, Gondi
Malayalam	Narivirayan, Verasham, Veri
Tamil	Sirunaruvuli
Marathi	Gondani
Telugu	Chinnabotuku, Chinnavirigi
Punjabi	Gondi
Sanskrit	Laghusleshatmaka

Morphology of Bark

a) Inner surface of *cordial gharaf* bark

b) Outer surface of *cordial gharaf* bark

Bark is curved to channeled in shape; young bark 10-20 cm in length, 12-20 mm in width and 1-2 mm in thickness with smooth greenish brown outer surface transverse by transversely elongated whitish lenticels. Older barks are thicker (3-5 mm) and with very rough external surface due to presence of rhytidoma. Inner surface is longitudinally striated, whitish in color when fresh. Certificated by Institutional animal ethical committee, Registration no. 1197/PO/c/08/ CPCSEA and Ref: SPCOP/IAEC/2012/2013/09.

Standardization of selected plant materials

These plant pieces were cleaned and shade dried at room temperature then subjected to physical evaluation with different parameters then these selected plant part (bark) were subjected to size reduction to get coarse powder, separately in a mechanical grinder and passed through sieve no.40 to get a desired particle size and stored in a well- closed air tight jars. This uniform powder was subjected to standardization with different parameter [9].

Preparation of Extraction [10]

Cordia gharaf barks were dried and powdered. The powder obtained was weighed, and then used for extraction. The Powder was extracted with ethanol (40-60 ºC) for 8 hrs. To remove fatty matter and then with methanol till exhaust by Sohxlet extraction method. The resulting extracts were concentrated transferred in Petri dishes and allowed to dry in hot air oven at 60 ºC for 24hrs. The amount of extracts were weighed and stored in airtight self-sealable pouch with Aluminum foil wrapping. The% yield, colors and nature of the extracts were observed.

Preliminary phytochemical tests

The ethanolic extracts of the crude drug were subjected to qualitative chemical tests in order to identify class compound present as per procedures. The chemical tests were performed for identifying the different chemical constituents present in the extracts [11] (Table No. 3)

Isolation of compound by column chromatography

Two mobile phases were used to separate different phytoconstituents. At first, column eluted with pet ether: acetone (90:10), elution shows green, yellow and brown colored bands (Fig no.3). Elution should be continuous until removal of green colored compound, fats and waxes. The second elution done continuously with mobile phase methanol: ethyl acetate (50:50), until a removal of brown colored spot. All the samples collected at the interval of 15ml in a cleaned glass vials marked up to 15ml. All the collected

fractions were subjected for TLC; (Fig.no.4) and fraction with similar Rf value combined together and evaporated for yield. (Table no.2)

Animals

Experiment were carried on Swiss albino mice mice (20-25g) (n=6 per group).Animals were kept in colony cages at 25±2 ºC, relative humidity 50-55% maintained under 12h light and dark cycle. The animals were fed with standard animal feed and water was applied ad *libitum*. Each animal was used only once. The animals were kept on fasting overnight prior to the experimentation and animals care.

Determination of acute toxicity studies [16]

Six mice were divided in to 3 groups as per dose 175mg/kg, 563mg/kg and 2000mg/kg. Mice were exceptionally died, so needed to be stopping dosing at 2000 mg/kg. One tenth of 2000mg/kg was considered as dose (200mg/kg) for isolated compound. *Cordia gharaf* bark extract was found to be safe up to 5000mg/kg. [12]

Antidiabetic activity

Induction of diabetes mellitus

Dexamethasone induced insulin resistance in mice

Mice were rendered hyperglycemic by daily administration of a pre standardised dose of Dexamethasone (1ml/kg, i.m.) for consecutive 7days and then divided into 6 groups of six each.[13] Animals were then kept under observation for a standard condition; following administration and blood glucose levels were subsequently determined. Mice with more than 3_/4-fold in creased in their blood sugar levels were considered diabetic and used for further test; Serum profile of H.D.L, L.D.L., V.L.D.L., T.G., and Total cholesterol.

Group 1: (Normal) received orally distilled water for 14 consecutive days fallowed by fasting on last day.

Group 2: (Diabetic control) received distilled water and feed for 14 consecutive days.

Group 3: (Dexamethasone+*C.G.B.I.F*.-100mg/kg) received orally and for 14 Consecutive days after overnight fasting.

Group 4: (Dexamethasone+ *C.G.B.I.F* -200mg/kg) received orally and for 14 Consecutive days after overnight fasting.

Group 5: (Dexamethasone+ *C.G.B.I.F* -300mg/kg) received orally and for 14 Consecutive days after overnight fasting.

Oral glucose tolerance test (OGTT)

All the animals were given glucose (2 g/kg) 30 min after daily dosing except normal. After the glucose loading mice were divided into 3 groups, *Cordia gharaf extract* and *C.G.B.I.F*. (100,200 and 300mg/kg) and normal control. Blood glucose levels were estimated**.** Blood samples were collected from the retro orbital plexus of the eye just prior (0 h) and 30, 60, 90,120 min. and 180minutes [14].

Collection of blood sample and blood glucose determination

Blood samples were drawn from tail tip of mice at weekly intervals till the end of study (i.e., for2 weeks). Fasting blood glucose estimation and body weight measurement were done on day 1, 7, and 14 of the study. Blood glucose estimation can be done by one touch electronic glucometer using glucose test strips. (SD Codefree, Made in Korea). On day 14, blood was collected from retro-orbital plexus under mild ether anesthesia from overnight fasted mice and fasting plasma glucose concentration was estimated. Serum was separated and analyzed for serum cholesterol, serum triglycerides by enzymatic DHBS colorimetric method, serum H.D.L., serum L.D.L, serum V.L.D.L. and Total cholesterol estimated [15].

Statistical analysis

All the values of fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.) and analyzed for One way ANOVA and post hoc Dunnetts *t*-test using Graph pad prism 6.0 version computer software. Differences between groups were considered significant at *($p<0.05$), **($p<0.01$), ***($p<0.001$), ****($p<0.0001$).

Results and Discussion

Phytochemical screening: Phytochemical screening revealed the presence of Carbohydrates, Gums, Mucilage, amino acid, Proteins, flavonoids, steroids, tannins and phenolic compounds. The microscopic characteristics of bark extract are showed in fig.2.

Acute toxicity studies: The CGFAE was found to be non-toxic even up to the highest dose (2000 mg/kg b.w.) tested in Swiss albino mice for the period 48h and no morbidity and/or mortality were recorded. The observation of the entire dose does not show any toxic effect on mice.

Dexamethasone induced insulin resistance in mice

In Dexamethasone induced insulin resistance, it was found that it was significantly controlled by treating with isolated compound at dose 200mg/kg. and compared with standard (positive control) Glibenclamide a marketed preparation. With three doses of isolated brown coloured compound treated to animals 100mg/kg, 200mg/kg, and 300mg/kg. At the dose 300mg/kg, it was resulted in significant decrease in blood serum glucose level. The overall study results are reported in table no.5 and in fig.no.5, 6, and 7.

The purpose of this work is to evaluate the scientific basis for its traditional claim uses of *Cordia gharaf* bark extracts isolated compounds. 200mg/kg was found as lethal dose.

Preliminary Phytochemical studies revealed that it shows positive test for carbohydrates, reducing sugar, glycoalkaloids, flavonoids, phytosterols, and phenolic compounds.

Isolated compound from *Cordia gharaf* bark extract evaluated for acute toxicity tests, lethal dose was found 200mg/kg. Also tested for Oral Glucose Tolerance Test (OGTT), and hypoglycemic activity. Positive tests for hypoglycemic activity and OGTT, indicates presence of anti-diabetic activity. The Oral glucose tolerance test results are reported in table no. 4.

Table 1: Macroscopy of Cordia gharaf Bark

Sr.no	Particular	Observation
1	Color	Inner bark is reddish brown and outer bark is blackish brown
2	Odor	Characteristic
3	Taste	Bland
4	Surface	Outer surface is thick, with numerous small fissures & inner surface is fibrous
5	Shape	Slightly curved

Table 2: Thin layer chromatography of fractions

Solvent system	Visualizing agent	No of spots	Rf value	Color of spots
Pet ether: acetone (90:10)	UV light	1	0.4	fluorescence
Methanol: ethyl acetate (50:50)	Phenol sulphuric acid reagent	1	0.7	brown

Table 3: Preliminary Phytochemical screening

Sr. No.	Chemical Test	remark
1	Test for Carbohydrates	+
2	Test for Protein/amino acid	-
3	Test for Fats/waxes	-
4	Test for Glycosides	+
5	Test for Flavonoids	+
6	Test for Alkaloids	+
7	Test for Terpenes	-
8	Test for Steroids	+
9	Test for Saponins	-
10	Test for Tannins/Phenolics	+

(+ mark positive reaction and – indicates negative reaction)

Table 4: Oral glucose tolerance test for *Cordia gharaf* bark extract

Sr.no	Animal marked As	Weight of animal	Dose of extract	Dose of glucose	0 min	30 min	60 min	90 min	120 min
1	Head	23 gm.	11 mg.	4 mg.	106	102	275	235	211
2	Body	24 gm.	12 mg.	4.8mg.	122	86	338	294	279
3	Tail	24 gm.	12 mg.	4.8mg.	126	159	122	92	87

Table 5: Effect of *C.G.B.I.F.* on plasma glucose in dexamethasone induced mice.

Sr.no.	Group	Plasma glucose concentration (Mean±S.E.M.)		
		0 day	7thday	14thday
1.	Normal control	90.67±1.97	89.83±11.81	90.0±1.06
2.	Diabetic control	236.3±10.51	253.0±11.81	282.5±7.21
3.	Dexamethasone+C.G.B.I.F. (100mg/kg)	208.2±6.51	158.2±11.51**	161.3±9.42****
4.	Dexamethasone+C.G.B.I.F. (200mg/kg)	220.7±9.59	177.5±10.93**	164.3±9.71***
5.	Dexamethasone+C.G.B.I.F. (300mg/kg)	242.2±14.09	188.2±6.93**	147.8±5.64****
6.	Dexamethasone+Glibenclamide (600µg/kg)	230.1±10.89	196.3±7.66**	158.7±11.03***

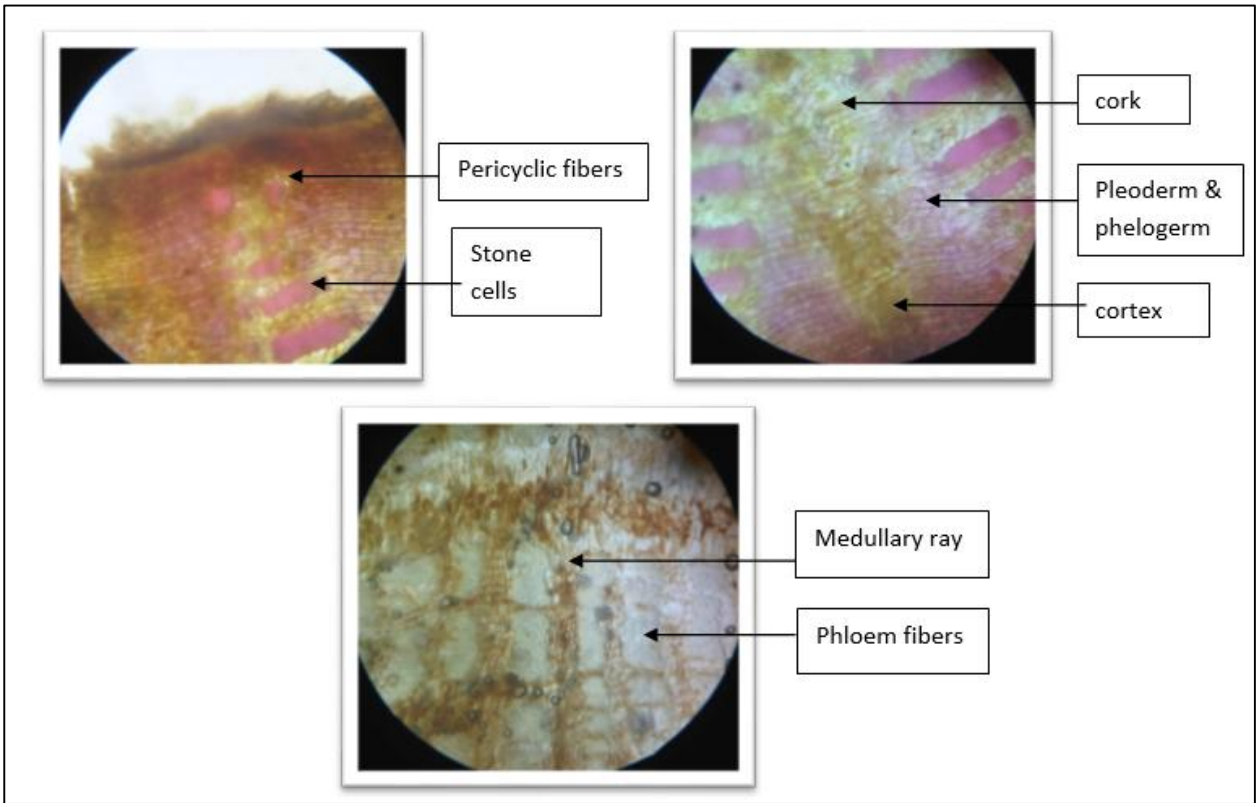

Fig 2: Microscopy of *cordial gharaf* bark

Fig 3: Column Chromatography

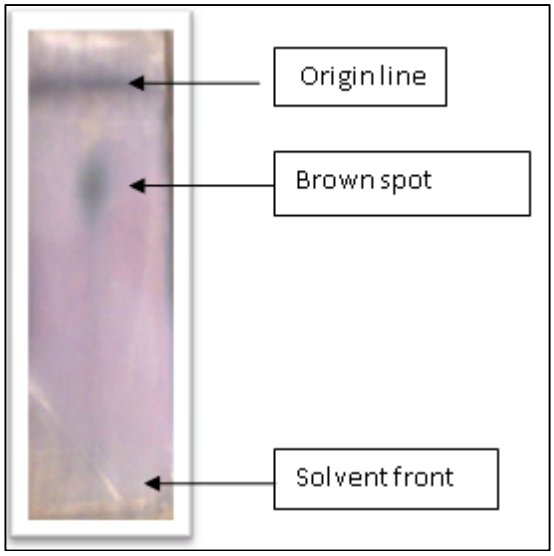

Fig 4: TLC

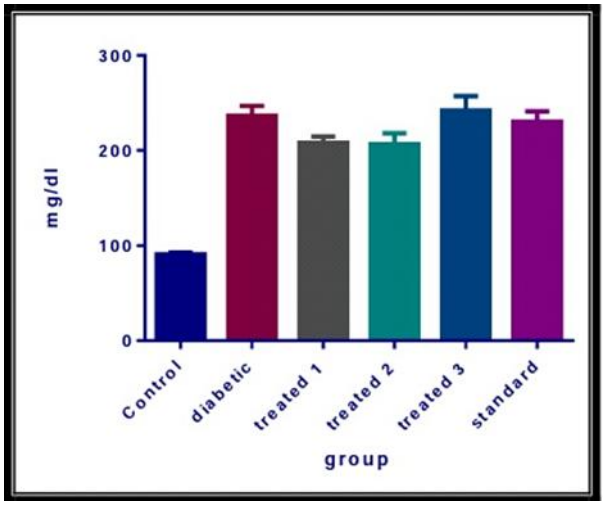

Fig 5: Effect of *C.G.B.I.F.* on plasma glucose concentration in Dexamethasone induced mice

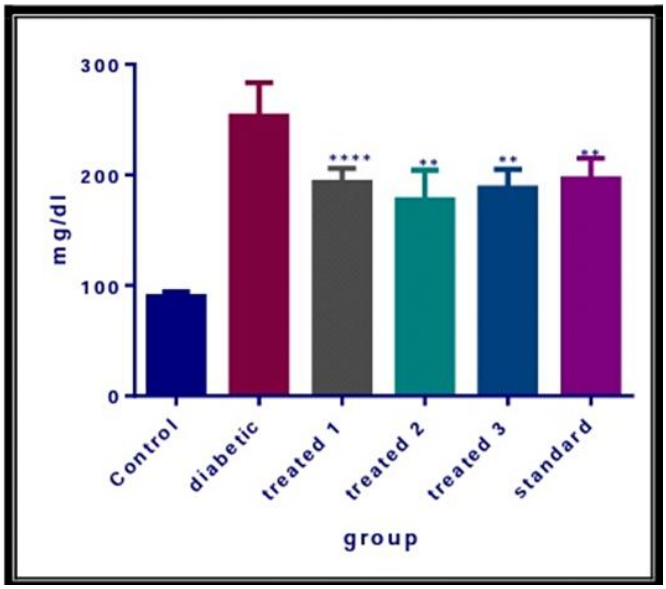

Fig 6: Effect of *C.G.B.I.F.* on plasma glucose concentration in Dexamethasone induced mice.

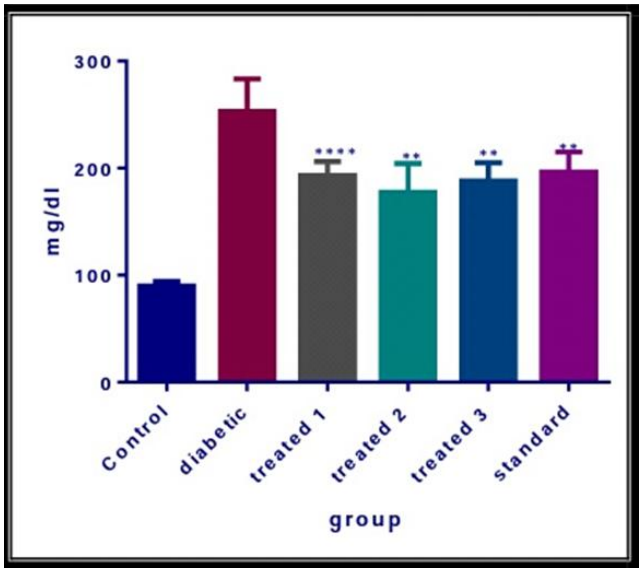

Fig 7: Effect of *C.G.B.I.F.* on plasma glucose concentration in Dexamethasone induced mice

Conclusion

Cordia gharaf barks were subjected for studying phytochemical and pharmacological evaluation. Finding of present study establish that the drug was found safe up to 2000mg/kg/po. 200mg/kg was found as lethal dose and 100, 200, and 300 mg/kg was used for evaluation of anti-diabetic activity. 300 mg/kg dose was found to be effective for anti-diabetic activity. *Cordia gharaf* bark may contain the active constituent Steroidal aglycone, amongst the alkaloid, sterol, glycosides, and carbohydrate. It has significant anti-diabetic activity as compared to standard drug (Glibenclamide). Further experimentation would necessary to elucidate the exact mechanism of action of *C.gharaf* bark.

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