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PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDY OF *AEGLE MARMELOS* FOR ANTI DIABETIC ACTIVITY

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ABSTRACT

The present work is focused to evaluate the Pharmacognostic features and pharmacological studies of the Aegle marmelos (Rutaceae) leaves. In This present study is been designed to investigate detailed morphological and microscopical features of the leaves of the plant and evaluation of Anti diabetic activity. In the present work, other identification parameters are also studied which includes powder characterization and fluorescence analysis of the leaves. In the present study albino wistar rats weighing about 200-250 gms were selected and divided into 6 groups containing 6 animals in each group. The experiment was started with the treatment of Alloxan monohydrate by intraperitoneal route in a dose of 150 mg/kg body weight to all rats except group-I animals for 3 days alternatively for induction of diabetes and biochemical parameters are estimated. it was observed that Diabetes produced by the 150mg of Alloxan is prevented by treatment of groups ethyl acetate extract of *Aegle Marmelos* decreases the levels of Blood Glucose, Cholesterol, Triglycerides, HDL, LDL, VLDL

Key Words: Aegle Marmelos, Anti diabetic activity, Alloxan monohydrate

INTRODUCTION:

Diabetes mellitus is characterized by alterations in the metabolism of carbohydrate, fat and protein, is caused by a relative or absolute deficiency of insulin secretion and different levels of insulin resistance and it is resulting from both genetic predisposition and favoring environmental factors.

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In the patients, late complications develop consisting of alterations and failure of various organs (especially the non-insulin sensitive ones) including the eyes (retinopathy with vision loss), kidneys (nephropathy leading to renal failure), nerves (peripheral and autonomic neuropathy), heart and blood vessels (precocious and severe cardiovascular, cerebrovascular and peripheral vascular atherosclerosis)[3]. Increasing interest in herbal medicine, more individuals are exploring the possibility of using natural medicines to complement conventional therapy, as is already the case in certain minority cultures [1].

Agele marmelos (L.,) Corr., (rutaceae) is a popular medicinal plant in the ayurvedic and siddha systems of medicine and folk

medicines used to treat a wide variety of ailments. The plant, popularly known as the bael tree[2]. Various parts of the tree,including the fruit, possess medicinal properties. The roots are useful for treating diarrhea, dysentery, and dyspepsia[31].The leaf is used for opthalmia, diabetes, and asthmatic complaints. Unripe fruit is useful for treating diarrhea, dysentery and stomachalgia. The aqueous extracts of the stem and root bark are used to treat malaria, fever, jaundice, and skin diseases such as ulcers, urticaria, and eczema.

Material and method:

The plant leaf was collected during the month December to January and was identified pharmacognostically by a botanist. Then it was dried in shade, powdered, weighed and stored in a clean, dry and air tight container. The powder was subjected for successive extraction with solvents n-Hexane and ethanol.

Powder extraction:

Powder was packed in a round bottom flask and extracted with n-Hexane at 70^0C temperature by soxhlet apparatus to remove the chlorophil content from palnt. After that the powder is extracted with ethyl acetate at 80^0C by Soxhlet extactor. After extraction the residue was dried on water bath at 100^0C to get a solid mass.

PHARMACOLOGICAL ACTIVITIES

Based on the references of previous acute toxicity studies of the plant, 250mg/kg & 500mg/kg doses were selected as low and high dose[69].

Experimental animals

Adult male or female Wister rats, weighing 200 to 250g were used in the study. The study protocol was reviewed and approved by the institutional animal ethical committee and conforms to the Indian national science academy guidelines for the use and care of experimental animals in research. Animals were obtained from the Sri Venkateswara Enterprises, Bangalore. Rats were housed in polyacrylic cages (38X23X10 cm) with not more than four animals per cage. They were housed in an air conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (approximately 14 h light/ 10 h dark) and maintained humidity 60±5% and an ambient temperature of 25±2^{o}C. All experiments were performed between 9:00am and 4:00pm. the animals were free access to standard diet and tap water *ad libitum* and allowed to acclimatize for one week before the experiments. Commercial pellet diet contained 22 % Protein, 4% Fat, 4% Fiber, 36% Carbohydrates and 10% Ash w/w, supplied by Sri Venkateswara Enterprises, Bangalore was used.

Experimental protocol

Rats were randomly divided into five groups, each consisting of six animals

Group 1: Normal group animals are untreated animals.

Group 2: Positive Control group animals treated with 1ml normal saline orally.

Group 3: Vehicle control group animals treated 0.5 ml of DMSO.

Group 4: Test group-I animals treated with 250 mg/kg *Aegle Marmelos* extract orally.

Group 5: Test group-II animals treated with 500 mg/kg *Aegle Marmelos* extract orally.

Note: All test agents were dissolved in DMSO and given orally for a period of 7 days for treatment.

CHEMICAL SUBSTANCES

Alloxan monohydrate was procured from the Avra Synthesis, Hyderabad. And all the other chemicals are procured from Merck laboratories, nice chemicals, Sd fine chemicals from local market.

Drug solutions

Ethyl acetate extract of *Aegle Marmelos* is very sparingly soluble in aqueous solutions, to solubilise the extract DMSO was used as a vehicle and desired concentration of drug solutions were prepared. Alloxan was freshly prepared by dissolving in normal saline.

EXEPERIMENTAL PROCEDURE

In the present study albino wistar rats weighing about 200-250 gms were selected and divided into 6 groups containing 6 animals in each group. The experiment was started with the treatment of Alloxan monohydrate by intraperitoneal route in a dose of 150 mg/kg body weight to all rats except group-I animals for 3 days alternatively for induction of

diabetes. After induction of diabetes **Group-I** animals served as untreated control. **Group-II** animals were treated with 1ml normal saline orally for 7 days. Similarly **Group-III** and **Group-IV** animals were treated with 250mg and 500mg of *Aegle Marmelos* extract for 7days respectively. And after 7 days animals were fasted for 48 hrs and anaesthetized with thiopentone sodium and sacrificed. Blood samples were collected by carotid artery, then immediately blood glucose was measured with glucometer and centrifuged at 4500 rpm for 15 minutes. The supernatant was separated and used for estimation of various biochemical parameters by autoanalyser.

BIOCHEMICAL ESTIMATIONS

Estimation of Blood sugar levels, Cholesterol, Triglycerides, High density lipoprotein, Low density lipoprotein, Very low density lipoprotein

Estimation of serum glucose

To 1500 µl of the reagent, 1500 µl of purified water, 20 µl of standard glucose (100 mg/dl) was added and incubated for 5 min at 37^{o} C. This incubated mixture was aspirated and concentration of standard was calibrated to show a value of 100 mg/dl. The serum glucose was estimated by adding 20 µl of the serum sample to 1500 µl of the reagent, 1500 µl of purified water mixed well and incubated at 37^{o} C for 5 min. This incubated mixture was aspirated and absorbance recorded against a reagent blank at 505 nm using Clinical Chemistry Analyzer.

Estimation of triglycerides

To 1000 µl of the reagent, 10 µl of standard triglyceride (200 mg/dl) was added and incubated for 10 min at 37^{o}C. This incubated mixture was aspirated and concentration of standard was calibrated to show a value of 200 mg/dl. The serum triglyceride was estimated by adding 10 µl of the serum sample to 1000 µl of the reagent, mixed well and incubated at 37^{o} C for 10 min. This incubated mixture was aspirated and absorbance recorded against a reagent blank at 505 nm using Clinical Chemistry Analyzer.

Biochemical Parameters

Blood Glucose:

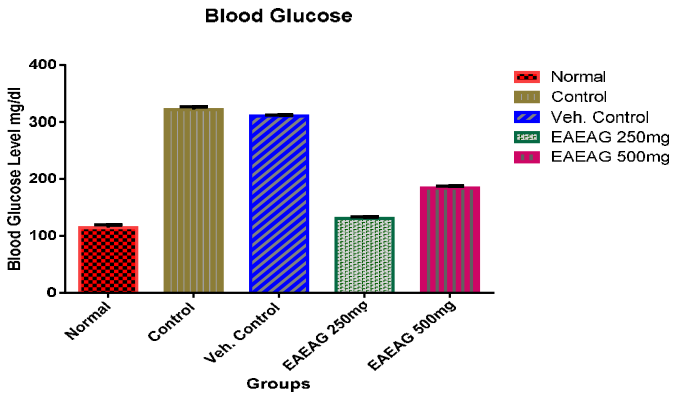
With normal group Blood glucose levels were found to be **114.6 ± 5.25** with Control (Saline) Blood glucose levels were found to be **321.9 ± 4.88** and with vehicle control (DMSO) Blood glucose was reduced to **311.0 ± 1.24.** Hence all the values of treatment groups were compared with control group. When EAEAM was administered orally at the dose of 250mg/kg Blood glucose was significantly reduced to **130.81 ± 2.89 (*p<0.0001*)**. When EAEAM was administered orally at the dose of 500mg/kg Blood glucose was significantly reduced to **184.18 ± 3.56 (*p<0.0001*)**. There was a significant decrease in Blood glucose with EAEAM 250mg/kg.

Table No.**1 Nature, Percentage and Yield of the extract**

S.No	Name of the extract	Nature	Colour	% yield of extract in gms
1	Ethyl acetate extract of Aegle Marmelos	Sticky	Dark Green	20%

Table No. 2: Consolidated Table Showing the **Effect of EAEAM on Blood Glucose**

S.NO	Normal	Control	Veh.Control	EAEAM 250mg/kg	EAEAM 500mg/kg
1	100.19	317.2	312.4	133.4	187.6
2	120.40	330.6	310.1	126.2	181.5
3	112.15	320.4	309.5	130.1	179.3
4	118.20	319.2	310.9	134.3	182.4
5	109.35	324.5	312.6	131.2	186.9
6	119.17	319.6	310.6	129.7	187.4
Mean ± S.D.	**114.6 ± 5.25**	**321.9 ± 4.88**	**311.0 ± 1.24**	**130.81 ±2.89 *****	**184.18 ± 3.56 *****



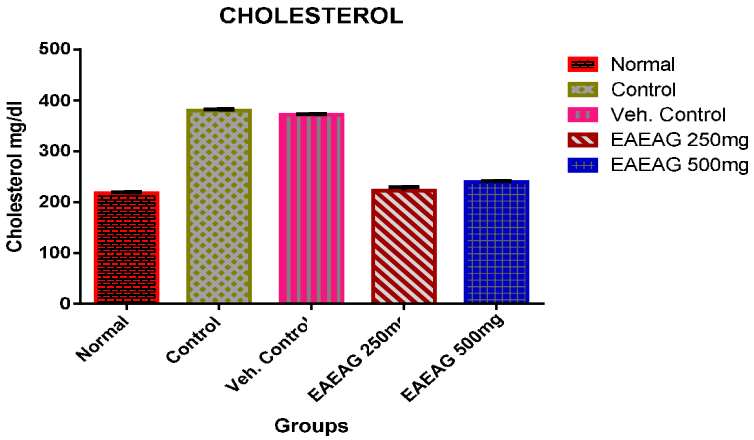
Graph-1

Cholesterol

With normal group Cholesterol levels were found to be **217.91± 1.77,** with Control (Saline) Blood Cholesterol levels were found to be **380.08 ± 2.71** and with vehicle control (DMSO) Blood Cholesterol was reduced to **371.5 ± 1.63.** Hence all the values of treatment groups were compared with control group. When EAEAM was administered orally at the dose of 250mg/kg Cholesterol was significantly reduced to **223 ± 6.83 (*p<0.0001*)**. When EAEAM was administered orally at the dose of 500mg/kg Cholesterol was significantly reduced to **239.75 ± 1.33 (*p<0.0001*)**. There was a significant decrease in Cholesterol with EAEAM 250mg/kg and 500mg/kg.

Table No. 4 **Consolidated Table Showing the Effect of EAEAM on Cholesterol**

S.NO	Normal	Control	Veh.Control	EAEAM 250mg/kg	EAEAM 500mg/kg
1	220.6	382.4	372.4	210.5	240.9
2	218.7	375.6	370.1	220.4	239.6
3	216.9	380.1	374.2	224.6	237.5
4	217.4	378.3	369.8	228.7	240.1
5	218.5	381.5	370.9	225.3	241.2
6	215.4	382.6	371.6	228.5	239.2
Mean ± S.D.	**217.91± 1.77**	**380.08 ± 2.71**	**371.5 ± 1.63**	**223 ± 6.83**	**239.75 ± 1.33 *****



Graph-2

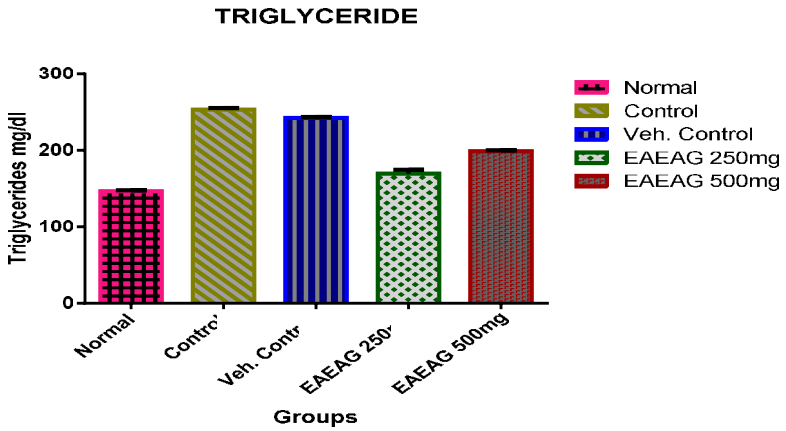
Triglycerides

With normal group triglyceride levels were found to be **146.43± 1.39** with Control (Saline) triglyceride levels were found to be **253.08 ± 1.93** and with vehicle control (DMSO) triglyceride was reduced to **242.31 ± 1.25.** Hence all the values of treatment groups were compared with control group. When

EAEAM was administered orally at the dose of 250mg/kg triglyceride levels was significantly reduced to **169.46 ± 5.03** (***p<0.0001***). When EAEAM was administered orally at the dose of 500mg/kg triglyceride levels was significantly reduced to **198.75 ± 1.46** (***p<0.0001***). There was a significant decrease in triglyceride with EAEAM 250mg/kg.

Table No. 5 **Consolidated Table Showing the Effect of EAEAM on Triglycerides**

S.NO	Normal	Control	Veh.Control	EAEAM 250mg/kg	EAEAM 500mg/kg
1	147.4	250.6	244.1	169.2	199.6
2	144.3	255.5	240.9	160.1	200.4
3	145.1	251.4	241.6	169.8	198.1
4	147.8	254.1	241.6	172.4	196.2
5	146.9	252.3	242.1	174.9	199.3
6	147.1	254.6	243.6	170.4	198.9
Mean ± S.D.	**146.43± 1.39**	**253.08 ± 1.93**	**242.31 ± 1.25**	**169.46 ± 5.03**	**198.75 ± 1.46** ***



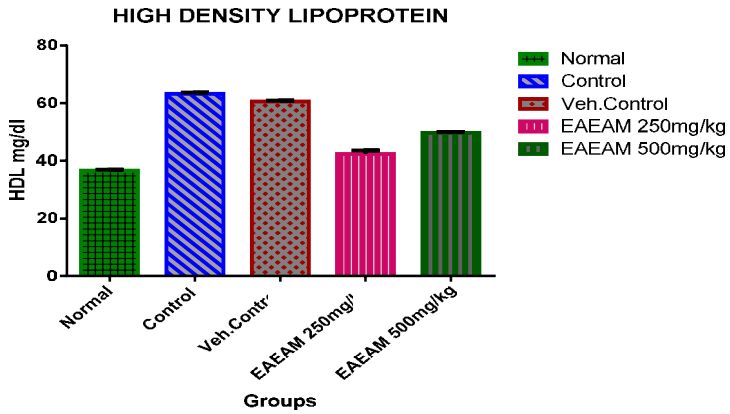
Graph-3

High Density Lipoprotein

With normal group HDL levels were found to be **36.60 ± 0.35** with Control (Saline) HDL levels was found to be **63.26 ± 0.48** and with vehicle control (DMSO) HDL was reduced to **60.58 ± 0.3.** Hence all the values of treatment groups were compared with control group. When EAEAM was administered orally at the dose of 250mg/kg HDL levels was significantly reduced to **42.36 ± 1.26** (***p<0.0001***). When EAEAM was administered orally at the dose of 500mg/kg HDL levels was significantly reduced to **49.68 ± 0.36** (***p<0.0001***). There was a significant decrease in HDL with EAEAM 250mg/kg.

Table No. 6 **Consolidated Table Showing the Effect of EAEAM on HDL**

S.NO	Normal	Control	Veh.Control	EAEAM 250mg/kg	EAEAM 500mg/kg
1	36.85	62.65	61.02	42.3	49.9
2	36.07	63.87	60.22	40.02	50.1
3	36.27	62.85	60.4	42.45	49.52
4	36.95	63.52	60.47	43.1	49.05
5	36.72	63.07	60.52	43.72	49.82
6	36.77	63.65	60.9	42.6	49.72
Mean ± S.D.	**36.60 ± 0.35**	**63.26 ± 0.48**	**60.58 ± 0.3**	**42.36 ± 1.26**	**49.68 ± 0.36** ***



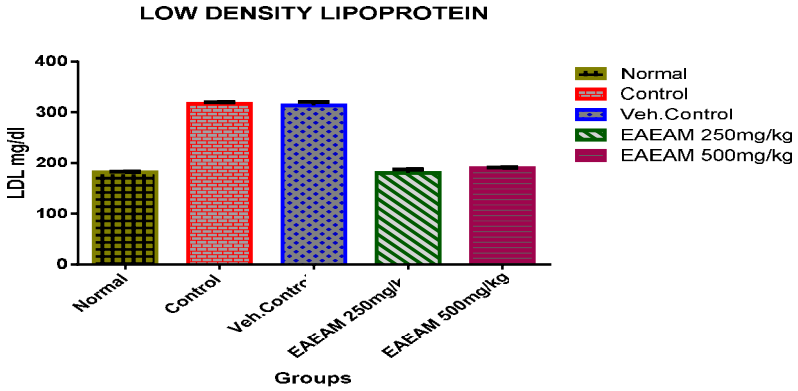
Graph-4

Low Density Lipoprotein

With normal group LDL levels were found to be **181.31 ± 1.80** with Control (Saline) LDL levels were found to be **316.81 ± 3.03** and with vehicle control (DMSO) LDL was reduced to **313.71 ± 6.77.** Hence all the values of treatment groups were compared with control group. When EAEAM was administered orally at the dose of 250mg/kg LDL levels was significantly reduced to **180.65 ± 6.49 (*p<0.0001*)**. When EAEAM was administered orally at the dose of 500mg/kg LDL levels was significantly reduced to **190.06 ± 1.31 (*p<0.0001*)**. There was a significant decrease in LDL with EAEAM 250mg/kg.

Table No. 7 Consolidated Table Showing the Effect of EAEAM on LDL

S.NO	Normal	Control	Veh.Control	EAEAM 250mg/kg	EAEAM 500mg/kg
1	183.75	319.75	311.38	168.2	191
2	182.62	311.73	309.88	180.39	189.5
3	180.63	317.25	313.8	182.2	187.98
4	180.45	314.78	309.33	185.61	191.05
5	181.78	318.43	327.18	181.62	191.38
6	178.63	318.95	310.7	185.92	189.48
Mean ± S.D.	**181.31 ± 1.80**	**316.81 ± 3.03**	**313.71 ± 6.77**	**180.65 ± 6.49**	**190.06 ± 1.31 *****



Graph- 5

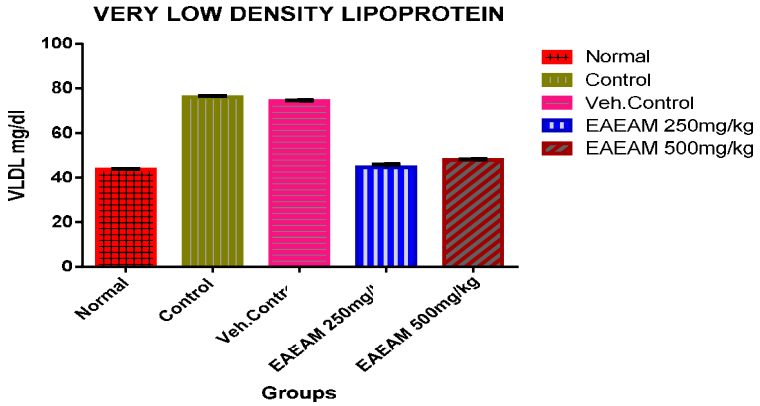
Very Low Density Lipoprotein

With normal group VLDL levels were found to be **43.58 ± 0.35** with Control (Saline) VLDL levels were found to be **76.01 ± 0.54** and with vehicle control (DMSO) VLDL was reduced to **74.3 ± 0.32.**

Hence all the values of treatment groups were compared with control group. When EAEAM was administered orally at the dose of 250mg/kg VLDL levels was significantly reduced to **44.6 ± 1.36 (*p<0.0001*)**. When EAEAM was administered orally at the dose of 500mg/kg VLDL levels was significantly reduced to **47.95 ± 0.26 (*p<0.0001*)**. There was a significant decrease in VLDL with EAEAM 250mg/kg.

Table No. 8 **Consolidated Table Showing the Effect of EAEAM on V LDL**

S.NO	Normal	Control	Veh.Control	EAEAM 250mg/kg	EAEAM 500mg/kg
1	44.12	76.48	74.48	42.1	48.18
2	43.74	75.12	74.02	44.08	47.92
3	43.38	76.02	74.84	44.92	47.5
4	43.48	75.66	73.96	45.74	48.02
5	43.7	76.3	74.18	45.06	48.24
6	43.08	76.52	74.32	45.7	47.84
Mean ± S.D.	**43.58 ± 0.35**	**76.01 ± 0.54**	**74.3 ± 0.32**	**44.6 ± 1.36**	**47.95 ± 0.26 *****



Estimation of cholesterol

To 1000 µl of the reagent, 10 µl of standard cholesterol (200 mg/dl) was added and incubated for 10 min at 37° C. This incubated mixture was aspirated and concentration of standard was calibrated to show a value of 200 mg/dl. The serum cholesterol was estimated by adding 10 µl of the serum sample to 1000 µl of the reagent, mixed well and incubated at 37° C for 10 min. This incubated mixture was aspirated and absorbance recorded against a reagent blank at 505 nm using Clinical Chemistry Analyzer.

STATISTICAL ANALYSIS

The data were represented as Mean ± S.D., and statistical significance between treated and diabetic control groups was analyzed using one-way ANOVA, followed by Tukey`s multiple comparison test. $P < 0.0001$ was considering statistically significant.

DISCUSSION

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted[74]. Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from Streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas[75]. Alloxan, a beta cytotoxin, destroys betacells of islets of langerhans of pancreas resulting in a decrease endogenous insuline secretion and paves the ways for the decreased utilization of glucose by the tissue. It results in elevation of blood glucose level. Expresion of elivated pasting blood glucose level conformed induction of diabetes in alloxan induced experimental rats, thereby inducing hyperglycaemia[76]. Experimental studies reveal that aqueous extract from *Ziziphus Jujuba* (250mg/kg&500mg/kg) orally administered for 7days produce a significant decrease in the

blood glucose level in the model of alloxan induced diabetes in rats. In the present investigation it was observed that Diabetes produced by the 150mg of Alloxan is prevented by treatment of groups' ethyl acetate extract of *Aegle Marmelos* decreases the levels of Blood Glucose, Cholesterol, Triglycerides, HDL, LDL, VLDL. This protective effect of ethyl acetate extract may be due to the presence of Alkaloids, Triterpenoids and Tannins. Further studies are needed to elucidate the particular Alkaloids, Triterpenoids and Tannins which is responsible for Anti-diabetic activity.

CONCLUSION

In the present investigation it was observed that Diabetes produced by the 150mg of Alloxan is prevented by treatment of groups ethyl acetate extract of *Aegle Marmelos* decreases the levels of Blood Glucose, Cholesterol, Triglycerides, HDL, LDL, VLDL. This protective effect of ethyl acetate extract may be due to the presence of Alkaloids, Triterpenoids and Tannins. Further studies are needed to elucidate the particular Alkaloids, Triterpenoids and Tannins which is responsible for Anti-diabetic activity.

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