EXTRACTION, ISOLATION AND CHARACTERIZATION OF COMPOUNDS FOR ANTIDIABETIC ACTIVITY IN *FICUS GLOMERATA*

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Abstract:
Objective: The main focus of the study was to extract, isolate and characterize the phytochemical compounds for its antidiabetic activity from various parts of ficus glomerata.
Methods: Extraction of the compounds were done by the method of soxhlation. Extract were subjected to isolation of compounds which were evaluated for its antidiabetic activity through invivo evaluation on male wistar rat by determining the glucose lowering effect after administration of isolated compound.
Result: The result obtained from the invivo evaluation of glucose lowering effect showed potential owering of blood glucose level to substantial level by administration of isolated compound with a dose of 20mg/kg as compared to standard glibenclamide drug.
Conclusion: the research found to conclude that the isolated compound have a substantial glucose lowering effect on the male wistar rat. The antidiabetic effect can be further enhanced by increasing the dose and improving bioavailability through various dosage form.

Keywords: Anti-diabetic effect, Ficus glomerata, glucose lowering, soxhlation, Isolation

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INTRODUCTION

Ficus glomerata, also known as the cluster fig tree, is a species of fig tree that belongs to the Moraceae family. It is native to South and Southeast Asia, including countries such as India, Sri Lanka, Bangladesh, Myanmar, Thailand, and Malaysia. The tree is a large, evergreen tree that can grow up to 30 meters tall. Its leaves are dark green, leathery, and about 10-20 cm long. The fruit of the Ficus glomerata is a fig, which grows in clusters on the trunk and branches of the tree. The figs are small and round, and about 1-2 cm in diameter. When ripe, they turn from green to yellowish-brown [1].

Ficus glomerata has been used for centuries in traditional medicine systems to treat a variety of ailments. The bark, leaves, and fruits of the tree are believed to possess medicinal properties and are used in the treatment of various conditions such as diabetes, diarrhea, dysentery, respiratory disorders, and skin diseases [2]. Apart from its medicinal properties, Ficus glomerata also has significant ecological importance. The tree is a host plant for several species of butterflies and moths. Its fruit is an important source of food for birds and small mammals, and its large, spreading canopy provides shade and shelter to many other species [3].

In addition to its traditional medicinal uses, recent research has also shown that Ficus glomerata has potential as a source of novel bioactive compounds, including flavonoids, triterpenoids, and steroids, which have been shown to possess a range of biological activities such as antioxidant, anti-inflammatory, and anticancer properties. Ficus glomerata is a valuable plant species with significant ecological and medicinal importance. Its potential as a source of novel bioactive compounds makes it a promising area of research for the development of new medicines and other applications [4].

Ficus glomerata has been found to contain a wide range of phytochemicals, many of which have been identified as having therapeutic properties. Recent research has provided evidence supporting the antidiabetic activity of Ficus glomerata. Several studies have investigated the potential of various parts of the plant, including the leaves, bark, and fruit, to lower blood glucose levels and improve other markers of diabetes.

Studies found that an aqueous extract of Ficus glomerata leaves significantly reduced blood glucose levels in diabetic rats. The extract was also found to improve insulin sensitivity and reduce oxidative stress, suggesting that it may have a protective effect against diabetic complications [6]. Another study investigated the effects of a methanol extract of Ficus glomerata bark on diabetic rats. The extract was found to significantly lower blood glucose levels and improve lipid metabolism, indicating its potential as an antidiabetic agent [7].

In addition to these studies, other research has identified various bioactive compounds present in Ficus glomerata that may be responsible for its antidiabetic activity. For example, a 2019 study published in the journal Natural Product Research found that a flavonoid compound isolated from the leaves of the plant showed significant antidiabetic activity in vitro [8]. The growing body of research supports the traditional use of Ficus glomerata in the treatment of diabetes and suggests that it may have potential as a source of new antidiabetic drugs. However, further studies are needed to fully understand the mechanisms of action of the plant's bioactive compounds and to evaluate their safety and efficacy in human trials [9].

MATERIALS AND METHODS:

Collection and Authentication Plant material:

Freshly collected Ficus glomerata leaves obtained from the local market in Bhopal were cleaned with sterile distilled water and then dehydrated in an oven at 40°C for 3-4 days. Authentication of the plant material was performed by Dr. Jaswinder Mehta from the Department of Botany at Carrier College in Bhopal, India. The leaves were then dried under shade, pulverized, and stored in an airtight container.

Drying and Size Reduction of Plant Material

Ficus glomerata (L.) leaves were dried in the laboratory under shade and then ground into moderately coarse powder. To ensure uniformity, the powder was passed through sieve No. 16 and stored in a cool, dry place for further study.

Physicochemical Evaluation

Loss on Drying

About 10 gm. of the powdered drug was weighed in a tarred Petridish. It was dried at 105°C for 1 hour in hot air oven and then reweighed. Loss on drying was determined from calculating the initial and final weight.

Total Ash Value

About 5 gm. accurately weighed powdered drug was incinerated in a silica dish at a temperature not exceeding 450°C until free from carbon in muffle

furnace. It was then cooled and weighed. The % *w/w* of ash with reference to the air-dried drug was calculated.

Acid Insoluble Ash Value

Accurately weighed 1 gm. ash was boiled for 5 minutes with 25ml hydrochloric acid by covering the crucible with a watch-glass on water bath then cooled. The watch-glass was rinsed with 5 ml of hydrochloric acid and this liquid was added in to the crucible. Then the content was filtered on a previously weighed Whattman filter paper and filtrate was dried and weighed. Acid insoluble ash value was determined by calculating the % content remaining after deducting the weight of filter paper.

Water Soluble Ash Value

Accurately weighed 1 gm. ash was boiled for 5 minutes with 25ml distilled water by covering the crucible with a watch-glass on water bath then cooled. The watch-glass was rinsed with 5 ml of distilled water and this liquid was added in to the crucible. The % of remaining content was deducted from initial % of ash taken (i.e. 100%) to determine the water soluble ash value.

Foaming Index

About 1 gm. coarse powder was weighted and transferred to a 500 ml conical flask containing 100 ml of water. It was maintained at moderate boiling for 30 minutes on water bath. It was cool and filtered in to a 100 ml volumetric flask. Volume was diluted by adding sufficient amount of water. The decoction was poured in test tube, and then shaken in a lengthwise motion for 15 seconds. They were allowed stand for 15 minutes and the height of foam was measured to determine the foaming index.

Extraction of *Ficus glomerata*

Preparation of plant extract of was *Ficus glomerata* carries out by the Soxhlet method. The solvent ethanol (250ml) was placed in a round bottom flask isolated with a Soxhlet extractor and condenser (Figure 1). The crushed leaves were placed in a thimble and the thimble was inserted into a Soxhlet extractor. The side arm was covered with glass wool.

The solvent is heated using a heating source (isojacket), travels through the device to the condenser and begins to evaporate. The condensate then drops into the reservoir with the sleeve. When the solvent reaches the siphon, it flows back into the flask and the cycle resumes. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined

Figure 1: Soxhlet extraction of Plant Material

Isolation of compound by column chromatography

The extract was subjected to column chromatography containing silica gel of 60-120 mesh as the stationary phase in the glass column (100 X 3 cms). The isolation was carried out through isocratic elution method.

Column size: Glass Column, 100 x 3 cms

Stationary Phase: Silica gel (60 to 120 mesh size)

Elution mode: Isocratic elution

Mobile Phase: *Ficus glomerata* leaves extract

Extract: extract of *Ficus glomerata* leaves extract

Visualized by: Short UV (254nm), long UV (365nm) and Normal light

Identification of similar fractions: Visualized by Short UV (254nm), long UV (365nm) and Normal light and Phytochemical Test

Procedure

Preparation of column

Column packing was done by wet packing method. Silica gel (activated at 105^{o}C) was taken; and suspended it in Mobile Phase then transfers it in Column; allowed to settle down. At the top of silica layer cotton plug was kept to avoid disturbance in silica layer during elution.

Preparation of sample

5gm of Extract of *Ficus glomerata* was taken in beaker; to it silica gel used for column chromatography (60-120 mesh size) and sufficient amount of mobile phase was added. The slurry was made and introduced from top of the silica gel column over cotton plug.

Isocratic elution technique

In order to fractionate the components of isocratic elution technique was used. *n*-butanol: acetic acid: water (3:1:7) mobile phase is used for elution. The 10 ml of each fraction were collected and the solvent was recovered by distillation. The fractions were collected, concentrated, stored and subjected to TLC.

Identification of similar fractions

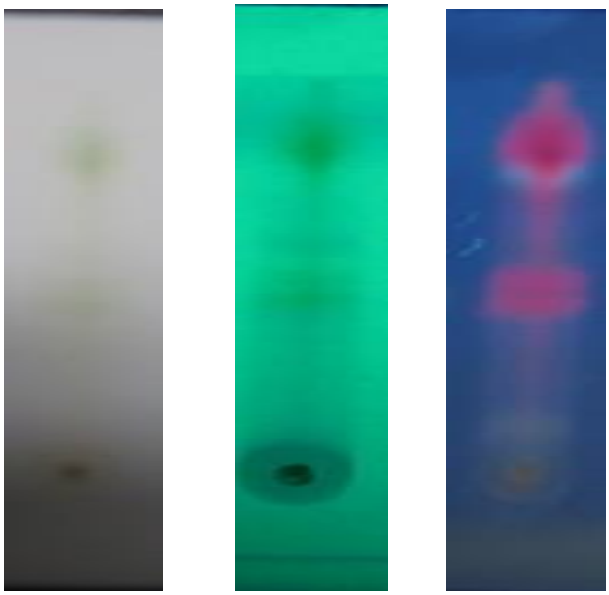
The different fractions of column chromatographic elution were monitored by TLC *n*-butanol: acetic acid: water (3:1:7); using UV chamber and derivatization with specific reagent for identification of single isolated compound with comparison of reference compounds. The fractions which show similar fingerprinting profile on TLC were collected and mixed. Fraction showed single compound and have similar R_f value as compared to reference compound were dried, compound were purified by recrystallization procedure.

Isolation of compound from *Ficus glomerata* leaves extract

Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1µl by using capillary at distance of 1 cm in the twin trough chamber with different solvent system used. After pre-saturation with mobile phase for 20 min for development were used. After the run plates are dried and sprayed freshly prepared iodine reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor (R_f), values were calculated for different samples.

Table 1: Optimization of Mobile Phase for isolation by column chromatography

Mobile Phase used	No. of Spot (UV-256)	No. of spot (UV-366)	Observation
Ethyl acetate: water (9:1)	2	2	No satisfactory resolution
Ethyl acetate: water (7:3)	2	2	No satisfactory resolution
Chloroform: MeOH (9:1)	1	2	No satisfactory resolution
Chloroform: MeOH (7: 3)	1	3	No satisfactory resolution
n-butanol: water (2:8)	2	3	No satisfactory resolution
n-butanol: water (3:7)	2	4	No satisfactory resolution
n-butanol: acetic acid: water (3:1:7)	**3**	**5**	**Satisfactory resolution**
n-butanol: acetic acid: water (4:1:6)	2	4	No satisfactory resolution
n-butanol: acetic acid: water (3:1:7)	3	4	No satisfactory resolution



Normal Light Short U.V Long U.V

Characterization of Isolated compound:

Table 2: Characterization of Isolated compound

No	No. of fractions	TLC UV spectra		Chemical Test
		UV-254	UV-366	Shinoda Test
1	1-12	No Spot	No Spot	No Color
2	12-26	1 Spot	2 Spot	No Color
3	28-32	1 Spot	3 Spot	No Color
4	33-44	0 Spot	3 Spot	No Color
5	45-70	2 Spot	3 Spot	No Color
6	71-86	2 Spot	4 Spot	No Color
.7	87-95	2 spot	3 Spot	No Color
8	96-107	1 Spot	1 Spot	+Ve
9	108-119	2 Spot	3 spot	No Color
10	115-134	1 Spot	2 Spot	No Color
11	135-152	No Spot	1 Spot	No Color
12	153-160	No Spot	No Spot	No Color

Compound 1: Fraction shows single spot at same Rf value as Quercetin and going to derivatized with $AlCl_3$ so it may be flavonoids in chemical nature.

Characterization of Isolated compound

160 fraction, each 10ml were collected and isolated compound was characterized by IR, NMR and Mass Spectra studies.

***IN VIVO* ANTI-DIABETIC ACTIVITY**

Animal care and handling

The experiment was carried out on Wistar albino rats of 4 months, of both sexes, weighing between 110 to 160 gm. They were provided from Oriental University, Indore, (M.P.). The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 44 –56% and light and dark cycles of 12:12 hours, fed with standard pallet diet and water *ad libitum* during experiment. The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no. IAEC/2019-20/RPO-07).

Acute oral toxicity studies

Oral Acute toxicity study was evaluated as per OECD guidelines (425) on Wistar albino rats. Three animals were selected for maximum tolerable dose (2000mg/kg) of Polyherbal preparation. Animals were observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression and mortality after dosing for 24 hours. All observations were systematically recorded with individual records being maintained for each animal. No toxic signs were noticed in animals till day 7. Hence administered dose was considered tolerable.

Streptozotocin induced diabetes

Procedure: Diabetes was induced in animals by a single intraperitoneal injection of a freshly prepared Streptozotocin (STZ). STZ solution of 10 mg/ ml was prepared in ice-cold citrate buffer 0.1 M, pH 4.5 kept in ice and was administered at a dose of 60mg/kg body weight on day 1st. Treatment was given after diabetes induction (day 3rd) for 21 days [10,11].

Table 3: *Grouping and dosing:* Animals were divided into five groups containing six animals in each.

Group	Dosing and treatment
I	Normal control (vehicle only, 1ml/100gm)
II	Diabetic control, Streptozotocin 60g/kg, i.p.
III	Standard, Diabetic rats were treated with Glibenclamide 0.25 mg/kg once daily for 21 days
IV	Diabetic rats were treated with Polyherbal preparation at 10 mg/kg once daily for 21 days.
V	Diabetic rats were treated with Polyherbal preparation at 20 mg/kg once daily for 21 days.

Physiological Parameters: Body weight of animals were measured using animal weighing balance.

Fig 2. Animal weight by weighing balance

Biochemical Parameters

Samples collection and storage

At the end of the experimental, animals were anaesthetized with intraperitoneal injection of Ketamine (50 mg/Kg i.p.) and blood was collected from retro-orbital puncture in blank (for serum) and EDTA containing apendorff tube (for plasma). The one drop of blood samples was immediately spread on the marked end of the gluco-strip. After few seconds the gluco-meter was display the blood glucose level. Serum and plasma were obtained by blood centrifugation at 3000rpm for 15 min. Animals were then sacrificed and pancreas were collected in 10% formalin for histopathology. All biological samples were store at −20 ◦C until analysis.

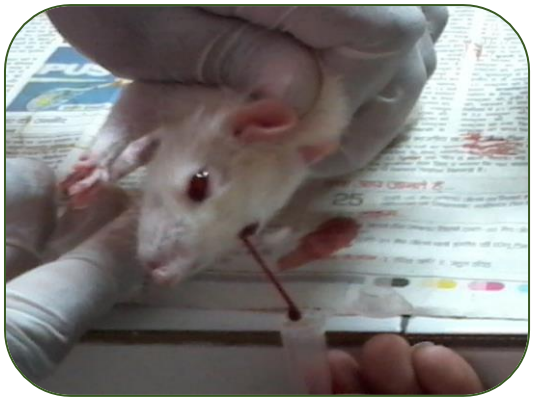


Fig 3. Blood collection from animals

Result and Discussion

Results of Isolation of compound by column chromatography

Characterization of pooled Fraction no. 96-107

Appearance-The fraction was Dark Yellow in color.

Solubility- Soluble in ethanol & acetone slightly soluble in water & di ethyl ether.

Melting point-315 ^{0}C

I.R. Analysis- The IR spectrum of compounds was recorded on (Bruker Alpha) using solid plate technique with KBr.

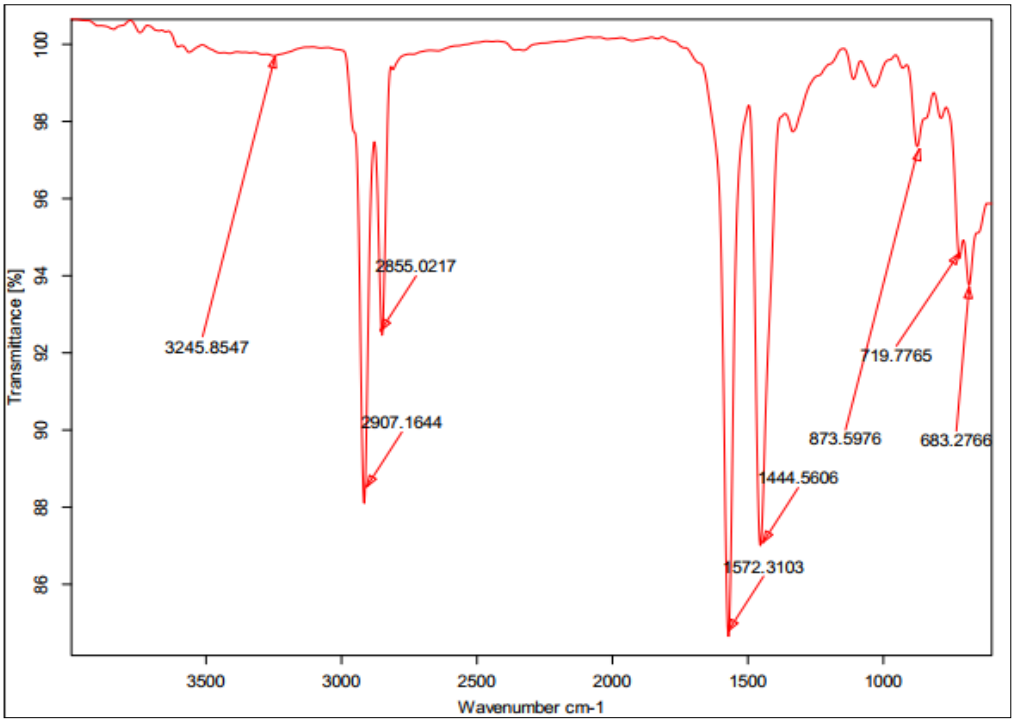


Figure 4: - I.R. Spectra of pooled Fraction no. 96-107

NMR Analysis- H^1NMR was recorded on Bruker DRX -300 (300 MHz FT-NMR) in $CDCL_3$ using TMS as internal standard.

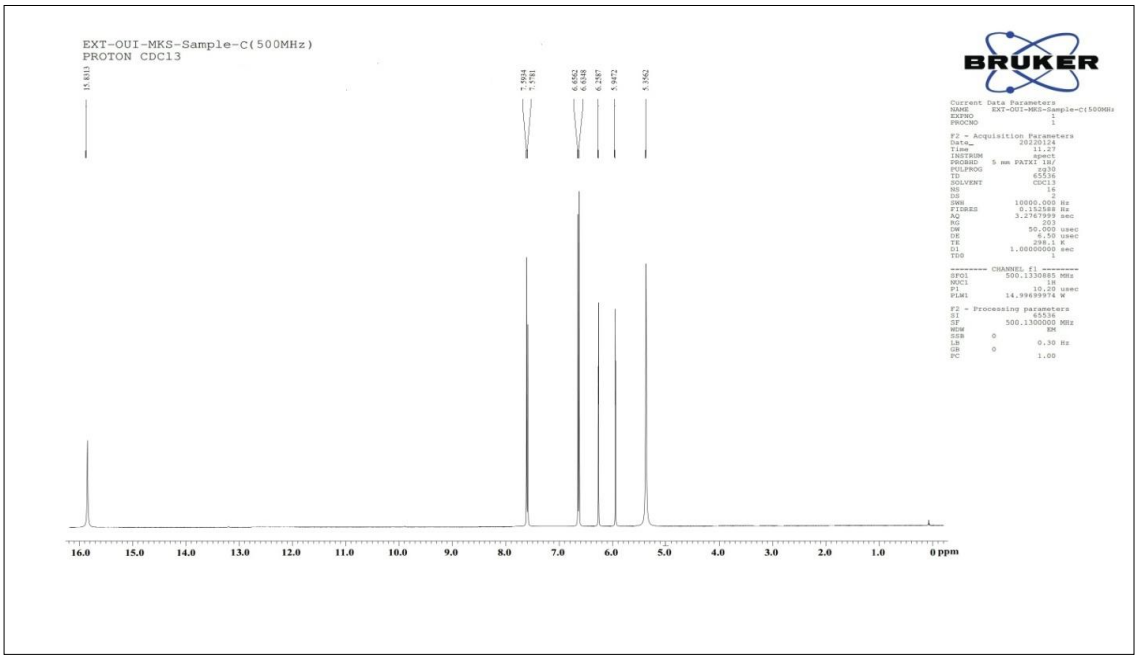


Figure 5: - N.M.R. Spectra of pooled Fraction no. 96-107

MASS Analysis

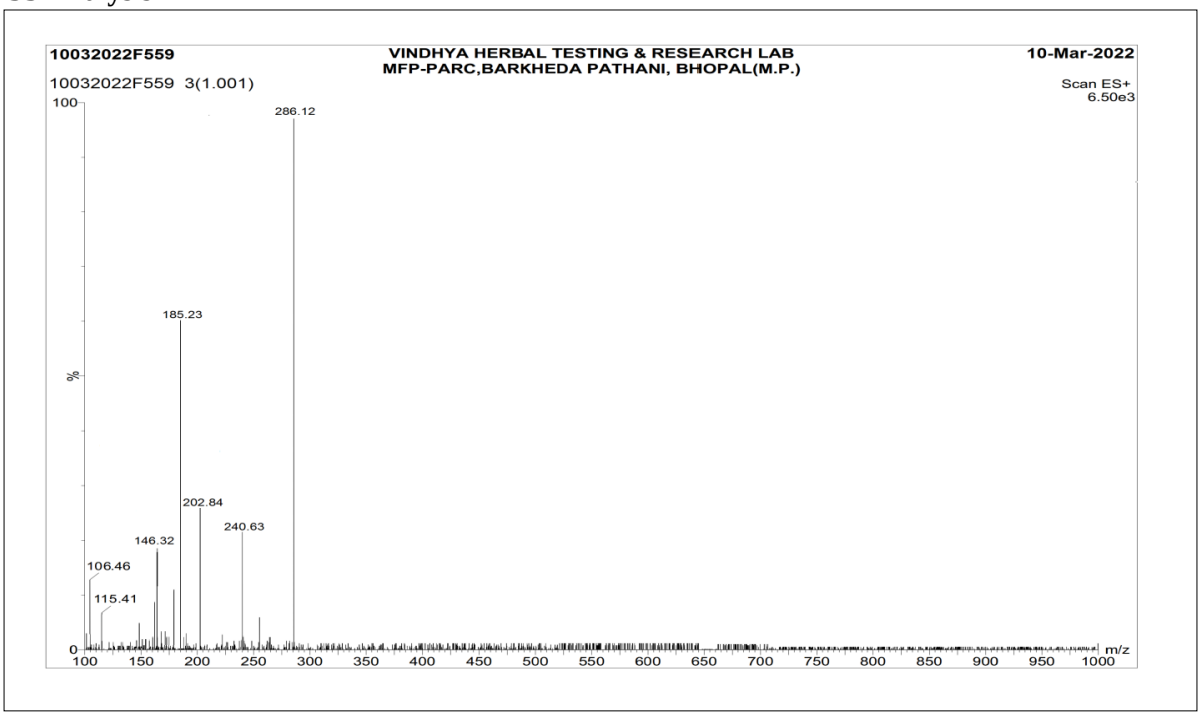


Figure 6: - MASS Spectra of pooled Fraction no. 96-107

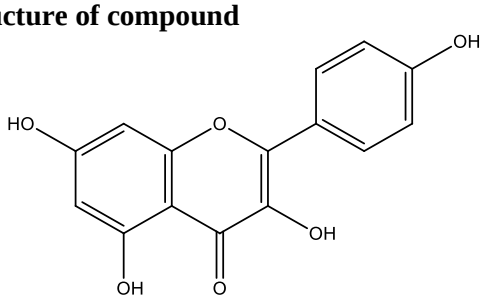
Interpretation of spectra

IR: O-H 3500-3000, C=C- 2907.1644, C=O- 1572.3103, C-O- 1444.5606,

^{1}H NMR ($CDCl_3$, 500 MHz): δ, 15.83 (s, 1H, OH), 7.59 (d, 2H, J= 7.65, CH), 6.65(d, 2H, J = 10.7, CH), 6.25(s, 1H, CH), 5.94(s, 1H, CH), 5.35(s, 3H, OH)

Mass (m/z): 286.12, 240.63, 202.84, 185.23

Structure of compound



Molecular formula: $C_{15}H_{10}O_6$

IUPAC Name: 3,5,7-trihydroxy-2-(4- hydroxyl-phenyl) chromen-4-one

Statistical Analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.). The results are analyzed for statistical significance using one-way ANOVA followed by Dunnett's test. $P < 0.05$ was considered significant.

Antidiabetic Activity of Isolated Compound:

Acute toxicity studies revealed the non-toxic nature of the polyherbal preparation. Experiment was carried out on normal healthy rats at 2000mg/kg. No mortality was observed at this dose in rats and behaviour of the treated rats also appeared normal.

In the present investigation, Diabetes was induced in animals by a single intraperitoneal injection of a freshly prepared Streptozotocin. Treatment was given after diabetes induction (day 3rd) for 21 days. Change in Body weight is one of the evaluation criteria in disease progression. The body weight of animals in different groups during the study period is shown in (Table 4). Body weight was monitored on initial, day 7, day 14 and day 21. Body weight continuously decline throughout the treatment majorly in diabetic control group animals while in the remaining groups overall no gain no loss was observed.

Assessment of diabetes by blood glucose level provides an excellent and simple tool to measure the anti-diabetic activity of the study drug. The evaluation of blood glucose level was significantly increased in diabetic control group animal. Animal treatment with polyherbal preparation at dose 250 mg/kg & 500 mg/kg showed a significant anti-hyperglycemic activity. The maximum reduction in blood glucose levels was observed in animals receiving glibenclamide (Table-5).

On the basis of results of this preliminary study it is concluded that polyherbal preparation has dose-dependent anti-diabetic activity. The present study shows that the polyherbal preparation at dose 500 mg/kg has significant anti-diabetic activity. Further studies may be carried out to confirm its anti-diabetic activity.

Table No. 4:- Effect of isolated compound *on* body weight of diabetic rats.

Groups	Treatment	Body weight (gm) (mean±SEM)			
		Initial	Day 7	Day 14	Day 21
I	Normal Control	126.3±5.36	127.7±6.36	128.5±6.73	129.4±5.83
II	Diabetic control	126.9±6.22 a***	124.4±6.48 a***	124.2±6.33 a***	123.6±6.73 a***
III	Standard (Glibenclamide 0.25 mg/kg)	128.3±5.27 a***	130.4±6.58 a***, b***	130.8±5.32 a***, b***	130.2±5.76 a***, b***
IV	Hydroalcoholic extract (250mg/kg)	127.0±3.24 a***	128.8±3.52 a***, b***, c***	127.7±3.44 a***, b***, c***	127.8±3.89 a***, b***, c***
V	Hydroalcoholic extract (500mg/kg)	127.9±7.56 a***	125.4±7.22 a***, b***, c***	128.5±7.74 a***, b***, c***	128.7±7.84 a***, b***, c***

Values are mean ± SEM from a group of six animals. *p<0.05, **p<0.01 and***p<0.001

a- Significance difference as compare to normal control group
b- Significance difference as compare to Diabetic control group
c- Significance difference as compare to standard treated group

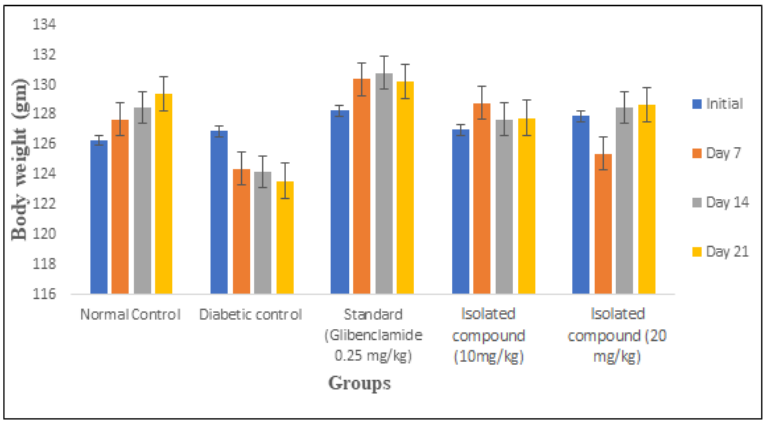


Figure 7: Effect of hydroalcoholic preparation on body weight in Streptozotocin induced diabetes in rats.

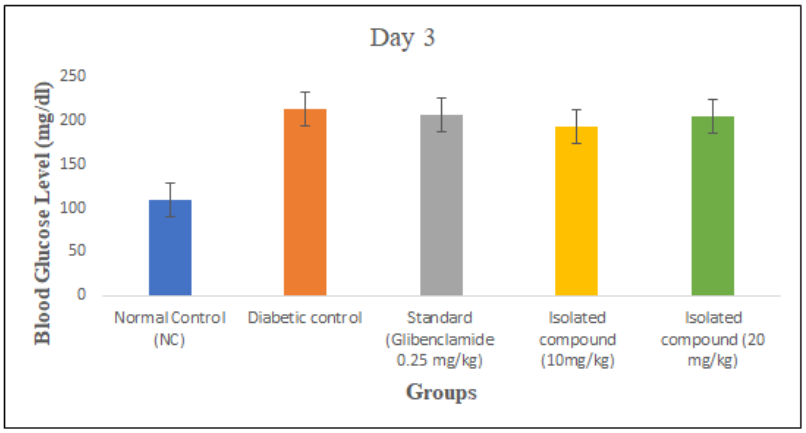
Table 5: Effect of Isolated compound (IC) on blood glucose level in Streptozotocin induced diabetes in rats.

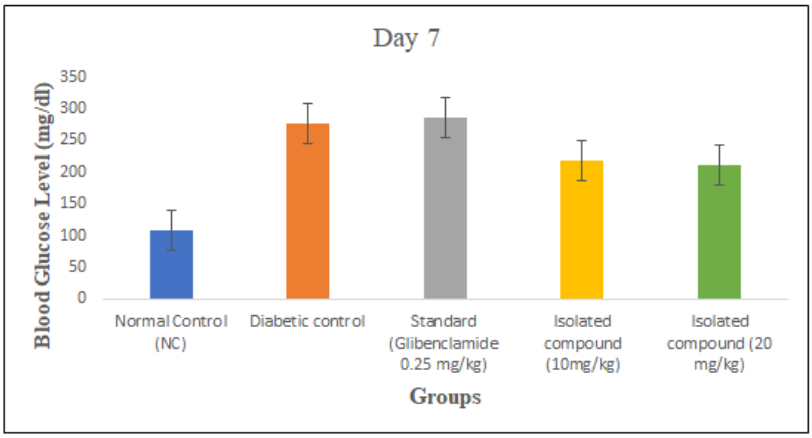
Groups	Treatment	Blood Glucose Level (mg/dl)			
		Day 3	Day 7	Day 14	Day 21
I	Normal Control (NC)	109.0±8.22	108.2±8.66	109.4±6.25	112.7±6.36
II	Diabetic control	213.0±7.59 ^{a***}	276.0±9.26 ^{a***}	318.7±9.55 ^{a***}	303.8±7.31 ^{a***}
III	Standard (Glibenclamide 0.25 mg/kg)	205.6±9.75 ^{a***}	286.0±6.72 $^{a***, b***}$	318.2±6.28 $^{a***, b***}$	284.3±5.32 $^{a***, b***}$
IV	Isolated compound (10mg/kg)	192.0±9.54 ^{a***}	218.0±6.67 $^{a***, b***, c***}$	212.2±9.24 $^{a***, b***, c***}$	196.3±8.36 $^{a***, b***, c***}$
V	Isolated compound (20 mg/kg)	204.0±8.54 ^{a***}	212.2±8.76 $^{a***, b***, c***}$	186.5±6.83 $^{a***, b***, c***}$	187.2±7.48 $^{a***, b***, c***}$

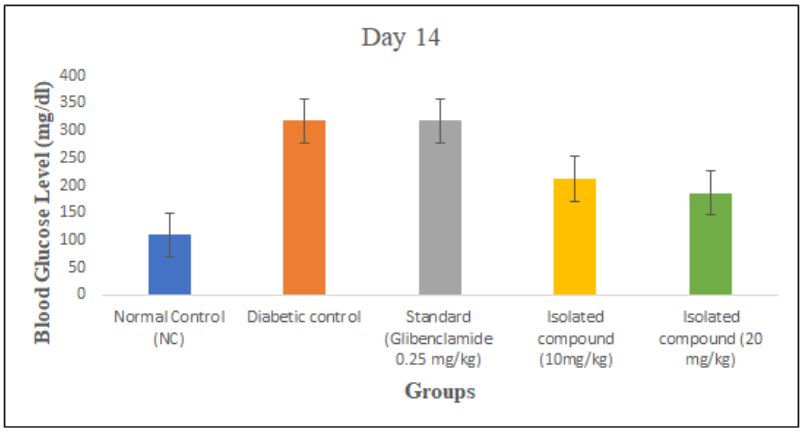
All values are mean ± SEM, n = 6. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

a- significant difference as compared to control

b- Significant difference as compared to standard







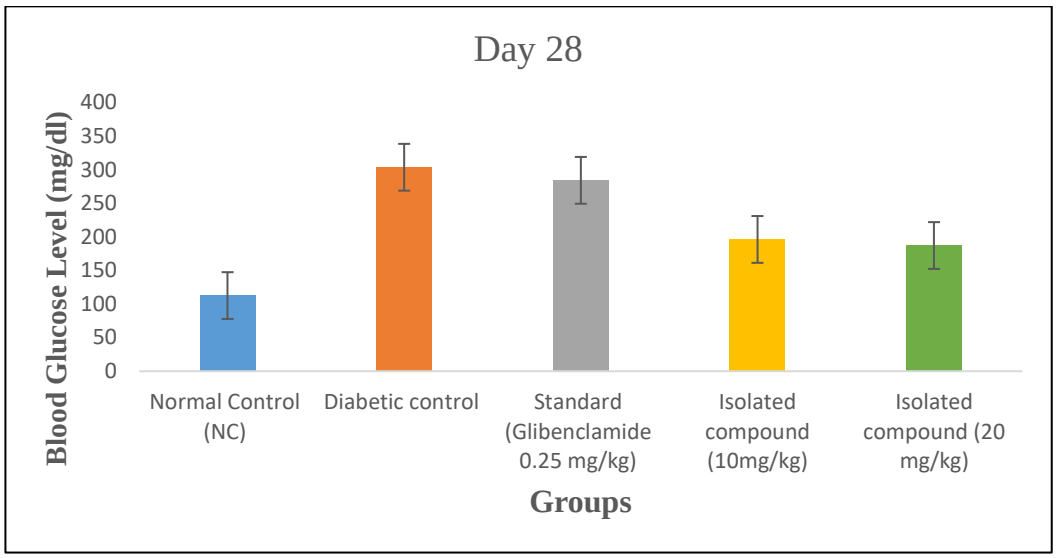


Figure 8: Effect of Isolated Compound on blood glucose level in Streptozotocin induced diabetes in rats.

Summary and Conclusion:

This present work deals with an attempt to develop a modified novel, simple method for extraction of ***Ficus glomerata*** **Roxb** for determination of antidiabetic activity. *Ficus glomerata* have been used since years for its various therapeutic activity by the tribal. Thus a particular method is required for easy and proper extraction of seed oil to get better therapeutic activity. The finalization of methods been used for extraction as well as methods used to evaluate the therapeutic activity was conducted after successful completion of literature survey.

IR spectral graph shown different stretching data with 3500-3000 (O-H str), 2907.1644 (C=C), 1572.3103 (C=O-), 1444.5606 (C-O-). ^{1}H NMR spectra showed different peaks at δ, 15.83 (s, 1H, OH), 7.59 (d, 2H, J= 7.65, CH), 6.65(d, 2H, J = 10.7, CH), 6.25(s, 1H, CH), 5.94(s, 1H, CH), 5.35(s, 3H, OH) and **Mass (m/z):** 286.12, 240.63, 202.84, 185.23. After successful interpretation of these data the extracted compound was interpreted to be 3,5,7-trihydroxy-2-(4-hydroxyphenyl) chromen-4-one.

Acute toxicity study revealed the non-toxic nature of the unknown sample. Experiment was carried out on normal healthy rats at 2000mg/kg. No mortality was observed at this dose in rats and behaviour of the treated rats also appeared normal. Table 5 shows the levels of blood glucose, glycosylated haemoglobin and plasma insulin in control and experimental groups of rats. In which result showed potent reduction in blood glucose level in animal who were administered with 20 mg/kg of isolated compound. From the present study, we conclude that isolated compound of ficus glomerata leave has shown more potent antidiabetic activity. This indicates the possibility of developing as a potent, safe and effective isolated compound for antidiabetic activity.

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