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EVALUATION OF ALLOXAN INDUCED ANTI-DIABETIC ACTIVITY OF *CROTON CAJUCARA* LEAF AND ROOT EXTRACTS

[1]*Rudrabhishek Yadav, [2]Ashutosh Mishra, [3]Rekha Gour, [4]Shailendra Modi

[1,2,4]Daksh Institute of Pharmaceutical Science, Chhatarpur.
[3]Daksh Pharmacy College, Chhatarpur.

***Corresponding Author: Rudrabhishek Yadav**
Daksh Institute of Pharmaceutical Science, Chhatarpur.

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ABSTRACT
The present study aimed to evaluate the in vitro and in vivo antidiabetic potential of ethanolic leaf and root extracts of *Croton cajucara* using alloxan-induced diabetic Wistar rats. Leaves and roots of *Croton cajucara* were sequentially extracted using petroleum ether, ethyl acetate, and ethanol. The extracts were screened for α-amylase inhibitory activity, followed by phytochemical analysis and thin-layer chromatography. Acute oral toxicity was evaluated according to OECD guideline 423. Antidiabetic activity was assessed in alloxan-induced diabetic rats at doses of 200 and 400 mg/kg, with glibenclamide (2 mg/kg) serving as the standard drug. Blood glucose levels were monitored during hypoglycemic, oral glucose tolerance, and 14-day antidiabetic studies. The ethanolic extract exhibited the strongest α-amylase inhibitory activity and contained flavonoids, phenols, alkaloids, glycosides, saponins, and terpenoids. In vivo studies demonstrated a significant, dose-dependent reduction in blood glucose levels, with the 400 mg/kg dose showing effects comparable to glibenclamide. The extract was found to be safe up to 2000 mg/kg in the acute toxicity study. The ethanolic extract of *Croton cajucara* possesses significant antidiabetic activity, likely mediated through α-amylase inhibition and phytoconstituents such as quercetin and kaempferol. These findings support its potential as a promising natural therapeutic candidate for diabetes management and warrant further phytochemical isolation and clinical investigation.

KEYWORDS: *Croton cajucara*, Diabetes mellitus, Alloxan-induced diabetes, α-Amylase inhibition, Antidiabetic activity.

INTRODUCTION
Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. It has emerged as one of the most significant global public health challenges, primarily due to sedentary lifestyles, unhealthy dietary habits, obesity, and increasing stress levels. According to the International Diabetes Federation, the global prevalence of diabetes continues to rise, with India being among the countries bearing the highest disease burden. The growing incidence of diabetes and its associated complications necessitate the development of safer, more effective, and affordable therapeutic agents.

Although conventional antidiabetic drugs, including sulfonylureas such as glibenclamide, effectively control blood glucose levels, their long-term use is often associated with adverse effects, including hypoglycemia, weight gain, and gastrointestinal disturbances. Consequently, there has been increasing interest in medicinal plants as alternative or complementary therapies due to their therapeutic efficacy, lower toxicity, and cost-effectiveness. *Croton cajucara*, a medicinal plant traditionally used for the management of diabetes and other ailments, has received limited scientific evaluation despite its ethnopharmacological importance. The presence of bioactive phytochemicals such as flavonoids, phenolic compounds, and terpenoids suggests its potential as a natural antidiabetic agent.

Therefore, the present study aimed to scientifically evaluate the antidiabetic activity of *Croton cajucara* leaf and root extracts using in vitro α-amylase inhibition and

alloxan-induced diabetic rat models. In addition, phytochemical screening, LC–MS analysis, and PASS bioinformatics prediction were employed to identify and characterize the bioactive constituents responsible for its antidiabetic activity, thereby providing scientific evidence for its traditional use and potential development as a novel phytopharmaceutical for diabetes management.

MATERIALS AND METHODS

Plant collection and authentification

The *Leaves and Root* s of *Croton cajucara* were collected from forest nursery chhatarpur and which was authenticated by Department of Botany, Govt Girls College, chhatarpur. (Gcc/Bot/23/2023)

Preparation of coarse powder and Extraction technique

The Leaves and Root were shade dried at room temperature for 10 days. Then these were milled into powder by mechanical grinder. This powder was sequentially extracted to their increasing polarity with Petroleum ether, Ethyl acetate, Ethanol respectively. About 500gm of powdered *Leaves and Root* was uniformly packed into a thimble in a soxhlet apparatus and extracted with 1000ml Petroleum ether, Ethyl acetate and Ethanol, respectively. Constant heat was provided by Mantox heater for recycling of the solvent. The process of extraction continues for 1-2 hours for each solvent. The excess solvent was evaporated and the dried extracts were kept in refrigerator at 4ºC for their future use in phytochemical analysis and pharmacological screenings.

Preparation and activation of TLC plates

Mixture of stationary phase and water forms slurry. TLC plates prepared by pouring, dipping, spraying or spreading. In pouring technique, slurry prepared and poured on the glass plate. Slurry spread uniformly on glass plate. Plates are dried in oven. In dipping technique two plates dipped in slurry, separated and dried. Disadvantage is that large quantity slurry required.

Procedure

Commercial sheet pre coated with silica gel are available. Select a solvent by testing out the samples in various solvents. Dissolve a small quantity of ethanolic extract of *Croton cajucara* Leaves and Root of the unknown in different flask containing solvents of different polarity. Place the TLC plates, the spotted side down in to the chamber so that the lower the pencil line about the solvents. Remove the plate from the development chamber and allowed to dry. Plate is placed under UV light, dark spots are observed.

Preparation of extract

Leaves and Root extraction used in *invitro* and *invivo* studies were prepared by using suitable solvents (Carboxy methyl cellulose).

In vitro antidiabetic activity of *Croton cajucara Leaves and Root* extracts Alpha-amylase inhibition assay

Chemicals or reagents

Potato starch, trichloroacetic acid, Folin-Ciocalteau reagents were purchased from SD Fine Pvt. Ltd., Mumbai, 3,5-dinitrosalicylic acid, Tris buffer, linoleic acid, ammonium molybdate, were purchased from Hi-Media Pvt. Ltd., Mumbai, α-amylase, α-glucosidase enzymes, xanthine oxidase, quercetin, hypoxanthine, pyrocatechol were purchased from SRL Pvt. Ltd., Mumbai. Glucose assay kit from Agappe diagnostic Pvt. Ltd., Kerala, Acarbose was obtained from Bicon Pvt. Ltd., Chennai, ferrozine, (2'2-azobis (2-amidino propane) dihydrochloride), butylated hydroxy toluene from Loba Cheme. All other chemicals used in the study were obtained commercially and were of analytical grade.

Instrument used

UV-visible Spectrometer (Systronic double beam- UV-2201).

Experimental procedure for α-amylase inhibition assay

A total of 500 µl of test samples and standard drug (100-1000µ g/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

Calculation of 50% inhibitory concentration (IC_{50})

The concentration of the plant extracts required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations of the extract.

Preliminary phytochemical screening of ethanolic Leaves and Root extract of *Crotoncajucara*

The ethanolic Leaves and Root extract of *Croton cajucara* was used for testing preliminary phytochemical screening in order to detect major chemical groups.

Test for carbohydrates

Molisch's test: Dissolved small quantity of 300mg alcoholic and dried *Leaves and Root* extract powder of Pimenta dioica separately in 4ml distilled water and filtered.The filtrate was subjected to Molisch's test.

Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution.

Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours.

Test for flavanoids
Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added.
Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added.

Test for tannins
Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solutionwas added.

Test for steroid/terpenoid
Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added.

Test for alkaloids
Draggendorf's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Draggendorf's reagent.
Hager's test: The extract was treated with few ml of Hager's reagen.
Wagner's test: The extract was treated with few ml of Wagner's reagent.

Tests for Glycosides
Legal's test: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution.

Test for Saponins
Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes.

Test for Anthraquinones
Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia.

Test for Amino acids
Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent.

Test for fixed oils and fats
Press small quantity of the petroleum ether extract between two filter paper.
Note: the results for the above experiments can be noted as follows.
If the response to the test is high it can be noted as +++ which indicates thatthe particular group is present as the major class.
If the response is average then note it as ++ indicates the presence in moderatequantity.
If the response is very small then note it as + indicating the presence of only intraces.
If no response is then negative.

Acute toxicity study
In a research study when a drug is administered to a biological system there will be some interactions may happen .In most case these are desired and usefull,but many effects are not advantageous. Acute, subacute and chronic toxicity studies are performed by the manufacturers in the investigation of a new drug. Acute toxicity is involved in estimation of LD50 (It is the lethal dose (cuasing death) to 50% of tested group animals).[54]

LD50 (median lethal oral dose)
LD 50 (median lethal oral dose) is a statistically derived oral dose of a substance that can be expected to cause death in percent of animals when administered by the oral route. The LD50 value is expressed in terms of weight of test substance per unit weight of animal (mg/kg)

In this study acute toxicity study was carried out in wistar albino rats. The procedure was followed by using OECD 423(Acute toxic class method).The rats are fasted overnight, prior to dosing. The three dose levels are administered by the help oforal feeding needle over the prior of 24 hours. After the drug has been administered, food may be with held for a further 3-4 hours in rats. The purpose of sighting study is to allow selection of the appropriate starting dose for main study. The test substance is administered to a single animal in a sequential manner following from the fixed dose levels of 5, 50, 300 and 2000mg/kg. The interval between dosing of each level is determined by the mortality/onset, duration and severity of toxic signs over the period of 24 hours, special attention given during the first 4 hours. Four hours after the drug administration, provide the food and water for 14 days and daily observed some parameters such as food intake, water intake, mortality, onset, Duration and severity of toxic signs. The animal weight is recorded on weekly once. On the day fourteen all the animals are sacrificed, to isolate the organs and observe the histopathological changes. Based on the mortality result of sighting is decided and carried out with five animals per dose level (5 or 50 or 300 or 2000mg/kg).Based on the mortality result on 14th day of observation, the doses for in vivo study are selected.

***In vivo* antidiabetic activity of *Croton cajucara* Leaves and Root extract in Alloxan induced diabetic wistar albino rats**
Wistar albino mice (25-35 g) bred in Central Animal facility of the Institute, were used. They were housed under standard conditions, maintained on a 12 h light/dark cycle and had free access to food and water up to the time of experimentation. The mice were acclimatized to the laboratory environment 1 h before the experiments. All experiments were conducted during the light period (08.00-16.00 h). During the experiments animals were free access to water only. All the protocols were approved by the Institutional Animal Ethical Committee (IAEC) and conducted according to the guidelines of CPCSEA (Committee for the Purpose of

Control and Supervision of Experiments on Animals).

Chemicals

Alloxan from Loba Chemie. Standard Glibenclamide (Daonil) from Aventis Pharma. Ethanol (Analytical grade) and 5% Dextrose solution Glucose Estimation Kit from Gluco Dr Super sensor.

Hypoglycemic Test

Groupings were done as follows: Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat), Group II served as Positive control – Glibenclamide (2mg /kg), Group III served as aqueous ethanolic extract of *Croton cajucara* – (200mg/kg), Group IV served as aqueous ethanolic extract of *Croton cajucara* – (400mg/kg). Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Oral Glucose Tolerance Test

Groupings were done as follows: Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat), Group II served as Positive control – Glibenclamide (2 mg /kg), Group III served as aqueous ethanolic extract of *Croton cajucara* – (200mg/kg), Group IV served as aqueous ethanolic extract of *Croton cajucara* – (400mg/kg). All the groups of animals were fasted for 24h and blood samples were collected before drug or solvent treatment. The drug, extract andsolvent, have been adminisratered to different groups and 30mins later all the groups feeding needle. Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Induction of diabetes to animals

A single dose (100 mg/kg b.w., i.p.) of Alloxan monohydrate dissolved in sodium citrate buffer was used for the induction of diabetes in rats after overnight fasting. After 1 hr of Alloxan monohydrate administration, the animals were given feed and libitum and 5% dextrose solution was also given in feeding bottle for a day to overcome early hypoglycaemic phase. The animals were stabilized for a week and animals showing blood glucose level more than 200 mg/dl were selected for the study.

Experimental design

Five groups of rats six in each groups received the followingtreatment schedule for 14 days.

GROUP I -Normal control (normal saline 10 ml /kg, P.O)

GROUP II -Alloxan treated control (100 mg/kg, I.P)

GROUP III - Alloxan (100 mg/kg, I.P) + Standard drug, Glibenclamide (2 mg/kg, P.O).

GROUP IV- Alloxan (100 mg/kg, i.p.) + EEBAL.(200 mg/kg,P.O)

GROUP V- Alloxan (100 mg/kg, i.p.)+ EEBAL. (400 mg/kg,P.O)

Plant Leaves and Root extract, standard drug and normal saline were administered with the help of oral feeding needle. Group I serve as normal control which received normal saline for 14 days. Group II to Group V were diabetic control rats. Group IV and Group V (which previously received Alloxan100mg/kg) were given fixed doses of ethanol Leaves and Root extract (200 mg/kg, P.O, 400 mg/kg, P.O) of *Croton cajucara* and group III received standard drug Glibenclamide (2 mg/kg,P.O) for 14 consecutive days. (EEBAL- Ethanolic extract of Croton *cajucara* Leaves and Root).

Collection of blood samples

Fasting blood samples were drawn from retro orbital puncture of rats at weekly intervals till the end of the study 1, 7, and 14 days.

Estimation of biochemical parameters Serum blood glucose

On 1, 7, and 14 days fasting blood samples were collected and analyzed the blood glucose.

Blood glucose level

The blood glucose level test measures the amount of glucose in the blood sample obtained from the animals. The test is usually performed to check for elevated blood glucose levels which can be an indication of diabetes or insulin inhibition.

Statistical analysis

Statistical analysis was done by using GRAPHPAD PRISM 5.0.All the values of Biochemical parameters and body weight were expressed as Mean ± Standard Error Mean (SEM). The values were analyzed for statistical significance using one- way analysis of variance (ANOVA), comparison was done by using Dunnett's t test. P values < 0.05 were considered as significant, P values < 0.01 were considered as very significant, P values < 0.001 were considered as highly significant and ns were considered as not significant.

RESULTS

Appearance and percentage yield of EEHC (Ethanolic Extract of *Crotoncajucara* Leaves and Root)

EEBAL was a semisolid brownish colour extract and the percentage yield wasfound to be 14.35%

***Invitro* antidiabetic study**

Table No. 1: α-Amylase Inhibition of Petroleum Ether extract of *Crotoncajucara* Leaves and Root.

Concentration(µg/ml)	Percentage Inhibition (%)
0	0
25	29
50	33
75	42
100	53
125	56

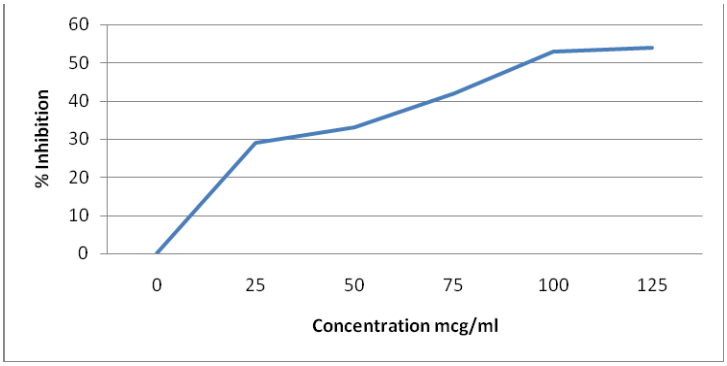


Fig No: 3α-Amylase Inhibition of Petroleum Ether extract of *Croton cajucara* Leaves and Root

Table no. 2: α-Amylase Inhibition of Ethyl acetate Extract of *Croton cajucara* Leaves and Root.

Concentration(µg/ml)	Percentage Inhibition(%)
0	0
25	34
50	41
75	48
100	56
125	60

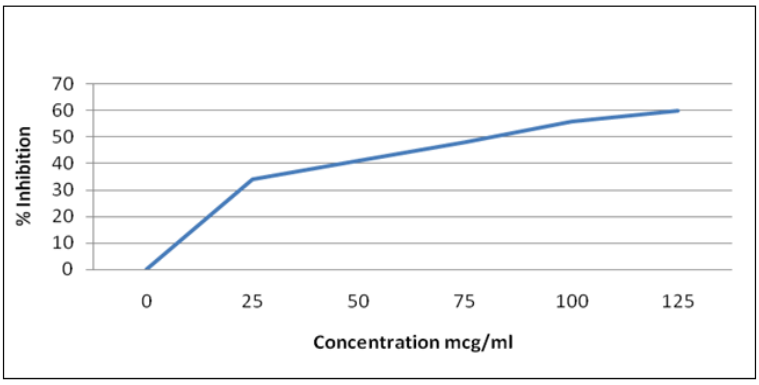


Fig No:4 α-Amylase Inhibition of Ethyl acetate Extract of *Croton cajucara* Leaves and Root

Table no. 3: α-Amylase Inhibition of Ethanolic Extract of *Croton cajucara* Leaves and Root.

Concentration(µg/ml)	Percentage Inhibition(%)
0	0
25	30
50	36
75	50
100	56
125	61

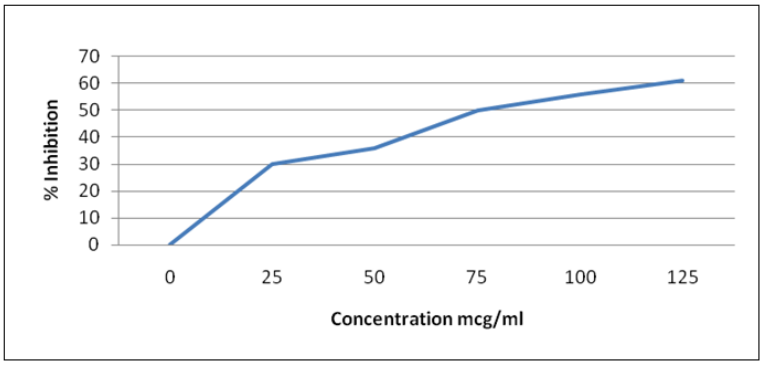


Fig. No. 5: α-Amylase Inhibition of Ethanolic Extract of *Croton cajucara* Leaves and Root.

Table no. 4: α-Amylase Inhibition of Acarbose (Positive control).

Concentration(µg/ml)	Percentage Inhibition(%)
0	0
25	25
50	45
75	53
100	56
125	65

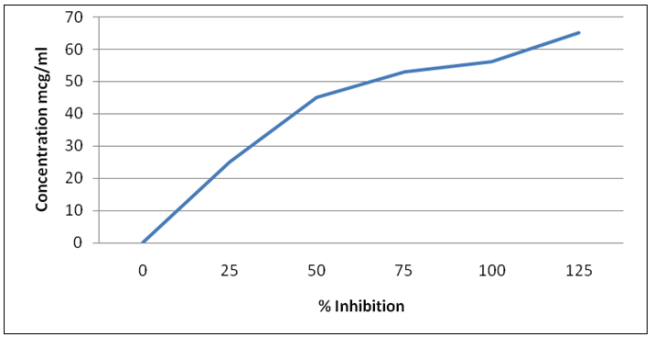


Fig No:6 α-Amylase Inhibition of Acarbose (Positive control).

Result

% Inhibition of Petrolium ether extract of *Croton cajucara Leaves and Root* = 91 µg/ml
% Inhibition of Ethyl acetate extract of *Croton cajucara* Leaves and Root = 80 µg/ml
% Inhibition of Ethanolic extract of *Croton cajucara Leaves and Root* = 75 µg/ml
% Inhibition of Acarbose (Positive control) = 61 µg/ml
Minimum % Inhibition was found in Ethanolic extract of *Croton cajucara Leaves and Root* which resemblance to %Inhibition of positive control, So Ethanolic extract of *Croton cajucara* contain active constituents of antidiabetic.

TLC Study

Table No. 5: Results of Ethanolic Extract of *Croton Cajucara* Leaves and Root.

S. No.	Solvents	Concentration	Rf value
1.	Toluene + Ethyl acetate	7:3	0.62
2.	Toluene + Ethyl acetate + Glacialacetic acid	5:5:1	0.76
3.	Petrolium ether+Chloroform	7:3	0.56
4.	Ethyl acetate + Methanol	1:1	0.88
5.	Hexane + Dichloro methane	1:1	0.67
6.	Ethyl acetate + Methanol	3:1	0.59
7.	Dichloro methane +Hexane	3:1	0.72

Rf Value range high-Polar substances present. Rf value range low-Low polar substance present.
Solubility of compounds depends upon the polarity of solvents. Obtained Rf values were confirmed by standard Rfvalues.
Rf values obtained for my extract ranges from 0.56 to 0.88, So my extract may contain compounds like Flavanoids, Glycosides and Alkaloids.
Phytochemical studies

Table No. 6: Results of ethanolic extract of *Croton cajucara* Leaves and Root.

Class of compounds	Tests performed	Results
Carbohydrates	Molisch's test, Fehling's test	+ -
Phenols	Phosphomolybdic acid test	+
Flavonoids	Shinoda test	+
	Lead acetate test	+
Tannins	Braemer's test	_
Alkaloids	Wagner's	+
	Mayer's Draggendrof's test	+
	Legal's test	+

Glycosides	Brontranger's test	+
Saponins	Foam test	+
Sterols	Salkowski's test	_
Amino acids	Ninhydrin test	_
Terpenoids	Lieberman Burchardt test	+

+Present in moderate amount
-Absence

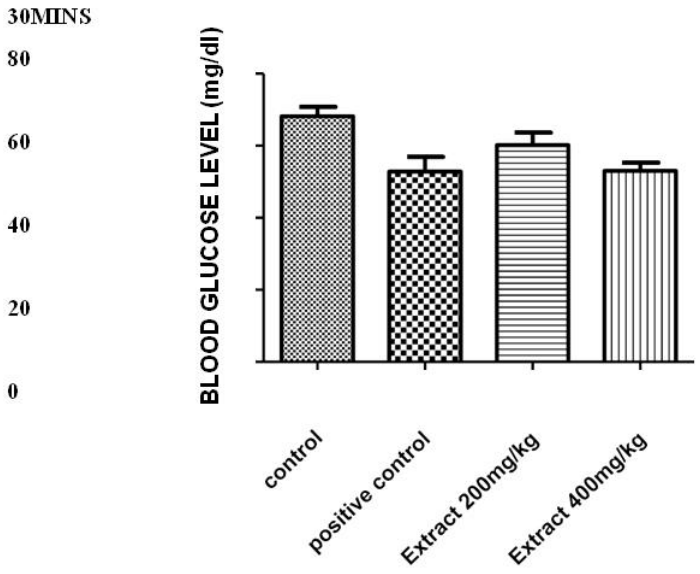
The phytochemical studies results revealed that the Molisch's test no characteristic observation indicated the absence of carbohydrates, by phosphomolybdic acid test Blue coloration of the spot indicated the presence of phenols. Shinoda test and Lead acetate test gave pink or red coloration of the solution indicated the presence of flavonoids Flocculent white precipitate also indicated the same. There is no dark blue or greenish grey coloration of the solution indicated the absence of of tannins in the drug. No characteristic observation for steroids and dark pink or red coloration of the solution indicated the presence of terpenoids. Orange coloration of the spot indicated the presence of alkaloids. Yellow or reddish brown precipitation precipitation indicated the presence of alkaloids. Pink to red colour solution indicates the presence of glycosides. No layer of foam formation indicates the absence of Saponins. If the response to the test is indicated table-1high it can be noted or which indicates that the particular group is present as the major class. If the response is average then note it as indicates the presence in moderate quantity and note it as indicating the presence of only in traces. If no response is then negative.

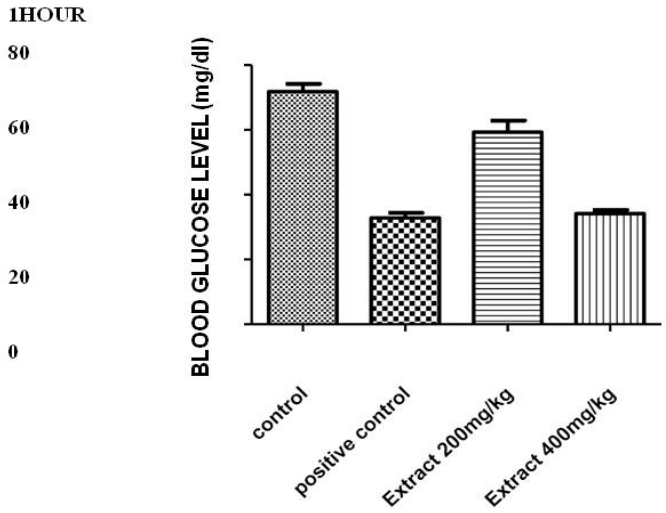
Table I: Hypoglycemic Test.

TREATMENT	DOSE mg/kg	BLOOD GLUCOSE LEVEL (mg/dl)		
		0 min	0.5hr	1 hr
CONTROL CARBOXYME THYL CELLULOSE(CMC)	0.5 %	68.00±2.429	68.17±2.587	71.83±2.372
POSITIVE CONTROL Glibenclamide	2	69.00±0.6325	52.83±4.037**	32.83±1.515***
AQUEOUS ETHANOLIC EXTRACT OF CROTON CAJUCARA	200	68.80±2.245	60.17±3.497*	59.33±3.584*
AQUEOUS ETHANOLIC EXTRACT OF CROTON CAJUCARA	400	68.00±2.429	53.00±2.309**	34.17±1.138***

The glucose levels were analyzed by using glucometer and each value is the mean ± standard error (n= each group consist of 6 animals)($p<0.05$)*, ($p<0.001$)**& ($p<0.0001$)*** as compared to control & positive control group evaluated by one way, ANOVA followed by Dunnet 't' test.

GROUPS





The hypoglycemic test results have shown Table No: I, which indicated aqueous ethanolic extract of *Croton cajucara* treated animals 200 & 400, significantly decreased in blood glucose level (0.84± 1093↓ & 18.83±3.879↓) (P<0.05)*, (P<0.001)**& (P<0.0001)*** when compared to control and positive control.

***Invivo* antidiabetic study**

Table No. 13: Results of the effects of ethanolic extract on blood Glucose levels.

S.No.	TREATMENT	Blood glucose level (mg/dl) day		
		Day 1	Day 7	Day 14
1	Normal control 10 ml/kg P.O	79.83±2.833	75.7±4.014	76.7± 4.944
2	Negative control	265.2±3.85	270.1±2.9	275.2±2.5
3	Possitive control (Glibenclamide2mg/kg) P.O	255.83±2.386	135.63±3.8***	112±2.8***
4	EEBAL 200 mg/kg P.O	260±3.5	250.3±3.138**	245.2±3.250**
5	EEBAL 400 mg/kg P.O	263±4.55	173.1±2.88***	162.1±1.8***

EEHC-Ethanoloc extract of *Croton cajucara* Leaves and Root

(The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

The experimental results have indicated on Table No:13. The negative control group glucose levels were significantly increased when compared to each other groups. All the groups of animals were affected in diabetes, which indicated blood glucose levels were slight changes in the blood glucose level (4.13±1.207↓& 1.±0.93↑) for normal control group at 7th and 14th days. On day 7th glucose levels were significantly decreased glibenclamide 2mg/kg treated group (120.2±1.414↓& 23±1↓) (P<0.05)*, (P<0.001)**& (P<0.0001)*** when compared with control group at 7th and 14th days. The ethanolic Leaves and Root extract of *Croton cajucara* treated groups

200 & 400 mg/kg were dose dependent manner decreased (P<0.001)**& (P<0.0001)*** (10±0.362↓& 90±1.67↓) when compared with control group but positive control have more anti diabetic activity at 7th day. The aqueous ethanolic Leaves and Root extract of *Croton cajucara* at the dose level 400mg/kg have equipotent activity (90±1.67↓&120.2±1.414↓when compared with positive control at 7th day. The ethanolic Leaves and Root extract of *Croton cajucara* 200 & 400 mg/kg have been expressed dose dependent anti diabetic action (P<0.001) **& (P<0.0001) *** when compared to control and positive control. On day 14th, ethanolic Leaves and Root extract of *Croton cajucara* treated animals 200 & 400 mg/kg significantly decreased and maintain the blood glucose level (5.1±0.07↓ & 11±1.08↓), (P<0.001) **& (P<0.0001) *** when compared to control and positive control.

Table- Oral Glucose Tolerance Test.

TREATMENT	DOSE mg/kg	Blood Glucose Level (mg/dl)						
		0 min	0.5hr	1 hr	1.5hr	2 hr	2.5hr	3hr
Control Carboxymethyl Cellulose (Cmc)	0.5 %	68.00±2.429	142.5±6.292	187.5±9.465	172.5±12.25	157.5±12.38	153.5±12.83	130.2±13.31
PositiveControl Glibenclamide	2	69.00±0.6325	104.2±7.323***	110.5±6.980***	93.67±1.308***	83.67±1.308***	77.17±4.070**	74.33±2.940***
Aqueous Ethanolic Extract Of *Croton Cajucara*	200	68.80±2.245	128.3±6.009	147.3±2.404*	138.5±5.667*	128.5±5.667*	113.8±6.760*	108.8±6.107**
Aqueous Methanolic Extract Of *Croton cajucara*	400	68.00±2.429	115.0±6.191*	121.2±6.188**	103.3±4.766***a	93.33±4.766*** a	86.67±3.602**a	83.67±2.906***a

The glucose levels were analyzed by using glucometer and all values are expressed as Mean±SEM (n=6), Group 2 was compared with group 1, Groups —3,4 were compared with group 2; *p<0.05, **p<0.01,p<0.001*** evaluated by one way, ANOVA followed by Dunnet 't' test.

DISCUSSION

The present study demonstrated the significant antidiabetic potential of the ethanolic leaf and root extract of Croton cajucara through both in vitro and in vivo investigations. The in vitro α-amylase inhibition assay revealed that the ethanolic extract exhibited the lowest IC_{50} value among the tested extracts, indicating a strong inhibitory effect on α-amylase, a key enzyme involved in the hydrolysis of dietary starch. Inhibition of this enzyme delays carbohydrate digestion and glucose absorption, thereby reducing postprandial hyperglycemia, which is an important therapeutic strategy in diabetes management.

Preliminary phytochemical screening confirmed the presence of phenols, flavonoids, alkaloids, glycosides, saponins, and terpenoids, all of which are known to possess antioxidant and antihyperglycemic properties. LC–MS analysis coupled with PASS bioinformatics identified quercetin and kaempferol as the major bioactive constituents with predicted antidiabetic activity. These flavonoids have been reported to improve glucose homeostasis through antioxidant effects, enhancement of insulin secretion, and modulation of carbohydrate-metabolizing enzymes.

Acute oral toxicity studies performed according to OECD Guideline 423 demonstrated that the extract was safe up to 2000 mg/kg, supporting its favorable safety profile. In the alloxan-induced diabetic rat model, administration of the ethanolic extract at 200 and 400 mg/kg produced a significant, dose-dependent reduction in fasting blood glucose levels, with the higher dose showing efficacy comparable to the standard drug glibenclamide. Since alloxan induces diabetes through selective destruction of pancreatic β-cells, the observed antihyperglycemic effect suggests that the extract may enhance residual β-cell function, improve peripheral glucose utilization, and inhibit intestinal carbohydrate digestion. Overall, these findings provide scientific evidence supporting the traditional use of Croton cajucara as an antidiabetic medicinal plant and highlight its potential as a promising source for the development of safe and effective phytopharmaceutical agents. Further studies on molecular mechanisms, bioactive compound isolation, and clinical evaluation are warranted.

CONCLUSION

The present study successfully demonstrated the antidiabetic potential of the ethanolic leaf and root extract of *Croton cajucara* through comprehensive in vitro and in vivo investigations. Sequential extraction using petroleum ether, ethyl acetate, and ethanol identified the ethanolic extract as the most active fraction based on its superior α-amylase inhibitory activity. Preliminary phytochemical screening revealed the presence of biologically active constituents, including phenols, flavonoids, alkaloids, glycosides, saponins, and terpenoids, which may collectively contribute to its therapeutic efficacy. LC–MS analysis, supported by PASS bioinformatics prediction, identified several phytoconstituents, including quercetin, kaempferol, rhamnetin, rhamnoxanthin, and luteolin, with quercetin and kaempferol being the principal compounds associated with antidiabetic activity.

Acute oral toxicity studies conducted according to OECD Guideline 423 confirmed the safety of the extract up to 2000 mg/kg, and doses of 200 and 400 mg/kg were selected for pharmacological evaluation. In alloxan-induced diabetic rats, the ethanolic extract produced a significant and dose-dependent reduction in fasting blood glucose levels, with the 400 mg/kg dose demonstrating

efficacy comparable to glibenclamide. These findings suggest that the extract exerts its antidiabetic effect through α-amylase inhibition and protection against alloxan-induced hyperglycemia. Overall, *Croton cajucara* represents a promising natural source of antidiabetic agents and warrants further studies to isolate its bioactive compounds, elucidate their molecular mechanisms, and evaluate their clinical efficacy and safety.

BIBILIOGRAPHY

1. American Diabetes Association Professional Practice Committee. Standards of Care in Diabetes—2025. *Diabetes Care*, 2025; 48(1): S1–S350.
2. International Diabetes Federation. *IDF Diabetes Atlas*. 11th ed. Brussels, Belgium: International Diabetes Federation, 2025.
3. World Health Organization. *Global Report on Diabetes*. Geneva: World Health Organization, 2024.
4. Tripathi KD. *Essentials of Medical Pharmacology*. 9th ed. New Delhi: Jaypee Brothers Medical Publishers, 2023.
5. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London: Chapman & Hall, 1998.
6. Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*. 58th ed. Pune: Nirali Prakashan, 2022.
7. Evans WC. *Trease and Evans Pharmacognosy*. 17th ed. London: Elsevier, 2020.
8. OECD. *Test No. 423: Acute Oral Toxicity – Acute Toxic Class Method*. OECD Guidelines for the Testing of Chemicals. Paris: OECD Publishing, 2022.
9. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. *J Ethnopharmacol*, 2002; 81(1): 81–100.
10. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pac J Trop Biomed*, 2012; 2(4): 320–330.
11. Kumar S, Narwal S, Kumar V, Prakash O. α-Glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacogn Rev*., 2011; 5(9): 19–29.
12. Tundis R, Loizzo MR, Menichini F. Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycemic potential in diabetes. *Mini Rev Med Chem*., 2010; 10(4): 315–331.
13. Li WL, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *J Ethnopharmacol*, 2004; 92(1): 1–21.
14. Bnouham M, Ziyyat A, Mekhfi H, Tahri A, Legssyer A. Medicinal plants with potential antidiabetic activity—A review of ten years of herbal medicine research. *Int J Diabetes Metab*., 2006; 14: 1–25.
15. Srinivasan K. Plant foods in the management of diabetes mellitus: Spices as beneficial antidiabetic food adjuncts. *Int J Food Sci Nutr*., 2005; 56(6): 399–414.
16. Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, Watal G. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves in experimental animals. *J Ethnopharmacol*, 2005; 99(1): 75–81.
17. Shukla R, Sharma SB, Puri D, Prabhu KM, Murthy PS. Medicinal plants for treatment of diabetes mellitus. *Indian J Clin Biochem*, 2000; 15(1): 169–177.
18. Kooti W, Farokhipour M, Asadzadeh Z, Ashtary-Larky D, Asadi-Samani M. The role of medicinal plants in the treatment of diabetes: A systematic review. *Electron Physician*, 2016; 8(1): 1832–1842.
19. Bailey CJ, Day C. Traditional plant medicines as treatments for diabetes. *Diabetes Care*., 1989; 12(8): 553–564.
20. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TPA. Indian herbs and herbal drugs used for the treatment of diabetes. *J Clin Biochem Nutr*., 2007; 40(3): 163–173.