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ORIGINAL ARTICLE **OPEN ACCESS**

Investigation of hypoglycemic, anticholesteremic, *in vivo* antioxidant and pancreatic beta cell protective effect of *Tecoma gaudichaudi DC* leaves in streptozotocin-induced diabetic rats

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ABSTRACT
Bignonia Linn (Bignoniaceae) is a monotypic genus of woody climbers, native to North America and mostly grown for ornament in the tropics of the old world. The antidiabetic potential of core species of Bignoniaceae was carried out on some species of Tecoma genus such as Tecoma gaudichaudi DC. In present study, in-vivo antidiabetic potential of isolated fraction of ethyl acetate extract of Tecoma gaudichaudi DC has been investigated. The identification of triterpenoid and their related functional group in bioactive fraction was categorized by using HRMS and IR. Oral administration of ethyl acetate extract of Tecoma gaudichaudi DC at dose 250 mg/kg & 500mg/kg) significantly increase in the body weight, decrease in the blood glucose and total cholesterol (P<0.05) and restore function of SOD and CAT enzymes. Histologically EATG (250 & 500mg/kg) treated group shows no significant effect on pancreatic β- cells while fraction rich with Ursolic acid treated group shows increased cell size of pancreatic β- cells. Insulin treated group shows normal density of islets of β- cells along with few areas showing necropsy. These finding reveals that ethyl acetate extract of leaves of Tecoma gaudichaudi DC shows significant antihyperglycemic, anti-cholesterolemic, in-vivo antioxidant activity and improved the cell density of β-cells of islets of langerhans in diabetic rats.
Keywords: *Tecoma gaudichaudi DC, Streptozotocin; Antihyperglycemic; Anti-cholesterolemic; Antioxidant*

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INTRODUCTION

Diabetes mellitus is a metabolic disease, characterized by hyperglycemia and impaired metabolism of glucose and other energy-yielding fuels, such as lipids and proteins and is the result of a deficiency of insulin secretion or a resistance to insulin action, or both [1]. Diabetes constitutes a worldwide public health problem [2]and according to International Diabetes Federation 382 million people get affected by diabetes in 2013 and recent projections suggests that this prevalence is likely to increase in the next 20 years, affecting 592 million people (10.1%) in 2035. Diabetes mellitus type 1 and type 2 are caused by damage due to chronic inflammation of pancreatic β-cell island. It causes abnormal insulin release, effects insulin receptor and post receptor events and ends with liver, kidney, eye damage [3]. Various complications get arises during diabetes from these vascular complications are the leading cause of morbidity and mortality among patients with type 1 and type 2 diabetes mellitus. These vascular abnormalities result of a chronic hyperglycemia state, which leads to an increase in oxidative stress and inflammatory responses [4].Herbal medicines overcome various side effects of synthetic drugs therefore the study of hypoglycemic plants is then encouraged [5,6].Plant families which are confirmed to show hypoglycemic activity include: Leguminoseae, Lamiaceae, Lilliaceae, Cucurbitaceae, Asteraceae, Moraceae, Rosaceae, Euphorbiaceae, Araliaceae, Polygalaceae, Asclepidaceae, Meliaceae etc [7]. The effect of the medicinal plants may delay the diabetic complications and rectify the metabolic abnormalities. Now a day's more focus on to isolate bioactive compounds and it shows hypoglycemic activity [8].From all secondary metabolite's pentacyclic triterpenes, are an important group of it considered as lupenyl, ursanyl, betulenyl or oleanyl. They are presented in plant species as the form of aglycone's saponin triterpenoids [4-5]. Previous reports state that species of Bignoniaceae family show presence of promising active constituents such as tannins, flavonoids, triterpenes, alkaloids, carbohydrates, etc. [6].

The phytochemical analysis of various species of Bignoniaceae family was not studied so far hence; the following research deals with to carry an out preliminary phytochemical analysis of various extracts of leaves of *Tecoma gaudichaudi* DC. However, no previous biological activities have been reported for *Tecoma gaudichaudi* DC leaf powder except some ethnomedicinal claims were reported such as in Bangladesh whole plant of *Tecoma gaudichaudi* DC use of a remedy for diabetes and infertility problems [3].The present study includes successive extraction of *Tecoma gaudichaudi* DC leaf powder with solvent of increasing polarity, phytochemical analysis for the successive extracts. High resolution mass spectrometric analysis of some selected extract as a rapid screening tool for extract activity, most potent of which were further assessed for antidiabetic activity. Finally, isolation attempt was made to isolate and identify the major chemical forms present in the leaf ethyl acetate extract are potential molecule for its antidiabetic activity.

MATERIAL AND METHODS

Chemicals and reagents

DPPH, Streptozotocin was purchased from Sisco Research Laboratories Pvt. Ltd. Insulin was purchased from Lantus Solostar of Sanofi India Limited. The biochemical estimations were carried out by using standard diagnostic kits of span diagnostics, Ahmedabad, India. Other chemicals and reagents used in the study were of high purity.

Collection of Plant material

The stems with leaves of *Tecoma gaudichaudi* DC(Family Bignoniaceae) were collected from Moshi area of Pune district (Maharashtra) in the month of September 2011 and the plant were authenticated at Botanical Survey of India, Pune. The specimen voucher number is KALKTEG1.

Animal details

Female Wistar rats weighing about 225-275gm were obtained from. The study protocol was approved by IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for purpose of control and supervision of experimentation on animals) with reference no.MCP/IAEC/167/2015, dated: 31/10/2015. Animals were kept for acclimatization at room temperature between 22±3^{o}C with relative humidity 55±5^{o}C with 12 hours each of dark and light cycles. Animals had free access to standard pellet and water adlibitum.

Extractionand preliminary phytochemical analysis for*Tecoma gaudichaudi* DC leaves:

The leaves of plant *Tecoma gaudichaudi* DC were used for extraction. Dried and coarsely powdered crude drugs were passed through sieves no. 40 and successive extraction has been carried out on plant material by soxhlet extraction. Preliminary chemical analysis was performed according to the established protocol mentioned in Rangari V.D [9].

In vitro antioxidant activity

Serial dilutions of pet ether, ethyl acetate and ethanol leaf extract from 10 to 80µg/ml were assessed for their antioxidant effect by using reported method [11]. 1 ml of extracts solution, 5ml of methanolic solution of DPPH were mixed and incubated at 370C for 20min. The absorbance was measured against methanol as a blank at 517nm. The absorbance of DPPH was taken as a control. The percent antiradical activity was calculated by using following formula [12].

% antiradical activity = (A Control. – A sample) / A Control X 100
where A Control: Control absorbance (DPPH).
A sample: Sample/standard absorbance.

Isolation process

The leaves of *Tecoma gaudichaudi* DC was collected, (500g) leaves powder was extracted with ethyl acetate (2.5 L) by Soxhlet assembly. Obtained ethyl acetate extract (3.1g) was further use for isolation. Ethyl acetate extract was selected based on preliminary phytochemical analysis.

Fractionation of EATG by column chromatography:

Column chromatography of ethyl acetate soluble extract:

Experimental:

Column Dimension	80 cm (length), 3 cm (Diameter)
Stationary phase	Silica particles of mesh size (#60-120).
Mobile phase	Ethyl acetate→ Toluene
Elution Mode	Gradient
Quantity of fraction	100 ml
Solvent for column packing	Ethyl acetate
Analysis of fraction	TLC

Fraction 1, by elution (90ml ethyl acetate: 10ml hexane), fraction 2 (70ml ethyl acetate: 30ml hexane), fraction 3 (50ml ethyl acetate: 50ml hexane) fraction 4 (30ml ethyl acetate: 70ml hexane), fraction 5 (100ml hexane). Fractions 1-5 were checked by TLC by using mobile phase hexane: ethyl acetate (3:7) [10]. So fraction 5 shows single spot and subjected to spectroscopic analysis and to obtain Compound-TG1

Acute toxicity studies (OECD, 2001)

The acute toxicity study for ethyl acetate extract of bark was performed by using female Wistar rats followed by OECD Guidelines No. 423, where different doses (30-2000 mg/kg of body weight) of ethyl acetate extracts were administered orally. After administration of the extracts orally, the animals were observed for their death and toxic effects after 24h treatment. The toxicological effects were observed in terms of mortality. Extracts were found to be safe in doses up to the 2000 mg/kg b.w. The animals were fasted over-night prior to the experiment and maintained under standard conditions.

***In vivo* antidiabetic activity determination**

Type-1 diabetes was induced in 48 rats by i.p injection of STZ (50 mg/kg) freshly prepared in 0.1 M sodium citrate buffer, pH 4.5 [13]. During the first 24 h of diabetes induction, STZ-treated animals were allowed to drink 5% glucose solution to overcome drug-induced hypoglycemia. 7 days after STZ administration, diabetes was confirmed by the presence of hyperglycemia. STZ-treated animals showed fasting blood glucose less than 400mg/dl were discarded [14].Rats were divided into five groups of six rats (n=6) each. Group I and II served as normal control and diabetic control respectively receives saline (0.2 ml oral). Group III serves as a standard and was treated with Insulin (6 IU/animal; subcutaneous; once in a day). Group IV and V were treated with ethyl acetate extract of *Tecoma gaudichaudi* DC at a dose of (250 mg/kg and 500 mg/kg per oral once in a day for 21 days). Body weight of each animal was monitored weekly during the period of the study. Fasting blood glucose measured on day 7, 14 and 21 using Glucose measurement kit (GOD POD method). Total cholesterol in serum was measured on 1 and 21 days [15].High levels of antioxidant level in serum are found to be associated with decreased risk of non-insulin dependent diabetes mellitus so in present study serum antioxidant level (Superoxide Dismutase and Catalase) was estimated in anti-diabetic study. At termination on day 21, blood was collected using cardiac puncture and centrifuged at 7500 rpm for 10 min to obtain serum. Superoxide dismutase (SOD) activity was determined by the reported method [16]. This method based on the specific principle such as pyrogallol autooxidises rapidly in aqueous or alkaline medium solution and this has been employed for the estimation of superoxide dismutase. SOD inhibits auto oxidation of pyrogallol and absorbance of samples was recorded at 420nm.Catalase (CAT) activity was determined according to the report method by Goth, 1991 [17]. According to this method, serum sample was incubated with substrate of hydrogen peroxide in sodium potassium phosphate buffer, pH 7.4 and the enzymatic reaction was stopped by adding ammonium molybdate. So stable yellow complex of molybdate and hydrogen peroxide was measured at 405nm.

Histopathological study

On the 21 day of the study, the animals were sacrificed. Pancreas was dissected and fixed in 10% neutral buffered formalin for 24 h. thin sections of tissue around 5µm, were cut and stained with haematoxylin and eosin. The sections were dehydrated by using increasing concentration of alcohol. They were then treated with diphenyxylene (DPX) and observed under microscope.

Statistical analysis

The results were expressed as mean SEM from six animals. The results were subjected to statistical analysis by using one way ANOVA followed by Tuckey's test $p<0.05$ was considered to be statistically significant.

Spectral analysis:

The IR Spectrometer used was 'Jasco FT/IR-4100' System. Mass spectrum was analyzed by Ultra-High-Resolution Time-of-Flight Mass Spectrometer Impact II (HRMS) (Bruker Daltonik GmbH, Germany).

RESULTS

Preliminary phytochemical by chemical test:

Initially all extracts were analyzed by chemical test shows presence of carbohydrate, proteins, steroids, triterpenoids, flavonoids and tannins. (Table 1)

Phytochemical analysis of Ethyl acetate extract of *Tecoma gaudichaudi* DC by HRMS:

Ethyl acetate and ethanol extracts of *Tecoma gaudichaudi* DC were analyzed by High resolution mass spectrometry (HRMS). HRMS of Ethyl acetate Extract shows m/z at 453.20, 461.19, 465.37, 471.22, 479.34, 487.36 & 495.34 (Fig. 2). Ethanol extracts note m/z at 403.30, 429.20, 445.20, 461.18, 477.17, 499.14, and 514.33 (Fig.3). So comparatively ethyl acetate note m/z at 479.34 ($C_{30}H_{48}NAO_3$) (Fig.2).

Isolation of compounds from *Tecoma gaudichaudi* DC:

Based on chromatographic data further fraction no. 5 was purified by using methanol, subjected to chromatographic analysis, thin layer chromatography was performed by using solvent system hexane: ethyl acetate (3:7v/v).; Single compound was present in fraction 5, the yield was noted as (8mg). For further isolation of this compound repeat same procedure again and isolated fraction checked by TLC, use for biological activity. The melting point was found to be 190-194^{0}C. The compound was subjected to HRMS and IR analysis, mass spectra shows a molecular peak at [m/z 456.70 (M+H) (C30H48O3), 479.35 (M+Na)+ (C30H48NAO3)] as view in spectra (Fig 2). The IR spectra reveal bands at 3710.37cm- (-COOH, O-H stretching, acidic), 2927.41 cm- (CH2-CH3 stretching), 1693.19 cm-(ketone stretching), 1514.81 cm-(C=C stretching), 1456.96 cm-(CH2-CH3 bending), 1000 cm-(C-O stretching), 900 cm-(C=C-H bending) Compound TG-1 was identified as ursolic acid (Fig 3)

***In vitro* antioxidant activity determination for successive leaf extract using DPPH assay:**

Results of scavenging free radicals are expressed as IC_{50} values (ug/ml):IC_{50} value of ethanol, ethyl acetate and ascorbic acid was found to be 33.15, 40.72, 28.03 respectively. (Table 2).

Acute toxicity determinations:

Acute oral toxicity studies shows that the non-toxic effect of ethyl acetate leaf extract of *Tecoma gaudichaudi* DC. Extracts were found to be safe in doses up to the 2000 mg/kg b.w. above the dose of 2000 mg/kg b.w., animal shows the signs as convulsions, weakness, and dizziness, loss of appetite, tremor and finally death.

In vivo antidiabetic activity determination

Effect of EATG on body weight

At the end of 21-day treatments, body weight of EATG (500mg/kg), Ursolic acid (10 mg/kg) and standard insulin treated group, shows significantly increased in weight ($p<0.05$). Body weights of rats in diseases control and EATG (250mg/kg) treated group shows lower body weight than other groups (Table 3)

There was significant change in body weight of the rat in disease control and all treatment group on day 7 when compare to day 0. On day 21, there was significant weight gain in animals treated with EATG (500mg/kg), Ursolic acid (10 mg/kg) and standard insulin treated group, when compared with the body weight data on day 7. (Table 3& Fig 4)

Effect of EATG on Blood glucose in normal rats:

On day 7 there was significant increase in fasting blood glucose level in all rats compare to normal control ,increased fasting blood glucose level indicates hyperglycemic state. On day 21, there was significant decrease in fasting blood glucose level in all treatment group when compare to disease control data. EATG (250mg/kg), EATG (500mg/kg) and ursolic acid (10mg/kg) treated group and standard treated group shows significantly decrease in glucose level ($p<0.05$) as compare to diseases control group. Insulin treated diabetic rats restored the glucose level on 14 and 21 days (Table 4& Fig 5).

Effect of EATG on Cholesterol levels:

21day diseases control group indicates significant increase in cholesterol ($P<0.05$) as compare with the normal control group. While extract treated EATG (250 &500mg/kg) and ursolic acid (10mg/kg), standard insulin treated shows a significant decrease ($P<0.05$) in cholesterol as compare with diseases control (Table 5&Fig. 6)

Effect of EATG on SOD and Catalase enzyme:

SOD and CAT levels were significantly decrease ($P<0.05$) in the serum of diseases control. Oral administration of EATG (250 &500mg/kg) and ursolic acid (10mg/kg), standard insulin treated group shows a significant increase in SOD and CAT antioxidant enzyme level ($p<0.05$) except the normal control (Table 6, Fig. 7 & 8).

Changes of Histopathology of the pancreas:

The histological investigation of the pancreas showed normal pancreatic β-islet cells in the case of a normal control group and observed destruction of ß-cells in diseases control.EATG (250& 500 mg/kg) treated group shows no significant effect on pancreatic ß-cells. While ursolic acid treated group shows increased cell size of pancreatic ß-cellls. Insulin treated group shows normal density of islet ß-cells along with few areas showing necropsy (Fig. 9)

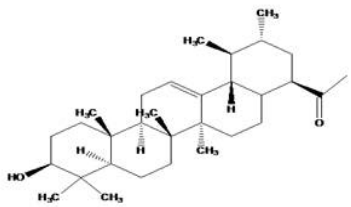


Fig. 1 Ursolic acid

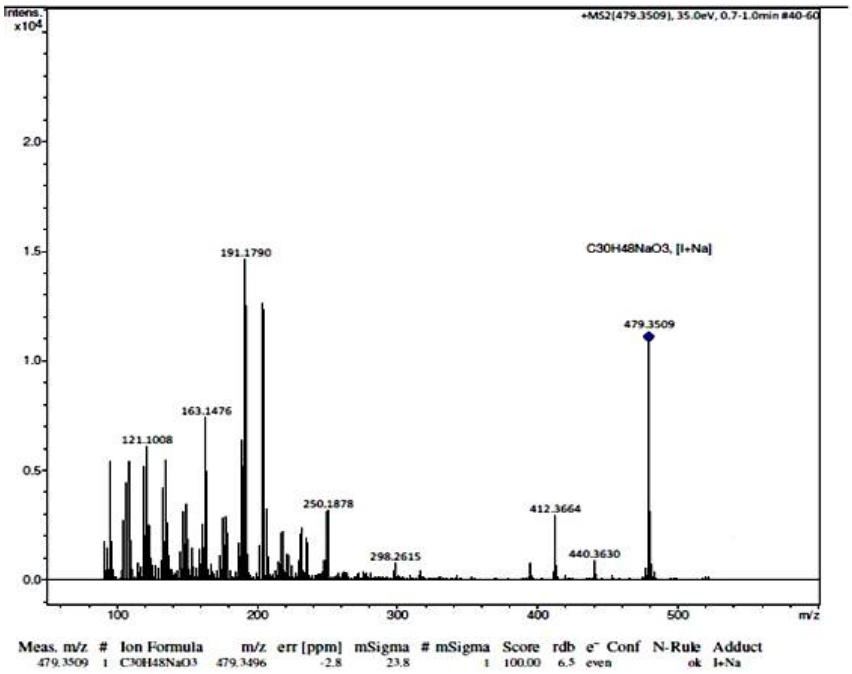


Fig. 2 spectrum: HRMS Spectra of TG-1

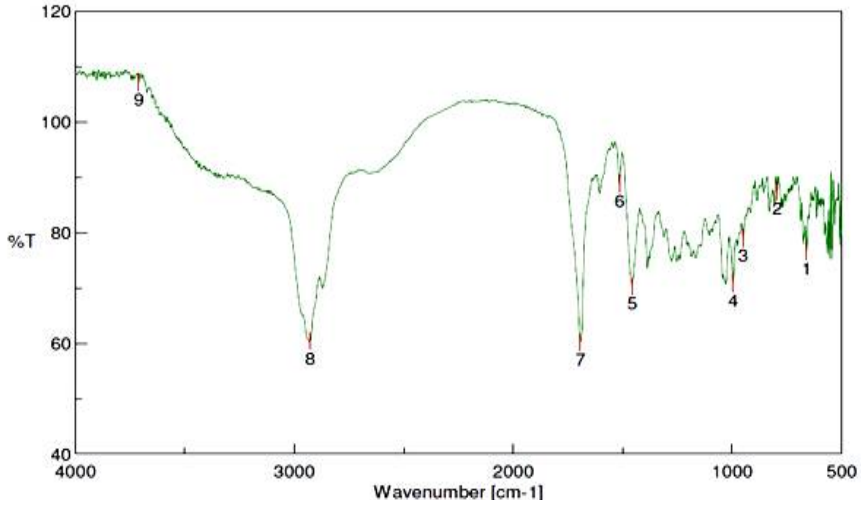


Fig. 3 Spectrum IR Spectra of TG-1

Table no. 1 Results of phytochemical analysis of *Tecoma gaudichaudi* DC leaf powder

Phytoconstituents	*Tecoma gaudichaudi* DC
Carbohydrates	+
Proteins	+
Alkaloids	-
Glycosides	-
Steroids	+
Triterpenoids	++
Flavonoids	++
Tannins	++
Volatile oil	-

"+" show presence of constituents "–" shows absence of constituents.

Table no. 2 DPPH radical scavenging activity of *Tecoma gaudichaudi DC*

Sr. no.	Test sample	Concentration (µg/ml)	Percentage inhibition	IC_{50} (µg/ml)
1	EATG	10 20 40 60 80	37.17 43.2 54.2 67.36 70.32	33.15
2	ETG	10 20 40 60 80	13.6 23.59 57.02 78.23 84.45	40.72
3	Ascorbic acid	10 20 40 60 80	23.61 39.66 74.52 91.42 94.86	28.03

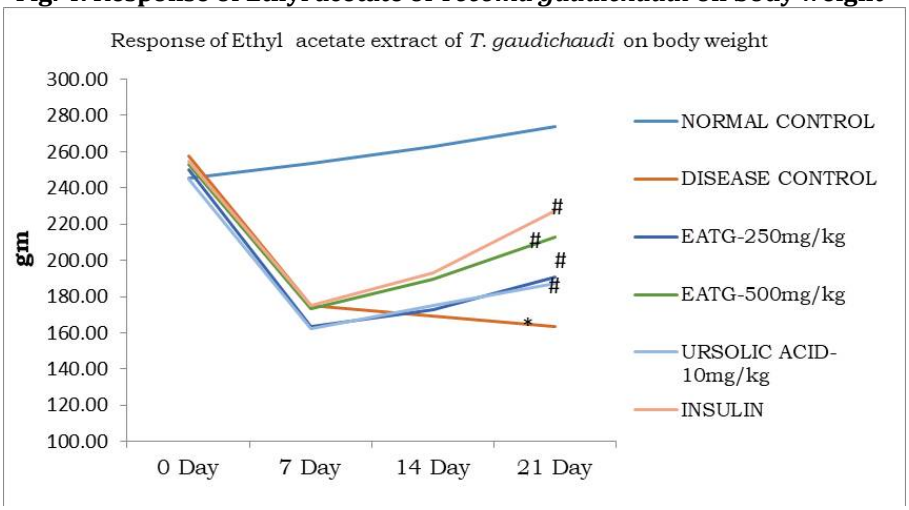
ETG and EATG- ethanolic and ethyl acetate extract of *T. gaudichaudi*, results are considered as ± SEM, n= 5, p value < 0.05.

Table. 3 Response of Ethyl acetate extract of leaf of *Tecoma gaudichaudi* DCon body weight of animals on diabetic rats

Groups	**Body weight (g)**			
	Day 0	**Day 7**	**Day 14**	**Day 21**
Control	245.33±4.75	253.83±4.07	262.67±3.21	273.67±4.72
Diabetic control	257.83±4.96	174.83±4.03*	169.50±4.49*	163.67±5.39*
DC+ EATG (250)	250.33±8.80	163.17±8.01	172.83±9.82**	190.67±12.24**
DC+EATG (500)	253.00±4.89	173.50±5.40	189.67±4.65**	212.83±10.04**
DC+ Ursolic acid	244.83±4.25	162.50±5.37	175.00±6.03	187.17±7.89**
DC+ Insulin(6 IU)	254.67±5.31	174.83±4.44	193.00±3.69	227.33±13.89**

The statistics are indicated as mean *± S.E.M.; each group contain (n = 6)*
Statistics treated by one way ANOVA pursue by Tuckey's test p<0.05,
*indicates symbolic decrease in data as correlated to 0-day Data, **indicates symbolic rise in data when correlate to 0 day Data.

Fig. 4. Response of Ethyl acetate of *Tecoma gaudichaudi* on body weight



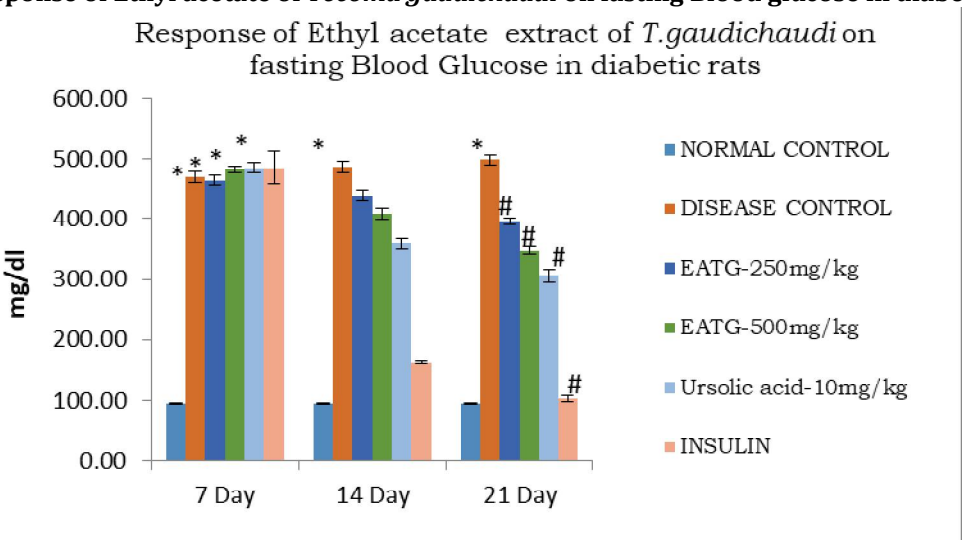
The statistics are indicated as mean ± SEM (n=6). Statistics treated by ANOVA (one way) pursue by Tuckey's test p<0.05, *indicates symbolic decrease in data as compared to 0 day Data, #indicates significant increase in data as compare to 0 day Data

Table no. 4. Effect of ethyl acetate extracts of leaf of *Tecoma gaudichaudi* DCon blood glucose level diseases treated group

Treatment and dose	Blood glucose level (mg/dl)		
	7 day	**14 day**	**21 day**
Control	93.67±1.15	93.83±0.75	95.00±1.24
Diabetic control	470.00±9.31	486.17±9.21*	498.83±8.54*
DC+ EATG (250)	464.00±8.73*	438.50±8.08	395.00±5.57**
DC+EATG (500)	483.17±5.71*	408.17±8.93	347.33±5.99**
DC+Ursolic acid	485.67±9.46*	358.50±9.04	306.17±10.57**
DC+ Insulin (6 IU)	485.67±27.84*	163.00±2.70	102.33±5.36**

The statistics are indicated as mean ± SEM (n=6). Statistics treated by ANOVA (one way) pursue by Tuckey's test p<0.05, * indicates symbolic rise in data as correlated to Normal control Data (p<0.05), ** mark symbolic decrease in data as compare to Disease control data except Normal control data (p<0.05).

Fig. 5. Response of Ethyl acetate of *Tecoma gaudichaudi* on fasting Blood glucose in diabetic rats.



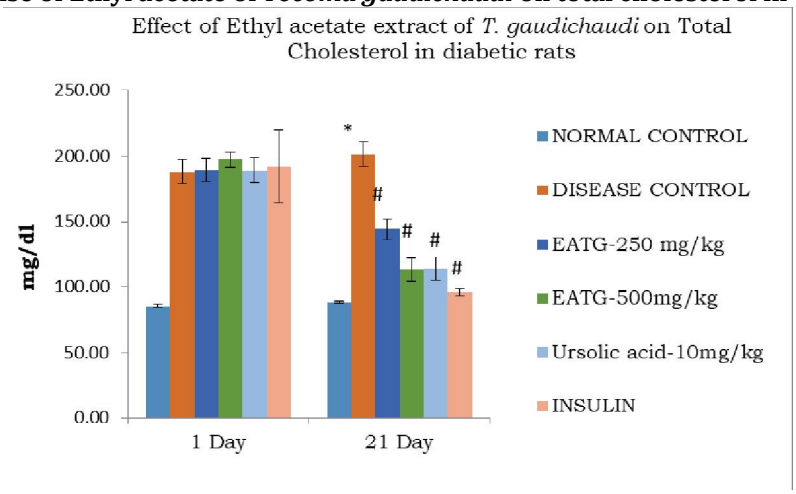
The statistics are indicated as mean ± SEM (n=6). Statistics treated by ANOVA (one way) pursue by Tuckey's test p<0.05, * mark symbolic rise in data as correlated to Normal control Data (p<0.05), # mark symbolic decrease in data as compare to Disease control data except Normal control data (p<0.05)

Table no 5. Response of ethyl acetate extracts of leaf of *Tecoma gaudichaudi* DCon cholesterol level in diabetic rats.

Treatment and dose	Total cholesterol level in mg/dl	
	1 day	**21 day**
Control	85.67±2.01	88.50±2.32
Diabetic control	188.17±2.59	201.17±2.81*
DC+ EATG (250)	189.33±1.56	144.00±5.62**
DC+EATG (500)	197.33±4.37	113.00±4.15**
DC+ Ursolic acid	188.83±2.26	113.83±5.28**
DC+ Insulin (6 IU)	191.83±2.18	95.83±1.38**

The statistics are indicated as mean ± SEM (n=6). Statistics treated by ANOVA (one way) pursue by Tuckey's test p<0.05, *indicates significant increase in data as compared to Normal control Data (p<0.05), ** indicates significant decrease in data as compare to Disease control data except Normal control data (p<0.05)

Fig. 6. Response of Ethyl acetate of *Tecoma gaudichaudi* on total cholesterol in diabetic rats.



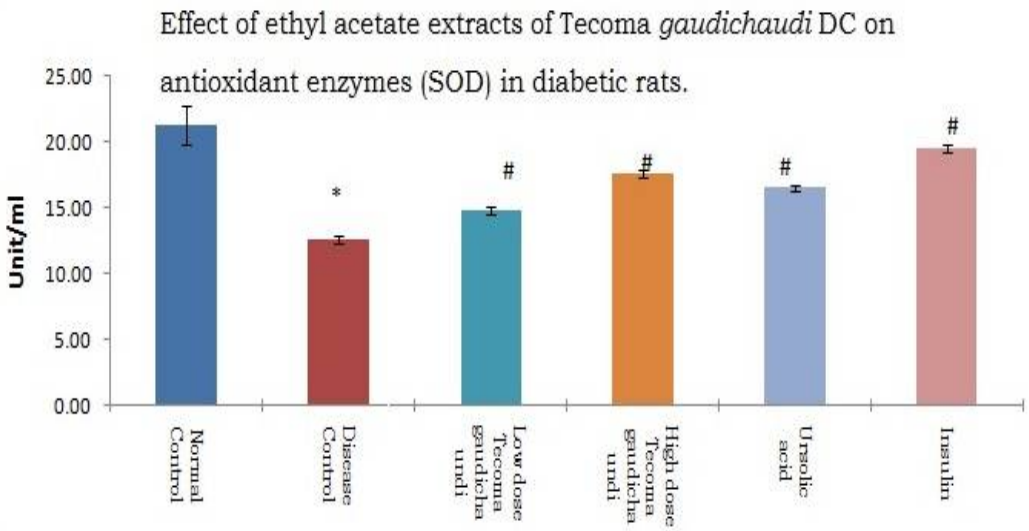
The statistics are indicated as mean ± SEM (n=6). Statistics treated by one way ANOVA followed by Tuckey's test $p<0.05$, * indicates significant increase in data as compared to Normal control Data ($p<0.05$),#indicates significant decrease in data as compare to Disease control data except Normal control data ($p<0.05$)

Table no. 6 Effect of ethyl acetate extracts of *Tecoma gaudichaudi* DCon antioxidant enzymes in diabetic rats.

Treatment and dose	Antioxidant enzyme	
	SOD (Unit/ml)	**CATALASE (Ku/l)**
Control	21.27±1.41	0.34±0.00
Diabetic control	12.66±0.27*	0.20±0.00*
DC+ EATG (250)	14.81±0.26**	0.23±0.00**
DC+EATG (500)	17.57±0.32**	0.27±0.00**
DC+ Ursolic acid	16.53±0.19**	0.25±0.00**
DC+ Insulin (6 IU)	19.51±0.24**	0.32±0.01**

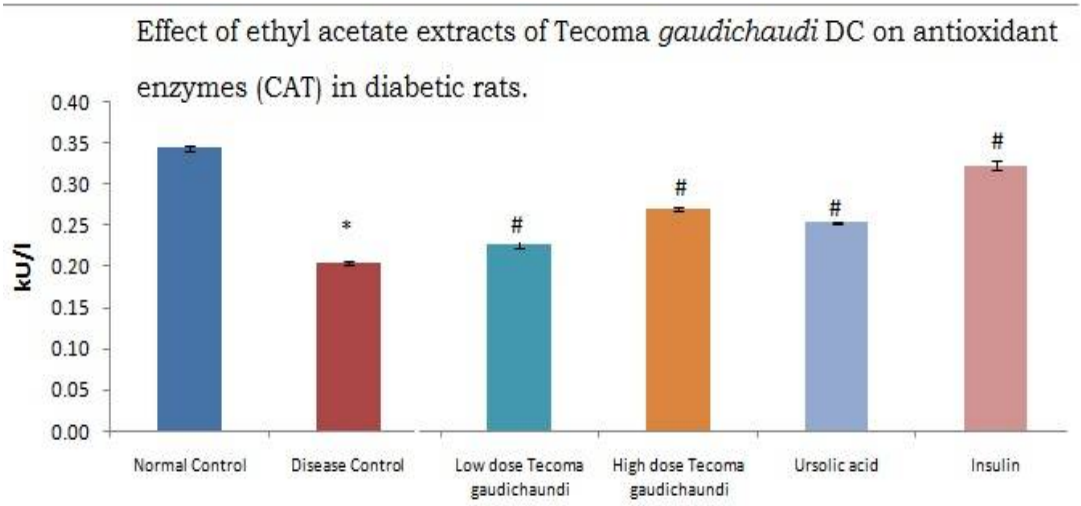
Results are expressed as ± SEM (n=6). Data processed by one way ANOVA followed by Tuckey's test $p<0.05$, * indicates significant decrease in data as compared to Normal control Data ($p<0.05$), **indicates significant increase in data as compare to Disease control data except Normal control data ($p<0.05$)

Fig. 7. Response of Ethyl acetate of *Tecoma gaudichaudi* on antioxidant enzymes (SOD) in diabetic rats.



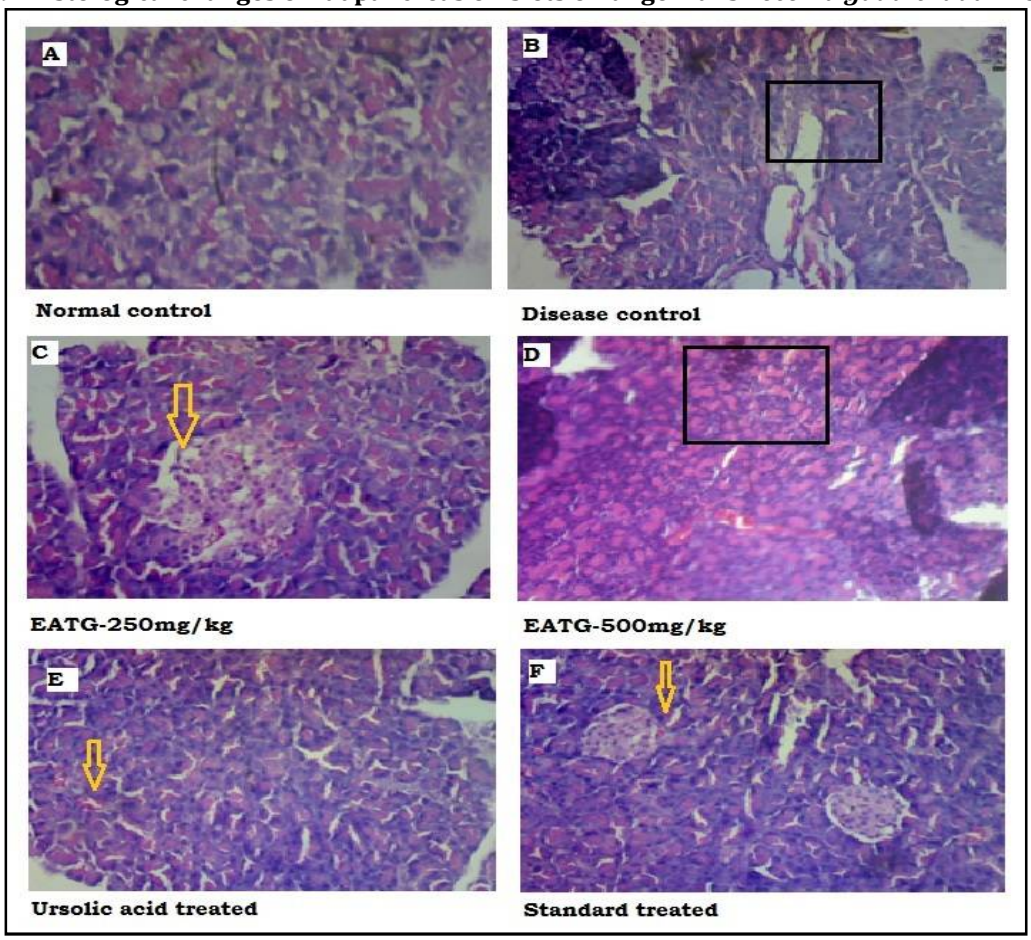
Results are expressed as ± SEM (n=6). Data processed by one way ANOVA followed by Tuckey's test $p<0.05$, *indicates significant decrease in data as compared to Normal control Data($p<0.05$),** indicates significant increase in data as compare to Disease control data except Normal control data ($p<0.05$)

Fig. 8. Response of Ethyl acetate of *Tecoma gaudichaudi* on antioxidant enzymes (CAT) in diabetic rats.



Results are expressed as ± SEM (n=6). Data processed by one way ANOVA followed by Tuckey's test $p<0.05$, *indicates significant decrease in data as compared to Normal control Data($p<0.05$), #indicates significant increase in data as compare to Disease control data except Normal control data ($p<0.05$)

Figure 9. Histological changes of rat pancreas of islets of langerhans *Tecoma gaudichaudi* DC



A) Non diabetic normal histological structure of rat pancreas showing normal pancreatic islet cells. B) Diabetic control rat showing irregular cells and necrosis of cell destruction of ß-cells (indicated by arrow and box) C) EATG (250mg/kg) showed destruction of ß-cells indicated by arrow D) EATG(500mg/kg) showed increased cell size of ß-cells indicated by colour box. E) Ursolic acid treated rats showed increase cell size (indicated by colored box) by slight regeneration of β-cells were seen when compare

with diabetic control. F) Insulin treated rat pancreas showing normal density of islet of β-cells along with few areas showing necropsy indicated by arrow.

DISCUSSION

Phytochemical analysis of *Tecoma gaudichaudi* DC

The present study has been originated to know the *Tecoma gaudichaudi* DC plant as potential antioxidant and antidiabetic agent. The preliminary phytochemical analysis revealed the presence of carbohydrate, proteins, steroids, triterpenoids, flavonoids, tannins in *Tecoma gaudichaudi* DC.As we know the therapeutic properties of medicinal plants are associated with their secondary metabolites. Selection of suitable solvent for extraction of phytoconstituents is vital step it gives higher yield of soluble phytoconstituents. Plants contain compounds and are numerous phytochemical constituents, mainly secondary metabolites which are known to be biologically more active compounds and responsible for exhibiting various pharmacological activities. Based on these results concentrated fractions or extract of leaves of *Tecoma gaudichaudi* DC is a rich source of ursolic acid and may be more useful for biological activity

In vitro antioxidant activity of successive leaf extracts:

There is correlation in between diabetes complications and antioxidants. The effects of antioxidants on oxidative stress are measured through by monitoring antioxidant enzymes such as enzymes superoxide dismutase (SOD), catalase (CAT). Antioxidant assay of successive *Tecoma gaudichaudi* DC leaf extracts showed promising antioxidant effects, comparatively ethyl acetate shows potent antioxidant activity (IC_{50})than that ethanol extract, so ethyl acetate extract further selected for in vivo antidiabetic activity.

In vivo antidiabetic activity determination:

In the present study reductions in body weight in diabetic rats these results agree with previous reports that have also reported loss of body weight. On 21 day of study a significant increase in body weight of diabetic rats was observed which treated with *Tecoma gaudichaudi* DC leaf extract (EATG500mg/kg) and ursolic acid (10mg/kg) as compare to *Tecoma gaudichaudi* DC leaf extract (EATG250mg/kg). In current study, the diabetic rat's shows higher glucose level when compare to normal control rats.The results of the present experimentwere observed that treatment with *Tecoma gaudichaudi* DC leaf extract (EATG500mg/kg) and ursolic acid (10mg/kg) decreased the blood glucose in STZ induced diabetic rats on 21 day as compare to diabetic rats. It is due to stimulation of insulin secretion from pancreatic β-cells, which in turn enhance glucose utilization by peripheral tissue of diabetic rats either by promoting glucose uptake and metabolism. These results agree with the previous observation that has also reported plant extracts and its biomolecules have ability to regeneration of β-cells of pancreas. This is confirmed by histopathological study, *Tecoma gaudichaudi* DC (EATG500mg/kg) and ursolic acid treated histopathological observations shows the structural integrity and improved cell density of β-cells of islets of langerhans in diabetic rats.On 21-day diseases control group indicate significant increase in cholesterol as compare with the normal control group. While extract treated EATG (500mg/kg), ursolic acid (10mg/kg) and standard insulin treated shows a significant decrease in cholesterol as compare with diseases control. Furthermore, in our study the activity of SOD, CAT decreased in the diabetic group, which could be due to the involvement in the elimination of ROS generated by STZ. Treatment diabetes in the *Tecoma gaudichaudi* DC leaf extract (EAPR500mg/kg), ursolic acid (10mg/kg) and standard insulin reverse the activity of this enzymatic antioxidant, which might be due to the involvement in decreased oxidative stress.

CONCLUSION

In conclusion, *Tecoma gaudichaudi* DC leaf ethyl acetate extract at a higher dose (EATG500mg/kg) and Ursolic acid (10mg/kg) shows hypoglycemic action which might mediate through due to stimulation of insulin secretion from pancreatic β-cells. Meanwhile, these extracts shows anticholesterolemic action and the level of endogenous antioxidant enzymes (CAT and SOD) were increased. These results are further supported by the pancreatic histology which showed protection of pancreatic β-cells. Thus, the antidiabetic potential of *Tecoma gaudichaudi* DC leaf ethyl acetate extract at a higher dose might be due to presence of active phytoconstituents.

ABBREVIATIONS

BSI, botanical survey of India; EATG, ethyl acetate extract of *Tecoma gaudichaudi* DC leaf; HRMS High resolution mass spectrometry; IR, Infrared spectroscopy; STZ, streptozotocin; i.p, Intraperitoneal; o.i.d, once in day; SOD, superoxide dismutase; CAT, catalase; IAEC, Institutional Animal Ethical Committee; CPCSEA, Committee for purpose of control and supervision of experimentation on animals; OECD,

Organisation for economic co-operation and development; ANOVA, Analysis of variance; ROS, Reactive oxygen species.

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PLANT AUTHENTICATION
The leaves of species of Bignoniaceae family i.e. *Tecoma gaudichaudi* DC were collected from Pune and authorized from BSI with reference number BSI/WRC/Iden./2015/576 on dated 18-12-2015. The specimen voucher number is KALKTEG1 Specimens of this plant species were deposited at the Botanical Survey of India, Pune

COMPETING INTERESTS
There are no conflicts of interest

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