International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 739-747 www.ijprajournal.com ISSN: 2249-7781

Antidiabetic activity of Syzygium samarangense and Luffa acutangula (leaves) On Streptozotocin Induced Diabetic Rats.

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Submitted: 25-01-2022 Accepted: 05-02-2022

ABSTRACT
The aim of the present study was screening of ethanolic extracts of Syzygium samarangense and Luffa acutangula leaves to determine anti-diabetic activity in Streptozotocin induced diabetic rats.Streptozotocin was used to induce diabetes mellitus. The antidiabetic potential was assessed by determining oral glucose tolerance test, fasting blood glucose levels, changes in body weight, serum triglyceride, total cholesterol and histopathological studies was done for the control and experimental rats.Ethanolic extracts of S. samarangense and L. acutangula was administered to normal and experimental diabetic rats for 21 days. Significant reduction in blood glucose level was observed in ethanolic extracts in treated diabetic animals from day 14 onwards. In Oral Glucose Tolerance Test, reduction in fasting blood glucose was noted at 30 mins of extracts administration. After 14 days of treatment with extracts of S. samarangense and L. acutangula (400 mg/kg & 800 mg/kg body wt.) and the body weight was maintained in treated rats as compared to diabetic rats. Streptozotocin leads to elevated levels of serum triglyceride, and total cholesterol. But, the treatment with ethanolic extract of L. acutangula showed more anti-diabetic effect as compared to S. samarangense. From the above results, screening of ethanolic extracts of L. acutangula and S. samarangense revealed that the extract of L. acutangula showed more Anti-diabetic effect as compared to S. samarangense.

KEYWORDS: S. samarangense, L. acutangula, Streptozotocin, Anti-diabetic activity.

I. INTRODUCTION

Diabetes mellitus is considered the commonest endocrine disorder and it is the sixth leading cause of death globally. Increase in the blood glucose damages many of the body's systems, in particular, the blood vessels and nerves. The hyperglycemia caused due to decreased insulin. It is estimated that diabetes in adults is over 170 million worldwide and its prevalence is likely to increase to over 300 million by the year 2025. Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature mentioned the use of plants in treatment of various human ailments. India has more than 45000 plants species and among them, several thousands have been claimed for medicinal properties. The commonly practiced treatment of diabetes includes oral anti-diabetic drugs, insulin injection and management through diet and physical exercise. Apart from currently available therapeutics for the treatment of diabetes, traditional plant medicines are also used throughout the world for the treatment of diabetes [1].

Luffa acutangula belongs to the family [Cucurbitaceae]. It has large monoeius annual climber. Petiole is brownish yellow coloured 3-8cm in length, somewhat wrinkled and angular while lamina having pale or light green in colour, 6,9cm long crumbles and broad. Different parts of the plant have been reported to have Anti-larvicidal, Anti-inflammatory activities. It has large number of health benefits which currently clinical research is supporting as well, it is used in weight loss, blood purifier, helps in preventing constipation problems, helps in boosting immune system, helps in curing jaundice, stomach worms and asthma[2].

Syzygium samarangense belongs to the family [Myrtaceae]. It is a tropical tree growing 12 meters tall with evergreen leaves 10-25cm long and 5-10cm broad. The leaves are oval but rounded at the base, they smell aromatic when crushed. The plant consists of several phytochemicals such as β-sitosterol, Quercetin, flavanol, steroids, and carotenoids [4]. Various parts of plant have been reported for Anti-microbial, Anti-inflammatory and Anti-bacterial activities. In the present study screening of ethanolic extracts of L. acutangula and S. samarangense leaves were used to evaluate the anti-diabetic activity and to establish its therapeutic

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 739-747 www.ijprajournal.com ISSN: 2249-7781

potential in the treatment of diabetes and its complications.

II. MATERIALS AND METHODS

PLANT MATERIALS

The leaf powder of Luffa acutangula and Syzygium samarangense were procured from local market of Kandivali, Mumbai. Both the plant powders were authenticated by Dr. Harshad Pandit, Department of Botany, Andheri West, and Mumbai with voucher number (Syzygium samarangense- #: ssmp 167191782, Luffa acutangula- #: ssmp 167191750).

PREPARATION OF EXTRACTS

The leaf powders were extracted by using Soxhlet Apparatus with 95% ethanol as the solvent. These extracts were further concentrated by using Rotary Evaporator. Gymnema sylvestre was selected as a standard drug formulation. The tablets consist of stem powder and leaf extract. The human dose was converted to animal dose based on body weight.

PRELIMINARY PHYTOCHEMICAL SCREENING

Extracts obtained from S. samarangense and L. acutangula were subjected to various qualitative tests for the identification of various phytoconstituents present in this species [3].

EXPERIMENTAL ANIMALS

Healthy male albino rats of Wistar strain weighing 150-200g were used for the present study. Animal were procured from Bharat Serum and Vaccine pvt Ltd. Wagle Industrial Estate Road No: 27, Thane 400604. These animals were housed in polypropylene cage and maintained under the standard laboratory condition (12hrs light/12hrs dark cycle; 25 ± 30◦ C; 35–60% humidity) they were fed with the standard diet and water. Permission from the Institutional Animal Ethics Committee (Regd No. 762/PO/Re/S/03/CPCSEA) was obtained prior to commencing the study.

SAMPLE COLLECTION

Blood samples were collected by the retro-orbital plexus puncture method from overnight fasted rats under light ether anesthesia and blood glucose levels were estimated using Glucometer testing kit. For histopathological studies, pancreas and the liver were dissected out immediately and transferred into 10% formalin.

ACUTE ORAL TOXICITY STUDY

The acute oral toxicity was carried out as per OECD guideline 425 (Acute Oral Toxicity: Up and Down Procedure). The study was approved by the Institutional Animal Ethics Committee (IAEC). No mortality and no signs of toxicity were found even after administration of a limit dose of 2000 mg/kg body weight of extract; hence 1/5th of the dose was taken as effective dose. Two doses, 400 and 800mg/kg [7] were selected for the present study to evaluate anti-diabetic activity.

ORAL GLUCOSE TOLERANCE TEST:

The Oral Glucose Tolerance test was performed in overnight fasted normal rats. The rats were divided into 6 groups, each group consisting of 4 rats. Group I served as control and received distilled water. Group II served as a Standard control, received Gymnema sylvestre extract (122 mg/kg body weight) [10, 11]. Group III and IV received 400 and 800 mg/kg of S. samarangense extract orally. Group V and VI received 400 and 800 mg/kg of L. acutangula extract orally. The rats of all groups were given glucose 2 g/kg body weight orally one hour after the administration of the extracts. Blood samples were collected from the retro orbital plexus just prior to glucose administration (i.e., at 0 min) and at 30 min, 60 min, and 120 min after the glucose loading. Rat's serums were separated and the fasting blood glucose levels were measured immediately [22].

EVALUATION OF EXTRACT OFS. samarangense and L. acutangulaON STREPTOZOTOCIN INDUCED DIABETIC RATS

Experimental diabetes was induced in overnight fasted rats by single intraperitoneal injection of Streptozotocin (45 mg/dl body weight) was dissolved in freshly prepared 0.1 M of cold citrate buffer. The rats were provided with 10% glucose solution after 6 hours of STZ administration for the next 24 hours to overcome hypoglycaemia. After a week, the rats marked with hyperglycemia (fasting blood glucose > 250 mg/dl) were selected and used for the study. The rats used for the study were classified into seven groups (n=8). Group I- Normal Control (received 1% CMC solution).Group II- Diabetic control untreated (received 1% CMC solution).Group III- Diabetic control treated with standard drug Gymnema sylvestre (122 mg/kg body weight) orally. Group IV- Diabetic control treated with S. samarangense extract (400 mg/kg) orally. Group V- Diabetic

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Volume 7, Issue 1 Jan-Feb 2022, pp: 739-747 www.ijprajournal.com ISSN: 2249-7781

control treated with S. samarangense extract (800 mg/kg) orally. Group VI- Diabetic control treated with L. acutangula extract (400 mg/kg) orally. Group VII- Diabetic control treated with L. acutangula extract (800 mg/kg) orally. After giving the following treatments, blood glucose levels were checked on 1st, 7th, 14th, 20th day by using glucometer testing kit. On 21st day the rats were sacrificed and blood was collected to estimate various parameters [8].

ESTIMATION OF BIOCHEMICAL PARAMETERS

On day 21blood was collected from retro-orbital plexus of the overnight fasted rats and the blood was kept for clotting. Serum was separated by centrifuging the samples for 20 mins at 6000rpm. The serum was analysed for total cholesterol (CHOD-POD method) and triglycerides (GPO method). And the estimation of biochemical parameters was done by using ERBA diagnostic kit.

HISTOPATHOLOGY

Pancreas and liver were isolated and stored in 10% v/v formaldehyde solution and sent for histopathological evaluation. It was observed under 400 x resolution [23].

STATISTICAL ANALYSIS

The statistical significance between the groups was analysed separately using One–way Analysis Of Variance (ANOVA), followed by Dunnett's multiple comparison tests. The significance was expressed by P values, as mentioned in the tables.P<0.05 was considered as significance.

III. RESULTS

Preliminary Phytochemical Screening

The qualitative phytochemical analysis of ethanolic extract of L. acutangula showed the presence of Saponins, carbohydrates, alkaloids and ethanolic extract of S. samarangense showed the presence of flavanol, tannins and steroids.

ACUTE ORAL TOXICITY STUDY

The result of acute toxicity study revealed that extracts were safe upto the dose of 2000mg/kg, so these two doses were selected (400mg/kg and 800mg/kg) [10].Both the plant extracts showed neither mortality nor toxicity signs like skin rashes, irritation, salivation, diarrhoea. The histopathological observation of six vital organs did not reveal any abnormalities.

ORAL GLUCOSE TOLERANCE TEST:

OGTT test carried out for the In-Vivo study the values are expresses as mean ± SEM (n=6 since the study was carried out with 6 animals in each group). The statistical analyses of the results were carried out with one way ANOVA followed by Dunnett's test. Oral administration of the extract, 30 min prior to glucose load showed improved glucose tolerance in normal rats

EFFECT OF L. acutangula and S. samarangense EXTRACTS ON STREPTOZOTOCIN INDUCED RATS

In Streptozotocin treated rats, the rise in the blood glucose level reached its peak value on 5th day and then remained stable throughout the study period. Treatment with the two doses (400mg/kg & 800 mg/kg) of the two extracts of S. samarangense and L. acutangula produced a significant reduction in the blood glucose level. But the maximum reduction of blood glucose levels was observed in L. acutangula extract at the dose of 800 mg/kg. The peak reduction in the blood glucose level with all the seven groups were observed at the end of 21st day.

BIOCHEMICAL PARAMETERS

Significant difference was observed in serum lipid profile (Total cholesterol and triglyceride) in the ethanolic extracts of L. acutangula and S. samarangense (400 mg/kg & 800 mg/kg). But the maximum reduction was observed in the ethanolic extract of L. acutangula at a dose of 800 mg/kg.

HISTOPATHOLOGY

Figure 1(A-E) depict the islet of pancreas of rats in different groups. Photomicrograph (A) depicts the pancreas of healthy rat which showed the normal islet cells. In the present study, damage of pancreas was observed in Streptozotocin treated diabetic rats (Fig 1B). Gymnema sylvestre treated group showed regeneration of β- cells (Fig C). The moderate regeneration was observed in S. samarangense extract (Fig E). But the maximum regeneration of islet cells was observed by L. acutangula extract at a dose of 800 mg/kg.

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TABLE 1: Effect of ethanolic extracts of L. acutangula and S. samarangense on Oral Glucose Tolerance test in Streptozotocin induced diabetic rats.

GROUP	BLOOD GLUCOSE LEVEL (mg/dl)			
	0 min	**30 min**	**60 min**	**120 min**
1% CMC	85.50 ± 0.97	84.50 ± 4.06	84.33 ± 2.45	83.50 ± 1.91
G. sylvestre (122 mg/kg)	76.3 ± 2.48	160 ±2.60[a]	143.2 ± 2.05[a]	133.1 ± 3.16[a]
S. samarangense (400 mg/kg)	74.31 ± 0.88	168.41 ± 3.82	159.91± 3.15	125.9 ± 2.40
S. samarangense (800 mg/kg)	72.7 ± 0.63	155.51±3.29	142 ± 1.78	115.28 ± 4.12
L. acutangula (400 mg/kg)	64.21 ± 2.64	132 ± 2.84	130.1 ± 4.12	113.20 ± 1.5
L. acutangula (800mg/kg)	62.7 ± 0.94	120 ± 2.69[b]	129.3 ± 1.68[b]	110 ± 3.13[b]

The statistical analysis of the results were carried out with one way ANOVA followed by Dunnett's test when compared with standard [a] p<0.01 and [b] p<0.001

FIGURE 1: Effect of ethanol extracts of S. samarangense and L. acutangula on OGTT. Values are given as mean ± S.E.M, in each group. [a] P < 0.001, [b] P < 0.01, [c] P < 0.05 when compared with corresponding values of the standard group

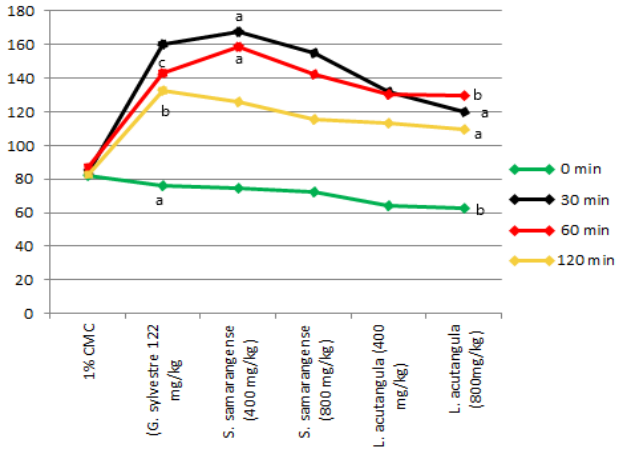

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FIGURE 2: Effect of ethanol extracts of S. samarangense and L. acutangula on OGTT. Values are given as mean ± S.E.M, in each group. **p<0.01 and ***p<0.001 when compared with standard and disease

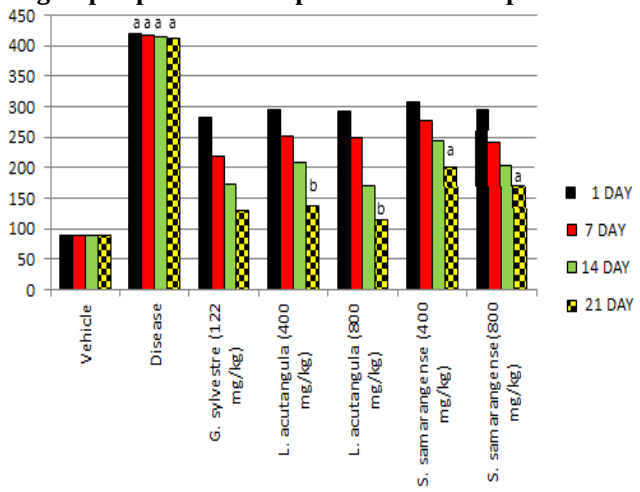

TABLE 2: Effect of ethanolic extracts of L. acutangula and S. samarangense on fasting blood glucose levels in Streptozotocin induced diabetic rats.

GROUP	BLOOD GLUCOSE LEVEL (mg/dl)			
	DAY 1	DAY 7	DAY 14	DAY 21
Vehicle	88.3 ± 3.77	88.8 ± 1.38	89.7 ± 2.31	90.4 ± 1.92
Disease	419.16 ± 2.77**	416.18 ±2.03**	414.5 ± 1.77**	413.6 ± 2.13**
G. sylvestre (122mg/kg)	283 ± 2.99	219 ± 1.48	172 ± 1.85	130 ± 1.69
L. acutangula (400 mg/kg)	296 ±3.88**	252.8 ± 1.9***	208.16 ± 2.37***	172.8 ± 3.5***
L. acutangula (800mg/kg)	293.6 ±3.13***	248.8 ±1.38***	183.16 ± 1.84***	138.8 ±1.38***
S. samarangense (400 mg/kg)	308.6 ±1.68**	276.8 ± 1.60**	245.5 ± 1.69**	202.5 ± 1.58**
S. samarangense (800 mg/kg)	294.6 ± 1.50**	241.8 ± 3.53**	202.5 ± 2.16**	171.8 ± 1.45**

The statistical analysis of the results were carried out with one way ANOVA followed by Dunnett's test when compared with standard and disease *p<0.05 **p<0.01 and ***p<0.001

TABLE 3: Effect of ethanolic extracts of leaves of L. acutangula and S. samarangense on lipid profile in Streptozotocin induced diabetic rats on 21st day of experiment.

GROUPS	Test results (mg/100 ml)	
	Total Cholesterol	Triglyceride
1% CMC	52 ± 0.83	48 ± 0.82
Disease	105 ± 0.70*	108 ± 1.03*

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G. sylvestre 122 mg/kg	75.3 ± 0.74**	72.3 ± 0.99**
S. samarangense (400mg/kg)	83.21 ± 1.39	82.20 ± 1.29
S. samarangense (800 mg/kg)	79.25 ± 0.81	70.25 ± 0.71
L. acutangula (400 mg/kg)	70.05 ± 0.75	64.05 ± 0.85
L. acutangula (800 mg/kg)	62.5 ± 0.53***	56.5 ± 0.53***

The statistical analyses of the results were carried out with one way ANOVA followed by. Dunnett's test when compared with standard and disease *p<0.05 **p<0.01 and ***p<0.001

TABLE 4: Effect of Ethanolic extracts of S. samarangense and L. acutangula leaves on body weight of rats at 2000 mg/kg after 14 days.

Group	Treatment	Body weight (Grams)	
		Before Treatment	After Treatment
Control	1% CMC solution	184 ± 6.90	189 ± 5.95
Treatment 1	2000 mg/kg Ethanolic extract of S. samarangense	185 ± 6.80	190 ± 5.87
Treatment 2	2000 mg/kg Ethanolic extract of L. acutangula	185 ± 6.25	190 ± 5.75

The statistical significance between the groups was analysed separately using One–way Analysis Of Variance (ANOVA), followed by Dunnett's multiple comparison tests. The significance was expressed by P values, as mentioned in the tables.P<0.05 was considered as significance.

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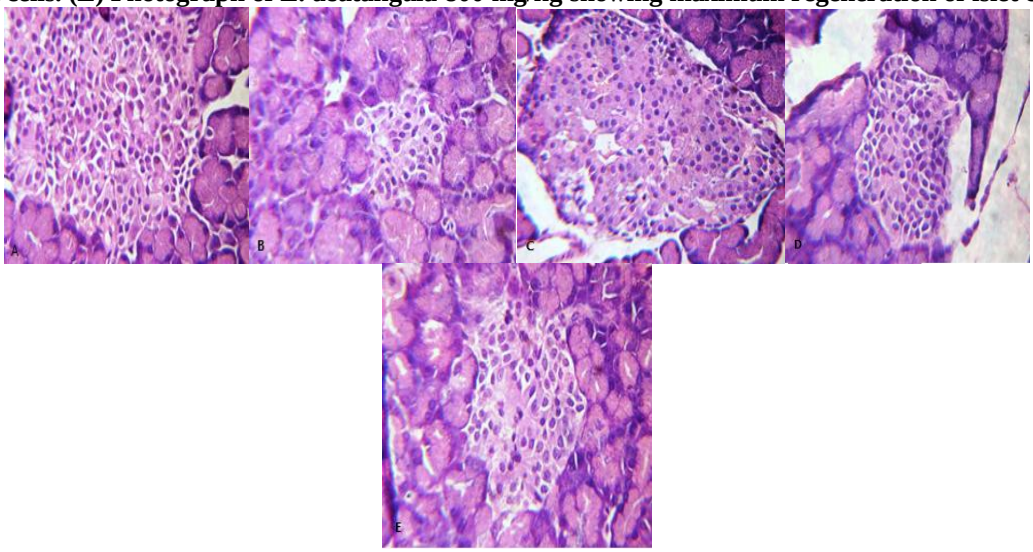
FIGURE 3: A) Photograph of normal control group showing normal islet cells. (B) Photograph of diabetic control showing damaged islet cells. (C) Photograph of standard G. sylvestre 122 mg/kg showing regeneration of β cells. (D) Photograph of S. samarangense 800 mg/kg showing moderate regeneration of islet cells. (E) Photograph of L. acutangula 800 mg/kg showing maximum regeneration of islet cells.



DISCUSSION:

The aim of the present study was screening of the ethanolic extracts of S. samarangense and L. acutangula to determine anti-diabetic activity of Streptozotocin induce diabetes mellitus in rats. The result of the current study showed significant lowering of the blood glucose levels in diabetic rats. The investigation of the study indicates that the ethanolic extracts of S. samarangense and L. acutangula (leaves) have anti-diabetic activity and it can be used for the treatment of diabetes mellitus. Phytochemical analysis of the Ethanolic extracts of S. samarangense showed the presence of tannins, flavonoids and steroids and L. acutangula showed the presence of Alkaloids, Saponins, Carbohydrates, amino acids, flavonoids and steroids. Steroids, glycosides, Saponins, Alkaloids are known to be an active phytochemicals present in both the plant extracts. Flavonoids are known to regenerate the damaged beta cells in the Streptozotocin induced diabetic rats. In the present study, the hypoglycaemic activity of a different dose of the ethanolic extracts was evaluated in Streptozotocin induced diabetic rats. However the diabetic rats treated with the ethanolic extracts of S. samarangense and L. acutangula 400 mg/kg and 800 mg/kg body weight. Animals showed significant lowering of blood glucose level, the maximum anti-diabetic activity was observed in high dose 800 mg/kg body weight in both the plant extracts (Samarangense and L. acutangula). The reduction in the blood glucose levels after the administration of both the extracts is time dependent as wells as dose dependent which is similar to that of Gymnema sylvestre treated rats.

Streptozotocin induced diabetes in the experimental model of rats increased cholesterol and triglyceride levels in the body of rats. The abnormally high concentration of lipids in serum in diabetes is mainly due to mobilization of free fatty acids from the peripheral fat deposits, since insulin inhibits the hormone sensitive lipase. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as consequence of the uninhibited action of lipolytic hormones of the fat deposit. In the present study, serum total cholesterol, triglycerides were significantly decreased in treated diabetic rats as compared to untreated diabetic rats. All lipid parameters tested were improved after the treatment with the extracts and Gymnema sylvestre. Histopathological studies also support our findings. Streptozotocin was suspected to destroy the pancreas partially. Diabetic rats showed reduced islet cells, which were restored to nearly normal upon treatment with the ethanolic extracts of S. samarangense and L. acutangula, two doses were selected 400 mg/kg and 800 mg/kg body weight. High dose of both the plant extracts i.e. 800 mg/kg showed better effect as compared to low dose 400 mg/kg body weight. Later, in the extended study Luffa acutangula leaves extract showed increase in secretory

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Volume 7, Issue 1 Jan-Feb 2022, pp: 739-747 www.ijprajournal.com ISSN: 2249-7781

granules as compared to Syzygium samarangense leaves extract.

CONCLUSION:

Studies have revealed that both the plants contain phytoconstituents having anti-diabetic activity, such as β-sitosterol,Quercetin and Carotenoids in S. samarangense and Anthraquinone glycosides in L. acutangula. Later, 0screening of ethanolic extracts of Luffa acutangula and Syzygium samarangense revealed that the extract of L. acutangula showed more Anti-diabetic effect as compared to S. samarangense at a dose of 800 mg/kg.

In Streptozotocin induced diabetes model in Wistar rats, the treatment with ethanolic extract of Luffa acutangula showed reduction in blood glucose levels at a dose of 800 mg/kg. The histopathological examination also confirmed that the Luffa acutangula leaves extract showed increase in secretory granules as compared to Syzygium samarangense leaves extract. On the basis of the results obtained from the present study, it can be extended further in future as follows: The extracts of L. acutangula can be fractionated to evaluate the anti-diabetic activity and the active phytoconstituents responsible for anti-diabetic activity can be isolated and subjected to complete characterization and structural elucidation

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