Evaluation of *in-vitro* anti-diabetic activity of *Luffa acutangula* (leaves) ethanolic extract and its Ethyl acetate and n-butanol fraction

Dr. Pallavi A. Patil[1]*, Aishwarya A. Kharpe[2], Dr. Heenu Dhar[3], Navya Dutta[4], Siddhesh Keni[5], Abhishek Joshi[6]

[1*]Assistant Professor, Bharati Vidyapeeth's College of Pharmacy, Navi-Mumbai, Maharashtra, India.
Email id. Pallavi.patil@bvcop.in
Contact no. 9766721459

[2]Research Scholar, Bharati Vidyapeeth's College of Pharmacy, Navi-Mumbai, Maharashtra, India.
Email id. kharpeaishwarya83@gmail.com
Contact no. 7028541319

[3]Associate Professor Department of Pharmacology, FMHS Sgt University. Haryana.
Email id. heenu.dhar@gmail.com
Contact no. 7011054541

[4]Research Scholar, Bharati Vidyapeeth's College of Pharmacy, Navi-Mumbai, Maharashtra, India.
Email id. navyadutta1208@gmail.com
Contact no. 9650583769

[5]Research Scholar, Bharati Vidyapeeth's College of Pharmacy, Navi-Mumbai, Maharashtra, India.
Email id. siddheshakeni@gmail.com
Contact no. 9920768382

[6]Research Scholar, Bharati Vidyapeeth's College of Pharmacy, Navi-Mumbai, Maharashtra, India.
Email id. abxisx3k@gmail.com
Contact no. 9324356366
***Corresponding author: Dr. Pallavi A. Patil, Assistant Professor, Bharati Vidyapeeth's College of Pharmacy, Navi-Mumbai, Maharashtra, India.**
Email id. Pallavi.patil@bvcop.in Contact no. 9766721459

Abstract

People of all ages are affected by Diabetes Mellitus (DM), a metabolic illness that is very prevalent. The development of the illness is significantly influenced by environmental variables and changes in lifestyle. The anti-diabetic activity of ethanolic crude extract of L*uffa acutangula* and its active fractions were evaluated by *in-vitro* assays. Extraction of *Luffa acutangula* leaves was carried out using ethanol and further, Ethyl-acetate and n-Butanol fractions were obtained. To evaluate the potential hypoglycemic effects of ethanolic extract of *Luffa acutangula* (EELA), Ethyl acetate fraction of *Luffa acutangula* (EALA), n-butanol fraction of *Luffa acutangula* (NBLA) and Acarbose (standard drug), (100-1000 μg/ml) concentrations were used for the α-amylase and α-glucosidase enzyme inhibitory study. EELA, EALA, NBLA & Metronidazole were also studied for glucose transport across yeast cells at 5mM, 10mM, and 25mM concentrations of glucose. From the above studies carried out, the active fraction was subjected for the MIN-6 cell line study. The insulin secretagogue actions of EELA, EALA & NBLA were investigated utilizing a mouse insulinoma beta cell line (MIN6-β). The study has revealed that the EALA shows the highest anti-diabetic potential in comparison to the EELA & NBLA.

Key Words Antidiabetic, *Luffa acutangula*, α-Amylase, α-Glucosidase, MIN6-β Cell line, Insulin Secretagogue

DOI: 10.53555/ecb/2022.11.12.344

Introduction

Diabetes mellitus is the collective term for a group of metabolic abnormalities caused mostly by a deficiency in insulin hormone secretion by the pancreatic islets. It is most commonly expressed as increased blood glucose levels (hyperglycemia). Insulin resistance causes a variety of problems in the biochemistry and physiology of glucose, lipid, and protein metabolism[1,2]. According to the World Health Organization, diabetes perished over 1.5 million people globally in 2019. There are currently 537 million diabetics patients living in the world, according to estimates. As per predictions, there will be 783 million diabetics worldwide by 2045[3]. Huge sums of money are spent in technologically sophisticated nations on the development of new synthetic or natural medications as well as the prevention and treatment of diabetes. Globally, an estimated 966 billion U.S. dollars was spent on diabetes-related healthcare in 2021, meaning almost 40 percent of the global expenditures for the treatment of diabetes was spent in the United States. Global healthcare spending for the condition is projected to grow to an estimated one trillion U.S. dollars by 2045[4]. In the *Ayurvedic* system of medicine and other Indian writings, plants were used to heal a variety of human illnesses. More than 45000 plant species have been recorded in India, and thousands of these have been deemed to possess therapeutic qualities. Diabetes is often treated with oral anti-diabetic medications, insulin injections, and management through diet and exercise. Traditional plant medicines are also used around the world for the treatment of diabetes in addition to the already accessible treatments[5]. *Luffa acutangula*, often known as ridge gourd, is a medicinal herb, belong to family Cucurbitaceae. In India, Southeast Asia, China, Japan, Egypt, and other countries in Africa, the plant is widely farmed. This plant reproduces through seed, which are seeded in February-March or June-July[6,7]. The leaves of

Luffa acutangula are triangular to widely spherical, 5-7 angled, and simple, alternating, with an orbicular shape and a length of 15-20 cm. They are a light green color. It is common to see veins and vein islets[7]. There have been reports of anti-larvicidal and anti-inflammatory properties in several plant components. It is used for weight loss, blood purification, reducing constipation issues, strengthening immune system, treating asthma, jaundice, stomach worms, and constipation, among many other health advantages that clinical research is currently confirming[8]. In light of this, a study was designed to assess the anti-diabetic activity of active fractions of *Luffa acutangula* leaves for their *in vitro* antidiabetic properties. The literature review indicates that there have been very few systematic attempts to assess the antidiabetic potential of *Luffa acutangula* leaves. Studies have reported that over 50 chemical compounds have been isolated from the plant, mainly comprising flavonoids, anthraquinones, proteins, fatty acids, saponin triterpene, volatile components, and other phytoconstituents[7]. According to investigations, 10 flavonoids in total have been isolated from several Luffa species. Only two of these flavonoids, luteolin-7-glucoside and apigenin-7-glucoside, were found in the leaf and flower of *Luffa acutangula*[9]. Using a bioassay-guided method, an anthraquinone derivative such as 1,8-dihydroxy-4-methylanthracene 9,10-dione was isolated from the ethanolic extract of aerial portions[10]. From the aerial portions of Luffa acutangula, seven saponins of the triterpene oleanane-type were extracted. The triterpene saponins that were discovered were given the names oleanolic, 3-O-β-D-glucopyranosyl-(1→2)- β-D-glucopyranoside (Acutoside-A), 28-O-[O-β-Dxylopyranosyl-(1→4)-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester (Acutoside-B), etc[11]. Thus, the active fraction of *Luffa acutangula* leaves can be outlined to study antidiabetic effect. The Ethanolic extract of *Luffa acutangula* was used in this work to investigate the ethyl acetate and n-butanol fractions for antidiabetic activity using various *in-vitro* techniques.

Materials and Methods

Plant Material Collection

Fresh Luffa acutangula leaves were collected from Kavthemahankal, District Sangli, India from December to February. Alarsin Pharmaceuticals, Mumbai, identified and validated the plant.

Preparation of extracts

The leaves were gathered, dried in the shade, roughly pulverized, and extracted with ethanol using Soxhlet extractor. The extracts were dried under decreased pressure with a rotary evaporator and stored at 0-4°C, away from sunlight. The ethanol (EELA) extract yield was determined to be 9.86% w/w per 50gm of leaves powder[12].

Preliminary Phytochemical Screening

Luffa acutangula extract were subjected to a variety of qualitative assays in order to identify the numerous phytoconstituents found in this species[13].

Fraction Preparation with different solvents

The Slurry was formed by combining the concentrated ethanol extract with water. 50 ml of ethyl acetate was poured in to the slurry in a separating funnel. The funnel was shaken vigorously and permitted to stand for a few minutes. The ethyl acetate (upper layer) was collected and the process was carried out twice more, obtained fraction was concentrated using rotary evaporator. Following the extraction with ethyl acetate, 50ml of butanol was then added to the aqueous solution and forcefully agitated in a separating funnel. After that, the funnel was left for a few minutes in order to separate layers completely. The n-butanol (organic layer) was collected. The process was carried out twice more. A rotatory vacuum evaporator will be used to condense the resultant fraction at 40°C[14].

High Performance Thin Layer Chromatography (HPTLC)

The extract (EELA) and its fractions ethyl acetate (EALA) and n-butanol (NBLA) were concentrated under reduced temperature and pressure before being submitted to HPTLC (CAMAG HPTLC system (Muttenz, Switzerland). At 408 nm, these experiments were carried out on silica gel 60f 254, 50x50 mm HPTLC plates (Merck, Germany) with Toluene: methanol: n-Butanol [9:0.5:0.5 (v/v)] as a mobile phase. The sample solution of 5µl with a concentration of 1000 µg/ml was applied to the plates as 10 mm bands using a CAMAG-Linomat V automated spray on band applicator equipped with a 100 µl syringe and operated with the following settings: band length 5 mm, application rate 10 sec/µl, distance between 6.2 mm, distance from the plate side edge 10 mm, and distance from the plate bottom 9 mm. Using CATS software, the CAMAG TLC Scanner IV was utilised to densitometrically quantify the bands. The scanner's working parameters were as follows: (Mode: absorbance/reflection; Slit dimension: 4x0.45 mm; scanning rate: 20 mm/s; and monochromator band width: 20 nm at an optimized wavelength of 408 nm in the visible range)[15,16].

In-Vitro Antidiabetic activity

Alpha Amylase Inhibition assay

The 3, 5-dinitrosalicylic acid (DNSA) technique was used to perform the α-amylase inhibition assay. The ethanolic crude extract of *Luffa acutangula* and its solvent fractions of ethyl acetate and n-butanol were diluted in buffer (Na2HPO4/NaH2PO4 (0.02 M), pH 6.9) to obtain concentrations ranging from 100 to 1000 µg/ml. A volume of 500 µL of α-amylase solution (0.5 mg/mL) was combined with 500 µL of extract and solvent fractions and incubated at 30°C for 10 minutes. Following that, 500 µL of starch solution [1% in water (w/v)] was then added to each tube and incubated for 3 minutes. The reaction was ceased with the addition of 1mL DNSA reagent and boiling for 10 minutes in an 85°C water bath. The sample solutions were cooled to room temperature and diluted with 10mL of distilled water before measuring absorbance at 540nm with a UV-Visible spectrophotometer. By substituting vehicle for extract, the control indicates 100% enzyme activity[17].

$$Inhibition\ of\ \alpha\text{-}Amylase\ (\%) = \frac{Abs\ (sample) - Abs\ (control)}{Abs\ (sample)} \times 100$$

Alpha Glucosidase Inhibition assay

The inhibitory activity of α-glucosidase enzyme of test substances was evaluated using a conventional technique described by Hanh *et al.* with minimal modifications[18]. The ethanolic crude extract of *Luffa acutangula* and its solvent fractions (ethyl acetate and n-butanol) was prepared in dimethyl sulfoxide (DMSO) to give concentrations ranging from 100-1000 µg/ml and 0.5U/ml α-glucosidase (40 µl) were mixed in 120 µl of 0.1M phosphate buffer (pH 7.0). The resultant solution was incubated for 5 minutes at room temperature. After 5 minutes of pre-incubation, 5mM p-nitrophenyl-a-D-glucopyranoside solution (40 µl) was added. The solution was further incubated at 37°C for 1 hour. After the incubation period, the optical density of test samples was measured at 405 nm by using a microplate reader. The well containing only α-glucosidase was used as negative control. Acarbose was used as standard control[19].

$$Inhibition\ of\ \alpha\text{-Glucosidase}\ (\%) = \frac{Abs\ (sample) - Abs\ (control)}{Abs\ (sample)} \times 100$$

Glucose uptake assay

This assay was carried out in accordance with Cirillo's well-defined technique[20]. To make a 1% suspension, commercial baker's yeast was dissolved in distilled water. The suspension was stored at room temperature (25^0C) overnight. On subsequent day, the yeast cell suspension was centrifuged for 5 minutes at 4200 rpm (Microfuge 16 Centrifuge, FX241.5P Rotor, 50/60 Hz and 220-240 V). By adding distilled water to the pallet, the process was repeated until a clear supernatant was achieved. To obtain a 10% v/v yeast cell suspension, 10 parts clear supernatant fluids were combined with 90 parts distilled water. Various concentrations of extract and its fractions (1–5 mg/ml) were added to 1mL of glucose solution (5, 10 and 25mM) and incubated together for 10 min at 37°C. Reaction was started by adding 100µl of yeast suspension, vortex and further incubated at 37°C for 60 min. After 60 min, the tubes were centrifuged (2500×g; 5 min) and glucose was estimated in the supernatant. Metronidazole was taken as standard drug[21].

The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

$$Increase\ in\ glucose\ uptake\ (\%) = \frac{Abs\ (sample) - Abs\ (control)}{Abs\ (sample)} \times 100$$

Where, Abs (control) is the absorbance of the control reaction (containing all reagents except the test sample), and Abs (sample) is the absorbance of the test sample. All the experiments were carried out in triplicates.

Cell Line study for antidiabetic activity

MIN6- β Cells Culture

Mouse Insulinoma Cells (MIN6-β) cells were grown in Dulbecco's modified Eagle's medium (DMEM), 2 mM glutamine, supplemented with 10% foetal calf serum, 10,000 units/mL penicillin, and 10 mg/mL streptomycin at 37^0C in a 5% CO2 environment. To prepare working solutions, the stock solution was prepared by dissolving ethanolic extract of *Luffa acutangula* (EELA) and ethyl acetate fraction of *Luffa acutangula* (EALA) in Dimethyl sulphoxide (DMSO) and further diluting with Kreb's Ringer buffer (KRB)[22].

Bioassay for Insulin secretion

With a few minor modifications, the aforementioned procedure was used to perform the β-Cell insulin secretion test, which involved planting around 30,000 MIN6-β cells per well into 96-well plates. Cells were grown for 60 minutes using only KRB under hyperglycemic (11.1 mM) conditions. To determine the impact on insulin secretion, an ethanolic extract of *Luffa acutangula* and its active fraction (ethyl acetate fraction) were used at a dose of 1000 μg/mL. The standard in the studies was glibenclamide (10 M). Following 60 minutes of incubation, aliquots from each well were taken, the contents were placed in Eppendorf tubes, and the tubes were centrifuged (4000 g, 5 minutes, 4 °C) for the purpose of analyzing the insulin using the Chemluminence ELISA[22 -24].

Results

Yield of Crude Extract and Fractions

From 50 grams (11.86 % yield) of 95% leaves crude extract (EELA), and a yield of ethyl acetate (EALA) and n-butanol (NBLA) fractions of leaves of *Luffa acutangula* were 9.69 % w/w and 5.88 % w/w, respectively.

Preliminary Phytochemical Screening

The Preliminary Phytochemical Screening was done for the ethanolic extract of *Luffa acutangula* which shows the presence of Carbohydrates, Flavonoids, Steroids, Cardiac and Saponin glycosides, Tannins, Phenolic compounds and Alkaloids (Table 1.)

High Performance Thin Layer Chromatography (HPTLC)

Five different mobile phases previously described for the separation of compounds were tested using silica gel TLC plates, namely chloroform: hexane: methanol: formic acid (6.4:3.9:2.0:0.5 v/v)[25], chloroform: hexane: methanol: formic acid (4:1:1:1 v/v)[25], acetic acid: conc. HCl: H_2O (30:3:10 v/v), n-butanol: acetic acid: H_2O (4:1:5v/v), phenol: H_2O (3:1 v/v)[264]. The only mobile phase that allowed us to visualize difference among extracts studied was the newly developed

mobile phase toluene: methanol: n-butanol (9:0.5:0.5 v/v) at 408 nm, which showed R_f value as shown in table 2. The HPTLC densitogram of EELA, EALA and NBLA recorded at 408 nm after scanning at UV 408 nm are given in figure 1,2,3.

Alpha Amylase Inhibition assay

One crude extract and two solvent fractions were tested for potential α-amylase inhibitory effects in this investigation, along with acarbose as a positive control. Table 03 summarizes the α-amylase inhibitory activity of acarbose, crude extract, and fractions. The results demonstrated a dose-dependent rise in % inhibitory activity for various tested concentrations of extracts and standard against the α-amylase enzyme. The ethyl acetate fraction of *Luffa acutangula* extract has the highest α-amylase enzyme inhibitory activity (79.43 ± 0.456 at 1000 μg/ml) (Figure 4). Similarly, at 1000 μg/mL, the inhibitory activity of ethanolic extract and n-butanol fraction was 77.35 ± 0.236 and 75.09 ± 0.479, respectively. At 1000 μg/ml, the standard positive control Acarbose showed a percentage inhibition of 80.75 ± 0.199 as shown in table 3.

Alpha Glucosidase Inhibition assay

Alpha glucosidase is the dominant enzyme which involved in the digestion of the sucrose and starch. Inhibition of this enzyme will slow the process of carbohydrate digestion. Table 04 shows the glucosidase inhibitory activity of EELA, EALA, and NBLA. The ratio of inhibition of α-glucosidase enzyme increases with the increasing concentration of the extract and its fraction (Figure 5). Ethyl acetate fraction showed the highest percentage inhibition of α-glucosidase enzyme (84.70 ± 0.159) whereas the % inhibition of EELA and NBLA was found to be 79.18 ± 0.596 & 71.09 ± 0.145 respectively. The standard acarbose inhibits the enzyme at 1000 μg/ml about 92.62 ± 0.239 % as shown in table 4.

Glucose Uptake Assay

The rate of glucose transport through cell membrane in yeast cells system at 5 mM, 10 mM and 25 mM concentrations of glucose is presented in Figure 6, 7 & 8 respectively. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. The rate of uptake of glucose into yeast cells was linear in all 3 concentrations (5 mM, 10 mM, 25 mM). The ethyl acetate fraction (EALA) exhibited significantly higher activity than control at all concentrations (78.67 at 5 mM, 79.43 at 10 mM, 75.54 at 25 mM), when compared to standard (79.81, 80.75, 77.16 at 5 mM, 10 mM, 25 mM, respectively). However, the highest glucose uptake was seen in 10 mM glucose concentration.

Cell Line

Bioassay for Insulin secretion

A stimulation in insulin secretion was exhibited by Ethyl acetate fraction of *Luffa acutangula* (EALA) leaves at the dose 1000 μg/mL, 11 mM glucose, compared with respective glucose controls. The insulin release was about two folds at hyperglycemic conditions (11 mM).The maximum insulin secretion was observed at 1000 μg/mL of extract and ethyl acetate fraction as shown in figure 9. The glucose dependent

insulin release was significantly potentiated in the presence of ethyl acetate fraction of *Luffa acutangula* (EALA) at 1000 µg/mL, compared with ethanol extract of *Luffa acutangula* leaves extract(EELA).

Discussion

In this current study, antidiabetic activity of ethanol extract of *Luffa acutangula* leaves and its active fractions (ethyl acetate and n-butanol) were explored using in-vitro assays. It was observed that ethyl acetate fraction of *Luffa acutangula* exhibits more efficient antidiabetic activity than the leaves ethanolic extract and n-butanol fraction. Diabetes is one of the most emerging disorders with its mortality rate increasing gradually. The projected number of diabetics is expected to reach 783 million by 2045. Uncontrolled DM also causes severe consequences like blindness, renal failure, and heart failure. In traditional medical systems like *Ayurveda* and Traditional Chinese Medicine, numerous plants have been reported to treat DM. Despite the availability of synthetic oral antidiabetic medications and insulin preparations, there is a critical need to discover and create novel antidiabetic medications because of the rise of drug resistance and the side effects associated with their prolonged usage. Over the past few years, the field of herbal medicine has experienced exponential growth in popularity in both developing and developed countries due to their natural origin and fewer side effects[27,28]. The studies conducted in research were based on extensive literature review and data. Since the plant exhibits potential blood glucose lowering effect, the study was planned to evaluate ethyl acetate and n-butanol fractions. The extract was prepared using Soxhlet extraction method. Phytochemical analysis of the ethanolic extract of *Luffa acutangula* leaves showed the likely presence of phytoconstituents like carbohydrates, steroids, cardiac and saponin glycosides, flavonoids, tannins, phenolic compounds and alkaloids, that are essential for antidiabetic activity[29]. The extraction was followed by fractionation using ethyl acetate and n-butanol. HPTLC analysis of *Luffa acutangula* ethanolic extract along with the solvent fractions showed that the samples contain different phytoconstituents. Ethyl acetate fraction had the greatest number of phytoconstituents with R_f values of 0.287, 0.393, 0.440, 0.703 and 0.890. n-Butanol fraction shows the least number of phytoconstituents with their R_f values being 0.170 and 0.690. These results can be further studied for structural elucidation and in-depth identification of the compounds. In our study, ethyl acetate fraction showed robust inhibition of the digestive enzymes alpha amylase and alpha glucosidase. The highest % inhibition activity (79.43%) was observed in the ethyl acetate fraction while the lowest was observed in n-butanol fraction (75.09%). Acarbose drug was used as standard for comparative analysis which showed % inhibition of 80.75. The result of the α-amylase enzyme inhibition activity of *Luffa acutangula* extract showed a dose-dependent inhibition activity. The α-glucosidase inhibitory activity a potent concentration-dependent effect was demonstrated for the ethyl acetate extract. α-glucosidase enzyme is located in the intestinal brush-borders membranes. It is an important enzyme in catalyzing the ultimate step in digestion of carbohydrates. The *Luffa acutangula* extracts tested in this study have significantly inhibited the enzyme. The highest % inhibition activity (84.7%) was observed in the ethyl acetate fraction while the lowest was observed in n-butanol fraction (71.09%). Acarbose drug was used as standard for comparative analysis which

showed % inhibition of 92.62%. Therefore, we can assume from this study that one or more phytoconstituents present in the extracts can play an important role in the inhibition of α-glucosidase enzyme[30]. The process of glucose transport through the yeast cell membrane has received interest as an in vitro testing approach for the antidiabetic effect of different compounds/medicinal plants. Current research suggests that some metabolizable glycosides and non-metabolizable sugars are transported across the yeast cell membrane by stereospecific membrane transporters. Glucose transport in yeast cells occurs through a facilitated diffusion mechanism. Certain facilitated carriers are used to move solutes along concentration gradients. This indicates that the elimination of intracellular glucose is necessary for efficient transport to occur[28].The ethyl acetate extract exhibited significantly higher activity than ethanolic extract and n-butanol fraction at all concentrations. However, the highest uptake of glucose was seen in 10mM Glucose concentration. The use of cell lines in anti-diabetic investigations has proven to be quite advantageous. Animal studies can be effectively replaced by cell lines for evaluating the anti-diabetic potential of various synthetic and natural substances. Since the tissue may be directly accessed and evaluated, cell culture is very reliable. Based on in vitro assays carried out on ethanolic extract of *Luffa acutangula* and its ethyl acetate and n-butanol fractions, ethyl acetate fraction exhibited highest activity when compared to n-butanol fraction, thus ethyl acetate fraction was selected for the cell line study. A MIN6 cells retains glucose-stimulated insulin secretion (GSIS) as isolated islets. In the cell line studies performed, the glucose dependent insulin release was significantly potentiated in the presence of ethyl acetate fraction of *Luffa acutangula* (06.70 µg/mL), compared with ethanol extract of *Luffa acutangula* leaves extract (02.10 µg/mL), demonstrating the significant role of cell glucose metabolism in the ethyl acetate fractions ability to secrete insulin[31].

Conclusion

This study has revealed that the ethyl acetate fraction of *Luffa acutangula* is effective as α-amylase and α-glucosidase inhibitors, which may help to reduce the postprandial glucose level, in comparison to the ethanolic extract and its n-butanol fraction. The results also revealed the % glucose uptake by yeast cells and also insulin secretion by MIN-6 β cells was exhibited by ethyl acetate fraction of *Luffa acutangula*. In addition, research should concentrate on the bioactive components of *Luffa acutangula*, which are the sources of the plant's health advantages, in order to identify potential novel medication targets for a range of chronic conditions. The findings provide valuable insights for the plant's usage in traditional medicine for the treatment of diabetes and its complications.

Future perspective

Additional preclinical and clinical research is needed to fully comprehend the mechanism of action and establish the safety and efficacy of the ethyl acetate fraction of the ethanolic extract of *Luffa acutangula in vivo*. This study represents an important step in the identification and

characterization of bioactive fractions from *Luffa acutangula* and demonstrates the promise of herbal remedies as a source of novel and effective treatments for chronic illnesses like diabetes.

References

1. Craig, M. E., Hattersley, A., and Donaghue, K. C., "Defnition, epidemiology and classifcation of diabetes in children and adolescents," *Pediatric Diabetes*., **2009**, *10(12)*, 3–12.
2. Craig, M. E., Jeferies, C., Dabelea, D., Balde, N., Seth, A., and Donaghue, K. C., "Defnition, epidemiology, and classifcation of diabetes in children and adolescents," *Pediatric Diabetes*., **2014**, *15(20)*, 4–17.
3. *Statista*. (**2021**, December 15). Diabetes worldwide 2021, 2030 and 2045. https://www.statista.com/topics/1723/diabetes/#topicOverview
4. https://www.statista.com/statistics/495352/top-10-countries-diabetes-health-expenditure/
5. International Diabetes Federation, Media Information IDF's Diabetes Atlas 5th edition **2011**.
6. Munshi, A., Behera, T., Sureja, A., and Kumar, R., Occurrence and preliminary characterization of gynoecious ridge gourd (*Luffa acutangula (L.) Roxb*) in a natural population. *Cucurbit Genet. Coop. Rep*. **2010**, *33-34*, 57–59.
7. Shendge, P. N., Belemkar, S. Therapeutic Potential of *Luffa acutangula*: A Review on Its Traditional Uses, Phytochemistry, Pharmacology and Toxicological Aspects. *Front Pharmacol*. **2018**, *9*, 1177. http://doi:10.3389/fphar.2018.01177
8. Manikandaselvi, S., Vadivel, V., Brindha, P. Review on *Luffa acutangula* L: Ethnobotany, Phytochemistry, Nutritional Value and Pharmacological Properties, *Int. J. Curr. Pharm.*, **2016**, *7(3)*, 151-154.
9. Schilling, E., and Heiser, C. Flavonoids & the systematics of *Luffa*. Biochem. *Syst. Ecol*., **1981**, **9**, 263–265. http://doi:10.1016/0305-1978(81)90006-5
10. Vanajothi, R., and Srinivasan, P. Bioassay-guided isolation and identification of bioactive compound from aerial parts of *Luffa acutangula* against lung cancer cell line NCI-H460. *J. Recept. Signal Transduct. Res.* **2015**, *35*, 295–302. http://doi:10.3109/10799893.2014.977451
11. Nagao, T., Tanaka, R., Iwase, Y., Hanazono, H., and Okabe, H. Studies on the constituents of *Luffa acutangula Roxb*. I. Structures of acutosides A–G, oleanane-type triterpene saponins isolated from the herb. *Chem. Pharm. Bull.*, **1991**, *39*, 599–606.
12. Ulaganathan, I., Divya, D., Radha, K., Vijayakumar, T. M., & Dhanaraju, M. D. Protective Effect of *Luffa acutangula (var) amara* against Carbon Tetrachloride-Induced Hepatotoxicity in Experimental Rats. *Research Journal of Biological Sciences*, **2010**, *5(9)*, 615–624.

13. Khandelwal, K. R., Practical pharmacognosy. *Tenth Edition Nirali Publication*, Pune **2003**, 127-55

14. Bulbul,I. J., Zulfiker, A. H. M., Hamid, K., Khatun, M. M., & Begum, Y. Comparative Study of *In vitro* Antioxidant, Antibacterial and Cytotoxic Activity of Two Bangladeshi Medicinal Plants- *Luffa cylindrica L.* and *Luffa acutangula*. *Pharmacognosy Journal*, **2011**, *3(23)*, 59–66.
15. Nile, S. H., Park, S. W., HPTLC Analysis, Antioxidant and Antigout Activity of Indian Plants. *Iran J Pharm Res*., **2014**, *13(2)*, 531-539.
16. Yadav, B. S., Yadav, R., Yadav, R. B., Garg, M., Antioxidant activity of various extracts of selected gourd vegetables. *J Food Sci Technol*., **2016**, *53(4)*, 1823-33
17. Kifle, Z. D., Yesuf, J. S., & Atnafie, S. A., Evaluation of *in vitro* and *in vivo* anti-diabetic, anti-hyperlipidemic and anti-oxidant activity of flower crude extract and solvent fractions of *hagenia abyssinica (Rosaceae)*. *Journal of Experimental Pharmacology*, **2020**, *12*, 151–167.
18. Hanh, T. T. H., Chau, N. M., Tram, L. H., Luyen, N. T., Binh, P. T., Minh, C. V., Nam, N. H., Dat, N. T., Inhibitors of alpha-glucosidase and alpha-amylase from *Cyperus rotundus*. *Pharm Biol*., **2014**, *52(1)*, 74–77.
19. Telagari, M., & Hullatti, K. K. *In-vitro* α-amylase and α-glucosidase inhibitory activity of *Adiantum caudatum Linn*. and *Celosia argentea Linn*. extracts and fractions. *Indian Journal of Pharmacology*, **2015**, *47*(4), 425. https://doi.org/10.4103/0253-7613.161270
20. Cirillo, V. P., Mechanism of glucose transport across the yeast cell membrane, *Journal of Bacteriology*, **1962**, *84(3)*, 485– 491.
21. Rehman, G., Hamayun, M., Iqbal, A., Islam, S., Arshad, S., Zaman, K., Ahmad, A., Shehzad, A., & Lee, I. *In Vitro* Antidiabetic Effects and Antioxidant Potential of Cassia nemophila Pods. *BioMed Research International*, **2018**, 1–6. https://doi.org/10.1155/2018/1824790
22. Umamahesh Balekari, Ciddi Veeresham, *In Vivo* and *In Vitro* Evaluation of Anti Diabetic and Insulin Secretagogue Activities of *Capparis zeylanica*, *Pharmacology & Pharmacy*, **2015**, *6*, 311-320.
23. Keller, A. C., Ma, J., Kavalier, A., He, K., Brillantes, A.M. and Kennelly, E.J. Saponins from the Traditional Medicinal Plant *Momordica charantia* Stimulate Insulin Secretion in Vitro. *Phytomedicine*, **2011**, *19*, 32-37. http://dx.doi.org/10.1016/j.phymed.2011.06.01

24. Menichini, F., Tundis, R., Loizzo, M. R., Bonesi, M., Liu, B., Jones, P. B., Persaud, S. J., Mastellone, V., Lombardi, P., Houghton, P. J., Avallone, L., & Menichini, F. C. medica cv Diamante peel chemical composition and influence on glucose homeostasis and metabolic parameters. *Food Chemistry*, **2011**, *124*(3), 1083–1089. https://doi.org/10.1016/j.foodchem.2010.07.083

25. Yadav, B. S., Yadav, R., Yadav, R. B., & Garg, M. Antioxidant activity of various extracts of selected gourd vegetables. *Journal of Food Science and Technology*, **2016**, *53(4)*, 1823–1833. https://doi:10.1007/s13197-015-1886-0
26. Harborne, J. B., Textbook of Phytochemical Methods. *A Guide to Modern Techniques of Plant Analysis*. 5th Edition, Chapman and Hall Ltd, London, **1998**, 21-72.
27. Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S., & Devasagayam, T. P. Indian herbs and herbal drugs used for the treatment of diabetes. J*ournal of clinical biochemistry and nutrition*, **2007**, *40(3)*, 163–173.
28. Alam, S., Sarker, M. M. R., Sultana, T. N., Chowdhury, M. N. R., Rashid, M. A., Chaity, N. I., Zhao, C., Xiao, J., Hafez, E. E., Khan, S. A., & Mohamed, I. N. Antidiabetic Phytochemicals From Medicinal Plants: Prospective Candidates for New Drug Discovery and Development. *Frontiers in endocrinology*, **2022**, *13*, 800714.
29. Das, S. U. S. M. I. T. A., & Basu, P. S. Effect of cephalexin on flowering, fruit setting and development of *Luffa acutangula* Roxb. *Indian journal of Plant Physiology*, **1997**, *2*, 18-20.
30. Daksha, G., Lobo, R., & Nilesh, G. (n.d.). *In-vitro Antidiabetic activity of stem bark of Bauhinia purpurea Linn*. www.scholarsresearchlibrary.com
31. Latha, M., Pari, L., Sitasawad, S. and Bhonde, R. Insulin-Secretagogue Activity and Cytoprotective Role of the Traditional Antidiabetic Plant Scoparia dulcis (Sweet Broomweed). *Life Sciences*, **2004**, *75*, 2003-2014. http://dx.doi.org/10.1016/j.lfs.2004.05.012

Preliminary Phytochemical Screening

Sr. No	Chemical Test	Test	Ethanolic extract of *Luffa acutangula*
1.	Test for Carbohydrate	Fehling's Test	+
		Benedict's Test	+
2.	Test for Protein	Biuret test	-
		Million's Test	-
3.	Test for Amino acid	Ninhydrin Test	-
4.	Test for Steroids	Salkowski Test	+
		Liebermann-Burchard Test	+
5.	Test for Cardiac Glycosides	Baljet Test	+
6.	Test for Saponin Glycosides	Foam Test	+
7.	Test for Flavonoid	Sulphuric acid Test	+
		Lead acetate	+
		Alkaline reagent	+
		Zn + HCl	+
8.	Test for tannins and phenolic compound	5% $FeCl_3$	+
		Lead acetate	+
9.	Test for alkaloid	Dragendroff Test	+
		Mayers Test	+

Table 1. Phytochemical Screening of ethanolic extract of *Luffa acutangula*

High Performance Thin Layer Chromatography (HPTLC)

Sr. No.	EELA		EALA		NBLA	
	R_F	Area	R_F	Area	R_F	Area
1	0.393	66.46	0.190	4.16	0.433	55.53
2	0.583	29.88	0.397	57.99	0.647	44.47
3	0.943	3.66	0.443	19.17	-	-
4	-	-	0.620	18.69	-	-

Table 02.HPTLC data of Ethanolic extract and its ethyl acetate and n-butanol fractions of *Luffa acutangula*

Alpha Amylase Inhibition assay

Concentration (µg/ml)	% Inhibition of α-amylase enzyme			
	STD (Acarbose)	EELA	EALA	NBLA
100	64.52 ± 0.812	61.88 ± 0.711	63.25± 0.489	60.37 ± 0.125
250	67.16 ± 0.745	66.60 ± 0.569	68.03 ± 0.854	63.77 ± 0.254
500	73.01 ± 0.569	70.56 ± 0.265	70.56 ± 0.763	68.67 ± 0.488
750	77.73 ± 0.687	74.15 ± 0.148	76.03 ± 0.296	71.88 ± 0.259
1000	80.75 ± 0.199	77.35 ± 0.236	79.43 ± 0.456	75.09 ± 0.479

Table 03. Percentage inhibition activity of STD, EELA, EALA and NBLA against α-amylase enzyme

Alpha Glucosidase inhibition assay

Concentration (µg/ml)	% Inhibition of α-Glucosidase enzyme			
	STD (Acarbose)	EELA	EALA	NBLA
100	60.98± 0.752	54.03 ± 0. 549	60.98 ± 0.485	55.68 ± 0.153
250	75.99 ± 0.845	60.99 ± 0.259	66.85 ± 0.894	58.89 ± 0.589
500	83.83± 0.599	65.99 ± 0.653	70.95 ± 0.146	62.96 ± 0.745
750	89.85 ± 0.874	72.89 ± 0.841	75.86 ± 0.258	67.05 ± 0.985
1000	92.62 ± 0.239	79.18 ± 0.569	84.70 ± 0.159	71.09 ± 0.145

Table 04. Percentage inhibition activity of STD, EELA, EALA and NBLA against α-Glucosidase enzyme

.

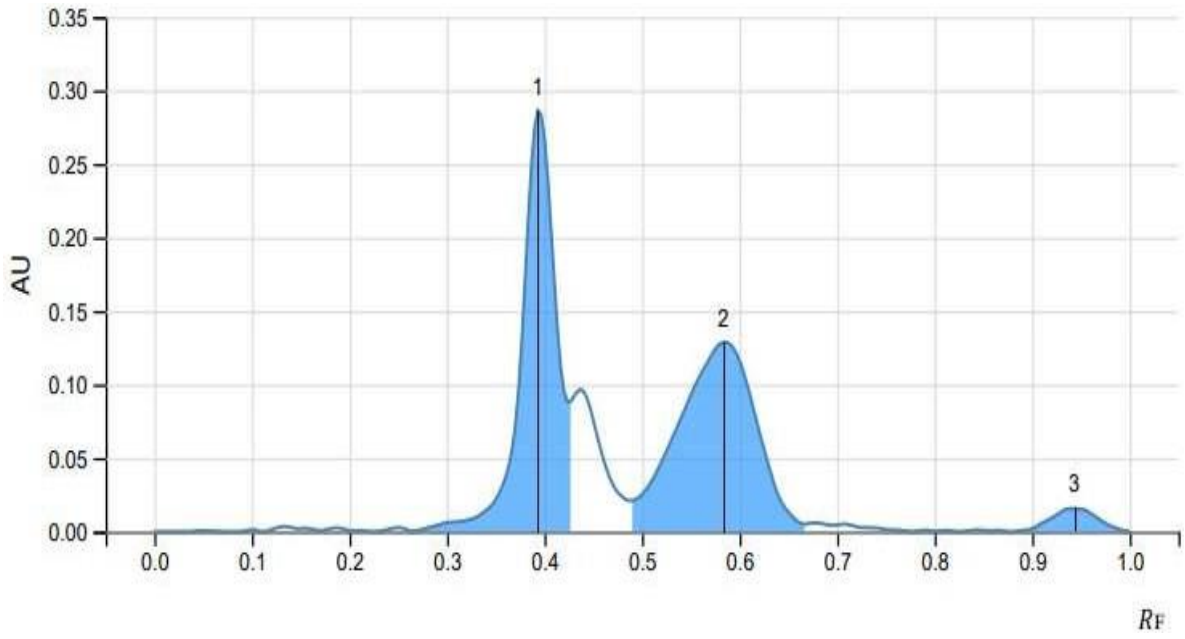

Figure 1. High Pressure Thin Layer Chromatography (HPTLC) densitogram of Ethanolic Extract of *Luffa acutangula (*EELA) at 408 nm

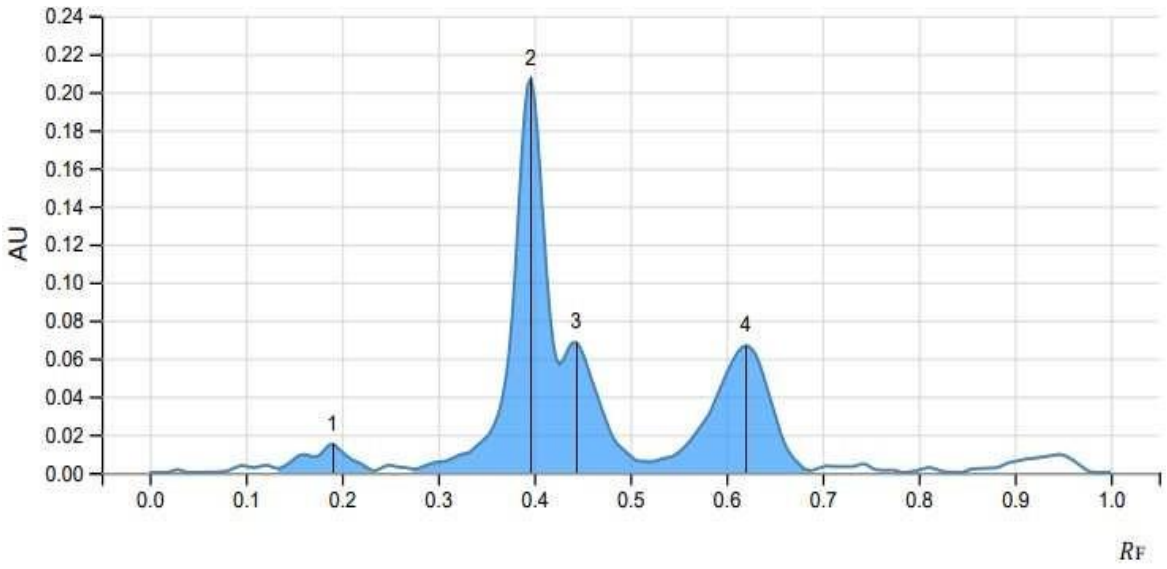

Figure 2. High Pressure Thin Layer Chromatography (HPTLC) densitogram of Ethyl acetate Fraction of *Luffa acutangula* (EALA) at 408 nm

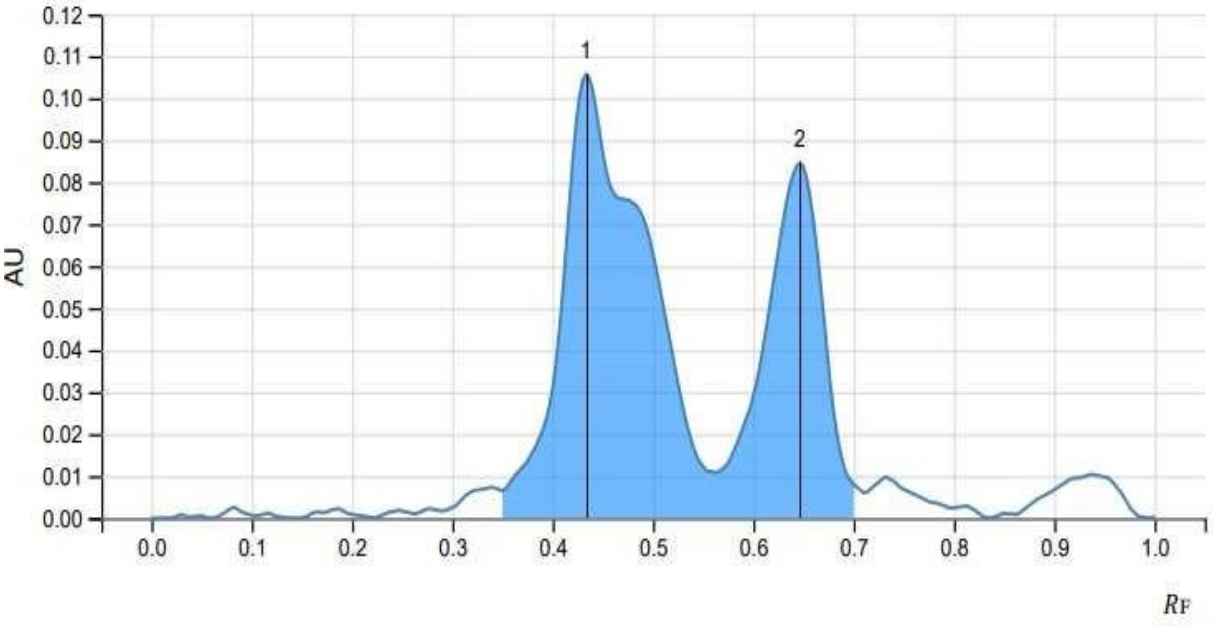

Figure 3. High Pressure Thin Layer Chromatography (HPTLC) densitogram of n-Butanol Fraction of *Luffa acutangula* (NBLA) at 408 nm

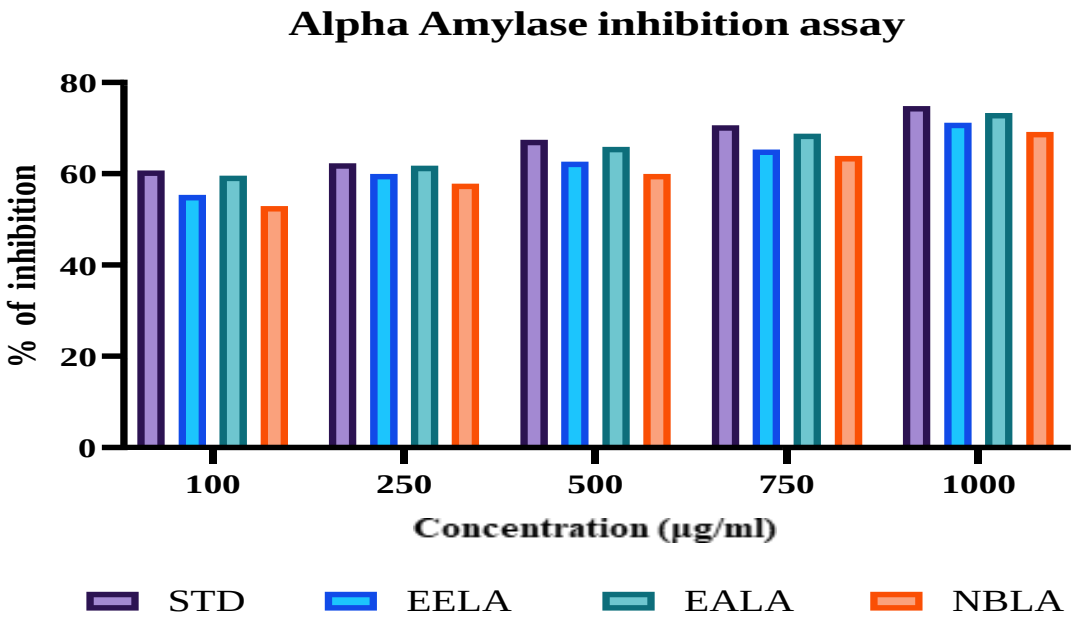

Figure 4. α-Amylase inhibitory activity of Ethanolic extract and fractions of *Luffa acutangula* and its comparative analysis

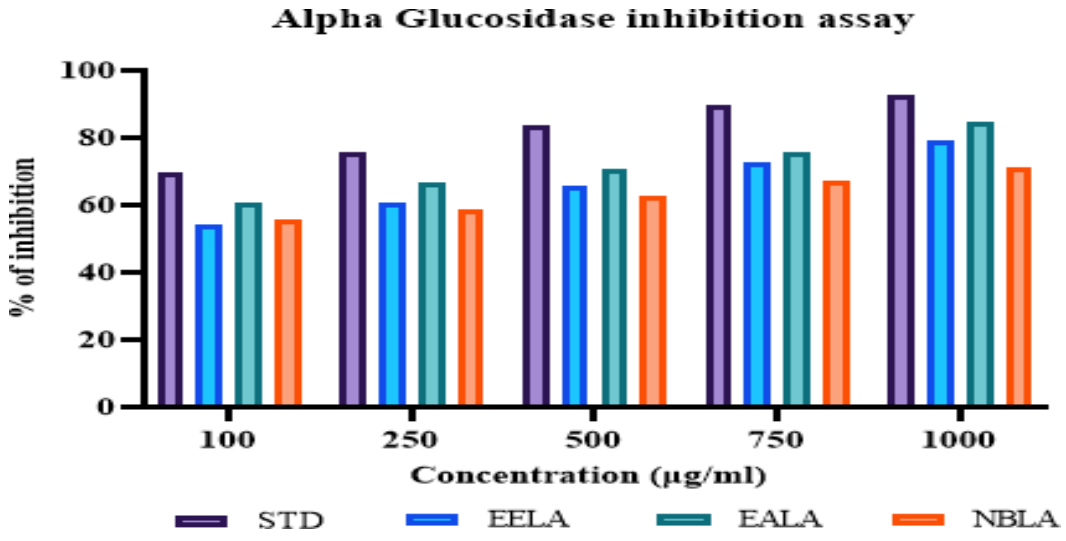

Figure 5. α-Glucosidase inhibitory activity of Ethanolic extract and fractions of *Luffa acutangula* and its comparative analysis

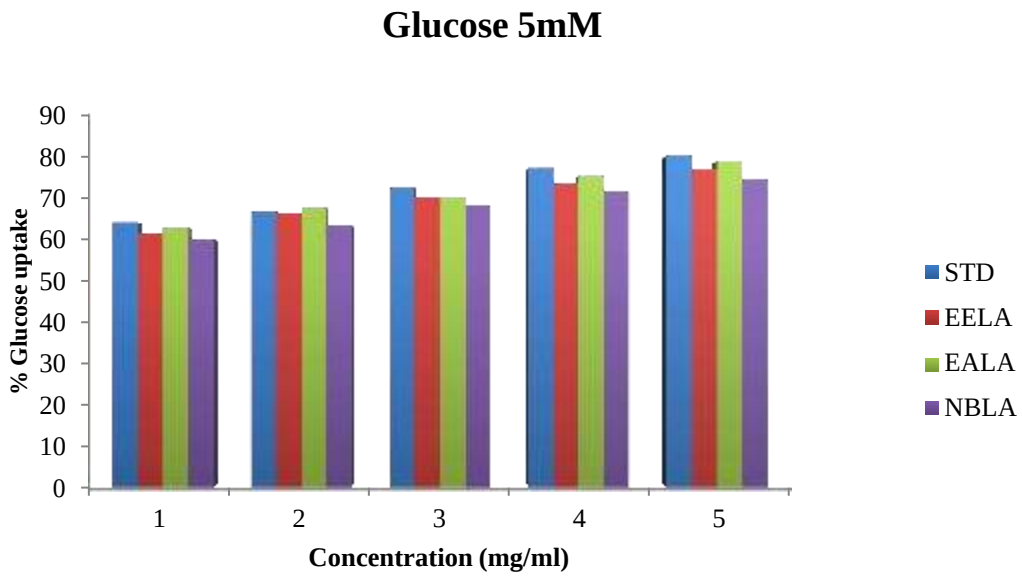

Figure 6. % Glucose uptake in 5mM glucose concentrations

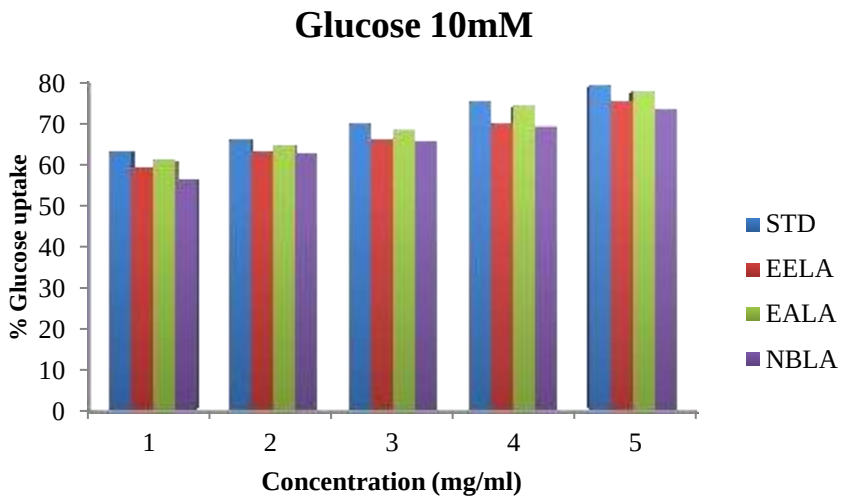

Figure 7. % Glucose uptake in 10 mM glucose concentrations

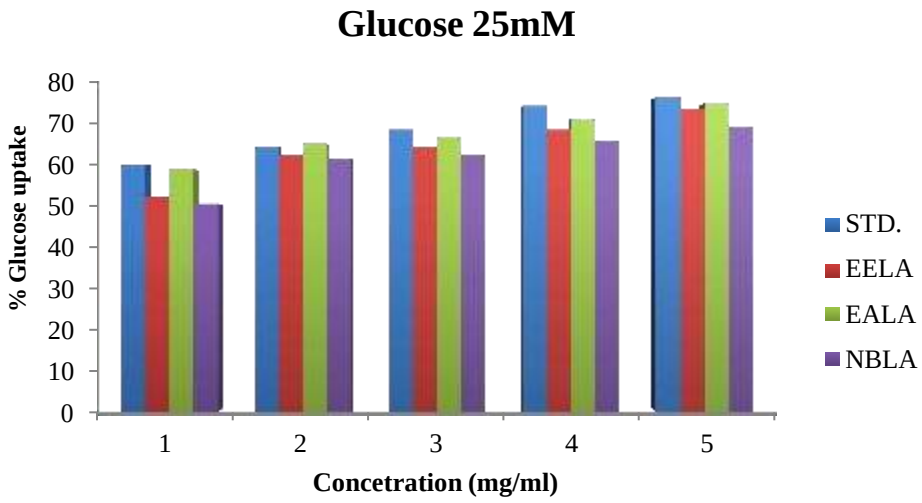

Figure 8. % Glucose uptake in 25 mM glucose concentrations

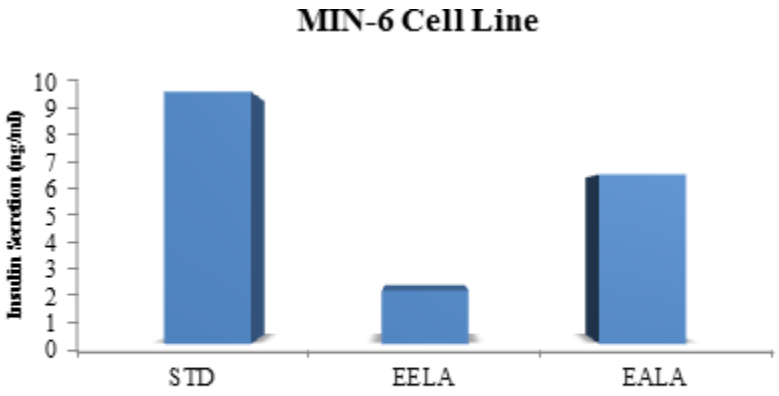

Figure 9. Comparison of insulin secretions observed in MIN6-β Cell line