Section A-Research paper
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In vivo studies of fraction isolated from chloroform extract of *Abelmoschus esculentus L* (Malvaceae) stem as potential antidiabetic agent in alloxan induced diabetic rats

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Abstract:

Background: *Abelmoschus esculentus L* (Malvaceae), commonly named Lady finger is one of the commonly used medicinal plants. Historically it is found that stem of *A. esculentus L* contain a variety of compounds that have been linked to diabetes mellitus. Okra stem contained the greatest concentration of phenolic and flavonoid compounds. Okra had a lot of fibre. **Objective:** The present study was carried out to investigate the traditional use of *Abelmoschus esculentus L* stem in alloxan monohydrate -induced diabetes in rats alongwith antidiabetic chemical constituents in stem and was tested for in vivo biological activities. **Methods**: The ethanolic, aqueous and chloroform extract was obtained by the Reflux extraction method, and fractionation was done with column chromatography and TLC. The antidiabetic chemical constituent has been confirmed by Gas Chromatography-Mass Spectroscopy (GC-HRMS) analysis. For in vivo activities, rats with diabetes mellitus caused by alloxan monohydrate were selected and the anti-diabetic indicators assessed were body weight, blood glucose level, insulin and urine volume. **Results:** Fraction of the Chloroform extract showed presence of Lysine, Leucine, Dodeanoic acid, Pentanoic acid in GC-HRMS Study**.** In GC-HRMS analysis determine Leucine is present in highest area of 2565054.20 with molecular weight 265. This Chemical constituent decreased blood glucose levels and has positive effects to cure diabetes. The extracts and fraction with oral dose were compared with standard drug metformin (150 mg/kg b. w). *A.esculentus* chloroform extract Fraction (AECEF) showed better effect than other extracts used for study. **Conclusion:** From the results we can conclude that *Abelmoschus esculentus L* plant, is having various antidiabetic compounds with effective activity.

INTRODUCTION

Diabetes mellitus is a metabolic disorder that causes an increase in blood glucose levels in the body**.** (1) (2) Diabetes mellitus, commonly referred to as diabetes, is a chronic disorder of the body's ability to process sugar that is characterized by hyperglycaemia due to a defect in insulin secretion, insulin function, or both, together causing vascular and tissue damage and a number of complications with the kidney, eyes, and nerves**.** (3) Eating a balanced diet, staying away from foods high in sugar, exercising frequently and taking medications as prescribed and if necessary, using insulin therapy are all parts of general diabetes management. Insulin is used to the control of diabetes People in developing or low-income countries always intend to treat the effects or symptoms of diabetes in a more natural manner because treatment with antidiabetic drugs is expensive. Diabetes has a significant negative impact on the liver and insulin-dependent organs that are essential for maintaining healthy levels of glucose and lipids. Additionally, insulin regulates cholesterol metabolism and stimulates the production of fatty acid molecules that result in atherosclerosis. (4) (5)

There are the two type of diabetes mellitus type 1 diabetes and type 2 diabetes. This form of diabetes affects 5%-10% of people with diabetes and is brought on by the death of pancreatic beta cells. According to the International Diabetes Federation (IDF), 497100 children and adolescents (0 to 14 years old) were diagnosed with type 1 diabetes globally in 2013. Type 1 diabetes accounts for 80% to 90% of all diabetes in children and adolescents. In 1988, type 2 diabetes was first identified as a part of the metabolic syndrome. The most prevalent type of DM, type 2 (previously known as non-insulin dependent DM), is marked by hyperglycaemia, insulin resistance, and relative insulin deficiency. The interplay of behavioural, environmental, and genetic risk factors leads to type 2 diabetes**.** (6) (7)

Recently, some medicinal plants have been used empirically in anti-diabetic and anti-hyperlipidemic remedies and have been found to be helpful in treating diabetes globally. The primary mechanisms by which plants exhibit antihyperglycemic activity include their capacity to raise insulin output, inhibit glucose absorption from the intestine, or promote the activity of metabolites in insulin-dependent processes. Although there are more than 400 plant species with hypoglycaemic activity documented in literature, finding novel anti-diabetic medications derived from natural plants is still appealing because they contain compounds that have different and safe effects on diabetes mellitus. Glycosides, alkaloids, terpenoids, flavonoids,

carotenoids, etc. are found in the majority of plants and are commonly implicated as having antidiabetic effects. (1)

Abelmoschus esculentus L is a member of the Malvaceae family is the plant *A esculentus L* (Malvaceae). It has been naturalised in every tropical nation and is widely grown in India. The plant prefers acid, neutral, and low-nutrient. It can thrive in extremely alkaline soil. Basic (alkaline) soils a solid, cylindrical, tall, aerial, herbaceous, or woody stem that is typically branching. The woody portion of the stem is fibrous, while the herbaceous portion is covered in scaly hairs. In addition to this, it serves a number of additional functions, such as replacing jute with fibre from plant stems and producing paper and textiles. (8) Local populations have long used fruits from the A. esculentus plant as food and herbal remedies for a variety of ailments. Fruits are used as stew by people in Pakistan and India. Fruits are either processed into broth or eaten raw by Indonesians. In order to improve digestion, locals in India's Jalgaon region chew raw fruits.

Stem of *A. esculentus L* contain a variety of compounds that have been linked to DM use historically. These functional substances were divided into four groups: volatile oils, polyphenols, glycosides and alkaloids. The most prevalent and widespread family of secondary metabolites in plants, polyphenol, has a number of subclasses, some of which contain flavonoids. Either the flavonoids are unbound or they are bound to the sugar moiety. From the fruits of *A. esculentus L*, researchers extracted myricetin, a free, naturally occurring flavonoid. Similarly, the fruits' seeds were used to extract flavonoid and its derivatives. (9)

Okra stem contained the greatest concentration of phenolic and flavonoid compounds. Okra had a lot of fibre, which slows down how quickly the body assimilates sugar and thus serves to stabilise blood sugar. Okra's fibre and other minerals have the ability to lower diabetics' blood sugar levels. Only by delaying the uptake of sugar through the intestine, this fibre also aids in blood sugar regulation. In this study, in vivo methods will be used to perform experiments on okra stem extract as an anti-diabetic. In vivo experiments included evaluation of antihyperglycemic after administration of several carbohydrates and antidiabetic evaluation in insulin deficiency and insulin resistance animal model the results of this study were anticipated to give an overview of the okra fruit extract's anti-diabetic mode of action . (10)

2. MATERIAL AND METHODS

2.1 Authentication and Collect Plant Material:

The Stem of *Abelmoschus esculentus L* was obtained from village area of Rabata, Ahmednagar district, Maharashtra, India and was authenticated by Dr. A. S. Wable, Research Guide, Department of Botany and Research Centre, PVP College of Arts, Science and Commerce, Pravaranagar, Maharashtra, vide letter number PVPC/Bot/2022-23/60 dated 11/09/2022, as shown in fig 1.

2.2 Drug and Chemicals:

Alloxan monohydrate (Sigma Chemical Company, USA) was used to induce diabetes in rats. Metformin was used as a standard hypoglycaemic drug. Ethanol, chloroform and distilled water were used for extraction of the plant materials. The following chemicals were used for phytochemical screening test: chloroform, sulphuric acid , Dragendroff's reagent, ammonia, acetic acid, ferric chloride, copper acetate, million reagent, Fehling's solution, sodium hydroxide etc. n hexane, chloroform, methanol, toluene and ethyl acetate used for Thin layer TLC. And toluene and ethyl acetate used for column chromatography. All the chemicals were of analytical grades.

2.3 Extraction of Plant Material:

The Stem of *Abelmoschus esculentus L* was wash and cut and they air-dried for one week under the shade at room temperature. Then dried plant material was manually powdered finely and used for extraction. In their three different extract (Aqueous extract ethanolic extract . chloroform extract) In reflux extraction method 50g of fresh immature stem powder of Abelmoschus esculentus Add 500 ml of different solvent (Aqueous ,ethanolic, chloroform) at 10℃ for 3 hr .The Ethanolic & chloroform extracts were filtered and the filtrate was concentrated by natural evaporation at solid residue. Aqueous extract filtrate was evaporated in heating mental for 10℃at solid residue. And Calculated the Extract yield. (9) (11) (12) (13)

2.3.1 Determination of % yield of extract

The percentage yield was obtained using this formula:

$$W_2\text{-}W_1/W_0 \times 100.$$

Where, W2 is the weight of the extract with container.W1 the weight of the empty container. and W0 the weight of the dried sample/Powder**.** (14) (15)

2.4 Preliminary phytochemical screening

Phytochemical Screening Chemical tests were carried out on the aqueous, ethanolic and chloroform extracts to identify the chemical constituents the extracts of *A. esculentus L* stem

were tested to determine the presence of secondary metabolites like alkaloids, carbohydrate, glycosides, saponins, phenol, flavonoids, protein, terpenoids, and tannins**.** (16) (17)

1. **Test for alkaloids:**

   Dragendroff's Test: Filtrates were treated with Dragendroff's reagent Formation of red precipitate indicates the presence of alkaloids.

2. **Test for Tannin :**

   Ferric Chloride test: to 2-3ml 0f filtrates extract add few drops of Fecl3 solution . And it show deep blue-black colour.

3. **Test for Saponin :**

   Froth test: Shake the drug extract with water. Persistent stable foam observed.

4. **Steroids Test :**

   Salkowski Test : 5 mL of *A. esculentus* extract was treated with 2 mL of chloroform followed by the addition of 3 mL of concentrated sulphuric acid (H2SO4). The development of a reddish-brown coloration at the contact proved that terpenoids exist. **.** (18)

5. **Test of Flavonoids :**

   Sodium hydroxide test: A 2 mL of each *A. esculentus* extract was treated with a few drops of 1% ammonia (NH3). The formation of intense yellow colouration showed the presence of flavonoid compounds**.** (18)

6. **Test of Cardiac Glycosides :**

   Keller-Killiani test : 2 mL of *A. esculentus* extract were added with 2 mL of glacial acetic acid, a few drops of 5% ferric chloride (FeCl3) and 1 mL of concentrated sulphuric acid (H2SO4). Brown ring appearance at interphase confirmed the presence of cardiac glycosides. (18)

7. **Test of Carbohydrates :**

   Fehling's Test : To 0.5 mL of plant extract, 1mL of water, and 5-8 drops of Fehling's solution were added and heated. It was indicated brick red precipitation. (19)

8. **Test of Proteins &Amino acid :**

   Million tests : 2mL filtrate add few drops of Millon's reagent the presence of Protein &Amino acid was indicate by the appearance of white precipitate. (20)

9. **Test of Phenol :**

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Ferric chloride test : To 2 – 3 ml of filtrate extract ,add few drops of ferric chloride test . The presence of Phenol was indicating by the appearance of Dark green/bluish black colour. (20)

10. Test of Coumarins :

Sodium Chloride test : 3 mL of 10 % NaOH was added to 2 mL filter extract extract and yellow colour was observed in positive results**.** (21)

2.5 TLC Studies

Chromatography was performed in the following solvent systems: chloroform & methanol (2.5 :2.5) for aqueous extracts. (22) toluene & ethyl acetate (3:1) for chloroform extracts. (23) & n hexane, chloroform, methanol (8:2:1) for ethenol extract. (24) all solvent used and performed TLC.

2.5.1 Method

In TLC there are two phase first mobile phase and second stationary phase. In stationary phase used silica gel G. TLC plate is coated with Silica gel slurry. Dry TLC plate for 5 min in room temperature. then in oven for 10 to 20 min. After remove the TLC plate in the oven. And Drawing a light line on the plate. Then Applied the extract dots by using capillary tubes. The TLC plate is deep in mobile phase and examined under ultraviolet light. For the development of the separated bands, they were subsequently sprayed with iodine vapor, and the retention factor (Rf) values were calculated for various samples to represent the movement. (25) (26)

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

2.6 Column Chromatography

One of the most essential methods for the purification and sorting of both liquids and solids is column chromatography. It is a small-scale experiment. A glass column was used for column chromatography on a Chloroform extract. In this method silica gel is a stationary phase and Toluene and ethyl acetate is a mobile phase. In a three ratio first is toluene (10 concentration) . toluene and ethyl acetate (7.5:2.5) are second ratio and Ethyl acetate (10 concentration) is third ratio, first Add a silica gel in column with Toluene and set the silica gel . then add 1g of Chloroform extract dissolve in 2g silica gel loaded in a column. first add toluene in column

.sample is run and collect the 3 fractions. Then add second ratio toluene and ethyl acetate (7.5:2.5) collect 2 fractions. last add ethyl acetate and collected 2 fractions. Total collected 7 fractions in column. then this fraction is analysis for GC-MS. (27) (28)

2.7 GC-HRMS Analysis

The Chloroform stem extract of *Abelmoschus esculentus L* were subjected to GC-HRMS analysis. GC-HRMS method is used for the identification biologically active natural chemical constituents. the sample was sent to SAIF IIT Bombay Powai, Mumbai (Maharashtra) for GCMS analysis. And identify active antidiabetic compound from selected fractions, identify active antidiabetic compound in fraction 1 is n-Hexatriacontane, n-Tetracosane,, Nonadecane, lysin, Leucine, Oxalic acid, Dodecanoic acid (lauric), Pentanoic acid, Fumaric acid, Acetic acid, Trichloroacetic acid .This Active compound is used for Antidiabetic activity. (29) (30) (31)

2.7 Acute Toxicity Study for Safety and Selection of Dose

According to previous studies with normally change in dose. ethanolic extract of *A. esculentus L* was studied for acute toxicity at dose 1000, 2000 mg /kg b. w**.** (32) Aqueous extract of *A. esculentus L* was studied for acute toxicity at dose 1000, 2000, 3000 mg/kg b. w (33) Chloroform extract of *A. esculentus L* was studied for acute toxicity at dose 300, 500,2000 mg/kg b. w**.** (34)LD_{50} of ethanolic extract of *A. esculentus* is 2000 mg/kg b. w. LD_{50} of aqueous extract of *A. esculentus* 2500 mg/kg b. w. and LD_{50} of chloroform extract of *A. esculentus L*.is 2000 mg /kg b. w. and it was found to be safe. Three dose levels-200 mg/kg b. w. , 250 mg/kg b. w. , and 200 mg/kg b. w. were chosen for the study in accordance with the standard procedure.

2.8 Induction of experimental diabetes

Male wistar rats were given a single intraperitoneal injection of newly prepared alloxan monohydrate solution (150 mg/kg body weight) after being fasted for 12–14 hours. This caused the rats to develop diabetes. Weight and fasting blood glucose levels were then measured using a glucometer. According to the weight of each particular animal, alloxan was prepared. After giving the animals alloxan, they were given food and drink 30 minutes later. The plasma blood glucose level of each animal was assessed by drawing blood from the tail and the animals 4 to 5 days after alloxan injection. Fasting blood sugar readings of 99 mg/dL or less are regarded as normal, 100 mg/dL to 125 mg/dL as prediabetic readings, and 126 mg/dL or higher as diabetes readings. (35) (36)

2.9 Experimental design

Male Wistar Rats will be dividing into nine groups. each group contains of six animals (n=6) and treated for 21days**.** (37) Diabetes was induced by alloxan with normal saline in experimental animals. Group I was normal control (NC) with non-diabetic animals kept on regular food and drinking water ad libitum. Group II & Group XI was diabetic control (DC) in which diabetes was induced by injecting Alloxan Monohydrate (150 mg/kg b. w) and was an untreated group given regular food and drinking water ad libitum. Group III Positive Group (PC), received standard drug metformin. orally administered at 150 mg/kg b. w. Group IV to Group IX is a treatment group and Group IV is Treated with *Abelmoschus esculentus* ethanolic stem extract is orally administered at 200 mg/kg b. w. (32) Group V is treated with A. *esculentus* aqueous extract 250mg/kg b. w. (33) Group VI is treated with *A. esculentus* chloroform extract 200mg/kg b. w. (34)Group VII is treated with *A. esculentus* chloroform extract Fractions 50 mg /kg b. w. Group VIII is treated with *A. esculentus* chloroform extract fractions(AECEF) 100 mg/kg b. w Group IX is treated with *A. esculentus* chloroform extract fractions 200 mg/kg b. w. Measure the blood glucose level on day 1,7, 14, 21 during treatment rat blood was taken from the vein part of the tail and measured using glucometer**.** (38)

2.10 Blood sample collection

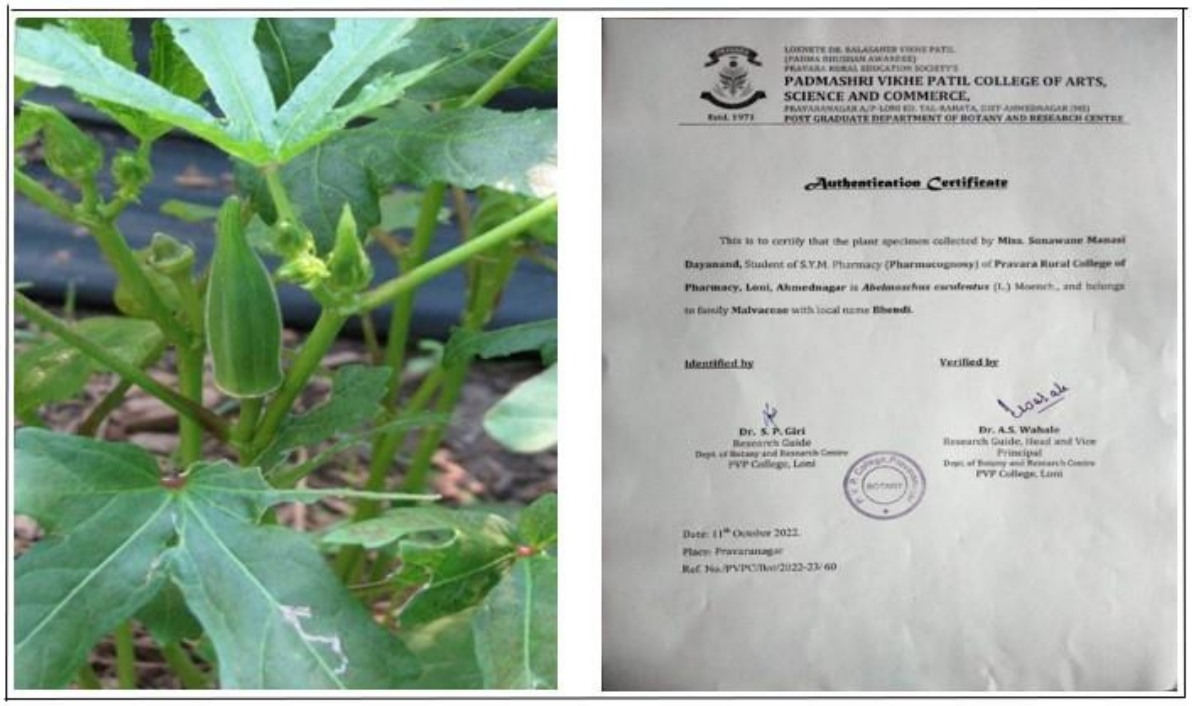
Blood sample was taken using the tail vein technique. This technique is advised for drawing up to 2 cc of blood per withdrawal, which is a significant amount of blood. The temperature in the restrainer is kept between 24 and 27 °C to make the beast comfortable. Rubbing the tail from the base to the tip will cause leukocytosis, so avoid doing it. If the vein cannot be seen, the tail is submerged in 40°C tepid water. 30 minutes prior to the trial, local aesthetic cream must be applied to the surface of the tail. Blood is drawn from the blood vessel using a capillary tube or a syringe with a needle after a 23G needle is introduced**.** (39) (40)

2.11 Histopathology study of Pancreas and Liver

After the animals had been killed by cervical dislocation, the pancreas and the liver of each group were separated, eliminated and washed with an ice-cold saline solution. Both were dissected and preserved in 10% formalin solution. Haematoxylin and eosin were used to colourize paraffin slices of both tissues for histopathological investigations under a light microscope. (41) (38)

3 Result

3.1 Authentication and collection of plant material



LOKNETE DR. BALASAHEB VIKHE PATIL
(PADMA BHUSHAN AWARDEE)
PRAVARA RURAL EDUCATION SOCIETY'S
PADMASHRI VIKHE PATIL COLLEGE OF ARTS, SCIENCE AND COMMERCE,
PRAVARANAGAR A/P-LONI BK. TAL-RAHATA, DIST-AHMEDNAGAR (MS)
Estd. 1971 **POST GRADUATE DEPARTMENT OF BOTANY AND RESEARCH CENTRE**

Authentication Certificate

This is to certify that the plant specimen collected by **Miss. Sonawane Manasi Dayanand,** Student of S.Y.M. Pharmacy **(Pharmacognosy)** of **Pravara Rural College of Pharmacy, Loni, Ahmednagar** is ***Abelmoschus esculentus*** (L.) Moench., and belongs to family **Malvaceae** with local name **Bhendi.**

Identified by

Dr. S. P. Giri
Research Guide
Dept. of Botany and Research Centre
PVP College, Loni

Verified by

Dr. A.S. Wahale
Research Guide, Head and Vice Principal
Dept. of Botany and Research Centre
PVP College, Loni

Date: 11th October 2022.
Place: Pravaranagar
Ref. No./PVPC/Bot/2022-23/ 60

Fig.1. Authentication of *A. esculentus L*

3.2 Extraction of Plant Material

The extraction yield is a metric for how effectively a solvent extracts particular component from the starting substance. It will provide information on the plant's capacity to be extracted under various circumstances the results were reported in Table 1. Our results showed that maximum percent yield was obtained when *Abelmoschus esculentus L.* was extracted by Reflux technique with Aqueous (11.4%), followed by chloroform (29.26%) and finally by ethenol (4.4%).

Table 1. Percentage yield of plant extracts in different solvents

Sr. No	**Extract**	**Extract value (%w/w)**
1	Aqueous extract	11.4 %
2	Chloroform Extract	29.26 %
3	Ethanolic Extract	4.4 %

3.3 Preliminary Phytochemical study

Phytochemical test was performed for the three different extract of *Abelmoschus esculentus L* stem. Aqueous extract show the presence of Alkaloid, saponin, steroids, flavonoids,

carbohydrate, phenol, coumarins. The chloroform extract shows the presence Tannins, Steroids, Flavonoids, Cardiac Glycosides, Coumarins. The ethanol extract shows the presence Tannin, Steroids, Flavonoids, Carbohydrate, Protein & Ammonia , Phenol . The result was shown in Table 2

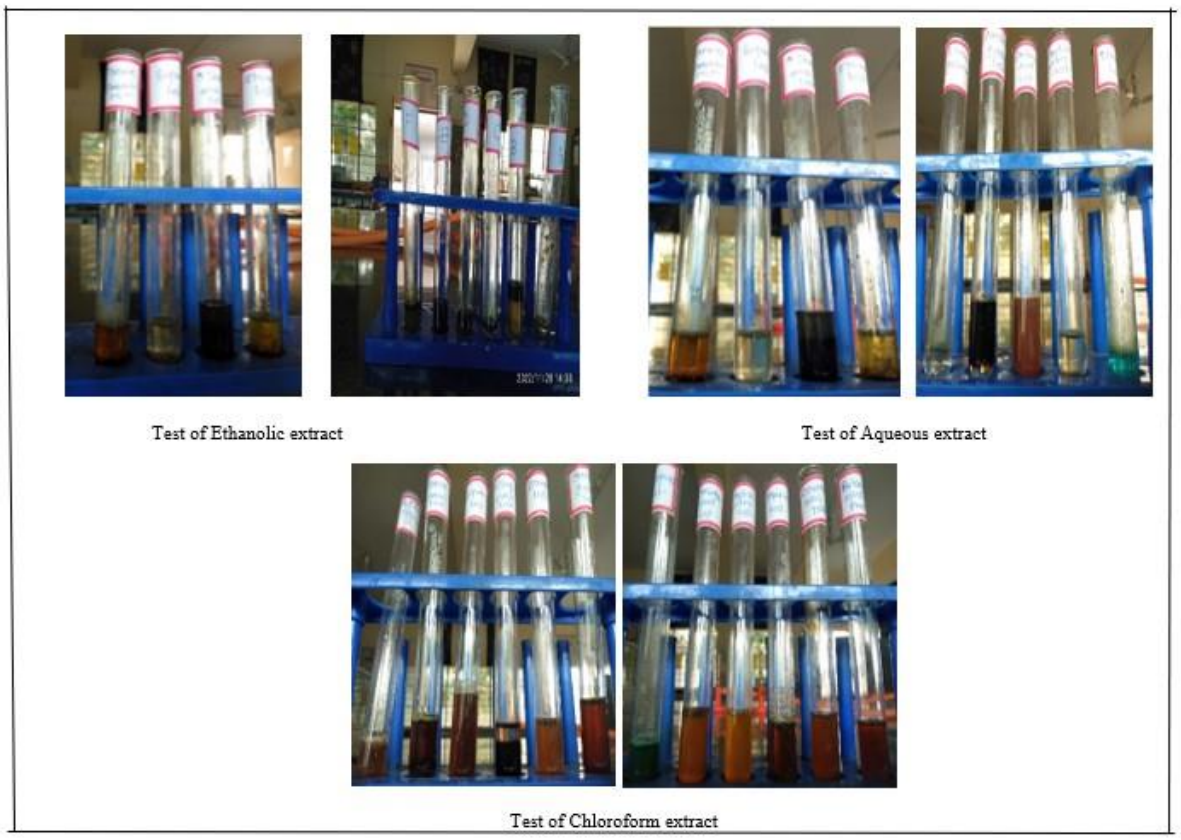


Fig 2. Preliminary Phytochemical study

Table 2. Phytochemical study of *Abelmoschus esculentus L.* (**+** present **, -** absent)

Phytochemical constituent	**Aqueous extract**	**Chloroform extract**	**Ethanol extract**
Alkaloids	+	-	-
Tannin	-	+	+
Saponin	+	-	-
Steroids	+	+	+
Flavonoids	+	+	-
Cardiac Glycosides	-	+	-
Carbohydrate	+	+	+
Protein & Ammonia	-	-	+
Phenol	+	-	+
Coumarins	+	+	-

3.4 TLC Studies

The thin layer Chromatographic separation techniques were performed for aqueous extract, chloroform extract, ethenol extract . The results show in the form of Rf values in table 3

Fig 3. TLC studies

Table 3. TLC of *Abelmoschus esculentus L.*

Extract	**Mobile Phase**	**RF value**
Aqueous extracts	Chloroform & Methanol (2.5 :2.5)	0.9
Chloroform extracts	Toluene & Ethyl acetate (3:1)	0.7
ethenol extract	n Hexane, Chloroform, Methanol (8:2:1)	0.1

3.5 Column Chromatography

The results of column chromatography chloroform extract of stem of *Abelmoschus esculentus L* obtained 3 fractions. Fractionation of column chromatography ethanol extract of Abelmoschus esculentus stem using column mobile phase solution which is different in polarity Toluene and ethyl acetate used as mobile phase. Results fractionation of vacuum column chromatography Chloroform extract of Abelmoschus esculentus stem is presented in Table 4.

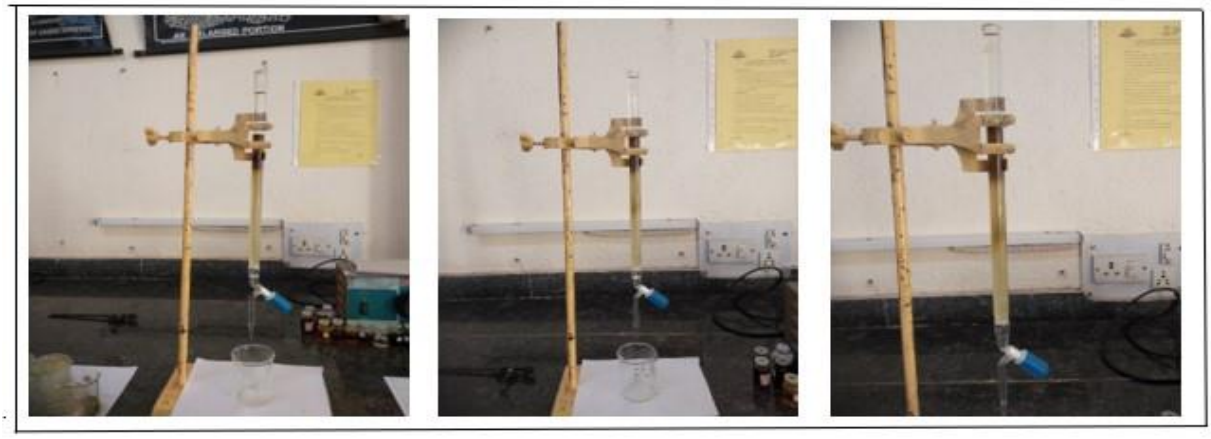


Fig 8. Column Chromatography of Chloroform extract

Table 4. Results of fractionation of chromatography of vacuum column chloroform extract of Abelmoschus esculentus *l* .

Fraction	No Vial	Column Mobile Phase
F1	1-3	Toluene(10 concentration)
F2	4-5	Toluene: Ethyl acetate (7.5:2.5)
F3	6-7	Ethyl acetate(10 concentration)

3.6 GC–HRMS analysis

The GC–MS chromatogram of chloroform extract from *Abelmoschus esculentus L* observed 14 bioactive compound-related peaks, which were identified by comparing their mass spectra to those listed in Table 5 and identifying them. The major components present in *Abelmoschus esculentus L* were ,n-Hexatriacontane, n-Tetracosane, Lysine Leucine, Oxalic Acid, Dodeanoic acid(Lauric), Pentanoic acid, fumaric acid, Acetic acid Trichloroacetic acid. This bioactive compound shows Antidiabetic property.

Table 5. results of Gas chromatography-mass spectrometry (GC–MS) analysis *Abelmoschus esculentus L.*

Sr. No	**Peak No.**	**Name of Constitution**	**Mol. Formula**	**Mol. Weight**	**Time**	**Area**
1	1	Lysine	C22H26N206	414	4.71	2565054.20
2	1	Leucine	C14H19NO4	265	4.71	2565054.20
3	5	Dodeanoic acid	C19H26F1202	514	9.74	924025.04
4	5	Pentanoic acid	C13H14F1202	430	9.74	924025.04
5	6	Fumaric acid	C20H35ClO4	374	11.68	327616.64
6	6	Acetic acid	C16H21CL3O3	366	11.68	327616.64
7	7	Oxalic acid	C13H2404	244	11.84	963520.35
8	7	Trichloroacetic acid	C8H13CL302	246	11.84	963520.35

9	29	n-Hexatriacontane	C36H74	506	35.00	3517186.59
10	29	n-Tetracosane	C24H50	338	35.00	3517186.59

Figures 5 & 6 depict the chromatogram from the GC-MS spectral study of A. esculentus L. which reflects the peaks of the various compounds, their elution, and their retention times.

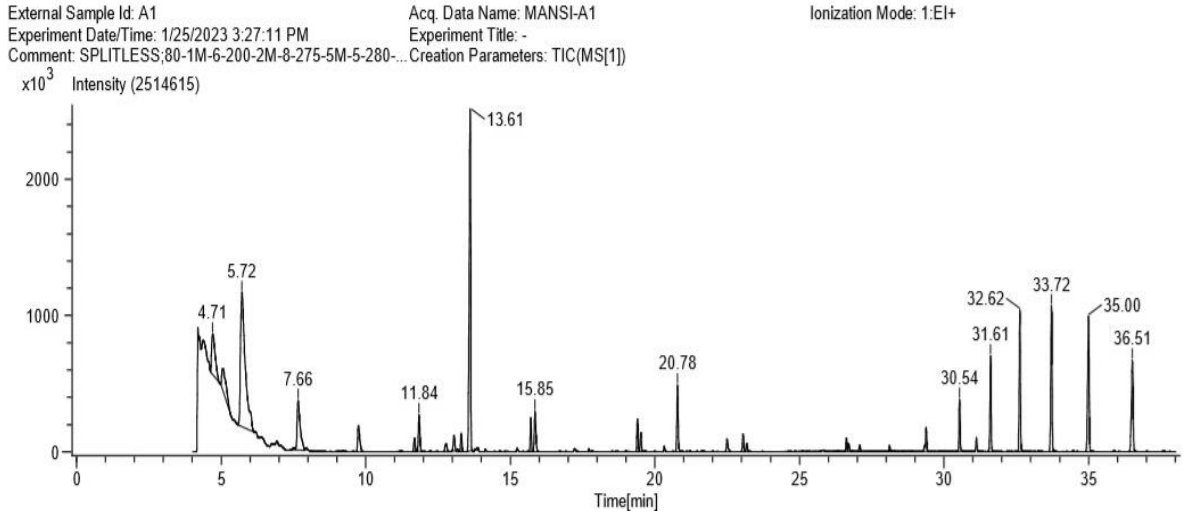
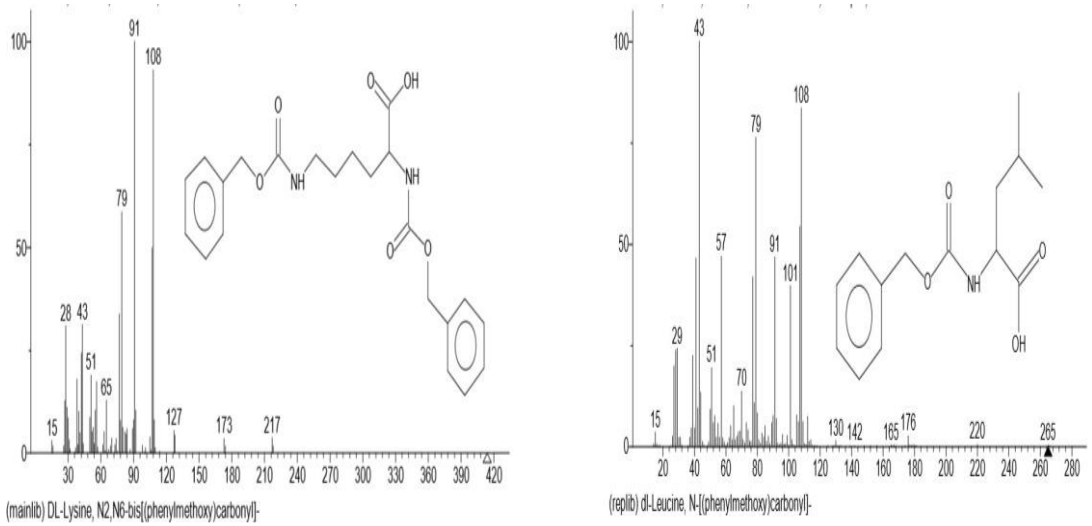
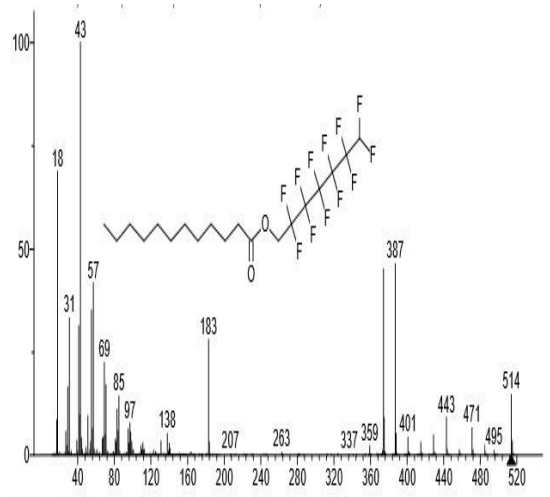
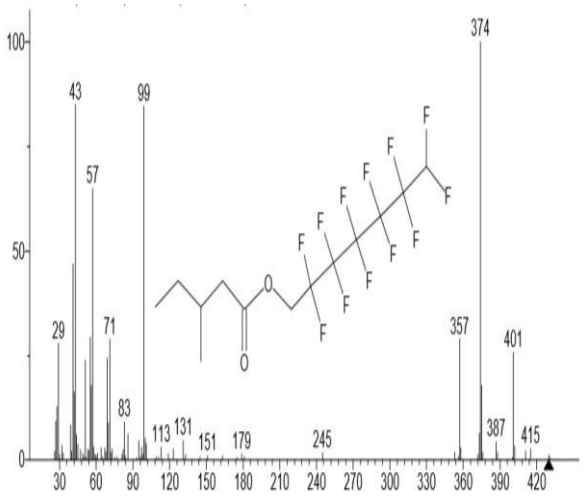


Fig 5.Chromatograms of chloroform extract of *A. esculentus* by Gas Chromatography-Mass Spectrometry

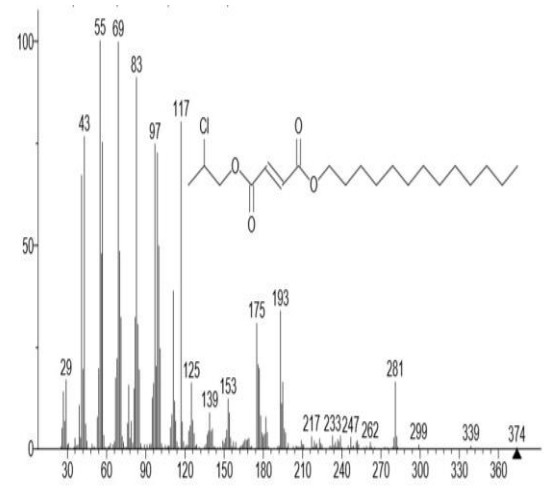




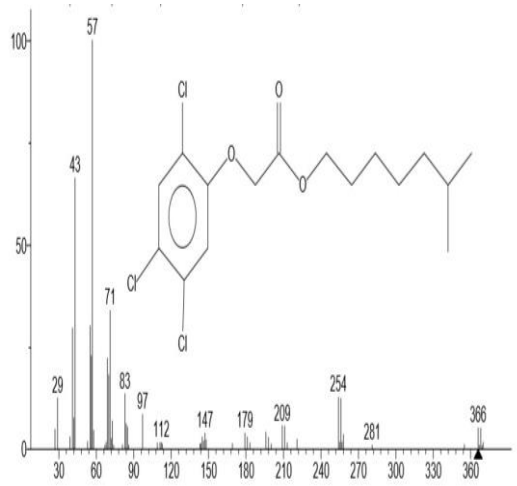
(mainlib) Dodecanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7-dodecafluoroheptyl ester



(mainlib) Pentanoic acid, 3-methyl-, 2,2,3,3,4,4,5,5,6,6,7,7-dodecafluoroheptyl ester



(mainlib) Fumaric acid, 2-chloropropyl tridecyl ester



(mainlib) Acetic acid, (2,4,5-trichlorophenoxy)-, isooctyl ester

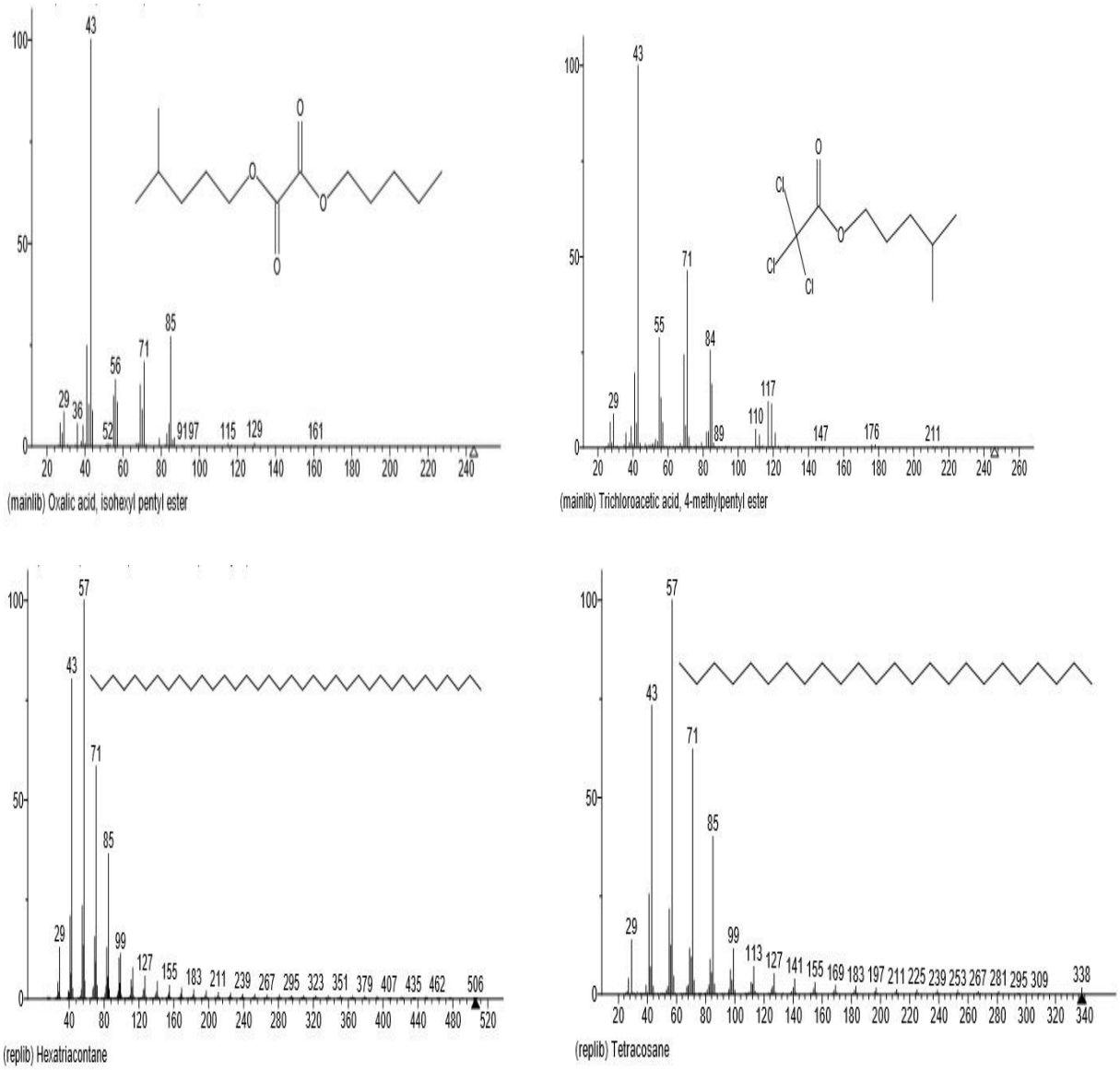


Fig 6. Chromatograms of Chloroform extract of A. esculentus by Gas Chromatography-Mass Spectrometry.

Anti-diabetic activity

In order to monitor the antidiabetic potential of the extract and fractions, the effect of the extract and fractions on all the parameters were compared to that of the PC (metformin-treated), NC and DC groups. The effect of extract and its fractions on BGL, INs, BW, and Vu are depicted in table 6. In this experimental study show the less body weight of diabetic rats. Diabetic group rats expanded less body weight than the normal control (group I) rats. In normal group blood glucose level is less than the other diabetic group. Because reaming 8 groups giving alloxan monohydrate. Group DC show the proper BGL then other because they giving standard drug. three extract were given three different group for the treatment. and that found the AECE

group show the good blood glucose level then other two. AECE show the good glucose level. so, we give the AECEF dose to the animal. and they show blood glucose level of AECEF is better than AECE. Results show in table 6.

Table 6. The effect of extract, its fractions, on BGL (mg/dl), , INs (µU/mL), BW (g), and Vu (mL) NC- Normal Control. DC – Diabetic control, PC -Positive control, AEEE – A, esculentus ethanolic extract , AEAE – A, esculentus Aqueous extract . AECE – A, esculentus Chloroform extract . AECEF – A, esculentus Chloroform extract fraction.

Parameters	**NC**	**DC**	**PC**	**AEEE**	**AEAE**	**AECE**	**AECEF**	**AECEF**	**AECEF**
				Dose (mg/kg b. w.)					
				200	**250**	**200**	**50**	**100**	**200**
Parameter for Anti-diabetic potential									
BGL-day1	82.62 ± 2.8	277.63 ± 2.73	274.13 ± 2.17	272.97 ± 2.95	282.87 ± 2.05	278.31 ± 4.15	278.72 ± 4.05	277.58 ± 4.88	275.62 ± 4.16
BGL-day21	81.59 ±3.2	329.83 ±4.22	111.78 ±3.6***	190.21 ±3.52	160.95 ±1.32	140.8 ±2.2**	200.77 ±3.17*	167.68 ±19.83*	131.56 ±3.09***
INs-day1	16.79 ±0.35	6.86 ±0.11	6.96 ±0.14	7.96 ±0.17	6.84 ±0.13	7.15 ±0.16	7.15 ±0.33	7. 26 ±0.21	7.10 ±0.12
INs-day21	17.66 ±0.35	7.01 ±0.06	16.44 ±0.3***	19.63 ±0.5	8.14 ±0.40	19.02 ±0.2**	9.31 ±0.57**	9.13 ±0.46*	18.11 ±0.36***
BW-day1	273.01 ±5.24	265.56 ±3.62	267.5 ±2.41	265.13 ±3.65	269.93 ±4.87	266.90 ±4.11	267.7 ±4.33	267.11 ±5.33	261.55 ±5.91
BW-day21	295.66 ±5.52	209.74 ±3.22	310.33 ±7.87***	242.63 ±7.99	235.9 ±11.9	260.77 ±9.18*	244.42 ±2.98	236.28 ±7.63*	295.16 ±9.01***
Vu-day1	1.3 ±0.12	1.44 ±0.1	1.4 ±0.2	1.65 ±0.2	1.65 ±0.15	1.5 ±0.22	1.55 ±0.08	1.68 ±0.11	1.3 ±0.2
Vu-day21	1.32 ±0.12	10.37 ±0.08	5.47 ±0.20***	8.67 ±0.38	8.75 ±0.35	9.27 ±0.2**	8.62 ±0.32**	8.58 ±0.34**	5.45 ±0.32***

3.7 Histopathological Studies

To evaluate the extract's and its fractions' potential as an antidiabetic, pancreatic histopathological investigations have been conducted. The picture show morphology and particular part of pancreases. The group 1 is NC that show the Beta cell, islets and Acinus cell.

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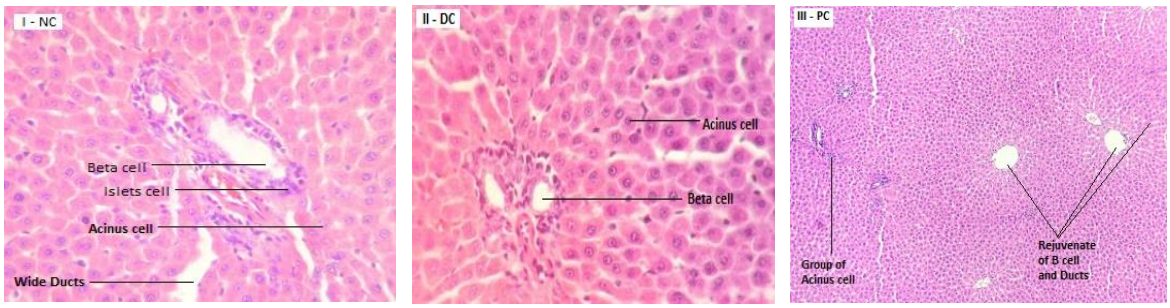
The group 2 is DC is group that show the Acinus and beta cell. Group 3 is PC that show the Rejuvenate of Beta cell and Group of Acinus . Then remaining 6 Group is treatment Group. Group 4 is AEEE (A .esculentus ethanolic extract) and that show the wide ducts , Acinus cell and Beta cell . Group 5 is AEAE(A. esculentus Aqueous extract) and that show the normally presence of Ducts and Acinus cell. Group 6 is AECE (A. esculentus Chloroform extract) and that show the Normal growth of islets cell and ducts. Then last 3 group is some Group but dose is different. Group 7 is AECEF (A. esculentus Chloroform extract fraction) at dose 50 mg/kg b. w . and that show normal growth of islets and ducts. Group 8 is AECEF at dose 100 mg/kg b. w. and that show the Growth of Acinus and some ducts. last Group 9 is AECEF at dose 200 mg /kg b. w. that show the better development of islets, ducts, beta cell. The histopathologic images of the pancreases are represented in fig 6

To evaluate the extract's and its fractions' potential as an antidiabetic, liver histopathological investigations have been conducted. the picture show morphology and particular part of pancreases. The group 1 is NC that show the Central vein and Hepatic Lobule. The group 2 is DC is group that show the Sinusoide , Hepatic inflammation, Hepatic lobules . Group 3 is PC that show the wide sinusoide and central vein . Then remaining 6 Group is Treatment Group. Group 4th is AEEE (A .esculentus ethanolic extract) and that show very diminute protection of hepatocyte burning . Group 5th is AEAE(A. esculentus Aqueous extract) and that show the less protection of hepatocyte injury. Group 6 is AECE (A. esculentus Chloroform extract) and that show the Good protection of hepatocyte injury. Then last 3 group is some Group but dose is different . Group 7 is AECEF (A. esculentus Chloroform extract fraction) at dose 50 mg/kg b. w .and that show the Hepatic lobules and wide sinusoide. Group 8 is AECEF at dose 100 mg/kg b. w and that show less separation of Hepatocyte. last Group 9 is AECEF at dose 200 mg /kg b. w that show the separation of Hepatocyte . The histopathologic images of the Liver are represented in fig 7.



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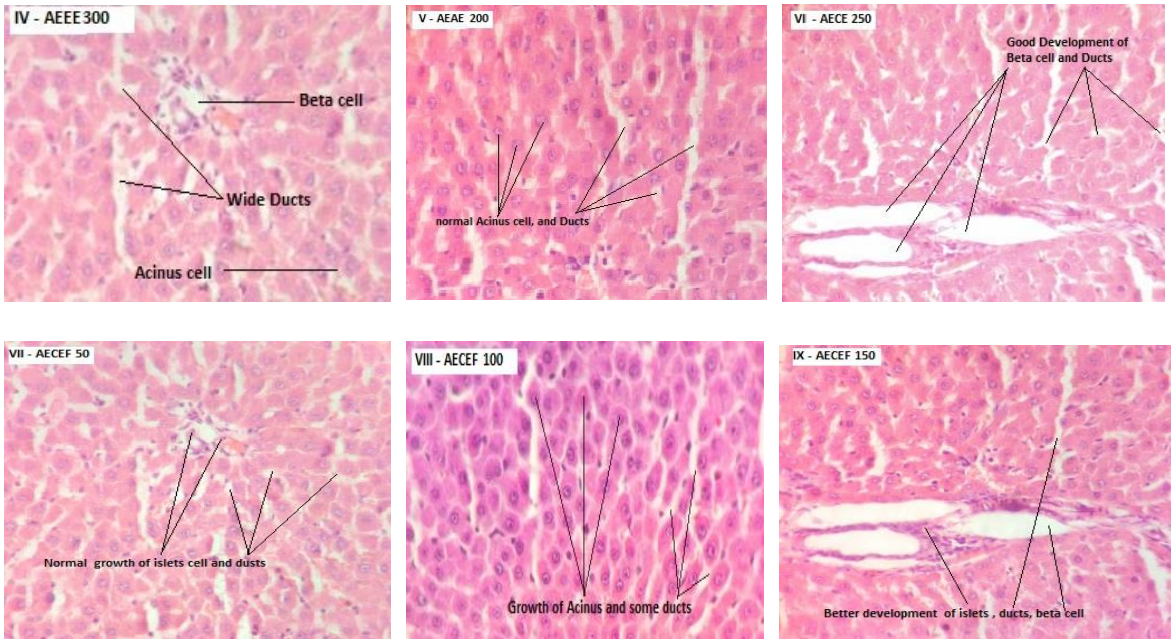
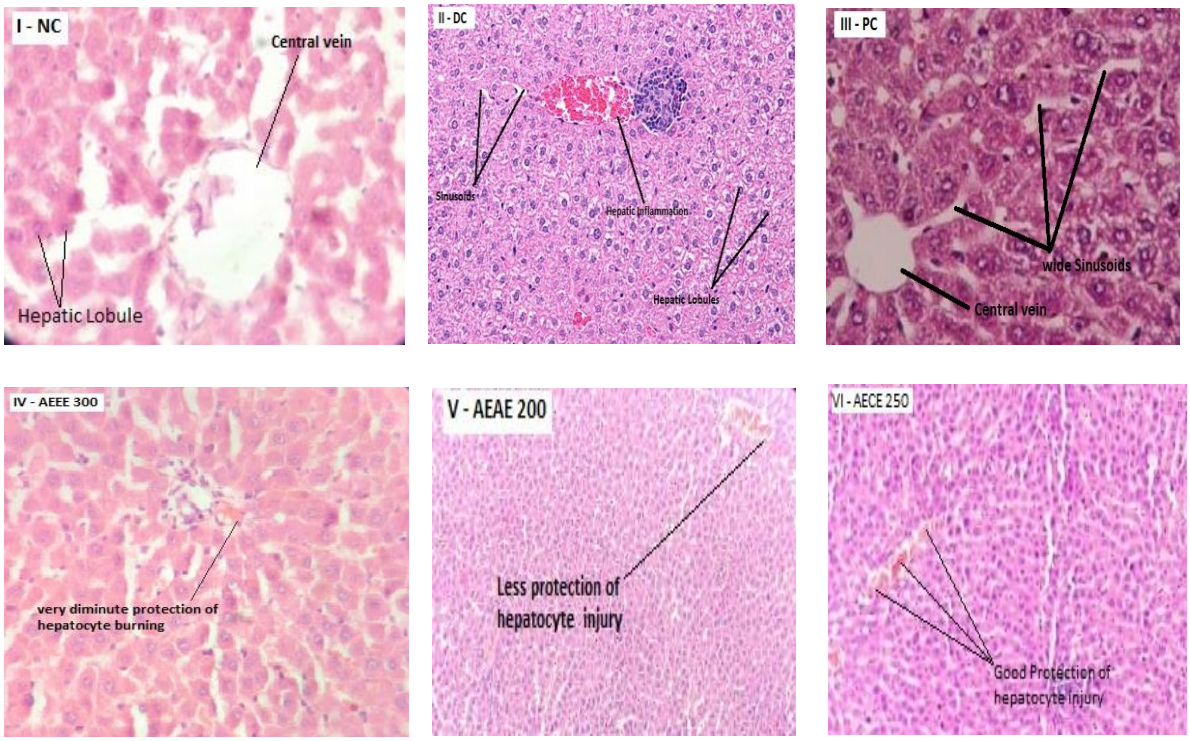


Fig 6. The pancreatic histopathologic pictures to evaluate the extract's and its fractions' antidiabetic potential



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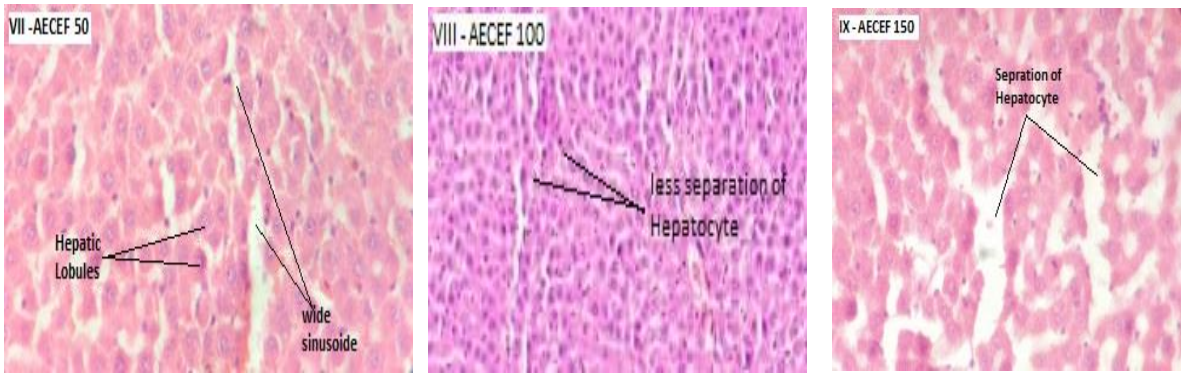

Fig 7. The liver histopathologic picture to evaluate the extracts and its fractions Antidiabetic potential

Discussion

The present study was performed to determine the antidiabetic activity of *Abelmoschus esculentus L*. stem. Diabetes is a disease of the metabolism of carbohydrates, fats and proteins. In this disease various medicinal plants are used for treatment. In traditional literature, *A. esculentus(L) stem* is preferred for diabetic disorders (1) **.** Extraction method is first step of this study**.** And this method was used to obtain bioactive compound through medicinal plant. In *A. esculentus (L)* stem Extraction, three different solvents were used for three different extracts. The chloroform extract yield is higher than Aqueous and ethanolic extract. (9)

The Preliminary phytochemical screening method is used to identify chemical constituents in plant material and various test is used to determine chemical constituents. In three extracts, various chemical constituents are present or absent like alkaloids, tannin, flavonoids, and glycoside. Flavonoid is important constituent for antidiabetic activity. (16) and they are present in Aqueous and Chloroform extract. Thin layer chromatography (TLC) is an important technique for the identification and separation of a mixture of organic compounds. It is useful in identification of components of a mixture. In three extracts, chloroform extract Rf value is 0.9 . and they are higher than aqueous and ethanolic extract but no proper confirmation for which bioactive compound present in Chloroform extract. Hence we performed Colum Chromatography of Chloroform extract. In this method three fractions were collected and sent for GC-HRMS analysis. In GC-HRMS analysis it was found that Leucine was present in high area. So the same fraction was used for antidiabetic activity.

Previous studies about phytoconstituents of the plant stem has revealed the presence of tannins, glycosides, flavonoids, steroids, phenols, coumarins, alkaloids and triterpenoids. Apart from this, no any specific phytochemical investigation regarding the presence of antidiabetic

chemical constituent in the stem of the plant. But GCHRMS analysis confirmed the antidiabetic bioactive compound. This fraction was selected for the pharmacological activity. (30)

For in vivo antidiabetic activity, Male Wistar Rats were divided into nine groups. Each group comprised six animals (n=6) and was treated for 21days. Diabetes was induced by alloxan with normal saline in experimental animals. In this study, Group DC show the proper BGL then other because we give standard drug. three extract were given three different group for the treatment group . and that found the AECE group show the good glucose level then other two. AECE show the good glucose level. hence, we given the AECEF dose to the animal. and AECEF group is show better blood glucose level then AECE group. (38)

In histopathological observations of pancreas and liver tissue sample are given in fig 6 and fig 7. In pancreatic tissue, group PC show that rejuvenate of Beta cell and group of Acinus. Group AECE show that show the normal growth of islets cell and ducts. And Group AECEF at dose 200 mg/kg b. w. that show the better development of islets, ducts, beta cell. In liver tissue, group PC show that wide sinusoide and central vein. Group AECE show that good protection of hepatocyte injury. And Group AECEF at dose 200 mg/kg b. w. show that separation of hepatocyte. (40)

Conclusion

The extracted stem of Abelmoschus esculentus was used in the current study. It has been discovered that the extraction of plant components depends primarily on solvents used. *A. esculentus L.* stem extracts were subjected to a phytochemical screening, which revealed the existence of various phytochemical components, including alkaloids, carbohydrates, glycosides, saponins, phenol, flavonoids, proteins, terpenoides, and tannins. *A. esculentus L* stem extracts in chloroform with isolated phytoconstituent Leucine had an anti-diabetic impact on rats. Histopathological studies revealed that treatment with chloroform extract fraction cured the diseased organs of rats.

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