In-Vitro Screening of anti-diabetic activity of *Pracecitrullus fistulous (*leaves) ethanolic extract and its fraction ethyl acetate and n-butanol.

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Abstract

Since the beginning of the medicinal plants have been used to treat a variety of illness. A systemic disorders called Diabetes mellitus is characterized by hyperinsulinemia, significantly increased blood lipid and fat concentrations and hyperglycaemia. The anti-diabetic activity of ethanolic crude extract of *Pracecitrullus fistulous (P. fistulous)* and its active fraction were

evaluated by *in-vitro* assays. Extraction of *P. fistulous* leaves was carried out using ethanol and further, Ethyl acetate and n-butanol fractions were obtained. To investigate the hypothesis of hypoglycemic effects of ethanolic extract of *P. fistulous* (EEPF), Ethyl acetate fraction of P. fistulous (EAPF), n-butanol fraction of *P. fistulous* (NBPF) and Acarbose (Standard drug), (100-100µg/ml) were used for the α-amylase and α-glucosidase enzyme inhibition study. EEPF, EAPF, NBPF were also studied for DPPH, Reducing power assay, Hydrogen peroxide scavenging assay, HPTLC. From the above the studies carried out the active fraction were subjected for the MIN-6β cell line study. The insulin secretagogue actions of EEPF, EAPF and NBPF were investigated utilizing a mouse insulinoma beta cell line (MIN6-β). The study has revealed that the EAPF showed the highest anti-diabetic potential in comparison to the EEPF and NBPF.

Keywords Diabetes mellitus, *P. fistulous*, α-Amylase, α-Glucosidase, MIN6-β Cell line, Insulin Secretagogue

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Introduction

Diabetes mellitus (DM) is a major chronic disease in the world, DM is characterised by a loss of glucose homeostasis as well as changes in the metabolism of carbohydrates, fats, and proteins as a result of deficiencies in insulin production and activity [1]. This happens as a result of insufficient insulin production, factors that counteract insulin's actions on the tissues, or a combination of the two. Although diabetes mellitus is typically irreversible, the patient can lead a relatively normal lifestyle, but complications greatly limit life expectancy [2]. Hyperglycaemia, alterations in the metabolism of lipids, carbohydrates, and proteins, and a higher risk of vascular disease consequences are all characteristics of the group of disorders known as DM. There are modern medications available to treat diabetes, including biguanides, sulfonylureas, and thiazolidinediones [3]. Numerous herbal medications have been suggested for the treatment of diabetes in condition to the already existing therapeutics alternative for the condition such as oral hypoglycaemic drugs each of which their have own drawbacks [4].

DM is a set of metabolic illnesses characterised by persistently elevated blood sugar levels. The symptoms of this elevated blood sugar include excessive urination, increased appetite, and increased thirst. Diabetic ketoacidosis and nonketotic hyperosmolar coma are examples of acute complications. Cardiovascular disease, cerebrovascular disease, renal disease, foot ulcers, and eye injuries are serious long-term consequences [5].

Pracecitrullus fistulous (P. fistulous), belonging to the family *Cucurbitaceae* is also known as Indian baby Pumpkin, Round gourd or Tinda [6]. *P. fistulous* is origin is north western India, mostly found in India in Punjab, Uttar Pradesh, Mumbai and Rajasthan. It is a creeping or climbing herb [6]. A medicinally plant herb used for its range of pharmacological effects including its antimicrobial, anthelmintic, antioxidant and treatment of DM [7]. *P. fistulous* reported to contains carbohydrates, protein and cardiac glycosides in addition to alkaloids, flavonoids, saponins, polyphenols, phytosterol, tannins, ascorbic acid and diterpenes. The reported chemical constituents is present in these leaves are stearic acid, oleic acid and linoleic acid [8]. This chemical constituents has been revealed various antidiabetic activity [9]. Diabetes mellitus, proposed a study in which plant *P. fistulous* were selected for antidiabetic activity.

METHOD & MATERIALS

The fresh leaves of *P. fistulous* were collected from medicinal plant garden Konkan Krishi Vikas Pratishtha, Kharghar, Navi Mumbai, Maharashtra 400709. The crude leaves was authenticated by the Department of Botany "ALARSIN PHARMACEUTICALS" Andheri west, Mumbai: 400093

Preparation of plant material & Extraction

The plant leaves were shade dried under room temperature for 15 to 20 days. The dried material was crushed by electronic grinding and stored in a dry place for until use. The leaves powder was extracted by using Soxhlet apparatus with 70% ethanol as the solvent. These extracts were further concentrated by using Desiccator.

Preliminary Phytochemical testing

The extracts were dissolved in 2-5ml of suitable solvent, the filtrate was subjected to various preliminary chemical tests, to detect the presence of different phytochemical constituents in extracts.

Organoleptic properties and Microscopical Characteristics of *P. fistulous* EEPF and their fraction (EAPF and NBPF)

- **Study of Organoleptic Properties**
  - Appearance
  - Color
  - Odour
  - Taste

Fractions Preparation with different solvents

The Slurry was formed by combining the concentrated ethanol extract with water. 50 ml of ethyl acetate was poured into 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 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 and the n-butanol (organic layer) was collected. The process was carried out twice more.

High Performance Thin Layer Chromatography (HPTLC)

P. fistulous extract (EEPF) and its fractions ethyl acetate (EAPF) and n-butanol (NBPF) were concentrated under reduced temperature and pressure before being submitted to HPTLC. HPTLC (Camag, Switzerland) is equipped with a sample applicator-Linomat-5, twin development chambers, TLC scanner-3 and integration software, documentation system Reprostar-3. Extracts was concentrated to 1 ml and used for HPTLC analysis. The adsorbent for HPTLC was an aluminium sheet that had been pre-coated with silica gel 60 (1.05547 E Merck). As the mobile phase, a mixture of methanol and chloroform (11:0.5) was utilised. The mobile phase was allowed to saturate the chromatographic development chamber for 10 minutes before the plates were added. The plates was derivate (10% H_2SO_4 in methanol) and run up to an 8 cm height. The derivative plates was heated for two minutes at 100 °C, bands were seen, at 366 nm and each analysis was repeated three times [10].

In-Vitro Antioxidant activity

DPPH radical scavenging assay [11]

2 ml of DPPH in methanol (0.33%) and 10–100 µl of each extract or standard were added to the test tube. After giving the mixture a good shake, it was incubated for 30 mins at 37°C. Following incubation, a spectrophotometer was used to calculate each solution absorbance at 517 nm. The remaining DPPH was then determined after taking the equivalent blank reading. The sample and the reference solution completed the test in three separate batches. The amount of free radical scavenging activity increases with decreasing reaction mixture absorbance.

Hydrogen Peroxide scavenging assay [12]

In phosphate buffer, a hydrogen peroxide solution (2 mmol/l) was added (pH 7.4). Extracts of 10-100 µg/ml were added to a 0.6 ml hydrogen peroxide solution. After 10 minutes, hydrogen peroxide's absorbance at 230 nm was measured in comparison to a blank solution made of phosphate buffer without hydrogen peroxide. For background subtraction, a distinct blank sample was utilised for every concentration.

Reducing power assay [13]

This test was run in three separate runs. 1.0 ml of various extract concentrations (10, 20, 40, 60, 80, and 100 µg/ml) were combined with 2.5 ml of potassium ferricyanide (1%), 2.5 ml of phosphate buffer (0.2 M, and 6.6 as the pH) and 2.5 ml of phosphate buffer. After that, the mixture was incubated for 20 minutes at 50°C (When the mixture had been incubated, 2.5 ml of trichloroacetic acid (10%) was added and it was centrifuged at 3000 rpm for 10 mins) after the separation of the supernatant. After that, 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%) were added to the supernatant (upper layer of the solution). The absorbance at 700 nm was determined and compared to a standard ascorbic solution.

In-Vitro Antidiabetic activity

Alpha Amylase Inhibition assay [14]

- Inhibition of salivary amylase activity by *P. fistulous* of crude extract and their fractions Ethyl acetate and n-butanol was performed according to method described by Gillard *et.al*, with some modifications.
- 50 ml saliva was used for performing the following assay. Saliva sample was refrigerated within 2hr of collection and left overnight at 4°C.
- Sample was centrifuged for 30 mins at 2000 rpm and supernatant was used for the study.
- Reaction mixtures consisted of 500µl: 0.02M phosphate buffer (pH 6.9; 6Mm of NaCl) ,1 ml of saliva (Salivary amylase), different concentrations of *P. fistulous* of crude extract and their fractions Ethyl acetate and n-butanol (10-100µg/ml) or distilled water or (blank).
- After 15 mins incubation at room temperature, 1 ml of 3,5-dinitrosalicyclic acid (10M) were added to it.
- Reaction mixture was incubated at 95°C in water bath for 1 hr and then brought to room temperature.
- Inhibition of α-amylase activity was determined spectrophotometrically at 540nm. Blank reaction represented 100% enzyme activity and relative activity was determined as per following equation.

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

α-Glucosidase Inhibition Assay [15,16]

- α -Glucosidase inhibitory activity was performed using a previously published method with some modifications.
- Sample solution of Ethanol extract crude of *P. fistulous*, Ethyl acetate and n-butanol Fraction of *P. fistulous*,100 µg/ml was mixed with glutathione (50 µL), α-glucosidase solution (50 µL) in phosphate buffer (pH= 6.8) and *p*NPG99 (4-Nitrophenyl β-D-glucopyranoside) (50µ L) in a 96-well microplate and incubated for 15 minutes at 37°C.
- Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (α - glucosidase) solution.
- The reaction was stopped with the addition of sodium carbonate (50 µL, 0.2 M). The sample and blank absorbance was read at 400 nm.

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

Cell Line study for antidiabetic activity

MIN6- β Cells Culture

Cell line use for anti-diabetic activities have proven to be quite beneficial. Cell lines can successfully substitute animal experiments when assessing the antidiabetic efficacy of various synthetic and natural compounds. Cell cultures are particularly dependable since the tissue may be readily accessed and examined. MIN6-β cells were cultured at 37˚C under atmosphere of 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with10% fetal calf serum, 2mM glutamine, 10,000 units/ml of penicillin and 10 mg/ml of streptomycin. Above all extract and fraction stock solution was prepared by dissolving extract and fraction in DMSO and further diluted with Krebs Ringer buffer (KRB) to prepare working solutions [17].

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 *P. fistulous* and its fraction (ethyl acetate fraction) was used at a dose of 1000 µg/ml. The standard was used in the study 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 [17-19].

Results

From 50 grams of leaf powder extract obtained EEPF 08.69 %w/w yield, EAPF and NBPF were 6.35 % w/w and 5.45% w/w respectively.

Preliminary Phytochemical Screening

The Preliminary Phytochemical Screening was done for the ethanolic extract of P. *fistulous* which shows the presence of Carbohydrates, Flavonoids, Steroids, Proteins, Phenolic compounds and Alkaloids (Table 01).

Sr. No	Chemical test	Test	*P. fistulous*
1.	**Test for Alkaloids**	Wagner's test	+
		Drangendoff's test	+
		Hager's test	+
2.	**Test for glycosides**	Killer Killani test	-
	Test for Phenolic Compounds	Ferric chloride test	+
		Lead acetate test	+
3.		Acetic acid test	-
4.	**Test for steroids**	Salkowski test	-
5.	**Test for flavonoids**	Shinoda test	+
6.	**Test for Carbohydrate**	Molisch test	-
		Barford test	+
7.	**Test for Proteins**	Millions test	+
		Biuret test	-

Table 01. Phytochemical screening of ethanolic extract of *P. fistulous*

High Performance Thin Layer Chromatography (HPTLC)

Mobile phase previously described for the separations of compounds was tested using silica gel TLC plates, namely Methanol: Chloroform (11:05 v/v). This mobile phase has allowed us to visualize difference among extracts at 366 nm, which showed Rf value as shown in (table 02). The HPTLC of EEPF, EAPF, NBPF are recorded at 366 nm after scanning UV 366 nm was given in figure 1,2,3.

Sr.No.	EEPF		EAPF		NBPF	
	Rf	**Area**	**Rf**	**Area**	**Rf**	**Area**
1	0.112	25.33	0.157	45.75	0.167	5.69
2	0.203	36.77	0.203	23.20	0.233	87.20
3	0.936	37.90	0.379	31.05	0.862	3.16
4	-	-	-	-	0.969	3.95

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

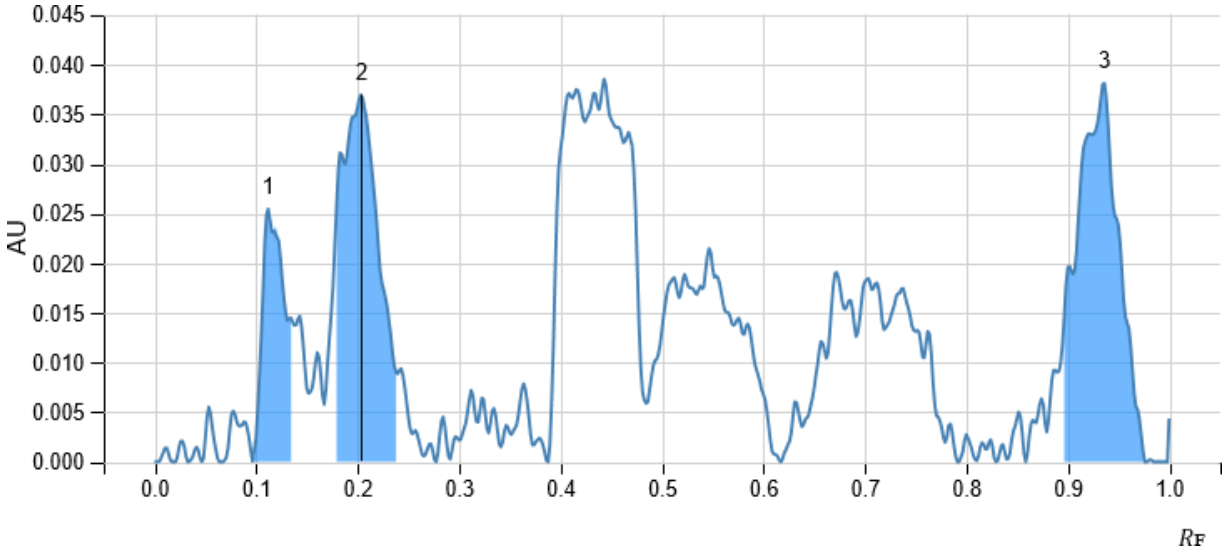

Figure 01. High Pressure Thin Layer Chromatography (HPTLC) densitogram of ethanolic Extract of *P. fistulous* (EEPF) at 366 nm

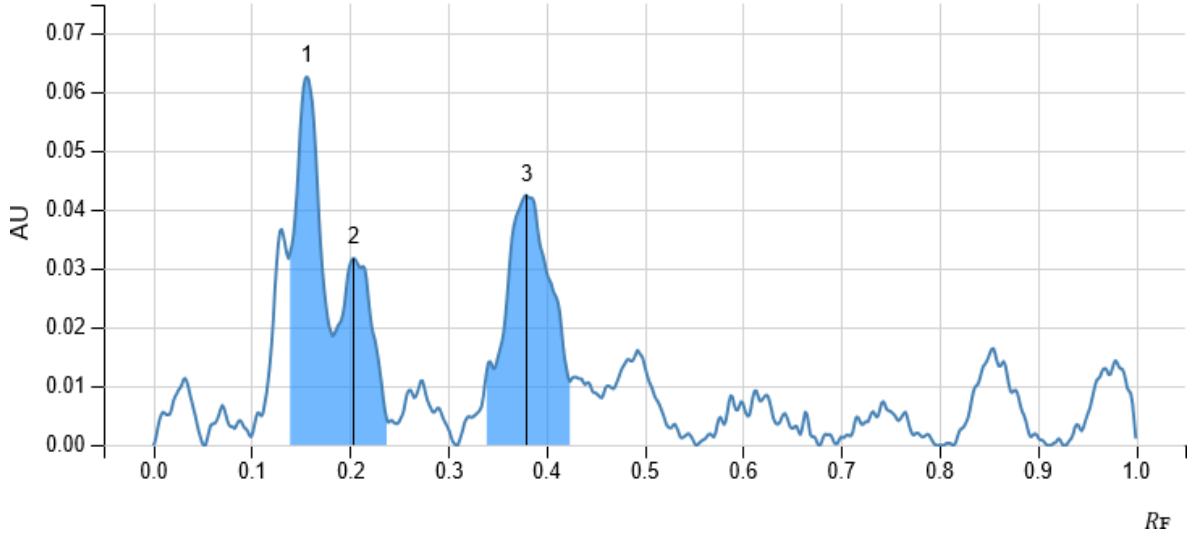

Figure 02. High Pressure Thin Layer Chromatography (HPTLC) densitogram of ethyl acetate fraction of *P. fistulous* (EAPF) at 366 nm

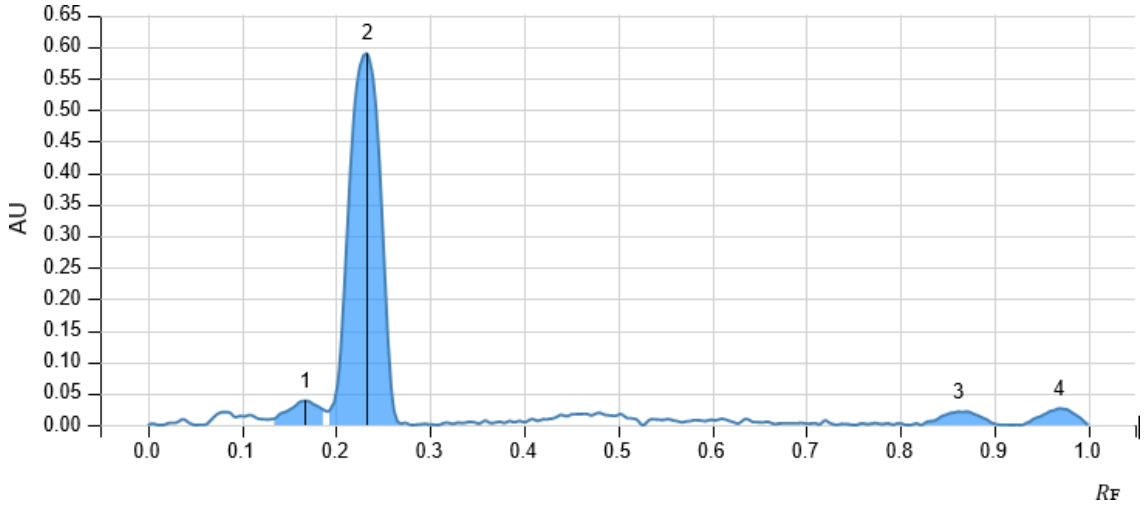

Figure 03. High Pressure Thin Layer Chromatography (HPTLC) densitogram of N-Butanol fraction of *P. fistulous* (NBPF) at 366 nm

DPPH radical scavenging assay

The results of DPPH radical scavenging assay of various concentrations of extract and ascorbic acid (STD) is depicted in (table 03). EAPF showed highest concentrations at 100µg/ml as given in figure 04.

Concentration (µg/ml)	% Antioxidant of *P. fistulous*			
	EEPF	EAPF	NBPF	Ascorbic acid (STD)
10	11.15 ± 0.35	18.2 ± 0.48	11.26 ± 0.26	24.82 ± 0.17
20	22.6 ± 0.65	28.58 ± 0.59	20.48 ± 0.32	32.62 ± 0.55
40	35.88 ± 0.72	39.5 ± 0.62	32.9 ± 0.84	48.5 ± 0.69
60	48.48 ± 0.85	55.6 ± 0.42	40.89 ± 0.72	62.84 ± 0.85
80	60.89 ± 0.72	69.82 ± 0.35	50.72 ± 0.19	75.89 ± 0.22
100	76.65 ± 0.69	82.6 ± 0.85	70.89 ± 0.20	85.19 ± 0.96

Table 03. DPPH data of EEPF, EAPF and NBPF fractions of *P. fistulous*

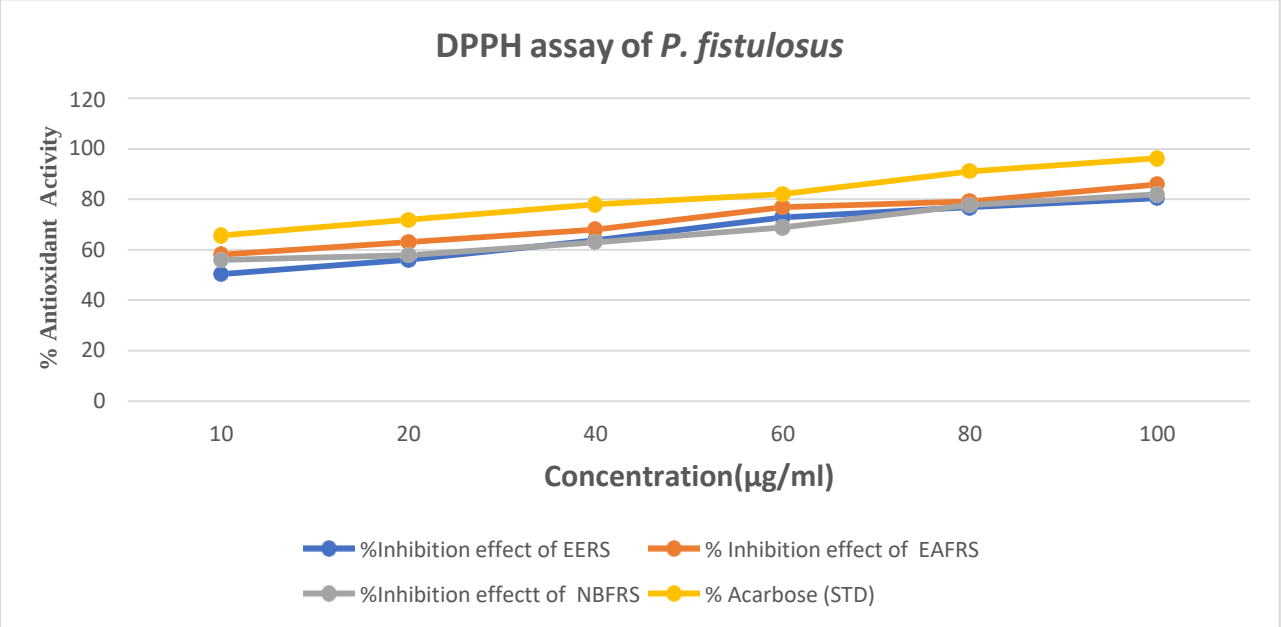

Figure 4. DPPH radical scavenging assay of *P. fistulosus*

Hydrogen Peroxide scavenging assay

The results of hydrogen Peroxide scavenging assay of various concentrations of extract and ascorbic acid (STD) is depicted in (table 04). EAPF showed highest concentrations at 100µg/ml as given in figure 05.

Concentration (µg/ml)	% Scavenging effect of *P. fistulous*			
	EEPF	EAPF	NBPF	Ascorbic acid (STD)
10	16.28 ± 0.148	24.18 ± 0.159	19.48 ± 0.225	40.6 ± 0.960
20	24.38 ± 0.260	35.26 ± 0.295	20.19 ± 0.320	59.7 ± 0.860
40	30.52 ± 0.315	45.16 ± 0.350	26.65 ± 0.554	65.48 ± 0.845
60	38.69 ± 0.295	52.84 ± 0.616	37.96 ± 0.614	70.96 ± 0.748
80	48.85 ± 0.420	65.96 ± 0.716	47.72 ± 0.413	80.88 ± 0.619
100	62.96 ± 0.525	84.69 ± 0.925	61.84 ± 0.748	92.89 ± 0.525

Table 04. Hydrogen Peroxide scavenging effect of EEPF, EAPF, NBPF fraction of *P. fistulous*

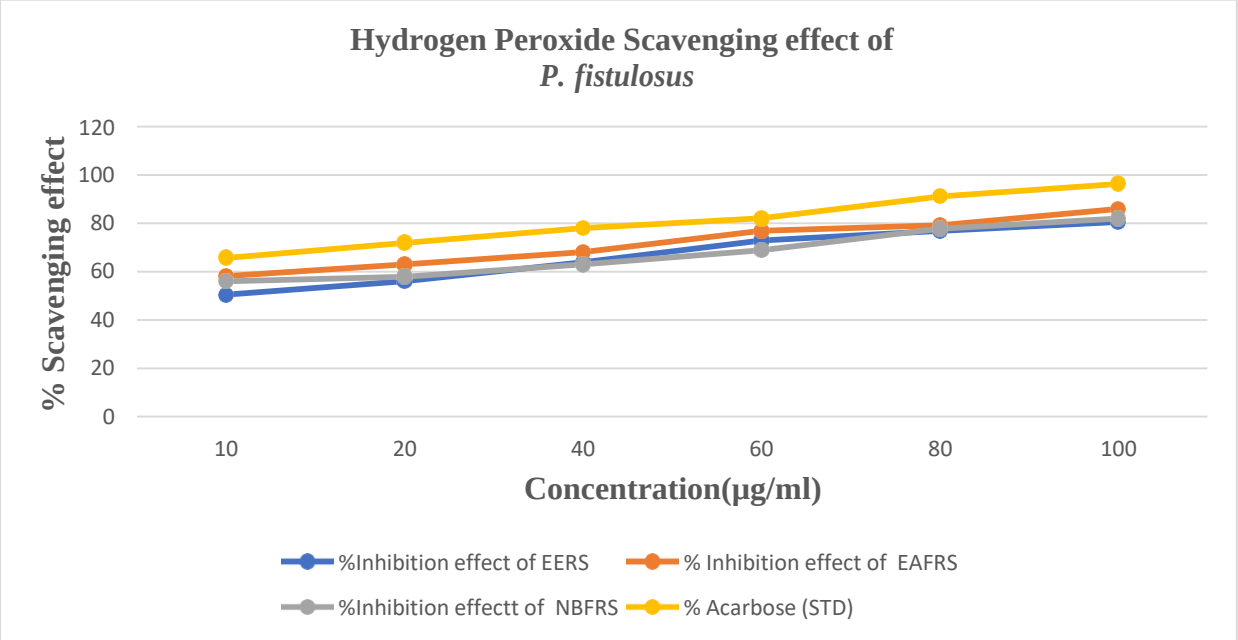

Figure 05. Hydrogen peroxide Scavenging assay of *P. fistulous*

Reducing power assay

The result of reducing power assay of various concentrations of extract and ascorbic acid (STD) is depicted in (table 05). EAPF showed highest concentrations at 100µg/ml as given in figure 06.

Concentration (µg/ml)	Absorbance of *P. fistulous*			
	EEPF	EAPF	NBPF	Ascorbic acid (STD)
10	0.108 ± 0.004	0.115 ± 0.005	0.105 ± 0.006	0.225 ± 0.006
20	0.115 ± 0.004	0.122 ± 0.006	0.115 ± 0.004	0.26 ± 0.005
40	0.295 ± 0.003	0.305 ± 0.006	0.28 ± 0.003	0.37 ± 0.003
60	0.514 ± 0.005	0.525 ± 0.004	0.504 ± 0.005	0.56 ± 0.005
80	0.616 ± 0.005	0.64 ± 0.003	0.606 ± 0.006	0.695 ± 0.004
100	0.78 ± 0.004	0.845 ± 0.003	0.77 ± 0.002	0.883 ± 0.003

Table 05. Reducing Power absorbance of EEPF, EAPF, NBPF of *P. fistulous*

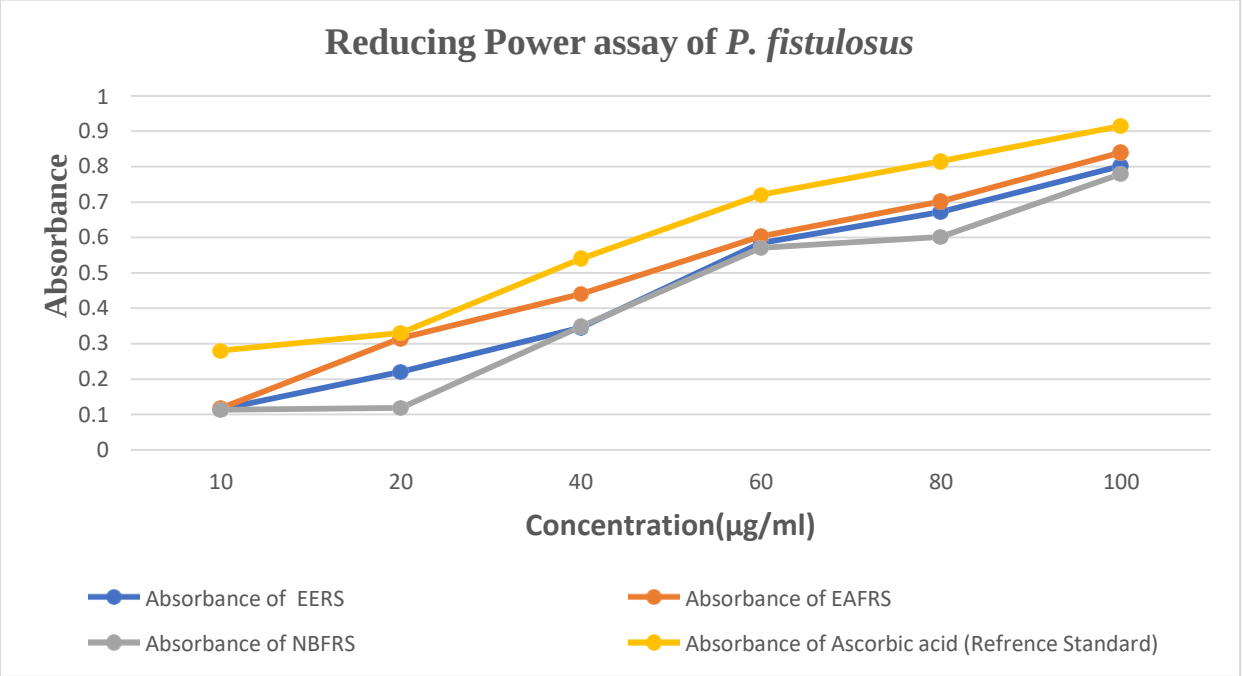

Figure 06. Reducing Power assay of *P. fistulosus*

Alpha Amylase Inhibition assay

The plant extract is EEPF and their fractions EAPF and NBPF tested for potential α-amylase inhibitory effects in this investigation, along with acarbose as a positive control (Table 6). 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 EAPF extract has the highest α-amylase enzyme inhibitory activity 75.20 ± 0.945 at 100 µg/ml (figure 07). Similarly, at 100 µg/mL, the inhibitory activity of EEPF and NBPF was lowest concentration of α-amylase enzyme inhibitory, respectively. At 100 µg/ml, the standard positive control acarbose showed a percentage inhibition of 85.27 ± 0.912 as shown in (table 06)

Concentration (µg/ml)	**% Inhibition of α-amylase enzyme**			
	EEPF	**EAPF**	**NBPF**	**Acarbose (Reference Standard)**
10	12.85 ± 0.485	23.85 ± 0.345	10.12 ± 0.245	35.6 ± 0.616
20	26.9 ± 0.375	39.45 ± 0.545	20.85 ± 0.385	43.72 ± 0.512
40	37.45 ± 0.670	43.67 ± 0.434	31.35 ± 0.525	51.85 ± 0.545
60	49.78 ± 0.537	56.93 ± 0.675	43.85 ± 0.612	68.72 ± 0.345
80	57.95 ± 0.735	72.28 ± 0.825	54.86 ± 0.685	78.18 ± 0.754
100	68.42 ± 0.925	75.2 ± 0.945	67.55 ± 0.725	85.27 ± 0.912

Table 06. α-amylase Inhibition assay of EEPF, EARPF, NBPF of *P. fistulous*

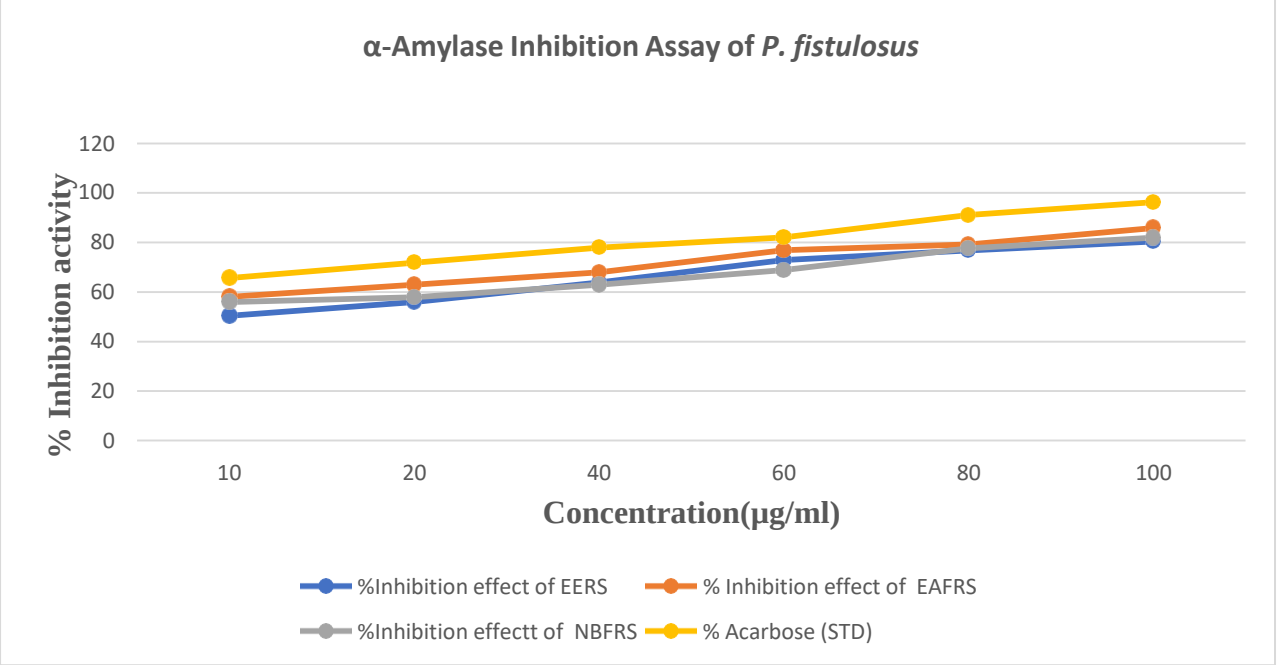

Figure 07. α-amylase Inhibition assay of *P. fistulous*

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 07) showed the glucosidase inhibitory activity of crude extract and fractions (ethyl acetate and n-butanol). The ratio of inhibition of α-glucosidase enzyme increases with the increasing concentration of the extract and its fraction (figure 08).

The EAPF showed the highest percentage inhibition of α-glucosidase enzyme (75.82 ± 0.666 and) whereas the % inhibition of EEPF and NBPF was found to be lowest inhibition of α-glucosidase enzyme respectively. The standard acarbose inhibition of α-glucosidase enzyme at 100 µg/ml about 91.25 ± 0.368 % as shown in (table 07).

Concentration (µg/ml)	**% Inhibition of α-Glucosidase enzyme**			
	EEPF	**EAPF**	**NBPF**	**Acarbose (Reference Standard)**
10	48.38± 0.682	53.26 ± 0.448	50.88 ± 0.587	60.25 ± 0.196
20	50.91 ± 0.543	58.59 ± 0.332	52.35 ± 0.445	68.29 ± 0.569
40	58.33± 0.683	62.59 ± 0.587	60.92 ± 0.445	72.78 ± 0.722
60	62.75 ± 0.730	66.85 ± 0.758	63.66 ± 0.220	79.65 ± 0.729
80	66.82 ± 0.336	72.16 ± 0.588	68.77 ± 0.650	85.65 ± 0.953

100	70.45± 0.520	75.82 ± 0.666	71.80 ± 0.963	91.25 ± 0.368

Table 07. α- Glucosidase Inhibition assay of EEPF, EARPF, NBPF of *P. fistulous*

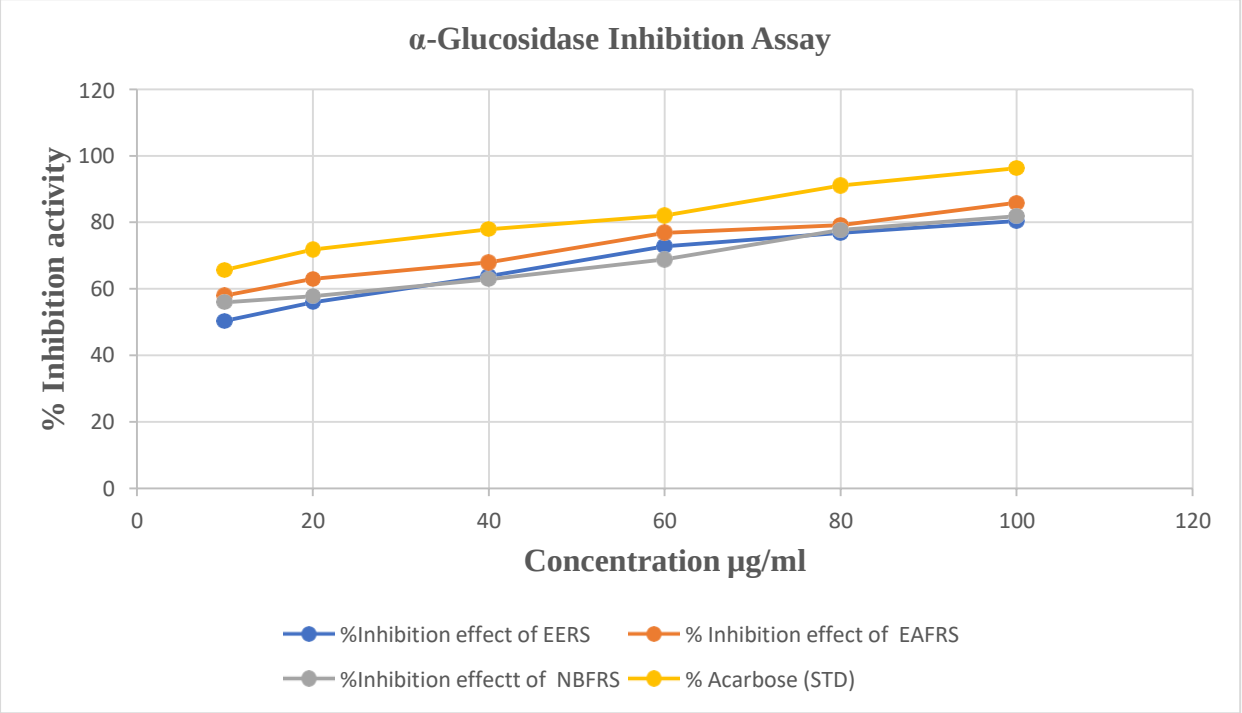

Figure 08. α-Glucosidase Inhibition Assay of *P. fistulous*

Cell Line

Bioassay for Insulin secretion

A stimulation in insulin secretion was exhibited by EAPF leaves at the dose 1000 µg/mL, 11 mM glucose, compared with respective glucose controls. The insulin release was about two folds at hyperglycaemic conditions (11 mM). The maximum insulin secretion was observed at 1000 µg/mL of extract and EAPF as shown in figure 9. The glucose dependent insulin release was significantly potentiated in the presence of EAPF at 1000 µg/ml, table 09 compared with EEPF.

CELL LINE -MIN6-β Cell line

Sr. No	Compounds	Concentration	Insulin secretion (ng/ml)
1.	Control	-	01.46
2.	Standard (Glibenclamide) (STD)	10µM	09.97
3.	Ethanol extract of *P. fistulous* (EEPF)	1000 µg/ml	4.40
4.	Ethyl acetate fraction of *P. fistulous* (EAPF)	1000µg/ml	7.10

Table 10. MIN6-β Cell line Data of STD, EEPF, EAPF of *P. fistulous*

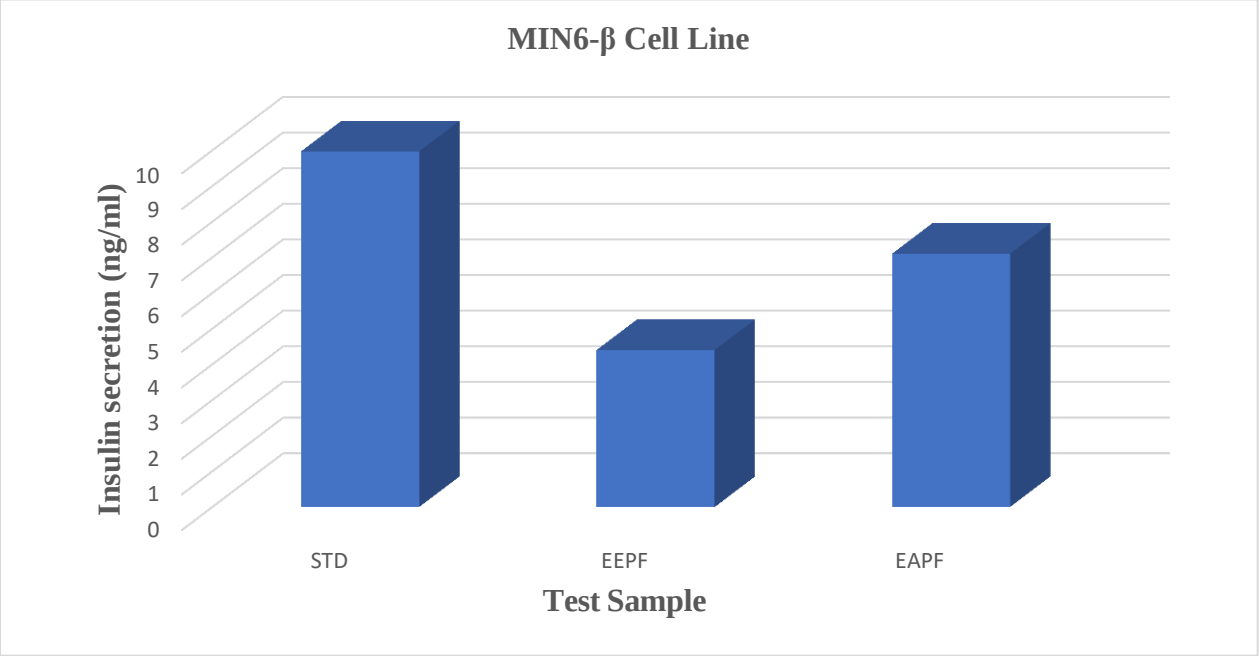

Figure 09. Comparison of insulin secretions observed in MIN6-β Cell line

Discussion

In this current study, antidiabetic activity of EEPF leaves and its fractions EAPF and NBPF was explored using *in-vitro* assays. It was observed that EAPF of exhibits more efficient antidiabetic activity than the leaves ethanolic extract and NBPF. Lack of insulin affects glucose, proteins and fat are metabolised and significantly affects the equilibrium of water and electrolytes. Type 2 diabetes has emerged as a major global health issue in recent years. According to the World Health Organisation, this condition affects more than 176 million people worldwide [20]. Newer pharmacological drugs have been developed as a result of recent developments in our understanding of the function of intestinal enzymes (α-amylase and α-glucosidase both play critical roles in the breakdown of carbohydrates and glucose absorption) [21]. The therapeutic strategy of preventing or reducing carbohydrate absorption after food consumption is one used to reduce postprandial hyperglycemia in diabetic patients. Before being absorbed in the duodenum and upper jejunum, complex starches, oligosaccharides and disaccharides must be broken down into monosaccharides by α-amylase and α-glucosidases [22]. The studies conducted in research were based on extensive literature review and data. Since the plant exhibits potential blood glucose lowering effects, the study planned to evaluate crude extract, ethyl acetate and n-butanol fractions. The ethanolic extracts was prepared using Soxhlet extraction method. Phytochemical analysis of the EEPF leaves showed the likely presence of phytoconstituents like carbohydrates, steroids, flavonoid, proteins etc., The extraction were followed by fractionation using ethyl acetate and n-butanol. Antioxidant activity of DPPH, Hydrogen peroxide Scavenging assay of *P. fistulous*, Reducing Power assay showed antioxidant activity at highest concentration of EAPF. HPTLC analysis of EEPF along with the solvent fraction showed the sample contain different Rf values. EAPF showed the greatest number of Rf values is 0.157, 0.20, 0.379 and EEPF and NBPF showed least number of Rf values is 0.112, 0.203, 0.936 and 0.167, 0.233, 0.862, 0.969 respectively. In our studies showed robust inhibition of the digestive enzyme α-amylase and α-

glucosidase. The highest % inhibition activity of α-amylase and α-glucosidase is 75.2% and 75.82 was observed in the EAPF while the least was observed in EEPF and NBPF (68.42% and 67.55), (70.45 % and 71.80%) respectively. Acarbose drug was used as standard for comparative analysis which showed % inhibition. The result of the α-amylase enzyme inhibition activity of *P. fistulous* extract showed a dose-dependent inhibition activity. The α-glucosidase inhibitory activity a potent concentration-dependent effect was demonstrated for the ethyl acetate fraction. The *P. fistulous* extracts tested in this study have significantly inhibited the enzyme. 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. 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. Based on *In-vitro* assays carried out on EEPF and its EAPF and NBPF exhibited highest activity when compared to n-butanol fraction, thus ethyl acetate fraction was selected for the cell line study. A MIN6-β 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 EAPF compared with EEPF leaves extract demonstrating the significant role of cell glucose metabolism in the ethyl acetate fractions ability to secrete insulin.

Conclusion

This study has revealed that the EEPF 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 percentage of insulin secretion by MIN6-β cells were exhibited by EAPF. In addition, research should concentrate on the bioactive components of *P. fistulous*, 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.

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