In Vitro Studies On Alpha Amylase, Alpha Glucosidase Inhibitory And Antioxidant Activities Of Methanolic Leaf Extract Of Abelmoschus Ficulneus(L) Wight& Arn

SHAIK YASMIN [1], VANAPATLA SWAROOPA RANI [2]*

Address:
Department of pharmacognosyUCPSc, kakatiya university, Hanumakonda 506001, Tealangana, India.

Corresponding author : vanapatla swaroopa rani, swarooparanivanapatla@kakatiya.ac.in

Professor, Department of pharmacognosy, UCPSc, kakatiya university, Hanumakonda 506001, Tealangana, India.

ABSTRACT

Diabetes is a metabolic dysfunction in which body has elevated blood sugar levels. The inhibition of α-amylase and α-glucosidase enzymes reducing postprandial blood sugar levels by delaying the breakdown and uptake of carbs.In the current study methanolic leaf extract and its various fractions (Butanol, Ethyl acetate, Toluene, & aqueous) of *Abelmoschus ficulneus* determine their ability to inhibit α -amylase & α -glucosidase as well as to observe their antioxidant activity. The crude extract & its fractions was evaluated at different concentrations to determine the concentration that produced the highest level of inhibition. Phytochemical investigation of the crude extract & its fractions confirmed the occurrence of flavonoids, steroids/terpenoids, carbohydrates, phenolic compounds and tannins. Among all the fractions tested,the butanolic fraction exhibit the highest poentcy against both α-amylase (IC_{50} = 6.25mg/ml) and α-glucosidase (IC_{50}= 5.90mg/ml), this values comparable to those of acarbose(IC_{50} =5.15mg/ml α-amylase and IC_{50}=5.01mg/ml α-glucosidase).The butanolic fraction of *Abelmoschus ficulneus* also demonstrated moderate to good antioxidant activity, along with strong reducing power assay. Furthermore, this fraction showed the highest TPC 97.5µg/ml and TFC 20µg/m among all the extracts tested. These results indicate that the elevated TPC and TFC in the Butanolic fraction contribute to its potent enzyme inhibition and antioxidant effect, suggesting potential for controlling postprandial hyperglycemia in diabetes management**.**

Keywords: Diabetes, *Abelmoschusficulneus,* α-amylase, α-glucosidase, Acarbose

INTRODUCTION

Diabetes is a chronic illness that causes the body sugar to be elevated. It occurs due to a shortage of insulin from pancreatic beta cells(or) by the pancreas or the body failing to utilize the insulin produced by the pancreas. Diabetes is noncurable, and controllable. When not treated or managed it would cause severe health issues[6]. Diabetes is classified into 3 types :Type IDDM(requires insulin), Type NIDDM (no insulin is required immediately), and diabetes during gestational period.Most frequently occurring metabolic dysfunction and its frequency is increasing swiftly among smoking, unhealthy diet, physical inactivity, and obesity **[Fathima, A., Dev, A.2022].**

According to IDF2025 diabetes mellitus can cause severe complications, including kidney failure and heart failure. 58.99 core Adults (20-28yrs) are affected by diabetes in 2025 this population size is envisaged to rise to 85.3core by 2050 (**www.diabetesatlas.org**).

The polysaccharides are broken & gets absorbed in the intestine with the help of α-Amylase and α-glucosidase.α-Amylase digests carbohydrates into simpler saccharides, while α-glucosidase breakdown into absorbable monosaccharides like glucose. Inhibition of these two enzymes is believed to diminish postprandial glucose in blood circulation after intake of Diet/nutrition packed with carbohydtrates [**Al-Zuhair S, Dowaidar A 2010]**. Enzyme inhibitors that are currently recommended in the treatment of adult-onset diabetes mellitus include acarbose,However, patients taking acarbose frequently experience gastrointestinal after effects, comprising abdominal swelling, Eructation, Tympanites, & diarrhea. These after effects are attributed to abundant inhibition of α-amylase that leads to incomplete polysaccharide digestion in small intestine. As a result, undigested carbohydrates reach the colon, where they undergo excessive bacterial fermentation [**Al-Zuhair S, Dowaidar A 2010**]. The inhibition of these enzymes is facilitated with the antioxidants specifically flavonoids & phenolics [**Al-Zuhair S, Dowaidar A 2010**]. Natural antioxidants such as phenolic compounds and flavonoids have been shown to inhibit oxidative stress, and in consequence, delay the onset and prognosis of diseases, including diabetes mellitus not to mention being able to inhibit oxidative stress display digestive enzymes inhibitory activities**[Nagamani Mukka *et., al* 2020& Tunna TS et.,al 2015,.20].**

Wight and Arn. *Abelmoschus ficulneus* (L.). It is (Family: Malvaceae), native rosella or jangli bhindi.and may be occuerd in wilderness & cultured fields of many Indian (including Tamil Nadu, Kurnool district, Kadapa, Andhra Pradesh state) and other countries (Africa and northern Australia)[**Swetha urugonda and swaroopa rani V.2021**].

Its flowers are small white to creamish,often with a rose or reddish pink center. The leaves palmately lobed with 3-5,often coarsely textured/rough on both surfaces,with serrated margins.Ground seeds have been used in respiratory issues, including asthma and breathing problems, leaves as constipation, roots regarded as a heart tonic and aid for calcium deficiency. Its paste is used in treating cuts & bruises. Root powder is used in jaundice and GIT problems[**Swetha urugonda and swaroopa rani V.2021**].Leaves contain phytochemicals such as beta-sitosterol & beta-D-glucoside derivatives.These steroidal compounds are common in many plants and often linked to anti-inflammatory cholesterol modulating effects. Flowers are rich in anthocyanins (pigments responsible for red/purple/blue colors,known for strong antioxidant properties)**[Swetha urugonda and swaroopa rani V.2021**].Petals contain betasitosterol along with various flavonoids[**Swetha urugonda and swaroopa rani V.2021].** Seeds have an essential oil[**Sinha.S and Osman.S.M.1982**]. The leaves are reported with antimicrobial activity [**Kalpana.S and Thangapandian. V.2017**].The composition of nutraceuticals as well as antioxidant activity were evaluated [**Ashwini.V.M2019**] and hepatoprotective has been reported from the root extract [**Swetha urugonda and swaroopa rani V. 2021**]. However, the α-amylase and α-glucosidase inhibitory activity of *Abelmoschus ficulneus* not been investigated in previous studies.Therefore, this study aimed to evaluate the inhibitory activity against α- Amylase and α-Glucosidase.

MATERIALS AND METHODS

Drugs, chemicals and solvents:

α-Amylase is extracted from the Porcine pancreas, α-Glucosidase from yeast, Para-nitro phenyl α-D-glucopyranoside, 3,5- Dinitrosalicylic acid, Sodium potassium tartarate, Acarbose, Potassium dihydrogen phosphate, Sodium dihydrogen phosphate, Disodium hydrogen phosphate and di potassium hydrogen phosphate, 1,1Diphenyl-1-picryl hydrazyl(DPPH), Thiobarbituricacid(TBA), Griessreagent, Nitro blue tetrazolium(NBT), Nicotinamide adenine methosulphate(NADH), Phenazine buffer saline(PBS), Phenazine methosulphate (PMS) were procured Taranath scientific's Hanamakonda, Telangana, India. All other reagents and solvents employed were of analytical reagent(AR).

Collection of plants materials: Leaves of *Abelmoschus ficulneus* were collected from Maripeda banglow village, Mahabubab dist, Telangana,India. Plant material was authenticatedbyDr.MD.Mustafa[Taxonomist],Botany Department, Kakatiya University. The plant specimen [KU/UCPSC/57] was stored in the Pharmacognosy Department, UCPSc, kakatiya university, Hanumakonda, India.

Preparation of methanolic extracts and its various fractions: The methanolic extracts and different fractions were prepared by taking the fresh leaves of *Abelmoschus ficulneus* (120grams) was collected,washing them in running tap, Air dried in the shade crushed into small granules and the crushed material were macerated in Erlenmeyer flask by using methanol for 1 week of time period. The filtarate were pooled, then evaporated to yield blackish green crude extract.The crude extract obtained was dissolved in deionized water and partitioned with Ethyl acetate, Butanol, Toluene &deionized water one after the other.

Phytochemical screening: The meathanolic extract and its different fractions of *Abelmoschus ficulneus* was subjected to phytochemical tests were conducted as per standard protocol for the detection of secondary

metabolites like Alkaloids, flavonoids, steroids/terpinoids, carbohydrates, tannins, glycosides, saponins and phenolic compounds.

Estimation of Total phenolic content

Phenolic content, was estimated in terms of Colorimetric method that involved the use of the enzyme to oxidize an H_2O_2 solution in the presence of titrite solution to produce a blue color in the solution [**Fathima, A *et .,al* 2022&.Marinova D 2025**].To measure the total poly phenolic content was measured by performing, Folin-ciocalteu assay which was depicted in literature [[**Fathima, A., *et .,al* 2022&.Marinova D 2025**]. The 25 ml of volumetric flask containing 9ml of deionized water was taken, to this 3,4,5-trihydroxy benzoic acid extract (100-1000microgram/ml) or reference solution (10-1001000microgram/ml) was added.The sample is replaced with the blank solution that was prepared using deionized water which contain 1ml folin cio calteau phenol reagent mixture &swirled. The mixture was then mixed with 10 ml of 7% solution of aqueous sodium carbonate after 5 min. The dilution mixture was mixed with a 50 ml of the double distilled water. The absorption at 750nm(Shimadzu1800UVVisibleSpectrophotometer) was then established after incubating the preparation with a prepared reagent blank after 90 min at room temperature. The amount of phenolics present in a gram of extract is expressed as equivalent wt of gallic acid [**Oyaizu, M. (1986)].**

Estimation of Total flavonoid content

The TFC was ascertained using $AlCl_3$ colourimetric method.
The 4ml of double deionised water is taken in 10ml volumetric flask to this 10-100 Mcg/ml extract or rutin reference solution 2.5-20Mcg/ml was added[**Fathima, A *et., al* 2022&, sayyada saleha2022**].To this 0.3ml of 5%$NaNO_2$ solution was added & incubated for 5min. Later 0.3ml of 10% $AlCl_3$ was added & incubated for 6minutes followed by addition of 2ml of 1M NaOH and make up the volume upto10ml using deionised water by mixing homogeneously. Later the measured absorbance was recorded at 510nm against reagent blank preparation[**Nagamani Mukka *et.,al* 2020].**
The amount of flavonoids present in a gram of extract is expressed as equivalent wt of rutin.

α- Amylase enzyme inhibiton Assay:

This assay was conducted as per McCue and Shetty[**McCue PP and Shetty K 2004**] with minor modifications. Extracts (1.25-10mg/mL) were put in a tube followed by the addition of 250µL of 0.02M sodium phosphate buffer (pH6.9) containing 0.5mg/ml α-amylase solution. And the given solution was pre incubated up to 37° C at 15 mins after which 250µL of 1% starch solution in 0.02M sodium phosphate buffer (pH6 9) was added at specific time period and the solution was moreover incubated at 37° C at 15 mins. 500µl of DNS reagent solution was to terminate the reaction. They were then left to cool at room temperature by putting the tubes into boiling water 5min. It was added to 5mL of demineralized water. The absorbance of the reaction mixture was recorded at 540nm using (Shimadzu1800UVVisibleSpectrophotometer). A control sample was prepared by using above procedure with the replacement of extract with demineralized water.

α-Glucosidase enzyme inhibition assay:
Inhibition of alpha-glucosidase was measured respectively by following the procedure mentioned by Apostolidis with certain modifications [**Apostolidis E,2007**]. The extracts was dissolved in DMSO at different concentrations (1.25-10mg/mL) the 50 µL each extracts (1.25-10mg/mL) was taken in different test tubes, then add the 100µL of yeast α-glucosidase solution containing 0.02M in phosphate buffer (pH 6.9) was incubated for 15mins at 37ºC[**sayyada saleha *et.,al* 2020**]. After which 50 µL of 0.1M phosphate buffer containing P-NPG were added. Incubation was then done in the reaction mix at 37ºC within 5min and pouring 3mL of 100Mm Na_2CO_3 solution to retard the reaction. The intensity of yellow colour of P-nitrophenyl was determined by measuring its absorbance at 405nm using (Shimadzu1800UV-Visible Spectrophotometer).To evaluate the activity of sample acarbose used as positive control & the α- amylase and α-glucosidase inhibition was calculated using the following formulae[15].
% inhibition= (Abs(control) -Abs (extract) /Abs (control) x 100

Determination of antioxidant activity

A.DPPH free radical-scavenging assay method

The DPPH 1ml of 0.1mmol/Liter In CH_3OH)scavenging activity of methanolic extract of leaves of abelmoschus ficulneus & its fractions 2.5ml (10-200Mcg/ml) were evaluated as per previously reported method[**Blois, M.S. (1958)**]. In comparison with Ascorbic acid (0.25–6 µg/mL as control.At 517nm absorbance was measured using Shimadzu1800UV Visible Spectrophotometer. Dpph assay was calculated using following formula
% scavenging = abs(control) - abs (sample)/ abs (control)*100

B.Superoxide Radical Scavenging Assay

The assay was done according to the method described in the literature with minor modification (**Nishikimi *et al.,* 1972**). Rutin was used as a positive control in the assay. 1.0 ml each of NBT solution (156 µm in 0.1 M PBS, pH 7.4), NADH solution (468 µM in 0.1 M PBS. pH 7.4) and test extract (dissolved in 0.1 M PBS, pH 7.4) of different concentrations (25-600 µg/ml) and the standard, rutin in different concentrations (0.25-6 µg/ml) were mixed. Then 1 ml of PMS solution (60 µM in 0.1 M PBS, pH 7.4) was added and incubated at 25°C for 5 min. The absorbance (A1) of all these and the samples (As) containing PBS instead of NBT was measured against a blank (Ao) (0.1 M PBS. pH 7.4) at 560 nm.
% scavenging activity = $\{[A_0-(A_1-A_2)] / A_o\}$ x 100
A_0:negative control of absorbance,
A_1: Absorbance of test substance
A_2: Absorbance of sample under identical conditions as A with PBS instead of NBT solution.

C.Nitric oxide scavenging assay :

Nitric oxide scavenging activity was determined as described previously [**GreenL.C,wagner,D.A,1982].** 10 mL of 10mM [Na_2 [Fe(CN)NO]in phosphate-buffered saline (pH 7.4) was mixed with 1 mL of crude extract (10-200 µg/mL). The mixture was incubated at 25 °C for 150 min. After incubation, 1.0 mL of Griess reagent was added and the solution was kept at 25 °C for 30 min. The absorbance of the pink chromophore formed was measured spectrophotometrically at 540 nm.using a blank sample as a reference. As a standard, ascorbic acid was used.

D.Ferric reducing power assay method

Colorimetric method was used in determining a total reducing power as as previously described [**Oyaizu, M. (1986)**]. Stock solutions were prepared by dissolving 10mg of the extract in 1ml of DMSO and working concentrations (10-100 µg/ml) were prepared in PBS (0.2 M). 2.5 ml of the testextract at different concentrations was mixed with 2.5 ml of 1% potassium ferricyanide. After incubating the mixtures at 50°C for 20 mins, 2.5 ml of 10% TCA was added and centrifuged at 1,036 rpm for 10 min. Then 2.5 ml of upper layer of reaction mixture was moved with 2.5ml of distilled water and 0.5 ml of 0.1% freshly prepared $FeCl_3$ solution The absorbance of the reaction mixture was of 0.1% freshly prepared $FeCl_3$, solution. The absorbance of the reaction mixture was measured at 700 nm. Ascorbic acid was used as positive control. The absorbance of the reaction mixture is directly proportional to the antioxidant capacity of the test sample.

Thin layer chromatography : The occurance of the active constituents (flavonoids) in the leaves extracts of the *A. ficulneus L.* was identified through the application of thin-layer chromatography (TLC) on a silica gel 60 TLC plate. TLC was done by spotting 5 µl of each extract onto chromatographic plates with $C_4H_8O_2$:H_2O:CH_2O2:$C_2H_4O_2$(100:26:11:11, v/v/v/v) as the solvent system for the extracts. Flavonoid were detected by performing TLC studies where spraying withPEG forms the of yellowish brown colour.These spots were visualized under UV light at 254nm and 365nm [**Wagner HBS(1996**].
R_f = (Distance from baseline to spot centre)/(Distance from baseline to solvent front)

RESULTS

Phytochemical analysis: The results of preliminary phytochemical screening of methanolic leaf extract and its various fractions of *Abelmoschus ficulneus* were as in table 1 which indicated the presence of carbohydrates,steriodas/terpinoids,flavonoids, glycosides, tannins and phenolic compounds.

Total Phenolic Content and Flavonoid Content Estimation:
The equilibrium of the polyphenols concentration in the test extracts quantified against gallic acid standard curve[**Fathima, A *et.,al* 2022**]. The phenolic content of methanolic leaf extract of *Abelmoschus ficulneus* and its fractions(TLAFME,BLAFME,EAAFME,AQAFME)was found to be 24.2mg,20.5mg,97.5mg,95.2mg, and 26mg of GA equivalent per gram of extract respectively.
The yield of flavonoid content available in the respective extracts is estimated using a standard curve made of Rutin [**Fathima, A *et.,al* 2022**]. The amount of total flavonoid of Methanolic extractandfractions (TLAFME,BLAFME,EA-FME,AQ-AFME) of *Abelmoschus ficulneus* was found to be 9.42mg,7.47mg,20mg,19.64mg,and 8.50of Rutin equivalent per gram of extract respectively. BN-AFME fraction showed higher levels of phenolics and flavonoid contents compared to the methanolic extract and its different fractions of *Abelmoschus ficulneus* [**Fathima,A *et.,al*2022**].

Table1: **preliminary phytochemical screening**

Phytocompounds	AFME	T-AFME	EA-AFME	BN-AFME	AQ-AFME
Alkaloids	-	-	-	-	-
Flavonoids	+	+	+	+	+
Stoids/terpinoids	+	+	+	+	+
Phenolic compounds	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Glycosides	+	-	+	+	+
Tannins	+	+	+	+	+
saponins	-	-	-	-	-

' +' present ' –' absent

α -Amylase and α -Glucosidase Enzyme Inhibitor Assays:

In the current study, the in-vitro method was used for testing of methanolic leaf extract of *Abelmoschus ficulneus* along with its different fractions (TL-AFME, BL-AFME, EA-AFME, AQ-AFME) and acarbose was analyzed on the basis of inhibitory activity against the α-Amylase & α-Glucosidase enzymes. Methanolic leaf extract of *Abelmoschus ficulneus* and its various fractions((TL-AFME,BL-AFME,EA-AFME,AQ-AFME)(at a concentration of 10mg/ml)exhibited 67.9%,60.3%,78.5%, 72.3%and 63.5% Fig.1 present the α-amylase inhibitory activity and 70.5%,65.5%80.5%, 77.3% and 68.9% Fig2 present the α-glucosidase inhibitory activity. Acarbose showed 92.5% α-amylase inhibitory activity & 95.5% α- glucosidase inhibitory activity at same concentration with IC_{50} value of α-amylase 5.15mg/ml & IC_{50} value of 5.01mg/ml α- glucosidase. However, the butanol fraction of *Abelmoschus ficulneus* exhibited the most significant dose dependent-inhibitory activity against carbohydrate hydrolyzing enzymes with the IC_{50} values of 6.25 mg/ml (αamylase) and 5.90mg/ml (α-glucosidase).Its potential to comparable to reference drug acarbose[**Fathima, *A et.,al* 2022**].

Table 2 : Effect of TPC ,TFC of AFME, TL-AFME, BL-AFME, EA-AFME AND AQ-AFME & IC_{50}values of Acrbose &α -Amylase and α -Glucosidase

Extract/ fractions	TPC(mgofGAE/g of extract)	TFC(mgofRE/g of extract)	α-Amylase(IC_{50} mg/ml)	α-Glucosidase(IC_{50} mg/ml)
Methanolic extract(AFME)	24.2	9.42	7.47±0.021	7.00±0.035
Toluene fraction (TL-AFME)	20.5	7.47	8.44±0.025	7.73±0.038
Butanol fraction (BL-AFME)	97.5	20	6.25±0.055	5.90±0.012
Ethyl acetate fraction (EA-AFME)	95.2	19.64	6.90±0.044	6.34±0.056
Aqueous fraction (AQ-AFME)	26	8.50	8.01±0.01	7.30±0.001
Acarbose	N/A	N/A	5.15±0.011	5.01±0.006

Values represented mean±standard deviation of triplicate experiments

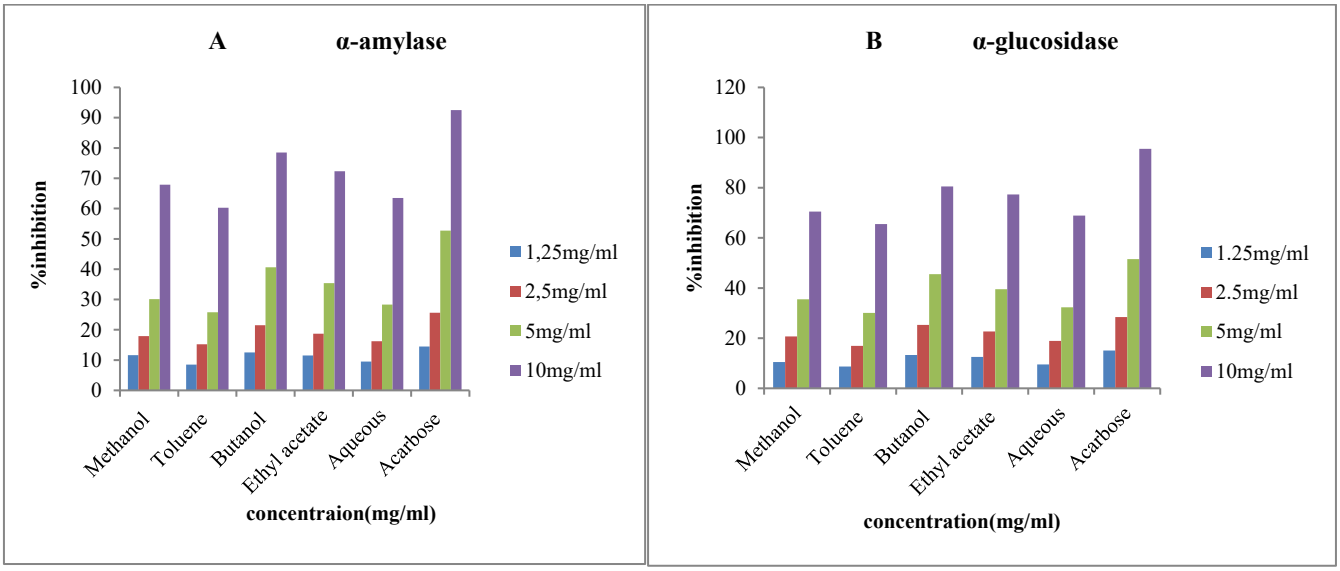


Fig-1 :A: Procine pancreatic α-amylase inhibitory activity of methanolic leaf extract of Abelmoschus ficulneus and its various fractions. Standard: Acarbose

B: Yeast glucosidase inhibitory activity of methanolic leaf extract of Abelmoschus ficulneus and its various Standard: Acarbose Standard: Acarbose fractions. Standard: Acarbose. Data expressed as mean ±SD,(n=3).

In vitro antioxidant activity

A.DPPH radical scavenging assay

The antioxidant property of methanolic leaf extract of *Abelmoschus ficulneus* and different fractions were evaluated based on their ability to eliminate free radicals of DPPH.The reaction of antioxidant compounds with the DPPH radicals comprise of exchange of hydrogen atom or an electron to convert the DPPH radicals into stable1,1-diphenyl-2-picrylhydrazine. This reduction causes a color change to be observed turning purple to pale yellow, which is the evidence of the ROS scavenging potential [**Blois, M.S. (1958)**].

The DPPH was applied to the methanolic leaf extract of *Abelmoschus ficulneus* and different fractions,as well as the standard antioxidant (ascorbic acid) with a concentration of 10 to 200 ug/ml, to compare them. The crude methanolic extract and its fractions viable had Concentration-dependent ROS reactive oxygen species scavenging activity was compared with standard $C_6H_8O_6$(vitamin C) Overall, they displayed moderate to good antioxidant activity. The IC_{50} values of DPPH assay for the *Abelmoschus ficulneus* methanolic leaf extract and its fractions(TLAFME,BLAFME,EAAFME,AQAFME)199µg/mL,22µg/mL,23 µg/mLand159µg/mL,this values were contrast with standard vitamin-C showed half maximul inhibitory activity with 3mcg/ml (result table4). When IC_{50} is lower the antioxidant activity is stronger and therefore the butanol fraction (BL-AFME) shown elevated antioxidant potential the midst tested samples.

The percentage inhibition versus concentration curves for the standard ascorbic acid and the plant extracts are presented in figure 3 and figure 4, respectively.

B.Superoxide Radical Scavenging Assay

Some of the most dangerous of the free radicals generated in living things include superoxide (O_2^-) and hydroxyl (OH•) radicals. One of the significant sources of the ROS is superoxide & these radicals have the potential to harm cells by oxidizing other molecules. Hence, oxidative stress is significant in the body defense by the capacity to counter the effects of superoxide[**Estévez, A. G., & Jordán, J. (2002)**].

The superoxide radical scavenging activity of the methanolic leaf extract and its fractions of *Abelmoschus ficulneus* was investigated, as shown in Table 8. The extract and its fractions exhibited moderate scavenging activity.Among all the fractions tested butanolic fraction (IC_{50} = 156 µg/mL) exhibited the maximum superoxide radical scavenging activity and the ethyl acetate fraction (IC_{50} = 163 µg/mL), displayed mild activity. The activity is probably attributed to phenolic compounds whose free hydroxyl groups have the capacity of donating electrons to neutralise superoxide.

The graphs showing the percentage inhibition over variable concentrations of the standard, the methanolic extract, and its fractions are presented in Graph 5 and Graph 6, respectively.

The graphs showing the percentage inhibition over variable concentrations of the standard, the methanolic extract, and its fractions are presented in Graph 5 and Graph 6, respectively.

C. NO radical scavenging activity

The NO is not stable in the air conditions and reacts with O_2 to generate nitrate and NO_2^-. Nitrite can be quantified by measuring the NO amount through the use of the Griess reagent[18].As a compound scavengers the nitrous acid is reduced as it is constructed, and this reduces the level of nitrite, which represents scavenging[**Sharan Suresh Volluri 2011**].

The IC_{50} values for nitric oxide scavenging activity were determined for the methanolic leaf extract of *Abelmoschus ficulneus* and its fractions (TL-AFME,BL-AFME, EA-AFME, AQ-AFME). These values were 180 µg/mL for the crude methanolic extract,23mcg/ml, 25 mcg/ml and 88mcg/ml these results were compared with the reference drug ascorbic acid, which exhibited an IC_{50} value of 8 mcg/mL (as shown in Table 4).Since a lower IC_{50} value indicates higher antioxidant (scavenging) potency, the butanol fraction (BL-AFME) demonstrated the highest nitric oxide scavenging activity among all the tested fractions and the crude extract.

The percentage inhibition versus concentration for the standard ascorbic acid is presented in figure 7, while the corresponding data for the plant extracts/fractions are shown in figure 8.

D.Reducing capacity assay

The reducing capacity is a measure of the capacity of the compound to give electrons and to break the free radical chains and is an antioxidant. This assay can be performed by the reduction of ferric to ferrous in the ferricyanide complex to form Fe 2+ to produce Prussian blue of Perl at 700 measures percyanide[**Uma shanker sharma 2011**].

The reducing capacity of all the solvent extracts demonstrated rising reducing power with rise in concentration of the extract and this activity was similar to that of the standard ascorbic acid[**Sharan Suresh Volluri 2011**]. The concentration required for 50% inhibition (or effective concentration providing equivalent reducing power) was determined to be 47 microgram/mL for the methanolic leaf extract of *Abelmoschus ficulneus*, and 71, 26, 65, and 52 microgram /mL for its various fractions (BL-AFME, TL-AFME, EA-AFME, and AQ-AFME, respectively), as compared to 80microgram /mL for the reference ascorbic acid. Among all the fractions, the butanol fraction (BL-AFME) exhibited the highest reducing capacity, which was superior to that of the reference drug ascorbic acid. Butanol fraction (BL-AFME) exhibited the highest reducing capacity, which was superior to that of the standard ascorbic acid.

Thin layer chromatography

The thin-layer chromatogram of the methanolic leaf extract of *Abelmoschus ficulneus* and its fractions was presented in Figure. 3, with the results aresummarized in Table 4. Based on the R_f values, the spots in the butanol, methanol, aqueous, ethyl acetate, and toluene fractions were observed at 0.33, 0.32, 0.31, 0.32, and 0.30 respectively. These R_f values were compared with the standard R_f value of the flavonoid rutin(0.35), resulting in detection of rutin as the flavonoid compound occured in the extract of *A.ficulneusL.*

Table 3: Thin layer chromatography of methanolic leaf extract of *Abelmoschus ficulneus* and its various fractions

Phytochemical	R_f value	Result	standard
Butanol extract	1.5/4.5	0.33	Rutin
Ethyl acetate extract	1.6/4.9	0.32	Rutin
Methanol extract	1.7/5.2	0.32	Rutin
Aqueous extract	1.4/4.4	0.31	Rutin
Toluene extract	1.5/4.9	0.30	Rutin

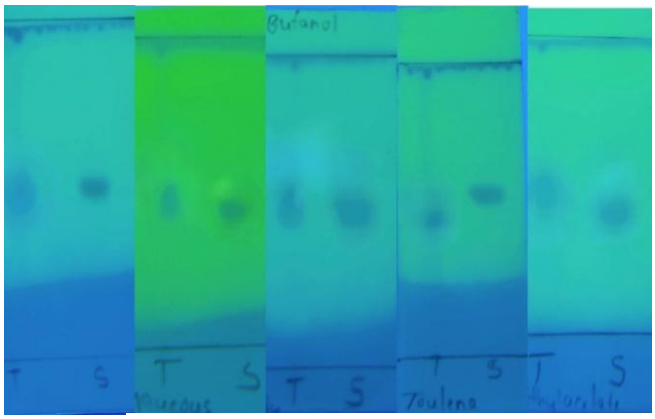


Fig2: TLC of standard, methanolic leaf extract and its fractions

DISCUSSION

Diabetes is a metabolic dysfunction in which body has elevated blood sugar levels. α- Amylase is an abundant enzyme which is found in pancreatic juice, saliva and several other tissues. Salivary amylase separates coarse, sticky molecules into smaller and soluble molecules. Those lesser portions are made maltose and dextrins[**Magaji, U *et.,al* 2019**]. The hydrolysis of starch is carried out by pancreatic amylase, which results in a dextridin is a mixture of dextrins, maltose and maltotriose[**Magaji, U *et.,al* 2019**].

On the other hand alpha-glucosidase present on the surface of small intestinal cell. It primarily act on α-(1,4)- Glycosidic bonds bonds and converts starch and disaccharides to glucose that could be consumed by the body[**Magaji, U *et.,al* 2019**].The suppression of α–Amylase & alpha-glucosidase enzymes are important in reducing the level of sugar in the blood following the intake of meals by reducing the rate at which carbohydrates are digested and absorbed. Thus, they are key targets to treat type 2 diabetes particularly to manage post-parandial high blood sugar[**Magaji, U *et.,al* 2019**].

Table 3: IC_{50} values of DPPH,nitric oxide& super oxide scavenging activities of methanolic leaf extract of *Abelmoschus ficulneus* and its fractions

Extract/ fractions/standards	DPPH (IC_{50} µg/mL)	Nitric oxide (IC_{50} µg/mL)	superoxide IC_{50} (µg/ml)	% inhibition of Reducing power in mg/gra of extract
Methanolic extract(AFME)	199.97	180	531	47.33
Toluene fraction (TL-AFME)	-	-	-	26.0
Butanol fraction (BL-AFME)	**22.91**	**23.8**	**156**	**71.73**
Ethyl acetate fraction (EA-AFME)	23.50	25.3	163	65.89
Aqueous fraction (AQ-AFME)	159.13	88.50	386	65.89
Ascorbic acid (std)	3.01	8.4	N/A	80.1
Rutin (std)	N/A	N/A	4	N/A

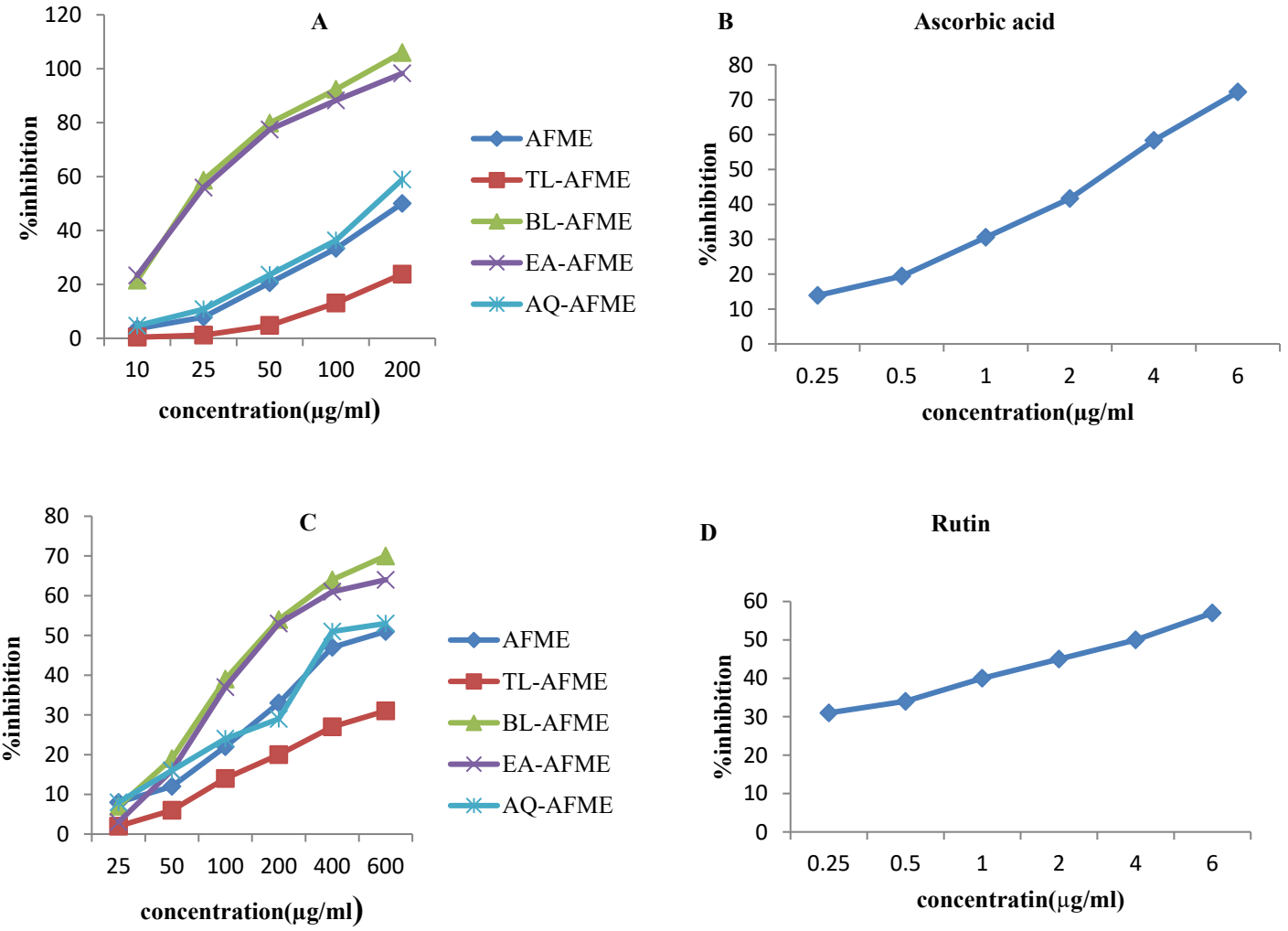


Fig 3: A : DPPH scavenging activity of methanolic extract and its fractions B : DPPH radical scavenging activtyof Ascorbic acid

C: Superoxide anion scavenging activity of methanolic extract and its fractions D : Superoxide anion scavenging activity of Rutin

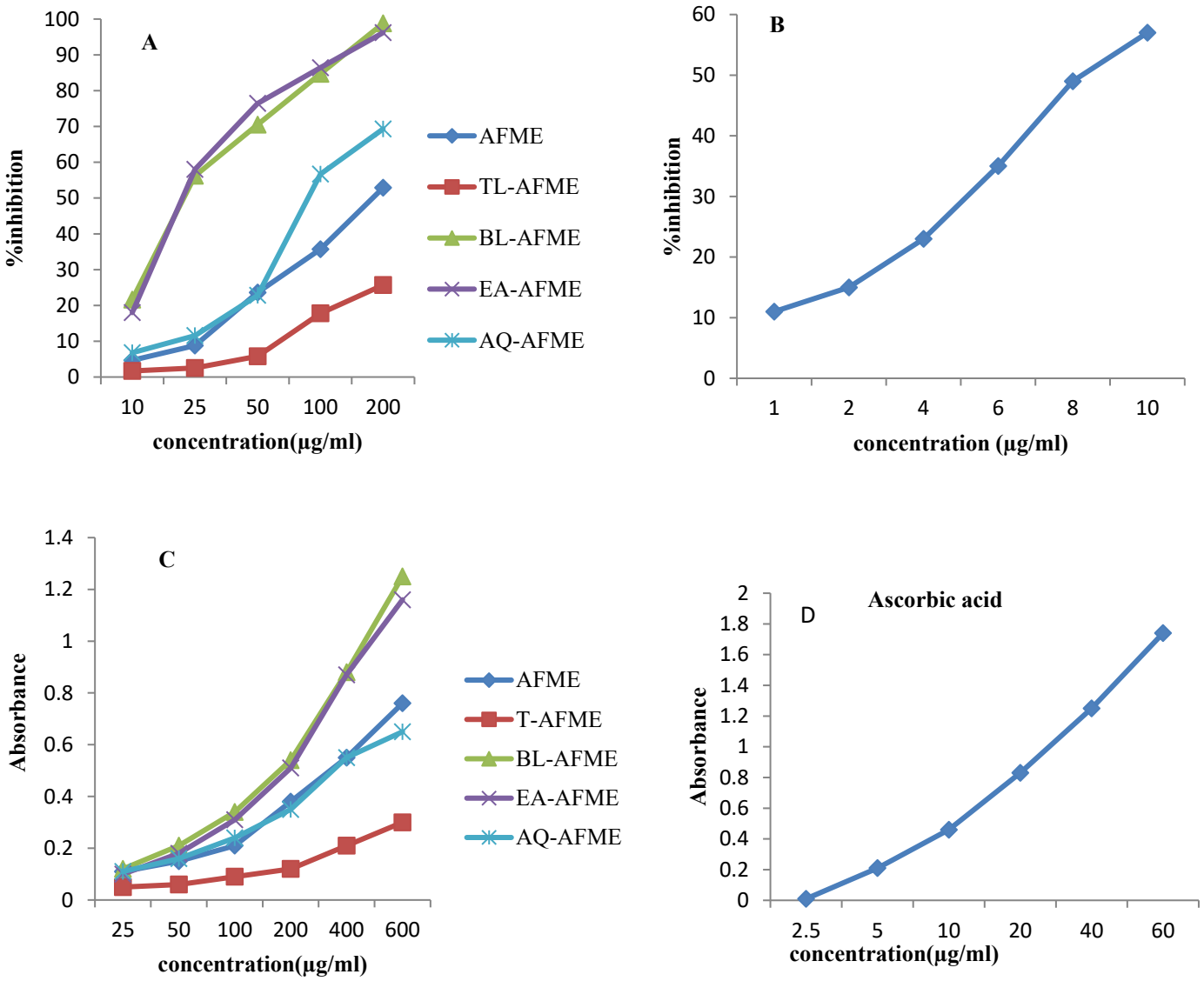


Fig 4: A: Nitric oxide scavenging activity of methanolic extract and its fractions of *Abelmoschus ficulneus* **B:** Nitric oxide scavenging activity oAscorbic acid

C: Reducing power of extract and its fractions of *Abelmoschus ficulneus* **D:** Reducing power of Ascorbic acid

The inhibitory effects of methanolic leaf extract of *Abelmoschus ficulneus* and its various fractions was analyzed on the basis of inhibitory activity against the α-Amylase & α-Glucosidase enzymes. Activity of the extracts was compared with acarbose[**Nagamani Mukka *et., al* 2020**].The methanol extract of *Abelmoschus ficulneus* and its fractions showed selective inhibitory activity against alpha-amylase and alpha-glucosidase enzymes.The methanolic leaf extract of *Abelmoschus ficulneushave* the mild inhibitory activity against α-amylase & α-glucosidase IC_{50} of 7.47 and 7.00mg/ml. Toluene fraction were not much inhibited, and the IC_{50} was 8.44mg/mL(α-amylase)and 7.73mg/mL(α-glucosidase).Both enzymes were strongly inhibited by the butanol fraction and the alpha-amylase give IC_{50} of 6.25mg/mL, and the alpha-glucosidase an IC_{50} of 5.90mg/mL. The reduced effect was on the Ethyl acetate fraction where the IC_{50} values were 6.90 and 6.34mg/mL respectively. The aqueous fraction were not much inhibited,and IC_{50} of 8.01and and 7.30 and against α-Amylase & α-Glucosidase, respectively[**sayyada saleha *et.,al* 2020**].Further it was observed that methanolic extract and its fractions exhibited more enzyme inhibition towards alpha-amylase than that of alpha-glucosidase.

The total flavonoid content was estimated in terms of milligrams of Rutin equivalent per gram of dry extract based on linear equation (Y=mx+C y = 0.0073x+0.0094 R^2 = 0.9979) derived from the standard graph. Flavonoid content of the extracts was found to be in the order: BL-AFME >EA-AFME > AQ-AFME >AFME >TL-AFME.Total phenolic content was estimated in terms of milligrams of gallic acid equivalent per gram of dry extract based on linear equation (y = 0.0055x + 0.0011 R^2 = 0.9964) derived from the standard graph. Phenolic contents of the extracts was found to be in the order: BL-AFME >EA-AFME > AQ-AFME >AFME >TL-AFME. One of the ways the plant can reduce blood sugar is enzyme inhibition test because it demonstrates the enzyme inhibitory capacity of the extracts[**Nagamani Mukka *et., al* 2020**]. Flavonoids have the ability to suppress enzymes, regulate elevated blood glucose levels, and maintain glucose equlibrium [**Tunna TS *et.,al* 2015**].

phytochemical analysis and TLC studies of the methanolic leaf extract of *Abelmoschus ficulneus* and its fractions showed the detection of steroids, triterpenoids, phenolics,flavonoids(rutin),glycosides,carbohydrates and tannins. The reason behind the intense inhibition of the enzymes is probably due to the elevated flavonoid and phenolic concentration were observed in the plant *Abelmoschus ficulneus*[**Nagamani Mukka *et., al* 2020&, sayyada saleha *et.,al* 2020**].

The antioxidant potential of medicinal plants has been associated with their phenolic constituents in multiple studies [**Shah Alam 2016**]. These free-radicals prevent age related diseases and assist in the general well being. Due to the complexity in antioxidant activities, one will prefer a combination of tests when quantifying the antioxidant activity[**Shah Alam 2016].**

The antioxidant potency of the methanolic leaf extract and its various fractions of *Abelmoschus ficulneus* was evaluated by reducing power assay as well as in vitro methods, including DPPH,superoxide and nitric oxide radical scavenging. The positive controls (asorbic acid and rutin) eliminated the radicals and enhanced the reducing power with increase in concentration[**Sharan Suresh et.,al 2011**]. Although all the tested extract/fractions showed moderate to good antioxidant activity, butanol fraction being the strongest. This enhanced activity is likely due to its higher phenolic and flavonoid contents in the butanol fraction.Therefore, the observed differences in antioxidant activity among the fractions can be attributed to variations in their TPC and TFC levels. The butanol fraction exhibited particularly high antioxidant activity, due to its rich phenolic compounds. Thus, it can be concluded that the *Abelmoschus ficulneus* is a natural source of antioxidants due to a large amount of phenols in it.

It was revealed that the butanol fraction was a potent alpha-amylase as well as alpha-glucosidase inhibitor. This could be due to its higher phenolic and flavonoid content was found in this fraction.The order of inhibition of the methanolic leaf extract of *Abelmoschus ficulneus* and its fractions was: BL-AFME >EA-AFME > AQ-AFME>AFME>TL-AFME.The butanol fraction was able to establish the fact that the plant was a good source of dietary antioxidants due to the high level of total phenols in the plant. It had a superior solvent ability as compared to other fractions in extracting phenolics and antioxidant potential.

CONCLUSION

The findings revealed that the Butanol fractions of *Abelmoschus ficulneus* are particularly rich in phenolic compounds and flavonoids, including rutin and quercetin. These bioactive constituents demonstrate potent antioxidant activity and with a high ability to prevent alpha-amylase and alpha-glucosidase.These results support the traditional use of the plant and support its potential as a natural therapeutic agent for controlling postprandial hyperglycemia in diabetes.

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Conflict of interest: There is No conflict of interest

ABBREVIATIONS

AFME: *Abelmoschus ficulneus* methanolic extract,**TL-AFME:** Toluene fraction of *Abelmoschus ficulneus* methanolic extract, **EA-AFME**:Ethyl acetate fraction of *Abelmoschus ficulneus* methanolic extract, **BL-AFME:** Butanolic fraction of *Abelmoschus ficulneus* methanolic extract, **AQ-AFME:**Aqueous fraction of *Abelmoschus ficulneus* methanolic extract.**TFC:** Total flavonoid content ,**TPC:**Total phenolic content**DPPH:**1,1-diphenyl -2-picryl –hydrazyl**,NADH:**Nicotinamide adenine dinucleotide,**NBT:** Nitro blue tetrazolium chloride **,PMS**:Phenazine metho sulphate ,**PBS:**Phosphate buffer saline.

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