https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27(4s) (December 2024); 15488-15494
Research Article

Phytochemical Characterization And *In Vitro* Evaluation Of Antidiabetic Activity Of *Abelmoschus Ficulneus* Extracts

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Abstract:
The present study investigates the phytochemical composition and *in-vitro* antidiabetic activity of an *Abelmoschus ficulneus* leaves extract. Different physical parameters like ash values, moisture content, Loss on drying, etc. were evaluated for powdered drug. The extracts were obtained from Soxhlet, Maceration and maceration with ultrasonication method by using ethanol 50%, Aqueous and Ethanol 95% as solvents for extraction and subjected for preliminary phytochemical evaluation and antioxidant studies. Preliminary phytochemical analysis acknowledged the presence of secondary metabolites constituents such as alkaloids, phenolic, flavonoids, carbohydrate, saponins and glycosides. Total phenolic and flavonoids content were also analysed. The aqueous extract exhibited a higher content of phenolic compounds (27.15%) and flavonoids (17.553%) compared to the ethanol extracts. The 100% aqueous extract of *Abelmoschus ficulneus* exhibited the highest antioxidant activity, with a radical scavenging rate of 65.67%. This was significantly greater than that of the 50% and 95% ethanol extracts, indicating superior efficacy of the aqueous extract. Each extract was subjected to in-vitro assays to evaluate antidiabetic activity, specifically through α-amylase inhibition. Results indicated that the aqueous extract exhibited significant enzyme inhibitory effects, suggesting the presence of potent antidiabetic compounds. These findings support the traditional use of the plant in diabetes management and provide a scientific basis for further phytochemical isolation and characterization of active constituent's pharmacological evaluation.

Keywords: *Abelmoschus ficulneus*, Ethanol and Aqueous Extract, α-amylase Inhibition assay, Antioxidant activity, Phytochemical screening.

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Received - 2024-12-11 Acceptance- 2024-12-16

DOI: https://doi.org/10.53555/AJBR.v27i4S.7720

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INTRODUCTION:

Diabetes mellitus is a long-term metabolic condition that occurs when the body either doesn't produce enough insulin or can't use it effectively. This leads to elevated blood sugar levels, which can cause serious health issues over time. As a growing global health concern, diabetes is expected to affect millions more in the coming years. By 2030, India, China, and the United States are projected to have the highest numbers of people living with this condition [1]. Synthetic antidiabetic medications, while effective in managing blood glucose levels, may also be associated with significant side effects, which can impact a patient's overall health and quality of life. [2]. Researchers are continually exploring

plant-based remedies to help manage diabetes. Even the World Health Organization supports the use of certain herbal treatments as part of diabetes care [3]. Many medicinal plants have long been known for their antidiabetic properties. People in different countries have traditionally used these plants as natural remedies to help control blood sugar levels [4]. Certain plants have natural compounds that can slow down the activity of the α-amylase enzyme, which helps reduce the breakdown and absorption of starch from food. By doing this, they help keep blood sugar levels more stable and may also support the management of type 2 diabetes and obesity [5].

Medicinal plants usually contain a variety of natural compounds, known as phytochemicals, which can work together to support better health and improve overall quality of life [6]. *Abelmoschus ficulneus* (L.) Wight and Arn is one of the traditionally important plants used for the treatment of various diseases. The *Abelmoschus ficulneus* (L.) Wight and Arn. Plant is exhibits various pharmacological activities. It has been used as traditional herbal medicine in asthma, spasm, disorders of spleen, pectorial lesions, inflammation, stress, insect bites, scorpion and snake bites, fever, and stomachic [7]. Various scientific studies have confirmed the beneficial effect of plants with antidiabetic activity in the traditional management of diabetes mellitus [8] on streptozotocin and alloxan-induced diabetic animal models [9].

Previous studies have demonstrated that various plant extracts, particularly those obtained from leaves using ethanol and aqueous, exhibit considerable antidiabetic potential in vitro. In this context, the ethanol extract of *Abelmoschus ficulneus* leaves was chosen for detailed phytochemical screening and an in vitro assessment of its antidiabetic properties.

MATERIALS AND METHODS:

A. Plant material:

The Leaves of *Abelmoschus ficulneus* were collected from an area around local forest area in Nanded district in Maharashtra and were shade dried. The identification of the plant parts was confirmed by Dr. V. R. Marathe, a botanist in the Department of Botony, N.E.S Science College, SRTM University, Nanded.

B. Chemicals and reagents: Organic solvents used for extraction of the plant material all purchased from Rankem. Alpha-amylase enzyme and DNSA (3,5-dinitrosalicylic acid) was procured from Sigma Aldrich, Banglore, India, Starch from Loba chemicals. All other chemicals used were of analytical grade.

C. Physicochemical Evaluation

i. Determination of the moisture content: The percent moisture content was calculated by the following formula:

$$\% \text{ Moisture} = 1 - \frac{\text{Weight dry sample}}{\text{Weight wet sample}} \times 100$$

ii. Determination of ash content: Percent ash was calculated by following formula:

$$\% \text{ Ash} = \frac{\text{Difference in weight of ash}}{\text{Weight of medicinal plant powder}} \times 100$$

iii. Determination of acid/alcohol/water soluble ash: The soluble ash was determined by using different solvents such as 5N HCl, alcohol and distilled water. The ash obtained was digested with 25 ml of solvent for 20-30 minutes in a boiling water bath. The content in the crucible was filtered by using ash less filter paper (Whatman filter paper No: 42). The filter paper with residue was removed carefully without any loss, folded, put in the same crucible, dried in hot air oven and ignited in muffle furnace at 600°C for 1 hour. Then it was cooled in a desiccator and weighed. The soluble ash value was calculated by the following formula. [10].

$$\% \text{ of soluble ash} = \frac{\text{Weight of soluble ash}}{\text{Total weight of ash}} \times 100$$

D. Extraction of Plant Material: The choice of extraction method plays a crucial role in isolating bioactive compounds from plant materials [11]. For this study, the extraction technique was selected based on the nature of the phytochemicals targeted, the polarity of solvents, and the efficiency of the method in preserving the integrity of the constituents. Both aqueous and ethanol extraction methods for selected *Abelmoschus ficulneus* plant were employed to ensure a broad range of phytochemicals, from highly polar to moderately polar compounds, were effectively extracted. Water, being a polar solvent, was selected for its ability to extract phenolic compounds, flavonoids, tannins, and glycosides, while ethanol was chosen for its intermediate polarity, making it suitable for extracting a wider range of bioactive constituents, including certain alkaloids and flavonoids.

The extraction process was carried out under controlled conditions to minimize degradation of sensitive compounds, ensuring the reliability of subsequent phytochemical.

E. Development of TLC Profile for Plant Extracts

Thin Layer Chromatography (TLC) was employed to analyze the various extracts obtained from the selected plant material [12]. Multiple solvent systems were used to achieve optimal separation of phytoconstituents present in the extracts. The retention factor (Rf) values of the separated compounds were recorded for each extract. Observations were made under UV light and after treating the chromatoplates with appropriate derivatizing reagents, allowing for visualization of the chemical constituents. The TLC analysis revealed the presence of compounds with a wide range of polarities, which were distinctly separated based on the solvent systems used. Preliminary phytochemical screening supported the TLC findings, indicating the presence of flavonoids, Phenols, and glycosides in the extracts.

F. Determination of phytochemicals:

a. Qualitative screening of phytochemicals:

A phytochemical screening of phenolics, alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, cardiac glycosides, glycosides/reducing sugars present in plant extracts was performed using standard methods. Phenolics were determined as per the method given by Krishnaiah *et al*., 2009 [13]. Saponins indicates its presence as per methods given by Houghton and Raman, 1998 and Jigna and Sumitra, 2007 [14]. The terpenoids were screened by method of Trease and Evans, 2002. Cardiac glycosides were screened in the plant material by Krishnaiah *et al*., 2009 method

b. Quantitative phytochemical analysis.

i. Total Phenolic content

Principal: The Total phenolic content of the extract was determined by the Folin–Ciocalteu method using Gallic acid as Standard. This test is based on oxidation of phenolic groups with phosphomolybdate and phosphotungstate. After oxidation a green-blue complex formed which measure at 760 nm.

Chemical required: Dilute Folin Ciocalteu reagent by taking 10 mL to 20 mL with diluent 7% Na_2CO_3 Reagent, Diluent: Water.

Standard Preparation: Dissolve 2.5 mg Gallic acid Reference Standard in 10 mL diluent.

Sample Preparation: Transfer an accurately weighed 15 mg of the sample in to a 10 mL volumetric flask, add about 80 mL of diluent and heat on a water bath at 80°c for 60 minutes, cool and dilute to volume with diluent and mix.

Procedure: Pipette out 1 mL from Standard solution and 1 mL from Sample solution in a separate 25 mL Volumetric flask, add 2 mL Folin ciocalteu reagent in each flask, mix well add 11 mL of 7% Na_2CO_3 solution in each flask and make up the volume with diluent. Shake well and allow the solution to stand in a dark place for 30 min. Measure Absorbance after 30 min at 760nm. Prepare Blank as per sample preparation method without adding sample. The % of Total Polyphenol content in the portion of sample taken by using following formulae. The results were expressed as mg of Gallic acid equivalent per g dry weight.

$$\text{Total Polyphenol} = \frac{A_t}{As} x \cdot \frac{w_t}{10} \times \frac{1}{25} \times \frac{10}{Wt.spl} \times \frac{25}{1} \times \frac{P}{100} \times 100 \quad (\%w/w)$$

Where, A_t = Average absorbance of sample Solution, A_s= Average absorbance of standards Solution Wt. std.= Weight of standard in mg Wt. spl.= Weight of Sample in mg. P= Purity of working Standard on as is basis

ii. Total Flavonoids Content:

Principle: This method is based on the nitration of any aromatic ring bearing a catechol group with its 3-4 position unsubstituted or not sterically blocked. After addition of $AlCl_3$ a yellow solution of complex was formed which then turned immediately to red after addition of NaOH and the value of absorbance is measured at 533 nm.

Procedure: The total flavonoid content (TFC) of the extract was determined using aluminium chloride colorimetric method with quercetin as standard. Briefly, the crude extracts (1 mg) were diluted with water (4 mL) in a 10 mL volumetric flask. Initially, 5% sodium nitrate solution (0.3mL) was added to each volumetric flask then 10% aluminium chloride (0.3 mL) was added into the flask and followed by 1.0M NaOH (2ml). Water (2.4mL) was then added to the flask and mixed well. Absorbance of the mixture was read at 533 nm. TFC values were determined as quercetin equivalents (QE) mg/g of extract weight. All samples were analyzed in duplicate.

$$\text{Total Flavonoids} = \frac{A_t}{A_s} \times \frac{Wt.std}{10} \times \frac{1}{10} \times \frac{10}{Wt.spl} \times \frac{P}{100} \times 100 \quad (\%w/w)$$

Where, A_t = Average absorbance of sample Solution, A_s = Average absorbance of standards Solution Wt. std.= Weight of standard in mg Wt. spl.= Weight of Sample in mg.

P = Potency of working Standard on as is basis

G. Pharmacological Screening of Plant Extracts:

***a. In vitro* antioxidant activities of the plant extracts:**

i. Free radical scavenging activity by DPPH assay:

The free radical scavenging activities of the medicinal plants was determined by 11-diphenyl-2-picrylhydrazyl (DPPH) method according to Brand-Williams *et al*. 1995 [15]. The scavenging activity was calculated by using the formula:

$$\% \text{ activity scavenging} = \frac{\text{Absorbance of the blank- Absorbance of the sample}}{\text{Absorbance of the blank}} \times 100$$

The blank contained all reagents except the medicinal plant extract. Ascorbic acid at a concentration of 1 mg/ml was used as reference.

b. In vitro method employed in anti-diabetic studies:

i. Inhibition assay for α-amylase activity: Plant extract were dissolved in distilled water. A 100 µL of α-amylase (8U /mL) was mixed with plant extract and incubated at 25 °C for about 30 min. A 100 µL of this mixture was mixed with starch (0.5 % w/v) solution (100µL) and incubated at 25 °C for 3 min. 3,5-dinitrosalicylic acid (DNSA reagent) (100 µL) was added incubated at 85 °C for 15 min in a water bath, allowed to cool and then diluted with distilled water (900µL). Blanks were conducted in the same manner. Negative controls were prepared by adding DNSA reagent prior to the addition of starch solution kept in 85 °C water bath for 15 min and then diluted with distilled water (900 µL) as before. Absorbance was measured at 540 nm.

OBSERVATION AND RESULTS:

A. Pharmacognostic microscopy

The pharmacognostic microscopy of the leaf of *Abelmoschus ficulneus* L is given in images

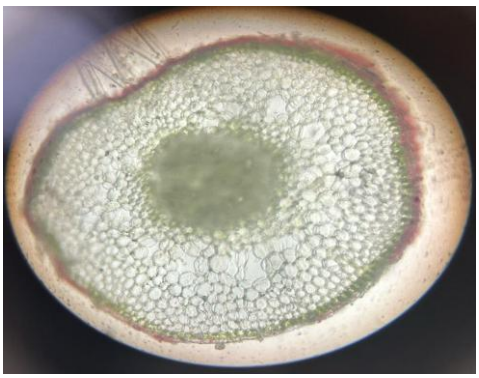


Figure a

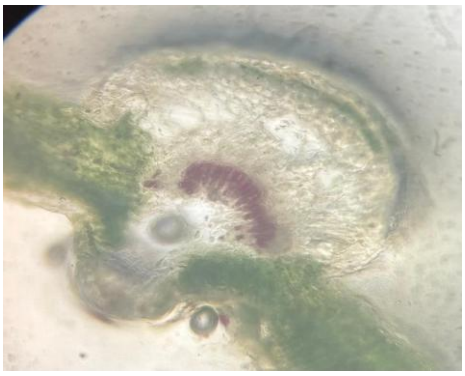


Figure b

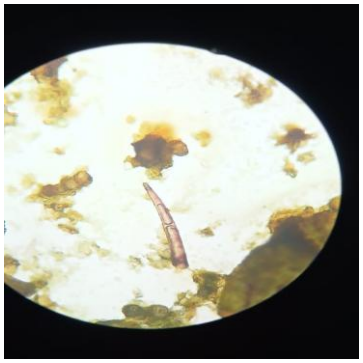


Figure c

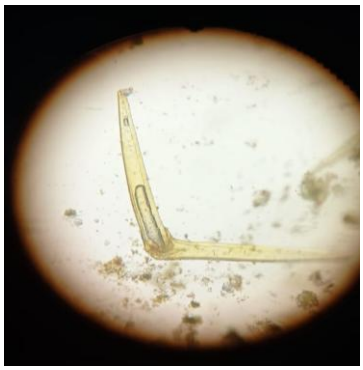


Figure d



Figure e

Figure a-e: Pharmacognostic microscopy of the leaf of *Abelmoschus ficulneus a)* upper epidermis of the leaf, b) Transverse section of the leaf, c) irregular shape of calcium oxalate d) Trichomes and e) Vascular Bundles

B. Proximate composition of plant:

Table 1: Physicochemical parameters

Standardization parameter	**Results**
Moisture Content	12.95%
Total Ash (w/w)	10.70%
% acid insoluble ash (w/w)	0.26%
% water soluble ash (w/w)	1.14
Loss on drying	3.61%

C. Thin Layer Chromatography

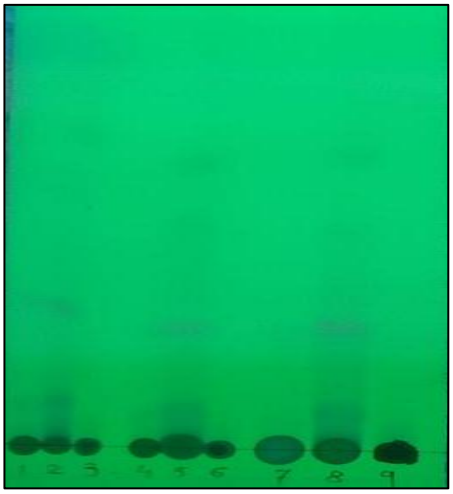
Thin Layer Chromatography of *Abelmoschus ficulneus* L. leaves extracts.

Table 2: Optimized Chromatographic Condition & Track of extract

Parameter	Condition
Mobile Phase	Toulene : Ethyl acetate (8:2)
Stationary phase	Precoated silica plates
UV wavelength	254nm
Diluent	Methanol
Concentration	100µg/ml

Track no.	Batch no.
1.	AF03
2.	AF06
3.	AF09
4.	AF02
5.	AF05
6.	AF08
7.	AF01
8.	AF04
9.	AF07





Extraction of Plant Material: All Plant materials were extracted by soxhlation method, simple maceration and ultrasonication with maceration by using variation percentage of ethanol and with water. The yields were calculated on dry weight basis. AF Stand for *Abelmoschus ficulneus* extract.

Table 3: Yield of crude plants extracts (mg/g dry weight)

Extract Batch	Raw Material	Solvent	Method of extraction	Time	Volume of Solvent	Yield mg/g	% Yield
AF001	5	50% Ethanol	Maceration	3 days	30	0.151	3.02
AF002	5	50% Ethanol	soxhlation	6 hrs × 3	30	0.190	3.8
AF003	5	50% Ethanol	Ultrasonication & Maceration	45 min & 6 hrs	30	0.146	2.91
AF004	5	95% Ethanol	Maceration	3 days	30	0.016	0.32
AF005	5	95% Ethanol	soxhlation	6 hrs × 3	30	0.059	1.18
AF006	5	95% Ethanol	Ultrasonication & Maceration	45 min & 6 hrs	30	0.053	1.06
AF007	5	100 % Water	Maceration	3 days	30	0.151	3.02
AF008	5	100 % Water	soxhlation	6 hrs × 3	30	0.138	2.76
AF009	5	100 % Water	Ultrasonication & Maceration	45 min & 6 hrs	30	0.150	3.0

Phytochemical present in the plants leaves extracts: The phytochemicals detected in the extracts of plants were flavonoids, phenols, alkaloids saponins, terpenoids, steroids, tannins, cardiac glycosides and carbohydrate.

Table 4: Qualitative screening of the phytochemicals in the medicinal plants extracts.

Phytochemicals	**Chemical Test**	**AF01**	**AF02**	**AF03**	**AF04**	**AF05**	**AF06**	**AF07**	**AF08**	**AF09**
Alkaloids	Dragendorff's test	+	+	+	+	+	+	+	+	+
Glycosides	Borntrager's test	+	+	+	+	+	+	-	-	-
	Keller-killianin test	+	+	+	+	+	+	-	-	-
Phenols	Ferric Chloride Test	+	+	+	+	+	+	+	+	+
Carbohydrates	Molisch's test	+	+	+	+	+	+	+	+	+
	Barfoed's test	+	+	+	+	+	+	+	+	+
Saponins	Foam test	-	+	+	-	-	-	-	+	-
Terpenoids	Salkowski test	+	+	+	-	+	+	-	+	-
Flavonoids	Shinoda Test	+	+	+	+	+	+	+	+	+
	Lead Acetate	+	+	+	+	+	+	+	+	+
Steroids	Libermann-burchard test	-	+	+	-	-	+	+	+	+
Tannin	Ferric Chloride Test	-	-	+	-	-	-	-	+	+

(positive +, negative -)

Above observation table shows the presence of phytoconstituents in the *Abelmoschus ficulneus* L. leaves extracts. It reveals that most of the chemical constituents present Aqueous (AF06-AF09) and (AF01-AF03) 50% Ethanol extract. Phytoconstituents such as alkaloids, flavonoids, phenols and carbohydrate present in all extract.

Phytochemical Quantitative analysis of extracts:

Total Phenolic content: Water extract shows more phenolic content than ethanol as per comparative evaluation of phenolic content of extracts. The Gallic acid were used as reference Standard.

Table 5 Total phenolic content of *Abelmoschus ficulneus* leaves Extracts

Extracts	Sample Absorbance	Standard Absorbance	% Content
AF02: Extract: 50% Ethanol	1.136	0.743	25.2275
AF05: Extract: 95% Ethanol	0.951	0.743	21.1191
AF09: Extract: 100% Water	1.223	0.743	27.1595

Total Flavonoid Content: Water extract shows more flavonoid content than ethanol as per comparative evaluation of flavonoid content of extracts. The quercetin were used as reference Standard.

Table 6 Total phenolic content of *Abelmoschus ficulneus* leaves Extracts

Extracts	Sample Absorbance	Standard Absorbance	% Content
AF02: Extract: 50% Ethanol	0. 847	0.614	12.071
AF05: Extract: 95% Ethanol	0. 681	0.614	10.636
AF09: Extract: 100% Water	1.189	0.614	17.553

Free radical scavenging activity by DPPH assay of powders: The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. 100% aqueous extract of *Abelmoschus ficulneus* extract had the highest radical scavenging activity of 65.67 % which was higher than other two 50% ethanol extract and 95% ethanol extract. The observed activities indicate that the plant Extract have a strong proton-donating ability and could serve as free radical scavengers, acting perhaps as primary antioxidants.

Table 7: Percentage free radical scavenging activity by DPPH assay

Extract	Sample Absorbance	Control Absorbance	% Content
AF02: Extract: 50% Ethanol	0.521	0.973	46.4543
AF05: Extract: 95% Ethanol	0.361	0.973	62.8983
AF09: Extract: 100% Water	0.334	0.973	65.6732

Inhibition assay for α-amylase activity: (DNSA): The results of the DNSA study are summarized in Table 8. All the above extract showed varying effect on glucose utilization. AF09: Extract: 100% Water showed maximum inhibition of the enzyme with the highest value of 21.5% seen at 100 mg/ml concentration of plant extract.

Table 8: % Inhibition of α-amylase enzyme brought about by different extracts of *Abelmoschus ficulneus leaves.*

Extract	Sample Absorbance	Control Absorbance	% Inhibition
AF02: Extract: 50% Ethanol	1.192	1.184	No activity
AF05: Extract: 95% Ethanol	0.773	0.702	No activity
AF09: Extract: 100% Water	0.775	0.988	21.5 %

DISCUSSION

The present investigation assessed the in vitro antidiabetic and antioxidant activities of aqueous extracts of *Abelmoschus ficulneus*, with particular emphasis on α-amylase inhibition and free radical scavenging potential. The extract demonstrated significant α-amylase inhibitory activity, indicating its potential to modulate postprandial hyperglycemia by slowing the enzymatic breakdown of complex carbohydrates. This effect is consistent with earlier studies on medicinal plants such as *Amaranthus spinosus* and *Avicennia alba*, which have shown similar inhibitory effects on carbohydrate-hydrolyzing enzymes [16-17].

Phytochemical analysis revealed the presence of various bioactive compounds, including phenols, alkaloids, flavonoids, tannins, terpenoids, glycosides, and carbohydrates. These constituents are known to contribute to enzyme inhibition and possess antioxidant properties. Flavonoids and phenolic compounds, in particular, are capable of interacting with digestive enzymes and neutralizing free radicals, suggesting a dual mechanism of antidiabetic action.

The antioxidant activity, as measured by the DPPH assay, reinforces the therapeutic value of *A. ficulneus*. Oxidative stress is a major contributor to the onset and progression of diabetes, especially through damage to pancreatic β-cells and promotion of insulin resistance. The free radical scavenging activity of the extract suggests its potential in reducing oxidative burden and improving metabolic stability.

The combined α-amylase inhibitory and antioxidant activities support the use of *Abelmoschus ficulneus* as a promising natural candidate for diabetes management. However, further in vivo studies and detailed molecular analyses are necessary to validate these findings and understand the pharmacological mechanisms involved.

SUMMARY AND CONCLUSION

The present study successfully demonstrated that the Aqueous extracts of *Abelmoschus ficulneus* (L.) Wight and Arn leaves contain significant phytochemicals, including phenolics, flavonoids, alkaloids and carbohydrates. Physicochemical parameters such as ash value, moisture content and loss on drying confirmed the quality and purity of the plant material. The Soxhlet extraction method yielded extracts rich in bioactive

compounds, with preliminary phytochemical screening confirming the presence of primary and secondary metabolites. Total phenolic and flavonoid contents were analyzed, revealing that the aqueous extract possessed the highest levels 27.15% and 17.55%, respectively. This correlated with its superior antioxidant activity, highlighting its potential as an effective natural source of antioxidants. The 100% aqueous extract of *Abelmoschus ficulneus* exhibited the highest antioxidant activity, with a radical scavenging rate of 65.67%. This was significantly greater than that of the 50% and 95% ethanol extracts, indicating superior efficacy of the aqueous extract. The α-amylase inhibitory activity showed by *Abelmoschus ficulneus* leaves aqueous extract has a significant role in management of diabetes. These overall activities due to bioactive phytochemicals were present in the extract. The leaves of *Abelmoschus ficulneus* could be a source of natural antidiabetic agent, which has contribution of most significant therapeutic agents responsible for prevent and management of Type-II diabetes. These findings suggest that *Abelmoschus ficulneus* leaves, particularly its aqueous extracts, can serve as an in vitro antidiabetic activity as well as potential natural source of antioxidants and bioactive phytochemicals for future pharmacological applications. The more investigative study is proposed to validate these claims by separation, isolation, and identifying bioactive components with potential therapeutic value.

ACKNOWLEDGMENT:
The authors are grateful to the Research Institute, School of Pharmacy, SRTMU, Nanded as well as Nanded Pharmacy College for proving Facilities.

CONFLICTS OF INTEREST
Authors declare no conflicts of interest.

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