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Antidiabetic potential of pentacyclic triterpenoids from tender Jackfruit: An *In vitro* comparison with medicinal plants

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Abstract

To understand the potential of antidiabetic compounds such as pentacyclic triterpenoids and other compounds from tender Jackfruit (*Artocarpus heterophyllus* Lam.) along with other known medicinal plants used in the treatment of diabetes was carried out. The tender Jackfruit is used as vegetable and it is a rich source of minerals, dietary fiber and other antioxidant compounds. It's usage has significant effect on lowering the blood glucose levels in diabetic patients. Seven medicinal plants (seven in total, including Jackfruit as the focal species) were used for isolation of crude extracts. The plants used were *A. heterophyllus*, *Tinospora cordifolia* (Thunb.) Miers, *Murraya koenigii* L. *Moringa oleifera* L., *Cyamopsis tetragonoloba* (L.) Taub., *Allium sativum* L. and *Costus pictus* D. Don. Total phenols, flavonoids and triterpenoids were extracted using suitable protocols and GC-MS analysis was carried out to identify various categories of triterpenoids in samples. α-amylase and α-glucosidase inhibition assays were carried out using the extracts of Jackfruit and other medicinal plants. The antioxidant potential of the extracts was also carried out using DPPH radical scavenging assay. The GC-MS analysis identified several pentacyclic triterpenoids in the plant samples, with *A. heterophyllus* and *T. cordifolia* containing the highest number of compounds (seven each). Among all plants screened, *A. heterophyllus* tender fruit showed the highest phytochemical content (phenols: 226.10 ± 4.46; flavonoids: 555.22 ± 7.9; triterpenoids: 627.09 ± 78.23 mg/100 g DW) and strongest enzyme inhibitory activity, with IC_{50} values of 74.05 ± 0.81 µg/ml (methanol) and 68.63 ± 2.51 µg/ml (DCM) for α-amylase, and 54.77 ± 0.23 and 53.86 ± 0.57 µg/ml for α-glucosidase (comparable to acarbose: 49.56 ± 1.71 µg/ml). Pearson correlation analysis revealed significant positive correlations between triterpenoid content and a-amylase inhibition (R = 0.860, p<0.05) and α-glucosidase inhibition (R= 0.757, p<0.05), and between flavonoid content and enzyme inhibition (R = 0.792 and 0.671, p<0.05), supporting the role of these phytochemicals in the antidiabetic activity of the plants studied.

1. Introduction

Nature has long been recognized as a valuable source of medicinal compounds, including plants, animals, and microorganisms, which provide a foundation for the treatment of various human diseases. Throughout history, medicinal plants have been utilized by civilizations such as China, Greece, Egypt, and India to address a wide range of ailments (Mulgundu *et al*., 2022). With over 17,000 species of higher plants, approximately 8,000 are believed to possess medicinal properties and are used in traditional systems by indigenous communities (Kumar *et al*., 2016).

Diabetes mellitus, a metabolic disorder characterized by persistent hyperglycemia, has been the focus of medicinal plant studies. According to the 10th edition of the International Diabetes Federation (IDF) Diabetes Atlas, which was released by the IDF, there are 537 million adults in the 20-79 age range worldwide living with diabetes as of 2021; by 2045, that number is expected to rise to 784 million.

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Diabetes mellitus kills one patient every six seconds, which is more than the combined mortality rates from HIV (1.5 million), TB (1.5 million) and malaria (0.6 million) (Dianna, 2021). Presently, diabetes is managed with six main oral medications (DPP-4 inhibitors, metformin, meglitinides, thiazolidinediones, sulfonylureas, and alpha glucosidase inhibitors) and two injectables (insulin and incretin mimetics). Despite their effectiveness, these treatments can have serious, sometimes life-threatening side effects (Mishra *et al*., 2023). Traditional remedies for diabetes have been utilized since ancient times and numerous plants have been reported to be beneficial for their management. Based on ethnobotanical data, approximately 800 plants have the potential to prevent diabetes (Goanker *et al*., 2020). Medicinal plants have been found to contain a diverse range of bioactive phytochemicals including alkaloids, terpenes, polyphenolic compounds, glycosides, and saponins, which contribute to their therapeutic properties including antidiabetic, antiviral, antibacterial, and anticancer effects. These natural remedies offer several advantages over synthetic medications, including reduced side effects, increased accessibility, and perceived safety (Siddiqui *et al*., 2023).

In particular, pentacyclic triterpenoids have emerged as promising lead molecules for antidiabetic research. These compounds have demonstrated significant antioxidant properties and have the ability

to regulate glucose transport, uptake, absorption, and insulin secretion. They have also been found to ameliorate diabetic complications such as retinopathy, kidney disease, and vascular dysfunction (Oboh *et al*., 2021). Pentacyclic triterpenoids, such as oleanolic acid and ursolic acid, have been shown to enhance insulin sensitivity and regulate glucose metabolism, thereby addressing insulin resistance associated with diabetes (Sharma *et al*., 2018; Alqahtani *et al*., 2013). They also exhibit anti-inflammatory effects, which are beneficial in mitigating diabetic complications like retinopathy and nephropathy (Banerjee *et al*., 2019). Based on these findings, a study was conducted to screen selected medicinal plants known for their antidiabetic properties in the presence of pentacyclic triterpenoids.

2. Materials and Methods

2.1 Plant materials

Seven medicinal plants with known medicinal value on the basis of literature (Table 1) (Plate 1 to 7 original photos), were selected based on (Gaonkar *et al.* 2020; Ghosh, 2020; Nazaruk *et al*. 2015) review papers on antidiabetic potential of the selected medicinal plants. The samples were collected from the Medicinal and Aromatic plants section, Department of Horticulture in the main campus of the University. Fresh parts of all the plants were collected during the period January-February 2024. The samples were dried in shade, powdered and used for further analysis.

Table 1: List of medicinal plants and their parts used with supporting review on their medicinal property (Gaonkar *et al.*, 2020; Ghosh, 2020; Nazaruk *et al*., 2015)

Treatment	Scientific name	Common name	Plant part used	Plant authentication for antidiabetic potential
T_1	*T. cordifolia*	Amruta balli	Matured leaves	Adisakwattana *et al*., 2012; Ardiles *et al*., 2012; Choudhary *et al*., 2013; Devprakash *et al*., 2011
T_2	*M. koenigii*	Curry leaves	Matured leaves	Bhandari, 2012; Gahlawat *et al*., 2014; Wani *et al*., 2018
T_3	*M. oleifera*	Drumstick	Dried seeds	Ferreira *et al*., 2008; Ho *et al*., 2020; Kumar *et al*., 2010
T_4	*C. tetragonoloba*	Cluster bean	Dried seeds	Kaushal *et al*., 1982; Sharma *et al*., 2017
T_5	*A. sativum*	Garlic	Matured bulbs	Bozin, 2008; El *et al*., 2021; Tudu *et al*., 2022; Wani *et al*., 2018
T_6	C. *pictus*	Insulin plant	Matured leaves	Prejeena *et al*., 2016;Prejeena *et al*., 2018; Remya *et al*., 2012; Selvakumarasamy *et al*., 2021
T_7	*A. heterophyllus*	Jackfruit	Two-week tender fruit	Baliga *et al*., 2011;Chavez Santiago *et al*., 2021; Moke *et al*., 2017;Paul *et al.,* 2017; Shafiq *et al*., 2017

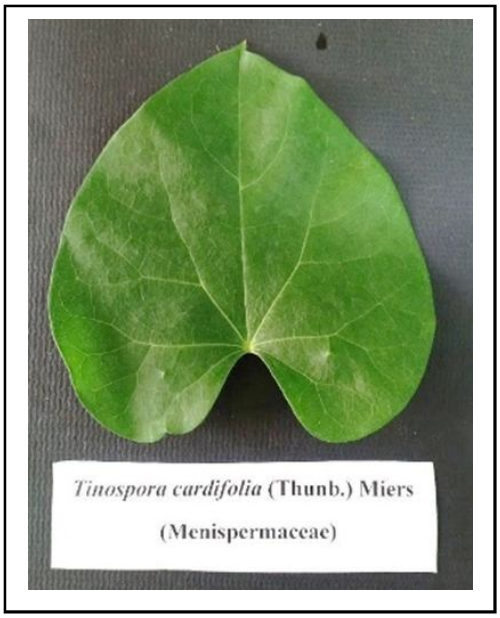

Plate 1: *T. cordifolia* leaf.

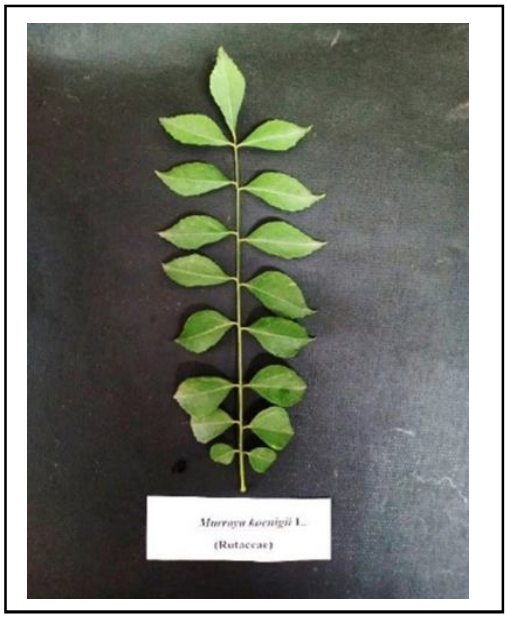

Plate 2: *M. koenigii* leaves.

Plate 3: ***M. oleifera*** seeds.

Plate 4: ***C. tetragonoloba*** seeds.

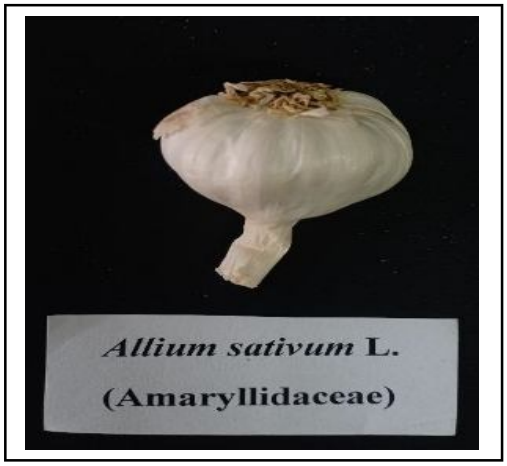

Plate 5: ***A. sativum*** bulbs.

Plate 6: ***C. pictus*** **leaf**.

Plate 7: ***A. heterophyllus.***

2.2 Quantitative phytochemical analysis

2.2.1 Estimation of total phenols

Total phenol content was estimated by spectrophotometric method using Folin-Ciocalteu reagent (FCR) (AOAC, 2005). Approximately one gram of plant sample was homogenized 2-3 times with 20 ml of methanol (80%) in a mortar and pestle. The resulting extract was made up to 50 ml. Took approximately 0.5 ml of the extract into a test tube, added 0.2 ml of FCR, then added 3.3 ml of distilled water and mixed well. After 2 min, added 1 ml of sodium carbonate solution and mixed well. Incubated the mixture at room temperature for 30 min and the intensity of blue colour was read at 700 nm in a spectrophotometer. Phenolic standard curves were generated using

gallic acid standards and results were expressed in gallic acid equivalents (GAE).

2.2.2 Estimation of total flavonoids

Total flavonoid content was determined using the aluminum chloride method (Shraim *et al*., 2021). Homogenized one gram of dried samples of selected medicinal plants with 20 ml of methanol (80%) in a mortar and pestle was grinded 2-3 times. The extracts were mixed and made up the volume to 50 ml. Took 1.0 ml of final extract in a test tube and added 0.3 ml of 5% $NaNO_2$. After 2 min, added 0.3 ml of 10% $AlCl_3$ followed by 3.4 ml of 4N NaOH. Incubated the mixture at room temperature for 10 min. The intensity of the red brick colour was measured at 510 nm relative to the blank. The standard curve of flavonoid compounds was drawn using rutin as the chemical standard and the results were expressed in terms of rutin equivalent (RE).

2.2.3 Estimation of total triterpenoids

Total triterpenoid content was estimated by vanillin method (Pedrosa *et al*., 2020). The tubes containing samples in methanol were placed in a water bath at 85°C until completely dry. After drying, 250 µl of Vanillin solution (50 mg/ml) and 500 µl of sulfuric acid were added to each tube. The mixture was then heated in a water bath at 60 ± 1°C for 30 min, after which the tubes were cooled in an ice bath and 2500 µl of acetic acid was added. The resulting solutions were kept on ice for 20 min and then allowed to sit at room temperature for an additional 20 min. A blank solution was prepared in the same manner, and absorbance was measured at 548 nm using a spectrophotometer.

2.3 Analysis of PTCs through GC-MS

2.3.1 Extraction and derivatization

The plant samples were dried at 35ºC for 2 weeks before being powdered. 1 g of the powdered plant material was weighed and 50 ml of dichloromethane (DCM) was added to the plant material in the flask. The mixture was heated to reflux for 3-4 h at 39ºC. Filtering was done to get fine extract using Whatman No.1 filter papers into a clean flask. Repeated the steps for a total of 2-3 extractions. Combined the filtrates and concentrated the solvent using vacuum concentrator.

The concentrated extract was further purified using column chromatography with silica gel of grade 74-149 mm (100-200 mesh) and elution solvents such as hexane-ethyl acetate gradient by loading the concentrated sample onto a silica column (450 mm x 18 mm) after optimizing the column. Sample was allowed to be absorbed into the column, then hexane-ethyl acetate gradient (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10) elution was performed to collect column elutes with 10 ml of volume for each gradient. Column elutes are dried in vacuum concentrator. Dried elutes and each of the standards (1 mg) were separately dissolved in 120 µl pyridine and then derivatized to trimethylsilyl ethers/esters (TMS) with 80 µl of BSTFA + 1% TMCS at 60ºC for 30 min.

2.4 GC-MS conditions

GC-MS conditions were maintained by using the method described previously (Dumandan and Kagaoan, 2022). Separation and identification of compounds was carried out in GCMS Model-QP2010 (Shimadzu) having SH-Rxi-5Sil MS capillary column. The initial oven temperature was held at 190°C for 1 min then raised to 300°C at 15°C/min and kept constant for 10 min. Helium was used as the carrier gas with a constant flow rate of 1 ml/min. The injector, MS ion source and MS interface temperatures were set at 310, 230 and 280°C, respectively. A sample injection of 1 µl was performed in a split mode of 10:1 and peaks were detected in full scan acquisition mode from m/z 50 to 500. Identification of the individual components was performed by NIST mass spectral library on the basis of the mass fragment and m/z values of each component.

2.5 Evaluation of antioxidant activity and antidiabetic activity of extracts

2.5.1 DPPH radical scavenging assay

The assay is based on the reduction of DPPH radicals in methanol by the antioxidants which cause a reduction of DPPH and absorbance are determined at 517 nm (Kang and Saltveit, 2001). One gram of powdered sample was crushed with 50 ml of 80% methanol and filtered. About 0.2 ml of extract was taken in test tube and 0.3 ml of acetate buffer was added followed by addition of 2.5 ml of 0.2 mM DPPH solution and mixed well. Read the absorbance of the solution at 517 nm after 30 min of incubation (A1). Read the absorbance of DPPH solution without sample (A2). The difference in the absorbance of DPPH solution with and without sample (A2 - A1) was calculated. The decrease in absorbance with sample addition was used for calculation of antioxidant activity. Preparation of standard curve was done using different concentrations of ascorbic acid (20-100 µg/ml). The results were expressed as ascorbic acid equivalent antioxidant capacity.

2.5.2 *In vitro* α-amylase inhibition assay

The α-amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid method (Alqahtani *et al*., 2021). Different plant extracts were prepared with 5% dimethyl sulfoxide (DMSO). For the assay, 500 µl of a 100 µg/ml concentration of the extract was mixed with 500 µl of a 20 mM sodium phosphate buffer (pH 6.8) containing 20 µl of amylase (1 mg/ml) and pre-incubated at 25ºC for 10 min. Following pre-incubation, 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated for 15 min. The reaction was halted by adding 1.0 ml of dinitrosalicylic acid (DNS reagent). The test tubes were then placed in a boiling water bath for 5 min and allowed to cool to room temperature. Afterward, the reaction mixture was diluted by adding 10 ml of distilled water, and the absorbance (Abs) was measured at 540 nm. Mean values were derived from triplicate experiments. The negative control was prepared without any plant extracts, while the positive control used acarbose. The percentage inhibition of amylase activity was calculated using the following formula:

$$\text{Inhibition } \% = \frac{\text{Abs control} - \text{Abs compound}}{\text{Abs control}} \times 100$$

2.5.3 *In vitro* α-glucosidase inhibition assay

The α-glucosidase inhibition assay was performed using the method described previously with some modifications (Alqahtani *et al*., 2020). The different fractions of the plant extract were prepared using 5% dimethyl sulfoxide (DMSO). Phosphate buffer (100 mM, pH 6.8) and 80 µl of the fraction/compound of different concentration was mixed with 20 µl of α-glucosidase (0.1 mg/ml) and incubated at 37ºC for 10 min in 96 well microplate. Next, 20 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) was added to the mixture to start the reaction. The reaction mixture was incubated at 37ºC for

60 min and stopped by adding 80 µl of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the absorbance (Abs) at 400 nm. Mean values were obtained from triplicate experiments. The negative control was prepared without any plant extracts. The positive control was prepared with acarbose. The percent inhibition of α-glucosidase activity was calculated using the following formula:

$$\text{Inhibition } \% = \frac{\text{Abs control} - \text{Abs compound}}{\text{Abs control}} \times 100$$

2.6 Statistical analysis

Mean ± SD values are based on two step hierarchial approach. Firstly, mean of technical replicates (n=3) for each biological sample was drawn. Using these resulting means, final Mean ± SD were calculated for biological samples (n=3).

2.6.1 Analysis of variance (ANOVA)

The analysis of variance was carried out for replication values of all the parameters by using Microsoft Excel. The level of significance was tested at 1%. One-way ANOVA was used for all parameters; pairwise comparisons between means were performed using the least significant difference (LSD) post-hoc test (α = 0.01). Means bearing different alphabetical superscripts within a column differ significantly ($p<0.01$). Mean ± SD values are based on a two-step hierarchical approach: the mean of technical replicates (n = 3 per biological sample) was first calculated, and these means were then used to compute the final Mean ± SD across three independent biological replicates (n = 3 independent experiments, each prepared from separately weighed plant material). Correlation analysis (Pearson correlation) was also carried out using OPSTAT webserver.

3. Results

3.1 Quantitative phytochemical analysis

The plants extracts were subjected to phytochemical analysis for estimation of total phenols, flavonoids and triterpenoids (Table 2.). The results revealed that the tender fruit sample extracts of *A. heterophyllus* contained significantly higher quantities of total phenols (226.10 ± 4.46 mg/100 g dry weight) followed by total flavonoids (555.22 ± 7.9 mg/100 g dry weight) and total triterpenoids (627.09 ± 78.23 mg/100 g dry weight). Further leaf extracts of *M. koenigii* also recorded higher quantities of total phenols (201.62 ± 6.83 mg/100 g dry weight) and total flavonoids (459.33 ± 7.0 mg/100 g dry weight) followed by *T. cordifolia* with total phenols (162.9 ± 0.46 mg/100 g dry weight), total flavonoids (249.27 ± 2.8 mg/100 g dry weight) and total triterpenoids (346.93 ± 42.31 mg/100 g dry weight). The total triterpenoid content was also found to be higher (334.01 ± 39.17 mg/100 g dry weight) in *A. sativum*.

Table 2: Quantitative phytochemical analysis

S. No.	Plants	mg/100 g dry weight		
		Total phenolic content	Total flavonoid content	Total triterpenoid content
1.	*T. cordifolia*	162.9 ± 0.46^c	249.27 ± 2.8^c	346.93 ± 42.31^b
2.	*M. koenigii*	201.62 ± 6.83^b	459.33 ± 7.0^b	240.14 ± 40.91^c
3.	*M. oleifera*	43.42 ± 1.71^e	188.10 ± 3.5^d	122.04 ± 31.47^d
4.	*C. tetragonoloba*	177.11 ± 6.22^c	148.08 ± 1.7^d	100.71 ± 26.54^d
5.	*A. sativum*	81.66 ± 0.86^d	163.96 ± 2.0^d	334.01 ± 39.17^b
6.	*C. pictus*	167.37 ± 6.05^c	196.35 ± 4.7^d	238.23 ± 33.26^c
7.	*A. heterophyllus*	226.10 ± 4.46^a	555.22 ± 7.9^a	627.09 ± 78.23^a

Results are expressed as Mean ± SD.

3.2 GC-MS identification of pentacyclic triterpenoids

Identification of pentacyclic triterpenoids present in the plant sample extracts were done through GC-MS and NIST library. The major pentacyclic triterpenoids identified in the dichloromethane extract of samples are listed in Table 3. Higher number (seven compounds) of pentacyclic triterpenoids were identified in *A. heterophyllus*. The major pentacyclic triterpenoids identified in GC-MS belong to oleanane, ursane, lupane and hopane classes and they are reported for the first time in all the plant samples used in the study.

Table 3: List of pentacyclic triterpenoids identified through GC-MS in the plants

S. No.	Plant	Compounds	Retention time	Molecular weight	Chemical formula
1.	*T. cordifolia*	Lupeol	18.84	426	$C_{30}H_{50}O$
		Epilupeol	18.845	426	$C_{30}H_{50}O$
		24-Norursa-3,12-diene	21.780	394	$C_{29}H_{46}$
		Urs-12-en-23-oic acid, 3-(acetyloxy)-, (4. beta.)-, TMS	17.765	570	$C_{35}H_{58}O_4$
		24-Noroleana-3,12-diene	17.765	394	$C_{29}H_{46}$
		Olean-12-ene-3,28-diol, (3. beta.)-	17.865	442	$C_{30}H_{50}O_2$
		Oleanan-3-yl acetate	21.115	470	$C_{32}H_{54}O_2$

2.	*M. koenigii*	-	-	-	-
3.	*M. oleifera*	12-Hydroxyoleanan-3-yl acetate	18.155	510	$C_{32}H_{54}O_3$
		D: A-Friedooleanan-7. alpha. -ol	18.155	486	$C_{32}H_{54}O_3$
4.	*C. tetragonoloba*	Oleanan-3-ol	18.780	428	$C_{30}H_{52}O$
		Oleanan-2-ol	18.780	428	$C_{30}H_{52}O$
5.	*A. sativum*	Oleanan-3-yl acetate	23.170	470	$C_{32}H_{54}O_2$
		3,12-Oleandione	23.170	440	$C_{30}H_{48}O_2$
		Ursane-3,12-diol	16.235	444	$C_{30}H_{52}O_2$
6.	*C. pictus*	Lup-20(29)-en-28-ol	15.82	426	$C_{30}H_{50}O$
		Friedelan-3-one	19.945	426	$C_{30}H_{50}O$
		A-Friedooleanan-3-ol	19.945	428	$C_{30}H_{52}O$
		(3.alpha.)-2,2,4a,6a,8a,9,12b,14a-Octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,12,12a,12b,13,14,14a,14b-eicosahydropicene	19.945	410	$C_{30}H_{50}$
		Ursane-3,12-diol	10.685	444	$C_{30}H_{52}O2$
7.	*A. heterophyllus*	Oleanan-3-yl acetate	20.115	470	$C_{32}H_{54}O2$
		17.alpha.(H),21.beta.(H)-Homohopane	19.965	426	$C_{31}H_{54}$
		A'-Neo-17. alpha.-gammacerane	19.965	412	$C_{30}H_{52}$
		17.alpha.H-Trisnorhopane	18.095	370	$C_{27}H_{46}$
		Nor-17.alpha.(H)-hopane	18.095	398	$C_{29}H_{50}$
		A-Friedooleanan-3-ol,	22.240	428	$C_{30}H_{52}O$
		(3.alpha.)-2,2,4a,6a,8a,9,12b,14a-Octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,12,12a,12b,13,14,14 a,14b-eicosahydropicene	22.240	410	$C_{30}H_{50}$

3.3 *In vitro* antidiabetic potential of extracts

The enzyme α-amylase converts starch into maltose and glucose by cleaving the internal alpha 1-4 glycosidic linkages in starch. Increase in its activity releases more glucose to blood in humans. Similarly, the enzyme α-glucosidase specifically hydrolyzes α-D-glucosidic linkages, breaking down oligosaccharides and disaccharides (like maltose and sucrose) into monosaccharides. Similar to α-amylase, increase in its activity increases blood glucose level in humans. Consequently, in individuals with diabetes, these enzyme inhibitors are administered as oral hypoglycemic agents to mitigate elevated blood glucose levels.

Dose-response curves for all extracts were generated across a concentration range of 25-400 µg/ml (six data points per curve) for α-amylase and 12.5-400 µg/ml for α-glucosidase, in triplicate across three independent biological replicates. IC_{50} values were calculated by linear regression using Microsoft Excel. In the present study, the methanol and dichloromethane extract of *A. heterophyllus* showed least IC_{50} values for α-amylase (74.05 ± 0.81 µg/ml and 68.63 ± 2.51 µg/ml respectively), indicating great potential of *A. heterophyllus* in diabetes management. Similarly, dichloromethane extracts of *T. cordifolia*, *C. pictus* and *A. sativum* showed IC_{50} values of 74.45 ± 0.21, 102.62 ± 1.13 and 111.30 ± 2.77 µg/ml, respectively, indicating better inhibition on α-amylase compared to *M. koenigii* (IC_{50}: 292.44 ± 13.54 µg/ml), *M. oleifera* (IC_{50}: 252.62 ± 10.09 µg/ml) and *C. tetragonoloba* (IC_{50}: 242.32 ± 5.87 µg/ml). Similarly, methanol extracts of *C. pictus*, *T. cordifolia* and *M. koenigii* showed IC_{50} values of 101.64 ± 1.67, 104.19 ± 0.32 and 107.32 ± 3.99 µg/ml indicating moderate potential towards inhibiting α-amylase.

Methanol and dichloromethane extract of *A. heterophyllus* also showed least IC_{50} values for α-glucosidase (54.77 ± 0.23 µg/ml and 53.86 ± 0.57 µg/ml), respectively, indicating greater potential of *A. heterophyllus* in inhibiting α-glucosidase activity. Dichloromethane extracts of *M. koenigii* (IC_{50}: 58.42 ± 0.29 µg/ml), *C. pictus* (IC_{50}: 84.10 ± 1.01 µg/ml) and *T. cordifolia* (IC_{50}: 121.78 ± 2.36 µg/ml) also showed good inhibition activity on α-glucosidase. Similarly, methanol extracts of *T. cordifolia*, *C. pictus* and *M. oleifera* showed moderate IC_{50} values (92.01 ± 0.91, 102.11 ± 0.47 and 121.38 ± 1.29 µg/ml) indicating potential towards inhibiting α-glucosidase. The IC_{50} values of extracts for inhibition of α-amylase and α-glucosidase activity are listed in Table 4.

Table 4: The IC_{50} values of extracts for inhibition of α-amylase and α-glucosidase activity (Tender Jackfruit extracts in comparison to other medicinal plant samples)

IC_{50} value (µg/ml)					
		α-amylase		α-glucosidase	
S. No.	Plants	Methanol extract	DCM extract	Methanol extract	DCM extract
1.	*T. cordifolia*	104.19 ± 0.32^{b}	74.45 ± 0.21^{b}	92.01 ± 0.91^{a}	121.78 ± 2.36^{b}
2.	*M. koenigii*	107.32 ± 3.99^{b}	292.44 ± 13.54^{d}	156.43 ± 1.75^{a}	58.42 ± 0.29^{a}
3.	*M. oleifera*	296.74 ± 16.37^{d}	252.62 ± 10.09^{d}	121.38 ± 1.29^{a}	210.30 ± 0.53^{c}
4.	*C. tetragonoloba*	300.41 ± 25.75^{d}	242.32 ± 5.87^{d}	267.43 ± 1.08^{b}	843.54 ± 102.61^{e}
5.	*A. sativum*	135.83 ± 3.64^{c}	111.30 ± 2.77^{c}	318.59 ± 1.46^{c}	283.97 ± 3.03^{d}
6.	*C. pictus*	101.64 ± 1.67^{b}	102.62 ± 1.13^{c}	102.11 ± 0.47^{a}	84.10 ± 1.01^{a}
7.	*A. heterophyllus*	74.05 ± 0.81^{a}	68.63 ± 2.51^{a}	54.77 ± 0.23^{a}	53.86 ± 0.57^{a}
8.	Acarbose (Standard)	32.07 ± 2.34	49.56 ± 1.71		

Results are expressed as Mean ± SD. Values with different alphabetical superscripts within the same column differed significantly.

3.4 *In vitro* antioxidant potential of extracts

Antioxidant capacity and radical scavenging ability were determined for the tender Jackfruit sample and selected medicinal plants by DPPH method. Radical scavenging activity was expressed in both total antioxidant content and inhibition percentage. The total antioxidants were expressed in terms of milligram per 100 g ascorbic acid equivalents antioxidant capacity (AEAC). Radical scavenging ability of selected medicinal plants ranged between 47.65 ± 0.60 to 439.52 ± 0.30 mg/100 g of dry weight. The higher radical scavenging ability was observed in leaves of *M. koenigii* (439.52 ± 0.30 mg/100 g DW) with inhibition percentage of 73.10 ± 0.18, followed by tender fruit of *A. heterophyllus* (431.99 ± 1.83 mg/100 g DW) with inhibition percentage of 71.84 ± 0.44. While least antioxidant activity was noticed in seeds of *M. oleifera* (47.65 ± 0.60 mg/100 g DW) with inhibition percentage of 7.33 ± 0.62 and *A. sativum* (72.35 ± 14.47 mg/100 g DW) with inhibition percentage of 11.48 ± 2.10. The data on antioxidant content in selected parts of medicinal plants are presented in Table 5.

Table 5: Total antioxidant content and radical scavenging activity in tender Jackfruit extracts and selected medicinal plant samples

S. No.	Medicinal plants	Total antioxidants (mg AEAC/100 g DW)	Inhibition (%)
1.	*T. cordifolia*	370.54 ± 0.60^{b}	61.53 ± 0.18
2.	*M. koenigii*	439.52 ± 0.30^{a}	73.10 ± 0.18
3.	*M. oleifera*	47.65 ± 0.60^{e}	7.33 ± 0.62
4.	*C. tetragonoloba*	211.20 ± 0.60^{c}	34.78 ± 0.42
5.	*A. sativum*	72.35 ± 14.47^{d}	11.48 ± 2.10
6.	*C. pictus*	193.73 ± 0.90^{c}	31.85 ± 0.47
7.	*A. heterophyllus*	431.99 ± 1.83^{a}	71.84 ± 0.44

Table 6: Pearson correlation (r) between phytochemical contents and α-amylase and α-glucosidase inhibition activity

Phytochemical parameter	α-Amylase inhibition (r)	*p*-value	α-Glucosidase inhibition (r)	*p*-value
Total phenolic content	0.673	0.097 ns	0.612	0.145 ns
Total flavonoid content	0.792*	0.034*	0.671*	0.049*
Total triterpenoid content	0.860*	0.013*	0.757*	0.048*
Antioxidant activity	0.795*	0.032*	0.641	0.121 ns

* Significant at $p<0.05$ (Pearson correlation, OPSTAT webserver); ns = not significant. Inhibition activity expressed as percentage inhibition at equivalent concentration.

3.5 Pearson correlation analysis

Pearson correlation analysis was carried out to statistically evaluate the relationship between phytochemical contents (phenols, flavonoids, and triterpenoids) and enzyme inhibition activity. The results are presented in Table 6.

Total triterpenoid content showed the strongest and most consistent positive correlation with both α-amylase inhibition (R = 0.860, $p<0.05$) and α-glucosidase inhibition (R = 0.757, $p<0.05$), indicating that triterpenoids are the primary drivers of the observed antidiabetic activity. Flavonoid content was also significantly correlated with

both α-amylase inhibition (R = 0.792, $p<0.05$) and α-glucosidase inhibition (R = 0.671, $p<0.05$). Antioxidant activity showed a significant positive correlation with α-amylase inhibition (R = 0.795, $p<0.05$). These findings statistically support the synergistic contribution of multiple phytochemical classes to the overall enzyme inhibitory activity.

4. Discussion

From the study, it can be understood that availability of pentacyclic triterpenoids can vary with the different plant parts. Fruit peel, leaves, and stem bark of plants contain abundant pentacyclic triterpenoids, as highlighted in the research utilizing ESI-QTOFMS/MS for structural-fragmentation analysis (Uddin *et al*., 2022). Identified compounds like lupeol and oleanan-3-yl acetate found to inhibit β-glucosidase (Sohay *et al*., 2022; Sun *et al*., 2006). Lupeol effectively inhibits carbohydrate-digesting enzymes (α-glucosidase and α-amylase), leading to reduced postprandial hyperglycemia (Lee *et al*., 2021). It enhances insulin signaling in adipose tissues, decreasing pro-inflammatory cytokines and improving insulin resistance in high-fat diet-induced diabetic rats (Daniel, 2023). D: A-friedooleanan-7. alpha. -ol and A-friedooleanan-3-ol, (3. alpha.)- have shown antidiabetic effects by inhibiting aldose reductase in the previous researches (Ardiles *et al*., 2012). The compounds of 24-noroleanolic acid derivatives exhibited potential antidiabetic effects, although specific activity (Zakirova, 2021).

The pharmacological properties of pentacyclic triterpenoids suggest that they could serve as promising candidates for clinical trials aimed at diabetes management. Pentacyclic triterpenoids like oleanolic acid and ursolic acid enhance insulin sensitivity by regulating transcription factors and protein kinases involved in glucose uptake and metabolism. They also possess anti-inflammatory properties, which can mitigate insulin resistance, a key factor in diabetes (Nistor *et al*., 2022). Pentacyclic triterpenoids have emerged as significant inhibitors of α-amylase and α-glucosidase, enzymes crucial in carbohydrate metabolism and diabetes management. These compounds, including oleanolic acid, ursolic acid, and corosolic acid, exhibit potent inhibitory activities, with IC_{50} values ranging from 12.1 µm to 94.1 ìm for α-glucosidase and 22.6 µm to 94.1 µm for α-amylase. Combinations with acarbose enhance inhibitory effects, suggesting a multifaceted approach to diabetes treatment (Zhang *et al*., 2012). Their mechanisms involve non-competitive inhibition, disrupting enzyme conformation through hydrogen bonding and hydrophobic interactions (Shen *et al*., 2023).

Additionally, plant samples of *A. heterophyllus, M. koenigii, T. cordifolia* and *C. pictus* were found to be rich source of phenols, flavonoids and triterpenoids that can attribute to the synergic effect on inhibition of enzymes like α-amylase and α-glucosidase. The inhibitory effects of phenolic compounds on α-glucosidase and α-amylase are well-documented. The potency of enzyme inhibition by these compounds, which can involve mixed, uncompetitive, or competitive mechanisms, is strongly linked to their chemical structures (Di Stefano *et al*., 2018; Tadera *et al*., 2006). Previous research has established a positive relationship between phenolic content and enzyme inhibition activity (Ramkumar *et al*., 2010; Xiao *et al*., 2011; Nouri *et al*., 2014) Furthermore, many studies have been conducted on the inhibitory effects of flavonoids on α-amylase and α-glucosidase. Results from *In vitro*, *in vivo* and *in silico* models have been reported (Adisakwattana *et al*., 2012; Kazeem *et al*., 2013; Toma *et al*., 2014; Adefegha *et al*., 2012). Studies indicate that when it comes to inhibiting these enzymes, flavonoids outperform other phenolic substances. The presence of more hydroxyl groups in the flavone structure is probably the cause of this increased activity, since it contributes to the greater inhibitory effects on enzymes (Di Stefano *et al*., 2018; Tadera *et al*., 2006).

Both methanol and dichloromethane extract of *A. heterophyllus* showed inhibition on α-glucosidase on par with the standard drug acarbose. Enzyme inhibition potential of tender fruit of *A. heterophyllus* is reported for the first time. It is around two-fold higher for α-amylase inhibition and on par with acarbose values for α-glucosidase, suggesting the importance of tender Jackfruit extract in management of blood glucose level. The acarbose IC_{50} value of 49.56 ± 1.71 µg/ml obtained in this study is consistent with values reported for commercially sourced yeast α-glucosidase (*Saccharomyces cerevisiae* origin) in colorimetric assays; comparable values (40-120 µg/ml) were reported by Alqahtani *et al*. (2019) and Elya *et al.* (2008) under similar experimental conditions. Values in the sub-µg/ml range cited in some literature apply to mammalian intestinal α-glucosidase or alternative substrate systems, not to the yeast enzyme used here. The higher IC_{50} for acarbose against yeast α-glucosidase is therefore expected and does not represent a methodological error. Previous studies reported that IC_{50} (half-maximal inhibitory concentration) values of oleanolic acid (5 µm) were 8-fold lower than that of acarbose (Javed *et al*., 2022). Another study mentioned that IC_{50} value for ursolic acid was 10-fold less than acarbose (Ding *et al*., 2018). They also mentioned that IC_{50} value changes due to the difference in the experimental conditions and methods of measurement as well as the source of inhibitor. Further changes in IC_{50} value can also attributed to source of enzyme.

Antioxidants may enhance insulin receptor activity and promote β-cell regeneration, leading to better insulin sensitivity (Ghosh *et al*., 2023). Studies show that combining antioxidant therapy with standard treatments can further reduce oxidative stress and improve metabolic outcomes in diabetic patients (Chadra *et al*., 2019). The previous studies reported that aqueous and ethanol extracts of *T. cordifolia* showed IC_{50} values of 97.68 ± 1.08 and 106.86 ± 0.80 µg/ml (Jain *et al*., 2018). It is also reported that the antioxidants in aqueous ethanol extract (1:1) of *M. koenigii* exhibited a scavenging activity of 82% at a concentration of 10 µg/ml, whereas gallic acid, used as a control, demonstrated 92% scavenging activity at the same concentration. The study found that the aqueous ethanol extract was the most effective system for achieving maximum antioxidant activity. Additionally, the temperature during extraction negatively impacted the DPPH scavenging activity (Sasidharan *et al*., 2011). Further, antioxidant capacity of different parts of *M. oleifera* studied in ethanolic extracts showed 20% inhibition activity at 1.25 mg/ml (Santos *et al*., 2022). Similarly, methanol extracts of *C. tetragonoloba* had 22.5% inhibition activity in DPPH radical scavenging assay (Sharma *et al*., 2017). It is reported that green onion extract exhibited low scavenging activity at 35.9%, while fresh garlic demonstrated higher scavenging abilities, with percentages 91% for garlic (Benkeblia, 2005). The ethanolic extract of Jackfruit seeds exhibited a DPPH scavenging activity with an EC_{50} value of 76.71 µg/ml, indicating strong antioxidant properties (Barbalho *et al*., 2015). The total extract from Jackfruit leaves demonstrated an IC_{50} value of 73.5 µg/ml in the DPPH assay, showcasing its effective radical scavenging ability (Loizzo *et al*., 2010). In the present study lower inhibition activity

of garlic may due to drying which would have evaporated volatile phenols. Further, it is reported that IC_{50} value of DPPH assay or FRAP value exhibited good linear correlations between antioxidant activity and contents of total flavonoids or phenolics suggesting their potency in scavenging radicals (Hong *et al*., 2008).

5. Conclusion

GC-MS analysis identified several pentacyclic triterpenoids in the plant samples, with *A. heterophyllus* and *T. cordifolia* containing the highest number of compounds (seven each). The tender fruit of *A. heterophyllus* demonstrated the strongest antidiabetic potential, with IC_{50} values for α-glucosidase (53.86-54.77 µg/ml) comparable to the reference drug acarbose (49.56 ± 1.71 µg/ml) representing the first report of enzyme inhibitory activity for this tissue. Pearson correlation analysis confirmed statistically significant positive correlations between total triterpenoid content and α-amylase inhibition ($R = 0.860$, $p<0.05$) and α-glucosidase inhibition ($R = 0.757$, $p<0.05$), and between flavonoid content and both enzyme activities ($R = 0.792$ and $R = 0.671$, $p<0.05$). These findings provide scientific evidence supporting the traditional use of Jackfruit and selected medicinal plants in diabetes management. Future studies should focus on isolation of specific bioactive pentacyclic triterpenoids from A. heterophyllus for targeted pharmacological evaluation and *in vivo* validation using appropriate diabetic models.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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