EXPLORING THE ANTI-DIABETIC AND ANTI-OXIDANT POTENTIAL OF DIFFERENT FRACTIONS OF AMORPHOPHALLUS PAEONIIFOLIUS LEAF EXTRACT THROUGH IN VITRO ASSAYS

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ABSTRACT

AIMS AND OBJECTIVES: - Diabetes mellitus is a metabolic disorder characterized by persistent high blood glucose level. The global incidence of DM is about 22.9 million**.** Thus the present study evaluated the antidiabetic activity of different fractions (Chloroform, Methanol and Water) of the leaves of *Amorphophallus paeoniifolius D.* by using *in vitro* assays.

MATERIALS AND METHODS: - *In vitro* methods like α-Amylase and α-Glucosidase inhibition assays were performed to determine antidiabetic activity. For the antioxidant activity, DPPH free radical scavenging activity and H_2O_2 scavenging activity were also performed on each fraction of *Amorphophallus paeoniifolius.*

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RESULTS: - In *in vitro* assays for anti-diabetic activity, α-amylase inhibition assay and α-glucosidase inhibition assay were performed. In case of both the assays the Acarbose was taken as the standard drug and was treated as the positive control. For α-amylase inhibition assay, the IC50 value of Acarbose was found to be 97.495±16.94 µg/ml. For α-glucosidase inhibition assay, the IC50 value of Acarbose was found to be 103.789±23.73µg/ml. The IC50 value of Ascorbic acid was found to be 19.105±7.9 µg/ml in DPPH Antioxidant Activity. The chloroform, methanol and water extract of *Amorphophallus paeoniifolius* have also shown significant activity against the standard drug like that of acarbose and ascorbic acid.

CONCLUSION: - *Amorphophallus paeoniifolius possess* inhibition effect on the two enzymes and also shown anti-oxidant effect. The findings offer valuable insights into the pharmacological actions of this plant in relation to diabetes.

Keywords: Antidiabetic, Anti-oxidant, *Amorphophallus paeoniifolius*, α-Amylase, α-Glucosidase, DPPH, H_2O_2 scavenging assay.

INTRODUCTION

Blood sugar (glucose) levels are unusually excessive in people with diabetes mellitus because the body does not create enough insulin or respond to it correctly. A condition known as diabetes mellitus is characterized by high blood sugar levels. Among the negative consequences of diabetes are increased thirst and urination, as well as the possibility of weight loss even if the patient is not making an effort. Diabetes impairs nerve function and results in sensory issues. [1]

Diabetes raises the risk of heart attack, stroke, chronic kidney disease, and eyesight loss by damaging blood vessels. The blood vessels, nerves, eyes, and kidneys are among the numerous major long-term issues that people with diabetes mellitus experience. In 2017, type 2 diabetes impacted around 462 million people, or 6.28% of the global population (4.4% of people aged 15–49, 15% of people aged 50–69, and 22% of people aged 70+). This translates to a prevalence rate of 6059 cases per 100,000. [2,3] Diabetes alone is the ninth greatest cause of death, accounting for over 1 million fatalities annually. By 2030, the prevalence of type 2 diabetes is expected to climb to 7079 cases per 100,000 people worldwide, representing a steady increase in cases in every part of the world. According to the National Institute for Health and Care Excellence (NICE), typical blood sugar levels for most healthy people are as follows: .[4,5]

Between 4.0 to 5.4 mmol/L (72 to 99 mg/dL) when fasting

Up to 7.8 mmol/L (140 mg/dL) 2 hours after eating. [6]

The tuberous plant Amorphophallus paeoniifolius (Dennst.), which belongs to the Areaceae family, is frequently employed in Indian tribal and Ayurvedic medicines. Elephant foot yam is the usual English name for this plant, and "Oal" is the Bengali name. The plant is a member of the order Alismatales and the phylum Magnoliophyta. Every year, they generate one or two big leaves that can reach a height of two meters. [7, 8] The enormous underground tuber, which can weigh up to 25 kg, is where the leaves grow. It develops with purple corm inflorescence during the rainy season.

This hardy plant thrives in tropical climates with rich, well-drained soil that is humid and protected.The plant has been used extensively as traditional medicine for a variety of conditions, including arthralgia, elephantiasis, tumors, inflammations, hemorrhoids, hemorrhages, bronchitis, asthma, anorexia, dyspepsia, flatulence, colic, constipation, helminthiasis, hepatopathy, splenopathy, amenorrhea, dysmenorrhea, fatigue, anemia, and general weakness. [9, 10, 11]

MATERIALS:-

Chemicals :- Chloroform, Methanol, Distilled Water, α-Amylase, α-Glucosidase, Phosphate Buffer Solution (pH-6.8), 2,2-diphenyl-1-picryl- hydrazyl-hydrate (DPPH), L-Ascorbic Acid, Hydrogen Peroxide

Equipment: - Hot Water Bath, UV Spectrophotometer, Micropipette- 10, 100, 1000 µl, Centrifuge, Vortex Spinner, Digital Balance

METHODS

Collection of plant materials and preparation of extract of different fraction of *Amorphophallus paeoniifolius* D. leaves. [12, 13, 25]

The leaves of *Amorphophallus paeoniifolius* D. were collected from the locality of district North 24 Parganas, West Bengal in the month of August. Around 2 kg of the fresh leaves were collected along with the stalks. Some of them preserved for herbarium. The collected leaves were allowed to dry avoiding direct sunlight. These were shade dried to maintain a temperature of below 55°C in order to preserve the phytochemical constituents of the leaves. The leaves were kept moisture free and allowed to dry until they become brown in color and brittle. After drying of the leaves they were grinded using laboratory mill maintaining a temperature below 55°C and were converted into fine powder ready for extraction. 1. The powder was weighed and transferred in a 1000 ml conical flask and defatting was done by immersing the ground leaves in sufficient Petroleum ether to remove the non-polar compounds from the powdered material. 2. The first extraction was done with Chloroform by adding sufficient quantity of Chloroform so that the powder is completely dissolved in it. 3. This mixture was allowed to stand under dark conditions for a period of 72 hours, a process called maceration.4. After that filtration was done to separate the chloroform extract by carefully pressing the marc. 5. The filtrate (chloroform extract) was made to evaporate firstly in open air followed by heating on water bath at a temperature of 55-60°C. 6.After complete drying a semi-solid to dry residue was obtained which was immediately transferred to eppendorf tubes and stored at freezing temperature of about 4-8°C. 7. The marc which was left behind after filtration was again dissolved in methanol and followed by water and the same process was repeated as mentioned in steps 3.

***In Vitro* Anti-diabetic Assays:-**

α- Amylase Inhibition Assay:-[14, 15, 16]

Principle: The delay in glucose absorption caused by blocking enzymes that hydrolyze carbohydrates, such as pancreatic amylase delay carbohydrate digestion and protract overall carbohydrate digestion time, resulting in the reduction in glucose absorption rate and consequently dulling the postprandial plasma glucose rise.

Hence this assay is performed in vitro with the test compound to check its efficacy.

Methods: Stock solutions of the leaf extract with Chloroform, Methanol and Water was dissolved in Phosphate Buffer Solution of pH 6.8. The concentration of the stock solutions were 25, 50, 100, 150, 200, 250 and 300 µg/ml respectively. The stock solution of the standard drug Acarbose (500 mg in 50 ml PBS) was prepared in Phosphate Buffer Solution (pH 6.9). Inhibition of porcine α-amylase activity was determined using dinitrosalicylic acid. The leaf extract at various concentration or Acarbose (100 µl of 2 to 20 mg/ml) was added to 100 µl of α-amylase (1 U/ml) and 200 µl of phosphate buffer solution (pH 6.9). The samples were pre-incubated at 25 °C for 10 min, and 200 µl of 1 % starch prepared in phosphate buffer solution (pH 6.9) was added.
The reaction mixtures were incubated at 25 °C for 10 min. The reactions were stopped by incubating the mixture in a boiling water bath for 5 min after adding 1 ml of dinitrosalicylic acid (DNS). The reaction mixtures were cooled to room temperature and absorbance was measured in a UV spectrophotometer at 540 nm. Percentage of inhibition of enzyme activity was calculated as
% Inhibition = $[(A_{540}^{Control} - A_{540}^{Test}) / A_{540}] \times 100$

Wherein $A_{540}^{Control}$ is absorbance at 540 nm in control sample without the leaf extract and A_{540}^{Test} is absorbance at 540 nm in treatment with leaf extract

α-Glucosidase Inhibition Assay:- [17,18]
Principle: The enzyme α-glucosidase, which is membrane-bound and found in the small intestine's epithelium, speeds up the breakdown of oligosaccharides and disaccharides into simple glucose, which is then absorbed and released into the bloodstream. Delaying the breakdown of carbohydrates can lower blood glucose levels by inhibiting the α-glucosidase enzyme.

Methods: Phosphate buffer solution (pH 6.9) was used for generating leaf extracts of various solvents at varied concentrations (25, 50, 100, 150, 200, 250, and 300 µg/mL). Ten microliters of the α-glucosidase enzyme solution (1 U/mL) were then added, and the mixture was incubated for 20 minutes at 37 °C. After 20 minutes, 20 µL of 1 M pNPG (substrate) was added to initiate the reaction, and the mixture was incubated for 30 minutes. 50 µL of 0.1 N Na2CO3 was added to stop the reaction, and a UV spectrophotometer was used to detect the final absorbance at 405 nm. As a positive control, several quantities of acarbose (13, 25, 50, 100, 200, and 300 µg/mL) were employed.
Enzyme activity was calculated as:
% Inhibition = $[(A_{540}^{Control} - A_{540}^{Test}) / A_{540}] \times 100$
Wherein $A_{540}^{Control}$ is absorbance at 540 nm in control sample without the leaf extract and A_{540}^{Test} is absorbance at 540 nm in treatment with leaf extract
***In vitro* Antioxidant Activity: - [**[19]
DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay:- [20,21,26]
50 µL of sample solution of various concentrations (50, 100, 150, 200, 250, 300 and 350 µg/mL) were mixed with 50 µL of methanolic solution of DPPH (1000 µl).

The reaction mixture was incubated at 37°C for 1 h in the dark. The free radical scavenging potential of the extracts were expressed as the disappearance of the initial purple color. The absorbance of the reaction mixture was recorded at 517 nm using UV–Visible spectrophotometer. Ascorbic acid was used as the positive control.

DPPH scavenging capacity was calculated by using the following formula:

Scavenging Activity (%) = [(Absorbance ^{Control} – Absorbance ^{Test}) /Absorbance ^{Control}] x 100

Where A ^{control}: absorbance of the control solution; A ^{sample}: absorbance of the extract.

A linear regression analysis was used to calculate the IC50 (minimum inhibitory Concentration) value (µg/mL). The lower the IC50, the higher the antioxidant power.

H_2O_2 Scavenging Assay:- [22,23]

0.1 mL of extracts (50, 100, 150, 200, 250, 300 and 350 µg/ mL) was transferred into the test tubes and their volume was made up to 2.4 mL with phosphate buffer (pH 7.4) followed by the addition of 0.6 mL of H2O2 solution (0.43 mM). The reaction mixture was vortexed and after 10 min of reaction time, its absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. The ability of the extracts to scavenge the H2O2 was calculated using the following equation:

H2O2 scavenging activity percentage = [(A0 – A_1)/ A_0] x 100 where:

A0 = Absorbance of control, A1 = Absorbance of sample

STATISTICAL ANALYSIS [24]

Statistical analysis of the data was performed using Graph Pad Prism Software Version 5. The data was analysed using one-way ANOVA parametric test followed by Tukey's multiple comparison test that compares all columns of data among themselves and Dunnet test that compares all columns of data with the control column respectively as per the requirement of the tests. Also in some cases unpaired t test was performed. The value $p<0.05$ is statistically significant. [*$p<0.05$, **$p<0.001$ and ***$p<0.0001$]

RESULT:-

ALPHA AMYLASE INHIBITION ASSAY

Standard Acarbose Concentration (µg/ml)	**% Inhibition**			**Interpolated IC50 Value (µg/ml)**	**Average IC50 Value**
	Set 1	**Set 2**	**Set 3**	97.495±16.94	NIL
0	0.000	0.000	0.000		
10	18.750	16.183	12.149		
20	22.410	19.883	14.862		
40	29.060	21.009	16.249		
60	38.740	33.479	25.112		
80	47.270	42.129	36.789		

Water extract Concentration (µg/ml)	**% Inhibition**			**Interpolated IC50 Value (µg/ml)**	**Average IC50 Value**
	Set 1	**Set 2**	**Set 3**		
0	0	0	0		

40	14.986	11.672	8.112	125.040±18.10	144.602±17.81
60	25.824	19.293	12.118		
80	31.649	27.116	21.094	145.249±17.50	
100	39.118	32.087	28.994		
120	48.349	43.245	39.461	163.518±17.83	

Methanolic extract Concentration (µg/ml)	**% Inhibition**			**Interpolated IC50 Value (µg/ml)**	**Average IC50 Value**
	Set 1	**Set 2**	**Set 3**		
0	0	0	0		148.347±17.85
40	12.329	9.213	7.591	131.754±18.15	
60	21.989	17.684	14.998		
80	29.684	25.129	21.921	145.219±18.12	
100	37.496	32.118	27.649		
120	46.273	44.198	37.623	168.068±17.30	

Chloroform extract Concentration (µg/ml)	**% Inhibition**			**Interpolated IC50 Value (µg/ml)**	**Average IC50 Value**
	Set 1	**Set 2**	**Set 3**		
0	0	0	0		162.479±17.74
40	10.281	6.744	2.189	142.898±17.79	
60	19.288	15.849	11.567		
80	27.246	21.998	18.349	160.762±17.63	
100	34.098	31.761	27.228	183.778±17.82	
120	42.982	37.655	32.724		

ALPHA GLUCOSIDASE INHIBITION ASSAY

Standard Acarbose Concentration (µg/ml)	**% Inhibition**			**Interpolated IC50 Value (µg/ml)**	**Average IC50 Value**
	Set 1	**Set 2**	**Set 3**		
0	0	0	0	103.789±23.73	NIL
15	41.889	36.216	30.189		
30	49.908	42.119	37.162		
60	55.261	49.984	41.762		
120	71.396	62.493	57.118		
250	80.206	74.326	69.467		

Water extract Concentration (µg/ml)	% Inhibition			Interpolated IC50 Value (µg/ml)	Average IC50 Value
	Set 1	**Set 2**	**Set 3**		
0	0	0	0		
60	38.247	31.992	28.776	111.001±27	
90	56.184	51.294	47.662		124.004±25.87
120	69.249	63.492	59.772	124.953±25.87	
200	72.821	70.005	67.614		
250	79.000	74.856	70.198	136.058±24.74	

Methanolic extract Concentration (µg/ml)	% Inhibition			Interpolated IC50 Value (µg/ml)	Average IC50 Value
	Set 1	**Set 2**	**Set 3**		
0	0	0	0		
60	31.791	27.442	21.994	135.390±24.33	
90	49.776	42.189	38.661		150.605±23.363
120	54.213	50.943	47.843	149.444±23.50	
200	66.846	62.749	58.219		
250	72.493	69.843	63.549	166.982±22.26	

Chloroform extract Concentration (µg/ml)	% Inhibition			Interpolated IC50 Value (µg/ml)	Average IC50 Value
	Set 1	**Set 2**	**Set 3**		173.757±21.733
0	0	0	0		
60	26.119	21.649	19.482	155.351±22.95	
90	40.592	37.163	31.824		
120	50.691	46.789	41.346	172.794±21.78	
200	59.761	54.129	50.498		
250	68.226	63.713	58.227	193.126±20.47	

Table 1: Alpha Amylase & Alpha Glucosidase Inhibition Assay- Percentage Inhibition and IC50 Value

DPPH ANTIOXIDANT ACTIVITY

Ascorbic Acid standard Concentration (µg/ml)	**% Inhibition**			**Interpolated IC50 Values**	**erage IC50 Value**
	Set 1	**Set 2**	**Set 3**		19.105±7.97
10	30.925	29.174	26.355	13.971±7.67	
25	57.162	54.652	52.191		
50	73.484	68.301	73.644	20.540±7.89	
75	83.191	85.124	83.087		
100	87.046	86.350	89.066	22.594±8.36	
125	88.939	88.027	88.494		
150	89.355	89.307	90.157		

Water extract Concentration (µg/ml)	**% Inhibition**			**Interpolated IC50 Values**	**erage IC50 Value**
	Set 1	**Set 2**	**Set 3**		176.189±18.053
50	30.178	27.533	30.497	173.570±17.84	
100	35.544	33.231	35.903		
150	44.156	42.102	44.533	183.429±18.48	
200	54.002	52.398	54.392		
250	65.098	63.847	65.534	171.569±17.84	
300	72.686	71.727	72.950		
350	81.107	80.508	81.424		

Methanolic extract Concentration (µg/ml)	**% Inhibition**	**rpolated 0 Values**	**erage IC50 Value**

	Set 1	Set 2	Set 3		217.358±19.343
50	17.763	14.695	18.194	215.048±19.08	
100	27.799	25.171	28.123		
150	37.002	34.673	37.411	224.013±19.88	
200	49.505	47.728	49.887		
250	58.013	56.599	58.412	213.014±19.07	
300	65.566	64.424	65.967		
350	74.318	73.476	74.683		

Chloroform extract Concentration (µg/ml)	**% Inhibition**	**rpolated 0 Values**	**erage IC50 Value**

	Set 1	Set 2	Set 3		287.118±16.08
50	16.374	13.360	16.808	284.464±15.84	
100	20.437	17.580	20.880		
150	30.352	27.839	31.190	295.588±16.63	
200	37.072	34.835	37.480		
250	45.598	43.635	46.075	281.304±15.77	
300	48.376	46.610	48.830		
350	62.858	61.630	63.281		

H_2O_2 FREE RADICAL SCAVENGING ASSAY

Standard Ascorbic Acid Concentration (µg/ml)	**% Inhibition**	**rpolated IC50 Values**	**erage IC50 Value**

	Set 1	Set 2	Set 3		17.358±8. 21
10	41.904	42.034	36.894	14.804±7.96	
25	52.294	48.353	53.327		
50	69.545	67.598	70.277	19.789±8.33	
75	79.675	76.217	81.132		
100	89.848	93.743	90.458	17.481±8.34	
125	98.311	97.886	97.867		
150	98.506	99.606	99.203		

Water extract Concentration (µg/ml)	% Inhibition			rpolated IC50 Values	erage IC50 Value
	Set 1	**Set 2**	**Set 3**		142.796±14.95
50	33.614	36.730	34.352	148.571±15.29	
100	37.575	40.667	38.466		
150	54.242	56.308	54.770	134.374±14.69	
200	57.878	59.850	58.410		
250	69.134	70.623	69.739	145.443±14.88	
300	75.389	76.590	74.456		
Methanolic extract Concentration (µg/ml)	**% Inhibition**			**rpolated IC50 Values**	**erage IC50 Value**
	Set 1	**Set 2**	**Set 3**		192.417±16.32
50	23.982	27.304	24.596	197.602±16.59	
100	32.922	35.943	33.620		
150	37.164	40.004	37.777	184.984±15.86	
200	49.653	51.957	50.226		
250	56.623	58.649	57.075	194.665±16.51	
300	73.852	75.201	74.348		
Chloroform extract Concentration (µg/ml)	**% Inhibition**			**rpolated IC50 Values**	**erage IC50 Value**
	Set 1	**Set 2**	**Set 3**		293.954±14.82
50	13.160	17.112	14.430	300.221±15.26	
100	21.471	24.984	22.162		
150	22.900	26.310	23.540	284.745±14.21	
200	32.510	35.550	33.211		
250	40.779	43.484	41.438	296.898±14.99	
300	53.528	55.666	54.102		

Table -2: DPPH Antioxidant Activity & H_2O_2 Free Radical Scavenging Assay- Percentage Inhibition and Average IC50 Value

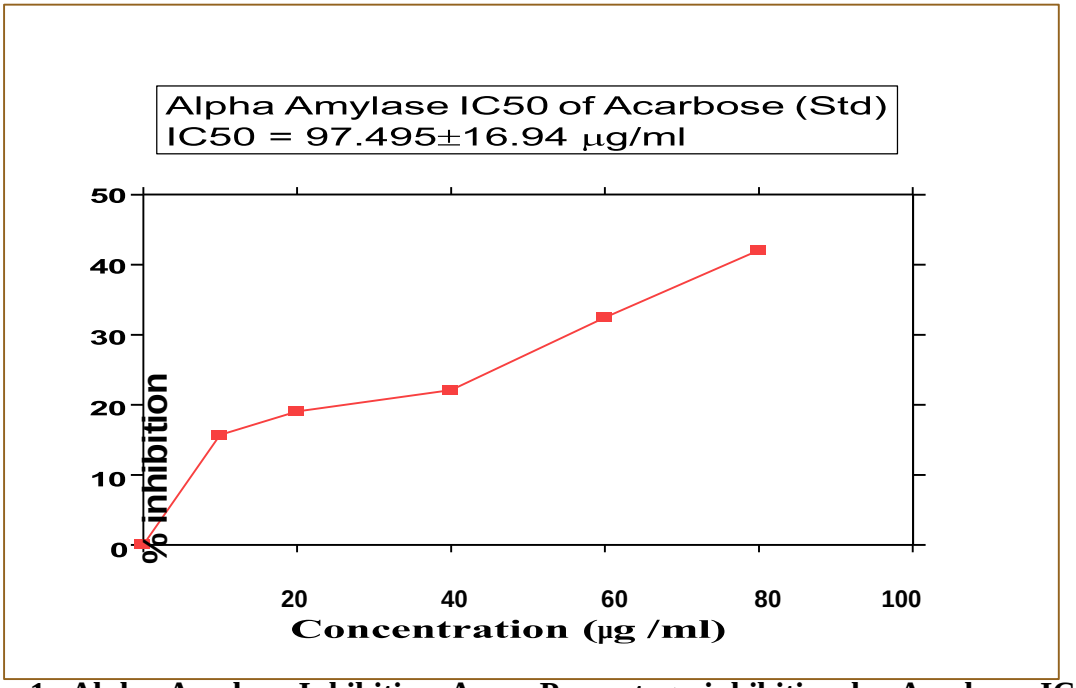

Figure 1: Alpha Amylase Inhibition Assay-Percentage inhibition by Acarbose. IC50= 97.495±16.94 µg/ml

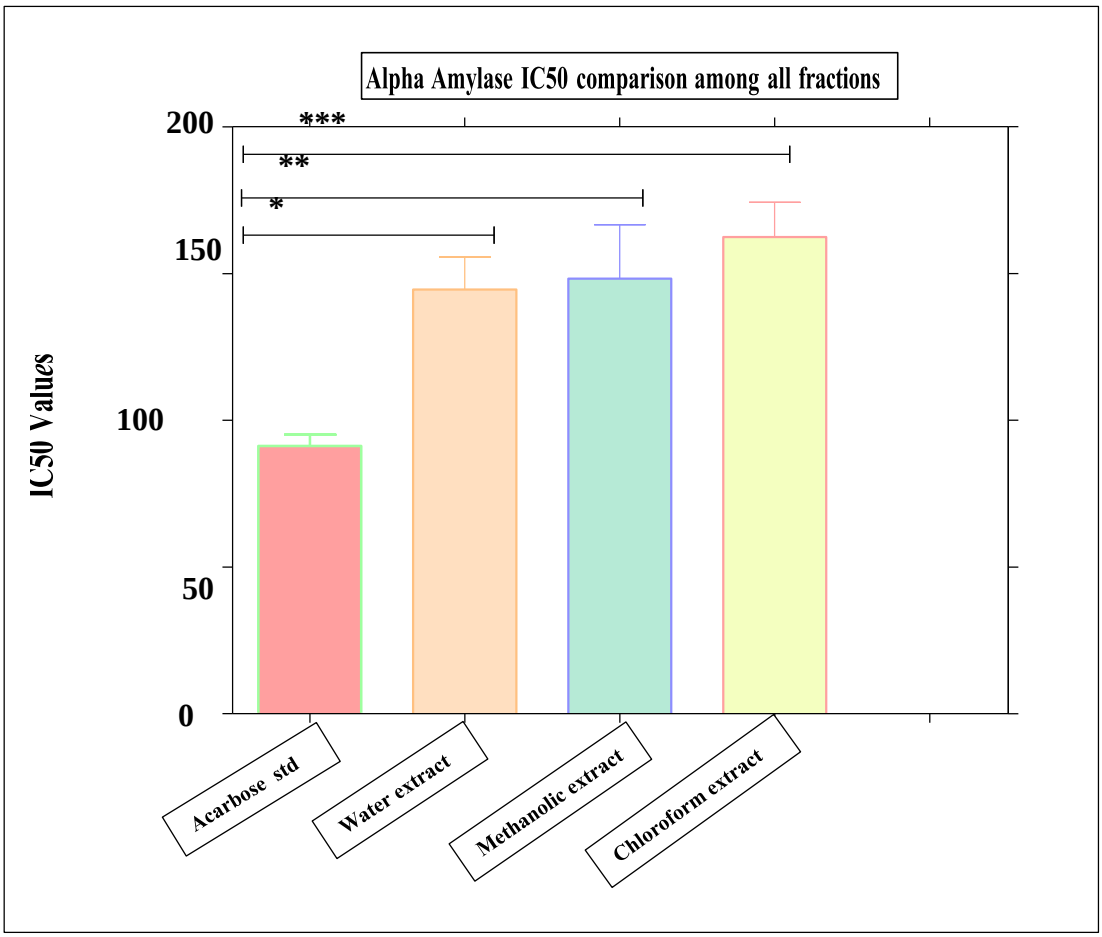

Figure-2 IC_{50} comparison of water, methanol, and chloroform fraction of test drug against Acarbose

All values are taken for triplicate analysis. The values of one-way ANOVA followed by Dunnet Post Test are statistically significant at *p<0.05 and **p<0.001

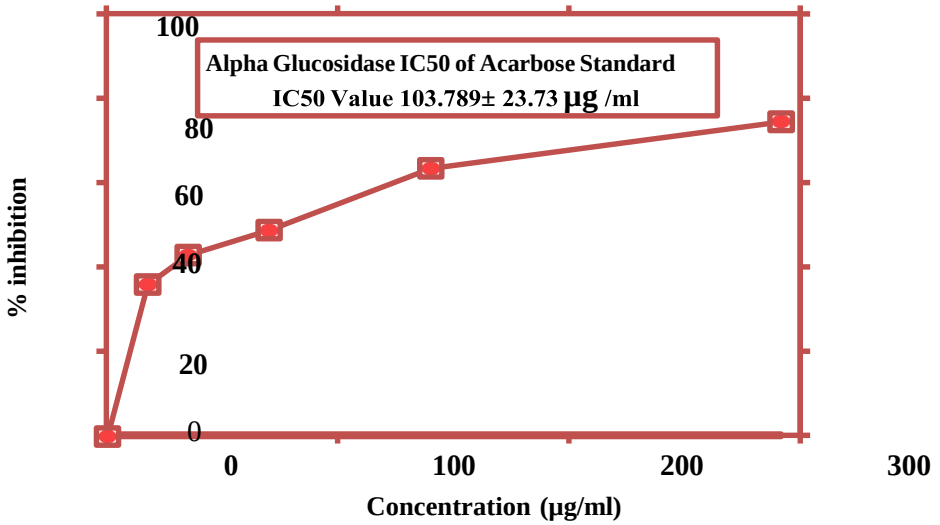

Figure 3: Alpha Glucosidase Inhibition Assay-Percentage inhibition by Acarbose. IC50= 103.789±23.7 µg/ml

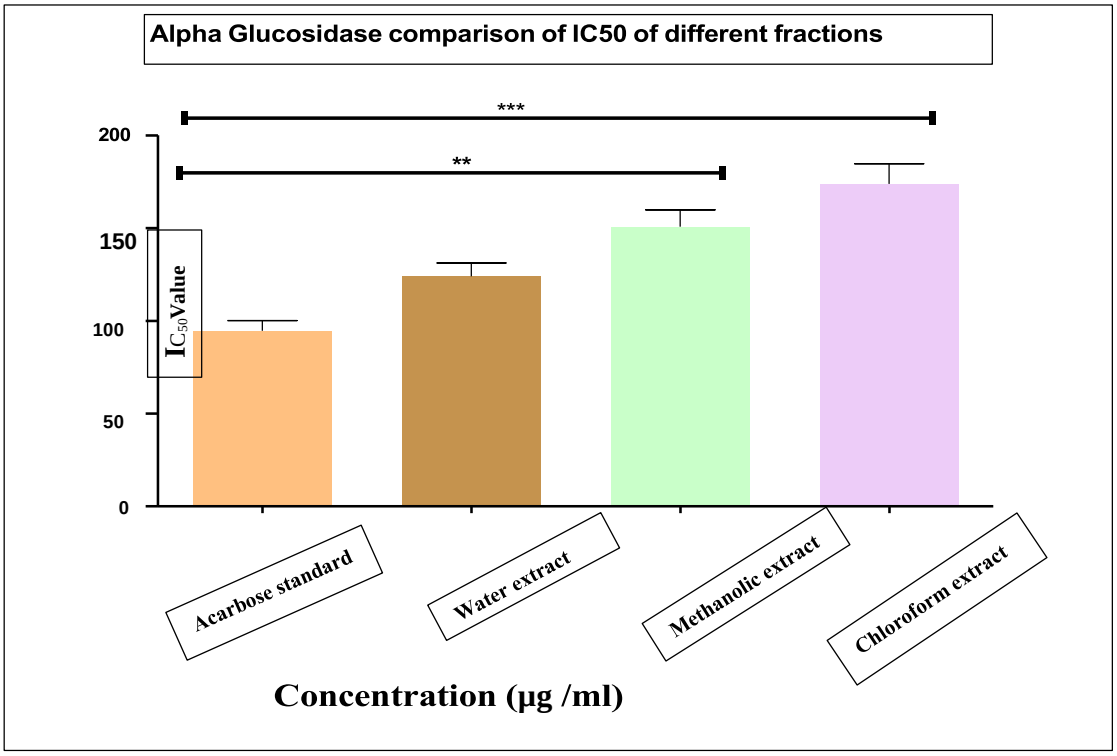

Figure-4 IC_{50} comparison of water, methanol, and chloroform fraction of test drug against Acarbose.

All values are taken for triplicate analysis. The p values of one-way ANOVA followed by Dunnet Post Test are statistically significant at **p<0.001 and *** p<0.0001

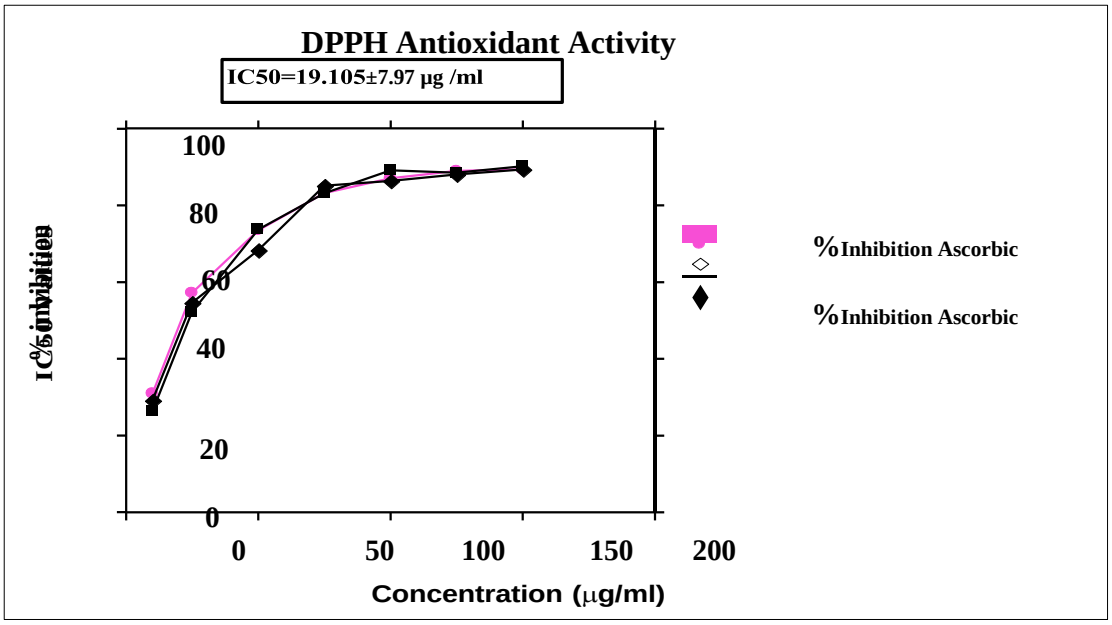

Figure-5 DPPH Antioxidant Activity Percentage inhibition by Ascorbic Acid. IC50=19.105±7.97µg/ml

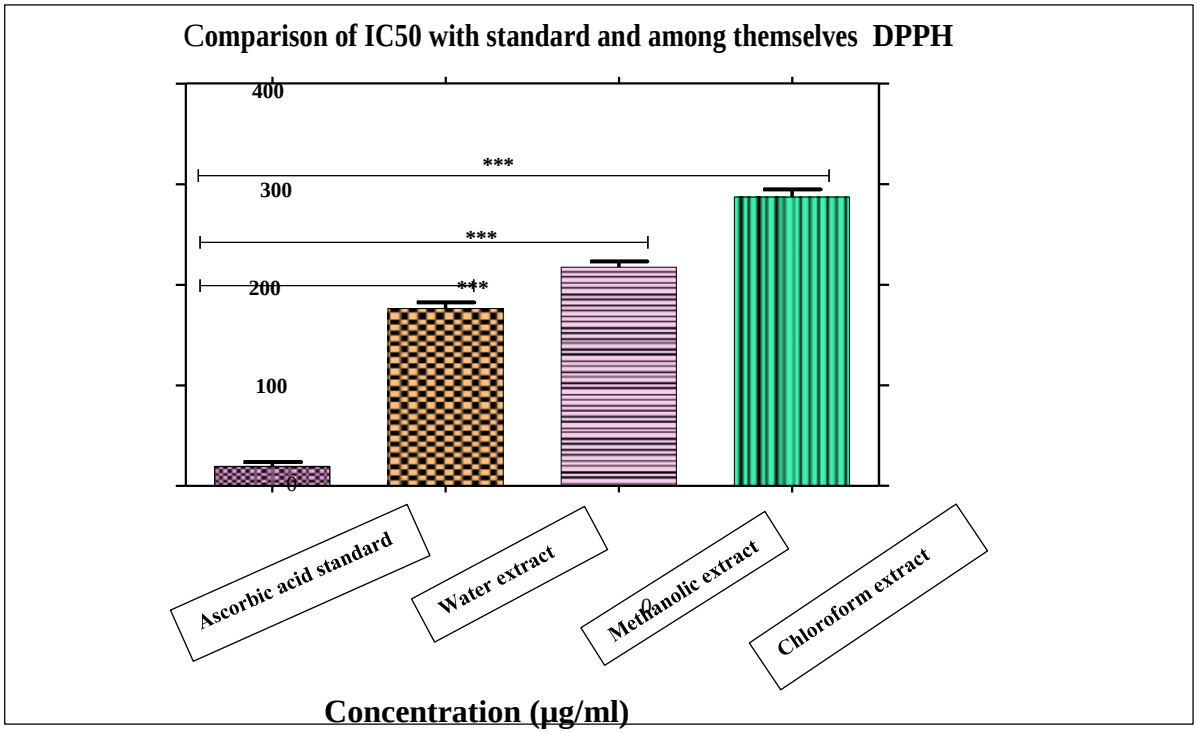

Figure-6 IC50 comparison of water, methanol and chloroform fraction of test drug against Ascorbic acid.

All values are taken for triplicate analysis. The p values of one-way ANOVA followed by Dunnet Post Test are statistically significant at *** p<0.0001

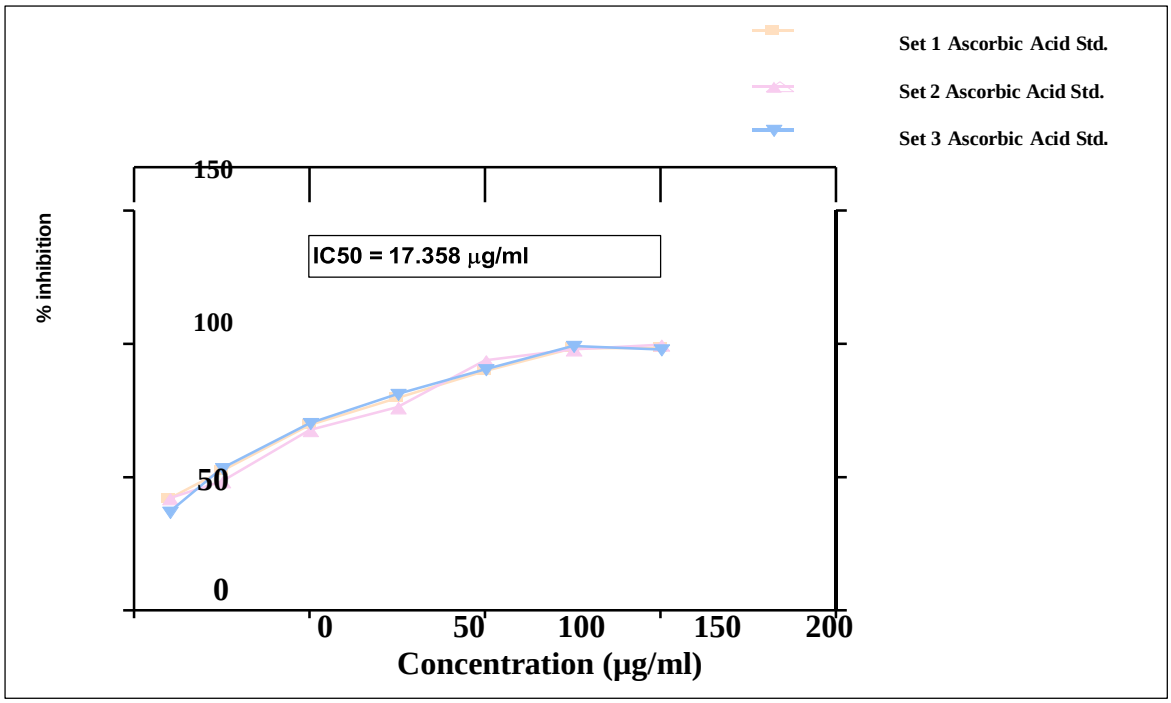

Figure-7 H_2O_2 Free Radical Scavenging Activity-Percentage inhibition by Ascorbic Acid. IC50= 17.358±8.21 µg/ml

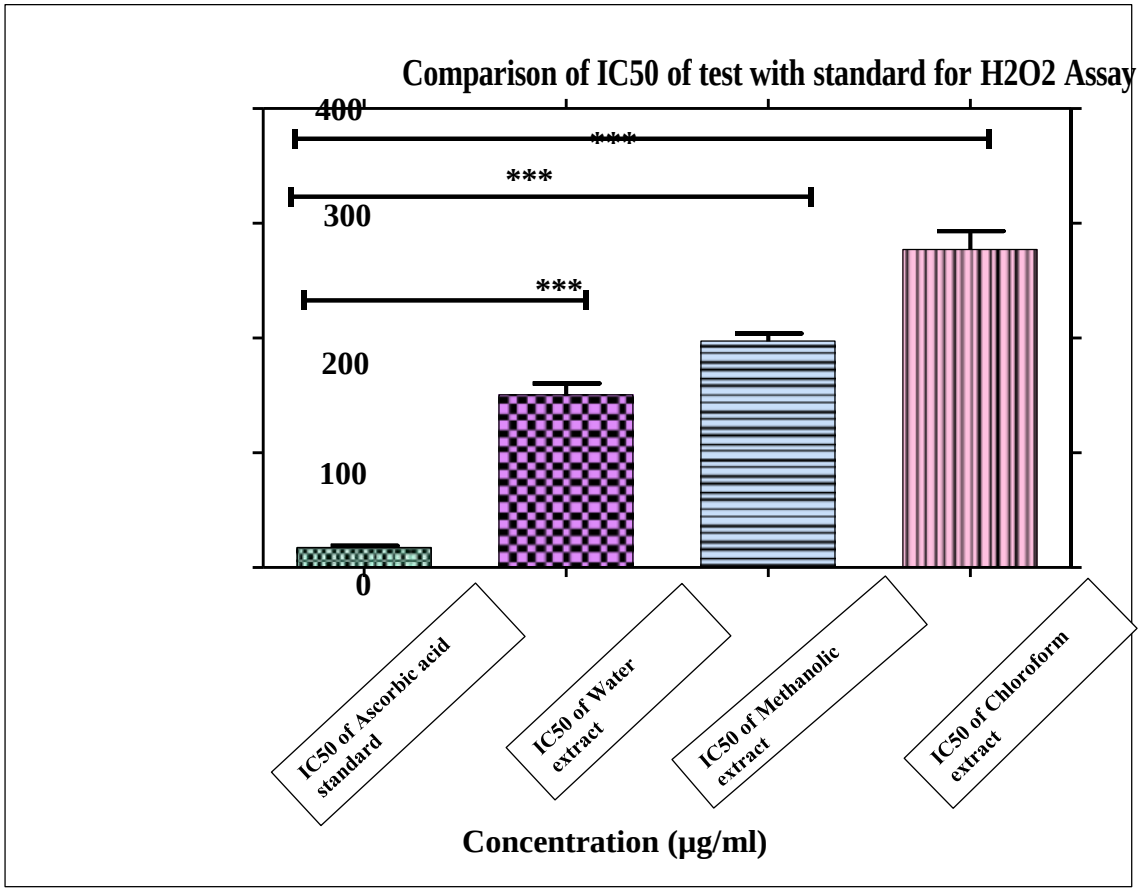

Figure-8 IC50 comparison of water, methanol and chloroform fraction of test drug against Ascorbic acid for H_2O_2 assay.

All values are taken for triplicate analysis. The p values of one-way ANOVA followed by Dunnet Post Test are statistically significant at *** p<0.0001

DISCUSSION

The leaves of *Amorphophallus paeoniifolius* possess a wide variety of phytochemicals like flavonoids, alkaloids, glycosides, saponin, inulin, protein and carbohydrates. Different plants have long been used for different pharmacological uses. The corm of this plant already has significant anti-diabetic activity. In *in vitro* assays for anti-diabetic activity, α-amylase inhibition assay and α- glucosidase inhibition assay were performed. **Table 1** shows α-Amylase & α- Glucosidase inhibition Assay- Percentage Inhibition and IC50 Value. In case of both the assays the Acarbose was taken as the standard drug and was treated as the positive control. For α-amylase inhibition assay, the IC50 value of Acarbose was found to be 97.495±16.94 µg/ml. **[Figure 1]** The Water, Methanolic and the Chloroform Extracts exhibited IC50 values at 144.60±17.81 µg/ml, 148.35±17.85 µg/ml and 162.48±17.74 µg/ml respectively. **[Figure 2]** For α-glucosidase inhibition assay, the IC50 value of Acarbose was found to be 103.789±23.73µg/ml. **[Figure 3]** The Water, Methanolic and the Chloroform Extracts exhibited IC50 values at 124.004±25.87 µg/ml, 150.60±23.36 µg/ml and 173.76±21.73 µg/ml respectively. **[Figure 4]** It can be conferred that the water extract has the lowest IC50 value followed by the methanolic extract in both the assays. The water extracts showed significant anti-diabetic activity followed by the methanolic extract. Since oxidative stress is an enormous drawback for diabetic patients, some *in vitro* free radical scavenging assays have also been performed with the test drug. This includes DPPH Antioxidant Activity and H_2O_2 Free Radical Scavenging activity. **Table 2** shows DPPH Antioxidant Activity & H_2O_2 Free Radical Scavenging Assay- Percentage Inhibition and Average IC50 Value. Ascorbic acid was used as the standard and the positive control in both cases. The IC50 value of Ascorbic acid was found to be 19.105±7.9 µg/ml in DPPH Antioxidant Activity. **[Figure 5]** The water, methanolic and chloroform extract of the test drug exhibited the IC50 values at 176.19±18.05 µg/ml, 217.36±19.34 µg/ml and 287.12±16.08 µg/ml respectively for DPPH Antioxidant Activity**. [Figure 6]** The IC50 value of Ascorbic acid was found to be 17.358±8.21 µg/ml in H_2O_2 Free Radical Scavenging Activity**. [Figure 7]** The water, methanolic and chloroform extract of the test drug exhibited the IC50 values at 142.79±14.95 µg/ml, 192.41±16.32 µg/ml and 293.95±14.82 µg/ml respectively for H_2O_2 Free Radical Scavenging Activity. **[Figure 8]** It can be conferred that the water extract has the lowest IC50 value followed by the methanolic extract in both the assays. The water extract showed significant antioxidant activity followed by the methanolic extract.

CONCLUSION

From this study it can be concluded that *Amorphophallus paeoniifolius* leaf possess anti-diabetic property particularly the Aqueous Fraction and Methanolic Fraction of the Extract in *in vitro* models. The Chloroform Fraction does not possess significant anti-diabetic activity. The Phytochemical Screening exhibits the presence of a wide variety of phytochemicals which are responsible for such activity. It also possesses antioxidant activity which is an essential factor in management of Diabetes Mellitus.

The Aqueous fraction followed by the Methanolic fraction exhibited a decent amount of antioxidant activity. After this study we want to conclude that among these three fractions the Water Fraction is the superior in all aspects and the dose can be optimized keeping in mind the safety and efficacy level of the fraction. The findings offer valuable insights into the pharmacological actions of this plant in relation to diabetes.

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