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Evaluation of Anti Diabetic Activity (In-vitro) of *Psidium guajava* Unripe Fruits Aqueous Extract

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Original Research Article

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Abstract: Diabetes Mellitus is a major endocrine disorder caused by the failure of the body function properly carbohydrate metabolism as well as changes in lipid and protein metabolism, thus contributing to hyperglycemia (increased blood sugar levels above normal), glycosuria (the presence of sugar in the urine), polyuria (the need to urinate frequently), polydipsia (always thirsty) and polyphagia (increase appetite). Numerous herbal plants and their formulations are therefore used widely for the various forms of diseases. One well-known herb of this kind is *Psidium guajava* Linn (Family: Myrtaceae) is a semi deciduous tropical tree commonly known as guava or '*Amrood*' in north India and is widely grown throughout India. The present study was undertaken to evaluate the Anti diabetic activity (In-vitro) of *Psidium guajava* unripe fruit aqueous extact. The Evaluation of anti-diabetic activity of extract by In-vitro method by glucose uptake by yeast cells the rate of uptake of glucose into the yeast cells was linear in all the 3 glucose concentrations. The extract of *Psidium guajava* exhibited significantly activity than the control. However, the percent increase in the glucose uptake by the yeast cells was observed to be inversely proportional to the glucose concentration and was found to decrease with increase in the molar concentration of the glucose solution. The effect of the plant extracts on retarding glucose diffusion across the dialysis membrane is shown in Table 3. The rate of glucose diffusion was found to increase with time from 1 hour to 5 Hours In the present study, the movement of glucose across the Biological membrane was monitored once in 1 Hour till 5 Hours and it was found that, both the samples of plant extracts and Standard Acarbose was demonstrated significant inhibitory effects on movement of glucose into external solution across the biological membrane compared to control.

Keywords: *Psidium guajava,* Diabetes Mellitus, Anti Diabetic Activity, blood sugar.

INTRODUCTION

Diabetes Mellitus is a major endocrine disorder caused by the failure of the body function properly carbohydrate metabolism as well as changes in lipid and protein metabolism, thus contributing to hyperglycemia (increased blood sugar levels above normal), glycosuria (the presence of sugar in the urine), polyuria (the need to urinate frequently), polydipsia (always thirsty) and polyphagia (increase appetite). Hyperglycemia caused by the failure of the pancreas secrete insulin insatiety, insulin resistance and the reduction of glucose by cells [1] There are lots of chemical agents available to control and to treat diabetic patients but total recovery from diabetes has not been reported up to this date. Alternative to these synthetic agents, plants provide a potential source of hypoglycemic drugs and are widely used in several traditional systems of medicine to prevent diabetes [2].

Numerous herbal plants and their formulations are therefore used widely for the various forms of diseases. One well-known herb of this kind is *Psidium guajava* Linn (Family: Myrtaceae) is a semi deciduous tropical tree commonly known as guava or '*Amrood*' in north India and is widely grown throughout India. More recent ethno pharmacological studies show that *Psidium guajava* is used in many parts of the world for the treatment of a number of diseases, e.g. as an anti-inflammatory, for diabetes, hypertension, caries, wounds, pain relief and reducing fever. Some of the countries with a long history of traditional medicinal use of guava include Mexico and other Central American countries including the Caribbean, Africa and Asia [3, 4].

The present study was undertaken to evaluate the Anti diabetic activity(In-vitro) of *Psidium guajava* unripe fruit aqueous extact.

Objectives of the study

The objectives of the present studies were:

- Collection of the plant

- Preparation of extract
- Evaluation of anti-diabetic activity of extract by In-vitro method
    a) Evaluation of anti-diabetic activity by glucose uptake by yeast cells
    b) Evaluation of anti-diabetic activity by Glucose diffusion method.

MATERIALS AND METHODS

Collection of Plant

The unripe fruits of *Psidium guajava* were collected from the surrounded of Mother Teresa Pharmacy College, Sathupally. The collected fruits are authenticated by Dr.N.Dorababu, Professor, Department of Pharmacognosy, Mother Teresa Pharmacy College.

Fig-1: *Psidium guajava* Fruit

Aqueous extraction of unripe fruits of *Psidium guajava* by soxhlation

Fresh unripe fruits are washed with tap water then which are wiped with towel and the fruits are sliced into small pieces, the small pieces are crushed in the mortar from that 30 grams are weighed accurately then it was packed in thimble flask and 150ml of Distilled water was taken into round bottom flask. Then the Soxhlet assembly was set at 55^{o}c and extraction process was continued till the colour of packed material changed to colorless, the total procedure was continued for 8 hours. After that obtained extract was filtered and it was concentrated under evaporation and the concentrated product was air dried it was transferred into clean container and stored in the refrigerator.

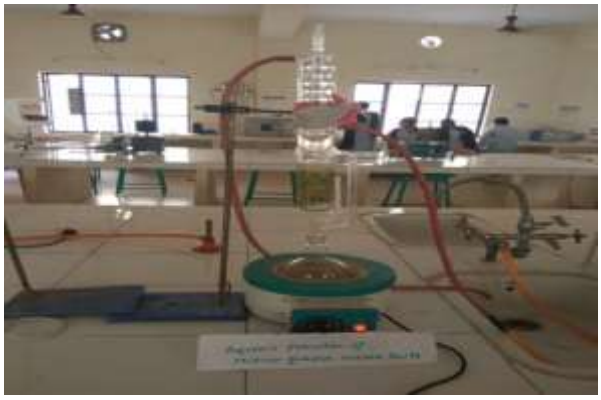

Fig-2: Soxhlet extraction of unripe fruits of *Psidium guajava*

Evaluation of anti-diabetic activity of extract by In-vitro method

a) In-vitro evaluation of anti-diabetic activity by glucose uptake by yeast cells [5-6]:

Preparation of glucose solution

The commercial Baker's yeast was dissolved in the distilled water and subjected to repeat centrifugations at 3000 rpm for 5minutes until clear supernatant fluids were obtained and a 10%(v/v) of the suspension was prepared in distilled water.

Various concentrations of plant extracts was prepared (20-500µg/ml) were added to 1ml of glucose solution (5,10,25Mm) and incubated together at 37°C for 60 minutes. After 60 minutes the tubes were centrifuged (2,500rpm for 5 minutes) an the amount of glucose was estimated in the supernent.

Preparation of standard drug solution

Metronidazol is used as an standard drug and prepare the different concentrations of standard drug i.e,(20-100µg/ml) to this concentrations add 1ml

glucose solutions (5,10,25Mml) and 1ml of yeast solution were incubated together at 37°C for 60 minutes.

After 60 minutes the tubes were centrifuged (2,500rpm for 5 minutes) and amount of glucose was estimated in the supernent layer was calculated using the formula:

Increase in glucose uptake (%)= (Abs.control–Abs.sample)/ Abs.control ×100

Fig-3: Glucose uptake by yeast cell

In- vitro evaluation of anti-diabetic activity by glucose diffusion assay [7-8].

Which involved the use of sealed chicken ileum into which 15ml of a solution of a glucose and Nacl (0.14M) was introduced and the appearance of glucose in the external solution was measured .The model used in the present experiment consists of sealed chicken ileum into which 2ml of 0.14M Nacl containing 0.22M glucose solution was added. Keep at room temperature. The movement of glucose into the external solution was monitored at set of time intervals from 1 hour to 5 hours with one hour time interval. The effects of plant extracts on glucose diffusion were compared with control tests conducted in the absence of plant extracts at the en of the experimental period the concentrations of glucose within the chicken ileum were measured. All tests were carried out in triplicate. Glucose dialysis retardation index (GDRI) was calculated by using the following formula:

$$GDRI\% = \frac{\text{Glucose content with addition of sample (mg/dL)}}{\text{Glucose content of the 100- control (mg/dL)}} \times 100$$

Fig-4: Glucose diffusion assay

RESULTS

Effect of Plant extracts on Glucose uptake by yeast cells

Increase in Glucose uptake by Yeast cells was calculated from the formula

Increase in glucose uptake (%)= (Abs.control–Abs.sample)/ Abs.control ×100

The rate of glucose transport across cell membrane in yeast cells system is presented in Table.

Table-1: Effect of Plant extracts on Glucose uptake by yeast cells

S. No	Group	TREATMENT	GLUCOSE CONCENTRATION		
			5mM	10mM	25mM
1	I	CONTROL			
2	II	STD(20Mcg/ml)	20.99±0.01	6.06±0.01	0.55±0.50
3	III	STD(40Mcg/ml)	30.81±0.01	11.26±0.01	9.22±0.50
4	IV	STD(60Mcg/ml)	41.09±0.01	21.21±0.01	16.45±1.32
5	V	STD(80Mcg/ml)	47.17±0.01	40.69±0.01	28.01±0.87
6	VI	STD(100Mcg/ml)	68.68±0.01	51.95±0.01	32.64±0.50
7	VII	STD(500Mcg/ml)	71.01±0.01	54.11±0.01	44.78±0.50
8	VIII	PGAFE(20Mcg/ml)	26.13±0.01	3.03±0.01	0.26±0.87
9	IX	PGAFE(40Mcg/ml)	31.74±0.01	19.05±0.01	6.04±0.50
10	X	PGAFE(60Mcg/ml)	60.26±0.01	32.03±0.01	15.29±0.50
11	XI	PGAFE(80Mcg/ml)	64.47±0.01	41.99±0.01	29.17±0.50
12	XII	PGAFE(100Mcg/ml)	75.69±0.01	53.68±0.01	34.08±1.50
13	XIII	PGAFE(500Mcg/ml)	78.49±0.01	60.61±0.01	41.89±1.50

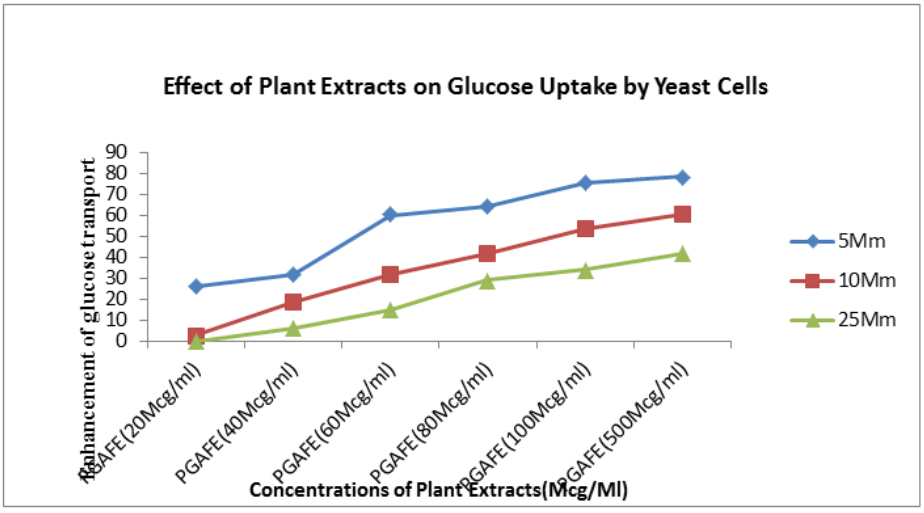

Fig-5: Effect of Plant Extracts on Glucose Uptake by Yeast Cells

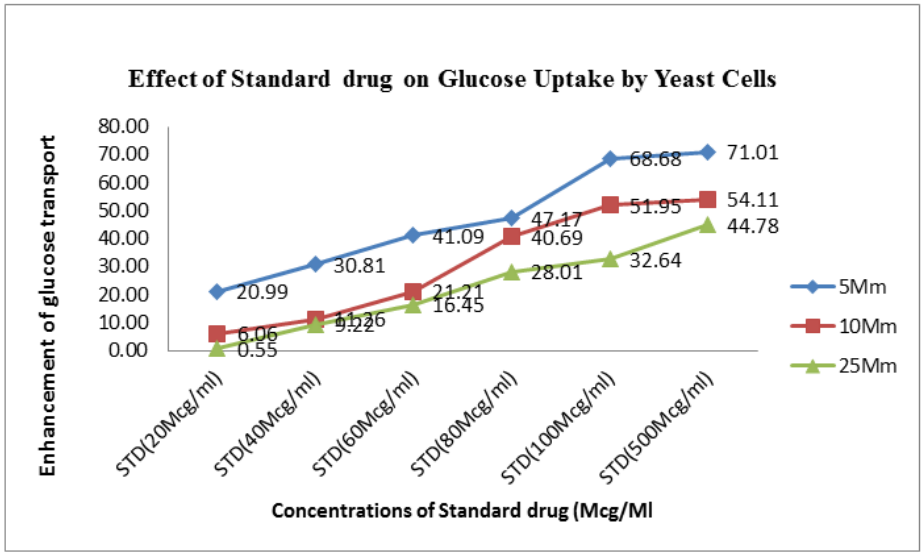

Fig-6: Effect of Standard drug on Glucose Uptake by Yeast Cells

The rate of glucose transport across cell membrane in yeast cells system is presented in table-1 The amount of glucose remaining in the medium after a specific time interval serves as an indicator of the glucose uptake by the yeast cells. The rate of uptake of glucose into the yeast cells was linear in all the 3

glucose concentrations. The extract of *Psidium guajava* exhibited significantly activity than the control. However, the percent increase in the glucose uptake by the yeast cells was observed to be inversely proportional to the glucose concentration and was found to decrease with increase in the molar concentration of the glucose solution.

Effect of PGAFE extracts on in vitro glucose diffusion

The effect of the plant extracts on retarding glucose diffusion across the dialysis membrane was performed, results are tabulated.

Table-2: Effect of PGAFE extracts on in vitro glucose diffusion

Group	Treatment	SEM Glucose content Mg/Dl			
		1Hour	2Hour	3Hour	5Hour
1	CONTROL	105.19	179.26	236.30	242.22
2	Test(6.5Mcg/ml)	62.96±1.28	81.85±1.70	169.63±1.28	214.07±1.28
3	TEST(12.5Mcg/ml)	54.81±1.28	88.89±2.22	205.93±5.59	212.59±2.57
4	TEST(25Mcg/ml)	30.37±2.57	56.30±1.28	148.89±3.85	151.11±2.22
5	TEST(50Mcg/ml)	28.89±0.00	41.48±2.57	145.93±4.63	147.41±5.13
6	STD(50Mcg/ml)	29.26±3.57	33.70±0.64	94.07±5.59	96.30±3.39

With the distinctive traditional medical opinions and natural medicines mainly originated in herbs, traditional medicine offers good clinical opportunities and shows a bright future in the therapy of diabetes mellitus and its complications. The effect of *P.guajava* unripe fruits as antidiabetic agents has been studied. All extracts showed varying effect on glucose utilization. These extracts caused a significant decrease in glucose concentration during the experiment. The effects of *P. guajava* unripe fruit extracts on glucose diffusion inhibition were summarized in Table5.3.a At the end of 5 hrs, glucose movement of control (without plant extract) in the external solution had reached a maximum with a mean glucose concentration above 242mg/dl. It was evident from the table that the aqueous extracts were found to be potent inhibitors of glucose diffusion compared to control. The standard drug Acarbose was found to be more potent than other extracts showing the lowest mean glucose concentration of 96.30±3.39mg/dl at the end of 5 hrs (Table-2).

Effect Extracts on GDRI

Glucose dialysis retardation index (GDRI) was calculated by using the following formula:

GDRI%= Glucose content with addition of sample (mg/dL)/Glucose content of the 100- control (mg/dL) ×100

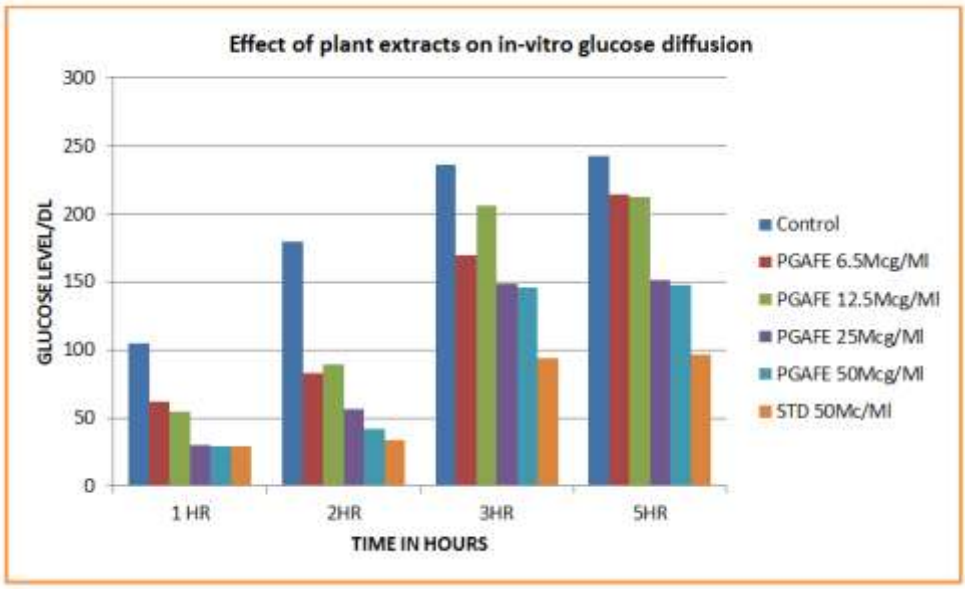

Fig-7: Effect of plant extracts on in-vitro glucose diffusion

Table-3: Effect Extracts on GDRI

Group	Treatment	SEM GDRI			
		1Hour	2Hour	3Hour	5Hour
1	Test(6.5Mcg/ml)	40.14±1.22	54.3±1.22	28.21±0.54	11.62±0.53
2	TEST(12.5Mcg/ml)	47.88±1.22	50.4±1.22	12.85±2.37	12.23±1.06
3	TEST(25Mcg/ml)	71.13±2.44	68.6±2.44	36.99±1.63	37.61±0.92
4	TEST(50Mcg/ml)	72.53±0.00	76.9±0.00	38.24±1.96	39.14±2.12
5	STD(50Mcg/ml)	72.18±3.40	81.2±3.40	60.19±2.37	60.24±1.40

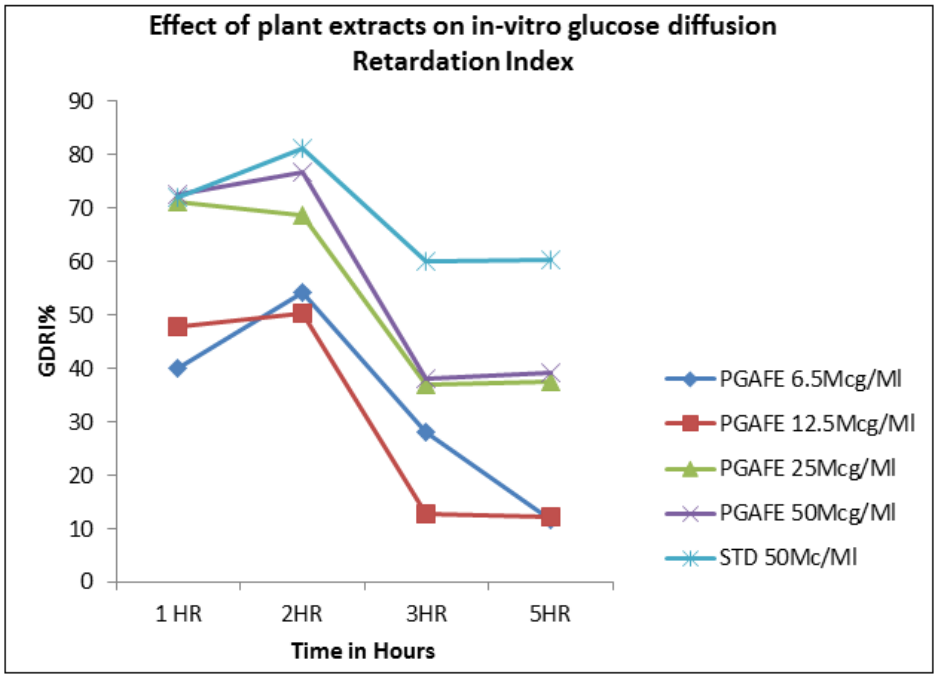

Fig-8: Effect of plant extracts on in-vitro glucose diffusion Retardation Index

The effect of the plant extracts on retarding glucose diffusion across the dialysis membrane is shown in Table 3. The rate of glucose diffusion was found to increase with time from 1 hour to 5 Hours In the present study, the movement of glucose across the Biological membrane was monitored once in 1 Hour till 5 Hours and it was found that, both the samples of plant extracts and Standard Acarbose was demonstrated significant inhibitory effects on movement of glucose into external solution across the biological membrane compared to control.

DISCUSSION

Diabetes mellitus is a debilitating and often life threatening disorder with increasing incidence throughout the world. There is a steady rise in the rate of incidence of Diabetes mellitus and estimated that 1 in 5 may be diabetic by 2025 [9]. In the present study, research has been carried out to evaluate the potential of aqueous extract to additionally retard the diffusion and movement of glucose in the intestinal tract and uptake of glucose by the yeast cells [10].

The higher adsorption capacity of the extracts of *Psidium guajava* may be attributed to their constituents. The results also revealed that the plant extracts under study could bind glucose even at lower concentrations of glucose (5 mmol/L) thereby reducing the amount of glucose available for transport across the intestinal lumen, consequently blunting the postprandial hyperglycemia. GDRI is a useful in vitro index to predict the effect of a fiber on the delay in glucose absorption in the gastrointestinal tract [11]. A higher GDRI indicates a higher retardation index of glucose by the sample.

The rate of glucose diffusion was found to increase with time from 1 hour to 5 Hours In the present study, the movement of glucose across the Biological membrane was monitored once in 1 Hour till 5 Hours and it was found that, both the samples of plant extracts and Standard Acarbose was demonstrated significant inhibitory effects on movement of glucose into external solution across the biological membrane compared to control.

The effect of *P.guajava* unripe fruits as antidiabetic agents by glucose uptaken by yeast cells method has been studied. All extracts showed varying effect on glucose utilization. These extracts caused a significant decrease in glucose concentration during the experiment. The effects of *P. guajava* unripe fruit

extracts at the end of 5 hrs, glucose movement of control (without plant extract) in the external solution had reached a maximum with a mean glucose concentration above 242mg/dl. It was evident from the results that the aqueous extracts were found to be potent inhibitors of glucose diffusion compared to control. The standard drug Acarbose was found to be more potent than other extracts showing the lowest mean glucose concentration of 96.30±3.39mg/dl at the end of 5 hrs.

CONCLUSION

The present study demonstrates the ability of various doses of Aqueous extracts of *P.guajava* to inhibit glucose diffusion using an in vitro model of glucose absorption and Glucose uptake. The aqueous extracts represent potential inhibitory of glucose diffusion and increases the glucose uptake, the unripe fruit supplements that may be useful for allowing flexibility in meal planning in type 2 diabetes. Further studies are required to elucidate whether in vitro effects represent therapeutic potential by limiting postprandial glucose absorptions and for improving glycemic control in type 2 diabetic subjects.

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