WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 8, 685-692. Research Article ISSN 2277– 7105

INVITRO SCREENING OF ANTI-DIABETIC POTENTIAL OF MIRABILIS JALAPA FLOWERS AND ABELMOSCHUS ESCULENTUS LEAVES

Sunmathy Kanakamani[1], Saraswathi Uthamaramasamy[2]* and Malathi Mangalanathan[3]

Department of Biochemistry, PSG College of Arts & Science, Coimbatore-14.

Article Received on 18 February 2018,
Revised on 11 March 2018,
Accepted on 2 April 2018,
DOI: 10.20959/wjpr20188-11798

***Corresponding Author**
Saraswathi Uthamaramasamy
Department of Biochemistry, PSG College of Arts & Science, Coimbatore-14.

ABSTRACT

Phytochemical analysis of *Mirabilis jalapa* revealed the presence of alkaloids, flavonoids, phenols, tannins, saponins and lignins. It is used in the treatment of skin disease, as an anti-pyretic and anti-parasitic. *Abelmoschus esculentus* is the only vegetable crop in *Malvaceae* family. It possess diuretic and demulcent property. The present study was done with an objective to explore the anti-diabetic property of *Mirabilis jalapa* (Flowers) and *Abelmoschus esculentus* (Leaves).The in vitro Anti-diabetic activity was done by inhibiting alpha-amylase, glucose diffusion and non-enzymatic glycosylation. The results of the study revealed that *Mirabilis jalapa* flower extract exhibited increased anti-diabetic activity at a concentration of 1000 g/ml than the leaves of *Abelmoschus esculentus*. From the observed results, it is concluded that *Mirabils jalapa* flowers are good source of phytochemicals which is responsible for its anti-diabetic activity.

KEYWORDS: Abelmoschus esculentus, Anti-diabetic, Alpha-amylase inhibition, Glucose diffusion assay, Mirabilis jalapa.

INTRODUCTION

Diabetes mellitus (DM) is rapidly becoming one of the most common non-communicable diseases globally.[1] It is also said to be a devastating metabolic disorder.[2] DM is a diverse collection of disorders commonly resulting with episodes of hyperglycaemia and glucose intolerance, as a result of defective insulin secretion or action or both**.**[3] Insulin is a hormone produced in the pancreas that helps to transport glucose from the bloodstream into the cells that results in glucose breakdown. People cannot live without insulin.[4] Basically, DM is classified as insulin dependent diabetes (Type 1) and non insulin dependent diabetes(Type 2). The number of people with diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity.

The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030.[5] Hyperinsulinemia and insulin resistance have been hypothesized to be involved in colorectal carcinoma.[6] It is well known that hyperinsulinemia can result in increased reabsorption of sodium and water by kidney tubular cells and this can be associated with a volume-dependent hypertension and various other disorders.[7] Since there is long occurance and serious complications of DM there is a need to reduce the risk of this disease by designing alternative drugs that are free from any side effects.[8]

In the present study *Mirabilis jalapa* (Flowers) and *Abelmoschus esculentus* (Leaves) were selected for Anti-diabetic studies. *Mirabilis jalapa* belongs to Nyctaginaceae is a relatively small family (30 genus and 400 species) that occurs mainly in tropical and subtropical regions of world and it is found in India, Africa and Pacific islands.[9] It is commonly called as four o'clock plant. It has been reported that indigenous Mexican people uses various parts of *Mirabilis jalapa* for the treatment of dysentery.[10] This plant contains several compounds like alkaloids, glycosides, carbohydrates, flavonoids, phytosterols (beta-sitosterol and stigmasterol), ursolic acid, oleanolic acid, brassicasterol, trigonelline.

Abelmoschus esculentus is the only vegetable crop of significance in the Malvaceae family and is very popular in the Indo-Pak subcontinent. Abelmoschus esculentus ensures recovery from psychological and mental conditions like depression and general weakness due to its alkaline origin. It also guards the mucous membranes of the digestive system, by covering them with additional layer. Seed oil of Abelmoschus esculentus is rich in unsaturated fatty acid such as linoleic acid, which is essential for human nutrition. Its mature fruit and stems contain crude fibre, which is used in the paper industry.[11] Since there are no reports available on the Anti-diabetic activity of *Mirabilis jalapa* flowers and *Abelmoschus esculentus* leaves, the present study was undertaken.

MATERIALS AND METHODS

The flowers of *Mirabilis jalapa* and leaves of *Abelmoscus esculentus* were taken in fresh condition without any defect from Coimbatore, Tamil Nadu. They were shade dried and powdered using a blender.

PREPARATION OF EXTRACT

10 grams of dried powder was added to 100ml of 50% hydroethanol with occasional stirring for 3 days. After 3 days, it was filtered with the help of Muslin cloth and it was evaporated to dryness at low temperature in a rotary vacuum evaporator (<40ºC). Further the powder was stored in a refrigerator and it was used for further investigations.

Assessment of in vitro diabetic activity

1) Alpha-amylase inhibition assay

This assay was performed with slight modifications of Rammohan *et al*., (2008).[12] In α - amylase inhibition method, the enzyme solution was prepared by dissolving α - amylase in 20mM phosphate buffer (6.9) at the concentration of 0.5mg/ml. 1ml of the plant extract of various concentrations of 62.5,125,250, 500, 750, 1000 µg/ml and 1ml of enzyme solutions were mixed together and incubated at 25°C for 10min. After incubation, 1ml of starch (0.5%) solution was added to the mixture and further incubated at 25°C for 10min. The reaction was then stopped by adding 2ml of dinitrosalicylic acid, heating the reaction mixture in a boiling water bath for 5 mins. After cooling, the absorbance was measured colorimetrically at 565 nm.

$$\text{The percentage inhibition of } \alpha\text{-amylase activity} = \frac{\text{(Abs control- Abs sample)}}{\text{Abs of control}} \times 100$$

2) Non-enzymatic glycosylation of haemoglobin

Non-enzymatic glycosylation assay is performed by modifying Chandrasekar et al.,(2012) method.[13] Glucose (2%), haemoglobin (0.06%), and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of the above solutions was mixed with 1ml of the plant extract of varying concentrations of 62.5,125,250, 500, 750, 1000 µg/ml. The reaction mixture was incubated in dark at room temperature for 72hrs, then the degree of glycosylation of haemoglobin was measured colorimetrically at 520nm and percentage inhibition was calculated using the formula,

The percentage inhibition = (Abs control- Abs sample) x 100 of glycosylation Abs control

3) Glucose diffusion

A method described by Gallagher (2002) et al., was used to evaluate the effect of hydroethanolic extracts of *Mirabilis jalapa (flowers)* and *Abelmoscus esculentus (leaves)* on glucose movement in vitro.[14] The dialysis membrane was purchased from HI-media laboratory, Mumbai. 2 ml of 0.15 M NaCl containing 0.22mM D-glucose was loaded into a dialysis tube containing plant extracts (1000µg/ml) and the dialysis tube was sealed. The sealed tube was then placed in a centrifuge tube containing 45 ml of 0.15 M NaCl and kept in an orbital shaker at room temperature. The diffusion of glucose into the external solution was monitored by measuring the glucose in the external solution for every 30minutes, for a period of three hours.

Statistical analysis

The results are expressed as mean $\pm$ SD for triplicates.

RESULTS

The alpha-amylase inhibition of the *Mirabilis jalapa* flowers and *Abelmoschus esculentus* leaves extracts was depicted in Table 1. There is a linear relationship between the concentration of plant extract and inhibition of α –amylase activity. Maximum inhibition of 75.09% was observed for the hydroethanolic extracts of *Mirabilis jalapa* flowers and *Abelmoschus esculentus* leaves showed an inhibition of 51.12% at a concentration of 1000µg. The results for non-enzymatic glycosylation of haemoglobin for *Mirabilis jalapa* flowers and *Abelmoschus esculentus* leaves extracts was depicted in Table 2.From the table it is evident that, when the concentration of drug increases, formation of glucose-haemoglobin complex decreases and free haemoglobin increases. This results in the inhibition of glycosylated haemoglobin. Maximum inhibition of 64.91% and 50.92% was observed for the hydroethanolic extracts of *Mirabilis jalapa* flowers and *Abelmoschus esculentus* leaves respectively.

Table 1: Inhibition of Alpha-amylase on varying concentrations of Mirabilis jalapa (flowers) and Abelmoschus esculentus (Leaves).

Concentration (µg/ml)	% Inhibition of alpha amylase	
	Mirabilis jalapa	*Abelmoschus esculentus*
62.5	31.07±0.20	21.56±0.14
125	38.13±0.28	33.52±0.50
250	46.69±0.06	44.07±0.36
500	58.90±0.07	48.06±0.58

1000	75.09±0.49	51.12±0.10
IC50 (µg/ml)	430.09±2.09	540±4.83

The values are expressed as Mean ± SD. (n=3)

Table 2: Invitro Non-enzymatic glycosylation of haemoglobin Assay.

Concentration (µg/ml)	**%Inhibition on glycosylation**	
	Mirabilis jalapa	***Abelmoschus esculentus***
62.5	36.66±0.24	32.48±0.10
125	45.11±0.13	34.69±0.52
250	51.51±0.30	41.81±0.48
500	59.18±0.05	45.94±0.39
1000	64.91±0.21	50.92±0.42
IC50 (µg/ml)	245±2.56	315±3.54

The values are expressed as Mean ± SD. (n=3).

Diffusion of glucose across the membrane was recorded for every 30mins for a period of 180mins. As the time proceeds the entry of glucose into the external solution was inhibited. The results of the glucose diffusion assay are depicted in Table 3. The relative movement of glucose at 180mins for *Mirabilis jalapa* was 50% and for *Abelmoschus esculentus* it is 83.33%. This data clearly suggests that *Mirabilis jalapa* maximally inhibit glucose diffusion than *Abelmoschus esculentus* which in turn states that the plant is capable of regulating glucose movement out of the cells into the blood stream thereby controlling post prandial glucose levels. After 3 hours, in the control (without plant extract) glucose movement out of dialysis bag reached a peak level but the plant extracts exhibited a concentration-dependent inhibitory effect on glucose movement.

TABLE 3: GLUCOSE DIFFUSION ASSAY.

Time (in minutes)	**Control**	***Abelmoschus esculentus***	**Relative movement**	***Mirabilis jalapa****	**Relative movement**
30	0.160±0.34	0.146±0.38	91.25%	0.136±0.29	85%
60	0.148±0.52	0.139±0.46	93.92%	0.121±0.18	81.71%
90	0.126±0.13	0.117±0.34	92.85%	0.101±0.40	80.15%
120	0.112±0.42	0.108±0.22	96.42%	0.098±0.53	87.5%
150	0.091±0.20	0.086±0.23	94.50%	0.071±0.43	82.55%
180	0.006±0.12	0.005±0.13	83.33%	0.003±0.35	50%

***** *The values are expressed as Mean ± SD. (n=3)*

DISCUSSION

Diabetes is characterized by hyperglycemia, altered lipid, carbohydrate and protein metabolism which affect the patient quality of life in terms of social, psychological wellbeing as well as physical ill health.[15] The drugs like metformin and pioglitozone will ameliorate insulin resistance and control the hyperglycemia and abnormal lipid metabolism. These drugs have adverse effects such as lactic acidosis, gastrointestinal disturbance, liver toxicity and cardiovascular risk. So there is an urgent need for medicinal plants. Alpha-amylase is an enzyme involved in the metabolism of carbohydrates. Drugs which inhibits the enzyme alpha-amylase decreases the post prandial glucose level, delays the carbohydrate hydrolysis and absorption.[16]

Non-enzymatic glycation of proteins also known as Maillard reaction is increased in diabetes mellitus due to hyperglycemia and leads to several complications that includes blindness, hepatic and cardiovascular diseases, nerve damage and kidney failure.[17] Reduction in glycation can be justified by the fact that non-enzymatic glycation is the covalent binding of reducing sugars to α- or ε- amino groups on protein. This glycation lowering activity can be linked to the presence of various phenolic components in *Mirabilis jalapa* flowers and *Abelmoschus esculentus leaves*. In recent years, much interest has been focused on the role of viscous polysaccharides in the treatment of diabetes mellitus. The action of the polysaccharides in reducing the postprandial hyperglycemia is thought to be related to their viscosity. Viscous polysaccharides from plants may delay glucose absorption probably by impairing the access of luminal contents to the absorptive epithelium.[18] *Mirabilis jalapa* flowers showed the maximum decline in movement of glucose than the *Abelmoschus esculentus* and this activity might be due to presence of various phytochemicals in *Mirabils jalapa* and *Abelmoschus esculentus.*

CONCLUSION

The present investigation confirmed the Anti-diabetic activity of 50% hydroethanolic extract of *Mirabilis jalapa* flowers and *Abelmoschus esculentus* leaves and maximum activity was found with *Mirabilis jalapa flowers*. Further works on *in vivo* anti-diabetic activity of *Mirabilis jalapa* flowers extract needs to be assessed.

ACKNOWLEDGMENTS

The authors gratefully thank the Department of Biochemistry, PSG College of Arts & Science, Coimbatore for their support.

CONFLICT OF INTEREST

There is no conflict of interests.

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