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In-vitro Anti - Diabetic Activity and Qualitative Bio Chemical Analysis of 20 MEGATHUKKU KUDINEER - A Siddha Formulation

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Abstract: ***In recent times, there is a growing interest towards Siddha medicine for the treatment of Diabetes mellitus. 20 Megathukkukudineer (MK) is a Siddha classical polyherbal formulation that has been mentioned in Sarabenthirar Vaithiya Muraigal Neerizhivu Sikitchai for the treatment of diabetes mellitus. The aim of this study is to analyse the in-vitro antidiabetic potential and bio-chemical analysis of 20 Megathukkukudineer. 20 Megathukkukudineer was prepared by boiling the ingredients weighed 20g in 160ml of water and reduced to 20 ml. The test sample (MK) was prepared in the serial dilution of concentrartion ranges from 100, 200, 300, 400 and 500 µg /ml using DD water. The invitro alpha amylase and alpha glucosidase enzyme inhibition of MK sample was compared with standard drug acarbose and the IC 50 value was calculated. The data was statistically analysed and expressed as Mean +SD (n =3). The result showed that MK had maximum activity towards the inhibition of enzyme alpha amylase (52.82 ± 17.33) and alpha glucosidase (38.61 ± 2.74) when compared with standard acarbose 98.62 ± 1.655 and 99.15 ± 0.3842 respectively. The qualitative phytochemical test revealed that MK contains calcium, sulphate, chloride, starch, unsaturated compound and amino acid.***

Keywords: Siddha, Megathukkukudineer, Madhumegam, Diabetes, In vitro method

1. Introduction

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Constant elevated levels of blood glucose (hyperglycaemia) over the time leads to serious damage to the heart, blood vessels, eyes, kidneys and nerves. In recent times the prevalence of type 2 diabetes has raised drastically particularly in low and middle – income countries. About 422 million people worldwide have diabetes and 1.5 million deaths are directly attributed to diabetes and its complication each year. The prevalence of diabetes in India has risen from 7.1% in 2009 to 8.9% in 2019. India ranks second after China in the global diabetes epidemic with 77 million people with diabetes. According to The India State-level Disease Burden Initiative Diabetes study report Tamil Nadu had the highest prevalence in 2016.

The word 'Madhumegam' is correlated with that of diabetes mellitus. Mathumegam is one among the pitha type of Mega noi. Madhumegam is a clinical condition characterized by frequent and excessive passage of urine with 'sweetness' eventually leading to deterioration of seven body constituents. Over several centuries mankind have been successfully treated with traditional medicine for all diseases. In siddha classical texts, many herbal, mineral and herbo – mineral formulations have been mentioned for the treatment of madhumegam. On such classical text is ***Sarabenthirar Vaithiya Muraigal Neerizhivu Sikitchai***, in which 20 Megathukkukudineer is mentioned for the treatment of madhumegam. All the ingredients of 20 megathukkukudineer has been provedfor its antihyperglycemic activity individually. Hence this study was carried out to prove the anti diabetic activity of the drugscollectivelyby in vitro methods in Alpha Amylase and Alpha Glucosidase Enzyme inhibition assay and qualitative biochemical analysis.

2. Materials and Methods

2.1 Identification of raw drugs:

The herbal ingredients were authenticated from faculty of department of Gunapadam, Government Siddha Medical College, Palayamkottai.

2.2 Ingredients of 20 MegathukkuKudineer:

1) Elam (Elettariacardamomum , Linn)
2) Vilamichu vaer (Plectranthusvettiveroides , Jacob)
3) Vettivaer (Vettiveriazizanioides , Linn)
4) Korai kilangu (Cyperusrotundus , Linn)
5) Lavangapaththiri (Cinnnamomumtamala , Buch.-Ham)
6) Santhanam (Santalum album , Linn)

2.3 Methods of purification:

Raw drugs were collected and purifications were done as per the methods given in classical siddha literatures.

2.4 Methods of drug preparation:

MK was prepared according to the procedure mentioned in Siddha classical text *Sarabenthirar Vaithiya Muraigal Neerizhivu Sikitchai.*

2.5 In-vitro Alpha Amylase Inhibition Study

Method Adopted: The spectrophotometric assay method.

The enzyme α-amylase (0.5 U/ml) was prepared by mixing 3.24 mg of α-amylase in 100 ml of phosphate buffer (pH 6.9). Test Sample (MK) was prepared in the serial dilution of the concentration ranges from 100,200,300,400 and 500 µg/ml using DD water. Acarbose 100 µg/ml used as a reference standard. About 600 µl of test sample were added to 30 µl of α-amylase enzyme solution and incubated at 37°C for 15 min. To this reaction mixture, 370 µl of substrate, 2-Chloro-4-Nitrophenyl-αMaltotrioside ($CNPG_3$- 0.5 mg/ml) was added, mixed and for incubated 37°C for 10 min. Finally, absorbance was measured at 405 nm against blank in spectrophotometer. A control reaction was carried out without the test sample. Percentage inhibition was calculated by the following formula.

Percentage inhibition

$$\%\text{inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

2.6 In-vitro α-Glucosidase Enzyme Inhibition Study

Method Adopted: The spectrophotometric assay method.

Test Solution: Test Sample (MK) was prepared in the serial dilution of the concentration ranges from 100,200,300,400 and 500 µg/ml using DD water.

PNPG (p-nitrophenyl-α-D -glucopyranoside**)**: 20 mM PNPG prepared by dissolving 603 mg PNPG in 100 ml of PBS

Enzyme: The α-glucosidase enzyme solution was prepared by dissolving 0.5 mg α-glucosidase in 10 ml phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin. About 10 µl of each of the test sample at varying concentration along with Acarbose 100 µg/ml used as a reference standard were added to 250 µl of 20 mM p-nitrophenyl-α-D -glucopyranoside and 495 µl of 100 mM phosphate buffer (pH 7.0). It was pre-incubated at 37°C for 5 min and the reaction started by addition of 250 µl of the α-glucosidase enzyme solution prepared by 0.5 mg α-glucosidase in 10 ml phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin, after which it was incubated at 37°C for exactly 15 min. 250 µl of phosphate buffer was added instead of enzyme for blank. The reaction was then stopped by addition of 1000 µl of 200 mM Na_2CO_3 solution and the amount of p-nitrophenol released was measured by reading the absorbance of sample against a sample blank (containing PBS with no sample) at 405 nm using UV visible spectrophotometer.

2.7 Biochemical Analysis of 20 Megaththukku Kudineer

Preparation of the extract:
5gms of the drug was weighed accurately and placed in a 250ml clean beaker then 50ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100ml volumetric flask and then it is made to 100ml with distilled water. This fluid is taken for analysis.

3. Results

Percentage inhibition of test drug MK on Alpha Amylase enzyme Inhibition Study

Concentration (µg/ml)	% Inhibition of MK
100 µg/ml	7.017 ± 4.332
200 µg/ml	16.76 ± 5.685
300 µg/ml	25.82 ± 7.414
400 µg/ml	39.92 ± 10.58
500 µg/ml	52.82 ± 17.33
Standard Acarbose	98.62 ± 1.655

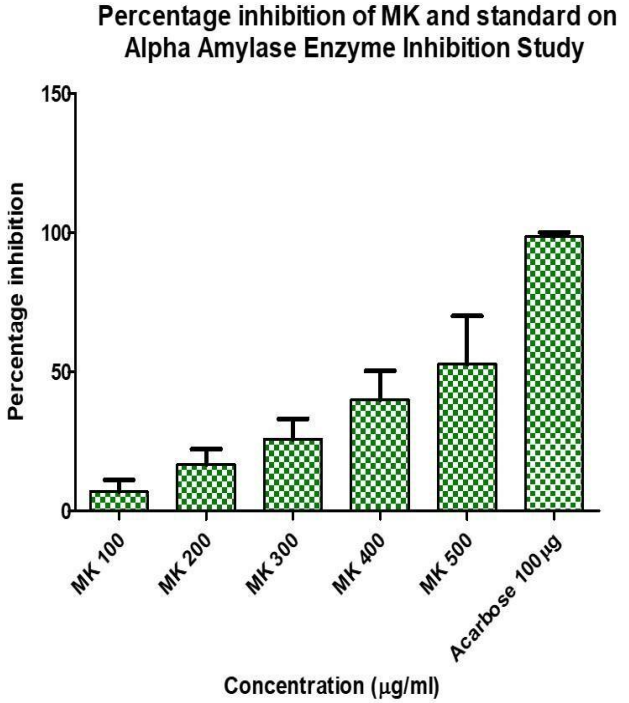
Data are given as Mean ± SD (n=3)

IC50 Values for Alpha Amylase Enzyme inhibition by MK and STD

Test Drug / Standard	IC50 Value of Alpha Amylase enzyme inhibition ± SD (µg /ml)
MK	517.9 ± 162
Standard- Acarbose	7.683 ± 5.419

Data are given as Mean ± SD (n=3)

Percentage inhibition of test drug MK and Standard on Alpha Amylase enzyme Inhibition Assay



Percentage inhibition of test drug MK and STD on α-Glucosidase enzyme Inhibition Study

Concentration (µg/ml)	% Inhibition of MK
100 µg/ml	8.908 ± 1.605
200 µg/ml	17.24 ± 1.51
300 µg/ml	21.31 ± 2.192
400 µg/ml	28.74 ± 3.207
500 µg/ml	38.61 ± 2.74
Standard- Acarbose	99.15 ± 0.3842

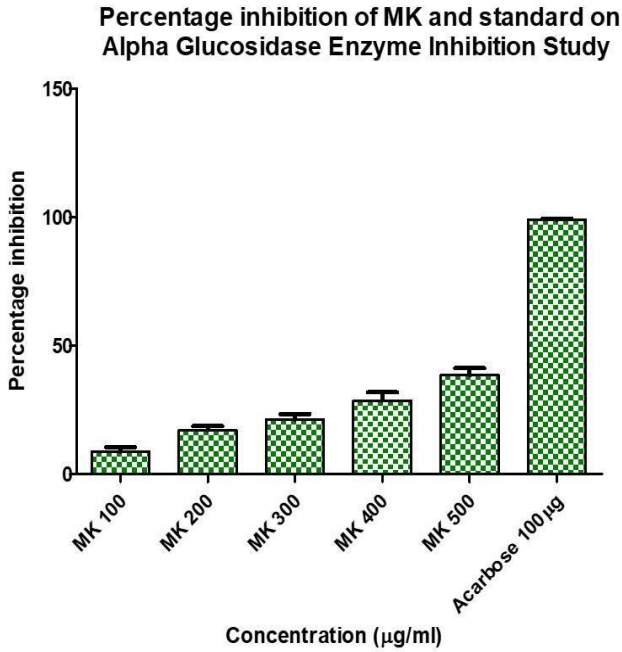
Data are given as Mean ± SD (n=3)

IC50 Values for α-Glucosidase enzyme Inhibition Assay by MK and STD

Test Drug / Standard	IC50 Value of α-Glucosidase enzyme inhibition ± SD (µg /ml)
MK	695.1 ± 50.16
Standard- Acarbose	26.83 ± 6.257

Data are given as Mean ± SD (n=3)

Percentage inhibition of test drug MK and Standard on Alpha Glucosidase enzyme inhibition Assay



Bio Chemical Analysis

S.No	Experiments	Observation	Inference
1	**Test for calcium:** 2ml of the above prepared extract taken in a clean test tube.To this add 2ml of 4% ammonium oxalate solution.	A white precipitate is formed	**Indicates the presence of Calcium**
2	**Test for sulphate:** 2ml of the extract is added to 5% barium chloride solution.	A white precipitate is formed	**Indicates the presence of Sulphate**
3	**Test for chloride:** The extract is treated with silver nitrate solution.	A white precipitate is formed	**Indicates the presence of Chloride**
4	**Test for carbonate:** The substance is treated with concentrated HCL.	No brisk effervessence is formed	Absence of carbonate
5	**Test for starch:** The extract is added with weak iodine solution.	Blue colour is formed	**Indicates the presence of Starch**
6	**Test for ferric iron:** The extract is acidified with glacial acetic acid and potassium Ferro cyanide.	No blue colour is formed	Absence of ferric iron
7	**Test for ferrous iron:** The extract is treated with concentrated nitric acid ammonium thiocyanate solution.	No blood red colour is formed	Absence of ferrous iron
8	**Test for phosphate:** The extract is treated with ammonium molybdate and concentrated nitric acid**.**	No yellow precipitate is formed	Absence of phosphate
9	**Test for albumin:** The extract is treated with Esbach's reagent**.**	No yellow precipitate is formed	Absence of albumin
10	**Test for tannic acid:** The extract is treated with ferric chloride.	Blue black precipitate is formed	Absence of tannic acid
11	**Test for unsaturation:** Potassium permanganate solution is added to the extract.	It gets decolourised	**Indicates the presence of unsaturated compound**
12	**Test for the reducing sugar:** 5ml of benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and add 8 to 10 drops of the extract and again boil it for 2 minutes	No colour change occurs	Absence of reducing sugar
13	**Test for amino acid:**	Violet colour is formed	**Indicates the presence of**

	One or two drops of the extract is placed on a filter paper and dried well. After drying, 1% ninhydrin is sprayed over the same and dried it well.		**amino acid**
14	**Test for zinc:** The extract is treated with potassium Ferro cyanide.	No white precipitate is formed	Absence of zinc

4. Discussion

- It was observed from the results of the present investigation that the formulation MK shown promising alpha amylase enzyme inhibition potential with the maximum inhibition of about 52.82 ± 17.33% and the corresponding IC50 is 517.9 ± 162 µg /ml. Standard acarbose exhibited significant inhibition in alpha glucosidase enzyme with the maximum inhibition of about 98.62 ± 1.655 % and the corresponding IC50 is 7.683 ± 5.419µg /ml.
- It was observed from the results of the present investigation that the formulation MK shown pronounced glucosidase enzyme inhibition activity with the maximum inhibition of about 38.61 ± 2.74% and the corresponding IC50 is 695.1 ± 50.16 µg/ml. Standard acarbose exhibited significant inhibition in alpha amylase enzyme activity with the maximum inhibition of about 99.15 ± 0.3842 % and the corresponding IC50 26.83 ± 6.257µg /ml.
- The qualitative bio chemical test revealed that MK contains calcium, sulphate, chloride, starch, unsaturated compound and amino acid.

5. Conclusion

From this study, it is concluded that 20 Megathukku Kudineer showed significant inhibition of alpha amylase enzyme and alpha glucosidase enzyme activity and it contains many biochemicals. Thus, this drug possesses anti diabetic activity and has beneficial effects on controlling hyperglycaemia. This can be further studied in experimental diabetic rats for potential future research.

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