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Evaluation of Madhumukthi Kudineer Chooranum for its Anti-diabetic properties using Alpha-amylase and Alpha-glucosidase enzyme inhibition assay -In Vitro Study.

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I. ABSTRACT:

Due to screening for phytochemicals that may slow or delay glucose absorption, there is great interest in discovering alternative therapies for type 2 diabetes. By showing in vitro evidence of possible inhibition of alpha-glucosidase and alpha-amylase enzymes and then conducting confirming in vivo research on rats, the current work attempts to bolster the biochemical case for more investigation. By suppressing alpha-glucosidase and alpha-amylase, two enzymes that break down carbs, patients with type 2 diabetes and those at risk of the condition may better control their blood glucose levels.

Objective: Alpha-Glucosidase and Alpha-Amylase Enzyme Inhibition Research conducted in vitro.

Methodology: The necessary bark and roots are collected from their native habitats on the slopes of Yelagiri and Jolarpettai. The pharmacognosy department of Siddha Central Research Institute, Chennai-106 is verifying the raw medications. Use the mentioned amounts after grinding these healing herbs to a coarse powder. Six grams of powder should be added to 240 milliliters of water, which should then be brought to a boil and reduced to 60 milliliters. (1/4)

Dosage: 60 milliliters BDS (taken in the morning and evening on an empty stomach)

Results:

With an IC50 value of 525.5 ± 100 µg/ml, the study shows that the MKC formulation effectively inhibits the alpha-amylase enzyme, with a peak inhibition of around 52.42%. At the same time, the alpha-glucosidase enzyme was significantly suppressed by traditional acarbose, with an IC50 value of 33.17 ± 17 µg/ml and a maximal inhibition of around 98.47 ± 1.86%.

Conclusion:

According to the study's findings, the MKC formulation significantly inhibited the Glucosidase enzyme, with an IC50 of 591.1 ± 232.2 µg/ml and a maximal inhibition of around 46.83 ± 15.95%. In contrast, the alpha-amylase enzyme activity was significantly reduced by conventional acarbose, with a maximal inhibition of 99.16 ± 0.3852% and an IC50 value of 24.97 ± 5.389 µg/ml.

Keywords Siddha, Polyherbal, Anti diabetic , The spectrophotometric assay method.,Madhumukthi kudineer chooranum (MKC)etc.,

INTRODUCTION

The wise people of old founded the medical and healing systems as a public service to humanity, which they saw as a general duty to the Lord of Lords. In all its perfection, this science seems to be a meaning-laden art, philosophy, and science since it is a by-product of Siddha's practice of attaining the ultimate, which explains why. [1,2]

Type 2 diabetes mellitus (T2DM), which is characterized by hyperglycemia resulting from progressive loss of adequate insulin production in the setting of insulin resistance, has been identified as a serious global health threat by WHO [3,4]. The International Diabetes Federation estimated that the prevalence of diabetes which was 10.5% in 2021, would increase to 11.3% by

2030 and 12.2% by 2040 [5] .Elevated blood sugar, or hyperglycemia, is one of the primary signs and symptoms of diabetes mellitus. An imbalance in the body's ability to utilize insulin, which may be inherited or acquired, is the cause of this complex and diverse set of metabolic illnesses. [6,7]Damage to the kidneys, eyes, nerves, heart, and blood vessels is linked to diabetes-related chronic hyperglycemia. The most common result is dyslipidemia, which may increase oxidative stress and change the body's antioxidant defense mechanism [8]. A complex and multifaceted etiology characterizes the disease that it is. Oxidative stress brought on by hyperglycemia is widely implicated as a contributing factor. This means that to treat the beginning, progression, and side effects of the illness, antidiabetic drugs with a multimodal mode of action must be developed. The majority of antidiabetic medications have just one known mechanism of action, and there's a chance they'll have some unintended adverse effects as well. The extensive range of bioactive secondary metabolites present in herbal treatments may give them an advantage over conventional medicine; each of these metabolites may have a distinct mechanism of action.

Plants have always been essential to traditional medical procedures, and their importance is still seen in contemporary healthcare systems. Conventional herbal medicines are still widely used, especially in underdeveloped countries, providing around 80% of the population's primary healthcare. However, empirical information about these techniques' efficacy, dosage, safety, and mechanisms of action is still lacking. Furthermore, they might be an excellent source of lead compounds for diabetic treatment. Despite their extensive use in treating metabolic diseases, including diabetes, there is a shortage of research on pharmacological drugs' antioxidant and antidiabetic characteristics.

MATERIAL AND METHODS

Plant Material

Procurement of plant material

The necessary bark and roots are collected from Jolarpettai and the Yelagiri foothills, where they are found in their native environment. The Department of Pharmacognosy at the Siddha Central Research Institute in Chennai-106 authenticates the primary pharmaceutical ingredients.

The Siddha Poly herbal formulation Madhumukthi kudineer chooranum was selected from the classical Siddha literature [9,10]

Purification

The drugs were taken raw and purified according to literatures of Siddha.

INGREDIENTS OF MKC

S.no	Botanical name	Tamil name	Parts used	Quantity
1	Salacia oblonga Wall.	Ponkoranti	Root	4 parts
2	Azadirachta indica A. Juss	Vembu	Stem bark	3 parts
3	Aegle marmelos(L). Correa	Vilvam	Root	3 parts

4	**Tinospora cordifolia (Willd) Miers ex Hook. f. &Thomson**	**Seenthil**	**Stem**	**2 parts**
5	**Cassia fistula L.**	**Sarakondrai**	**Stem bark**	**1 part**

Preparation of MKC

These therapeutic herbs must be taken in above mentioned proportions and ground into a coarse powder . Add 240 milliliters of water to six grams of powder; boil and reduce to 60 milliliters. (1/4th)

Dosage: In the morning and evening, take 60 milliliters of the kudineer and to be consumed empty-handed.

Note: After giving the medication, one should wait 20 minutes before eating.

1. **In Vitro Alpha Amylase enzyme inhibition assay**
   Spectrophotometric assay method was adopted.[11]

One hundred milliliters of phosphate and 3.24 milligrams of α-amylase were combined to create the enzyme with a 0.5 U/ml concentration.Primary buffer with an acidic pH of 6.9. A solution whose concentration ranges from 100, 200, 300, 400, and 500 µg/ml may be obtained by serially diluting the test sample MKC with DD water . At 100 µg/ml, acarbose is used as the reference standard. The 600 microliters of test sample was combined with about 30 microliters of α-amylase enzyme solution, and the mixture was incubated for 15 minutes at 37°C.

Subsequently, 370 microliters of 2-Chloro-4-Nitrophenyl-α-Maltotrioside (CNPG3 - 0.5 mg/ml) were added to the reaction mixture.

Once blended, it was incubated for ten minutes at 37°C. Analyzing the absorbance at 405 nm in comparison to a blank display on the spectrophotometer. There was a control answer in case the test material wasn't available. To find percentage inhibition, the following formula was used.

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

2. In Vitro Alpha Glucosidase enzyme inhibition assay
The Spectrophotometric Assay Method was used.[12]

Test Solution: A serial dilution of the concentration ranges of 100, 200, 300, 400, and 500 µg/ml was used to create the test sample (MKC) using DD water.

P-nitrophenyl-α-D-glucopyranoside, or PNPG, is the product of dissolving 603 mg of PNPG in 100 ml of PBS to produce 20 mM of PNPG.

Enzyme: To prepare the α-glucosidase enzyme solution, 0.5 mg was dissolved in 10 milliliters of pH 7.0 phosphate buffer and then supplemented with 20 milligrams of bovine serum albumin. A combination comprising 250 µl of 20 mM p-nitrophenyl-α-D glucopyranoside and 495 µl of

100 mM phosphate buffer (pH 7.0) was mixed with varying volumes of the test sample (10 µl). Acarbazole was the standard, and its concentration was 100 µg/ml.

To prepare the α-glucosidase enzyme solution, dissolve 0.5 mg of the enzyme in 10 ml phosphate buffer (pH 7.0) and mix it with 20 mg of bovine serum albumin (250 µl) to initiate the reaction. The reaction mixture was then incubated at 37°C for precisely fifteen minutes. Phosphate buffer (250 µl) was used as the blank control instead of the enzyme. 1000 µl of a 200 mM Na2CO3 solution was applied after incubation to stop the reaction. The amount of p-nitrophenol produced was estimated by measuring the sample's absorbance at 405 nm using a UV-visible spectrophotometer. After that, this was contrasted with a blank sample with no test material and phosphate buffer.

STATISTICAL ANALYSIS

Through data collection and interpretation techniques, statistical analysis seeks to identify patterns and trends in data. It's included in the data analytics category. Survey and study design, statistical modeling, and the interpretation of research results are only a few of the uses for statistical analysis. The five fundamental statistical analysis methods are mean calculation, standard deviation calculation, regression analysis, hypothesis testing, and sample size determination.

One often used program for statistical analysis is Microsoft Excel. Usually, the mean ± standard deviation is presented together with the sample size (n = 3). When doing statistical analysis, the Student's t-test is often used, and significance is assessed at a cutoff of $p < 0.05$.

Table 1 : Alpha-Amylase enzyme percentage inhibition by test substance MKC

Research on Inhibition

Concentration (µg/ml)	% Inhibition of MKC
100 µg/ml	15.48 ± 3.234
200 µg/ml	19.67 ± 2.497
300 µg/ml	31 ± 3.329
400 µg/ml	36.07 ± 1.715
500 µg/ml	52.42 ± 11.59
Standard Acarbose	98.47 ± 1.86

Data are given as Mean ± SD (n=3)

IC50 Values for MKC and STD Inhibition of Alpha Amylase Enzyme

Test Drug / Standard	**IC50 Value of Alpha-Amylase enzyme inhibition ± SD (µg /ml)**
MKC	525.5 ± 100
Standard- Acarbose	33.17 ± 17

Data are given as Mean ± SD (n=3)

Percentage of the test substance MKC and the reference in the Alpha Amylase Enzyme Inhibition Assay

Table 2 :The percentage of test drug MKC and STD inhibition on the α-glucosidase enzyme inhibition study

Concentration (µg /ml)	**%Inhibition of MKC**
100 µg /ml	13.22 ± 6.191
200 µg /ml	18.06 ± 5.18
300 µg /ml	30.39 ± 11.02
400 µg /ml	36.74 ± 10.16
500 µg /ml	46.83 ± 15.95
Standard –Acarbose	99.16 ± 0.3852

Data are given as Mean ± SD(n=3).

MKC and STD IC50 Values for the α-Glucosidase Enzyme Inhibition Assay

Test Drug/ Standard	**IC50 value of α-Glucosidase Enzyme Inhibition ± SD(µg /ml)**
MKC	591.1±232.2
Standard -Acarbose	24.97±5.389

Data are given as Mean ± SD(n=3)

Percentage of test material MKC and standard in the alpha-glucosidase enzyme inhibition assay

RESULTS

Fig. 1.Alpha Amylase Enzyme Inhibition Assay: Percentage of Test Drug MKC and Standard Inhibition

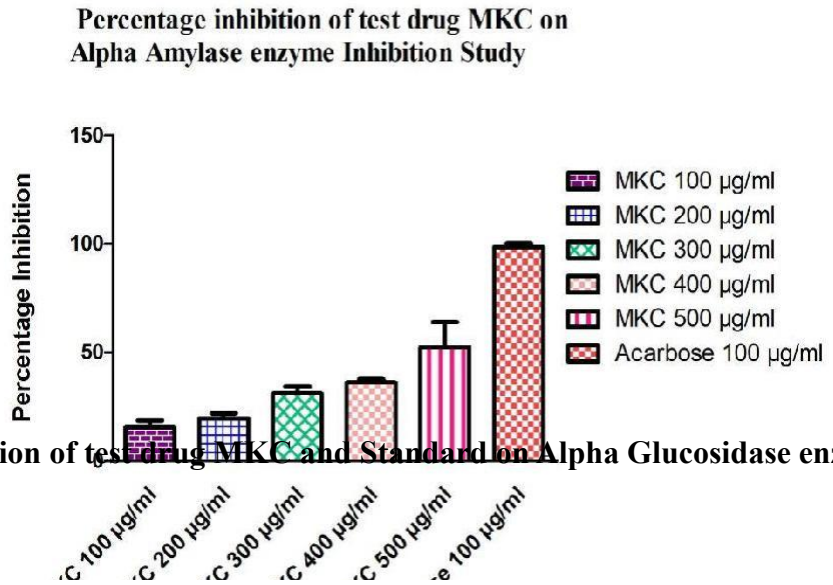
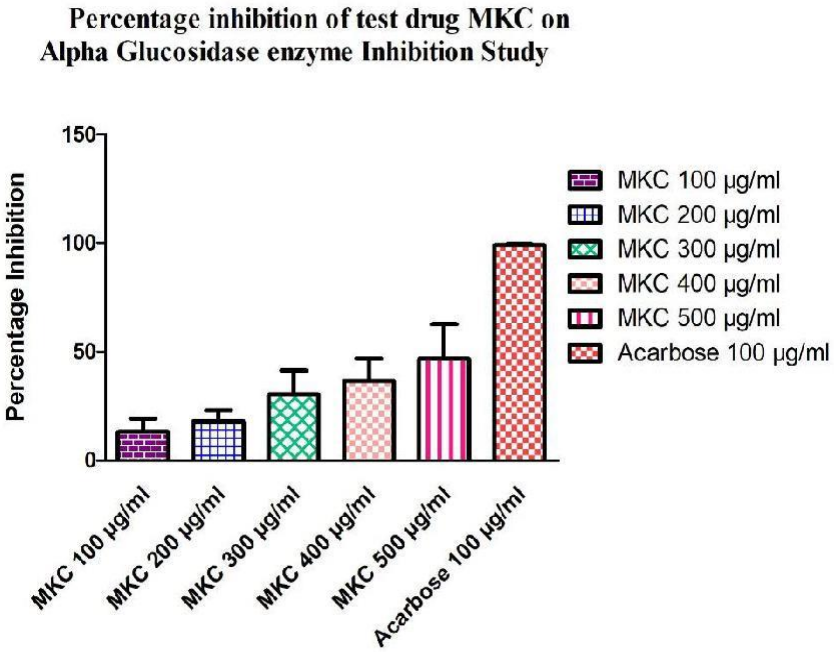


Fig.2 Percentage inhibition of test drug MKC and Standard on Alpha Glucosidase enzyme Inhibition Assay



DISCUSSION

IN-VITRO ASSAY

A-AMYLASE INHIBITORY ACTIVITY

The standard protocol was adhered to evaluate the extract and fractions' ability to inhibit α-amylase with minor adjustments. Acarbose in a range of concentrations (0.1–0.5 mg/ml) served as the standard. The material (extract and fractions) was prepared in parallel as a control, and each experiment was run in triplicate. The results were expressed as the % inhibition, calculated using the method.

A-GLUCOSIDASE INHIBITORY ACTIVITY

The conventional procedure was followed with slight modifications to determine the extract and fractions' α-glucosidase inhibitory activity. All experiments were run in triplicate, without the test material set up in parallel as a control. Using the following formula, the findings were reported as a percentage of inhibition:

Inhibitory activity (%) = $(1 - As/Ac) \times 100$

In this case, As represents the absorbance while the test drug is present, and Ac represents the absorbance of the control.

Worldwide, the use of herbal remedies as adjunctive therapies to conventional pharmaceuticals for managing diabetes and its complications is expanding, and several plants from various nations are recognized to have antidiabetic properties. More than 800 plants have been found to have antidiabetic qualities in ancient Indian literature, and research suggests that more than 1200 plants have hypoglycemic potential.

Conclusion :

The experiment results showed that the formulation MKC had a remarkable capacity to inhibit the alpha-amylase enzyme, with a maximum inhibition of around 52.42 ± 11.59% and a matching IC50 of 525.5 ± 100 µg/ml. The alpha-glucosidase enzyme was highly inhibited by conventional acarbose, with an estimated maximum inhibition of 98.47 ± 1.86 percent; the corresponding IC50 is 33.17 ± 17 µg/ml.

The current study's findings revealed that the formulation MKC exhibited significant inhibitory action against the glucosidase enzyme, with a maximal inhibition of around 46.83 ±14.95%. The corresponding IC50 was found to be 591.1 ± 232.2 µg/ml. Standard acarbose demonstrated significant suppression of alpha-amylase enzyme activity with a maximal inhibition of around 99.16 ± 0.3852 % and a corresponding IC50 of 24.97 ± 5.389 µg /ml.

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Statement of Ethical Approval and Informed Consent

This work does not involve animal testing and does not require ethical approval. Informed consent of all authors has been obtained for this work.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest concerning this article's research, authorship, and publication.

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