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In Vitro Antidiabetic Activity of Aqueous and Ethanolic Leaf Extracts from Various Medicinal Plants

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KEYWORDS

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ABSTRACT:

Momordica balsamina, and Salacia reticulata are recognized for their diverse biological properties, including antibacterial, anticancer, antioxidant, and anti-inflammatory activities. This study aimed to assess the in vitro antidiabetic potential of aerial parts of Momordica balsamina extracts and Slacia reticulata extract through digestive enzymes inhibition assay targeting α-amylase and α-glucosidase, crucial enzymes involved in carbohydrate digestion. Acarbose, aqueous, and ethanolic extracts of Momordica balsamina and Salacia reticulata were evaluated at concentrations ranging from 20 to 100 mg/ml.

The absorbance values for α-amylase and α-glucosidase enzymes were measured at 540nm and 400nm, respectively, using a spectrophotometer. Both aqueous and ethanolic extracts exhibited significant inhibition of α-amylase and α-glucosidase enzymes in a concentration-dependent manner. Moreover, the ethanolic extract displayed greater inhibitory activity compared to the aqueous extract.

INTRODUCTION

Diabetes mellitus represents a significant global health challenge, characterized by impaired glucose metabolism and insulin regulation. With its prevalence on the rise worldwide, there is an urgent need for effective and accessible treatments to manage this chronic condition. Traditional medicinal plants have long been recognized as potential sources of therapeutic agents for various ailments, including diabetes. In this context, Momordica balsamina (Balsam apple) and Salacia reticulata (Indian salacia) emerge as promising candidates for exploring their antidiabetic properties.[1, 2.]

Momordica balsamina, commonly known as Balsam apple, is a member of the Cucurbitaceae family, native to tropical regions of Africa and Asia. Traditionally, various parts of this plant have been used in folk medicine to treat diabetes and other ailments.[3] The plant is characterized by its bitter taste, which is attributed to the presence of bioactive compounds such as cucurbitane-type triterpenoids, flavonoids, and saponins. Previous studies have highlighted the potential of Momordica balsamina in managing diabetes due to its ability to enhance insulin secretion, improve glucose uptake, and regulate lipid metabolism.[4]

Salacia reticulata, commonly referred to as Indian salacia, belongs to the Hippocrateaceae family and is indigenous to India and Sri Lanka. [5] In traditional Ayurvedic medicine, various parts of this plant have been used for their medicinal properties, including their purported efficacy in managing diabetes. [6] Salacia reticulata contains bioactive compounds such as salacinol, kotalanol, and mangiferin, which have been shown to exert antidiabetic effects by inhibiting carbohydrate digestion and absorption, enhancing insulin sensitivity, and reducing blood glucose levels. [7]

Despite their traditional uses and documented pharmacological activities, the specific antidiabetic mechanisms of Momordica balsamina and Salacia reticulata remain to be fully elucidated. Furthermore, limited research has been conducted to evaluate their

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potential as therapeutic agents for diabetes management. Therefore, the current study aims to address this gap by investigating the in vitro antidiabetic activity of aerial parts extracts from Momordica balsamina and Salacia reticulata. [8,9]

Central to this investigation is the evaluation of the inhibitory effects of Momordica balsamina and Salacia reticulata extracts on key digestive enzymes involved in carbohydrate metabolism, namely α-amylase and α-glucosidase. [10] These enzymes play crucial roles in the breakdown of complex carbohydrates into absorbable monosaccharides, thus influencing postprandial glucose levels. By inhibiting these enzymes, plant-derived compounds may offer a natural approach to glycemic control in individuals with diabetes. [11,12]

In this study, we utilized aqueous and ethanolic extracts of Momordica balsamina and Salacia reticulata to assess their inhibitory activity against α-amylase and α-glucosidase enzymes. Acarbose, a commonly used antidiabetic medication that acts as an inhibitor of these enzymes, served as a reference compound. [13] The absorbance values for α-amylase and α-glucosidase enzymes were measured using a spectrophotometer to quantify the degree of enzyme inhibition by the plant extracts.

Preliminary findings from this study may provide valuable insights into the potential antidiabetic mechanisms of Momordica balsamina and Salacia reticulata extracts and pave the way for further research exploring their therapeutic utility in diabetes management. Understanding the pharmacological properties of these plants could contribute to the development of novel natural remedies for combating diabetes and improving the quality of life for individuals affected by this chronic condition. [14,15]

MATERIALS AND METHODS:

Chemicals and Reagents:

All the chemicals and reagents were of AR grade and were procured from Research Lab Mumbai, Maharashtra, India.

Plant Material and Extract Preparation: [16,17]

The fresh aerial parts of Momordica balsamina (Balsam apple), Salacia reticulata (Indian salacia) were collected from natural habitat. The specimens were authentically identified by Botanist. Aerial parts of Momordica balsamina and Salacia reticulata were air-dried at room temperature in the dark for approximately six weeks until fully dried. Subsequently, the dried plant material was ground into a fine powder.

10 grams of the powdered aerial parts were soaked in 100 ml of solvents (absolute ethanol and distilled water) at a ratio of 1:10 (w/v sample-to-solvent ratio) for 72 hours at room temperature with regular agitation to prepare ethanolic and aqueous extracts. After the extraction period, the mixture was filtered using Whatman filter paper (No. 1), and the solvents were evaporated under reduced pressure using a rotary evaporator. The resulting crude extracts were collected and stored at -20°C for further analysis.

A. Alpha-amylase Inhibitory Activity:[18, 19,20]

The alpha-amylase inhibitory activity was conducted following the method described by Ibrahim et al. (2017) with slight modifications. The reaction mixture consisted of 1 ml of Momordica balsamina (Balsam apple) or Salacia reticulata (Indian salacia) extracts (sample) at concentrations ranging from 100 to 500 µg/ml and 1 ml of alpha-amylase solution (0.5 mg/ml prepared in 0.20 mM phosphate buffer, pH 6.9). After pre-incubation for 30 minutes, 1 ml of starch solution (1%) in 0.02 mol/L sodium phosphate buffer (pH 6.9) was added, and the reaction was incubated at 37°C for 10 minutes. The reaction was stopped by adding 1 ml of 3,5-dinitrosalicylic acid reagent (DNS), followed by boiling the mixture for 5 minutes. Acarbose (100-500 µg/ml) was used as a standard (positive control). The absorbance of the reaction mixture was measured at 540 nm using a UV-vis spectrophotometer. All assays were performed in triplicate. The percentage of inhibition was calculated using the formula:

% Inhibition = [(Absorbance Control - Absorbance Sample) / Absorbance Control] x 100

B. Alpha-glucosidase Inhibitory Activity: [18, 19,20]

The alpha-glucosidase inhibitory activity of Momordica balsamina (Balsam apple) or Salacia reticulata (Indian salacia) extracts was determined by incubating 1 ml of starch solution (2% w/v maltose) with 0.2 M tris buffer (pH 8.0) and various concentrations of extracts (100-500

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mg/ml). The reaction mixture was incubated at 37°C for 10 minutes. Subsequently, the reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1 U/ml) to the mixture and incubating it at 35°C for 40 minutes. The reaction was then terminated by adding 2 ml of 6 N HCl. Acarbose was used as a positive control. All assays were conducted in triplicate. The absorbance of the mixture was measured at 400 nm using a spectrophotometer. The inhibitory effect was calculated using the formula:

% Inhibition = [(Absorbance Control - Absorbance Sample) / Absorbance Control] x 100

Statistical Analysis:

All assays were conducted in triplicate, and the results are presented as the mean ± standard error of the mean (SEM) using GraphPad Prism 9.0.2 (GraphPad Software, San Diego, USA). Additionally, the IC50 value was determined to ascertain the concentration of the plant extract required to inhibit 50% of alpha-amylase or alpha-glucosidase activity under the assayed conditions. Statistical differences between extracts were assessed using one-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

RESULTS:

Alpha-amylase and alpha-glucosidase inhibition assay:

The results of the research show the inhibitory effects of various compounds (AESR, Acarbose, AEMB, EEMB, and EESR) at different concentrations (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml).

AESR vs. Acarbose:

At all concentrations, Acarbose consistently exhibits higher inhibition percentages compared to AESR. This suggests that Acarbose might be a more potent inhibitor of the target substance across the concentration range tested.

AEMB vs. EEMB vs. EESR:

At lower concentrations (20 µg/ml and 40 µg/ml), EESR shows the highest inhibition percentages among these three compounds. However, as the concentration increases, EEMB gradually becomes more effective than both AEMB and EESR. This trend suggests that EEMB might have a stronger inhibitory effect at higher concentrations compared to AEMB and EESR.

Acarbose vs. EEMB:

At concentrations of 60 µg/ml and above, Acarbose demonstrates comparable or slightly lower inhibition percentages compared to EEMB. This indicates that EEMB might be as effective as, or potentially more effective than, Acarbose in inhibiting the target substance at higher concentrations.

Acarbose vs. EESR:

Across all concentrations, EESR consistently exhibits higher inhibition percentages compared to Acarbose. This suggests that EESR might be a more potent inhibitor of the target substance across the concentration range tested as shown in figure 1.

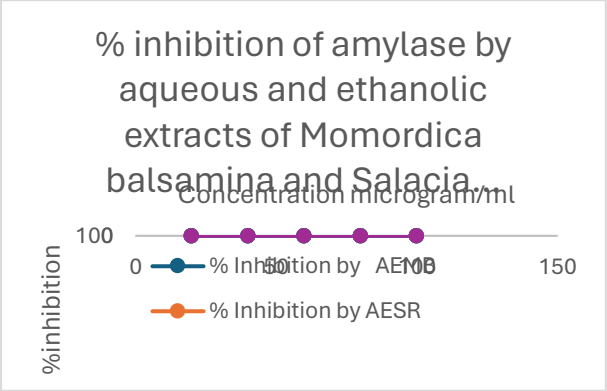

Figure 1. In vitro α-amylase inhibitory activity of aqueous and ethanolic extracts of Momordica balsamina and Salacia reticulata. Acarbose was used as a standard. Values are represented as Mean±SEM (n=3). *p<0.05, **p<0.01, ***p<0.001 vs. Acarbose (ANOVA, Tukey post hoc test).

we can observe changes in the percentage of inhibition by different compounds at varying concentrations.

At lower concentrations (20 µg/ml and 40 µg/ml):

In the first set of data, AESR shows slightly lower inhibition percentages compared to the second set.

Acarbose exhibits similar inhibition percentages in both sets, with slight variations.

AEMB and EEMB show comparable inhibition percentages in both sets.

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EESR shows slightly higher inhibition percentages in the first set.

At higher concentrations (60 µg/ml, 80 µg/ml, and 100 µg/ml):

AESR, AEMB, and EEMB show increased inhibition percentages in the second set compared to the first set.

Acarbose demonstrates similar or slightly increased inhibition percentages in the second set.

EESR exhibits similar or slightly lower inhibition percentages in the second set as shown in figure 2

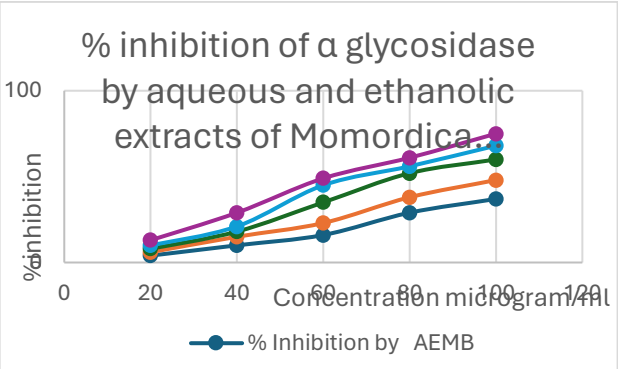

Figure 2. In vitro α-glucosidase inhibitory activity of aqueous and ethanolic extracts of Momordica balsamina and Salacia reticulata. Acarbose was used as a standard. Values are represented as Mean±SEM (n=3). *p<0.05, **p<0.01, ***p<0.001 vs. Acarbose (ANOVA, Tukey post hoc test)

Discussion:

The research work involves investigating the inhibitory effects of various compounds at different concentrations. The compounds tested include AESR, Acarbose, AEMB, EEMB, and EESR. These compounds are evaluated for their ability to inhibit a certain process or activity, which is not explicitly mentioned in the provided data.

The data presented in the table represent the percentage of inhibition achieved by each compound at various concentrations (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml). Each concentration is associated with the inhibition percentages of all tested compounds.

From the results, it's evident that the inhibitory effects of the compounds vary depending on both the concentration and the compound itself. Here's a detailed discussion of the findings:

Effectiveness of Compounds:

AESR: This compound shows moderate inhibition percentages across different concentrations. It exhibits a gradual increase in inhibition with higher concentrations.

Acarbose: Acarbose demonstrates relatively consistent inhibition percentages across concentrations. It shows effectiveness in inhibiting the process under investigation, with slightly higher inhibition percentages at higher concentrations.

AEMB and EEMB: Both AEMB and EEMB exhibit increasing inhibition percentages with higher concentrations. They show comparable effectiveness to AESR and Acarbose.

EESR: EESR shows varying levels of inhibition, with higher percentages observed at lower concentrations. However, its effectiveness diminishes at higher concentrations.

Concentration Dependence:

In general, higher concentrations of the tested compounds tend to result in higher inhibition percentages. This concentration-dependent effect is particularly noticeable for AESR, AEMB, and EEMB.

Acarbose, while showing consistent inhibition across concentrations, also demonstrates a modest increase in inhibition with higher concentrations.

Comparative Analysis:

Comparing the two sets of results provided, it's apparent that the second set generally shows higher inhibition percentages for all compounds at various concentrations compared to the first set.

This discrepancy suggests potential variations in experimental conditions, sample preparation, or assay methodologies between the two sets of data. Further investigation into these differences is necessary to ensure the reliability and reproducibility of the results.

Implications and Further Research:

The findings from this research provide valuable insights into the inhibitory effects of the tested compounds.

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Understanding the effectiveness of these compounds in inhibiting the target process or activity can have implications in various fields such as pharmacology, biochemistry, or agricultural science.

Further research could focus on elucidating the mechanisms underlying the inhibitory effects observed, exploring potential synergistic effects between different compounds, or optimizing the concentrations for maximum inhibition.

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