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α-Amylase Inhibitory Activity and Free Radical Scavenging Activity of *Murraya Koeingii* Leaves

Mathew George*[1], Lincy Joseph[1], Ajay Philip[2], Aleena Paul[2], Alen Thomas[2], Ashin Joseph[2], PK Sneha[2] and Vijisha George[2]

[1]Professor, Holy Queen College of Pharmaceutical Sciences and Research, Thrissur 680623, Kerala, India
[2]Research Scholars, Holy Queen College of Pharmaceutical Sciences and Research, Thrissur 680623, Kerala, India
*Correspondence for materials should be addressed to MG (email: holyqueencollege@gmail.com)

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Abstract

Murraya koenigii (L.) Spreng, commonly known as curry leaf, has a rich history in traditional medicine, with purported benefits in managing diabetes and other ailments. *Murraya koenigii* leaves are pinnate with 11 to 21 small, glossy and aromatic leaflets that are bright green in color. The fresh leaves are highly aromatic, with a distinct somewhat citrusy flavour and native to South Asia. This study investigates the dual potential of *M. koenigii* leaf extracts by evaluating their α-amylase inhibitory activity and free radical scavenging capacity, key factors in hyperglycemia management and combating oxidative stress. Methodology includes the preparation of aqueous and ethanolic extracts of *M. koenigii* leaves. α-Amylase inhibitory activity was assessed using a standard colorimetric assay with maltose as a positive control. Antioxidant potential was evaluated using FRAP method. The total phenolic and flavonoid content of the extracts were also identified. Aqueous ethanolic extracts of *M. koenigii* leaves demonstrated significant α-amylase inhibitory activity, with the ethanolic extract exhibiting superior inhibition compared to the aqueous extract, and approaching the activity of the positive control, maltose and α-amylase inhibitory activity was found to be 61.53%. Furthermore, extracts displayed potent free radical scavenging activity in the FRAP method it was about 1.49 reference standard was ascorbic acid indicating significant antioxidant potential. The extract shows positive phytochemicals like phenols and flavonoids. These findings highlight the promising dual therapeutic potential of *M. koenigii* leaves. The observed α-amylase inhibition suggests a potential role in managing postprandial hyperglycemia, while the strong free radical scavenging activity offers protection against oxidative stress-related complications often associated with diabetes. *M. koenigii* leaves warrant further investigation as a potential natural therapeutic agent for diabetes management and related oxidative stress.

Keywords: Murraya koenigii levaes; A-amylase inhibition; Free radical scavenging activity; Anti-oxidant; Anti-diabetes; Polyphenols; Flavonoids

Introduction

Murraya koenigii (L.) Spreng, commonly known as curry leaf, is a plant with a rich history in traditional medicine, particularly in India and Southeast Asia. Its leaves are widely used in culinary practices for their distinct aroma and flavor, but also hold a significant place in various traditional medicine systems. Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia or elevated blood glucose levels. This condition can lead to a cascade of complications, including cardiovascular disease, neuropathy, and retinopathy. One of the key enzymes involved in glucose metabolism is α-amylase, which is responsible for breaking down complex carbohydrates into simpler sugars. Inhibiting α-amylase activity can help slow down the digestion of carbohydrates and reduce the spike in blood glucose levels after meals, making it a potential therapeutic strategy for managing diabetes.

In addition to hyperglycemia, oxidative stress plays a significant role in the development and progression of diabetic complications. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these reactive intermediates. ROS can damage cellular components, including DNA, proteins, and lipids, contributing to the pathogenesis of diabetes and its complications.

Murraya koenigii leaves have been traditionally used to manage diabetes and related conditions. Recent scientific investigations have focused on exploring the potential of *M. koenigii* extracts to inhibit α-amylase activity and scavenge free radicals, thus providing a dual therapeutic approach for managing diabetes and oxidative stress.

α-amylase Inhibition (DNS Method)

The dinitrosalicylic acid (DNS) method is a widely used technique for measuring α-amylase activity. This method relies on the ability of DNS to react with reducing sugars, which are released during the enzymatic breakdown of starch by α-amylase. The amount of reducing sugars produced is directly proportional to the α-amylase activity. By measuring the absorbance of the reaction mixture at a specific wavelength, the α-amylase inhibitory activity of *M. koenigii* extracts can be determined.

Free Radical Scavenging Activity (FRAP Method)

The ferric reducing antioxidant power (FRAP) is a commonly employed method for assessing the antioxidant capacity of various substances, including plant extracts. This assay measures the ability of the antioxidants present in the extract to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The reduction of ferric ions is accompanied by a color change, which can be measured spectrophotometrically. A higher FRAP value indicates a greater antioxidant capacity.

Potential benefits of *Murraya koenigii* leaves

α-Amylase Inhibition

M. koenigii leaf extracts have demonstrated significant α-amylase inhibitory activity in various studies. This suggests that the extracts may help to regulate postprandial hyperglycemia by slowing down the digestion and absorption of carbohydrates.

Free Radical Scavenging

M. koenigii leaves are rich in bioactive compounds, including polyphenols and flavonoids, which possess potent antioxidant properties. These compounds can effectively scavenge free radicals, reducing oxidative stress and protecting against cellular damage. *Murraya koenigii* leaves exhibit promising α-amylase inhibitory and free radical scavenging activities, suggesting their potential as a natural therapeutic agent for managing diabetes and oxidative stress-related disorders. Further research is warranted to isolate and identify the specific bioactive compounds responsible for these activities and to explore their therapeutic potential in clinical settings.

Material and methods

Collection of crude drug

Green leaves plucked from our campus garden. The herbarium was prepared and authenticated at the State Medicinal Plant Board, Kerala by a Senior scientist. Organoleptic characters are observed and noted. The collected leaves were washed in running water to remove any organic or foreign particles if present. Dried in shade for 2 days and pulverized in mortar and pestle of the laboratory. The resultant powder was sieved to obtain a uniform particle-sized crude drug.

Extraction of chemical constituents

The chemical constituents are obtained by Soxhlet extraction method. The coarse powder was weighed and 15 gm was packed in an extraction chamber of the Soxhlet apparatus. The RBF was filled with aqueous alcoholic solvent 50% i.e. equal amounts of distilled water and ethanol. The condenser was attached and heated at 40^{O}C for six hrs. The obtained extract was concentrated by simple evaporation at 40^{O}C. % yield of the extract was determined.

α-amylase inhibition by DNS method

Materials

Starch solution: Took 1 g of potato starch and dissolved in 100 ml of 0.02 M phosphate buffer (pH 7).

DNS reagent: It can be prepared by dissolve at room temperature 1 g of 3, 5- Di Nitro Salicylic Acid in 20 ml of 2N NaOH, add 50 ml of distilled water followed by 30 g of Rochelle Salt make the volume up to 100 ml with distilled water. Protect this solution from CO_2 and store at 4°C.

α-amylase enzyme solution: Dissolve 6 mg of α-amylase in 200 ml of 0.2 M phosphate buffer (pH 7) containing 0.006 M NaCl. From this stock solution take 10 ml, dilute to 100 ml with same buffer solution. The final concentration of enzyme in the solution is 30 µg/ml.

Maltose standard solution: Dissolve 50 mg of maltose in 50 ml distilled water and store at 4°C.

NaOH (4.5%): Weigh 4.5 g of NaOH, dissolve it in approximately 80 ml of distilled water, and make the volume up to 100 ml with distilled water.

NaOH (2N): Weigh 8 g NaOH, dissolve in approximately 80 ml distilled water, and the final volume up to 100 ml with distilled water.

Phosphate buffer (0.2 M, pH 7): Take 39 ml of 0.2 M. monobasic sodium phosphate solution and mix with 61ml of 0.2M dibasic sodium phosphate solution and dilute to a total volume of 200 ml.

Phosphate buffer (0.02 M. pH 7): Take 10 ml of the above phosphate buffer (0.2 M) and dilute it to 100 ml with distilled water.

Preparation of maltose calibration curve

Pipette aliquots of 0.1 to 1.0 ml of maltose (100-1000 µg) solution into test tubes and make up the volume to 1ml with suitable addition of distilled water. To each tube add 2 ml of DNS reagent. Cover tubes with marbles. Keep the tubes in water bath for 10 minutes. Cool the tubes and add 10 ml of distilled water to each test tube. The orange red colour formed is measured at 540 nm against a reagent blank.

Determination of α-Amylase inhibitory activity

Pre-incubate the entire reagents for 15 minutes at 37° C in a water bath. Pipette 0.5 ml of 1% starch solution: add it to 0.25 ml of phosphate buffer (0.2M, pH 7) and 0.25 ml of α-amylase enzyme solution. Similarly, a second set of test tubes (blank) by using phosphate buffer in place of enzyme solution. Prepare a third set of test tubes containing 0.5 ml of starch solution, 2 ml of DNS reagent. 0.25 ml of α-amylase enzyme solution; this set is called the zero-time control. Incubate all the tubes at 37°C for three minutes. At the end of the incubation add 2 ml of DNS reagent to first and second set of tubes to stop the reaction and transfer all the tubes to water bath for 10 minutes. After cooling under cold water, add 10 ml of distilled water, mix thoroughly and take absorbance at 540 nm against the blank. Liberated reducing sugars are expressed as maltose equivalent using the calibration curve. One unit of enzyme activity is defined as that amount which liberates 1 **µ**mol of reducing sugars (calculated as maltose) /min from soluble starch at 37°C, pH 7, and. under the specified experimental condition.

Preparation of extract and quantification of α-amylase inhibitor activity

Weighed 1 g of the sample and extracted it with 75 ml of distilled water and 75 ml of ethanol for 2 hours at 40°C. Centrifuged the suspension at 5000 rpm and collected the supernatant. Took 0.25 ml of the supernatant and incubated it with 0.25 ml of enzyme solution for 15 minutes at 37°C. Ensured all reagents were also incubated at 37°C for 3 minutes. After incubation, added 2 ml of DNS reagent to the first, second, and sample tubes to stop the reaction. Transferred the tubes to a water bath for 10 minutes. After cooling with cold water, added 10 ml of distilled water and mixed thoroughly. Measured the absorbance at 540 nm, using the blank as a reference. The released reducing sugars were expressed as maltose equivalents based on the calibration curve. One unit of enzyme activity was defined as the amount that liberated 1 µmol of reducing sugars per minute from soluble starch at 37°C and pH 7, under the specified experimental conditions.

Free radical scavenging by FRAP method

Preparation of reagents

0.2M phosphate buffer (pH 6.6): 8 g of sodium chloride, 0.2 g of potassium chloride, 1.44 g of disodium hydrogen phosphate, 0.24 g of potassium dihydrogen phosphate was taken in a 1,000 mL standard flask and add 800 mL of distilled water and adjust the pH 6.6 using hydrochloric acid and adjust the volume with deionized water. Potassium ferricyanide (1%): 1 g of potassium ferricyanide

was dissolved in 100 mL of deionised water. Trichloroacetic acid (10%): 10 g of trichloroacetic acid was dissolved in 100 mL of deionised water. Ferric chloride (0.1%): 100 mg of ferric chloride was dissolved in 100 mL of deionised water. Ascorbic acid (0.1%): 1 mg of ascorbic acid was dissolved in 1 mL of water.

Methods

Different concentrations of the methanolic extract of *M. serratulum* and its various fractions (10-50 µg/mL) were added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution. The reaction mixture was vortexed well and then incubated at 50°C for 20 min using a vortex shaker. At the end of the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride. The colored solution was read at 520 nm against the blank with reference to the standard using a UV Spectrophotometer. Here, ascorbic acid was used as a reference standard, and the reducing power of the samples was comparable with the reference standard.

Results

Table 1. Maltose calibration curve

Concentration (µg/ml)	Absorbance
0	0
0.1	0.23
0.2	0.26
0.3	0.30
0.4	0.34
0.5	0.40
0.6	0.43
0.7	0.50
0.8	0.55
0.9	0.61
1.0	0.73

Table 2. α- Amylase inhibitory activity detection

Sample	Absorbance
Set 1 (E+S)	0.14
Set 2 (blank)	0.26
Set 3 (E+S+DNS)	0.39
Extract (E+S+DNS)	0.31

Where E = Enzyme, S = Starch, DNS = Di Nitro Salicylic acid reagent

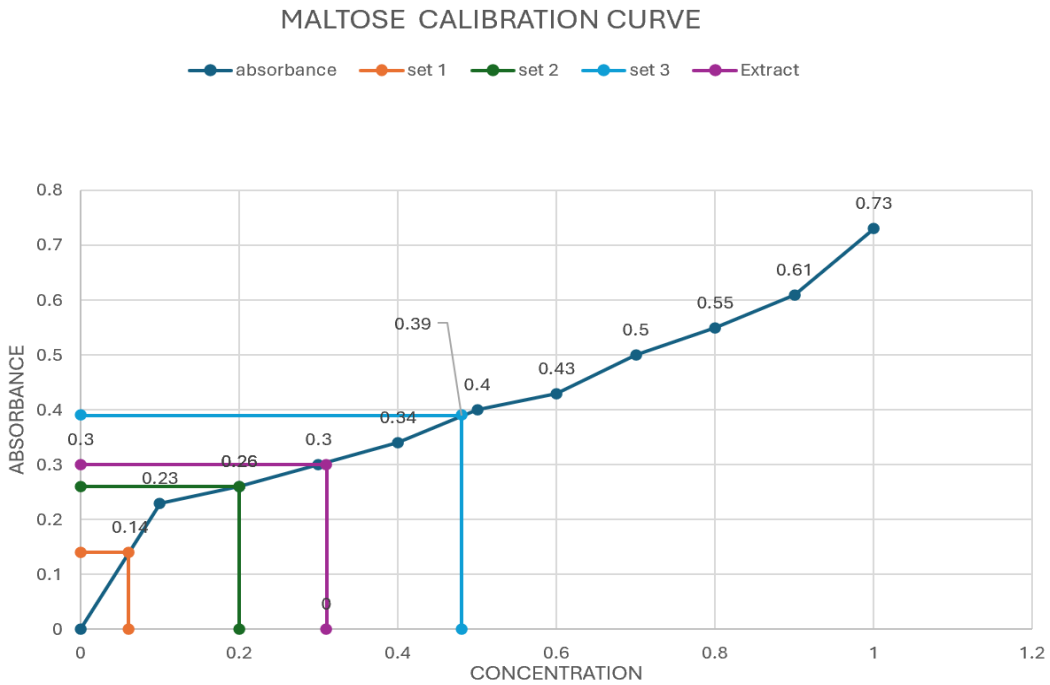

Fig. 1. Matose calibration curve

% Inhibition of α-Amylase

$$\%\ inhibitory\ activity = \frac{[A - C]}{[B - C]} \times 100$$

Where A= Absorbance of sample
B= Absorbance of blank
C= Absorbance of control

$$\%\ \text{inhibitory activity} = \frac{[0.31 - 0.39]}{[0.26 - 0.39]} \times 100$$
$$= 61.53\%$$

2 3

Fig.2 Soxhlet apparatus; **3** Maltose solution test tube series

Free radical scavenging by FRAP method

Table 3: Anti-oxidant activity by FRAP method observation

Sample	Absorbance
Blank	0
Reference Standard (Ascorbic acid)	1
Plant Extract (*Murraya koenigii)*	1.49

In this experiment, the yellow color changes to pale green and blue color depending on the concentration of antioxidants in the samples, by comparing the reference standard with plant extract is found to be 1.49, so the anti-oxidant activity in *Murraya koenigii* is more. The antioxidants such as phenolic acids and flavonoids were sent in considerable amounts in the extract of *Murraya koenigii.*

Discussions

Murraya koenigii leaves have a long history of use in traditional medicine, particularly in the management of diabetes. This study investigated the potential of *M. koenigii* leaf extracts to inhibit α-amylase activity and scavenge free radicals, two key factors in the management of diabetes and oxidative stress-related disorders.

α-Amylase Inhibition

The results of this study demonstrate that *M. koenigii* leaf extracts exhibit significant α-amylase inhibitory activity. This suggests that the extracts may have the potential to regulate postprandial hyperglycemia by slowing down the digestion and absorption of carbohydrates. The degree of α-amylase inhibition observed in this study is comparable to that of maltose, a commonly used α-amylase inhibitor drug. This finding supports the traditional use of *M. koenigii* leaves in the management of diabetes and suggests that the leaves may contain natural compounds with potent anti-diabetic properties. The percentage of α-amylase inhibition in flower was found to be 61.53 % because of this have α– amylase inhibitory activity this compound may have anti-diabetic activity and this compound has wide applications in future research fields.

Free radical scavenging activity

In addition to α-amylase inhibition, *M. koenigii* leaf extracts also exhibited significant free radical scavenging activity. This indicates that the extracts possess potent antioxidant properties, which can help to protect against oxidative stress-related damage. The free radical scavenging activity of *M. koenigii* leaves can be attributed to the presence of various bioactive compounds, including

polyphenols and flavonoids. These compounds have been shown to have strong antioxidant properties and can effectively scavenge free radicals, reducing oxidative stress and protecting against cellular damage. In this experiment, the yellow color changes to pale green and blue color depending on the concentration of antioxidants in the samples, by comparing the reference standard with plant extract is found to be 1.49, so the anti-oxidant activity in *Murraya koenigii* is more. The antioxidants such as phenolic acids and flavonoids were sent in considerable amounts in the extract of *Murraya koenigii.*

Potential therapeutic implications

The findings of this study suggest that *M. koenigii* leaves may have a dual therapeutic potential in the management of diabetes and oxidative stress-related disorders. The α-amylase inhibitory activity of the extracts may help to regulate postprandial hyperglycemia, while the free radical scavenging activity may protect against oxidative stress-related complications. These findings support the traditional use of *M. koenigii* leaves in the management of diabetes and suggest that the leaves may contain natural compounds with therapeutic potential.

Further research

While this study provides valuable insights into the α-amylase inhibitory and free radical scavenging activities of *M. koenigii* leaves, further research is needed to fully understand their therapeutic potential. Future studies should focus on isolating and identifying the specific bioactive compounds responsible for the observed activities, investigating the mechanisms by which these compounds exert their effects and evaluating the efficacy and safety of *M. koenigii* leaf extracts in clinical trials.

Murraya koenigii leaves exhibit promising α-amylase inhibitory and free radical scavenging activities, suggesting their potential as a natural therapeutic agent for managing diabetes and oxidative stress-related disorders. Further research is warranted to fully explore their therapeutic potential and to develop novel therapeutic strategies based on these findings.

Conclusion

Murraya koenigii, commonly known as curry leaf, is a plant native to India and Southeast Asia, known for its aromatic leaves used in culinary and traditional medicine. Phytochemical analysis of *Murraya koenigii* has identified several bioactive compounds such as alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds, all of which contribute to its medicinal properties, including antioxidant, anti-inflammatory, antimicrobial, and potential anticancer effects. The results of the current study demonstrate that *Murraya koenigii* leaf extracts exhibit significant α-amylase inhibitory activity, with an inhibition percentage of 61.53%. This suggests that the extracts may have the potential to regulate postprandial hyperglycemia by slowing down the digestion and absorption of carbohydrates. Furthermore, the extracts exhibited a strong free radical scavenging activity as evidenced by the FRAP value of 1.49. This indicates that *M. koenigii* leaves possess potent antioxidant properties, which can help to protect against oxidative stress-related damage.

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Author Contributions

MG, LJ, AP, AP, AT, AJ, PKS and VG conceived the concept, wrote and approved the manuscript.

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