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Original Research Article

α- Amylase Inhibitory Activity and Ferric Reducing Power of *Plumeria Pudica* Flowers

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Abstract: The α- Amylase inhibitory activity and Ferric reducing power of *Plumeria pudica* Flowers [1]. It have anti-inflammatory, antipyretic, antimicrobial, antioxidant and expectorant. Flowers of *Plumeria pudica* have been traditionally used to treat Fever, rheumatism, Skin conditions like acne, eczema, and dermatitis, respiratory problems like bronchitis, asthma, and coughs [2]. The *Plumeria pudica* (commonly known as white frangipani or bridal bouquet) is a species of flowering plant in the family Apocynaceae, which is native tropical America. Phytochemicals are naturally occurring chemicals in plants, such as flavonoids, alkaloids, phenols, and saponins. Phytochemical investigation is a systematic study of the chemical constituents present in plants, including their identification, isolation, characterization, and quantification. Amylase is a type of enzyme that catalyses the breakdown of starches and other complex carbohydrates into simpler sugars such as maltose and dextrin. It plays a crucial role in the digestive process, particularly in humans, where it initiates the breakdown of carbohydrates in the mouth and continues its action in the small intestine. Through the mentioned review of literature we understood about *Plumeria pudica* flower's phytochemicals and pharmacological activities. The articles include tests and methods to α amylase inhibitory activity of *Plumeria pudica* flowers. The ferric reducing antioxidant power (FRAP) is used to measure the antioxidant capacity of flower.

Keywords: *Plumeria pudica*, White frangipani, Bridal bouquet, Apocynaceae, Flowering plant.

INTRODUCTION

Plumeria pudica the importance of medicinal plants in drug development is known to us and humans have used them for different diseases from the beginning of human history. Traditional folk treatment from wild plants has always guided researchers to search for novel medications to develop healthy life for humans and animals. In addition, some medicinal plants are still obscured within the plant which needs to be scientifically evaluated. Plant derived medicines have been the first line of defence in maintaining health and combating diseases. Many secondary metabolites of plants are commercially important and find use in a number of pharmaceutical compounds. In the last century, roughly pharmaceutical products have been discovered based on the information obtained from the traditional healers. Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants. Medicinal plants, since times, have been used virtually in all cultures as a source of medicine. Substances derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma and other problems [3, 4].

Inhibition of α-amylase activity, α-amylase is the enzyme in humans that is responsible for the breakdown of starch to more simple sugars (dextrin, maltotriose, maltose and glucose). Carbohydrates are major constituents of human diet and polysaccharides are one of the main components of carbohydrates that play a role in the energy supply. The dietary carbohydrates break down to monosaccharides by some gastrointestinal enzymes, since only monosaccharides can be

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absorbed from intestinal lumen. Among monosaccharides, glucose can be readily absorbed from the gastrointestinal tract into blood stream after the hydrolysis of glycosidic bonds in digestible carbohydrate foods containing starch by the enzyme α-amylase and β-glucosidase. Inhibition of these enzymes reduced the high post prandial blood glucose peaks in diabetics. α-amylase inhibitory activity is done by DNS method and ferric reducing anti-oxdiant power of *Plumeria pudica* flowers is done by FRAP method.

MATERIALS & METHODOLOGY

➢ **Collection of Crude Drug**

Green leaves plucked from our campus garden. The herbarium was prepared and authenticated at State Medicinal plant board - Kerala by Senior scientist. Organoleptic characters are observed and noted. The collected leaves washed in running water to remove any organic or foreign particle 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 05 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 Inhibitory Activity by DNS Method

Materials

- **Starch solution:** Take 1 g of potato starch and dissolved in 100 ml of 0.02 M phosphate buffer of 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, dissolves in approximately 80 ml 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**

- Take 1 g of sample and extract with 75 ml of distilled water and 75 ml of ethanol for 2 hrs., at 40°C.
- Centrifuge the suspension at 5000 rpm. Collect the supernatant. Take 0.25 ml and incubate with 0.25 ml of enzyme solution for 15 minutes at 37°C.
- Incubate all the reagents also at 37°C for three minutes. At the end of the incubation add 2 ml of DNS reagent to first, second and sample 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 µmol of reducing sugars /min from soluble starch at 37°C, pH 7, and. under the specified experimental condition.

The % Inhibition of α- Amylase Enzyme activity is calculated using the equation: -

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

Ferric Reducing Power 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 deionised 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.

Method [5]

- Different concentrations of the methanolic extract of M. serratulum and its various fractions (10-50 µg/mL) was added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [K3Fe(CN)6] solution.
- The reaction mixture was vortexed well and then incubated at 50°C for 20 min using 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 standard using UV Spectrophotometer. Here, ascorbic acid was used as a reference standard, the reducing power of the samples were comparable with the reference standard.

RESULTS

α- Amylase Inhibitory Activity by DNS Method

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)	0.29

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

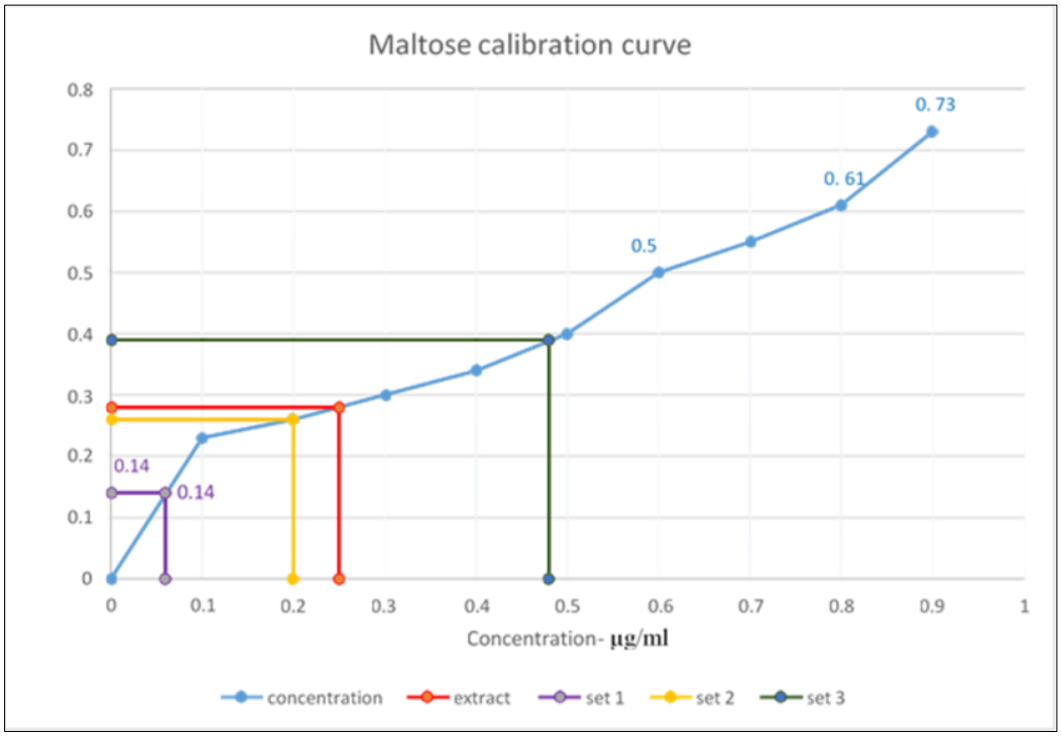

Figure 1: Matose calibration curve

% Inhibition of α- Amylase Enzyme: -

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

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

$$\%\ \text{inhibitory activity} = \frac{[0.29 - 0.39]}{[0.26 - 0.39]} \times 100$$
$$= 76.9\%$$

Ferric Reducing Power by FRAP Method

Sample	Absorbance
Blank	0
Reference Standard (Ascorbic acid)	1
Plant Extract (*Plumeria pudia* Flowers)	1.11

In this experiment, the yellow color changes to pale green and blue color depending on the concentration of antioxidants in the samples, by comparing reference standard with plant extract is found to be 1.11, so the anti-oxidant activity in *Plumeria pudia* Flowers is more. The antioxidants such as phenolic acids and flavonoids were present in considerable amount in the extract of *Plumeria pudica* Flowers.

DISCUSSION

α-Amylase inhibitory effect of *Plumeria pudica* Flowers Alpha amylase inhibitors block carbohydrate breakdown into simple sugars, reducing glucose absorption and showing potential in managing type 2 diabetes, obesity, and

cardiovascular health. Natural sources include plant extracts (e.g. *Plumeria pudica). Plumeria pudica* flowers exhibits significant alpha-amylase inhibitory activity. Bioactive compounds such as Plumericin, quercetin, kaempferol, and flavonoid glycosides may be responsible for to this effect, competitively inhibiting α-amylase and reducing rate of carbohydrate hydrolysis and glucose absorption. This mechanism regulates glucose levels, making *Plumeria pudica* flowers promising natural remedy for managing type 2 diabetes and preventing carbohydrate-related disorders. Further in vivo studies and structure-activity relationship investigations must perform to confirm the efficacy and develop phytochemical-based formulations. In this experiment, the yellow color changes to pale green and blue color depending on the concentration of antioxidants in the samples, by comparing reference standard with plant extract is found to be 1.11, so the anti-oxidant activity in *Plumeria pudica* flower is more. The antioxidants such as phenolic acids and flavonoids were present in considerable amount in the extract of *Plumeria pudica*.

CONCLUSION

Plumeria pudica flowers exhibits a range of pharmacological properties, including antioxidant, anti-inflammatory, antibacterial, antifungal, anticancer, analgesic, antipyretic, cardiovascular protective, neuroprotective, anti-diabetic, hepatoprotective, and immunomodulatory effects. These properties make it a valuable plant for medicinal research and traditional medicine practices, potentially alleviating conditions such as arthritis, skin disorders, infections, cancer, pain, fever, cardiovascular disease, neurodegenerative disorders, diabetes, and liver damage. Amylase is a type of enzyme that catalyses the breakdown of starches and other complex carbohydrates into simpler sugars such as maltose and dextrin. It plays a crucial role in the digestive process, particularly in humans. *Plumeria pudica* flowers extracts inhibit α-amylase activity, which is a key enzyme involved in carbohydrate digestion. The inhibition of α-amylase activity suggests potential anti-diabetic properties. This study investigates *Plumeria pudica's* α- amylase inhibitory activity. It identifies bioactive compounds and evaluates their therapeutic potential. The research uses DNS method for in vitro amylase inhibition assay. *Plumeria pudica* flowers shows promise for developing natural diabetes treatments. The study contributes to understanding the plant's pharmacological properties. α-Amylase inhibitory activity of the *Plumeria pudica* flowers extract also carried out according to the Dinitro Salicylic Acid method. % inhibition of α-Amylase enzyme is found to be 76.9%. This indicates that the flowers of *Plumeria pudica* flowers possess inhibitory action on Amylase enzyme. In future studies phytoconstituents will be isolated and will find out which constituent will be responsible for exhibiting the anti-diabetic property. The collected information can be used for further research as well as further development works. The research output can be used for isolation and characterization of bioactive compounds which would lead to suitable Phytochemical formulation after a detailed study.

In conclusion, the α-amylase inhibitory activity and ferric reducing power of *Plumeria pudica* flowers was successfully carried out, overcoming various challenges through careful planning and execution. The results are now part of a valuable scientific resource that will contribute to ongoing botanical research and anti-diabetic and anti-oxidant studies.

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