

Enzymatic in vitro Anti-diabetic Activity of *Homonoia retusa*

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Abstract: Using enzymatic inhibition of starch breakdown to control postprandial hyperglycemia is a successful treatment strategy for diabetes mellitus. In order to do this, *Homonoia retusa* was exposed to successive solvent extraction. The fractions obtained were then tested in vitro at four dosage levels for their ability to inhibit alpha amylase. When compared to the common medication Acarbose, the methanolic extract of *Homonoia retusa* exhibited the highest level of alpha amylase inhibitory action. An analysis of the extract's quantitative phytochemistry revealed that the flavonoid content was 44.59 ± 2.15 mg rutin equivalent g-1 and the phenol content was 58.68 ± 1.81 mg g-1 of gallic acid equivalent. According to the current study, *Homonoia retusa* may be used as an alpha-amylase inhibitor to treat diabetes.

Keywords: alpha amylase inhibitory activity, diabetes mellitus, post prandial hyperglycaemia.

INTRODUCTION:

The hallmark of the clinical syndrome known as diabetes is undesired hyperglycemia caused by a clinical resistance to insulin action or a relative or total lack of insulin⁴. A lower quality of life and increased risk factors for mortality and morbidity are associated with this illness. Persistent hyperglycemia is a major factor in the emergence and progression of micro- and macrovascular issues⁹. The Indian traditional health system includes some herbal preparations known as rasajanas, which have been used for more than a millennium⁵.

The major objective of diabetes treatment is to effectively control elevated blood sugar levels. The primary goals of diabetes treatments are to prevent the breakdown of dietary starch by carbohydrate-hydrolyzing enzymes like alpha-amylase and alpha-glucosidase, which lowers postprandial hyperglycemia, and to stimulate or increase insulin in target tissues by oral hypoglycemic agents. Reducing the rate at which glucose is absorbed by blocking two key digestive tract enzymes, alpha-amylase and alpha-glucosidase, to control postprandial hyperglycemia. The postprandial rise in plasma glucose is suppressed because this effect slows down the digestion of carbohydrates, which in turn slows down the rate of glucose absorption. Acarbose, miglitol, and voglibose are a few examples of such inhibitors that are presently available for purchase⁸.

Although numerous synthetic medications have been created for patients, it is a known fact that no one has ever been fully cured of diabetes. Current oral hypoglycemic medications have unfavorable side effects. Consequently, switching to different indigenous herbal and herbal preparations is urgently needed as an alternative form of treatment⁷.

MATERIALS AND METHODS

Chemicals:

α -amylase type VI-B from porcine pancreas (A3176-1MU) were purchased from Sigma Aldrich, Bangalore, India. DNSA (3,5-dinitrosalicylic acid), DMSO (dimethyl sulphoxide), sodium potassium tartrate, sodium hydroxide, Potato starch, disodium hydrogen phosphate, potassium dihydrogen phosphate was purchased from Gamut Scientific (SRL), Secunderabad, Telangana, India. Methanol, petroleum ether, ethyl acetate, aluminium trichloride, potassium acetate, sodium carbonate, tannic acid, Chloroform, Folin- Ciocalteu reagent were purchased from Taranath Scientific & Chemical Co. Acarbose was purchased from Hi-Media, Mumbai, India.

Plant material:

The plant specimen was gathered and verified by Dr. Md. Mustafa, a taxonomist and professor of botany at Kakatiya University, Warangal, Telangana, India. *Homonoia retusa* leaves were powdered, dried and stored in an airtight container after passing through sieve number 60. 1kg of powder was roughly crushed in separate round bottom flask and extracted with methanol (by maceration) for 7days at room temperature. The filtrate was concentrated under reduced pressure at 40 °C and stored at -4 °C.

Extraction and fractionation:

The plant material were dried, powdered and passed through sieve 60 and stored in an airtight container. 1kg of powder was roughly crushed and extracted with methanol (by maceration) in separate round bottom flasks (RBF) for 7days at room temperature. To ensure effective extraction, the contents of the round bottom flask were shaken frequently. The contents of each container were filtered after 7days and the Marc's were re-extracted with methanol for 3-5 days, until the plant chemical constituents were entirely extracted and then filtered. To obtain their extract and concentrated under reduced pressure (Rotavapour). The concentrated methanolic extract was collected separately and dried in desiccators. The methanolic extract was dissolved in 1000ml of water and fractionalized with n-hexane, chloroform, ethyl acetate and n-butanol. Under reduced pressure, the substances were separated from the fractions to provide the equivalent extract. All the fractions of *Homonoia retusa* were divided by separating funnel, evaporated in a rotavapour and used for forward studies.

α -Amylase inhibition test:

The colorimetric method developed by² was utilized to detect the inhibitory effect of chia extracts on α -amylase. After 15 minutes of boiling and stirring 0.25g of potato starch in 50 ml of deionized water, the starch solution (0.5% w/v) was produced. 0.001g of α -amylase, 6.7 mM sodium chloride, and 100 ml of 20 M sodium phosphate buffer (pH 6.9) were combined to create the enzyme solution (0.5 unit/mL). There are four concentrations of the extract available: 50 μ g/mL, 100 μ g/mL, 200 μ g/mL, and 500 μ g/mL. 5.31 M sodium potassium tartrate, 2 M sodium hydroxide (8 mL), deionized water (12 mL), and 96 mM 3, 5-dinitrosalicylic acid (20 mL) were combined to create the colorant solution.

A tube was filled with milliliters of each plant extract and one milliliter of an enzyme solution, and it was incubated for thirty minutes at 25°C. One milliliter of starch solution was added to one milliliter of this mixture, and the tube was then incubated for three minutes at 25°C. The closed tube was put in an 85°C water bath after 1 milliliter of the color reagent was added. Once the reaction mixture had cooled for fifteen minutes, it was taken out of the water bath, diluted with nine milliliters of distilled water, and its absorbance at five hundred and forty millimeters was taken. One milliliter of the matching extracted solvent was substituted for the plant extracts used in the controls. The positive control was acarbose solution at 25, 50, 75 and 100 μ g/mL.

The absorbance of the reaction mixture containing the enzyme is denoted by B in % inhibition, while the absorbance of the control reaction mixture is represented by A.

The formula for this is
$$\left(\frac{A - B}{A} \right) \times 100.$$

Analysis of phytochemistry:

Extracts exhibiting alpha amylase inhibition were subjected to standard chemical tests specified in the standard book to determine whether various phytochemicals were present or absent¹.

calculation of the phenolic content overall

The Folin-Ciocalteureagent was used, somewhat modified, in accordance with Singleton et al.'s methodology to ascertain the total phenolic contents of the plant methonolic extract. Each extract was dissolved in approximately 0.5 mL of 100 μ g/mL of methanol, and then 2.5 mL of 0.2 N Folin-Ciocalteu reagent was added. A full five minutes were spent shaking the mixture well and then adding two milliliters (75 grams per liter) of sodium carbonate solution. After a two-hour dark incubation period, the absorbencies were measured at 760 nm in relation to a water blank using a UVVis spectrophotometer. Gallic acid solutions, which served as the calibration curve's standard, underwent the same process once more. The standard was prepared with concentrations of 0.1, 0.02, 0.04, 0.06, 0.08 and 0.02/mL. Three runs of the analysis were performed and the results were stated as the gallic acid equivalent (GAE,mg/g) of the extract.

Determination of Total flavonoid content:

Following Sawadogo et al.'s description, the Dowd method with minor modifications was used to determine the total flavonoid contents of each plant methonolic extract. In summary, two milliliters of 2% AlCl_3 in methanol were mixed with two milliliters of each extract (100 μ g/mL), shaken well, and left to stand for ten minutes. Absorption was measured at 415 nm using a UV-vis spectrophotometer in relation to a blank sample that contained 2 mL of methanol and 2 mL of each extract that didn't contain AlCl_3 . Rutin solutions were utilized as the calibration curve's reference and the process was repeated with them⁹.

Concentrations of 0, 0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL were used to prepare the standard. The determination was done in triplicate, and the results were expressed in terms of rutin equivalents (mg of RE/g of extract).

Antioxidant activity:

Three ml of the mixture of the methanol from each of the 3 different plants were combined with one ml of a 0.1mM compound of DPPH in methanol– based extracts at various concentrations (10,25,50,75, & 100µg/mL). The each individual extract solutions were agitated forcefully and kept a side for 30 minutes at room temperature. A UV-VIS spectrophotometer was used to measure the absorbance at 517 nm. The reference standard utilised was ascorbic acid. High free radical scavenging activity was shown by reaction mixture with low absorbance values. The below formula was utilized to determine the ability of scavenging activity by DPPH method.

$$\text{Effect of DPPH scavenging (\% inhibition)} = \{(A_0 - A_1)/A_0\} * 100\}$$

Where A0 is the absorbance of the control response and A1 is the absorbance of the extract specimens. For each sample three different plant extracts were evaluated in triplicate and the results were averaged.

Statistically Analysis

Every experimental value was expressed as mean \pm using the standard error of mean (SEM). Following the application of one way analysis of variance (ANOVA) to ascertain the statistical significance between the groups, Turkey's multiple comparison test was carried out. A P-value of less than 0.05 is regarded as statistically significant.

RESULTS AND DISCUSSION

Alpha amylase inhibitory studies in vitro:

The IC50 values were computed by analyzing the plot of % alpha amylase inhibition as a function of extract concentrations. With an IC50 of 122.02 ± 2.12 µg/ml the nBHR extract had the lowest value. The IC50 values of the MEHR, nHHR and EAHR extracts were 231.04 ± 0.25 , 573.65 ± 1.14 and 462.71 ± 0.23 µg/ml respectively. Acarbose, the conventional positive control, with an IC50 of 30.12 ± 0.11 µg/ml. P value <0.05 did not indicate a significant difference in the butanol extract and acarbose IC50 in this investigation.

Total phenolic content:

Gallic acid equivalents are used to express the total phenolic content as milligrams per gram of extract (Table 1). Homonoia retusa's methanolic extract had a phenolic concentration of 9.63 ± 0.14 mg/g of extract, whereas the n-Hexane, n-Ethyl acetate and n-Butanol extracts had phenolic contents of 32.25 ± 0.23 , 7.42 ± 1.12 and 12.46 ± 0.11 mg/gram of extract, respectively.

DPPH radical scavenging activity

The extracts of ethyl acetate, methanol, n-hexane, and n-butanol demonstrated IC50 values of 270.30 ± 3.21 , 83.90 ± 1.02 , 143.50 ± 2.45 and 244.12 ± 2.13 µg/ml in relation to their DPPH radical scavenging capabilities (fig. Table). With an IC50 value of 83.90 ± 1.02 µg/ml, n-hexane had the highest activity, while ethyl acetate also demonstrates strong activity. There were notable variations seen in the IC50 values of every extract concerning their ability to scavenge DPPH.

Table 1:

The total phenol content, the IC₅₀ (μg/ml) of *Homonia retusa*'s DPPH scavenging activity and the IC₅₀ (μg/ml) of its alpha amylase inhibitory activity

Extract	Total Phenolic Content (Gallic equivalents acid mg/g extracts)	DPPH Scavenging Activity (IC ₅₀ (μg/ml))	Alpha amylase inhibitory activity IC ₅₀ (μg/ml)
Methanolic extract (MEHR)	9.63±0.14	270.30±3.21	231.04±2.13
n-hexane (nHHR)	32.25±0.23	83.90±1.02	573.65±1.25
Ethyl acetate (EAHR)	7.42±1.12	143.50±2.45	462.71±4.66
n-butanol (nBHR)	12.46±0.11	244.12±2.13	122.02±6.62

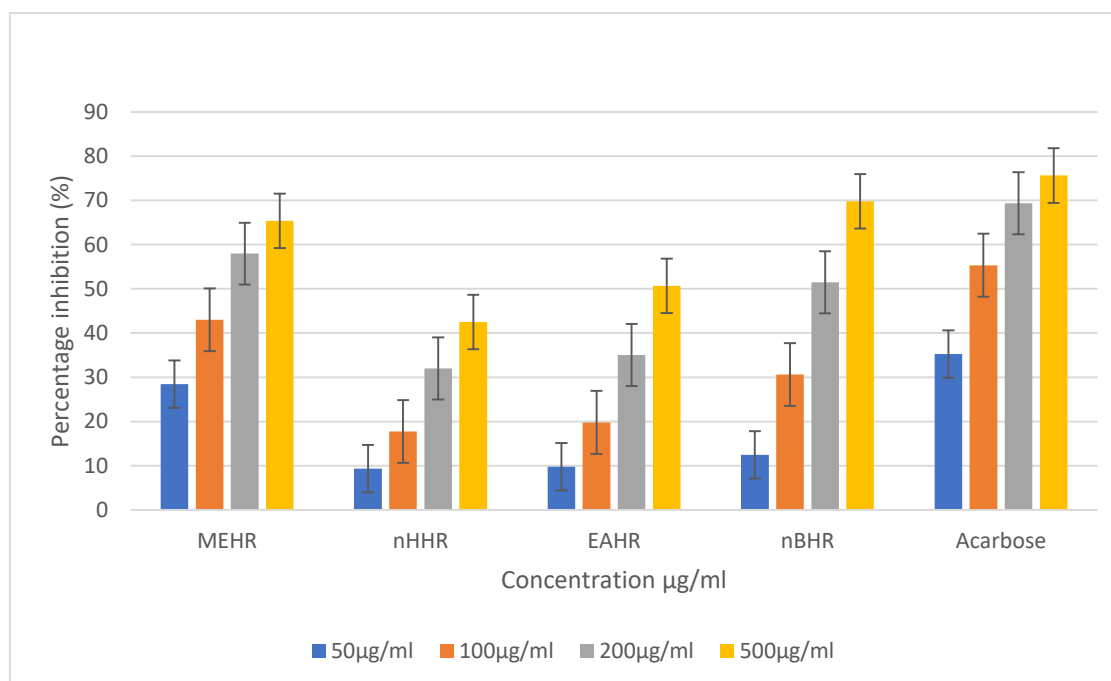


Figure: 1 Different fractions from the methanolic extract of *Homonia retusa* inhibiting alpha amylase

Phytochemical analysis:

To identify the most likely class of compounds in the extracts that are in charge of inhibiting alpha amylase, An initial qualitative analysis of the phytochemicals was done. The total phenolic content for the flavonoids was determined to be 44.59 mg of rutin equivalent g⁻¹ and 58.68 mg of gallic acid equivalent g⁻¹ using the Folin-Ciocalteu method. Due to their ability to scavenge free radicals and inhibit alpha amylase, the phenolic and flavonoidal compounds in the *Homonia retusa* methanolic extract were suggested by the previously mentioned information to be the potential causes of the observed activity¹⁰. The ability of these substances to bind with proteins enables them to inhibit the actions of digestive enzymes, thereby reducing postprandial hyperglycemia, according to published research³.

CONCLUSION

The results of the study indicate that the observed activity could be attributed to the flavonoid compounds present in the lead extract. A few conventionally used medicinal herbs in India, especially *Homonia retusa*, which exhibits promise as an alpha amylase inhibitor, may be helpful in the treatment of diabetes.

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