INVITRO ANTIDIABETIC ACTIVITY OF ETHANOL AND METHANOL EXTRACTS OF RHUS LONGIPES

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Abstract

Diabetes mellitus is a condition which impairs the ability of the body to take up glucose, otherwise known as blood sugar; the interest of using natural antidiabetic as a means of treatment exists. This study shows the in vitro analysis of the antidiabetic effect of methanolic and ethanolic extracts of Rhus longipes. Antidiabetic activity was determined by assessing the activities of alpha-amylase and alpha-glucosidase enzymes. The standard (acarbose) exhibited higher alpha amylase activity when compared to the methanolic and ethanolic plant extracts with IC_{50} values of 56.73µg/ml, 62.10µg/ml, and 68.79µg/ml, respectively. The alpha-glucosidase activities show that the standard (acarbose) exhibited higher activity when compared to the methanolic and ethanolic plant extracts with IC_{50} values of 29.27µg/ml, 51.11µg/ml, and 31.00µg/ml respectively. The antidiabetic effects were found to be dose-dependent. The results indicate that Rhus longipes possess antidiabetic properties to varying degrees. Therefore, it could be used to develop natural drugs which may be used in lieu of commonly used strong allopathic drugs which possess a number of harmful side effects.

Keywords*: Diabetes mellitus, alpha-amylase, alpha-glucosidase, acarbose. Rhus longipes*

Introduction

According to the American Diabetes Association (2014), diabetes is a set of metabolic illnesses defined by hyperglycemia caused by abnormalities in insulin action, insulin secretion or both. This disease's frequency has skyrocketed in recent years, owing to a demographic shift in its epidemiology. Previously untouched or minimally impacted populations are now reporting rising prevalence rates, posing a serious challenge to government and nongovernmental health financing. According to the International Diabetes Federation (IDF), there are 425 million people living with diabetes worldwide, with over half of them undiagnosed (International Diabetes Federation, 2017). Hyperglycemia weakens nerves and blood vessels over time, making diabetes a public health concern. As a result, serious medical complications such heart and kidney disease, stroke, blindness, nerve disorders, ulcers, gum infections, and amputation occur (Keerthana, 2013). Medicinal plants are still important in the management of diabetes mellitus, particularly in underdeveloped countries where many individuals lack access to standard antidiabetic medications. (Nwozo, 2019). In Ilaro, Southwest Nigeria, *Rhus longipes or Searsia longipes* (Engl.) is one of the plants often used in the treatment of asthma and malaria. It is a member of the Anacardiaceae family. In Yoruba, it's also referred as "ewe origin'. *Rhus longipes* is a small tree that grows up to 12 metres tall and has pale green leaves, whitish or greenish blooms, and dull red fruits. The plant can be found in the Savannah, thickets, various types of woodlands, and forests. Various elements of this plant, such as the roots extract, are used to treat infertility in women and enlarge the birth canal (Maroyi, 2011). Malaria and cancer are both treated with a decoction of the root (Burkil, 2014).). Research has therefore been performed on *Rhus longipes* for the discovery of a new antidiabetic drug as an alternative for synthetic drugs which can cause side effects like headache, vomiting, abdominal pain, low blood glucose and so on. Hence, the current study is focused to evaluate the antidiabetic potential of *Rhus longipes.*

Materials and Methods

Plant Collection, Authentication and Extraction

Fresh leaves of *Rhus longipes* were collected from district Oja-odan Area of Yewa- south Local Government, Ogun state, Nigeria, in the month of June, 2021. The plant samples were identified by a botanist and authenticated at the University of Lagos.

Preparation of Extracts

Fresh *Rhus longipes* plant leaves were collected, cleaned, weighed, and air-dried for two weeks. The plant sample of *Rhus longipes* was air-dried and coarsely powdered, and the extract phytochemical constituent was extracted using maceration method as described by Raaman, (2006) Powdered plant material (10 g) was taken in a conical flask and extracted with organic solvents (100 mL) methanol and ethanol in a mechanical shaker with Room temperature (28 ^{0c}) at constant stirring rate at 200 rpm. It was left for 24 h and solids were filtered using Whatman No. 1 filter. The extraction was repeated three times until complete extraction. The extract mixture was filtered, and the residue was extracted for additional five days with ethanol and methanol solvent before being filtered. The filtrates filtered extracts was concentrated in a rotary evaporator under decreased pressure at around 40°C before being lyophilized to produce the powdered extract. Inhibition experiments for -glucosidase and -amylase were performed on the extract.

Assays for Anti diabetic Activities

Alpha Amylase Inhibitory Assay

This experiment was carried out utilizing a modified McCue and Shetty technique (2004). 250 ml of extract (1.25–10 mg/ml) and 250ml of 0.02 M sodium phosphate buffer (pH 6.9) containing -amylase solution (0.5 mg/ml) were combined in a tube. This solution was pre-incubated at 25°C for 10 minutes before adding 250m l of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) at scheduled intervals and incubating at 25°C for another 10 minutes. By adding 500 ml of dinitrosalicylic acid (DNS) reagent, the process was stopped. After that, the tubes were boiled for 5 minutes and then cooled to room temperature (28 ^{0c}). The absorbance was measured after the reaction liquid was diluted with 5 mL distilled water. Spectrophotometer was used to measure the absorbance at 540 nm. A control was made by following the same technique but substituting distilled water for the extract. The α-amylase inhibitory activity was calculated as percentage inhibition:

Percentage of inhibition = Abs control –Abs extract/Abs control x 100

Alpha Glucosidase Inhibitory Assay

Using -glucosidase from Saccharomyces cerevisiae, the effect of plant extracts on -glucosidase activity was investigated using the method described by Kim et al. (2005). The p-nitrophenylglucopyranoside (pNPG) substrate solution was produced in 20mM phosphate buffer at pH 6.9. For 10 minutes, 100 ml of -glucosidase (1.0 U/mL) was pre-incubated with 50ml of the various concentrations of extracts. The reaction was then started with 50ml of 3.0 mM (pNPG) as a substrate dissolved in 20mM phosphate buffer (pH 6.9). The reaction mixture was incubated for 20 minutes at 37°C before being stopped with 2 mL of 0.1 M Na_2CO_3. The yellow colored paranitrophenol produced from pNPG was measured at 405 nm to evaluate the -glucosidase activity. The results were expressed as percentage of the blank control. Percentage inhibition is was calculated as

Percentage of inhibition = Abs control –Abs extract/Abs control x 100

Statistical Analysis

Data was treated by ANOVA (analysis of variance), data was expressed as mean ± SEM, and represented in form of scattered chart. All statistical analysis was carried out using graph pad prism, version 9.0

Results

The results of alpha amylase inhibitory assay of the plant extracts are presented in Table 1.The methanol and ethanol extracts showed a lower activity compared to Acarbose, the alpha-amylase inhibitory activities of the plant extracts is recorded in (Table 1).). The table shows the percentage inhibition of alpha amylase for ethanol extract of *Rhus longipes* and the methanol extract of *Rhus longipes* and that of the standard drug, acarbose has the highest activity with IC_{50} value of 56.73µg⁄ml followed by the methanol extracts of *Rhus longipes* which showed a lower activity compared to Acarbose with IC_{50}value of 62.10µg⁄ml and the ethanol extract of *Rhus longipes* has the least activity with IC_{50} value of 68.79µg⁄ml. The results of the experiment showed that, there was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme. Phytochemicals such as flavonoids, glycosides, tannins and phytosterols have been implicated in hypoglycaemic and antihyperglycemic action (Olasunkanmi et al.,2021;Nimenibo, 2013). Studies have also shown that flavonoids can act as an insulin mimetics (Collier et al.,1990).

The alpha-glucosidase inhibitory activities of the plants extracts are recorded in Table 2. The methanolic and ethanolic extracts of the plant were subjected to alpha-glucosidase inhibitory assay along with acarbose as the standard. The methanolic and the ethanolic plant extracts showed a lower activity compared to Acarbose. The table shows the percentage inhibition of alpha Glucosidase activity for the ethanolic and methanolic extract of *Rhus longipes* and of the standard drug (acarbose). Acarbose showed maximum activity with IC_{50}value of 29.272µg⁄ml, the ethanolic extract showed a lesser activity compared to acarbose with IC_{50} value of 31.002µg⁄ml. The methanolic extract showed the least activity with IC_{50} value of 51.119µg⁄ml compared to the ethanolic extract and the standard drug. The results of experiment showed that there was a dose-dependent increase in percentage inhibitory activity against α-glucosidase enzyme.

Table 1: Inhibitory of Alpha Amylase Activity of by Ethanol and Methanol Extracts of *Rhus longipes*

The table shows the percentage inhibition of alpha amylase for ethanol extract of *Rhus longipes* and the methanol extract of *Rhus longipes* and that of the standard drug, acarbose has the highest activity with IC_{50} value of 56.73µg⁄ml followed by the methanol extracts of *Rhus longipes* which showed a lower activity compared to Acarbose with IC_{50} value of 62.10µg⁄ml and the ethanol extract of *Rhus longipes* has the least activity with IC_{50} value of 68.79µg⁄ml. The results of the experiment showed that, there was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme. The extraction method also give a greater result, the methanol extract of the plant exhibited potent amylase inhibitory activity in a dose dependent manner. The presence of polyphenols and flavonoids in the plant's methanol extract showed the highest - amylase inhibitory activity (IC_{50} 62.10gml), which could be attributed to the presence of polyphenols and flavonoids because polyphenols can reduce oxidative stress as well as inhibit carbohydrate hydrolyzing enzymes due to their ability to bind with proteins. (Desai and Tatke, 2015).

Concentration (ug/ml)	**%Inhibitionof ethanol extract** ***Rhus longipes***	**%Inhibitionof methanol extract** ***Rhus longipes***	**Acarbose**
25	15.689±0.010	20.360±0.010	27.753±0.100
50	18.419±0.100	26.273±0.100	33.729±0.025
75	24.634±3.020	35.822±0.020	40.243±0.150
100	35.617±0.100	42.508±0.250	43.940±0.010
IC_{50}	68.79	62.10	56.73

Table 2: Inhibitory of Alpha Glucosidase Activity by Ethanol and Methanol Extracts of *Rhus longipes*

Concentration (ug/ml)	**%Inhibitionof methanolicextract *Rhus longipes***	**%Inhibitionof ethanolicextract *Rhus longipes***	**Acarbose**
10	10.764 ± 0.015	4.460 ± 0.050	98.615 ± 0.010
20	29.937 ± 0.050	34.523 ± 0.010	99.133 ± 0.015
50	46.774 ± 0.200	66.660 ± 0.015	99.218 ± 0.010
100	65.539 ± 0.020	72.728 ± 0.020	99.267 ± 0.005
150	73.553 ± 0.015	75.148 ± 0.010	99.388 ± 0.250
IC_{50}	51.119	31.002	29.272

Discussion

Plants are a great source of medicinal compounds. One of the biggest benefits of using plants as medication is that they rarely have the negative side effects that other allopathic pharmaceuticals have. The ability of *Rhus longipes* to function as effective anti-diabetic drugs was studied carried in this study. *Rhus longipes* has been shown to contain tannins, flavonoids, phenolics, terpenoids, steroids, alkaloids, and saponins, according to Olasunkanmi, Fadahunsi, and Adegbola (2021). The results in Table 1&2 shows that the percentage inhibition increases as the concentration of the sample increases. The increase in the percentage inhibition shows greater antidiabetic activity which is due to the ability of a compound in the sample test to inhibit or to delay hydrolysis of alpha amylase into the simple sugar. Acarbose has the highest inhibitory activity for against both the alpha amylase and alpha Glucosidase inhibitory assays with IC_{50} values of 29.272µg⁄ml for alpha Glucosidase inhibition and IC_{50} value of 56.73µg⁄ml for the alpha amylase inhibition, while the ethanol extract had an IC_{50} value of 31.00µg⁄ml and the methanol extract of *Rhus longipes* had the least activity with IC_{50} value of 51.11µg⁄ml. The activities of alpha amylase and alpha Glucosidase of both extracts of *Rhus longipes* was not significantly different when compared to that of the standard drug. This shows that the plant has the potential to be effective for diabetes mellitus management. It was observed that the ethanol extract of *Rhus longipes* showed more activity against alpha Glucosidase when compared with the methanolic extract of the plant. Ethanol is less polar than Methanol and could have extracted both the polar and non-polar Components of the plant, therefore, this might be due to the its strong scavenging potential (Olasunkanmi, Fadahunsi and Adegbola, 2021) also ethanol is reliable, effective, efficient, safe, consistently producing potent extractions with low fuss. Ethanol extract showed a lesser activity compared to acarbose with IC50 value of 31.002µg⁄ml, the methanol extract showed the least activity of IC_{50}value of 51.119µg⁄ml compared to the ethanol extract and the standard drug. The results of experiment showed that there was a dose-dependent increase in percentage inhibitory activity against α-glucosidase enzyme, the alpha amylase inhibitory assay showed that acarbose has the highest activity with IC_{50} value of 56.73µg⁄ml followed by the methanol extracts of *Rhus longipes* which showed a lower activity when compared to the activity of acarbose which has IC_{50} value of 62.10µg⁄ml and the ethanol extract of *Rhus longipes* has the least activity with IC_{50} value of 68.79µg⁄ml. The results of the experiment revealed a dose-dependent increase in percentage inhibitory activity against the α-amylase enzyme, as well as the type of solvent used for extraction. The methanol extract of *Rhus longipes* has a higher activity than the ethanol extract of *Rhus longipes*, which could be due to the presence of polyphenols and flavonoids. Because of its capacity to connect with proteins, polyphenols can reduce oxidative stress while also inhibiting carbohydrate hydrolyzing enzymes (Desai and Tatke, 2015). Methanol is also a strong solvent for extracting all primary and secondary metabolites because it penetrates the cell wall.

Long-chain carbohydrates are broken down by alpha amylase, while disaccharides and starch are broken down to glucose by alpha Glucosidase. The rate at which carbohydrate is digested is reduced by blocking both of these enzymes, which can prolong intestinal absorption and slow the abrupt rise in blood sugar levels that diabetic patients experience after a meal. Inhibiting the two enzymes lowers the amount of sugar available during digestion, making it a useful treatment for type 2 diabetes mellitus in its early stages. (Senthikumar, 2012).

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