Evaluation Of A -Amylase Inhibitory Action Of Annona Muricata (Soursop Leaves)

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ABSTRACT:
Annona muricata leaves are used in traditional medicine to treat diabetes and its complications. The purpose of this study is to identify the major phytochemical components and characterize the phenol-rich fractions for in vitro antioxidant and α-amylase inhibitory activity. Therby potential in vitro antidiabetic properties of Annonamuricata leaves Was to evaluate. Key strategies for reducing postprandial hyperglycemia in diabetes include inhibition of the carbohydrate hydrolase amylase. Therefore, this study evaluated the in vitro inhibitory ability of Annonamuricata leaves. Annona muricata has been used in the management of diabetes. A major strategy in decreasing postprandial hyperglycaemia in diabetes involves the inhibition of carbohydrate-hydrolysing enzymes-α-amylase. Thus, this study evaluated the in vitro inhibitory potentials of leaves of Annona muricata.

KEY WORDS :Hyperglycemia, α-amylase, Pancrelipase, Anti-inflammatory, α-glucosidase.

I. INTRODUCTION:
Annona muricata (Annona muricata) is one of the medicinal plants for that purpose. Therapeutic value. This vast tree is native to tropical America but is currently being planted World wide. It is mainly found in tropical and subtropical regions, including the south and south North America, India, Nigeria, Malaysia. Muricata is known by various local names, Soursop (English), Graviola (Portuguese), Sirsak (Indonesia), Guanabana (Latin America)[7]. muricata has therapeutic value for many illnesses. actually We report antidepressant[13] and anti-cancer [31]effects. The leaves work against the heat[25], Headache, sleep disorders, rheumatism[10], viral infections[18], breast cancer, bacterial infection [16]. The bark of the trunk has the potential for adaptogens [26] and antioxidants [24]. Moreover For plants, alkaloids [12] inorganic acetogenins [29], Cyclopeptides [17], megastigmene [20], flavonoids, triglycerides[21]and phenols.

Annona muricata (A. muricata) is a tropical plant species of the Annonaceae family. And it is known for its many ethnographic uses. All parts of sugar apples are used in natural remedies Tropical. It is considered an excellent source of natural antioxidants for a variety of diseases. Traditionally, leaves are used for headaches, insomnia, cystitis, liver problems, and diabetes. Anti-Tumor, anti-inflammatory. The health benefits of this plant are due to its uniqueness Phytochemical composition. [4]

The mechanism of action of this medicinal plant in their products is to delay absorption Pancrelipase and By inhibiting α-glucosidase and these enzymes, medicinal plants can be effectively controlled the Elevated blood sugar level after meals. [28]

Alpha amylase (α-amylase) is an enzyme that produces large alpha bonds. Alpha-binding polysaccharides like starch and glycogen that make shorter chains That, dextrin and maltose[5]. It is the main form of amylase found in humans, etc. Mammalian[33]. It is also found in starchy seeds as a food reserve and is excreted. By many mushrooms. It is a member of the glycose hydrolase family 13. Amylase is found in many tissues, but most often in pancreatic juice and saliva. Each has its own isoform of human α-amylase. They behave differently It can also be used for testing with specific monoclonal antibodies in isoelectric focusing, antibody. In humans, all amylase isoforms bind to chromosome 1p21 Pancreatic α-amylase erroneously cleaves the α (14) -glycosidic bond of amylase It can be dextrin, maltose or maltotriose. Uses a double shift mechanism Retention of anomer composition. In humans, salivary amylase That. [27]

Soursop leaves are the most useful part of this tree. It has Compounds containing acetogenin, namely bullatacin, asimicin and Squamosin. Phytochemical analysis of Annonamuricata leaves

The extract showed the presence of secondary metabolites such as tannins. Steroids and cardiac glycosides [18]. Soursop leaf nutrients it is also thought to stabilize blood sugar levels in the normal range. Very useful in treating diabetics. Some studies have shown that Annona muricata leaves have an antihyperglycemic effect. Showed islet regeneration[1]

II. MATERIAL AND METHODS:

PLANT MATERIAL: [FIG-1]
The plant material is composed of dried ground leaves of Annona Muricata. It belongs to the Annonaceae family.[We collected]

PREPARATION OF THE EXTRACT:

fresh leaves of Annona muricata and air-dried them in the shade at room temperature. The dried sheet material was mechanically driven and sifted through a # 40 mesh screen. The fine powder was stored separately in a closed container until it was used. Approximately grams of finely ground leaf material was soaked in methanol for 72 hours. The exact percentage yield was calculated.

REQUIRED CHEMICAL:

Potassium solution (5 g / 100 ml), sodium phosphate buffer, alpha amylase enzyme (0.5 mg / ml), potassium iodide and iodine solution, leaf extract.

CHEMICAL TEST:

ALKALOID MAYER TEST:
The extract was treated with Mayer's reagent. There is a yellow precipitate, indicating the presence of alkaloids.

BENEDICT'S TEST TO REDUCE SUGAR:
The extract was treated with Benedict's reagents and heated in a water bath. The formation of orange-red precipitates indicates the presence of reducing sugars.

FLAVONOID FERRIC CHLORIDE TEST:
The extract was treated with a few drops of fecl3 solution. Blackish red colour indicates presence of flavonoids.

TEST FOR TANNINS:

To 1 ml of extract add few drops of 1% fecl3 solution. The appearance of blue, black, green or blue green precipitate indicates presence of tannins.

TEST FOR STEROIDS:

2 ml of acetic anhydride was add to 0.5 gm of extract with 2 ml of H2so4, there is no change in colour from violet to blue indicates absence of steroids.

BORNTRAGER TEST FOR ANTHRAQUINONES:

Small portion of the extract was shaken well with 10 ml of benzene and filtered. 5 ml of 10% ammonia solution was added to the filtrate and stirred. If the pink, red, and purple colours are not formed, it indicates that anthraquinone is absent.

MOLISCH’S TEST OF CARBOHYDRATES:

The extract was treated in vitro with 2 drops of alcohol and 2 ml of concentrate. H2SO4 was carefully added to the sides of the test tube. The formation of a dull purple / red ring in interphase indicates the presence of carbohydrates.
TERPENOID SALKOWSWI TEST: 
Chloroform 2 ml was added to 1 ml of the solvent extract. Then 3 ml of concentrate. H2 SO4 I added it carefully to form a layer. The reddish-brown colour at the interface indicated the presence of terpenoids.

SAPONIN FOAM TEST:
Approximately 2 ml of distilled H2O and 1 ml of solvent extract were mixed and shaken vigorously. The formation of stable and persistent bubbles indicated the presence of saponins.

PRELIMINARY PHYTOCHEMICAL ANALYSIS:

IN-VITRO ANTI-DIABETIC ACTIVITY:

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PROCEDURE FOR PREPARATION OF BUFFER:
1. weigh accurate amount of disodium hydrogen phosphate, anhydrous sodium dihydrogen phosphate, dissolve it in distilled water.
2. Then adjust the pH to 7.4 by adding HCl or NAOH.
3. Then makeup to 100ml in a volumetric flask

PREPARATION OF ENZYME SOLUTION: Prepare the enzyme solution by weighing 0.4 g of alpha-amylase enzyme, dissolving it in distilled water, and then diluting to 100 mL in a volumetric flask with distilled water. The solution had a concentration of 2 units per millilitre.

PREPARATION OF STARCH SOLUTION: In a volumetric flask, weigh 100mg of starch and dissolve it in distilled water. Make up to 100ml with distilled water.

PROCEDURE:
• Mix 1 mL plant extract with 9 mL phosphate buffer.
• Add 0.1ml of enzyme solution to 100,200,300,400,500,600,700 µg of above solution.
• Incubate the aforementioned solution at 37°C for 10 minutes.
• Add 0.4 mL of starch solution to the aforementioned solution mixture after incubation.
• Incubate the solution mixture at 37°C for another 30 minutes.
• After incubation, add 1.5 ml of iodine solution and 0.2 ml of dilute HCL.
• Finally, look at the 580nm absorption.

\[ \% \text{inhibition} = \left( \frac{AC - AS}{AC} \right) \times 100 \]

Where, 
AC = absorbance of the control and AS = absorbance of tested samples.
III. RESULTS AND DISCUSSION:
Many herbal extracts have been shown to have anti-diabetic properties and are utilised in Ayurvedic medicine to treat diabetes. Many modern medicines have been made from herbal extracts, either directly or indirectly. An in-vitro inhibitory impact of Annona muricata extract on alpha-amylase activity was investigated in this work. The plant's leaves have strong alpha-amylase inhibitory action.

![FIG-2]

<table>
<thead>
<tr>
<th>S.NO</th>
<th>DOSE / CONCENTRATION</th>
<th>Absorbance</th>
<th>Percentage inhibition</th>
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<tbody>
<tr>
<td>1.</td>
<td>100</td>
<td>0.88</td>
<td>6.5%</td>
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<tr>
<td>2.</td>
<td>200</td>
<td>0.82</td>
<td>12.8%</td>
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<td>3.</td>
<td>300</td>
<td>0.76</td>
<td>19.3%</td>
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<tr>
<td>4.</td>
<td>400</td>
<td>0.68</td>
<td>27.8%</td>
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<td>5.</td>
<td>500</td>
<td>0.55</td>
<td>41.6%</td>
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<td>6.</td>
<td>600</td>
<td>0.48</td>
<td>49%</td>
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<td>7.</td>
<td>700</td>
<td>0.43</td>
<td>54%</td>
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IV. CONCLUSION:

Diabetes mellitus is a genetically and clinically heterogeneous group condition with similar aspects of glucose tolerance that is characterised by hyperglycemia. One of the early treatments for diabetes mellitus was the use of medicinal herbs. In vitro investigations have revealed that the alpha-amylase enzyme, which is involved in postprandial hyperglycemia, is inhibited. An alpha-amylase inhibitor is an anti-nutrient that prevents starch breakdown and glucose absorption. In conclusion, the in-vitro experiments have revealed an inhibitory effect on the alpha-amylase enzyme. Because of its hypoglycemic activity, Annona muricata may be useful in the treatment of diabetes mellitus, according to this study. Further research into the nature of the functional group would shed insight on the exact mechanism, allowing them to be used more effectively in the treatment of diabetes.

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