

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

Adepu.Santhi priya*, Nelluri Ajay kumar¹, kinjini prasanth².

*Assistant professor, Department of pharmacology, CRR college of Pharmaceutical Sciences, eluru-534007

^{1,2}[IV/VI Doctor of pharmacy], Department of Pharmacy Practice, CRR college of Pharmaceutical Sciences, eluru-534007

Submitted: 10-07-2022

Accepted: 21-07-2022

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, Thereby potential in vitro antidiabetic properties of *Annona muricata* 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 *Annona muricata* 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, Australia, 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]. Thebark of the trunk has the potential for adaptogens [26] and antioxidants [24]. Moreover For plants, alkaloids [12] inorganic acetogenins [29],Cyclopeptides [17],

megastigmane [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 glycoside 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 amylose 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 *Annona muricata* 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]



[FIG-1]

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 $FeCl_3$ solution. blackish red colour indicates presence of Flavonoids.

TEST FOR TANNIS:

To 1 ml of extract add few drops of 1% $FeCl_3$ solution. The appearance of blue, black, green or blue green precipitate indicates presence of tannins.

TEST FOR STEROIDS:

2 ml of acetic anhydride was added to 0.5 gm of extract with 2 ml of H_2SO_4 , there is no change in colour from violet to blue indicates absence of steroids.

BORTRAGER 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. H_2SO_4 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 SALKOWSKI 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 :[Table-1]
IN-VITRO ANTI-DIABETIC ACTIVITY:

[Table-1]

S.No	Plant constituents	Inference
1	Alkaloids	+ve
2	Flavonoids	+ve
3	Tannins	+ve
4	Reducing sugars	+ve
5	Steroids	-ve
6	Carbohydrates	-ve
7	Terpinoids	-ve
8	Anthraquinones	-ve
9	Saponins	+ve

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 make up 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.

$$\%inhibition = (AC - AS) /$$

AC × 100

Where, AC = absorbance of the control and AS = absorbance of tested samples.

[FIG-2]



[TABLE-2]

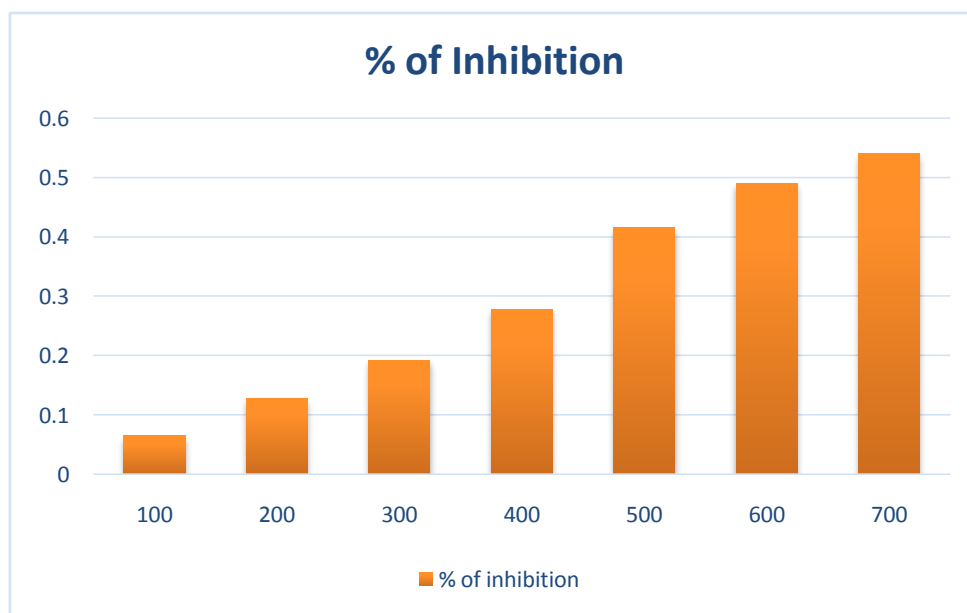
S.NO	DOSE / CONCENTRATION	Absorbance	Percentage inhibition
1.	100	0.88	6.5%
2.	200	0.82	12.8%
3.	300	0.76	19.3%
4.	400	0.68	27.8%
5.	500	0.55	41.6%
6.	600	0.48	49%
7.	700	0.43	54%

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-3]



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.

REFERENCES;

- [1]. Aderibigbe, K., Komolafe, O., Adewole, O., Obuotor, E., & Adenowo, T. (2010). Anti hyperglycemic activities of *Annona muricata* (Linn). African Journal Of Traditional, Complementary And Alternative Medicines, 6(1). doi: 10.4314/ajtcam.v6i1.57075
- [2]. Adewole, S., & Caxton-Martins, E. (2009). Morphological changes and hypoglycemic effects of *Annona muricata* linn. (annonaceae) leaf aqueous extract on pancreatic β -cells of streptozotocin-treated diabetic rats. African Journal Of Biomedical Research, 9(3). doi: 10.4314/ajbr.v9i3.48903
- [3]. Agu, K., Okolie, N., Eze, I., Anionye, J., & Falodun, A. (2017). Phytochemical analysis, toxicity profile, and hemomodulatory properties of *Annona muricata* (Soursop). The Egyptian Journal Of Haematology, 42(1), 36. doi: 10.4103/1110-1067.206431
- [4]. Agu, K., Okolie, N., Falodun, A., Erharuyi, O., Igbe, I., & Elekofehinti, O. et al. (2017). Isolation and Elucidation of 15-Acetylguanacone from Soursop (*Annona muricata* Linn) Fruit and Molecular Docking Experiments. Journal Of Applied Sciences And Environmental Management, 21(2), 236. doi: 10.4314/jasem.v21i2.3
- [5]. Amylase - Creative Biogene. (2022). Retrieved 8 May 2022, from <https://microbiosci.creative-biogene.com/similar/alpha-amylase-1135.html>
- [6]. Antipyretic Activity of AOF Herbal Formulation in Brewer's Yeast-Induced Pyrexia in Rats. (2019). Myanmar Health

- Sciences Research Journal, 31(2), 148-152. doi: 10.34299/mhsrj.00939
- [7]. Coria-Télez, A., Montalvo-González, E., Yahia, E., & Obledo-Vázquez, E. (2018). *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. *Arabian Journal Of Chemistry*, 11(5), 662-691. doi: 10.1016/j.arabjc.2016.01.004
- [8]. De Sousa, O., Vieira, G., De Pinho, J., Yamamoto, C., & Alves, M. (2010). Antinociceptive and Anti-Inflammatory Activities of the Ethanol Extract of *Annona muricata* L. Leaves in Animal Models. *International Journal Of Molecular Sciences*, 11(5), 2067-2078. doi: 10.3390/ijms11052067
- [9]. Effects Of *Annona Muricata* (Linn) On The Morphology Of Pancreatic Islet Cells Of Experimentally-Induced Diabetic Wistar Rats. (2008). *The Internet Journal Of Alternative Medicine*, 5(2). doi: 10.5580/229c
- [10]. El-Beltagy, A., Elsyayad, H., Abdelaziz, K., Madany, A., & Elghazaly, M. (2021). Therapeutic Role of *Annona muricata* Fruit and Bee Venom Against MNU-Induced Breast Cancer in Pregnant Rats and its Complications on the Ovaries. *Breast Cancer: Targets And Therapy*, Volume 13, 431-445. doi: 10.2147/bctt.s306971
- [11]. Endrini, S., Suherman, S., & Widowati, W. (1970, January 01). *Annona muricata* leaves have strongest cytotoxic activity against breast cancer cells: Semantic scholar. Retrieved May 8, 2022, from <https://www.semanticscholar.org/paper/Annona-muricata-leaves-have-strongest-cytotoxic-Endrini-Suherman/fc365a8b756e96f133d1e4211c3cb98832003f14>
- [12]. Europe PMC: a full-text literature database for the life sciences and platform for innovation. (2014). *Nucleic Acids Research*, 43(D1), D1042-D1048. doi: 10.1093/nar/gku1061
- [13]. Hasrat, J. A., De Bruyne, T., De Backer, J., Vauquelin, G., & Vlietinck, A. J. (1997). Isoquinoline derivatives isolated from the fruit of *annona muricata* as 5-HT_{1A} receptor agonists in rats: Unexploited antidepressive (lead) products. *Journal of Pharmacy and Pharmacology*, 49(11), 1145-1149. doi:10.1111/j.2042-7158.1997.tb06058.x
- [14]. Hossain, M., Mostofa, M., Awal, M., Chowdhury, E., & Sikder, M. (2014). Histomorphological and morphometric studies of the pancreatic islet cells of diabetic rats treated with aqueous extracts of *Momordica charantia* (karela) fruits. *Asian Pacific Journal Of Tropical Disease*, 4, S698-S704. doi: 10.1016/s2222-1808(14)60710-6
- [15]. Jiménez, Víctor M. et al. "Identification Of Phenolic Compounds In Soursop (*Annona Muricata*) Pulp By High-Performance Liquid Chromatography With Diode Array And Electrospray Ionization Mass Spectrometric Detection". *Food Research International*, vol 65, 2014, pp. 42-46. Elsevier BV, <https://www.sciencedirect.com/science/article/abs/pii/S0963996914003664?via%3Dihub>
- [16]. Kn, C., Shaji, C., & Thomas, B. (1970, January 01). [PDF] evaluation of major phytochemical constituents of two edible fruit yielding species of Annonaceae: *Annona muricata* L. and *Annona reticulata* L: Semantic scholar. Retrieved May 8, 2022, from <http://www.semanticscholar.org/paper/Evaluation-of-major-phytochemical-constituents-of-L-Kn-Shaji/e46217f796c165a6634bdf10c41da8adb483ab15>
- [17]. Kossouoh, C., Moudachirou, M., Adjakidje, V., Chalchat, J., & Figuéredo, G. (2007). Essential Oil Chemical Composition of *Annona muricata* L. Leaves from Benin. *Journal Of Essential Oil Research*, 19(4), 307-309. doi: 10.1080/10412905.2007.9699288
- [18]. Kumaran, D. (2021). A Review of Recent Studies on The Phytochemical and Pharmacological Activity of *Annona Muricata*. *Forestry & Agriculture Review*, 2(1), 1-5. doi: 10.47285/far.v2i1.31
- [19]. Kumaran, D. (2021). A Review of Recent Studies on The Phytochemical and Pharmacological Activity of *Annona Muricata*. *Forestry & Agriculture Review*, 2(1), 1-5. doi: 10.47285/far.v2i1.31
- [20]. Matsushige, A., Matsunami, K., Kotake, Y., Otsuka, H., & Ohta, S. (2011). Three new megastigmanes from the leaves of *Annona muricata*. *Journal Of Natural*

- Medicines, 66(2), 284-291. doi: 10.1007/s11418-011-0583-1
- [21]. Nawwar, M., Ayoub, N., Hussein, S., Hashim, A., El-Sharawy, R., & Wende, K. et al. (2012). Flavonol triglycoside and investigation of the antioxidant and cell stimulating activities of *Annona muricata* Linn. Archives Of Pharmacal Research, 35(5), 761-767. doi: 10.1007/s12272-012-0501-4
- [22]. Okolie, N., Agu, K., & Eze, G. (2013). PROTECTIVE EFFECT OF ETHANOLIC LEAF EXTRACT OF ANNONA MURICATA LINN ON SOME EARLY EVENTS IN CYCAS-INDUCED COLORECTAL CARCINOGENESIS IN RATS. Journal Of Pharmaceutical And Scientific Innovation, 2(4), 14-21. doi: 10.7897/2277-4572.02444
- [23]. Okwu, D., & Omodamiro, O. (2006). Effects of hexane extract and phytochemical content of *Xylopiya aethiopica* and *Ocimum gratissimum* on the uterus of guinea pig. Bio-Research, 3(2). doi: 10.4314/br.v3i2.28589
- [24]. Omale, J., & Olakunle, S. (2015). In vitro and in vivo evaluation of antioxidant activity of *Annona muricata* stem bark extracts in *Rattus norvegicus*. Planta Medica, 81(16). doi: 10.1055/s-0035-1565710
- [25]. Ong, H., & Norzalina, J. (1999). Malay herbal medicine in Gemencheh, Negri Sembilan, Malaysia. Fitoterapia, 70(1), 10-14. doi: 10.1016/s0367-326x(98)00023-9
- [26]. Padma, P., Chansauria, J., Khosa, R., & Ray, A. (n.d.). Effect of *annooa muricata* and *Polyalthia cerasoides* on brain neurotransmitters and enzyme monoamine oxidase following cold immobilization stress. Retrieved May 8, 2022, from <https://www.informaticsjournals.com/index.php/jnr/article/view/27>
- [27]. Perry, G., Dominy, N., Claw, K., Lee, A., Fiegler, H., & Redon, R. et al. (2007). Diet and the evolution of human amylase gene copy number variation. Nature Genetics, 39(10), 1256-1260. doi: 10.1038/ng2123
- [28]. Prashanth, D., Padmaja, R., & Samiulla, D. (2001). Effect of certain plant extracts on α -amylase activity. Fitoterapia, 72(2), 179-181. doi: 10.1016/s0367-326x(00)00281-1alpha-
- [29]. Rupprecht, J., Hui, Y., & McLaughlin, J. (1990). Annonaceous Acetogenins: A Review. Journal Of Natural Products, 53(2), 237-278. doi: 10.1021/np50068a001
- [30]. Sawant, T. (2014, May 27). Bio-chemical compositional analysis of *Annona muricata*: A miracle fruit's review. Retrieved May 8, 2022, from https://www.academia.edu/6961603/BIO_CHEMICAL_COMPOSITIONAL_ANALYSIS_OF_ANNONA_MURICATA_A_MIRACLE_FRUIT_S_REVIEW
- [31]. Sun, S., Liu, J., Sun, X., Zhu, W., Yang, F., & Felczak, L. et al. (2017). Novel Annonaceous acetogenins from *Graviola* (*Annona muricata*) fruits with strong anti-proliferative activity. Tetrahedron Letters, 58(19), 1895-1899. doi: 10.1016/j.tetlet.2017.04.016
- [32]. Sun, S., Liu, J., Zhou, N., Zhu, W., Dou, Q., & Zhou, K. (2016). Isolation of three new annonaceous acetogenins from *Graviola* fruit (*Annona muricata*) and their anti-proliferation on human prostate cancer cell PC-3. Bioorganic & Medicinal Chemistry Letters, 26(17), 4382-4385. doi: 10.1016/j.bmcl.2015.06.038
- [33]. Voet, D - Donald, J. G.: NEUF Paperback (2005) | Chapitre.Com : Livres Et Presse Ancienne". Abebooks.Com, 2022, available at <https://www.abebooks.com/biochimie-2e-%C3%A9dition-Voet-Donald-BOECK/15223816274/bd>.