Advances in Bioresearch
Adv. Biores., Vol 15 (5) September 2024: 264-271
©2024 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.5.264271

Advances in Bioresearch

ORIGINAL ARTICLE

Phytochemical Profiling and Antidiabetic Potential of *Annona squamosa*: Comparative Study of Extracts from Leaf, Stem, and Fruit.

Mahima, Trapti Gupta, Geeta Pandey
Department of Zoology, IIS (Deemed to be) University, Jaipur 302020

ABSTRACT

Diabetes mellitus poses a significant threat to individuals across all age groups, significantly impacting their everyday activities. Despite the wide array of synthetic antidiabetic drugs, there is a rising need for the creation of innovative medication due to the adverse reactions and the emergence of resistance to existing commercial treatment with prolonged use. Annona squamosa, a member of the Annonaceae family, contains diverse chemical compounds, including alkaloids, carbohydrates, tannins, phenolic compounds, isomeric hydroxyl ketones, cyclopeptides, and acetogenins, distributed in various parts of the plant. Its traditional medicinal use for treating various diseases and potential benefits in managing diabetes are well-documented. The aqueous and ethanolic extracts of several plant components, including stems, fruits, and leaves, were subjected to quantitative and qualitative phytochemical examination. As well as their α-amylase and α-glucosidase inhibitory activities was also evaluated to assess their antidiabetic activity. Leaf extract of Annona squamosa contains various phytochemicals such as phenol, flavonoid. Quantitative phytochemical analysis revealed varying phenolic and flavonoid content across plant parts, with stems exhibiting the highest phenolic content (2.182) and leaves displaying the highest flavonoid content (0.843). The ethanolic and aqueous leaf extracts exhibited maximum significant inhibition against α-amylase, with IC50 values of 0.026 mg/mL and 0.0052 mg/mL, respectively and significant inhibition for α-glucosidase, with IC50 values of 0.041 mg/mL and 0.109 mg/mL, respectively. The findings highlight the potential of leaf of Annona squamosa in diabetes management, as evidenced by their significant inhibitory effects on key enzymes. This offers a promising avenue for further research and development of natural remedies for diabetes.

Keywords; -Annona squamosa, Alpha-amylase, Alpha-glucosidase, Phytochemicals, Diabetes, Anti-diabetic.

Received 15.06.2024 Revised 21.07.2024 Accepted 26.09.2024

How to cite this article:
Mahima, Trapti G , Geeta P. Phytochemical Profiling and Antidiabetic Potential of *Annona squamosa*: Comparative Study of Extracts from Leaf, Stem, and Fruit. . Adv. Biores. Vol 15 [5] September 2024: 264-271.

INTRODUCTION

Diabetes mellitus is a long-lasting metabolic disorder characterized by hyperglycaemia, resultant from either insufficient insulin production or the body's reduced responsiveness to insulin, crucial for glucose absorption. Globally, the prevalence of diabetes stands at 9.3%, projected to rise to 10.2% by 2030 and 10.9% by 2045 [1]. There exist two primary types of diabetes: Type 1, an autoimmune condition wherein the body's immune system targets and destroys insulin-producing beta cells, and Type 2, more prevalent, associated with inadequate insulin production or reduced cellular sensitivity to insulin. Lifestyle factors such as poor diet, sedentary behavior, and obesity contribute significantly to Type 2 diabetes, which accounts for approximately 90% of diabetes cases and can lead to severe complications if untreated, including hyperlipidemia, oxidative damage, and protein glycation [1]. While Type 1 diabetes is typically managed through insulin replacement therapy, Type 2 diabetes treatments often involve oral hypoglycemic medications. Some examples of these medications include insulin-releasing drugs, biguanides, insulin sensitizers, incretin mimetics, amylin antagonists, and sodium-glucose co-transporter-2 (SGLT2) inhibitors. However, traditional drugs may pose adverse effects, prompting exploration into alternative remedies [2].Therefore, the investigation of medicinal plants as affordable and low-risk antidiabetic agents has gained traction, particularly in regions like India, where conventional treatments can be costly and have adverse effects. Herbal remedies are preferred for their documented efficacy, minimal side effects, and cost-effectiveness [3]. Among these plants, *Annona squamosa* (custard apple),

native to the West Indies but extensively cultivated in India for its nutritious fruit, has garnered attention [4]. Various parts of A. squamosa, including bark, roots, leaves, stems, fruits, peels, and seeds, contain phytochemicals traditionally used to treat ailments such as diarrhea, hemorrhage, nausea, and tumors [5] [6]. Consequently, the purpose of this research is to investigate the antidiabetic potential and phytochemical components of various Annona squamosa parts.The key objective of this study is to investigate phytochemical constituents along with antidiabetic potential of *Annona squamosa*, a renowned medicinal herb rooted in Ayurvedic principles.

MATERIAL AND METHODS

Plant Collection and Authentication

The leaves, fruit and stem of plant samples were collected in the month of September-October from the IIS University, Jaipur. Plant material was shade dried and grounded into fine powder. Leaves of *Annona squamosa* were authenticated by a professional taxonomist at the University of Rajasthan, and (RUBL no: RUBL21192) were submitted to the Herbarium Department of Botany.

Preparation of extract

Powders of different plant parts were homogenized in 95% ethanol/aqueous solution at a plant-to-ethanol/aqueous ratio of 1:10. The mixture was allowed to soak at 25°C for four days, with frequent stirring and mixing. Following that, the samples were filtered using a Whatman Filter paper no.1 and the filtrate was allowed to dry for a week before being utilized for phytochemical analysis and *in vitro* analysis.

Phytochemical Analysis of *Annona squamosa*

Qualitative phytochemical analysis

Photochemical analysis is crucial in determining a plant's antidiabetic activity by identifying bioactive compounds that affect glucose metabolism. It helps in isolation and purification of potential therapeutic compounds for the development of new antidiabetic drugs from natural sources. This approach holds promise for improving diabetes management and enhancing the quality of life for affected individuals.[7] Ethanolic/Aqueous extract were analysed for qualitative analysis of total phenols, tannins, flavonoids, saponins, glycosides, steroid, terpenoids and alkaloids. Phenols and tannins**-** 2 millilitres of a 2% $FeCl_3$ solution was combined with crude extract. When tannins and phenols were present, the colour became blue-green or even black. [8]

Flavonoids

Shinoda test- a small amount of magnesium ribbon pieces were combined with crude extract, then strong hydrochloric acid was applied dropwise. The presence of flavonoids was shown by the appearance of the pink scarlet color after a few minutes. [9]

Alkaline reagent test - 2 milliliters of a 2% NaOH solution were mixed with the crude extract. When a little amount of diluted acid was added, the formerly vibrant yellow hue became colorless, indicating the existence of flavonoids.

Saponins- The saponins were prepared by violently shaking 5 milliliters of distilled water into a test tube containing crude extract. We assumed that saponins were present because stable foam formed. [10]

Glycosides

Liebermann's test- Two milliliters of chloroform and two milliliters of acetic acid were combined to conduct Liebermann's crude extract test. The concoction was chilled by adding ice. The addition of H_2SO_4 was done with great care. The existence of a steroidal nucleus, or the glycoside's glycone component, was indicated by a shift in color from violet to blue to green.

Salkowski's test- 2 milliliters of chloroform was combined with crude extract. Then, 2 milliliters of concentrated H_2SO_4 were added with caution and mixed slowly. When the glycoside's glycone component a steroidal rings present, the substance takes on a reddish brown colour.

Keller-kilani test- mixture of crude extract and glacial acetic acid (2 ml) that contains 1-2 drops of a 2% solution of ferric chloride. A second test tube holding 2 milliliters of concentrated H_2SO_4 was subsequently filled with the combination. Interphase browning was a telltale sign of cardiac glycosides.

Steroid- After combining 2 milliliters of chloroform with steroid crude extract, concentrated H_2SO_4 was added in a side-by-side fashion. The presence of steroids was indicated by a reddish color in the bottom chloroform layer. To conduct an additional experiment, 2 ml of chloroform was mixed with the crude extract. Two milliliters of concentrated H_2SO_4 and acetic acid were then added to the concoction. The presence of steroids was confirmed by the development of a greenish tint. [11]

Terpenoids- The terpenoids were isolated by dissolving the crude extract in 2 milliliters of chloroform and then drying the mixture. Two milliliters of concentrated H_2SO_4 were added to this and left to heat for about two minutes. When terpenoids were present, the color became grayish.

Alkaloids**-** 2 milliliters of 1% hydrochloric acid were gently heated with crude extract. The combination was then supplemented with Mayer's and Wagner's reagents. Alkaloids were presumed to be present based on the turbidity of the precipitate that resulted.

Quantitative phytochemical analysis

Total phenolic content - The Folin-Ciocalteu reagent technique, with a few adjustments, was used to quantify the quantity of phenol in the water-based extract. 2.5 milliliters of Folin-Ciocalteu reagent (10%) and 2 milliliters of a Na_2CO_3 solution (2%) were added to 1 milliliter of plant extract. After that, the mixture was let to sit at room temperature for fifteen minutes. At 765 nm, the sample's absorbance was recorded. As a reference, gallic acid (1 mg/ml) was used. Triplicates of each test were run. The data were represented as gallic acid equivalent and were obtained using the standard curve. [11]

Total flavonoid content - The flavonoid content was determined using a modified version of the aluminum chloride colorimetric technique. Remaining at room temperature for half an hour, a mixture of 1 milliliter of plant extract sample, a mixture of 5.6 mL of distilled water, 0.2 mL of 10% aluminum chloride, and 3 mL of methanol was made. We tested the absorbance at 420 nm. An internal standard of 1 mg/ml quercetin was used. Triplicates of each test were run. The amount of flavonoids in the isolated product was measured using the standard curve and was represented as mg/g of quercetin equivalent. [11]

Determination of α-Amylase Inhibitory Activity

The chromogenic DNSA technique was used to perform the inhibition experiment. For 10 minutes at 37°C, the whole test combination was mixed with 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 1 ml of salivary alpha-amylase enzyme, and extracts at concentrations ranging from 0.5 to 1.5 mg/ml. Each tube was incubated at 37°C for 15 minutes after receiving 580µl of a 1% (v/v) starch solution in the same buffer, after pre-incubation. The addition of 1.0 ml DNSA reagent halted the reaction, which was followed by 5 minutes on a boiling water bath, cooling to room temperature, diluting, and measuring absorbance at 540 nm. The control method, which indicated full enzyme activity, did not include any plant extract. To eliminate the absorbance produced by plant extract, appropriate extract controls with the extract in the reaction mixture except for the enzyme were also included. [12]

***In Vitro* α-Glucosidase Inhibition Assay**

To test the inhibitory action, 1 ml of a starch solution containing 2% w/v maltose was incubated with 0.2 M tris buffer (pH 8), different concentrations of the sample (ranging from 100 to 500 mg/ml), and the results were recorded. For 10 minutes, the reaction mixture was kept at 37°C. One milliliter of the α-glucosidase enzyme (1 U/ml) was added to start the reaction, followed by incubation at 35°C for 40 minutes. After that, 2 cc of 6N HCl was added to stop the reaction. Spectrophotometer readings of the color's intensity were taken at 540 nm. [13]

Statistical Analysis-

Each study was carried out in triplicate in one of three sets. The information is described as mean ± standard error of the mean (SEM). The analytic program Graph Pad Prism 5 was used to conduct the difference and linear regression analyses. An analysis of variance was conducted using SPSS for statistical analysis.

RESULT

Qualitative Assessment of Bioactive Metabolites- Annona squamosa leaf extracts in both water and ethanol include tannins, phenols, alkaloids, and glycosides; however, the ethanolic leaf extract also contained terpenoids and saponins. Aqueous extract of *Annona squamosa* stem contains saponins, alkaloids and glycosides while ethanolic extract showed presence of flavonoids, alkaloids, terpenoids and glycosides. Ethanolic extract of *Annona squamosa* fruit contains only terpenoids and alkaloids, while aqueous fruit extract indicated presence of flavonoids, steroids, alkaloids and glycosides (Table 1). Results indicated that both extracts of leaves showed presence of maximum amount of phytoconstituents.

Quantitative Assessment of Bioactive Metabolites- Quantitative analysis of phytochemicals indicated the presence of total phenolic and flavonoid content in different parts of the plant, specifically the leaf, stem, and fruit. Total phenolic content of the leaf extract exhibited a range of 1.150 to 2.182, stem showing values of 1.219 and 1.132, and the fruit ranging from 1.071 to 1.150 (Figure 1). Interestingly, the leaf had the highest phenolic content among all plant parts with a peak value of 2.182. In terms of total flavonoid content, the leaf ranged from 0.064 to 0.630, while the stem showed values of 0.843 and 0.242, and the fruit exhibited values between 0.437 and 0.525. The stem displayed the highest flavonoid content, reaching 0.843 (Figure 1) (Table 2).

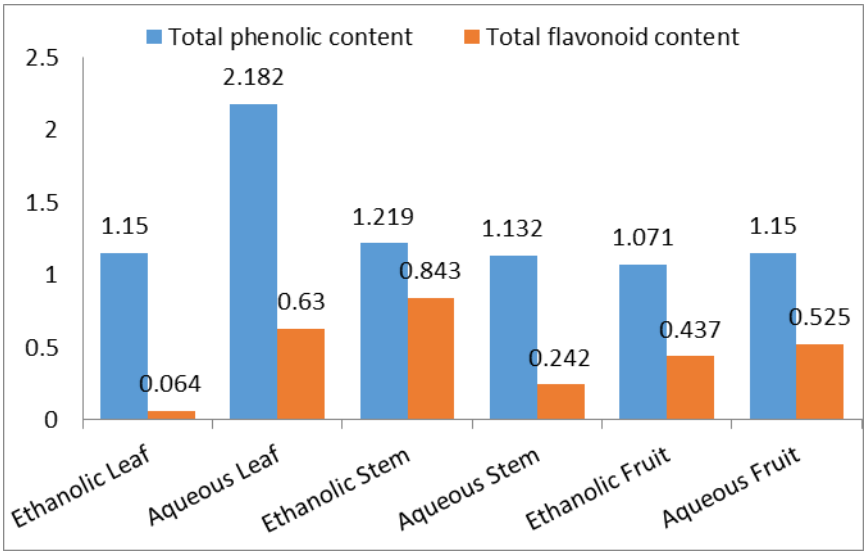


Figure 1 -Comparison of total phenolic content and total flavanoid content in leaf, stem and fruit of *Annona squamosa*

TABLE 1- Shows the qualitative phytochemical analysis of leaf, stem and fruit.

	Leaf		**Stem**		**Fruit**	
	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic
Test for Phenol and tannins-	+ve	+ve	-ve	-ve	-ve	-ve
Test for flavonoids-						
Shinoda test	-ve	-ve	-ve	-ve	+ve	-ve
Alkaline reagent	-ve	-ve	-ve	+ve	+ve	-ve
Test for Saponins-	-ve	+ve	+ve	-ve	-ve	-ve
Test for Steroids-	-ve	-ve	-ve	-ve	+ve	-ve
Test for terpenoids-	-ve	+ve	-ve	+ve	-ve	+ve
Test for alkaloids-	+ve	+ve	+ve	+ve	+ve	+ve
Test for glycosides						
Liebermann test	+ve	-ve	+ve	-ve	-ve	-ve
Salkowski's test	+ve	+ve	+ve	+ve	+ve	-ve
Keller-Kilani test	+ve	+ve	+ve	+ve	+ve	-ve

TABLE 2- shows the quantitative phytochemical analysis of leaf, stem and fruit.

	Leaf		**Stem**		**Fruit**	
	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous
Total phenolic content	1.150	2.182	1.219	1.132	1.071	1.150
Total flavonoid content	0.064	0.630	0.843	0.242	0.437	0.525

***In Vitro* α-Amylase Inhibition Activity -**In the α-amylase inhibition assay, the Ethanolic plant leaf extract exhibited a percentage inhibition of 71.8%, 73.71% and 76% at concentrations of 500,1000 and 1500µg/ml, respectively (Table 3), attaining IC50 value of 0.026 mg/ml (Table 3). The water fraction displayed 71.29%, 74.23%, and 77.96% inhibitions at the same tested concentrations (Table 3) with an IC50 value of .0052 mg/ml, respectively (Figure 2). The Ethanolic plant stem exhibited 86.05%, 89.15% and 90.18% inhibition of alpha amylase at concentrations of 500,1000 and 1500µg/ml, respectively (Table 3), attaining IC50 value of 0.129 mg/ml (Figure 2). The aqueous extract of stem displayed 79.2%, 80.1%, and 83.05% inhibitions at the same tested concentrations (Table 3) with an IC50 value of 0.082 mg/ml, respectively (Figure 2). The α-amylase inhibition assay showed 80.56%, 81.4% and 86.05%

inhibition respectively at 500,1000 and 1500µg/ml, concentration of ethanolic fruit extract (Table 3), attaining IC50 value of 0.055 mg/ml and 83.59%, 90.08%, and 92.45% inhibitions of alpha amylase was observed at the same tested concentrations with an IC50 value of 0.055mg/ml, for aqueous extract of fruit (Figure 2).

***In Vitro* α-Glucosidase Inhibition Activity** -In the α-glucosidase inhibition assay, the Ethanolic plant leaf extract exhibited a percentage inhibition of 82.06%, 86.56% and 89% respectively at concentrations of 100,200 and 300µg/ml, respectively (Table4), attaining IC50 value of 0.041 mg/ml (Figure3). The water fraction displayed 48.94%, 61.24%, and 63.84% inhibitions at the same tested concentrations (Table4) with an IC50 value of 0.109 mg/ml, respectively (Figure3). The Ethanolic plant stem extract exhibited a percentage inhibition of 82.05%, 86.42% and 88.15% inhibition of alpha-glucosidase of *Annona squamosa* respectively at 100,200 and 300µg/ml (Table4), attaining IC50 value of 1.991 mg/ml (Figure3). The water fraction of displayed 43.94%, 47.51%, and 62.18% inhibitions at the same tested concentrations (Table 4) with an IC50 value of 2.21 mg/ml, respectively (Figure3). In the α-glucosidase inhibition assay, the Ethanolic plant fruit extract exhibited a percentage inhibition of 70.72%, 72.14% and 77.8% respectively at concentrations of 100,200 and 300µg/ml, respectively (Table4), attaining IC50 value of 0.151 mg/ml (Figure3). The water fraction displayed 78.24%, 82.05%, and 84.74% inhibitions at the same tested concentrations (Table 4) with an IC50 value of 0.832mg/ml, respectively (Figure3).

Figure2: Percentage inhibitory effects on alpha amylase activity of aqueous and ethanolic extracts of plant leaf (A, A1). stem (B, B1), and fruit (C and C1).

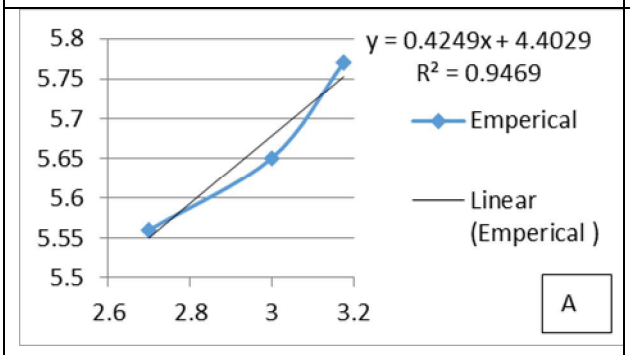
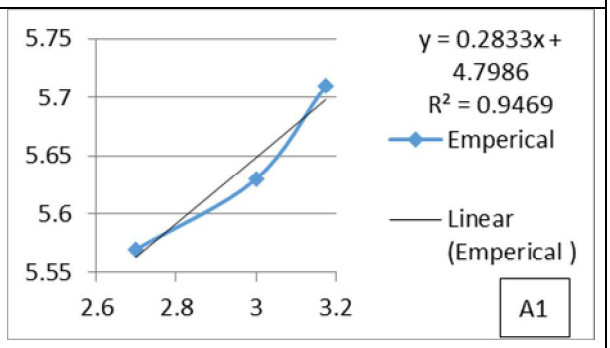
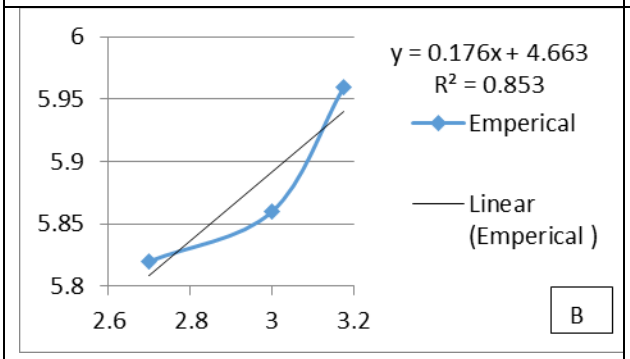
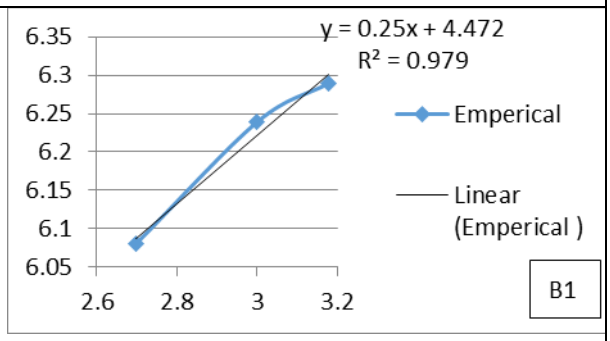
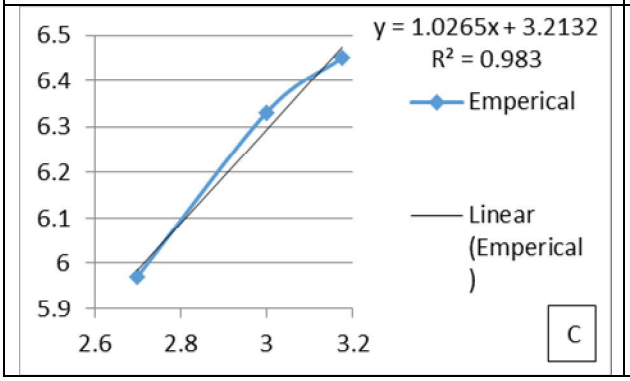
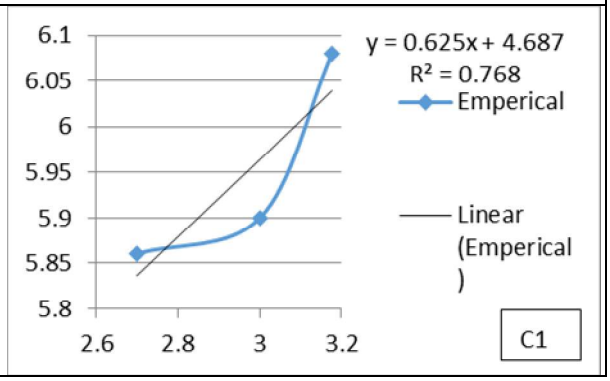
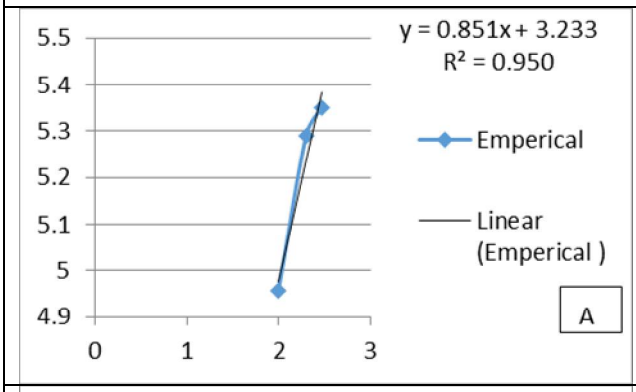
Aqueous Extract	**Ethanolic Extract**
	
	
	

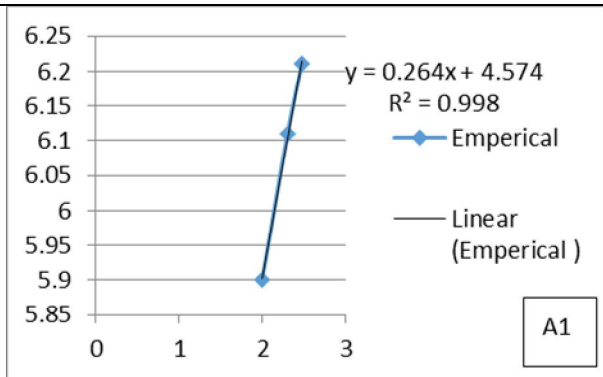
TABLE 3- Percentage inhibitory effects on alpha amylase activity of aqueous and ethanolic extracts of different parts of *Annona squamosa*

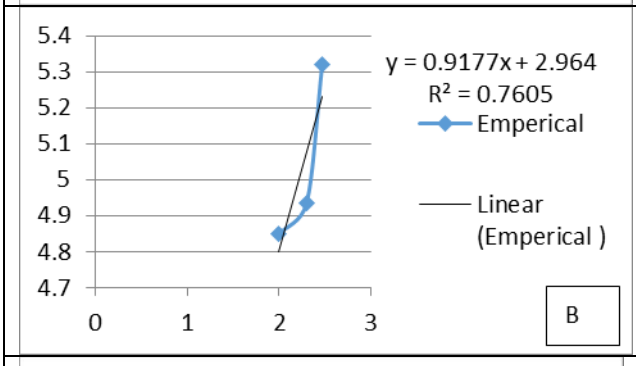
Annona Squamosa	Plant part	Conc. In µg/ml	% inhibition		Log dose		Empirical probit		Regression equation		IC50 in mg /ml	
			A	E	A	E	A	E	A	E	A	E
	LEAF	500	71.29	71.8	2.6989	2.6989	5.56	5.57	y= 0.424x + 4.402	y= 0.283x + 4.798	0.0052	0.026
		1000	74.23	73.71	3	3	5.65	5.63				
		1500	77.96	76	3.176	3.176	5.77	5.71				
	STEM	500	79.2	86.05	2.6989	2.6989	5.82	6.08	y=0.176x + 4.663	y=0.25x + 4.472	0.082	0.129
		1000	80.1	89.15	3	3	5.86	6.24				
		1500	83.05	90.18	3.176	3.176	5.96	6.29				
	FRUIT	500	83.59	80.56	2.6989	2.6989	5.97	5.86	y=1.026x + 3.213	y=0.625x + 4. 687	0.055	0.055
		1000	90.08	81.4	3	3	6.33	5.9				
		1500	92.45	86.05	3.176	3.176	6.45	6.08				

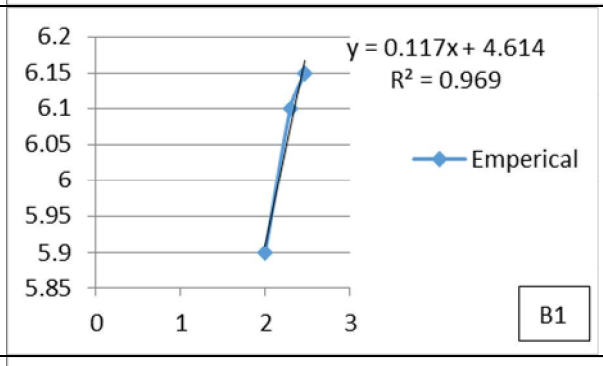
Figure3: Percentage inhibitory effects on alpha glucosidase activity of aqueous and ethanolic extracts of plant leaf (A, A1). stem (B, B1), and fruit (C and C1).

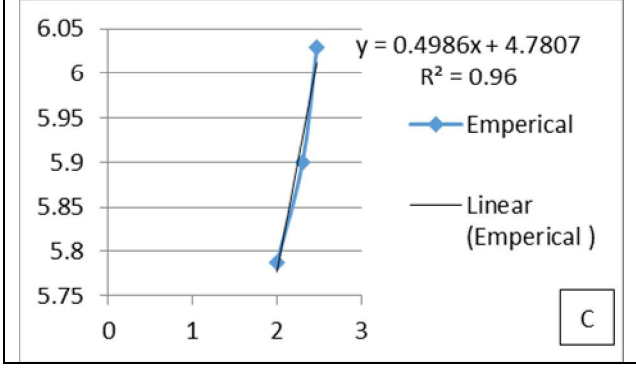
Aqueous Extract | **Ethanolic Extract**











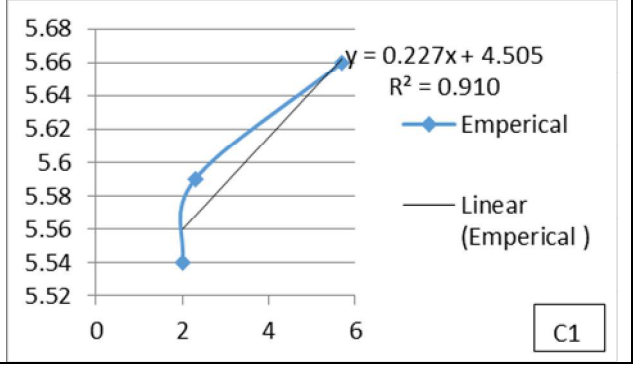


TABLE 4- Percentage inhibitory effects on alpha glucosidase activity of aqueous and ethanolic extracts of different parts of *Annona squamosa*

Annona squamosa	Plant part	Conc. in µg/ml	% inhibition		Log dose		Empirical probit		Regression equation		IC50 in mg /ml	
			A	E	A	E	A	E	A	E	A	E
	LEAF	100	48.1	82.06	2	2	4.95555	.5	y = 0.870x + 3.233	y = 0.264x + 4.574	0.109	0.041
		200	61.24	86.56	2.3	2.3	5.29	.6				
		300	63.84	89	2.47	2.47	5.35	6.21				
	STEM	100	43.94	82.05	2	2	4.85	5.9	y=1.190x+ 2.369	y=0.117x+ 4.614	2.21	1.991
		200	47.51	86.42	2.3	2.3	4.935	6.1				
		300	62.18	88.15	2.47	2.47	5.32	6.15				
	FRUIT	100	78.24	70.72	2	2	5.788	5.54	y=0.648x+ 4.461	y= 0.227x + 4.505	0.832	0.151
		200	82.05	72.14	2.3	2.3	5.9	5.59				
		300	84.74	77.8	2.47	2.47	6.03	5.66				

DISCUSSION

The increasing prevalence of diabetes is closely associated with technological advancements and lifestyle changes. It is characterized by abnormal glucose tolerance and disruptions in metabolic processes. It poses a significant global health challenge, particularly in developing countries like India. The escalating numbers of diabetes cases worldwide, particularly in countries such as China and India, underscore the urgent need for effective and accessible treatments. [14] Plant phytochemicals have physiological effects on a wide range of health conditions through diverse biological pathways. In different plant parts, the presence of phenols, tannins, saponins, steroids, and glycosides indicate a highly medicinal value to the plant. Phytochemical analysis of *Annona squamosa* extracts revealed varying levels of phenolic and flavonoid content in different plant parts. Notably, the stem exhibited the highest phenolic content, while the leaf contained the highest flavonoid content. These bioactive compounds have been implicated in various health benefits, including antidiabetic effects. [15] It is well known that flavonoids scavenge most of the free radicals that cause metabolic and degenerative diseases. In addition, they trap and scavenge reactive species and protect antioxidant defense by upregulating processes. Our results are in corroboration with. [16,17]. α-amylase and α-glucosidase enzymes are crucial for glucose metabolism. The study proved that Annona squamosa leaves may have antidiabetic properties, as both the ethanolic and water-based leaf extracts significantly inhibited the activities of α-amylase and α-glucosidase, as shown by the significant IC50 values. Annona squamosa may have antidiabetic properties due to the presence of flavonoids. [18] has reported that dietary flavonoids showed a positive role in diabetes through regulation of glucose metabolism hepatic enzyme activities and lipid profile. A number of studies have shown that inhibition of α-amylase and α-glucosidase activity of various plant extracts significantly decrease the postprandial increment in blood glucose and serve as a promising candidate as antidiabetic agent with increased potency and minor undesirable effects than existing synthetic drugs. [19]

CONCLUSION

In conclusion, this study provides compelling evidence of the potential α-amylase and α-glucosidase inhibitory activities of *Annona squamosa* extracts, particularly from the leaves and stems. These findings offer a foundation for further investigations into the molecular mechanisms underlying these inhibitory effects and the isolation of specific bioactive compounds. The potential application of *Annona squamosa* as a natural antidiabetic remedy warrants further exploration, potentially leading to the development of novel therapeutic interventions or functional foods. Additionally, exploring the molecular mechanisms underlying these inhibitory activities may provide valuable insights into the potential therapeutic applications of *Annona squamosa* in diabetes management. This study gives to the rising body of knowledge on the health benefits of *Annona squamosa* and encourages continued research in this field.

ACKNOWLEDGMENTS

Authors would like to thank IIS deemed to be University, Jaipur for providing all the necessary facilities. Authors are also acknowledged to WISE-KIRAN division of Department of Science and Technology, New

Delhi, India for providing infrastructural financial support to IIS (deemed to be University), Jaipur, India (File No. DST/CURIE-02/2023/IISU) under CURIE program.

REFERENCES

1. Anand, K., Tiloke, C., Naidoo, P., & Chuturgoon, A. A. (2017). Phytonanotherapy for management of diabetes using green synthesis nanoparticles. *Journal of Photochemistry and Photobiology B: Biology, 173*, 626-639.
2. Kooti, W., Farokhipour, M., Asadzadeh, Z., Ashtary-Larky, D., & Asadi-Samani, M. (2016). The role of medicinal plants in the treatment of diabetes: A systematic review. *Electronic Physician, 8*(1), 1832.
3. Amjad, S., Jafri, A., Sharma, A. K., & Serajuddin, M. (2019). A novel strategy of nanotized herbal drugs and their delivery in the treatment of diabetes: Present status and future prospects. *Journal of Herbal Medicine, 17,* 100279.
4. Rohilla, R., Garg, C., & Garg, M. (2017). In vitro hypoglycemic evaluation of fractions of hydroalcoholic extract of heartwood of *Tecomella undulata Linn*. *In Vitro, 10*(2).
5. Ogbole, O. O., Aliu, L. O., Abiodun, O. O., & Ajaiyeoba, E. O. (2016). Alpha-amylase inhibition and brine shrimp lethality activities of nine medicinal plant extracts from South-West Nigerian ethnomedicine. *Journal of Herbs, Spices & Medicinal Plants, 22*(4), 319-326.
6. Pandey, B. P., Pradhan, S. P., Adhikari, K., Joshi, P., & Malla, S. (2020). Extracts of leaves of six locally available plants from Bagmati Province of Nepal as potent inhibitors of alpha-amylase, lipase, tyrosinase, elastase, and cholinesterases. *Current Enzyme Inhibition, 16*(3), 214-223.
7. Parveen, M., & Some, S. (2021). Antimicrobial activity of medicinal plants: A weapon to combat multi-drug resistant pathogens. *International Journal of Plant and Environment, 7*(2), 133-141.
8. Suriyavathana, M., & Roopavathi, I. (2016). Phytochemical characterization of *Triticum aestivum* (wheat grass). *Journal of Pharmacognosy and Phytochemistry, 5*(1), 283-286.
9. Agidew, M. G. (2022). Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. *Bulletin of the National Research Centre, 46*(1), 1-22.
10. Poovitha, S., & Parani, M. (2016). *In vitro* and *In vivo* α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia L.*). *BMC Complementary and Alternative Medicine, 16*(1), 1-8.
11. Sekhon-Loodu, S., & Rupasinghe, H. V. (2019). Evaluation of antioxidant, antidiabetic and antiobesity potential of selected traditional medicinal plants. *Frontiers in Nutrition, 6*, 53.
12. Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*(3), 426-428.
13. Bhatia, A., Singh, B., Arora, R., & Arora, S. (2019). In vitro evaluation of the α-glucosidase inhibitory potential of methanolic extracts of traditionally used antidiabetic plants. *BMC Complementary and Alternative Medicine, 19*, 1-9.
14. Rayar, A., & Manivannan, R. (2016). In-vitro alpha-amylase and alpha-glucosidase inhibition activity of umbelliferone and beta-ionone isolated from *Coriandrum sativum Linn*. *World Journal of Pharmacy and Pharmaceutical Sciences, 5*(1), 1280-1289.
15. Nair, S. S., Kavrekar, V., & Mishra, A. (2013). In vitro studies on alpha-amylase and alpha-glucosidase inhibitory activities of selected plant extracts. *European Journal of Experimental Biology, 3*(1), 128-132.
16. Yadav, R. N., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology, 3*(12).
17. Agu, K. C., Eluehike, N., Ofeimun, R. O., Abile, D., Ideho, G., Ogedengbe, M. O., Onose, P. O., & Elekofehinti, O. O. (2019). Possible anti-diabetic potentials of *Annona muricata* (soursop): Inhibition of α-amylase and α-glucosidase activities. *Clinical Phytoscience, 5*, 1-3.
18. Al-Ishaq, R. K., Abotaleb, M., Kubatka, P., Kajo, K., & Büsselberg, D. (2019). Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. *Biomolecules, 9*(9), 430.
19. Kumar, M., Changan, S., Tomar, M., Prajapati, U., Saurabh, V., Hasan, M., Sasi, M., Maheshwari, C., Singh, S., Dhumal, S., & Radha. (2021). Custard apple (*Annona squamosa L.*) leaves: Nutritional composition, phytochemical profile, and health-promoting biological activities. *Biomolecules, 11*(5), 614.