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Computational and *Invitro* Evaluation of Aerial Part Extracts of *Barringtonia acutangula* in Preventing Postprandial Hyperglycemia

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Abstract

Polyphenols have the potential for therapeutic properties that may alleviate diabetes and exhibit inhibitory effects on certain pancreatic enzymes. The study aimed to screen the hypoglycemic effects of *Barringtonia acutangula* aerial part extracts. Cold maceration was used to extract coarsely powdered aerial parts with seven different solvents, which were studied. Quantitative estimation of aerial part extracts was done to determine the total flavonoid, phenolic, and alkaloid content. Further *in silico* and *in vitro* studies were carried out to assess the inhibitory action of *B.acutangula* on alpha-amylase and alpha-glucosidase pancreatic enzymes. The hexahydroxy flavone Gossypetin showed a good docking score. The highest total flavonoid (31.543±0.175mg Quercetin/gm extract) and phenolic content (19.127±0.311mg gallic acid/gm extract) were observed in the methanolic extract, and the chloroform extract showed the highest alkaloid content (23.570±0.090) when compared with a standard solution of Atropine. The phytochemical constituents satisfied drug-likeness properties, and the compound gossypetin showed the highest binding affinity of -7.197 and -7.420 kcal/mol with the active site of target proteins alpha-amylase (2QV4) and alpha-glucosidase (5NN8). The ethanolic extract showed a significant % inhibitory response of alpha-amylase and alpha-glucosidase of 80.955±0.023 and 73.250±0.033, respectively**.** The results indicate that polyphenols in the aerial extracts of *B. acutangula* play a significant role, and gossypetin can be a potential candidate in preventing postprandial hyperglycemia in diabetes patients.

Keywords: phytoconstituents, total content, docking studies, invitro hypoglycaemic estimation

Introduction

The etiology of several diseases is attributed to hyperglycemia, a disturbance to carbohydrate, fat, and protein metabolism resulting from defects in insulin biosynthesis, its action, or both, leading to changes in small and large blood vessels. Diabetes is one of the most common and growing health problems worldwide. According to a WHO report, about 537 million people suffer from diabetes mellitus, and this will reach 643 million by 2030 (1). India is the second most affected country, after China in the world. More than 7.2 % of the population of India lives with diabetes. Pancreatic abnormalities like pancreatectomy, pancreatitis, cystic fibrosis, and neoplasia also lead to DM. There are three main types of diabetes based on their causes: type 1, type 2, and type 3. Type 1 is known for insulin-dependent DM (inability to produce insulin) and juvenile-onset (it begins in childhood). Type 2 is called non-insulin-dependent or adult-onset DM; insulin produced by the

body is insufficient to meet needs. Type 3 diabetes occurs when there is insulin resistance in the brain (2,3).

The two major digestive enzymes metabolizing complex carbohydrates into simple sugar are alpha-glucosidase and alpha-amylase. α- glucosidase is an enzyme present on the brush border of the small intestine. It is necessary to break down oligosaccharides into simple sugars (before carbohydrates can be absorbed) in the intestinal lumen. Whereas α- amylase is present in pancreatic juice and saliva and helps break down large insoluble starch. Hyperglycemia can be managed by inhibiting these digestive enzymes, resulting in poor carbohydrate absorption (4-7).

Barringtonia acutangula is commonly known as a freshwater mangrove, Indian Oak, Itchy Tree, and Mango-Pine, and belongs to the family Lecythidaceae. The leaves and long hanging inflorescence make the plant *Barringtonia acutangula* a decorative shade tree or windbreak in gardens. It is an evergreen tree of height 9-12 m, commonly seen in the sub-Himalayan region (8).

The leaves, fruits and roots of *Barringtonia acutangula* are used in traditional medicine. It is widely used in folklore for conditions associated with kapha and pitta, including leprosy, arthralgia, dysmenorrhea, plumbago, skin diseases, diarrhoea, inflammation, flatulence, and haemorrhoids. In Ayurveda, the roots, leaves, and fruits of *Barringtonia acutangula* are used to treat jaundice, liver and stomach disorders, leprosy, and splenic disorders. The leaf extract is traditionally used in diarrhea as a CNS depressant, antidiabetic, antioxidant, and hepatoprotective (9,10). The leaf and fruit extract of *Barringtonia acutangula* shows significant reduction in glucose levels (11) and anti-diabetic properties have been explored in the roots of *Barringtonia acutangula* with effective blood glucose lowering capacity (12). Nowadays, several therapeutic agents are available to treat hyperglycemia. However, due to their side effect and high cost, it is difficult for the common man to receive the medication. Hence, there is a need to develop plant-based antidiabetic agents.

So, the present study was designed to explore the inhibitory potential of aerial part extracts of *Barringtonia acutangula* against alpha-glucosidase and alpha-amylase by *in silico* and *in vitro* studies. The quantitative estimation of total flavonoid, phenolic, and alkaloid content in the aerial parts has also been carried out.

Materials and Methods

Materials

Alpha amylase& alpha-glucosidase kit and acarbose were purchased from Sigma Aldrich Co. India. All other chemicals, aluminium chloride, Quercetin, Folin-ciocalteu reagent, gallic acid, Bromocresol green, and atropine, used were of pharmaceutical grade.

Methodology

Preparation of plant extracts

The aerial parts of *Barringtonia acutangula* were collected from Pilikula Botanical Garden, Mangalore, India. The Pilikula Nisargadhama, located in Vamanjoor, Mangalore city, Karnataka, is known for its arboretum, which features 6 acres of medicinal and therapeutic plants, including more than 460 varieties. The *Barringtonia acutangula* is part of this arboretum. The aerial parts were collected between July and October and authenticated by a taxonomist. The plant material was washed thoroughly to remove adhering debris and dirt. Then, it was shade-dried and ground into coarse powder. The extraction was done using the cold maceration procedure. Based on polarity, the solvents chosen for extraction are n-hexane, petroleum ether, ethyl acetate, chloroform, ethanol, methanol, and aqueous. 50g of powdered aerial extract was used for extraction with each solvent, and after seven days, the extracts were filtered using a muslin cloth, only the aqueous extract maceration was done for 24 hours, and the filtrate was collected and allowed to dry at room temperature. The residue was collected,

concentrated, and stored in desiccators for further use.

Quantitative analysis
Total flavonoid content

The Chang et.al aluminum chloride colorimetric method with slight modification (13) was used to determine the total flavonoid content of each extract. The calibration curve was plotted using quercetin and expressed as mg of quercetin equivalent per gram of dry weight of extract (mg QE/g DW). Quercetin was prepared in ethanol (concentration 2mg/ml) and diluted to get a working concentration of 20-100 µg/ml. One ml of each standard solution was mixed with 0.3ml of 5% sodium nitrite. After 5 minutes, 0.3ml of 10% aluminum chloride and 2ml of 1M NaOH were added and made up to volume in a 10 ml volumetric flask, incubated at room temperature for 30 minutes, and absorbance was measured at 415 nm against a blank (14,15). The same procedure is followed for standard quercetin. All samples were performed in triplicate.

Total phenolic content

The total phenolic content in the aerial part extract of *Barringtonia acutangula* was determined using Folin-ciocalteu reagent (FC) (16); Gallic acid was the standard(1mg/ml). The standard curve of gallic acid was prepared (20-100 µg/ml) To each ml of the standard solution, 2ml of 7% sodium carbonate and 200µl of FC reagent were added, shaken well, and incubated in the dark at room temperature for 30 min and the absorbance of the blue colored solution was read at 760nm against a blank solution (17). The procedure was followed with 1 ml extract in different solvents. The procedure was done in triplicates, and the phenolic content was calculated as gallic acid equivalents (GAE)/gm of the dry sample.

Total alkaloid content

Total alkaloid content was determined using the Fazel et al. method with slight modification (18). The plant extract (1 mg/mL) was dissolved in DMSO and filtered. To each ml of this solution in a separating funnel, 5 ml each of bromocresol green (BCG) solution and phosphate buffer were added to maintain the pH 4.7, mixed thoroughly. Further extraction of the complex was carried out using chloroform by vigorous shaking. The chloroform layer was collected in a 10 ml volumetric flask and diluted to volume with chloroform, and absorbance was measured at 470 nm. A standard solution of Atropine (1mg/10 ml) was prepared in distilled water. Aliquots of atropine standard solution (0.4,0.6,0.8,1.0 and 1.2 ml), transferred to different separating funnels, to these solutions 5 ml each of BCG and phosphate buffer (pH 4.7) and thoroughly shaken with 1,2,3, and 4 ml of chloroform and the absorbance of the mixture was measured at 470 nm (19). The total alkaloid content was expressed as mg of AE/g of extract.

In silico studies

Schrödinger (2020-4, LLC, New York) was used for computational analysis. Maestro 12.3 version (LigPrep, Glide XP docking, binding free energy calculations, ADMET)

Ligand and protein preparation

The compounds selected were based on reported pharmacognostic literature (20), and the structures of ten selected phytoconstituents were drawn using ChemDraw(21-23). The ionization states were at pH 7.0, and LigPrep generated the low energy conformations. The 3D crystal structures of alpha amylase (2QV4) and alpha glucosidase (5NN8) were downloaded from the protein data bank (24-27) and is depicted in figure 2. Active site water molecules (<3 hydrogen bonds) were removed, and hydrogen bonds at pH seven were incorporated. The protein preparation wizard processed and prepared the proteins following the energy minimization OPLS force field (28).

Receptor ligand docking

The receptor-ligand docking study helps find the ligand's best binding modes with

the target protein. Protein-Ligand docking was done using the Glide program. Glidescore SP and XP were used to rank the compounds in order. All ten phytocompounds were docked into the pocket of the target protein using the standard precision algorithm (SP) followed by the extra precision (XP) algorithm. Low energy conformations of all phytocompounds were docked into the catalytic pocket of the protein by flexible docking using Glide SP and Glide XP program (29,30).

The Prime module of Schrodinger evaluated the binding energy of the receptor-ligand complex. It calculates the total free energy in dGbind (kcal/mol) by considering the molecular mechanics energies and polar and non-polar solvation (31).

ADME and Physicochemical Properties

QikProp of Schrodinger software helps predict ligands' pharmacokinetic and physicochemical properties. The ADME features and pharmaceutically relevant properties of all the 10 ligands are predicted (32).

Invitro anti-diabetic activity
Alpha-amylase inhibition Assay

The α-amylase solution (20µL) was added to 200µL sodium phosphate buffer (0.02M). 200µL aerial part extracts (20,40,60,80,100µg/ml) were added to the above reaction mixture and incubated at 25°C for about 10 minutes. After the incubation, 200µl of 1% starch solution was added and again incubated at 25°C for about 10 minutes. The reaction was stopped by adding 400µl of di-nitro salicylic acid color reagent. Further incubated in boiling water bath at 70°C for 5 min. Similar procedure followed for standard, Acarbose (20- 100µg/ml). The absorbance of the mixture was recorded at 540nm. Without a test, the substance is set up in parallel as a control, and each experiment is performed thrice (33,34).

Alpha-glucosidase inhibition assay

To the 10µl of the enzyme, α-glucosidase, 50µL of phosphate buffer (100mM, pH-6.8), and 20µl of extracts of concentration 20-100µg/ml were poured in a 96-well plate and incubated for about 15 min at 37°C.To the above mixture, 20µl p-nitro phenyl-α-D-glucopyranoside solution (5Mm) was added as a substrate and incubated again for 15 min at 37°C. Further, 50µl sodium carbonate (0.1M) was added to cease the reaction. The absorbance was measured at 405 nm. Acarbose was the standard, with a 20 -100µg/ml concentration. Solution without test substance was taken as control, and each experiment must be repeated thrice (35,36).

Statistical analysis

All the analysis were done in triplicates. Average values were calculated and reported with ± Standard error of mean (SEM). Data were analyzed using GraphPad Prism 8.0.2 software. Statistical comparison was done by two-way analysis of variance, and p values < 0.05 were considered significant, and correlation coefficient (R^2) values were calculated.

Results and Discussion

Quantitative analysis of the various extracts shows secondary plant metabolites are known to produce synergistic action in treating different disease states; in the present study, the leaves and stem extract of *Barringtonia acutangula* are analyzed together. The effect of different solvents on phytoconstituents was observed by quantitative estimation of total flavonoid, phenols, and alkaloid content.

The aluminium chloride colorimetric assay method determined the total flavonoid content of various extracts, and data were measured at 415 nm. Values are expressed in quercetin equivalent mg/ml. The calibration curve of quercetin,gallic acid and Atropine are given in Figure 1. From the calibration curve, the total flavonoid content of the methanolic extract (31.543±0.175) was more than that of other extracts. The values decrease in following order methanol (31.543±0.175) > aqueous (27.593±0.330) > ethanol (23.110±0.24) > eth-

yl acetate (29.890±0.460) > petroleum ether (19.490±0.360)> chloroform (19.032±0.193) > hexane (11.480±0.173).

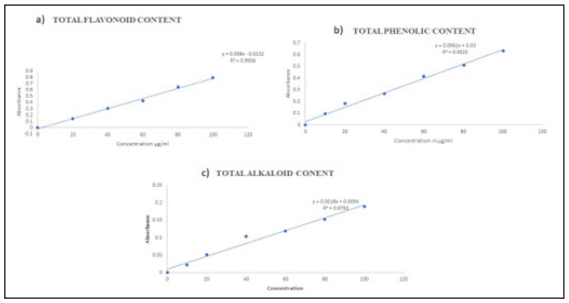

Fig. 1: Calibration curve of a) Quercetin, b) Gallic acid, c) Atropine sulphate

Table 1: Quantitative estimation for flavanoids, phenolic and alkaloidal content

Solvent system	Total flavonoid content mg of QE/gm*	Total phenolic content mg of GAE/gm*	Total alkaloid content mg of AE/gm*
Hexane	11.48±0.173	9.343±0.335	15.44±0.059
Pet. ether	19.49±0.360	8.950±0.160	12.02±0.250
Ethyl acetate	21.89±0.460	13.310±0.416	19.083±0.135
Chloroform	19.032±0.193	7.610±0.240	23.570±0.090
Ethanol	23.110±0.24	18.190±0.160	16.670±0.330
Methnol	31.543±0.175	19.127±0.311	14.960±0.190
Aqueous	27.593±0.330	16.180±0.210	19.943±0.385

**mean ± SEM (n=3)*

Total phenolic content was estimated by Folin ciocalteu method. Compared to other extracts, the total phenolic content of the methanolic extract was found to be higher and expressed as gallic acid equivalent mg/ml. The calibration curve of gallic acid is given in Figure 1, and the results are given in Table 1. Values were obtained from calibration curve y = 0.0061 x + 0.03 and R^2 =0.9925, Where x is absorbance and y concentration of a gallic acid solution (µg/mL). The highest phenolic content was obtained in methanolic extract (19.127±0.311), followed by ethanol (18.190±0.160), aqueous (16.180±0.210), ethyl acetate (13.310±0.416), hexane (9.343±0.335), petroleum ether (8.950±0.160), and chloroform (7.610±0.240).

Atropine was used as a standard for estimating the total alkaloid content. The calibration curve is given in Figure 1, and the results are in Table 1. The chloroform extract's alkaloid content was higher than other extracts. The highest alkaloid content was obtained in Chloroform (23.570±0.090) extract, followed by aqueous (19.943±0.385), ethyl acetate (19.083±0.135), ethanol (16.670±0.330), hexane (15.440±0.059), methanol (14.960±0.190), and petroleum ether (12.020±0.250).

The methanolic extract showed more flavanoid and and phenolic content whereas the chloroform extract showed highest alkaloid content than other solvent extracts.

Molecular docking studies were carried out to predict the antidiabetic activity of the phytoconstituent present in *Barringtonia acutangula*; results are depicted in Table 2 and 2D and 3D interaction of best-docked compound, gossypetin with targets alpha amylase (2QV4) and alpha-glucosidase (5NN8) depicted in Figure 3 and 4 respectively.

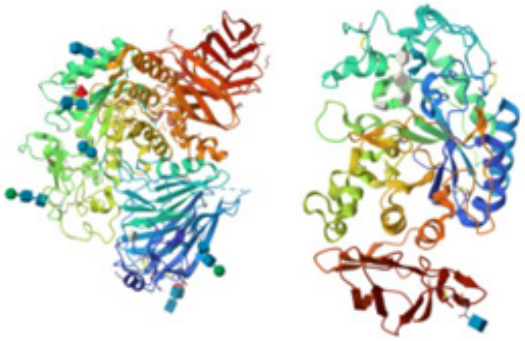

Fig 2: 3D structure of alpha amylase and alpha glucosidase

Table 2:Docking studies of the phytoconstituent with 2QV4 and 5NN8

Ligand	Protein	Docking Score	MMGBSA dG Bind	Hydrophobic interaction	Polar interaction with ligand	Hydrogen bonding	Pi-pi stacking
Acarbose	2QV4	-10.577	-62.53			*ALA 106 THR16 HIS 305 GLU 233 ASP 197 ARG 195 ASP 300*	
	5NN8	-9.919	-52.45	*TRP 618 ALA 284*		*TRP 618 ASP 616 ASP 518 ASP 282 ALA 284*	
Barringtogenic Acid	2QV4	-5.789	-10.76		*THR 163 HIE 299*	*ARG 195 HIE 299 THR 163*	
	5NN8	-3.005	-44.41	*TRP 481*		*ASP 404 ASP 518 TRP 481*	
Barringtogenol B	2QV4	-5.446	-56.67			*ASP 300 ASP 356*	
	5NN8	-3.445	-42.95	*ALA 284*		*ALA 284 ASP 282 ASP 616 ARG 600*	
Barringtogenol C	2QV4	-0.465	-51.81		*THR 163 HIS 305*	*ASP 300 THR 163 HIS 305*	
	5NN8	-4.292	-36.17			*ARG 600 ASP 616 ASP 282*	
Gallic acid	2QV4	-5.548	-16.35		*HIE 299*	*ASP 300 HIE 299 ARG 195*	
	5NN8	-5.912	-25.40			*ARG 600 ASP 616 ASP 404*	
Gossypetin	2QV4	-7.197	-37.25	*TYR 62*		*ASP 197 ASP 300 TYR 62*	*TYR 62*
	5NN8	-7.420	-39.08			*ASP 404 ASP 282*	

Melilotic Acid	2QV4	-3.648	-14.03		*HIE 299*	*ARG 195* *GLU 233* *HIE 299* *ASP 300*	
	5NN8	-4.982	-28.20			*ASP 616* *ARG 600* *ASP 282*	
P-Coumaric Acid	2QV4	-4.248	-23.20		*THR 163* *HIE 299*	*THR 163* *ARG 195* *HIE 299*	
	5NN8	-4.372	-26.83			*ASP 616*	
Syringic Acid	2QV4	-4.455	-30.89	*TRP 59*	*GLN 63*	*ASP 300* *TRP 59* *GLN 63*	
	5NN8	-4.543	-26.01	*TRP 481* *PHE 649*		*ASP 404* *ASP 616* *TRP 481* *PH-E 649* *ARG 600*	*TRP 481* *PHE 649*
Vanillic Acid	2QV4	-4.475	-20.55		*HIE 299*	*HIE 299* *ARG 195* *ASP 197*	
	5NN8	-4.783	-25.91			*ASP 616*	

The phytoconstituent gossypetin showed a docking score of -7.197 and -7.420 kcal/mol, comparable with the standard acarbose drug. The docking score of each phytoconsituent was compared with the standard acarbose (-10.577 kcal/mol) at the active site 2QV4. Gossypetin is the most active compound among the selected phytoconstituents, having a dock score of -7.197 kcal/mol. the binding mode shows that the hydroxyl group of gossypetin forms a hydrogen bond with ASP 197, ASP 300, and TYR 62. The phenolic ring of gossypetin forms the π–π stacking and hydrophobic interaction with TYR 62. 2D and 3D interactions of the compounds are given in Figure 3 to understand the interactions between ligands and the target protein.

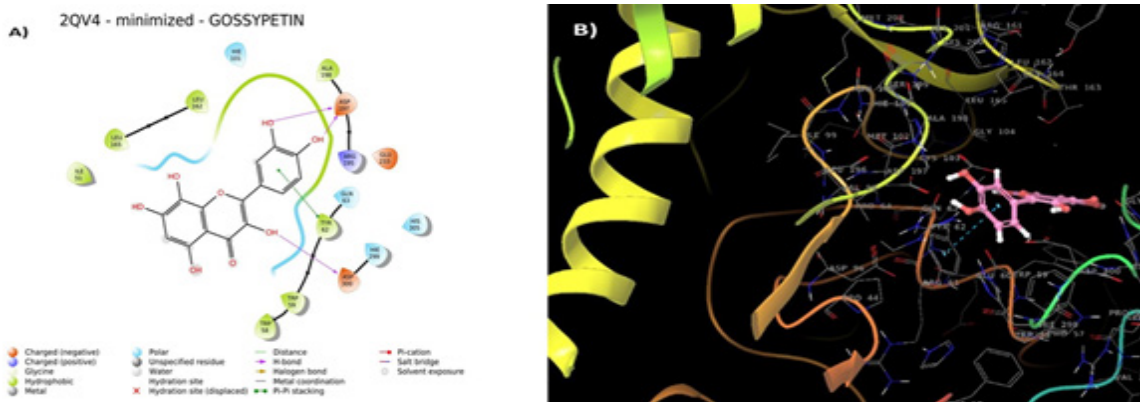

Fig. 3: a) 2D and b) 3D interaction of gossypetin with the active site of the target 2QV4

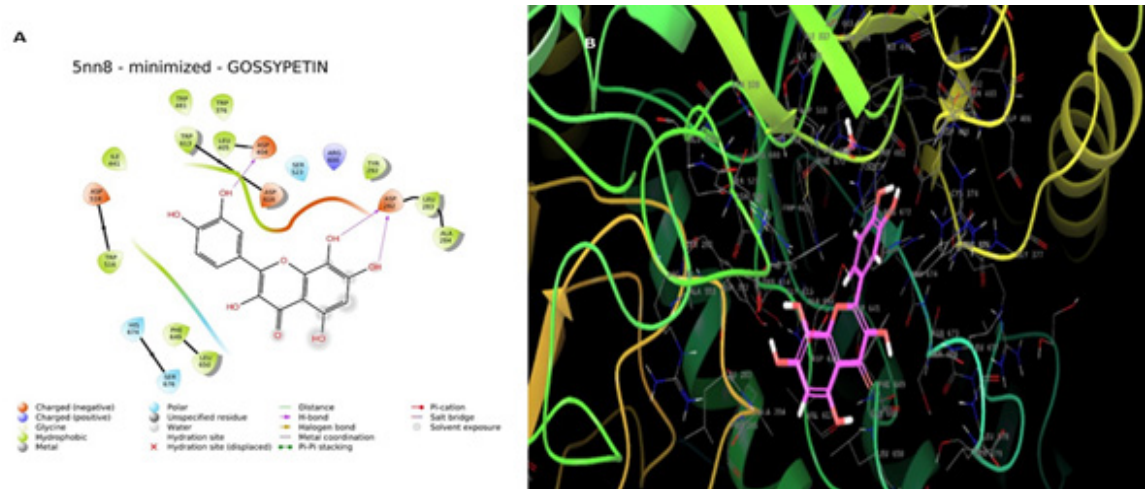

Fig. 4: a) 2D and b) 3D interaction of gossypetin with the active site of the target 5NN8

The compound gossypetin showed the highest dock score of -7.420 kcal/mol with the active site of target protein 5NN8. The standard reference acarbose showed a dock score of -9.919 kcal/mol. Gossypetin forms a hydrogen bond with ASP 404 and ASP 282. The compound with the highest docking score showed hydrogen bonding with the hydroxyl group. Hence, the presence of an electron donating group may be responsible for showing better inhibitory activity(37,38).

Table 3: Physiochemical and ADME properties of phytoconstituents present in *B. acutangula.*

Ligand	**Mol. weight**	**H-bond donor**	**H-bond acceptor**	**PSA***	**Human oral absorption**	**CNS**	**BBB**	**Qppcaco**
Acceptable range	≤500	≤5	≤10	7-200	1- low 2- medium 3- high	-2 - +2	-3 -+1.2	<25 >500
Acarbose	645.6	14	19	311.75	1	-2	-5.61	0.059
Barringtogenic Acid	502.7	4	6	121.27	2	-2	-1.50	7.338
Barringtogenol B	572.8	4	6	98.85	3	-1	-0.94	540.19
Barringtogenol C	490.7	5	5	118.47	1	-1	-0.93	826.07
Gallic acid	170.1	5	1	93.50	2	-2	-1.68	9.611
Gossypetin	318.2	6	8	115.81	2	-2	-2.73	8.774
Melilotic Acid	116.1	2	3	160.64	2	-1	-0.90	95.51
P-Coumaric Acid	164.1	2	3	72.92	3	-2	-1.08	62.03
Syringic Acid	198.1	2	5	74.19	3	-1	-0.97	79.67
Vanillic Acid	168.1	2	4	87.34	3	-1	-0.91	73.51

The physicochemical and ADME properties of the phytoconstituents are predicted using the Qikprop module of Schrodinger and given in Table 3. Physicochemical parameters help to predict the drug-likeness and pharmacokinetics of the compounds. Lipinski's rule of five predicts the compound's drug likeness properties, which specifies that the compound's Molecular weight should be ≤ 500 Dalton, which helps in easy transportation, absorption, and diffusion. The partition coefficient (Log P) value must be ≤5, the hydrogen bond acceptor should be ≤10, and the number of hydrogen bond donors should be ≤5. The compounds satisfying this rule are considered drug-like compounds and are orally active. Polar surface area (PSA) is the Van der Waals surface area of polar nitrogen and oxygen atoms (7 – 200 is the acceptable range), it is a descriptor used to correlate well with passive molecular transport through the membrane and, therefore, allow the prediction of absorption and transport properties of the drugs (37). All the reported phytoconstituents obey the rule of five with violations less than four. Thus, all the constituents are predicted as orally active and can act as a lead moiety. All the compounds showed molecular weight less than 500 Daltons, whereas barringtogenic acid and standard acarbose have molecular weight of 502.7 and 645.608 Dalton. The best-docked compound, gossypetin, has a hydrogen bond donor of more than 5, and standard acarbose showed a violation in several hydrogen bond donors and hydrogen bond acceptors. The PSA of all the compounds is within the recommended range (7 – 200 A˚).

The Caco-2 cell permeability has been commonly used to estimate the drug permeability in the human intestinal epithelium. It's an important parameter for oral drugs. The Caco-2 cell permeability of a compound can be expressed as log cm/s. A permeability of less than 25 means poor, and greater than 500 indicates good permeability. Barringtogenol C and Barringtogenol B show high permeability, whereas Melilotic acid, P-coumaric acid, Syringic acid, and Vanillic acid show intermediate permeability. The Caco-2 cell permeability of the highest docked compound, gossypetin, is 8.774 log cm/s. The reference standard acarbose showed the lowest permeability among all compounds at 0.059 log cm/s. Human oral absorption is an important parameter in determining the drug's efficacy; a molecule with absorbance three is considered good, two is considered medium, and one is considered poor. Most of the constituents showed medium to good predicted human oral absorption. The phytoconstituents like Barringtogenol B, P-coumaric acid, Syringic acid, and Vanillic acid showed good human oral absorption. Barringtogenic acid, Gallic acid, Gossypetin, and Melilotic acid showed moderate absorption. Barringtogenol C and acarbose showed poor absorption. The drugs that act on CNS need to cross BBB, but for the drugs acting on peripheral targets, there is no requirement to cross BBB because penetration may cause CNS side effects. The BBB penetration is expressed in cm/s. The recommended value for CNS active drugs is -2 inactive and +2 active. The recommended range for BBB permeability is -3 – +1.2. All the selected phytoconstituents are CNS inactive in nature. Hence, there is not much significance for BBB permeabilty. The ADME properties of the compounds are in an acceptable range. Hence, the slight modification in this compound will give us promising molecules for diabetes (39).

Invitro antidiabetic study

All seven extracts were evaluated for *in vitro* antidiabetic potential by alpha-glucosidase and alpha-amylase inhibitory assay method at a 20-100 µg/ml concentration range. Acarbose was used as the standard reference. The percentage inhibition with IC_{50} value of each extract is summarized in Table 4 and Figure 5.

Table 4: Alpha-amylase and alpha- gluccosidase inhibition assay

Solvents	Enzyme	% Inhibition*					IC_{50} Value
		20	40	60	80	100	(µg/ml)
n- Hexane	Alpha-amylase	20.053 ± 0.007	15.475 ± 0.048	26.713 ± 0.019	42.939 ± 0.016	46.333 ± 0.034	110.008
	Alpha-glucosidase	13.834 ± 0.007	17.837 ± 0.023	20.524 ± 0.008	29.737 ± 0.035	41.845 ± 0.008	134.339
Pet. Ether	Alpha-amylase	29.113 ± 0.008	37.526 ± 0.015	39.358 ± 0.073	43.076 ± 0.036	50.312 ± 0.014	102.2403
	Alpha-glucosi-dase	20.661 ± 0.024	25.322 ± 0.053	31.868 ± 0.024	37.520 ± 0.017	44.067 ± 0.027	121.369
Ethyl acetate	Alpha-amylase	23.287 ± 0.013	31.385 ± 0.027	33.275 ± 0.046	40.073 ± 0.046	53.130 ± 0.015	100.26
	Alpha-glucosi-dase	30.154 ± 0.030	57.909 ± 0.005	62.034 ± 0.038	63.863 ± 0.037	65.848 ± 0.033	44.584
$CHCl_3$	Alpha-amylase	28.911 ± 0.018	34.414 ± 0.017	38.996 ± 0.016	49.890 ± 0.013	50.416 ± 0.007	92.599
	Alpha-glucosi-dase	22.396 ± 0.0009	29.168 ± 0.023	38.650 ±0.031	42.901 ± 0.007	52.385 ± 0.015	94.989
EtOH	Alpha-amylase	58.427 ± 0.011	62.800 ± 0.019	63.745 ± 0.021	74.840 ± 0.018	80.955 ± 0.023	79.869
	Alpha-glucosi-dase	37.267 ± 0.018	56.058 ± 0.028	63.060 ± 0.027	68.145 ± 0.032	73.250 ± 0.012	37.383
CH_3OH	Alpha-amylase	33.560 ± 0.033	44.903 ± 0.007	48.955 ± 0.029	77.633 ± 0.017	79.108 ± 0.011	48.967
	Alpha-glucosi-dase	20.748 ± 0.006	50.160 ± 0.014	54.382 ± 0.024	56.811 ± 0.032	65.448 ± 0.177	68.013
Aqueous	Alpha-amylase	8.253 ± 0.042	23.809 ± 0.013	35.144 ± 0.020	39.483 ± 0.026	55.314 ± 0.007	90.0936
	Alpha-glucosi-dase	31.879 ± 0.012	37.738 ± 0.015	40.071 ± 0.031	41.940 ± 0.025	54.364 ± 0.033	95.786
Standard	Alpha-amylase	24.210 ± 0.011	34.331 ± 0.038	51.520 ± 0.043	63.031 ± 0.573	81.462 ± 0.473	58.7280
	Alpha-glucosi-dase	15.136 ± 0.013	32.832 ± 0.026	64.382 ± 0.074	71.477 ± 0.108	75.536 ± 0.156	57.653

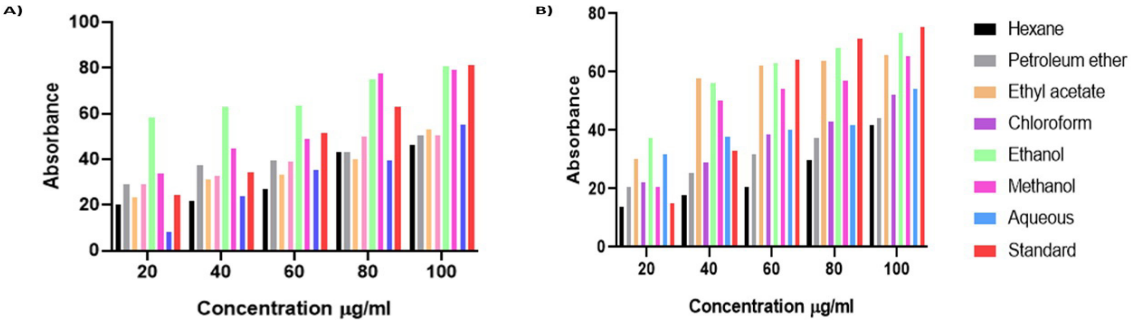

Fig. 5: *In vitro* anti-diabetic activity by a) alpha-amylase and b) alpha-glucosidase inhibition assay

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Alpha-amylase inhibition assay: The antidiabetic activity of the extract was analyzed using an alpha-amylase inhibition assay. The extract was tested at 20–100 µg/ml concentration, taking acarbose as standard. The extract showed inhibition in a concentration-dependent manner. The standard drug acarbose showed a maximum % inhibition of 81.462 ± 0.473 at a 100 µg/ml concentration with an IC_{50} value of 58.728 µM. These values were compared with the extracts. The ethanolic extract showed a comparable % inhibition of 80.955 ± 0.023 at a 100 µg/ml concentration with an IC_{50} value of 79.869 µM compared to standard acarbose. All the rest of the extracts showed comparable to moderate activity. The % inhibition of each extract as follows order acarbose (81.462 ± 0.473) > ethanol (80.955 ± 0.023) > methanol (79.108 ± 0.011) > aqueous (55.314 ± 0.007) > ethyl acetate (53.130 ± 0.015) > chloroform (50.416 ± 0.007) > petroleum ether (50.312 ± 0.014) > hexane (46.333 ± 0.034). The ethanolic extract showed significant alpha-amylase inhibitory activity.

Alpha-glucosidase inhibition assay was evaluated by acarbose used as a standard drug with a maximum inhibitory concentration of 75.536 ± 0.156 with an IC50 value of 57.653 µM All the extracts showed comparable activity with that of the standard. The ethanolic extract showed 73.250 ± 0.012 with an IC50 value of 37.383. The ethanolic extract showed almost similar activity to the standard. The inhibitory activity of extracts decreasing in following order standard (75.536 ± 0.156), ethanol (73.250 ± 0.012), methanol (65.448 ± 0.177), ethyl acetate (65.848 ± 0.033), aqueous (54.364 ± 0.033), chloroform (52.385 ± 0.015), petroleum ether (44.067 ± 0.027) and hexane (41.845 ± 0.008).

Therefore, from the above results, *Barringtonia acutangula* can be used for anti-diabetic activity, and further, the compound gossypetin can be isolated, characterized, and screened for *in vivo* antidiabetic activity. The *In silico* studies revealed that the compound gossypetin showed a good docking score with alpha-amylase (2QV4) and alpha-glucosidase (5NN8) and is a promising source as an antidiabetic agent for potential phytomedicinal development. Polyphenols are known to exert their action by binding to glucose transporters and by inhibiting digestive enzymes. It is evident that alpha-amylase and alpha-glucosidase inhibition is the probable mechanism in treating diabetes by enzyme inhibition, and the presence of flavonoids and phenolic compounds have insulin-sensitizing hypoglycemic mechanisms predicted in managing diabetes(40). Further *in vivo* study of these phytoconstituents could help develop novel, promising lead molecules for diabetes. Natural bioactive compounds are gaining more demand than synthetic drugs for treating diabetes due to their availability, efficacy, and lesser side effects (41). Flavanones, catechins, flavones, and flavanols are the classes of flavonoids that are naturally occurring phytoconstituents possessing antidiabetic potential, either by improving oxidative metabolism or altering glucose in the diabetic state (42). Phenolic compounds are known for their antioxidant activity; their total content is directly proportional to their antioxidant activity (43,44). Thus, phenolic compounds can prevent diabetic-related complications by inhibiting oxidative stress (45). Plant alkaloids are potent alpha-glucosidase inhibitors. The chemical nature of phytoconstituents can interact with several proteins involved in glucose homeostasis. Hence, it is receiving increasing attention as a reliable potential candidate for drug development (46).

Conclusion

Medicinally important plants such as *Barringtonia acutangula*, commonly referred to as the freshwater mangrove, have demonstrated considerable therapeutic potential in the management of metabolic disorders, particularly diabetes mellitus. In the present context, the plant has been systematically evaluated through quantitative phytochemical analyses, including the estimation of total alkaloid, total flavonoid, and total phenolic contents, which are widely associated with antioxidant and antidiabetic ac-

tivities. The in vitro antidiabetic assays further support its glucose-lowering potential, indicating the presence of bioactive constituents capable of modulating carbohydrate metabolism.

Notably, compounds such as gossypetin have been reported to exert hypoglycemic effects, suggesting their possible contribution to the observed biological activity. However, while these findings substantiate the traditional use of the plant, detailed molecular-level investigations are required to elucidate the precise mechanisms of action, target interactions, and efficacy. Advanced studies involving enzyme inhibition, cellular signaling pathways, and in vivo validation would provide a stronger mechanistic foundation and enhance the translational relevance of *B. acutangula* as a potential antidiabetic therapeutic agent.

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Authors contribution

Zakiya Fathima: Conceptualization of idea, experimentation, performing insilico studies. Jane Mathew: Interpreting data, draft editing, and supervision. Manoj Kumar: performing insilico studies & interpretation of data.

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Conflict of interest

The authors declare that there is no conflict of interest.

Abbreviation

FC: Folin-ciocalteu reagent; BCG: Bromocresol green; QE: Quercetin equivalent; SP: Standard precision; XP: Extra precision; PSA: Polar Surface Area.

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