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α-Amylase Inhibitory Property of major phytoconstituents of polyherbal formulation: An *In-Vitro* and Molecular Interaction Study

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ABSTRACT
The α-amylases are enzymes that hydrolyse starch molecules to allow numerous merchandises as well as dextrins and increasingly smaller polymers composed of aldohexose units that causes hyperglycaemia and also the development of type 2 DM. Presently Acarbose may be an advanced saccharide wont to lower plasma aldohexose levels by inhibiting the absorption of aldohexose by the internal organ. The foremost common adverse effects are GI symptoms, as well as flatulence, diarrhea, abdominal pain, and elevated body fluid transaminases might occur throughout acarbose medical aid. The event of inhibitors from natural merchandise offers another possibility for the management of hyperglycaemia. Therefore, the current study has been conducted screening of alpha-amylase repressing activity through in-vitro alpha amylase inhibitory effect and molecular docking of some commonly used nutraceuticals. The polyherbal formulation PHF_2 shows the highest α-amylase inhibition effect as compared to Voglibose, polyherbal formulation PHF1, and PHF3. Also, all polyherbal formulations illustrate a major synergistic impact as compared to individual extracts. Also in the Silico drug analysis model, the major phytoconstituents of A. Sativum, P. Granatum, Z. Officinale, and S. Cumini used in the preparation of this polyherbal formulation show significant inhibitory interactions with the human pancreatic alpha-amylase enzyme.
Keywords: *Human pancreatic α-amylases, Polyherbal Formulation, Diabetes Mellitus, Molecular Docking*

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INTRODUCTION

Diabetes mellitus (DM) may be a chronic, metabolic illness characterised by elevated blood sugar levels, that leads over time to serious harm to the heart, blood vessels, eyes, kidneys, and nerves. the foremost common is kind a pair of polygenic disease (T2DM), sometimes in adults, that happens once the body becomes proof against endocrine or does not create enough endocrine. regarding 422 million folks worldwide have polygenic disease, the bulk living in low-and middle-income countries, and 1.5 million deaths are directly attributed to polygenic disease every year. each the quantity of cases and also the prevalence of polygenic disease are steady increasing over the past few decades [1, 2, 11].

Several body fluid enzymes activities related with DM are often classified into four groups: Cluster I: Lysosomal enzyme-like β-glucuronidase N-acetyl-β-glucosaminidase, acid enzyme, and enzyme, these protein activities is also magnified with magnified glucose concentration. Cluster II: Alkaline Phosphatase and Trehalase, that are magnified however not related with glucose concentration, however might replicate with tissue metabolic disorders. Cluster III: Phosphohexose isomerase, Aminotransferases, and several other dehydrogenases, these protein activity will increase just in case of tissue harm caused by metabolic and circulatory alterations. enzyme, on the opposite hand, is cut. Cluster IV: Enzymes embrace the preceding enzymes with different enzymes, these are a lot of active in diabetics with complications like internal organ and excretory organ involvement and obesity [3, 12].

The α-amylases are enzymes that hydrolyse starch molecules to allow numerous merchandises as well as dextrins and increasingly smaller polymers composed of aldohexose units that causes hyperglycaemia and also the development of type 2 DM [4, 13].

Currently, T2DM manage with biguanides (metformin), sulfonylureas (Tolbutamide, Chlorpropamide, Glibenclamide, Glipizide, Gliclazide, Glimepiride), meglitinides (Repaglinide, Nateglinide), alpha-glucosidase inhibitors (Acarbose, Miglitol), thiazolidinediones (Rosiglitazone, Pioglitazone), glucagonlike-peptide-1 agonist (Liraglutide and Semaglutide), dipeptidyl enzyme IV (DPP-4) inhibitors (Sitagliptin), amylinomimetics (Pramlintide), and sodium-glucose transporter-2 (SGLT-2) inhibitors (Empagliflozin and Canagliflozin) [5, 6, 14]. The impact of those medication is aimed to lower the amount of blood sugar. One therapeutic approach for treating type-2 DM is to decrease the postprandial aldohexose levels. this might be done by retarding the absorption of aldohexose through the inhibition of the carbohydrates-hydrolyzing enzymes, α- glucosidase and α-amylase, gift within the little enteral brush border that's chargeable for the breakdown of oligosaccharides and disaccharides into monosaccharides appropriate for absorption [6].

Presently Acarbose may be an advanced saccharide wont to lower plasma aldohexose levels by inhibiting the absorption of aldohexose by the internal organ. It acts as a competitive, reversible substance of duct gland alpha-amylase and membrane-bound enteral alpha-glucoside hydrolase. The foremost common adverse effects are GI symptoms, as well as flatulence, diarrhea, and abdominal pain. Elevated body fluid transaminases might occur throughout acarbose medical aid. Post-marketing reports embrace cases wherever there have been rare occurrences of pneumatosis cystoid intestinalis with alpha-glucosidase substance use [7]. Therefore, the event of inhibitors from natural merchandise offers another possibility for the management of hyperglycaemia.

In Ayurveda, the standard Indian seasoning healthful system practiced for thousands of years have reports of medicine plants with no apparent glorious aspect effects, regarding 800 plant species are reported to possess anti-diabetic properties. Therefore, the current study has been conducted screening of alpha-amylase repressing activity through in-vitro alpha amylase inhibitory effect and molecular docking of some commonly used nutraceuticals.

MATERIAL AND METHODS

Drugs, Chemicals, and Instruments

The gift sample of hydroalcoholic extract of *Allium Sativum*, *Punica granatum*, *Zingiber officinale*, and *Syzygium Cumini* provided by Kisalaya Herbals Limited, Indore, India, 99.7%, Sodium Phosphate Dibasic Anhydrous extra pure AR, 99%, Sodium Phosphate Monobasic Dihydrate extra pure AR, were purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. **α**-amylase were procured from Sigma Aldrich (Co., St. Louis, USA), Sulphuric Acid, Methanol, Distilled Water, Spectrophotometer (UV-1800 Shimadzu), RX-50V Semi-Auto Biochemistry Analyzer, Micro Lab, Ahmedabad, India.

Development of Polyherbal Formulation

The polyherbal formulation was evolved by combining the dried hydroalcoholic extracts of the plant materials based on the oral glucose tolerance test of individual plant extracts (200 mg/kg each) in normal rats and total antioxidant capacity, advantageous in routine life (nutritional value) and reported activities of plants. The polyherbal formulation was made by mixing Hydroalcoholic Extract of *Allium Sativum* (bulb), *Punica granatum* (Fruit), *Zingiber officinale* (Shunt), and *Syzygium Cumini* (Seeds) in the proportion given in below Table 1.

Table 1: The hydroalcoholic extracts of *Allium Sativum* (A), *Punica granatum* (B), *Zingiber officinale* (C), and *Syzygium Cumini* (D) combinations

Prepared Combinations	
Name of Polyherbal Formulation	**Combination**
PHF_1	Drug A: Drug B: Drug C: Drug D, 1:1:1:1
PHF_2	Drug A: Drug B: Drug C: Drug D, 1.5:0.5:0.5:1.5
PHF_3	Drug A: Drug B: Drug C: Drug D, 1:1:0.5:1.5

In-vitro α-amylase inhibitor activity

The α-amylase restrictive activity of the polyherbal formulation and compounds was allotted exploitation the starch-iodide technique with slight modifications (M Setia 2017), (Hemlata Bhosale 2018).To 100 µl of Voglibose, every extract and polyherbal formulation (PHF_{1-3}) in a very tubing in triplicates, were preincubated with a 100 µl of α-amylase answer (02 units mg/mL) ready in 20mM phosphate buffer (pH 6.9) in a very total volume of 1000 µl for quarter-hour at 25°C. Thereafter take 0.4 millilitre of aliquots and add 3 millilitre of 1% Starch and 2 millilitres of twenty-millimeter phosphate buffer (pH 6.9). The reaction mixture was reincubated for forty-five minutes at 37°C. At zero time and the end of the

incubation period (45 minutes and after 24 hrs), 0.1 millilitre of the reaction mixture was withdrawn from every tube when mixture and discharged into 10 millilitres of 5mM Iodine solution. Solutions were completely mixed and also the absorbance was measured straightaway at 565 nm (Rawand S. Abu Soud 2004). Percentage inhibition was calculated consistent with the formula:

$$[(A_0\text{-}A_t)_{control} - (A_0\text{-}A_t)_{sample} / (A_0\text{-}A_t)_{control}] \times 100$$

Where A_0 and A_t are measure the absorbance values at zero time and also the end of the incubation, severally. Every experiment was repeated three to four times and also the average value was used for getting the relevant plots. Further, a study carried between a comparative study of α-amylase restrictive activity of Voglibose and optimized polyherbal Formulation (PF_2).

Molecular Docking Studies

Molecular docking is one amongst the foremost used ways in Structure-Based Drug Design Strategies (SBDD) because to its ability to predict, with a considerable degree of accuracy, the conformation of small-molecule ligands inside the acceptable target binding site. In the present study Molecular docking Studies has allotted to Exploration the mechanism of action and molecular interaction of major phytoconstituents of *A. Sativum, P. Granatum, Z. Officinale*, and *S. Cumini* with α-amylase by exploitation PyRx 0.8 Virtual Screening [8-10]. The grid box resolution was adjusted to outline the binding site as Centered at X: 8.635, Y: 58.6677, Z: 19.0723, A grid dimension at X: 55.7075, Y: 72.7494, Z: 55.583 Å.

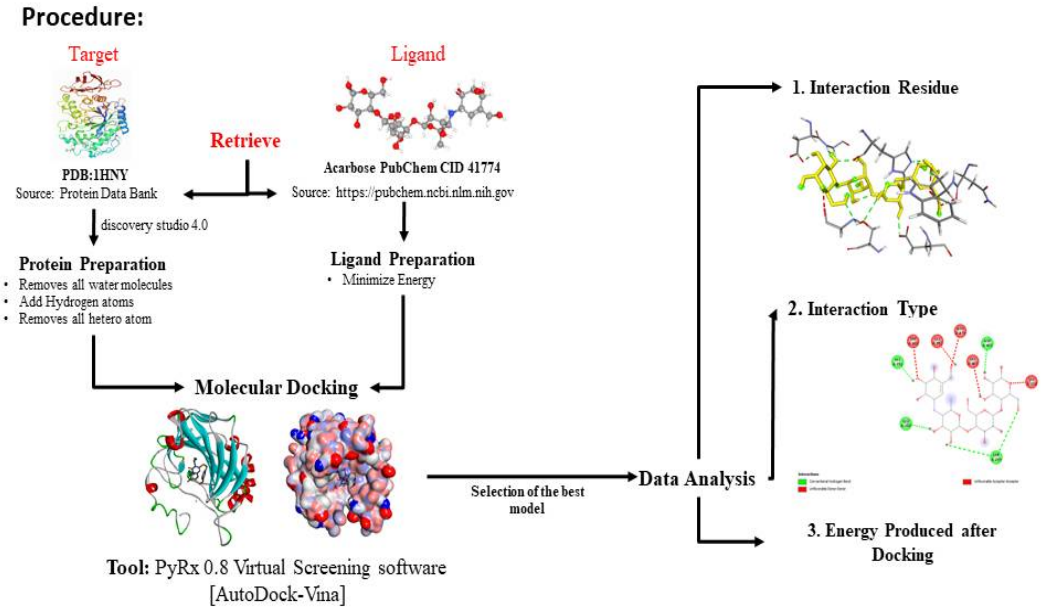


Figure 1: Schematic representation of Molecular Docking Studies for alpha-amylase (PDB: 1HNY) inhibitory activity

Statistical Analysis

All tests were carried out in triplicates. Data were presented as mean ± SD. To evaluate significant relationships between experimental parameters by correlation and regression analysis were used. Prism Graph Pad 8 and Microsoft Excel 2021 were used for the statistical and graphical evaluations.

RESULT

α-Amylase Inhibitor Activity

In-vitro α-amylase inhibition assay results of the chosen extracts, Voglibose, and polyherbal formulation ($PHF_{1\text{-}3}$) exploitation the starch-iodine methods are presented in terms of percentage of inhibition (**Table 2**) and inhibitory concentrations (IC_{50}) (**Figure 2**).The result discovered that hydroalcoholic extract of *Allium sativum* (6.68% and 22.61%), *Punica granatum* (9.41% and 24.63%), *Zingiber officinale* (20.35% and 30.42%), and *Syzygium Cumini* (30.11% and 41.12%), PHF_1 (47.45% and 67.94%), PHF_2 (68.99% and 85.90%), PHF_3 (61.27% and 70.83%) were compared to Voglibose (51.48% and 74.02. The IC_{50} value of final optimized polyherbal formulation (PHF_2) is 45.401 µg/ml and 37.601 µg/ml, whereas IC_{50} value of Voglibose is 32.19 µg/ml and 57.46 µg/ml at after 45 minutes and 24 hours of incubation with α-amylase enzyme respectively (**Table 03**). It considerably indicates all extracts, polyherbal formulation and Voglibose shows a significant α-amylase inhibition effect. The polyherbal formulation PHF_2 shows the highest α-amylase inhibition effect as compared to Voglibose, polyherbal formulation PHF1, and PHF3. Also, all polyherbal formulations illustrate a major synergistic impact as compared to individual extracts.

Table 2: *In-vitro* α-amylase inhibitory activity of Polyherbal Formulation (PHF_{1-3})

S. No	Treatment	Conc µg/ml	% of Inhibition of α-amylase activity (n=3)	
			After 45 minutes	After 24 hrs
1	Voglibose	100	51.48±1.5421	74.02±0.7453
2	*A. Sativum* Extract	100	6.68±1.4655	22.61±2.2036
3	*P. Granatum* Extract	100	9.41±1.3858	24.63±0.922
4	*Z. Officinale* Extract	100	20.35±2.5136	30.42±1.8228
5	*S. Cumini* Extract	100	30.11±2.064	41.12±2.9076
6	Polyherbal Formulation (PHF_1)	100	47.45±1.4843	67.94±0.7079
7	Polyherbal Formulation (PHF_2)	100	68.99±0.539	85.9±1.003
8	Polyherbal Formulation (PHF_3)	100	61.27±0.6275	70.83±0.2668

Figure 2: *In-vitro* α-amylase inhibitory activity of Polyherbal Formulation (PHF_{1-3})

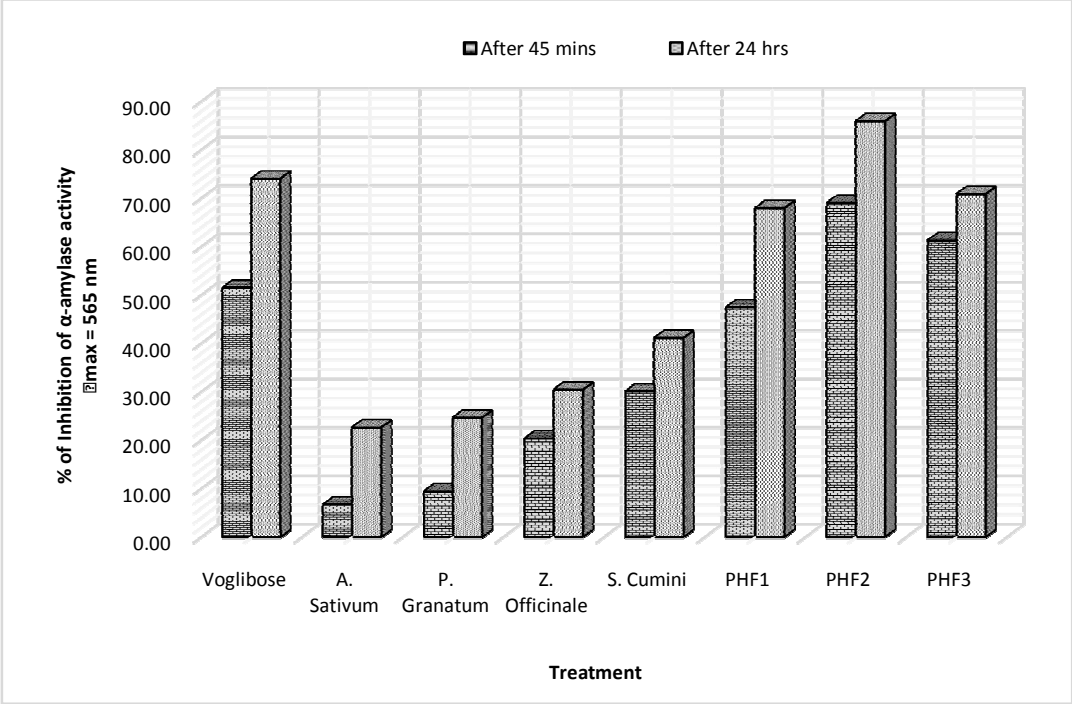
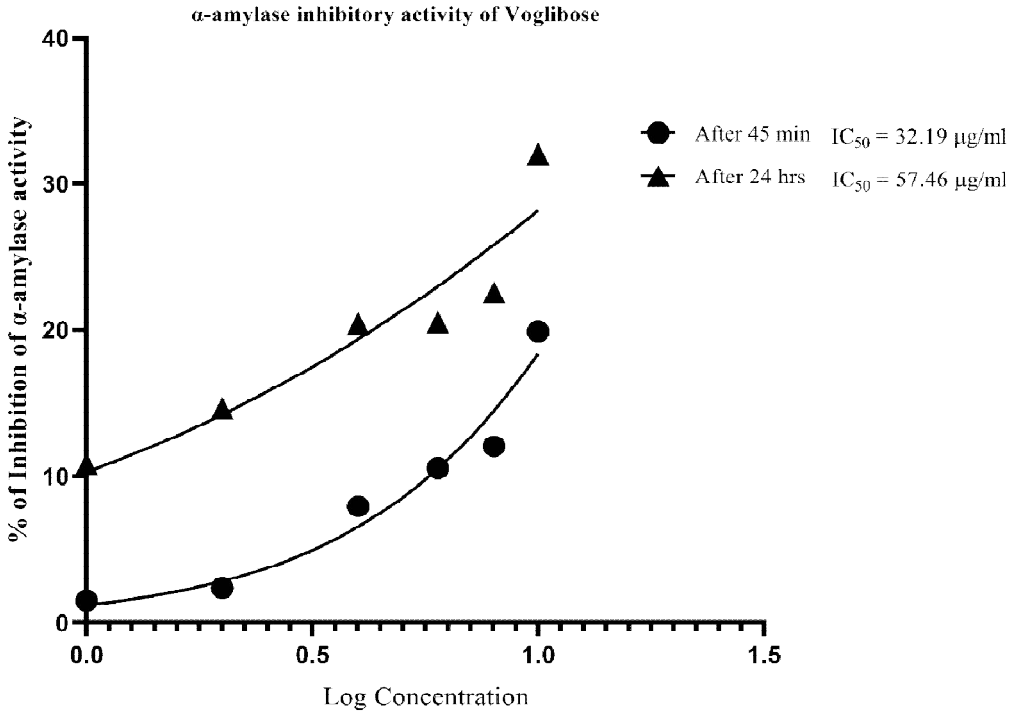


Table 3: Comparative study of α-amylase inhibitory activity of Voglibose and Polyherbal Formulation (PF_2)

Treatment	Conc µg/ml	α-amylase inhibitor activity			
		After 45 minutes		After 24 hrs	
		% of inhibition	IC_{50} Value	% of inhibition	IC_{50} Value
Voglibose	1	1.47	32.19 µg/ml	10.85	57.46 µg/ml
	2	2.35		14.66	
	4	7.95		20.45	
	6	10.56		20.53	
	8	12.05		22.59	
	10	19.88		32.05	
PHF_2	10	22.10	45.401 µg/ml	27.76	37.601 µg/ml
	20	27.27		29.55	
	40	45.78		49.10	
	80	59.49		63.67	
	100	61.68		66.98	
	150	69.75		73.77	

Figure 3a: Comparative study of α-amylase inhibitory activity of Voglibose



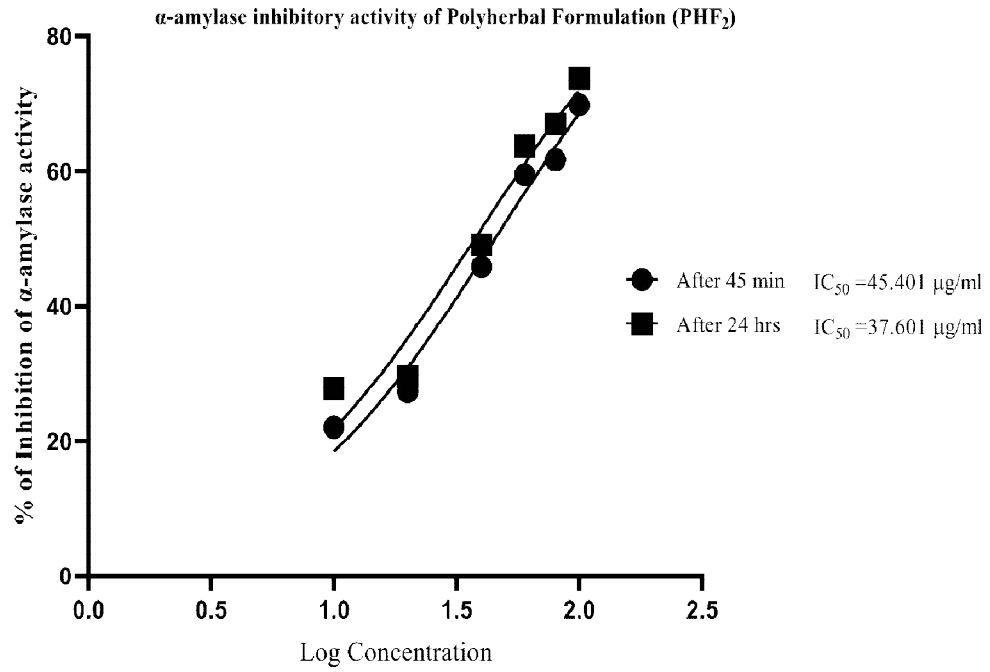


Figure 3b: Comparative study of α-amylase inhibitory activity of Polyherbal Formulation (PHF_2)

Molecular Docking Studies

In silico screening is an excellent approach for the screening of libraries of compounds in a very short time and therefore it minimizes the arduous work. Molecular docking may be a wide used technique to predict binding interactions between the 3D conformations of assorted ligands and receptor proteins that facilitate in improvement and leads toward drug development (Furqan Ahmad Saddique 2021). In the molecular docking experiment, most of the phytoconstituents of selected plants show binding energy lower than standard Acarbose with -8.1 KJ/mol and Voglibose -8.5 Kcal/mol (**Table 4**). Among these phytoconstituents shows binding energy Ursolic Acid (-10.1), Rutin (-8.9), Myricetin (-8.7), Quercetin (-8.6), Kaempferol (-8.4), Ellagic Acid (-8.2 respectively. The ligands Ursolic Acid (-10.1 KJ/mol), Rutin (-

8.9), Myricetin (-8.7 KJ/mol), Quercetin (-8.6 KJ/mol), Kaempferol (-8.4 KJ/mol), Ellagic Acid (-8.2 KJ/mol) have docking scores from -8.1 to -10.1 KJ/mol were ranked the most effective anti-diabetic molecule, Gingerenone (-6.9 KJ/mol), 6-Shogaol (-6.7 KJ/mol), 6-Gingerol (-6.2 KJ/mol), Gallic Acid (-5.9 KJ/mol) were also considered important due to good binding energy values in the range of having docking scores from -6.0 to -8.0 KJ/mol. The 2D interaction modes revealed that the binding interactions of Acarbose (-8.1 KJ/mol) shows H-bond type interaction with GLU 282, SER 289, HIS 331, ASP 402, and Voglibose (-8.5) shows H-bond type interaction with GLU 282, SER 289, HIS 331, PRO332, ASP 402 and CH bond with TRP 280 amino acid residue of human pancreatic alpha-amylase at 1.8 angstroms resolution α-amylase enzyme. While the phytoconstituents like Ursolic Acid (-10.1 KJ/mol) shows H-bond type interaction with ASP 197, GLY 306, Rutin (-8.9 KJ/mol) SER 3, THR 6, SER 289, ARG 421, Pi-Alkyl bond with PRO 4, CH-Bond with PRO 332 and Pi-Pi bond with ARG 252, PHE 335, Myricetin (-8.7 KJ/mol) H-bond with GLN 63, GLU 233, HIS 299, ASP 300, Pi-Pi bond with TRP 59, Quercetin (-8.6 KJ/mol) H-bond with TYR 62, GLN 63, Pi-Pi bond with TRP 59, Kaempferol (-8.4 KJ/mol) H-bond with TRP 59, GLN 63, Pi-Pi bond with TRP 59, Pi anion bond with ASP 300, and Ellagic Acid (-8.2 KJ/mol) H-bond with HIS 305, Pi-Pi bond with TRP 59 amino acid residue of human pancreatic alpha-amylase respectively.

Table 4: Binding energy of interactions between major phytoconstituents of *A. Sativum, P. Granatum, Z. Officinale, and S. Cumini* with alpha-amylase enzyme

S. No	Drug	Chem. Constituents	PubChem CID	Binding Affinity (KJ/mol)
1	Acarbose	-	CID_41774	-8.1
2	Voglibose	-	CID_444254	-8.5
3	A. Sativum	Alliin	CID_121922	-4.7
		DAS	CID_11617	-3.4
		Allicin	CID_65036	-3.9
		DATS	CID_16315	-3.5
		DADDS	CID_16590	-3.4
4	P. Granatum	Kaempferol	CID_5280863	-8.4
		Ellagic Acid	CID_5281855	-8.2
		Gallic Acid	CID_370	-5.9
5	Z. Officinale	Gingerenone-A	CID_5281775	-6.9
		6-Gingerol	CID_442793	-6.2
		6-Shogaol	CID_5281794	-6.7
6	S. Cumini	Ursolic Acid	CID_64945	-10.1
		Rutin	CID_5280805	-8.9
		Myricetin	CID_5281672	-8.7
		Quercetin	CID_5280343	-8.6

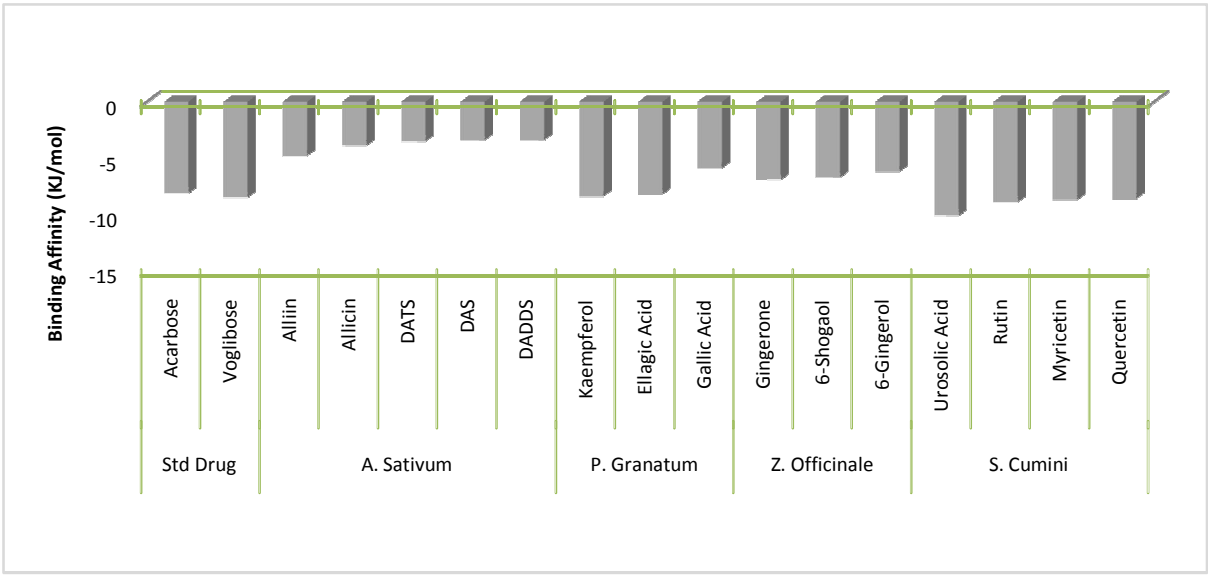


Figure 4: Binding energy of interactions between major phytoconstituents of *A. Sativum, P. Granatum, Z. Officinale, and S. Cumini* with alpha-amylase enzyme

Table 5: Binding affinity and binding interactions of Acarbose, Voglibose, major phytoconstituents of *A. Sativum, P. Granatum, Z. Officinale, and S. Cumini* with α-amylase

S. No	Ligand	Type of Bond	Amino Acid Residue
1	Acarbose (-8.1)	H-Bond	GLU 282, SER 289, HIS 331, ASP 402
2	Voglibose (-8.5)	H-Bond	GLU 282, SER 289, HIS 331, PRO332, ASP 402
		CH-Bond	TRP 280
3	***A. Sativum***		
	Alliin (-4.7)	H-Bond	ASN 301, ILE 312, THR 314, ARG 346
		CH-Bond	TRP 316
	Allicin (-3.9)	Pi-Alkyl	TYR 62, HIS 299
	DATS (-3.5)	Pi-Alkyl	TYR 62, LEU 165, HIS 299
	DAS (-3.4)	Pi-Alkyl	TYR 62, LEU 165, HIS 299
	DADDS (-3.4)	Pi-Alkyl	TYR 62, LEU 165, HIS 299
4	***P. Granatum***		
	Kaempferol (-8.4)	H-Bond	TRP 59, GLN 63
		Pi-Pi Stacked	TRP 59
		Pi-Anion	ASP 300
	Ellagic Acid (-8.2)	H-Bond	HIS 305
		Pi-Pi Stacked	TRP 59
		Pi-Pi T shaped	TRP 59
	Gallic Acid (-5.9)	H-Bond	ALA 128, LYS 178
5	***Z. Officinale***		
	Gingerone-A (-6.9)	H-Bond	ASP 197, ASP 300
		Pi-Alkyl	LEU 162, ALA 198, HIS 201
		CH-Bond	HIS 299
		Pi-Sigma	TYR 62
		Pi-Pi Stacked	TYR 62
	6-Shogaol (-6.7)	H-Bond	ASP 197, GLU 233
		Pi-Alkyl	TRP 59, LEU 162, ALA 198
		Pi-Anion	ASP 300,
		Pi-Pi-T-shaped	TYR 62
	6-Gingerol (-6.2)	H-Bond	GLU 233, ASP 300
		Alkyl	LEU 162, LEU 165
		Pi-H-Bond	TRP 59
		Pi-Pi Stacked	TRP 59
6	***S. Cumini***		
	Urosolic Acid (-10.1)	H-Bond	ASP 197, GLY 306
	Rutin (-8.9)	H-Bond	SER 3, THR 6, SER 289, ARG 421
		Pi-Alkyl	PRO 4
		CH-Bond	PRO 332
		Pi-Cation	ARG 252
		Pi-Pi-T-shaped	PHE 335
	Myricetin (-8.7)	H-Bond	GLN 63, GLU 233, HIS 299, ASP 300
		Pi-Pi Stacked	TRP 59
	Quercetin (-8.6)	H-Bond	TYR 62, GLN 63
		Pi-Pi Stacked	TRP 59

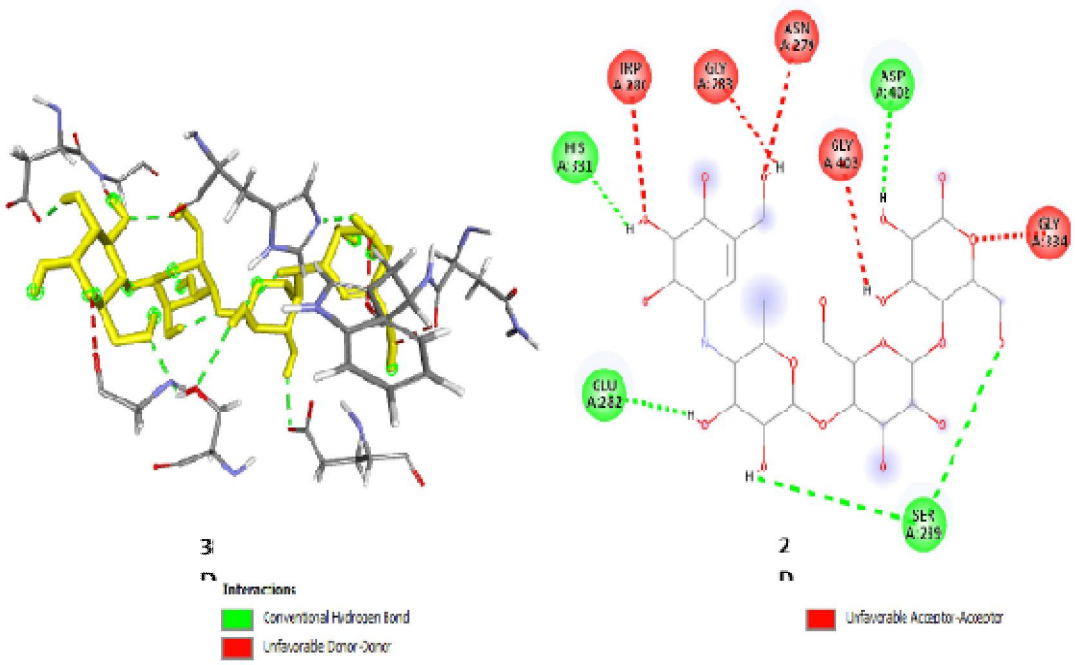


Figure 5a: Binding cavity of α-amylase with Acarbose (Binding Affinity (KJ/mol) -8.1)

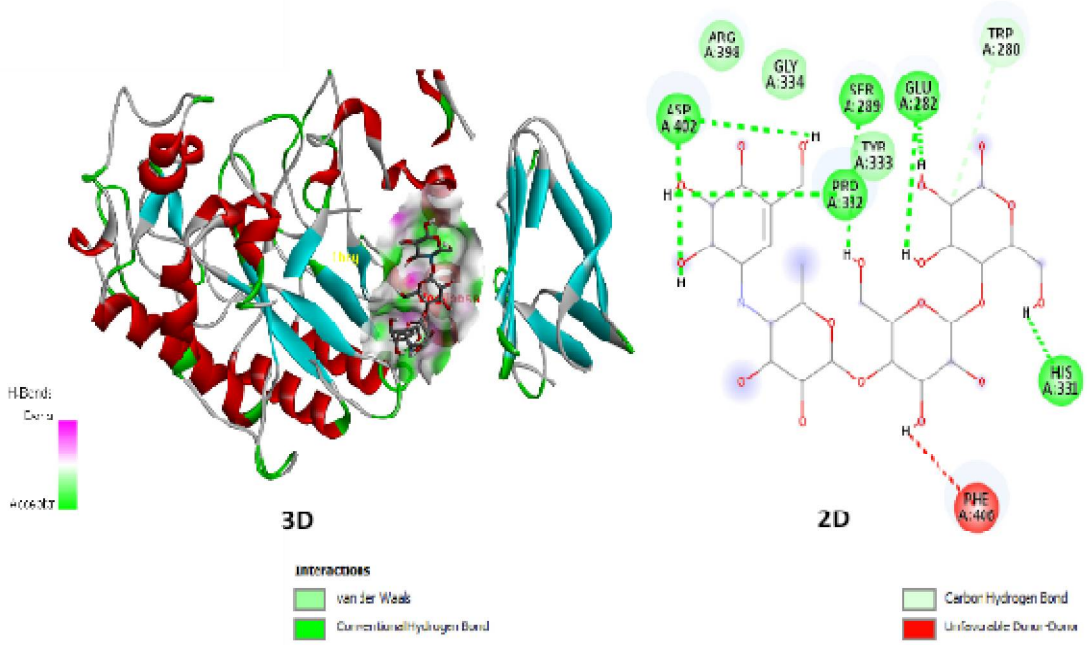


Figure 5b: Binding cavity of α-amylase with Voglibose (Binding Affinity (KJ/mol) -8.5)

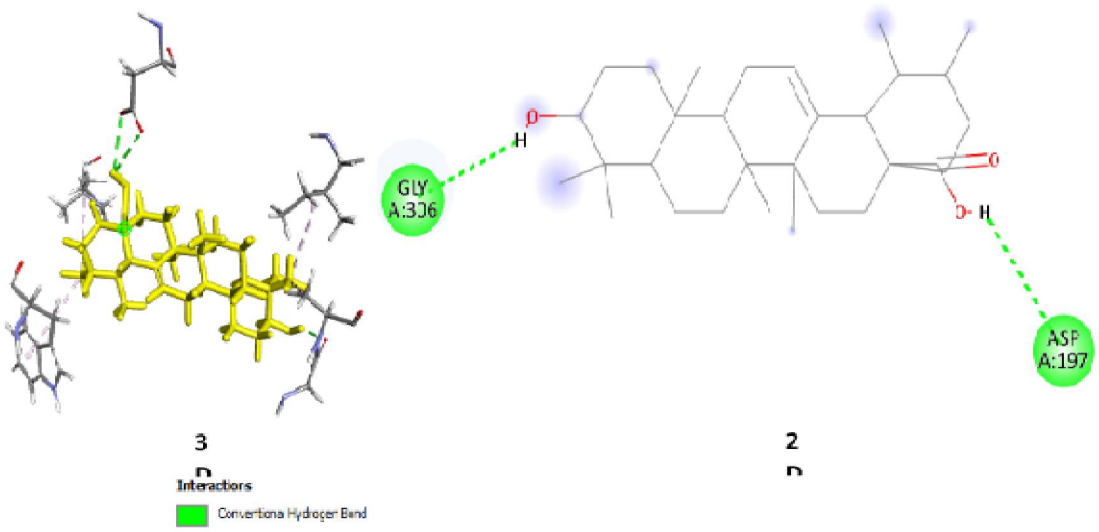


Figure 5c: Binding cavity of α-amylase with Urosolic Acid (Binding Affinity (KJ/mol) -10.1)

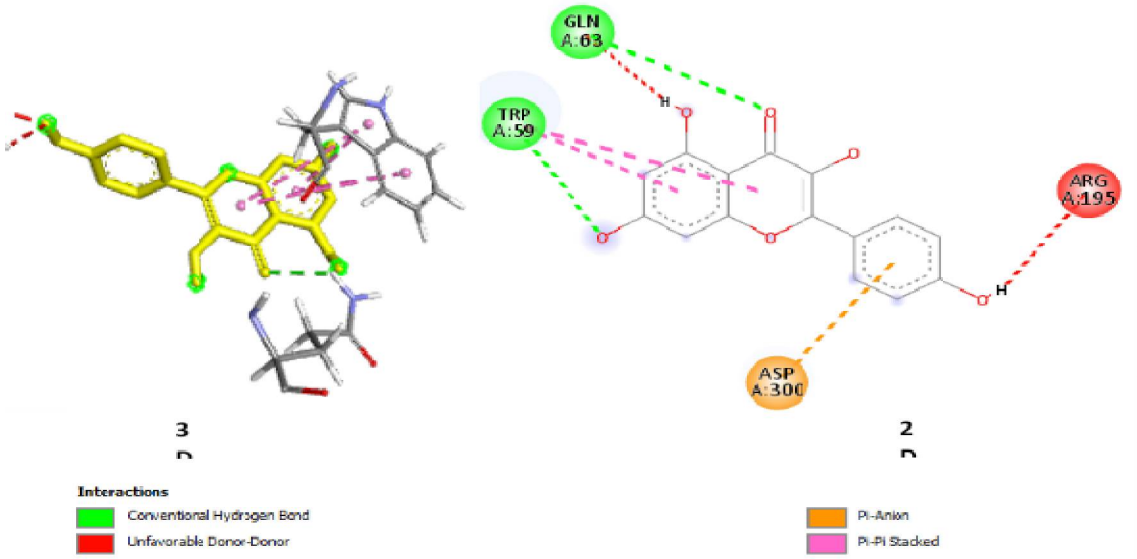


Figure 5d: Binding cavity of α-amylase with Kaempferol (Binding Affinity (KJ/mol) -8.4)

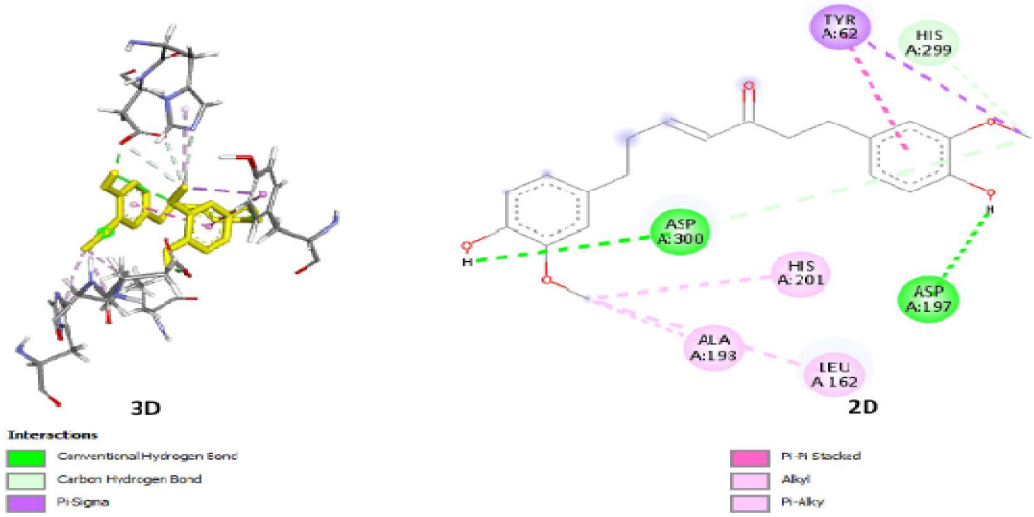


Figure 5e: Binding cavity of α-amylase with Gingerone (Binding Affinity (KJ/mol) -6.9)

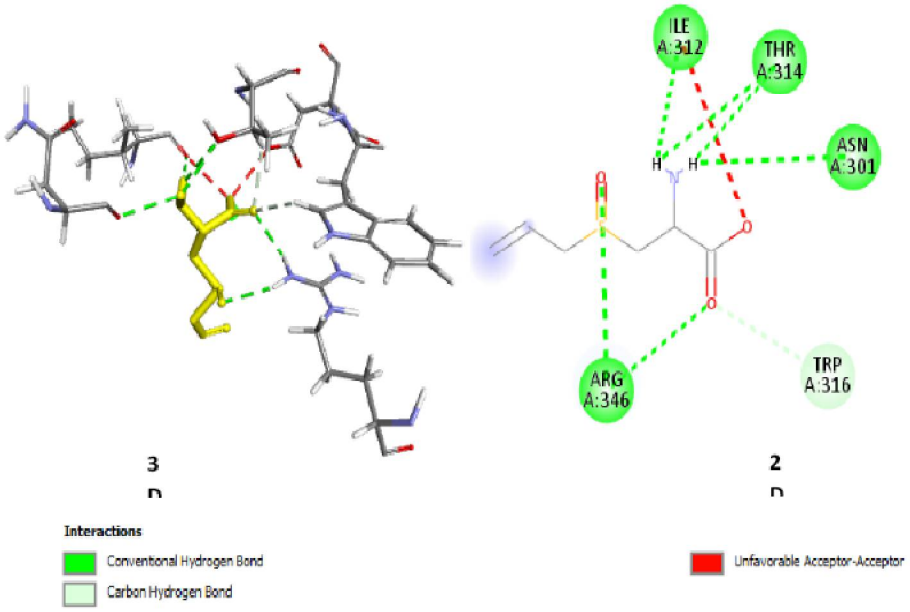


Figure 5f: Binding cavity of α-amylase with Alliin (Binding Affinity (KJ/mol) -4.8)

DISCUSSION

Currently used synthetic medication, that are well-known to guard against type 2DM and oxidative injury, have their adverse aspect effects. As a result, consumption of natural antioxidants, that are well-known to be effective scavengers of free radicals, through plants, food, or dietary supplements, interrupts the assembly of ROS and therefore helps within the bar of assorted diseases together with type 2DM [15]. The usage of medicative herbs within the management of diabetes and oxidative stress is accepted, and these herbs contain bioactive constituents like flavonoids, polyphenols, alkaloids, terpenoids, carotenoids, vitamins, and diverse different phytochemicals, which may act as antidiabetics and/or antioxidants/radical scavengers [16].

The α-Amylase, a salivary or pancreatic enzyme plays a very important role within the early breakdown of complicated carbohydrates into simple molecules. Modulation of α-amylase activity affects the use of carbohydrates as an energy supply and stronger is that this modulation; a lot of important is that the reduction within the breakdown of complicated carbohydrates. The majority of studies have centered on the anti-amylase phenolic compounds [6]. Because the intake of phenolic compounds is related to several helpful effects, it's conjointly necessary to contemplate the dose for humans, because it is possible to reduce α-amylase activity by overwhelming food or medicative herbs made in polyphenols with robust activity, if it takes in thought that this supply of polyphenols possess completely different forms of this compounds in variable concentration. The starch-iodine assay methodology is presently used as a fast methodology in screening plants for α-amylase inhibitors that would function lead compounds within the rummage around for more potent antidiabetic drugs or agents [17]. This study investigated the constraints of this methodology in screening plant extracts for α-amylase inhibition potential.

In the Silico drug analysis model, the major phytoconstituents of *A. Sativum, P. Granatum, Z. Officinale,* and *S. Cumini* used in the preparation of this polyherbal formulation show significant inhibitory interactions with the human pancreatic alpha-amylase enzyme.

CONCLUSION

In vitro alpha-amylase enzyme inhibitors activity all hydroalcoholic extracts of *A. Sativum, P. Granatum, Z. Officinale,* and *S. Cumini* shows strong inhibitor effect as compared to standard marketed drug Acarbose and Voglibose. The polyherbal formulation PHF_2 shows the highest α-amylase inhibition effect as compared to Voglibose, polyherbal formulation PHF_1, and PHF_3. Also, all polyherbal formulations illustrate a significant synergistic impact as compared to individual extracts. We hope the present work will benefit researchers to find novel phytoconstituents from these herbal plants to develop more active antidiabetic agents with no side effects.

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