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In Silico ADMET Analysis, Molecular Docking and in Vivo Anti Diabetic Activity of Polyherbal Tea Bag Formulation in Streptozotocin-Nicotinamide Induced Diabetic Rats

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***Abstract*---**Ichnocarpus frutescens, Ficus dalhousie, Crateva magna, Alpinia galangal, and Swertia chiraita are well-known plants available throughout India and they are commonly used for the treatment of various diseases including diabetes mellitus. The antidiabetic activity of the individual plant parts is well known, but the synergistic or combined effects are unclear. Polyherbal formulations enhance the therapeutic action and reduce the concentrations of single herbs, thereby reducing adverse events. The aim of the present study is to formulate a polyherbal tea bag formulation and evaluate its in vivo antidiabetic potential. The polyherbal formulation was formulated using the crude powder of the plants. The herbal formulation depicts hyperglycemic effects in both normal and experimentally induced hyperglycemia rats. The antidiabetic activity of the polyherbal formulation (100 mL/kg b.w.) was screened against streptozotocin-nicotinamide induced diabetes mellitus in rats. The infusion extract was administered for 21 consecutive days, and the effect of the polyherbal formulation on blood glucose levels was studied at regular intervals. At the end of the study, the blood samples were collected from all the animals for biochemical estimation (SGOT and SGPT). Polyherbal tea bag formulation showed significant antidiabetic activity

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and this effect was comparable with that of Metformin. The major phytoconstituents from each plant were screened through ADMET analysis and molecular docking was performed to understand possible molecular mechanism behind antidiabetic activity. The present study demonstrates that polyherbal tea bag formulation exhibits promising antidiabetic activity and helps to maintain good glycemic and metabolic control.

***Keywords*---**tea bag, polyherbal, aqueous, streptozotocin, molecular docking, alpha amylase.

Introduction

Diabetes mellitus affects almost 6 percent of the world's population, and in low- and middle-income nations, the disease's dynamics are shifting quickly. Around 80% of the world's diabetics would reside in low- and middle-income nations by 2030, according to the International Diabetes Federation (IDF)(Chaudhury et al., 2017; Endris et al., 2019). As per IDF 2011 report, China, India, and the United States of America have a diabetic population of 90.0, 61.3, and 23.7 million, which may be increased up to 129.7, 101.2, and 29.3 million, respectively, in 2030. Diabetes is one of the six leading causes of mortality worldwide, and it may also lead to a variety of systemic problems. Diabetes mellitus is treated by hormone treatment (insulin) or by delivering glucose-lowering medications such as alpha-glucosidase inhibitors, sulfonylureas, biguanides, and thiazolidinediones (El-Kaissi and Sherbeeni, 2011; Poretsky, 2010).

Diabetes patients' death and morbidity rates are rising over the globe, necessitating the development of a better therapy to keep the condition from deteriorating. Because of its effectiveness and safety, traditional medicine is becoming increasingly popular in the treatment of diabetes. The greatest antioxidant activity was discovered in the combination of chosen individual plants with green tea, which had a high concentration of phenolics and flavonoids. Polyherbs, rather than single herbs, are more often used in traditional methods to treat diabetes because of their synergy and less adverse effects(Firdous, 2014; Galicia-Garcia et al., 2020; Rupeshkumar et al., 2014). The conventional silver sulphadiazine diabetic wound cream was shown to be efficacious and safe in treating diabetic foot ulcers created utilising polyherbal formulation. One plant may contain several phytochemical components, therefore combining a number of these plants or herbs produces an efficient pharmacological effect. It's possible that this comprehensive approach might be safer and more tolerable if proven successful(“Diagnosis and Classification of Diabetes Mellitus,” 2004; Olokoba et al., 2012).

Monosaccharides are formed from the breakdown of oligo- and disaccharides by the enzyme referred to as a glucosidase. Because monosaccharides are the type of carbohydrates that are absorbed through the mucosal boundary in the small intestine, inhibiting these enzymes lowers blood glucose levels. Alpha-amylase inhibitors have been discovered to be effective with various enzymatic agents,

which has led researchers to express an interest in generating powerful alpha-amylase inhibitor compounds(Ameri et al., 2020; Okechukwu et al., 2020; Yang et al., 2019). The concept of polyherbal formulation is well documented in the ancient literature. Compared to the single herb, the polyherbal formulation has better and extended therapeutic potential. *Ichnocarpus frutescens, Ficus dalhousie, Crateva magna, Alpinia galangal*, and *Swertia chiraita* are well-known plants available throughout India and they are commonly used for the treatment of various diseases including diabetes mellitus(F.P. et al., 2022; Faheem et al., 2021; Faheem I P et al., 2021; FP et al., 2018). Hence, the present study was planned to formulate and standardize a polyherbal tea bag formulation using a plant having known antidiabetic activity and evaluate its therapeutic effects in rodents.

Material and Methods

Chemicals and Reagents

Alpha amylase, 3,5-dinitrosalicylic acid (DNSA reagent), starch, ethanol, conc. hydrochloric acid, sulphuric acid, different reagents used for qualitative analysis were purchased and procured from Lab Trading Laboratory, Aurangabad, Maharashtra, India. All the chemicals used were of analytical grade.

Collection of plants and authentication

The plants *Ichnocarpus frutescens, Ficus dalhousie, Crateva magna, Alpinia galangal*, and *Swertia chirata* were collected and authenticated from Dr. Madhava Chetty, Department of Botony, Sri Venkateswara University, Tirupati, India with voucher numbers 0448, 0879, 0550, 0911, and 0612 respectively.

Calculation of pharmacokinetic parameters

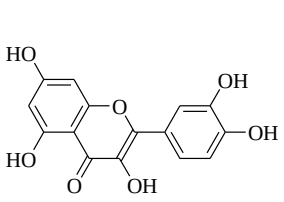
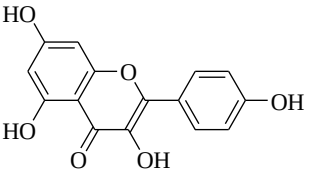
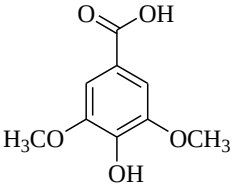
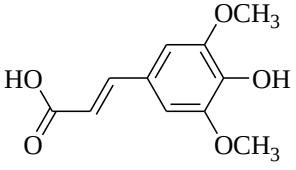
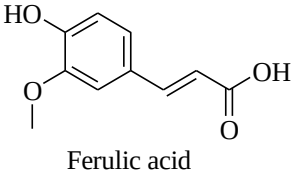
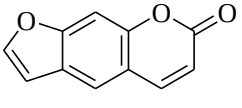
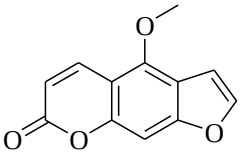
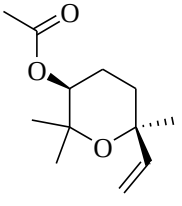
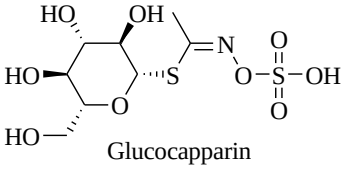
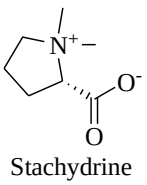
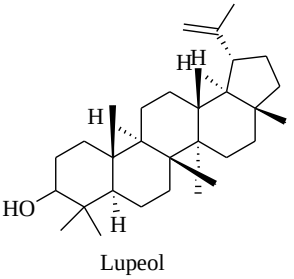
In order to further optimize the molecules, all the phytoconstituents were tested for violating Lipinski's rule of five along with their binding affinity with the alpha-amylase enzyme. The properties of all the phytoconstituents were calculated from SwissADME online tool (http://www.swissadme.ch/index.php)(Banerjee et al., 2018; Drwal et al., 2014).

Molecular docking studies

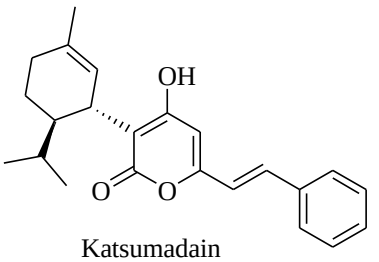
We conducted molecular docking (MD) on Lenovo ThinkPad T440p using PyRx-Virtual Screening Tool(Dallakyan and Olson, 2015). The structures of all the phytoconstituents and native ligand (.sdf File format) were downloaded from the National Center for Biotechnology Information PubChem (https://pubchem.ncbi.nlm.nih.gov/). The energy minimization (optimization) was performed by Universal Force Field (UFF)(Rappé et al., 1992). Autodock vina 1.1.2 in PyRx 0.8 was used to perform the MD studies of all the phytoconstituents and native ligand against the crystal structure of alpha amylase enzyme. The enzyme structures, with the aid of Discovery Studio Visualizer 2019, were optimized, purified and prepared for MD. Molecules (PDBQT Files), both ligands as well as

targets (human DPP-IV enzyme) were selected for MD(Chaudhari et al., 2020). For the purpose of MD simulation, the three-dimensional grid box (size_x = 63.6943A^0; size_y = 63.4700A^0; size_z = 62.1868A^0) was built using Autodock tool 1.5.6 with exhaustiveness value of 8(Dallakyan and Olson, 2015). The active amino acids in the protein were analyzed and illuminated using BIOVIA Discovery Studio Visualizer (version-19.1.0.18287)(San Diego: Accelrys Software Inc., 2012. The full MD process, the identification of cavity and active amino acid residues were performed as per the procedure described by S. L. Khan et al(Chaudhari et al., 2020; Khan, Sharuk L; Siddiui, 2020; Khan et al., 2022, 2020; S. Khan et al., 2021; S. L. Khan et al., 2021; Shntaif et al., 2021; Siddiqui et al., 2021).

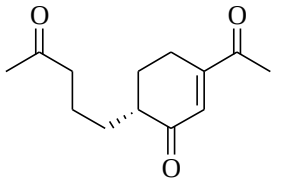
Table 1
The structure and names of selected phytoconstituents from each plant

Name and Structures of Phytoconstituents
Ichnocarpus frutescens
 Quercetin  Kaemferol  Syringic acid  Synapic acid
Ficus dalhousiae
 Ferulic acid  Psoralen  Bergapten  Pyranoid
Creteva magna
 Glucocapparin  Stachydrine  Lupeol

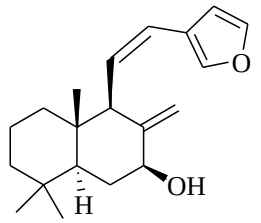
Alpinia galanga



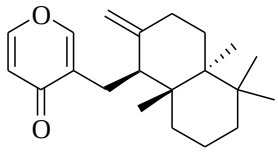
Katsumadain



Oxyphyllone A

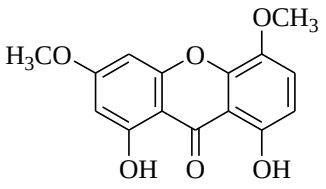


Coronarin A

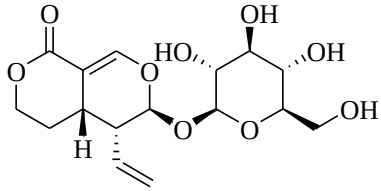


Ottensinin

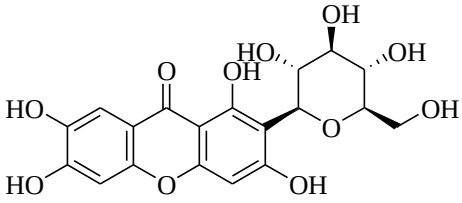
Swertia chirata



Swerchirin



Sweroside



Mangiferin

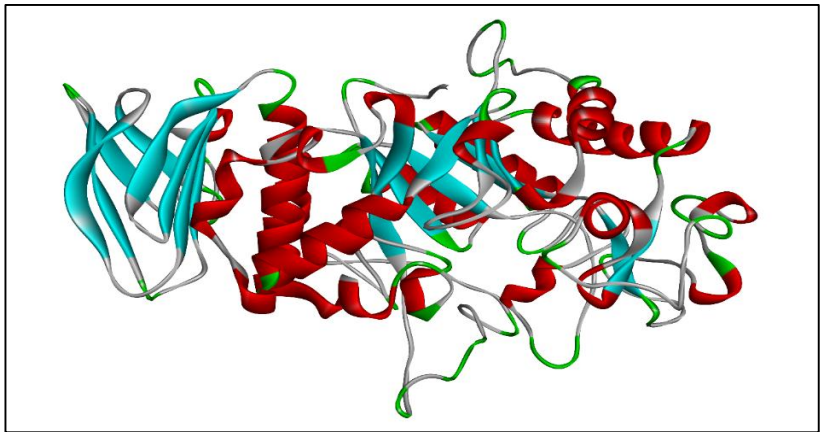


Figure 1. The crystal structure Human alpha amylase enzyme (PDB ID: 3IJ9)

Formulation of Polyherbal Tea Bag (T bag)

Methodology

The extracts (F1, F2, F3 and F4) were prepared in the tea bag form and tested for its antidiabetic activity.

Selection of tea bag paper

Fibre used in tea bag may be processed with synthetic polymers to form papers that are heat sealable. For a good quality filter paper there must be good tensile strength at sealing joints, wet strength should be high, it should not affect flavour infusion. They are made from nonwoven fibres which are based on cellulose obtained from the seeds of jute or abaca trees or cotton or stem fibres of hemp. Cellulosic tea bag filter paper has high tensile strength and is highly porous that provides high durability and protective layer for any adsorbent. In addition, cellulosic tea bag filter paper is cheap and nontoxic. Replacement of a polypropylene tea bag filter paper with the cellulosic tea bag filter paper is also done(Friesenhahn, 2009; Oduro et al., 2013; Pelden et al., 2014).

Tea bag paper shape

2 other shapes rectangular tea bags of diameter 8 × 6.1 cm and round tea bags of 7.9 cm was used to study the rate of active constituent release. It was seen that after the surface area of 49 cm^2 there is very little effect on the rate constants, which was obtained on 80°C. 4 sizes of tea bag were used 16, 36, 49, and 64cm^2 that is, for square tea bags and 49 cm^2 for rectangular and round tea bags. The tea bags were immersed in 400 mL distilled water placed in thermostat bath at a temperature of 80°C without any air bubble in the tea bag and it should remain flat on the wire mesh immersed in water(Jaganyi and Mdletshe, 2000). It was seen that there is increase in the extraction rate with the increase in the tea bag size as from 16 to 36 cm^2 area there was 25% increase was seen and less extraction rate was seen in tea bags larger then 36–64 cm^2 area. Laboratory studies were conducted for tea bag extraction efficiency on polyherbal drug, it was measured by full factorial experimental design and the comparison between loose herb and tea bag was done. Tea particle size was large (1.70–1.18 mm) with bag dimensions of 51 by 50mm and the agitation was dynamic whereas in the other set tea bag particle size taken was small (250–500 mm) with a bag dimensions of 44 by 45 mm) and static conditions were there. It is higher for those tea bags, which are continuously dunked (dynamic infusion) as compared to that which just floats in water (static infusion)(Jaganyi and Ndlovu, 2001).

Preparation of formulation

The collected plants have been washed with water to remove any dust or foreign particle present on it and shade dried for one week at room temperature to avoid excessive loss of volatile phytoconstituents. After drying, the plant material grinded individually and at least 100 gm of crude powder prepared from each plant. It was mixed thoroughly in different compositions as formulation-1 (F1),

formulation-2 (F_2), formulation-3 (F_3), and formulation-4 (F_4) of *Ichnocarpus frutescens, Ficus dalhousiae, Creteva magna, Alpinia galanga, Swertia chirata.* Table 2 illustrates the quantity of each plant taken for the formulation(Ansarullah et al., 2009).

Table 2
Formulation table for the preparation of polyherbal Tea bag

Name of plants	Batches (qnty)			
	F1	F2	F3	F4
Ichnocarpus frutescens	0.5	0.7	0.7	0.7
Ficus dalhousiae	0.5	0.7	0.5	0.7
Creteva magna	0.5	0.7	0.7	0.7
Alpinia galanga	0.5	0.5	0.5	0.7
Swertia chirata	0.5	0.5	0.7	0.7

Infusion time

40 samples of loose herbs and polyherbal tea bags were taken out of which 20 were loose herbs and 20 were tea bags from different formulations F1, F2, F3 and F4. Each sample was immersed in 240 mL distilled water and brewed at 80 °C for 5 min. and stirred twice whereas loose herb (2 g) was immersed in 240 mL at 80 °C for different times that is, 5, 10, 15, 20, and 30 min and the solution was bought to room temperature for analysis. Approximate infusion time of 10 min for loose herbs and 2 min for tea bags was enough for extraction of phenols and antioxidants. A lot of variations were seen in brewing and steeping of a tea bag in different countries, which was based on their own preference of choice and culture. Maximum infusion time for the tea bag was <30 s to 2 min(Yadav et al., 2017). Even the infusion times sometimes vary from 30 s to 5 min. Comparison between formulations F1, F2, F3 and F4 was done to see difference in their steeping times and its effect on total drug content and capacity of tea bags. Tea particle of large size (1.70–1.18 mm) packed in 51 × 50mm tea bags with dynamic agitation compared to other set tea bag of small particle size (500–250 µm) and bag dimensions 44 × 45 mm) with static agitation. The flow rate of herb solutes from the tea bag increases by decreasing the pore size of the tea bag(Yang et al., 2007).

Preliminary phytochemical screening of formulations

The extract was subjected for preliminary phytochemical screening by various qualitative tests to detect the presence of different class of phytoconstituents. The different tests such as test for alkaloids, carbohydrates, steroids, triterpenoids, cardiac glycosides, saponins, tannins, and flavonoids were performed(Chaudhari et al., 2020; Khandelwal K. R., 2005; Mukherjee, 2002).

In vivo *Antidiabetic activity*
Experimental animals and ethical considerations

Swiss Albino mice of either sex weighing between 20 and 30 g were used. The mice were acclimatized to the animal house condition for 1 week before carrying out any experimental work. The mice were fed with normal animal pellet diet and water *at- libitum*. They were housed at standard housing conditions of temperature (23 °C±12 °C), humidity (45 ± 5%), and 12-h light and dark cycle.In line with the Committee for the Purposes of Control and Supervision of Experiments on Animals' (CPCSEA) requirements all of the animals were observed and cared for in accordance with their needs. The animals were kept in polypropylene cages, and all procedures on them were carried out in an aseptic environment. With the approval of the Institutional Animal Ethics Committee (IAEC), the study's procedures were implemented (approval number CPCSEA/IAEC/JLS/16/07/21/37).

Acute toxicity studies

Safety Studies for dose titration were carried out according to of the Organization for Economic Co-Operation and Development (OECD 425) Guideline on normal mice with three different doses of the tea bag solution. The fasted mice were fed with single dose of 500, 1000 and 2000 mg/kg body weight by oral route to three different groups respectively. All the mice were keenly examined for 2 h to check any abnormalities in behaviour of the animals and further continued to monitor and examine the mice for 24 and 72 h(Kim et al., 2020; Piyachaturawat et al., 1983).

Induction of chronic diabetes & experimental design

Hyperglycemia was induced by a single intraperitoneal injection of freshly prepared streptozotocine (STZ)-nicotinamide (55mg/kg body weight, in 0.1M citrate buffer (pH 4.5) to a group of overnight fasted rats. To control drug-induced hypoglycemia, a solution of 5% glucose overnight was given to rats. Hyperglycemia was confirmed on the third day after STZ injection by estimating glucose level by Glucometer. The rats having a glucose level of 300 mg/dl were used for the study(Baydas et al., 2005).

On day 14 of post STZ injection, the diabetic rats were randomized based on their fasting blood glucose and regrouped into 8 groups, comprising 6 rats each. STZ-untreated rats served as non-diabetic control. Group I served as control. Group II served as diabetic control, group III and IV treated with an oral dose of 100 mL/kg and 200 mL/kg respectively(Dureshahwar et al., 2017; Islam, 2013). Group V was served as a standard group that received Metformin 120mg/kg (Oral route)(Ojha et al., 2014). The rats were allocated to different treatment groups and were administered with different treatments for next 14 days as tabulated in Table 3.

Table 3
Experimental design of the animal activity

Group ID	Group Details	Treatment (Dose & Route)	No. of animals
G1	Normal Control	Saline, 10ml/kg/day, Oral Route	6
G2	Diabetic control	Saline, 10ml/kg/day, Oral Route	6
G3	Diabetic + F1	100 mL/kg/day, Oral Route	6
G4	Diabetic + F2	100 mL/kg/day, Oral Route	6
F5	Diabetic + F3	100 mL/kg/day, Oral Route	6
F6	Diabetic + F4	100 mL/kg/day, Oral Route	6
G7	Diabetic + Metformin	Metformin, 120 mg/kg/day, Oral	6

Estimation of biochemical parameters in animal groups

Different biochemical parameters have been estimated to determine the anti-diabetic potential of the molecules. Few parameters were calculated, including body weight, serum glucose, glutamic-oxaloacetic transaminase (SGOT), as well as serum glutamic pyruvic transaminase (SGPT). The body weight and blood glucose level were estimated in rats at 0, 7^{th}, 14^{th}, and 21^{st} day of the treatment. The initial body weight were measured and compared with normal control rats. Estimation of blood glucose level before and after 3 weeks of treatment was done by Glucometer (One touch). All animals were monitored for body weight during the treatment period. Blood was collected from tip of the tail vein and fasting blood glucose levels were measured(Bafna and Mishra, 2004; Chaitanya et al., 2012; Sharma et al., 2013).

Results

The Lipinski rule of 5 and Veber's rule of the docked phytoconstituents are represented in Table 4. The pharmacokinetics and drug-likeness properties of phytoconstituents are tabulated in Table 5. The physicochemical radar of the molecules in which the colored zone is the suitable physicochemical space for oral bioavailability is illustrated in Fig. 3. The ligand energies (kcal/mol), binding free energy (kcal/mol), and the active amino residues, bond length (A^0), and type of interactions of phytoconstituents with alpha amylase enzyme are depicted in Table 6. The 2D- and 3D-docking poses of all the docked molecules are represented in Table 7.

Table 4
Lipinski rule of 5 and Veber's rule calculated for molecules

Compound codes	Lipinski rule of five					Veber's rule	
	Log P	Mol. Wt.	HBA	HBD	Violations	Total polar surface area (Å^2)	No. of rotatable bonds
Bergapten	2.16	216.19	4	0	0	52.58	1
Coronarin A	4.31	300.44	2	1	0	33.47	2
Ferulic acid	1.36	194.18	4	2	0	66.67	3
Glucocapparin	-1.92	333.34	10	5	0	199.79	5
Kaemferol	1.58	286.24	6	4	0	111.13	1
Katsumadain	4.79	350.45	3	1	0	50.44	4
Lupeol	7.27	426.72	1	1	1	20.23	1
Mangiferin	-0.77	422.34	11	8	2	F201.28	2
Ottensinin	4.80	314.46	2	0	0	30.21	2
Oxyphyllone A	1.80	222.28	3	0	0	51.21	5
Psoralen	2.12	186.16	3	0	0	43.35	0
Pyranoid	2.27	212.29	3	0	0	35.53	3
Quercetin	1.23	302.24	7	5	0	131.34	1
Stachydrine	-0.30	144.19	2	1	0	40.54	1
Swerchirin	2.16	288.25	6	2	0	89.13	2
Sweroside	-0.75	358.34	9	4	0	134.91	4
Synapic acid	1.31	224.21	5	2	0	75.99	4
Syringic acid	0.99	198.17	5	2	0	75.99	3

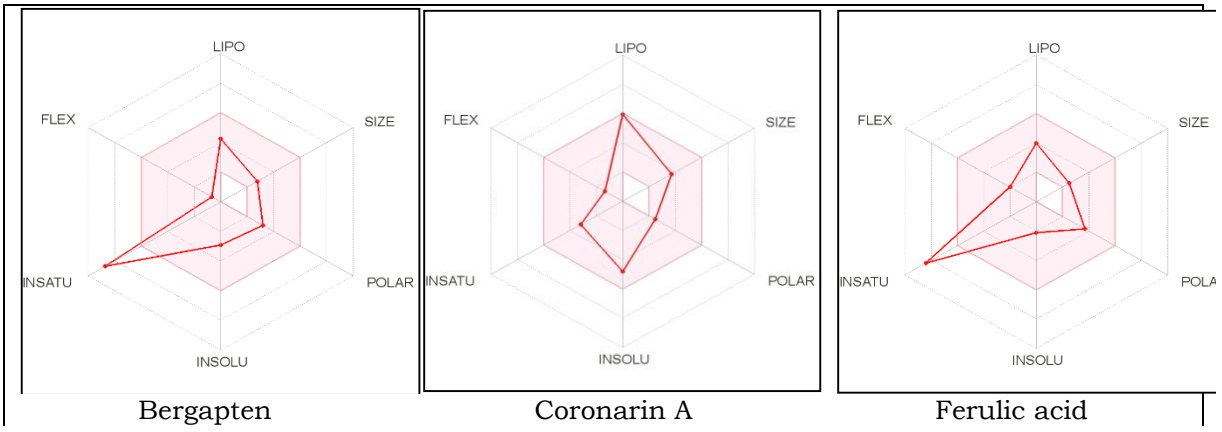
Where: Mol. Wt., molecular weight; HBA, hydrogen bond acceptors; HBD, hydrogen bond donors

Table 5
The pharmacokinetics and drug-likeness properties of phytoconstituents

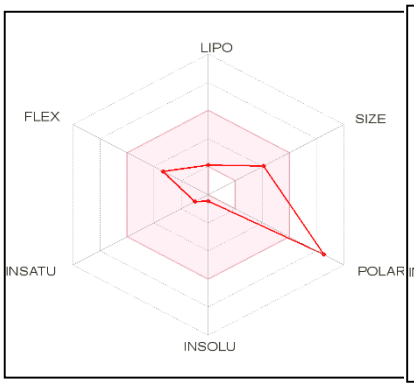
Compound codes	Pharmacokinetics									Drug-likeness			
	GI abs.	BBB pen.	P-gp sub.	CYP1A2 inhibitors	CYP2C19 inhibitors	CYP2C9 inhibitors	CYP2D6 inhibitors	CYP3A4 inhibitors	Log K_p (skin permeation, cm/s)	Ghose	Egan	Muegge	Bioavailability Score
Bergapten	H	Y	N	Y	N	N	N	N	-6.25	Y	Y	Y	0.55
Coronarin A	H	Y	N	N	Y	Y	N	N	-4.66	Y	Y	Y	0.55
Ferulic acid	H	Y	N	N	N	N	N	N	-6.41	Y	Y	Y	0.55
Glucocapparin	L	N	Y	N	N	N	N	N	-9.62	N	N	N	0.11
Kaemferol	H	N	N	Y	N	N	Y	Y	-6.70	Y	Y	Y	0.55
Katsumad	H	Y	N	N	Y	Y	N	Y	-4.61	Y	Y	Y	0.55

ain													
Lupeol	L	N	N	N	N	N	N	N	-1.90	N	N	N	0.55
Mangiferin	L	N	N	N	N	N	N	N	0.17	N	N	N	0.17
Ottensinin	H	Y	N	N	Y	N	Y	N	-4.39	Y	Y	Y	0.55
Oxyphyllone A	H	Y	N	N	N	N	N	N	-7.16	Y	Y	Y	0.55
Psoralen	H	Y	N	Y	N	N	N	N	-6.25	Y	Y	Y	0.55
Pyranoid	H	Y	N	N	N	N	N	N	-6.17	Y	Y	Y	0.55
Quercetin	H	N	N	Y	N	N	Y	Y	-7.05	Y	Y	Y	0.55
Stachydrine	H	N	N	N	N	N	N	N	-6.90	N	Y	N	0.55
Swerchirin	H	N	N	Y	N	Y	Y	Y	-6.11	Y	Y	Y	0.55
Sweroside	L	N	Y	N	N	N	N	N	-9.14	N	N	Y	0.56
Synapic acid	H	N	N	N	N	N	N	N	-6.63	Y	Y	Y	0.56
Syringic acid	H	N	N	N	N	N	N	N	-6.67	Y	Y	Y	0.56

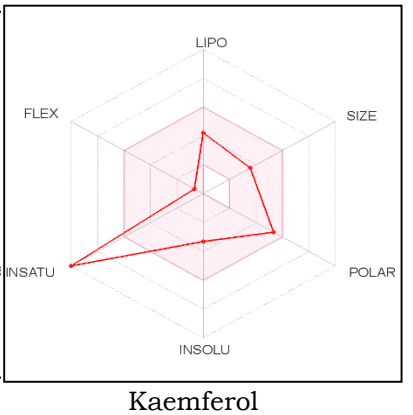
Where: NL, Native ligand; GI abs., gastrointestinal absorption; BBB pen., blood brain barrier penetration; P-gp sub., p-glycoprotein substrate



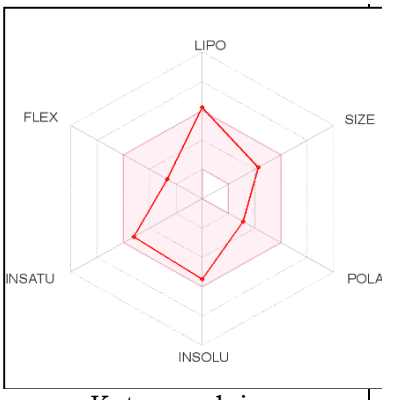
Bergapten Coronarin A Ferulic acid



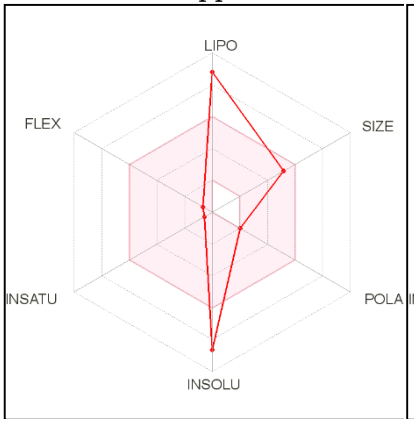
Glucocapparin



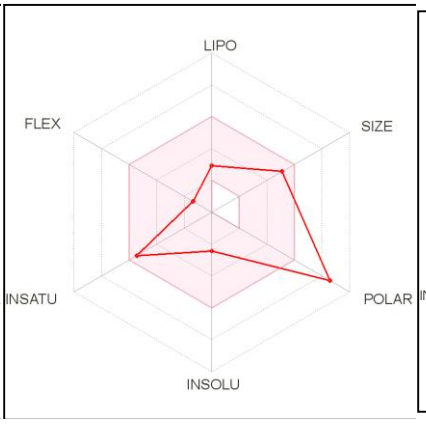
Kaemferol



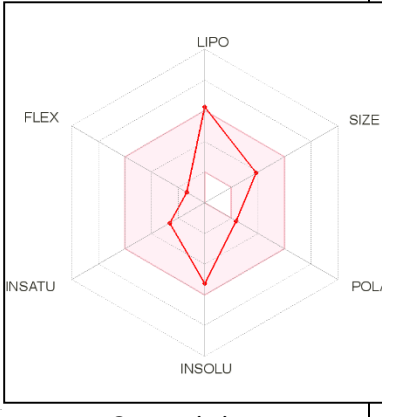
Katsumadain



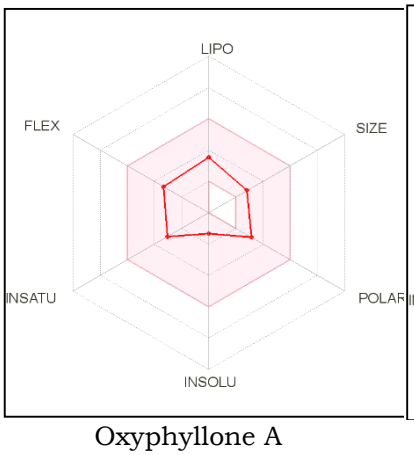
Lupeol



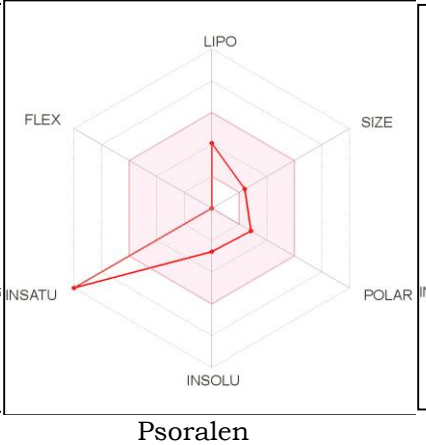
Mangiferin



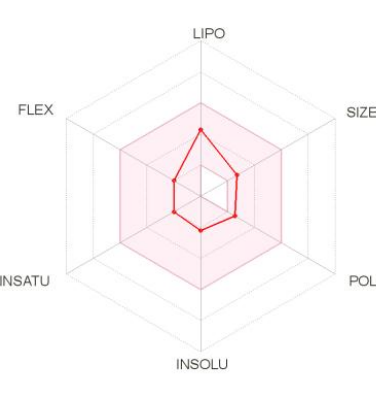
Ottensinin



Oxyphyllone A



Psoralen



Pyranoid

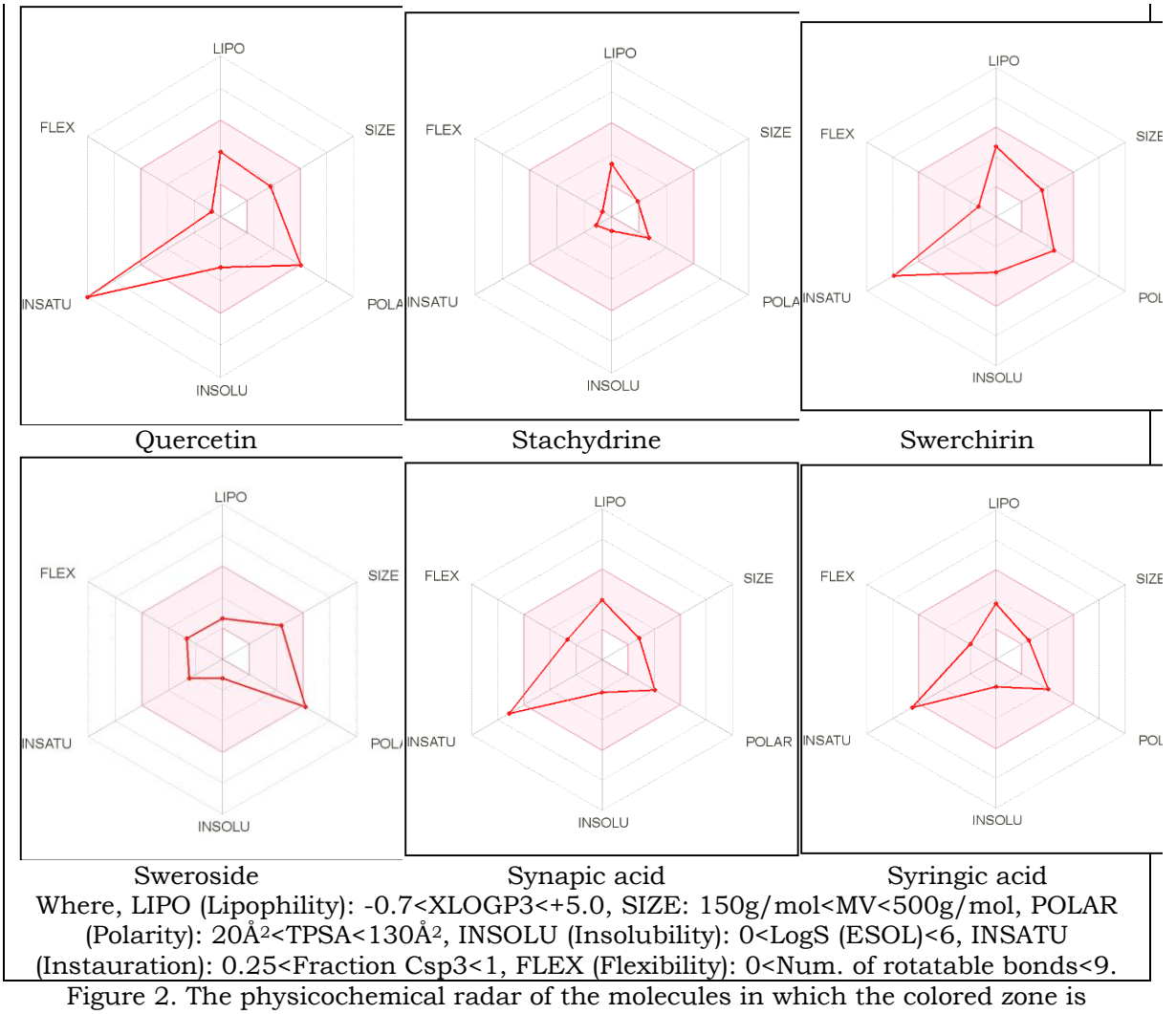


Figure 2. The physicochemical radar of the molecules in which the colored zone is the suitable physicochemical space for oral bioavailability

Table 6
The interaction involved in the molecular docking and docking scores of phytoconstituents with alpha amylase enzyme

Active amino acid residues	Bond length (A^0)	Bond type	Bond category	Ligand energy (kcal/mol)	Binding affinity (kcal/mol)
Bergapten					
ASP353	2.24835	Hydrogen Bond	Conventional Hydrogen Bond	330.12	-6.9
ARG346	3.60114	Electrostatic	Pi-Cation		
PHE348	4.88237	Hydrophobic	Pi-Pi T-shaped		
PHE348	5.16287				

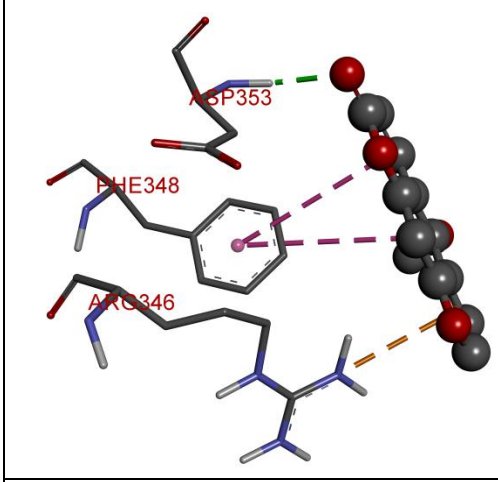
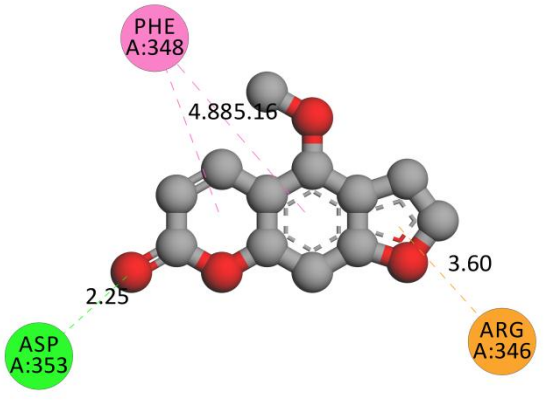
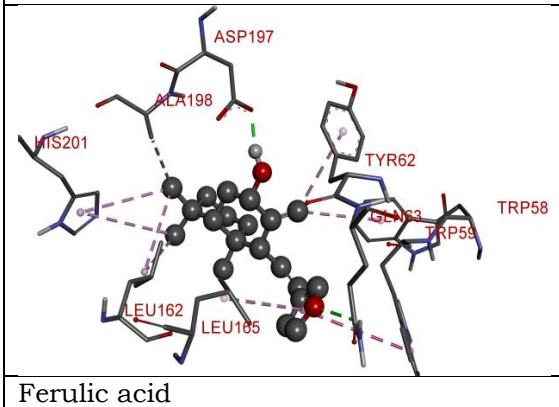
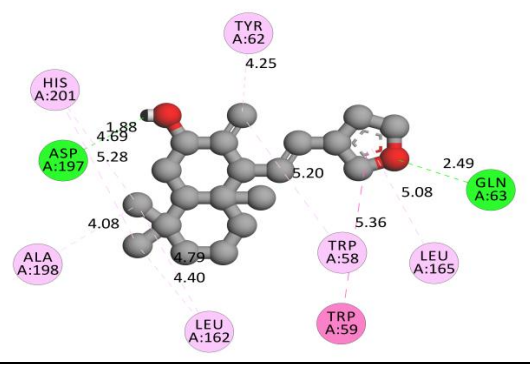
Coronarin					
ASP197	1.88409	Hydrogen Bond	Conventional Hydrogen Bond	351.49	-8.9
GLN63	2.48595				
TRP59	5.36007	Hydrophobic	Pi-Pi T-shaped		
LEU162	4.40127		Alkyl		
LEU162	4.7906				
ALA198	4.07741				
LEU165	5.07968		Pi-Alkyl		
TRP58	5.19862				
TYR62	4.24888				
HIS201	5.27859				
HIS201	4.68955				
Ferulic acid					
GLU233	2.31917	Hydrogen Bond	Conventional Hydrogen Bond	72.7	
TYR62	4.74622	Hydrophobic	Pi-Pi Stacked		-6.5
Glucocapparin					
HIS299	2.28723	Hydrogen Bond	Conventional Hydrogen Bond	684.58	
ASP300	2.18364				
ASP300	2.03198				-6.7
Kaemferol					
GLU233	2.59309	Hydrogen Bond	Conventional Hydrogen Bond	179.75	-8.6
GLN63	2.5179				
GLN63	2.49236				
ASP300	4.26756	Electrostatic	Pi-Anion		
TRP59	5.70239	Hydrophobic	Pi-Pi Stacked		
TRP59	4.14375				
TRP59	5.27155				
TRP59	4.01457				
Katsumadain					
ASP300	2.23996	Hydrogen Bond	Conventional Hydrogen Bond	215.54	-8.6
HIS305	1.94438				
ILE235	3.464	Hydrophobic	Pi-Sigma		
HIS201	4.36038		Pi-Pi T-shaped		
LEU165	5.0511		Alkyl		
LEU162	5.40137		Pi-Alkyl		
ALA198	5.42267				
LYS200	4.66232				
HIS305	4.6666				
Lupeol					
TRP59	3.76411	Hydrophobic	Pi-Sigma	659.96	-10.5
LEU162	5.37307		Alkyl		
LEU165	4.75354				
LEU165	4.47825				
LEU162	5.30571				
ALA198	3.69597				
TRP59	4.75675		Pi-Alkyl		

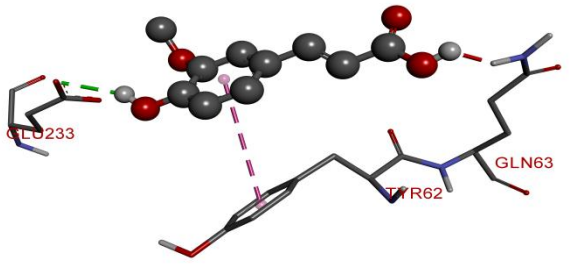
TRP59	4.49513				
TRP59	4.08603				
TRP59	4.68843				
TYR62	5.12162				
TYR62	4.22054				
HIS101	5.23772				
HIS299	5.31362				
HIS305	4.59792				
Mangiferin					
ASP317	2.30562	Hydrogen Bond	Conventional Hydrogen Bond	279.92	-9
ILE312	2.60931				
GLN302	2.76005				
ASP356	2.84299				
ARG267	3.04399				
ARG267	2.14129				
ARG346	2.04051				
ARG346	3.4459		Carbon Hydrogen Bond		
ASP353	2.95773		Pi-Donor Hydrogen Bond		
GLY304	3.57905	Hydrophobic	Pi-Sigma		
PHE348	4.80638		Pi-Pi T-shaped		
Ottensinin					
TRP59	4.35912	Hydrophobic	Pi-Pi Stacked	331.62	-9
TRP59	4.93388				
LEU162	4.45137		Alkyl		
TRP58	4.67678		Pi-Alkyl		
TYR62	5.27412				
TYR62	4.33823				
TYR62	4.51021				
HIS299	5.3624				
HIS305	5.10326				
HIS305	4.69189				
Oxyphyllone					
GLN63	2.7658	Hydrogen Bond	Conventional Hydrogen Bond	190.03	-6.3
HIS305	2.28099				
TRP59	3.62001	Hydrophobic	Pi-Sigma		
TRP59	5.31264		Pi-Alkyl		
Psoralen					
ASP300	3.42862	Hydrogen Bond	Carbon Hydrogen Bond	303.41	-6.9
ASP300	4.18844	Electrostatic	Pi-Anion		
LEU165	4.81504	Hydrophobic	Pi-Alkyl		
Pyranoid					
TYR62	3.79584	Hydrophobic	Pi-Sigma	2001.1	-6.8
LEU165	4.27502		Alkyl		
TRP59	4.68283		Pi-Alkyl		

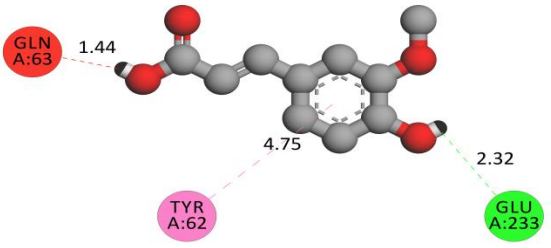
TRP59	3.8389				
TRP59	4.94421				
TRP59	5.44235				
TRP59	3.86894				
TRP59	4.01109				
Quercetin					
GLN63	2.59916	Hydrogen Bond	Conventional Hydrogen Bond		
TRP59	5.68867	Hydrophobic	Pi-Pi Stacked		
TRP59	4.14655				
TYR62	4.35875				
TRP59	5.27938				
TRP59	4.063				
Stachydrine					
ASP300	3.36163	Electrostatic	Attractive Charge	977.88	-5.1
ASP197	2.3402	Hydrogen Bond	Conventional Hydrogen Bond		
HIS305	3.56281		Carbon Hydrogen Bond		
Swerchirin					
ASP197	2.41617	Hydrogen Bond	Conventional Hydrogen Bond	195.17	-7.7
HIS305	3.39361		Carbon Hydrogen Bond		
TRP59	5.86656	Hydrophobic	Pi-Pi Stacked		
TRP59	5.52613				
LEU165	5.36866		Pi-Alkyl		
Sweroside					
HIS292	2.16907	Hydrogen Bond	Conventional Hydrogen Bond	1425.36	-8.2
ASP300	2.3411				
ASP300	2.58387				
HIS305	3.56214		Carbon Hydrogen Bond		
ASP197	4.83727	Electrostatic	Pi-Anion		
TYR62	5.34085	Hydrophobic	Pi-Pi Stacked		
LEU165	4.82278		Alkyl		
TRP59	4.29992		Pi-Alkyl		
TRP59	3.61002				
Synapic acid					
ARG39	2.70618	Hydrogen Bond	Conventional Hydrogen Bond	115.36	-6.8
ARG252	2.3418				
ARG252	2.58633				
ARG421	2.06568				
ARG10	3.59759		Carbon Hydrogen Bond		
SER289	3.61288				
PRO332	3.47419				
PHE335	5.06983	Hydrophobic	Pi-Pi T-shaped		

Syringic acid					
HIS305	3.07699	Hydrogen Bond	Conventional Hydrogen Bond	96.45	-5.9
HIS305	2.40205				
ASP197	3.73782		Carbon Hydrogen Bond		
TYR62	3.81397	Hydrophobic	Pi-Sigma		

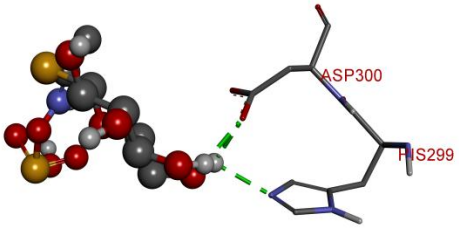
Table 7
The most potent compounds' 2D and 3D binding poses

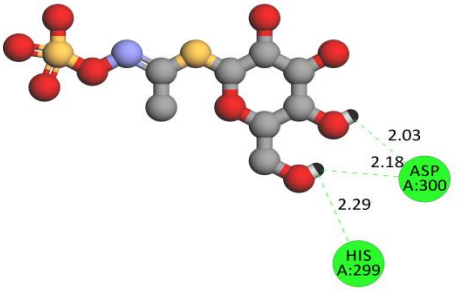
3D-binding pose	2D-binding pose
Bergapten	
	
Coronarin A	
	
Ferulic acid	



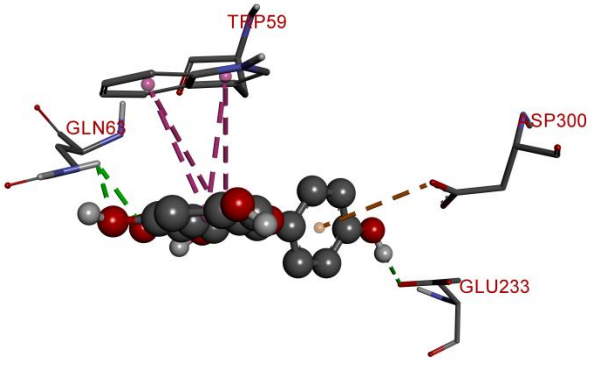


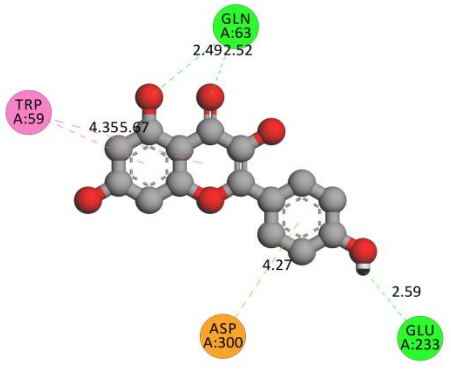
Glucocapparin



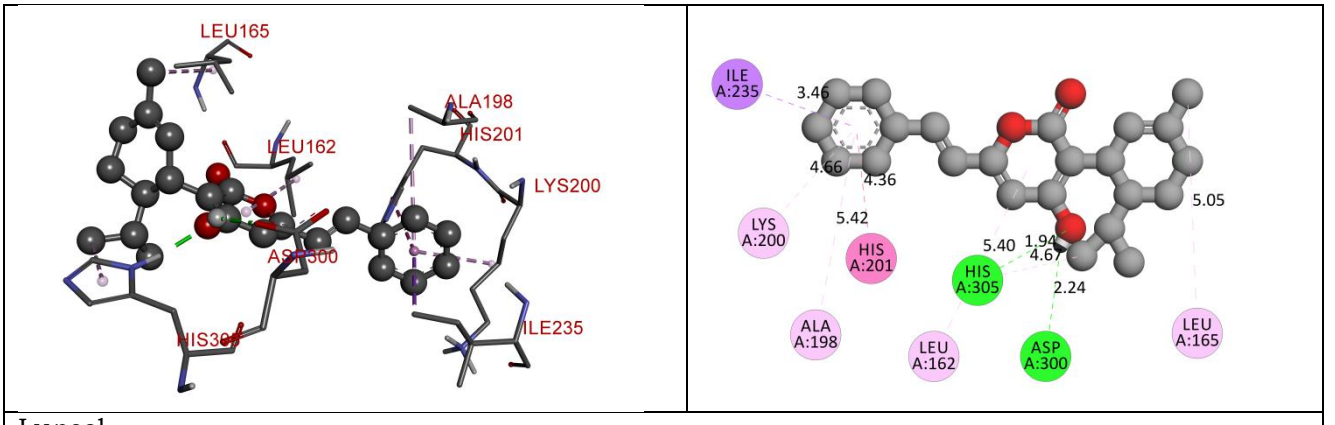


Kaemferol

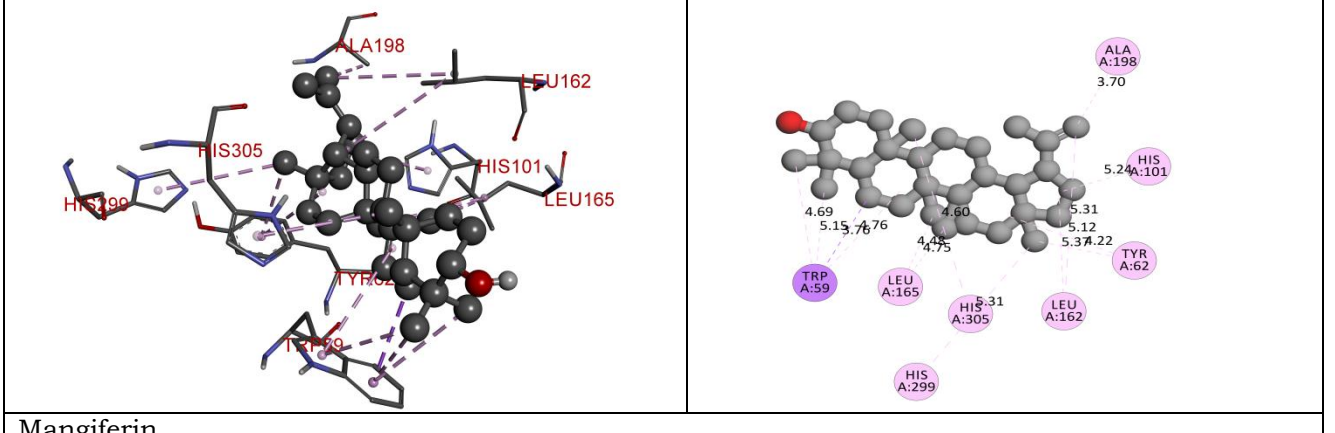




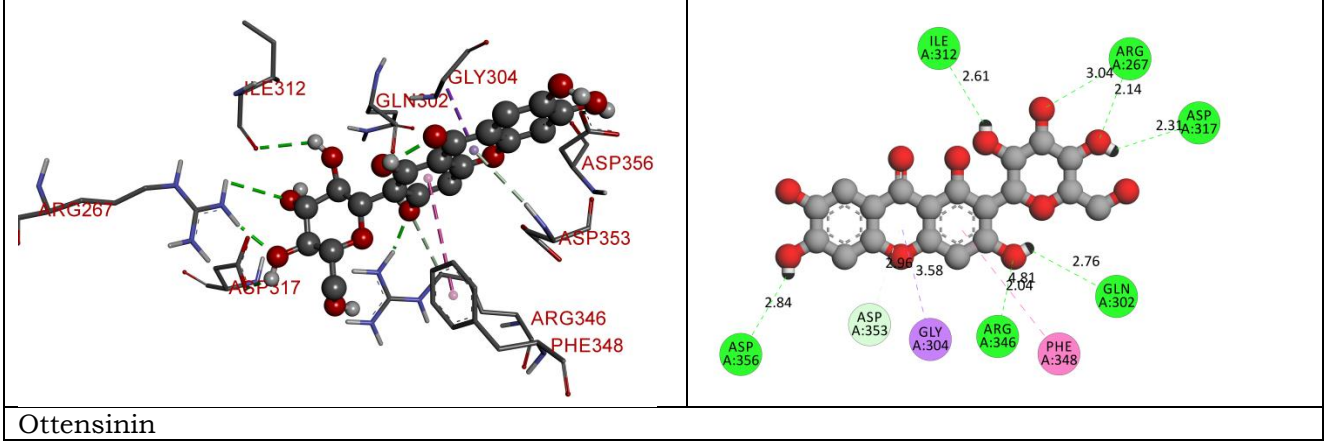
Katsumadain



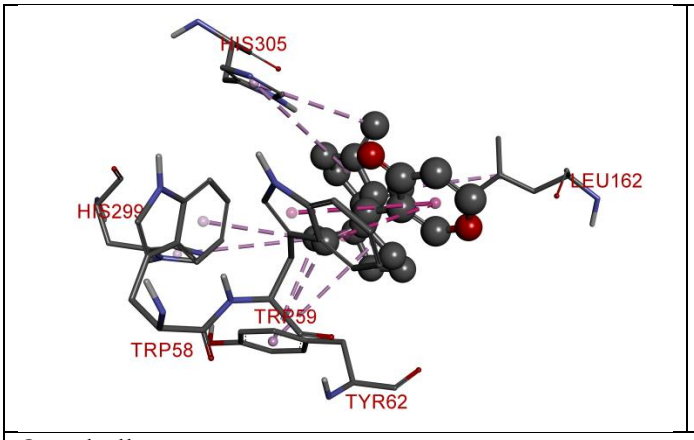
Lupeol

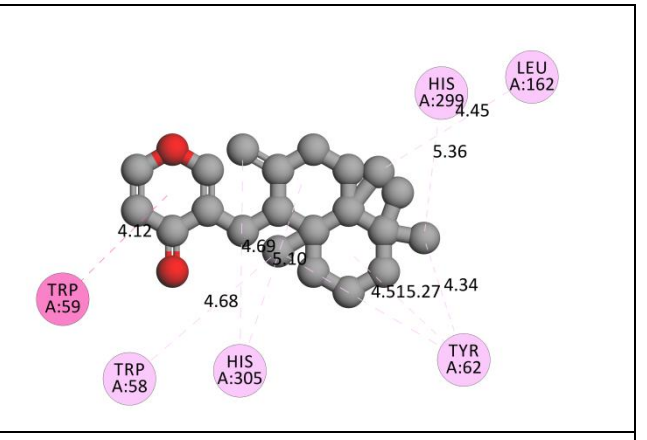


Mangiferin

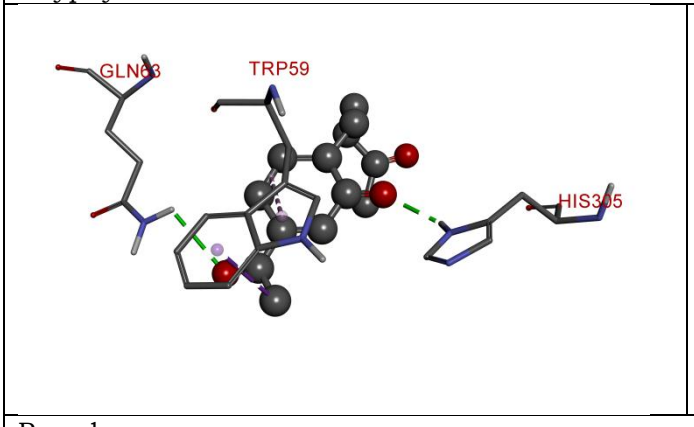


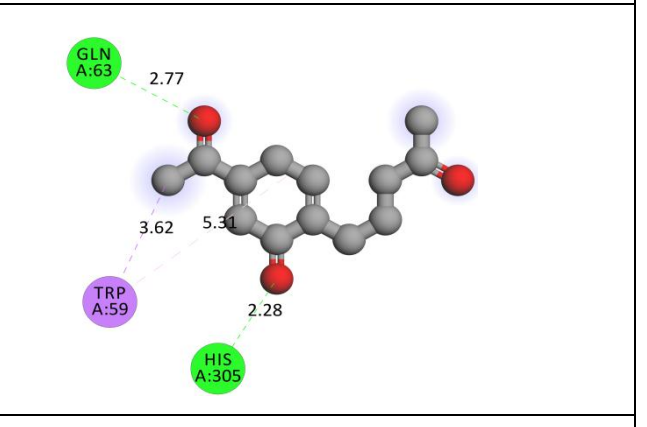
Ottensinin



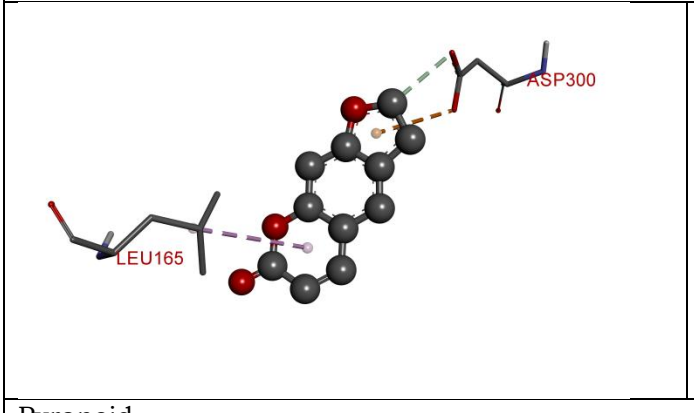


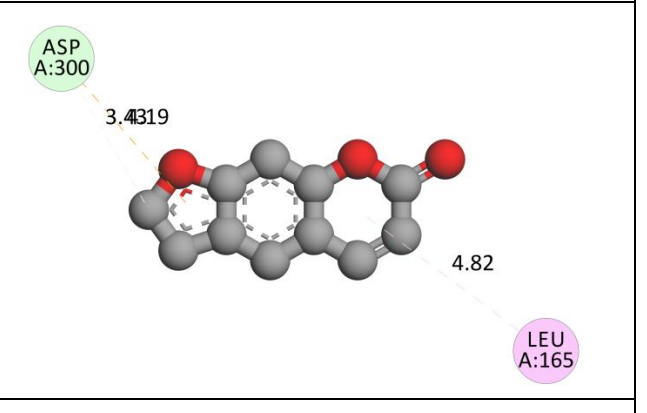
Oxyphyllone



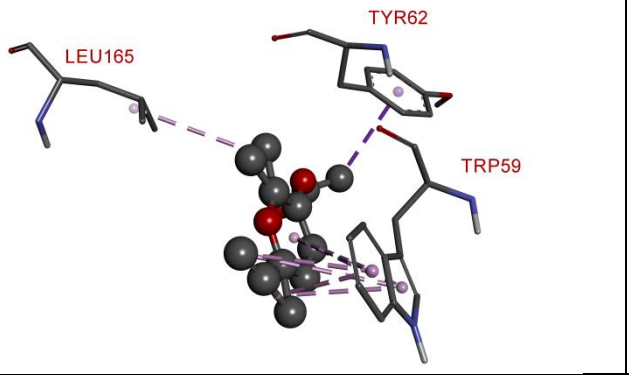


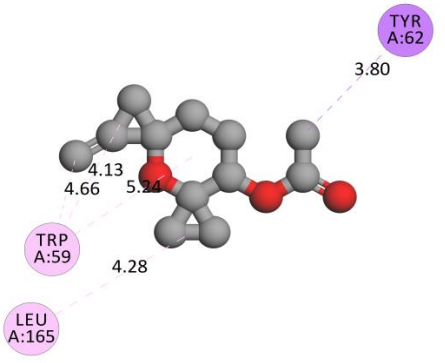
Psoralen



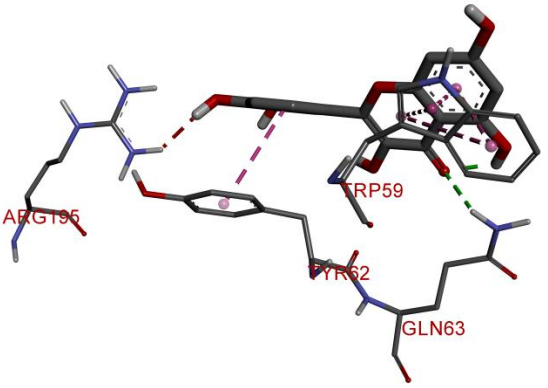


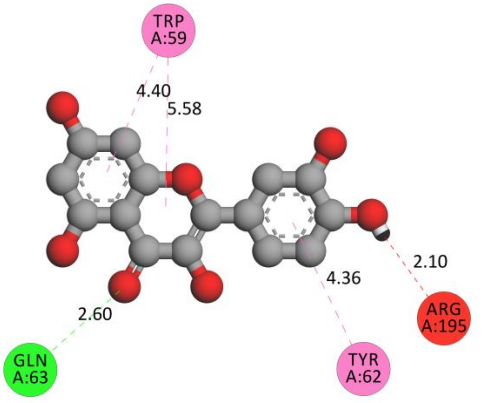
Pyranoid



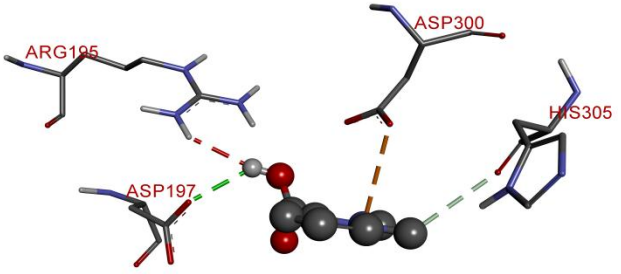


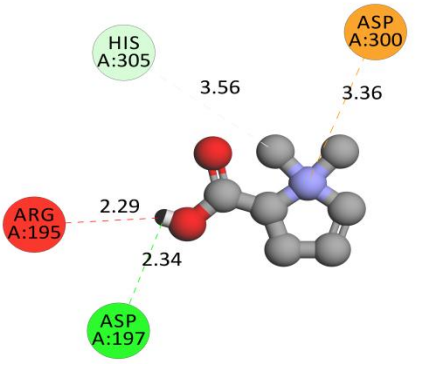
Quercetin



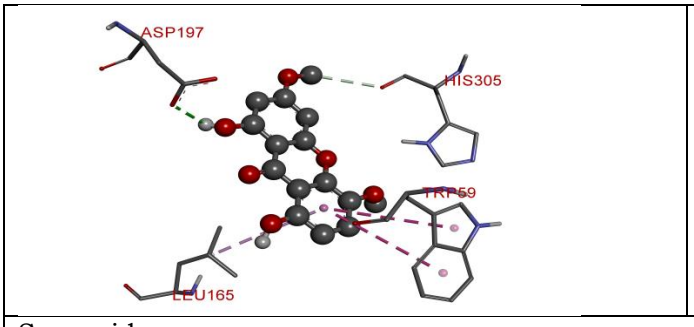


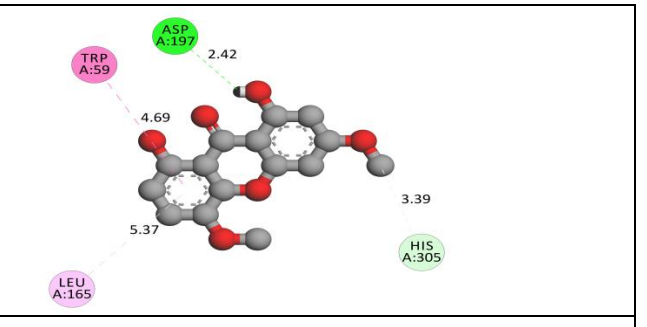
Stachydrine



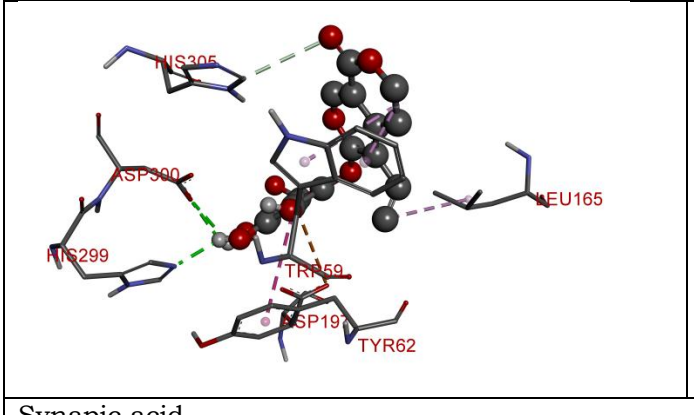


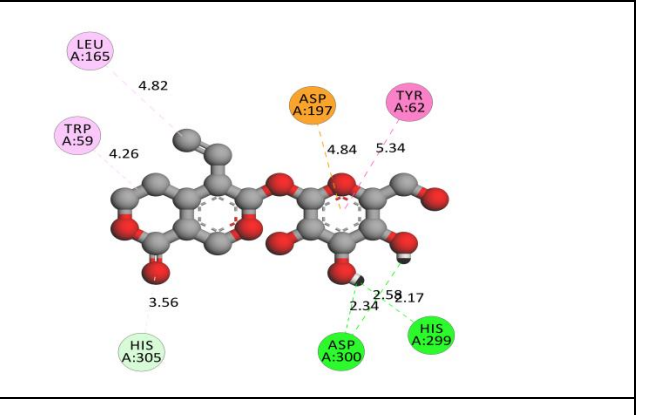
Swerchirin



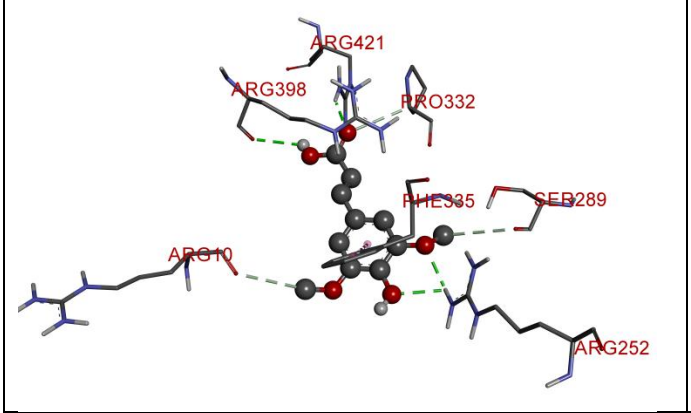


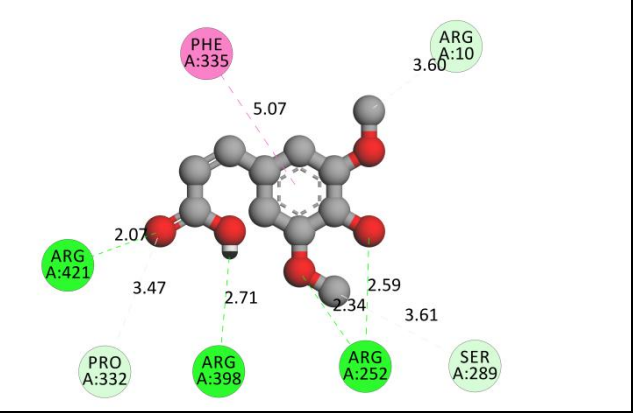
Sweroside





Synapic acid





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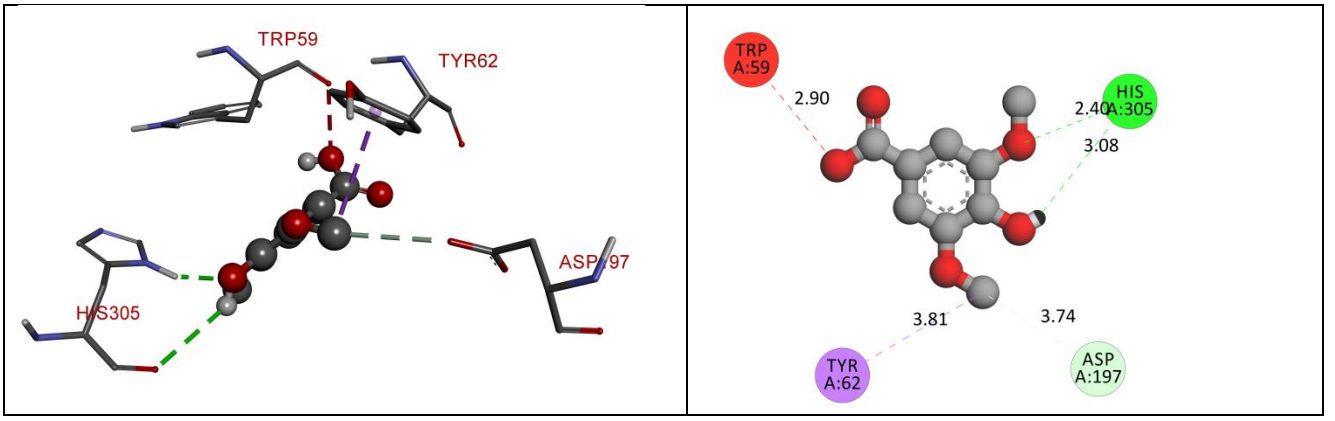

The *in vivo* antidiabetic activity of the formulations are tabulated in the tables given below.

Table 8
Effect of F1, F2, F3, and F4 on body weight and serum glucose levels

Day	Normal control group	Diabetic control group	F1 group	F2 group	F3 group	F4 group	Metformin group
Body weight (g)							
0	218 ± 1.7	216 ± 1.6	218 ±2.2	214 ± 1.8	215 ± 1.7	216 ± 1.5	216 ± 1.5
7	317 ± 1.8	304 ± 4.4$^{\gamma}$	319 ± 2.1^{c}	321 ± 1.1^{b}	318 ± 2.2^{c}	321 ± 2.0^{b}	323 ± 0.99^{a}
14	337 ± 2.5	252 ± 3.1$^{\alpha}$	319 ± 3.2^{a}	323 ± 3.0^{a}	317 ± 1.7^{a}	330 ± 2.1^{a}	351 ± 3.1^{a}
21	336 ± 2.9	227 ± 2.5$^{\alpha}$	294 ± 7.6^{a}	307 ± 2.5^{a}	286 ± 5.7^{a}	330 ± 2.9^{a}	356 ± 2.5^{a}
Serum glucose (mg/dl)							
0	91.0 ± 2.1	90 ± 2.2	89 ± 2.0	90 ±1.8	90 ± 1.4	90 ± 1.4	91 ± 2.2
7	110 ± 2.8	362 ± 1.6$^{\alpha}$	280 ± 4.4^{a}	258 ± 6.7^{a}	282 ± 4.8^{a}	239 ± 3.7^{a}	234 ± 3.8^{a}
14	127 ± 1.4	356 ± 1.8$^{\alpha}$	258 ± 3.9^{a}	246 ± 3.0^{a}	260 ± 4.4^{a}	236 ± 1.9^{a}	222 ± 3.0^{a}
21	124 ± 1.1	349 ± 1.1$^{\alpha}$	183 ± 2.5^{a}	173 ± 0.9^{a}	185 ± 2.3^{a}	166 ± 1.4^{a}	157 ± 5.0^{a}

Data were expressed as mean±SEM, n=6, and analyzed by ANOVA followed by Tukey's post hoc test. $^{\alpha}p<0.001$, $^{\gamma}p<0.05$, when compared to the normal control group; $^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$, when compared to diabetic control group.

Table 9
Effect of F1, F2, F3, and F4 on OGTT

Groups	0 min	30 min	60 min	120 min	AUC
Normal control	116 ± 6.0	161 ± 4.4	135 ± 2.7	117 ± 5.3	268 ± 5.0
Diabetic control	155 ± 2.5[α]	205 ± 3.2[α]	179 ± 2.9[α]	167 ± 4.3[α]	360 ± 2.5[α]
F1	130 ± 1.9[a]	162 ± 6.2[a]	149 ± 1.6[a]	136 ± 3.6[a]	292 ± 4.8[a]
F2	124 ± 2.4[a]	159 ± 5.6[a]	148 ± 1.6[a]	134 ± 3.2[a]	289 ± 4.5[a]
F3	128 ± 1.4[a]	162 ± 6.3[a]	149 ± 2.2[a]	135 ± 3.4[a]	280 ± 9[a]
F4	126 ± 2.5[a]	156 ± 5.1[a]	143 ± 2.2[a]	131 ± 3.3[a]	283 ± 5.0[a]
Metformin	118 ± 3.0[a]	150 ± 5.3[a]	140 ± 2.1[a]	120 ± 3.0[a]	269 ± 5.8[a]

Data were expressed as mean±SEM, n=6, and analyzed by ANOVA followed by Tukey's post hoc test. [α]$p<0.001$, when compared to the normal control group; [a]$p<0.001$, when compared to diabetic control group.

Table 10
Effect of F1, F2, F3, and F4 on SGOT and SGPT levels

Parameters	Normal control group	Diabetic control group	F1 group	F2 group	F3 group	F4 group	Metformin group
SGOT (U/L)	43 ± 1.9	75 ± 1.5[α]	55 ± 1.6[a]	55 ± 1.7[a]	55 ± 1.5[c]	56 ± 1.8[a]	54 ± 1.7[a]
SGPT (U/L)	38 ± 1.4	89 ± 1.8[α]	53 ± 1.5[a]	56 ± 1.5[a]	53 ± 1.6[b]	57 ± 1.5[a]	54 ± 1.2[a]

Data were expressed as mean±SEM, n=6, and analyzed by ANOVA followed by Tukey's post hoc test. [α]$p<0.001$, when compared to the normal control group; [a]$p<0.001$, [b]$p<0.01$, when compared to diabetic control group.

Discussion

The alpha-amylase enzyme is regarded as a good target for antidiabetic agents to design the drug and provide an alternate approach for the treatment of T2DM.We tried to identify the potential natural lead compounds from *tea bag* as human alpha amylase enzyme Inhibitors through their binding mode in the enzyme's allosteric site and binding free energies. Herbal teas are actually mixtures of several ingredients, and are more accurately known as 'tisanes'. Tisanes are made from combinations of dried leaves, seeds, grasses, nuts, barks, fruits, flowers, or other botanical elements that give them their taste and provide the benefits of herbal teas. Therefore from present investigation we have decided to formulate polyherbal tea bag containing *Ichnocarpus frutescens, Ficus dalhousiae, Crateva magna, Alpinia galangal*, and *Swertia chirata*. From qualitative phytochemical screening it was observed that the infusion extract possess numerous kind of chemicals such as alkaloid, carbohydrates, saponins, glycoside, fats and fixed oils, resins, phenols, tannins, diterpins, flavonoids, and proteins. The structure and names of selected phytoconstituents from each plant is given in Table 1. In accordance with Lipinski's and Veber's rule (Table 4) Lupeol & Mangiferin violated

Lipinski's rule whereas Glucocapparin & Mangiferin violated veber's rule .The log P values of all the molecules found between -0.30 to 4.38 which indicate optimum lipophilicity. Lipophilicity is a significant feature of the molecule that affects how it works in the body(S. Khan et al., 2021).

It is determined by the compound's Log P value, which measures the drug's permeability in the body to reach the target tissue(Krzywinski and Altman, 2013; Lipinski et al., 2012). The molecular weight of all the molecules was below 500 Da which indicates active better transport of the molecules through biological membrane. Fortunately, the Lipinski rule of 5 had not been compromised by the compounds, including native ligand, indicating improved absorption and/or lipophilicity(Khan et al., 2022; Shntaif et al., 2021). Further optimization of the compounds was done by calculating the pharmacokinetics and drug-likeness properties for each one. The compounds Glucocapparin , Kaemferol , Lupeol , Mangiferin , Quercetin , Stachydrine , Swerchirin , Sweroside , Synapic acid , and Syringic acid failed to penetrate the blood-brain barrier (BBB), which is not ideal for medications targeting the central nervous system. The log *Kp* (skin penetration, cm/s) and bioavailability values of all the compounds were within acceptable limits. All of the molecules passed the Ghose, Egan, and Muegge requirements (Table 5). Absorption in the gastrointestinal tract was high for all of the compounds tested. Many derivatives exhibited more than two hydrogen bonds with the target. The formation of hydrogen bonds with target can effectively modulate the activity of enzyme and exhibit potent pharmacological response. There are many compounds which exhibited more binding affinity but did not formed more than three hydrogen bonds.

Coronarin with ligand energy 351.49 kcal/mol formed 2 hydrogen bonds with Asp197 & Gln63. Along with Hydrophobic type of bond such as Pi-Pi T-shaped with Trp59, alkyl with Leu162, Ala198, Pi-alkyl with Leu165, Trp58, Tyr62, and His201. Kaemferol has binding affinity -8.6 with ligand energy of 179.75 kcal/mol, and formed 3 conventional hydrogen bonds with Glu233, Gln63 along with electrostatic interactions such as Pi-alkyl with Asp300. It also exhibited 4 hydrophobic interactions such as Pi-Pi stacked with Trp59. Katsumadain displayed -8.6 kcal/mol docking score and has 215.54 kcal/mol ligand energy. It formed 2 conventional hydrogen bonds with Asp300 & His305. It has developed hydrophobic interactions such as Pi-sigma, Pi-Pi T-shaped, alkyl, Pi-alkyl with Ile235, His201, Leu165, Leu162, Ala198, Lys200, and His305. Lupeol exhibited -10.5 docking score and ligand energy of 659.96 kcal/mol. It has formed hydrophobic interactions such as Pi-sigma, alkyl, Pi-alkyl with Trp59, Leu162, Leu165, Ala198, Trp59, Tyr62, His101, His299, and His305.

Mangiferin exhibited -9 kcal/mol binding affinity and showed 279.92 kcal/mol ligand energy. It has formed conventional, carbon- and Pi-donor hydrogen bond with Asp317, Ile312, Gln302, Asp356, Arg267, Arg346, and Asp353. It has also developed hydrophobic interactions such as Pi-sigma and Pi-Pi T-shaped with Gly304 and Phe348. Ottensinin showed docking score of -9 kcal/mol and having 331.62 kcal/mol ligand energy. It has developed hydrophobic interactions (Pi-Pi stacked, alkyl, Pi-alkyl) with Trp59, Leu162, Trp58, Tyr62, His299, and His305. Sweroside showed -8.2 kcal/mol binding affinity and formed three conventional

hydrogen bonds with His292, Asp300 and carbon-hydrogen bond with His305. It also showed electrostatic interactions (Pi-anion) with Asp197 and hydrophobic interactions (Pi-Pi stacked, alkyl, Pi-alkyl) with Tyr62, Leu165, and Trp59.

The MD studies was done of all the phytoconstituents and native ligand against the crystal structure of alpha amylase enzyme depicted in fig 1. The extracts (F1, F2, F3 and F4) were prepared in the tea bag form and tested for its antidiabetic activity shown in table 2. Approximate infusion time of 10 min for loose herbs and 2 min for tea bags was enough for extraction of phenols and antioxidants. Maximum infusion time for the tea bag was <30 s to 2 min. Even the infusion times sometimes vary from 30 s to 5 min. Hyperglycemia was induced by a single intraperitoneal injection of freshly prepared streptozotocine (STZ). Table 3 depicts the experimental design of the animal activity in which the basal body weight of animals of all the groups was found to be statistically equivalent. Diabetes induction caused significant decrease in the body weight during the experimental period when compared to NC group. The mean body weights of NC group, at the end of the treatment period was found to be 218 ± 1.7grams, this was significantly ($p<0.001$) decreased to 216 ± 1.6 grams in DC group. The decreased body weight was significantly ($p<0.001$) improved F1, F2, F3, F4, and metformin groups. The basal serum glucose level of animals of all groups was found to be statistically equivalent. Over the course of the trial, the DC group had a considerable rise in blood glucose levels, indicating that they were diabetic. When comparing the DC to the NC group, the blood glucose levels of the DC group were considerably ($p<0.001$) higher (from 90±2.2 to 349±1.1mg/dl) at the conclusion of the research. The increased serum glucose level was significantly decreased with treatment with the compounds F1, F2, F3, F4, and metformin ($p<0.001$).

Effect on OGTT

NC group serum **OGTT** levels were observed at specific time intervals of 0 min, 30 min, 60 min, 120 min along with the AUC curve. NC group level was 268 ±5.0, whereas DC group **OGTT** levels were considerably ($p<0.001$) higher at 360±2.6 mg/dl. Treatment with compounds F1, F2, F3, and metformin ($p<0.001$), F4 ($p<0.01$), and F5 ($p<0.05$) considerably reduced these elevated levels.

Effect of Compounds on Serum SGOT and SGPT

The mean serum SGOT level of NC group was 43±1.9U/L, which significantly ($p<0.001$) increased to 75±1.5 U/L in DC group, the increased levels were significantly decreased in F1, F2, F3 and metformin group ($p<0.001$), F4 group ($p<0.05$), when compared to DC group. Diabetes induction caused significant increase ($p<0.001$) in SGPT levels from 38±1.4U/L to 89±1.8U/L, when compared to the NC group. The increased SGPT levels were significantly decreased in F1, F2, F3 and metformin group ($p<0.001$), F4 group ($p<0.01$), when compared to DC group.

Conclusion

In present study, *in vivo* antidiabetic activity of polyherbal tea bag formulation have been performed in STZ-induced diabetic model in rats. The antidiabetic potential have been determined by estimating different biochemical parameters such as body weight, serum glucose level, SGOT (AST), and SGPT (ALT) levels. Amongst all the polyherbs, *Alpinia galanga* demonstrated significant antidiabetic when combined with *Creteva magna* along with this it showed high binding affinity. The antidiabetic potential of the polyherbal formulation is comparable with that of Metformin, which is evidenced by decreased levels of blood glucose, SGOT, and SGPT. Thus, our findings demonstrate that the polyherbal tea bag formulation possess significant antidiabetic activity and if optimize latter can be used clinically to overcome diabetes in a very convenient way.

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