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Natural Products as Prominent Source of Bioactive Components with Anti-diabetic Potential

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Abstract

Type 2 diabetes (T2DM) is a chronic metabolic condition characterized by elevated blood sugar levels. It is caused by a combination of insulin resistance and insulin production impairment. The nuclear transcription factor peroxisome proliferator-activated receptor gamma (PPAR-gamma) is essential for glucose homeostasis and lipid metabolism. PPAR-γ agonists are a family of medicines used to manage type 2 diabetes by improving blood sugar management and enhancing insulin sensitivity. For decades, natural materials have been utilized as traditional remedies, and many of them have been demonstrated to have anti-diabetic properties. Some natural compounds have been proven in recent investigations to activate PPAR-γ. We employed molecular docking and physicochemical screening in this investigation to discover natural compounds with the potential to be developed as novel anti-diabetic medicines. A library of more than 50 natural compounds was tested against the PPAR-γ ligand binding domain. We also assessed the ADMET and physicochemical features of the

compounds found to determine that they are drug-like. Our study shows the Drug-likeness, bioactivity score along with good ADMEt profile of various phytoconstituents with their high binding affinity toward PPAR-g (PDB ID: 2XKW) as a major target for T2DM. Physicochemical properties of selected compounds were done with SWISS ADME server while ADMEt screening was done by pkCSM server. The binding affinity and molecular interaction study of natural compounds with PPAR-γ was done by using Molegro Virtual Docker (MVD). The MolDock and Rerank score of the top one compound of each category was taken to check the binding interaction with tubulin.

Keywords: Type-2 diabetes, PPAR-γ, Molecular docking, Physicochemical properties, ADMET, Drug likeness.

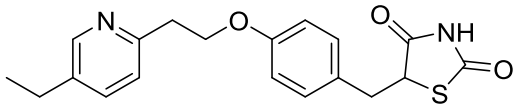
1. Introduction

Diabetes mellitus (DM) is an endocritic dysfunction condition that is inherited, chronic, potentially debilitating, and often fatal. It is caused by tissues' failure to perform normal protein, lipid, and carbohydrate metabolism. Diabetes is classified into two types: type 1 (insulin dependent) and type 2 (non-insulin dependent) (Qi et al., 2010). Type 1 diabetes mellitus (Type 1 DM) is distinguished by an absolute absence of insulin production, as well as auto-immune destruction of pancreatic cells (Merger, Leslie, & Boehm, 2013). Relatives of people who have the illness are more likely to get it (Baynes, 2015). Type 2 diabetes mellitus, which accounts for more than 90% of cases and is mostly caused by obesity and inactivity, is caused by insulin resistance as well as decreased insulin production (Olokoba, Obateru, & Olokoba, 2012). Long-term continuous hyperglycemia is known to affect numerous biological systems, including the nerves, blood vessels, heart, eyes, and kidneys. As a consequence, significant macrovascular and microangiopathy problems such as retinopathy, nephropathy, and peripheral neuropathy develop. Population expansion, ageing, urbanisation, lifestyle changes, and a growing incidence of obesity and physical inactivity have all contributed to an exponential global rise in the number of persons diagnosed with diabetes during the last two decades. In 1985, an estimated 30 million individuals globally developed diabetes (Bazzano, Joint, & Organization, 2005). According to the most recent data from the International Diabetes Federation (IDF) and World Health Organisation (WHO), diabetes now affects a staggering 246 million people worldwide, with 46% of those affected in the 40-59 age group during their economically most productive years, and this figure is expected to rise to at least 380 million by 2025 (Shaw, Sicree, & Zimmet, 2010). The prevalence of diabetes in Nauru is the highest in the world, while India has the biggest DM population with an estimated 41 million people (Khambalia et al., 2011). Diabetes, along with cancer, cardiovascular and cerebrovascular illnesses, is becoming the third "killer" of humanity's health due to its high incidence, morbidity, and death. In the year 2000, the extra worldwide mortality related to diabetes was projected to be 2.9 million fatalities or 5.2% of all deaths. Even

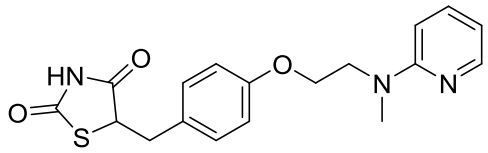
more, people die from cardiovascular disease, which is exacerbated by DM-related lipid abnormalities and hypertension. Primary diabetes and its complications are expensive to treat, not just for afflicted people but also for healthcare systems worldwide (Boutayeb & Boutayeb, 2005). According to the IDF, diabetes currently consumes 5- 10% of the overall healthcare budget in several countries. The United Nations designated 14 November as World Diabetes Day in 2007 to raise awareness of the rising issue of diabetes. Although oral hypoglycemic agents/insulin are the mainstay of DM treatment and are effective in controlling hyperglycemia, the treatment of DM with these chemical drugs is complicated by several disease-related factors, most notably insulin resistance, hyperinsulinemia, hypertension, impaired insulin secretion, and cholesterol abnormalities (Yach, Hawkes, Gould, & Hofman, 2004). The majority of them have substantial side effects and do not appreciably modify the course of diabetes problems. Because the currently available oral hypoglycemic medications and insulin treatment have limits in terms of systemic effectiveness, patient compliance, and side effects, it has sparked a massive global effort to find new therapeutic methods for this metabolic condition. Diabetes' complex pathogenicity necessitates a multimodal therapeutic strategy. Future therapy options may need the combination of multiple kinds of anti-diabetic medications, including a multicomponent-based natural product (NP) approach. The use of natural substances, especially plants, to heal ailments has been practiced for millennia (Tiwari & Rao, 2002). Many therapeutic plants continue to be significant in contemporary medicine, not only because they are still used as crude medication formulations and formulae, but also because they are rich sources of key pure compounds that have become mainstays of modern treatment.

1.1. **Peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist in T2DM management**

The nuclear transcription factor PPAR-gamma regulates metabolism, including glucose homeostasis, lipid metabolism, and inflammation. PPAR-gamma is found in adipose, liver, muscle, and pancreatic tissue. PPAR-gamma increases fat accumulation and insulin-sensitizing adiponectin production in adipose tissue (Pourcet, Fruchart, Staels, & Glineur, 2006). In the liver, PPAR-gamma suppresses glucose synthesis and increases lipid clearance. PPAR-gamma improves muscle glucose absorption and insulin sensitivity. PPAR-gamma supports beta cell insulin synthesis in the pancreas. PPAR-gamma dysfunction causes insulin resistance and other metabolic problems in type 2 diabetes (Israili, 2011). Thus, PPAR-gamma is a key target for type 2 diabetes medication research. This family of medicines activates the nuclear receptor PPAR-gamma. PPAR-gamma regulates glucose, cholesterol, and inflammation. To treat type 2 diabetes, PPAR-gamma agonists increase insulin sensitivity in adipose tissue, liver, and muscle. This lowers triglycerides, free fatty acids, and blood sugar (Zapata-Sudo et al., 2012). The most frequent type 2 diabetes PPAR-gamma agonists are TZDs like pioglitazone and rosiglitazone (**Fig. 1**) (Gerrits et al., 2007). Although TZDs reduce blood sugar, they may also induce fluid retention, weight gain, and heart failure. TZDs like pioglitazone and rosiglitazone increase insulin sensitivity in adipose tissue, liver, and muscle. This lowers blood triglycerides, free fatty acids, and blood sugar (Campbell, 2005). TZDs reduce blood sugar but may induce fluid retention, weight gain, and heart failure. Thus, not all type 2 diabetics should take TZDs.



Pioglitazone



Rosiglitazone

Fig. 1: FDA approved PPAR-γ agonist for effective management of T2DM

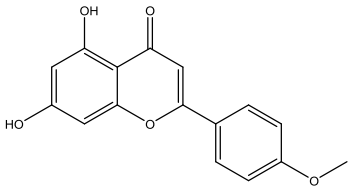
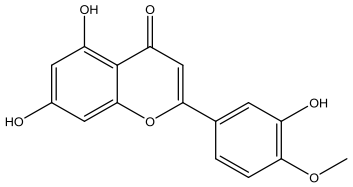
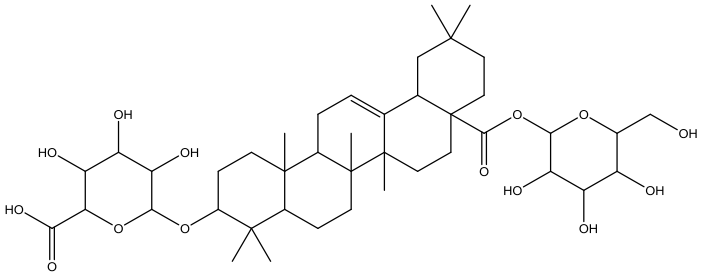
1.2. **Herbal treatment of diabetes mellitus**

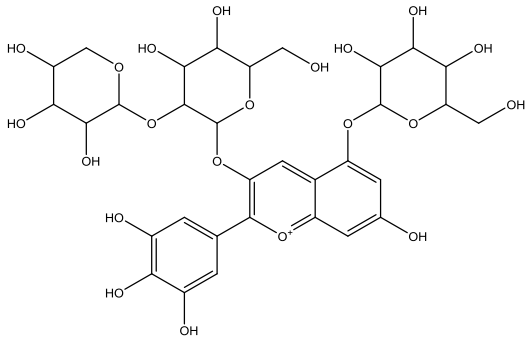
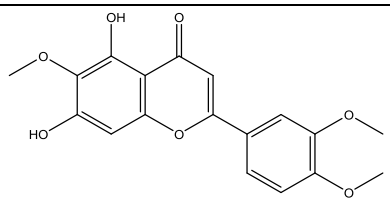
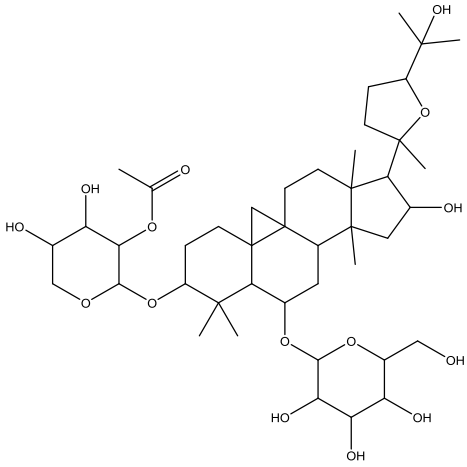
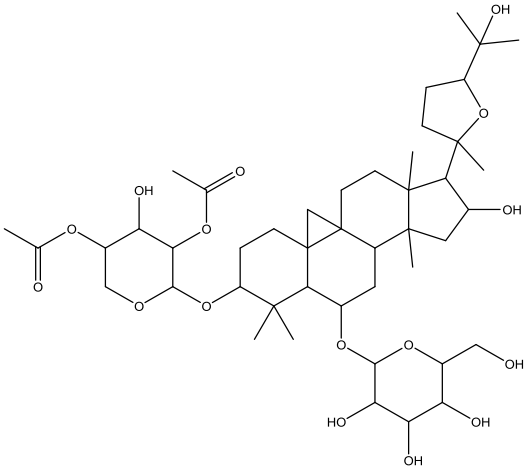
It is worth noting that around half of the medications on the market are of vegetable origin. Metformin, for example, was developed as a structure-modified natural substance from the plant Goat's Rue (*Galega officinalis*) to dramatically increase its potency. In recent years, a large range of NPs, including chemical entities, medicinal plants, and complex formulas including various plants, have been discovered to exhibit hypoglycemic action, making them potential anti-diabetic medicines. Since ancient times, humans have used plants for food, shelter, clothing, flavor, and fragrance. By 2025, the herbal extracts market will be worth USD 59.4 billion (Hudhud, 2007). Plant-based therapy has been used for thousands of years in India and China to cure various ailments. Plants are an infinite source of new molecules, and plant extracts and pure chemicals have a long history of treating numerous illnesses. There are 250,000 to 500,000 plant species on Earth, yet only 1 to 10% are eaten by humans and other animals, and only a limited number have been phytochemically and pharmacologically analyzed. Due to its numerous biologically active chemicals and endless structural variety, the plant has a long history of treating disorders and infectious diseases (Mustafa, Arif, Atta, Sharif, & Jamil, 2017). Indian, Chinese, Greek, Roman, and other traditional medicine systems include medicinal plants with their biological activity and applications. Herbal medicine is in significant demand in basic health care in developed and developing nations because to its lower risk of toxicity, larger safety margins, and lower cost. In developing countries, herbal medicine meets 80% of primary health care requirements. Plants are the wealthiest bio-resource of traditional medicines, nutraceuticals, contemporary medicine, and synthetic medications. Natural items may inspire new pharmaceutical compounds (Biharee et al.). Natural product research has increased in recent decades due to the failure of alternative drug discovery systems to deliver new therapeutic lead compounds to various therapeutic targets like anti-inflammatory, anticancer, immunosuppressant, antimicrobial, anti-diabetic, and other metabolic disorders. Due to the negative effects of oral hypoglycemic medicines for diabetes mellitus, herbal treatments are becoming more popular. Traditional herbal treatments made from plants are used to treat diabetes mellitus. Herbal hypoglycemic agents have grown in popularity recently (Rao,

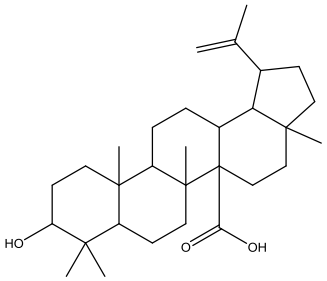
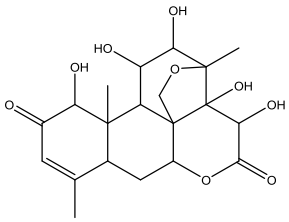
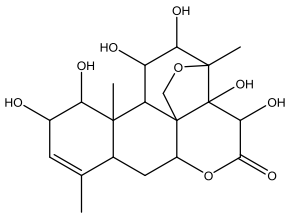
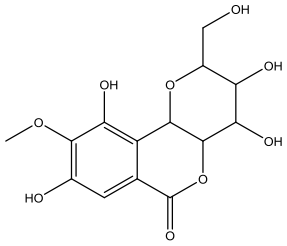
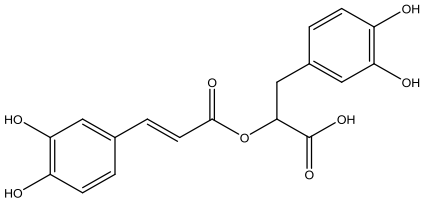
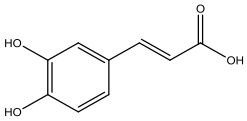
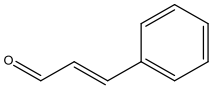
Sreenivasulu, Chengaiah, Reddy, & Chetty, 2010). Diabetes folk medicine involves around 1000 plant species. Chemical composition affects plant items used to treat diabetes. Herbal and plant products include phenolic chemicals, flavonoids, terpenoids, coumarins, and other blood glucose-lowering elements. In scientific and popular literature, some natural medications have antidiabetic properties (Shehadeh, Suaifan, & Abu-Odeh, 2021). Herbal medications are given due to their perceived efficacy, less adverse effects in clinical practise, and cheap cost. Many cultures have long employed medicinal and herbal plant materials to treat diabetes mellitus.

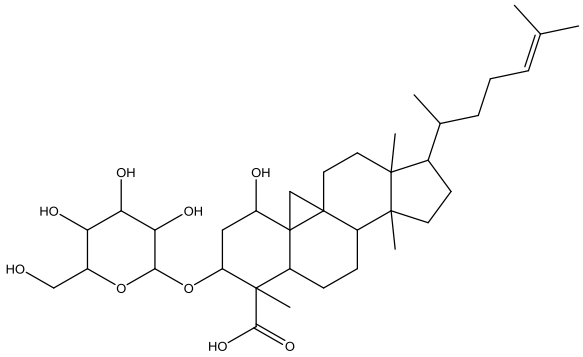
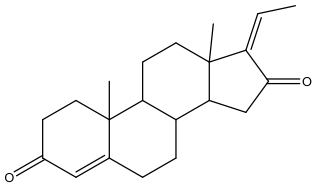
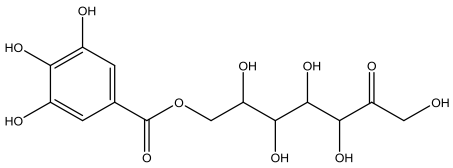
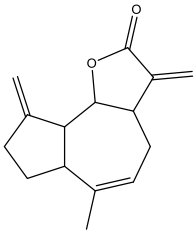
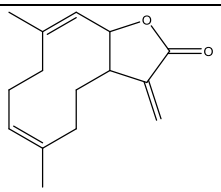
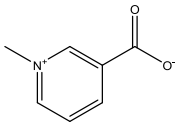
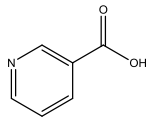
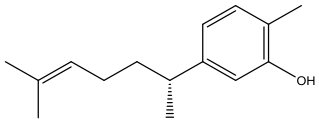
In current scenario search of alternative treatment by exploring natural products gained more attention of both academic and industrial researcher. To extend our urge we have selected 52 phytoconstituents reported in literature from various plants (**Table 1**). These 52 compounds were subjected to physicochemical screening and ADMET prediction to check their drug likeness. Furthermore, these compounds were docked with PPAR-γ protein that is a prominent target for type-2 diabetes mellitus. Their binding affinity toward PPAR-γ was determine in terms of MolDock score and the value obtained were compared with Rosiglitazone and Pioglitazone.

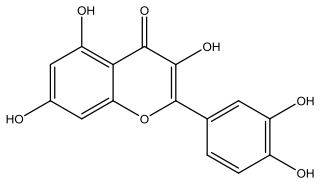
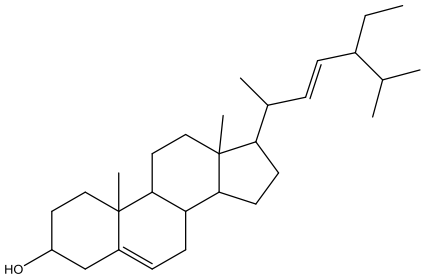
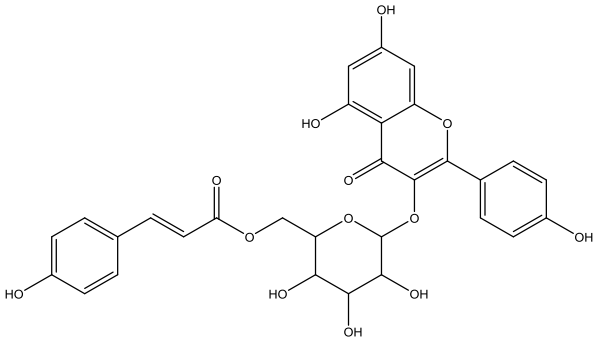
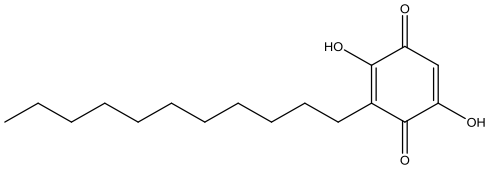
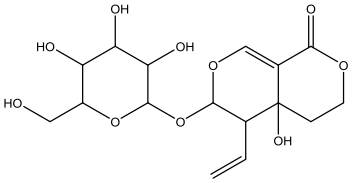
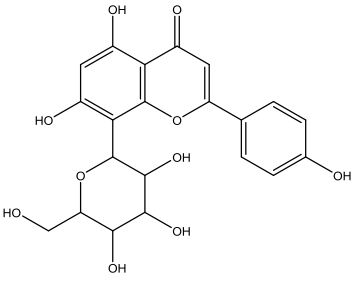
Table 1: List of Phytoconstituents selected for screening

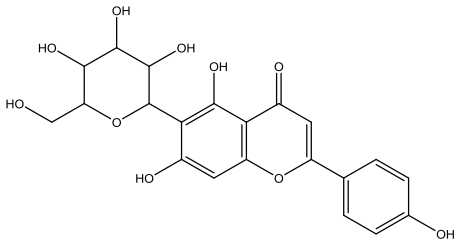
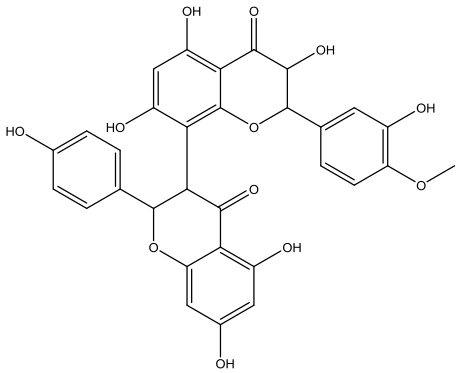
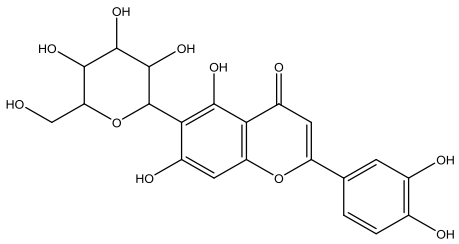
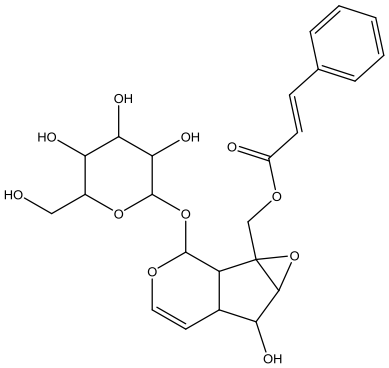
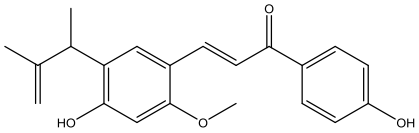
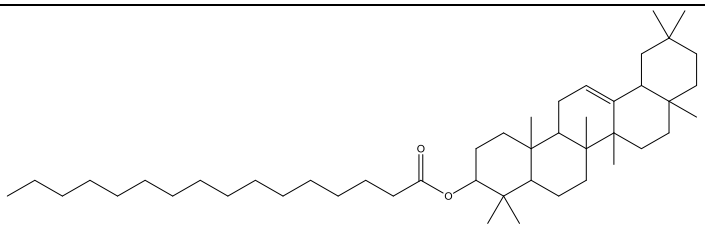
S.No	Active Compounds	Plant (Family)	Chemical structure
1	Acacetin	*Anoda cristata* (Malvaceae)	
2	Disometin		
3	Chikusetsu saponin IVa	*Aralia taibaiensis* (Araliaceae)	

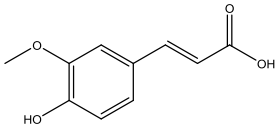
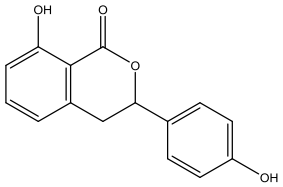
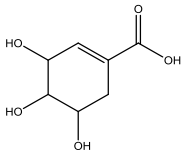
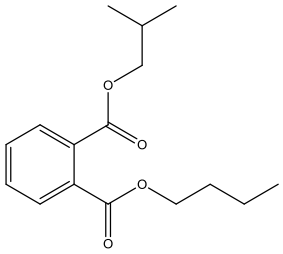
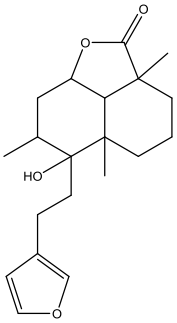
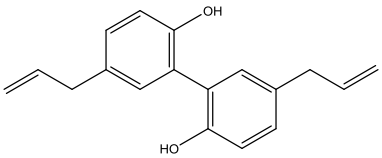
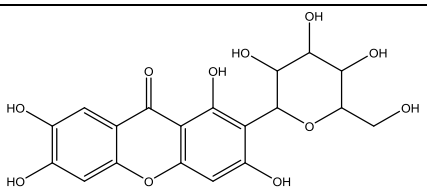
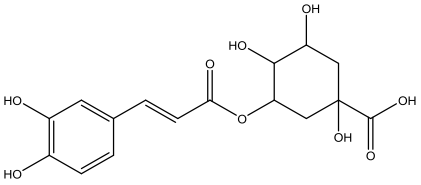
4	Delphinidin 3-sambubioside-5-glucoside	*Aristotelia chilensis* (Elaeocarpaceae)	
5	Eupatilin	*Artemisia ludoviciana* (Asteraceae)	
6	Astragaloside II	*Astragalus membranaceus* (Fabaceae)	
7	Isoastragaloside I		

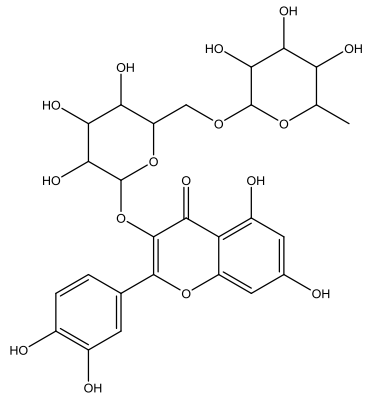
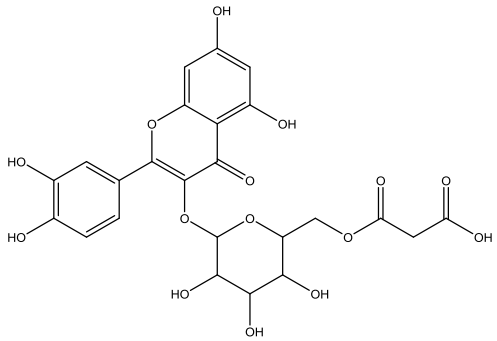
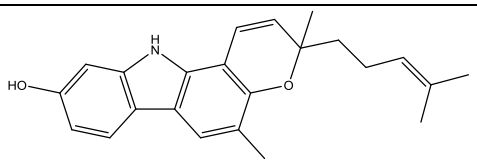
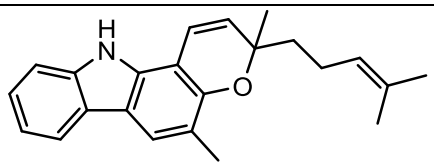
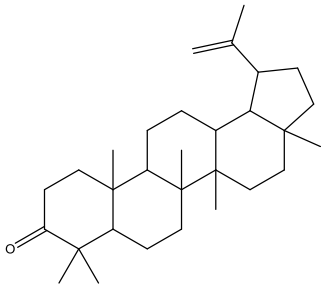
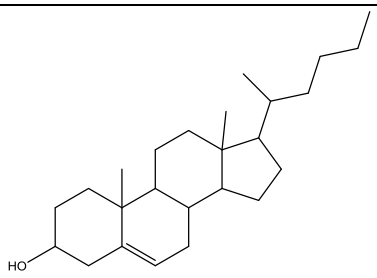
8	Bacosine	*Bacopa monnieri* (Scrophulariaceae)	
9	Bruceine D	*Brucea javanica* (Simaroubaceae)	
10	Bruceine E		
11	Bergenin	*Caesalpinia digyna* (Fabaceae)	
12	Rosmarinic acid	*Calamintha officinalis* (Lamiaceae)	
13	Caffeic acid		
14	Cinnamaldehyde	*Cinnamonum zeylanicum* (Lauraceae)	

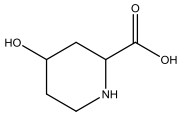
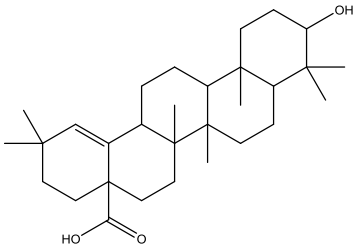
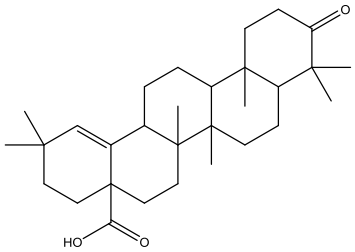
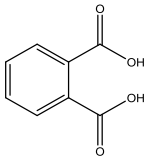
15	Mollic acid glucoside	*Combretum molle* (Combretaceae)	
16	Guggulsterone Z & E	*Commiphora mukul* (Burseraceae)	
17	7-O-galloyl-Dsedoheptulose	*Cornus officinalis* (Cornaceae)	
18	Eremanthin	*Costus speciosus* (Costaceae)	
19	Costunolide	*Costus speciosus* (Costaceae	
20	Trigonelline	*Cucurbita sp*. (Cucurbitaceae)	
21	Nicotinic acid		
22	Xanthorrhizol	*Curcuma xanthorrhiza* (Zingiberaceae)	

23	Quercetin	*Dillenia indica* (Dilleniaceae)	
24	Stigmasterol		
25	Tiliroside	*Edgeworthia gardneri* (Thymelaeaceae)	
26	Embelin	*Embelia ribes* (Myrsinaceae)	
27	Swertiamarin	*Enicostemma littorale* (Gentianaceae)	
28	Vitexin	*Ficus deltoidea* (Moraceae)	

29	Isovitexin		
30	Kolaviron	*Garcinia kola* (Clusiaceae)	
31	Isoorientin	*Gentiana olivieri* (Gentianaceae)	
32	Globularin	*Globularia alypum* (Globulariaceae)	
33	Licochalcone E	*Glycyrrhiza inflata* (Fabaceae)	
34	β-amyrin palmitate	*Hemidesmus indicus* (Asclepiadaceae)	

35	Ferulic acid	*Hibiscus mutabilis* (Malvaceae)	
36	Hydrangenol	*Hydrangea macrophylla* (Hydrangeaceae)	
37	Shikimic acid	*Juniperus oxycedrus* (Cupressaceae)	
38	Butyl isobutyl phthalate	*Laminaria japonica* (Laminariaceae)	
39	Marrubiin	*Leonotis leonurus* (Lamiaceae)	
40	Magnolol	*Magnolia officinalis* (Magnoliaceae)	
41	Mangiferin	*Mangifera indica* (Anacardiaceae)	
42	Chlorogenic acid	*Morus alba* (Moraceae)	

43	Rutin		
44	Quercetin 3-(6-malonylglucoside)	*Morus alba* (Moraceae)	
45	Mahanine	*Murraya koenigii* (Rutaceae)	
46	Mahanimbine	*Murraya koenigii* (Rutaceae)	
47	Lupenone	*Musa basjoo* (Musaceae)	
48	Nymphayol	*Nymphaea stellata* (Nymphaeaceae)	

49	4-Hydroxypipecolic acid	*Peganum harmala* (Zygophyllaceae)	
50	Morolic acid	*Phoradendron reichenbachianum* (Loranthaceae)	
51	Moronic acid		
52	Phthalic acid		

2. Materials and methods

2.1. Ligand preparation

The chemical structures of the selected 52 natural compounds be investigated by molecular docking study were drawn by using ChemDraw Professional 15.0 and saved as in .mol format. These structures were used for the prediction of physiochemical properties and ADMEt profile. The energy minimization of all the ligands used for docking study was done by Chem3D software via applying molecular mechanics (MM2) tool (Bajracharya, Paudel, Rajendra, & Shyaula, 2020). All the ligands were prepared by using preparation tab in MVD software to assign bond order and hybridization, detect flexible torsions and create explicit hydrogen if missing. These ligands were further docked with Tubulin-Colchicine complex by using Molegro Virtual Docker (Bitencourt-Ferreira & de Azevedo, 2019).

2.2. Prediction of physiochemical properties and bioactivity score

SWISS ADME (http://www.swissadme.ch/) (Daina, Michielin, & Zoete, 2017) servers were used to predict bioactivity score and physiochemical property of selected natural compounds. The SMILES of all the selected compounds were copied from ChemDraw Professional 15 and these SMILES were pasted in to SWISS ADME servers to predict the bioactivity score and physiochemical properties one by one. Various parameters like Molecular weight, Log P, hydrogen donor, hydrogen acceptor, total polar surface area, and number of rotatable bonds which shows the physiochemical property of compounds were estimated by SWISS ADME.

2.3. Prediction of ADMEt properties.

Assessment of absorption, distribution, metabolism, excretion and toxicity study is the crucial step in the early stage of drug discovery. The pkCSM server (http://biosig.unimelb.edu.au/pkcsm/prediction) was used to predict the ADMEt property of selected compounds (Pires, Blundell, & Ascher, 2015). The SMILES of these compounds were obtained from ChemDraw Professional 15.0 and pasted in pkCSM server to predict the ADMEt properties of selected compounds. Various parameters for absorption (like- Caco2 permeability, intestinal absorption, and skin permeability), distribution (like- VDss, BBB permeability and CNS permeability), metabolism (like- effect on cytochromes P450 and P-gp substrate), excretion (like- total clerance and renal OCT2 substrate), and toxicity (like- AMES toxicity and hepatotoxicity) were obtained from pkCSM server to predict ADMEt profile of the selected flavonoids.

2.4. Protein Preparation

The x-ray crystal structure of human PPAR-gamma (PDB ID 2XKW) in complex with the agonist pioglitazone has been obtained from protein data bank (https://www.rcsb.org/structure/2XKW) and the resolution of downloaded protein was 2.02 Å. This protein was imported in Molegro Virtual Docker software and warning error was repair by protein preparation tools available in software. The maximum five cavities were detected to determine where co-crystallize ligand can bind then the cavity with highest volume occupied (Vol=231.936) was selected and rest cavities were deleted.

2.5. Receptor grid generation

Generation of receptor grid is mandatory for the ligand docking. The docking study was performed on the basis of all the sites grids were generated to specify the binding site of the protein where ligand can bind. The grid spacing was 0.30 Å, the grid volume was 231.936 point and the binding site radius was 15Å (**Fig. 2**).

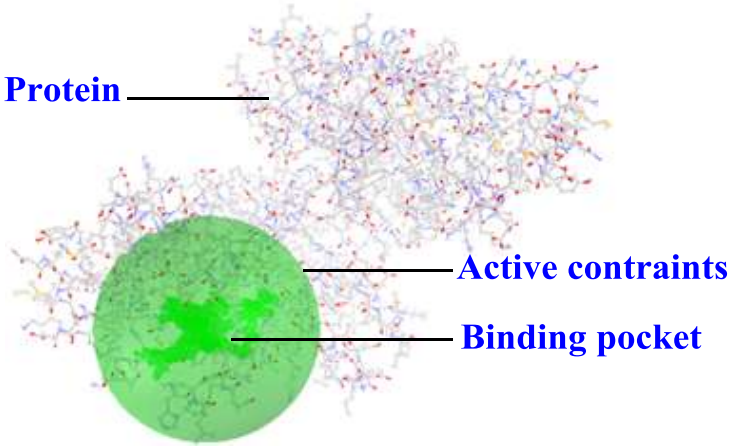


Fig. 2: Representation of PPAR-γ with its active binding site

2.6. Molecular docking

Molecular docking study was performed by using Molegro Virtual Docker. The *Molegro Virtual Docker* is developed by: Molexus IVS, Denmark which provides an integrated environment for predicting ligand with macromolecules like protein by flexible ligand docking. All the energy minimized and preprocessed ligand with optimal geometry were taken for molecular docking with well-prepared protein (PDB ID- 2XKW) and docking were performed by using docking wizard tool available in Molegro Virtual Docker, the MolDock SE algorithm was used for docking simulation with maximum population size 50 as well as 1500 iterations. Total 10 replication of each running were considered to validate the docking study of natural compounds with PPAR-gamma and best one pose was considered out of five poses obtained for result evaluation.

3. Result and Discussion

3.1. Physiochemical properties of investigated natural products

Physiochemical properties are an elemental segment of drug development to estimate the solubility and drug-like activity in the initial phase of drug discovery (Singh, Ahmad, Chatterjee, Bajpai, & Sengar, 2021). Lipinski's rule of five (Lipinski, Lombardo, Dominy, & Feeney, 1997) and Veber's rules (Veber et al., 2002) were used to monitoring the drug-likeness. According to the Lipinski rule, most drug-like compounds having logP < = 5, Molecular weight < = 500, number of hydrogen bond donors < = 5, and number of hydrogen bond acceptors < = 10. All the parameters require for Lipinski's rules and Veber's rules were estimated by SWISS ADME server (Daina et al., 2017). However, the Veber's rule is based on the number of rotatable bonds (No. of rotatable bond < = 10) and the total polar surface area (TPSA < = 140 $Å^2$) of compounds. Predicted physicochemical parameters are represented ion **Table 2**.

Table 2: Physicochemical parameters of selected phytoconstituents from different subcategories

S.No	Phytoconstituents Name	Physicochemical Parameters						Bioactivity Score
		Mole. Wt. (g/mol)	Log P	Hydrogen donor	Hydrogen acceptor	Total polar surface area ($Å^2$)	No. of rotatable bonds	Bioavailability Score
1	Acacetin	284.26	2.52	2	5	79.90	2	0.55
2	Disometin	300.266	2.5854	3	6	123.998	2	0.55
3	Chikusetsu saponin IVa	794.97	2.52	8	14	232.90	7	0.11
4	Delphinidin 3 sambubioside-5-glucoside	759.64	-4.26	14	21	351.74	9	0.17
5	Eupatilin	344.32	2.54	2	7	98.36	4	0.55
6	Astragaloside II	827.01	1.59	8	15	234.29	9	0.17
7	Isoastragaloside I	869.04	2.09	7	16	240.36	11	0.17

8	Bacosine	456.70	6.20	2	3	57.53	2	0.85
9	Bruceine D	410.42	-0.94	5	9	153.75	0	0.55
10	Bruceine E	412.43	-1.23	6	9	156.91	0	0.55
11	Bergenin	328.27	-0.72	5	9	145.91	2	0.55
12	Rosmarinic acid	360.31	1.52	5	8	144.52	7	0.56
13	Caffeic acid	180.16	0.93	3	4	77.7.6	2	0.56
14	Cinnamaldehyde	132.16	1.97	0	1	17.07	2	0.55
15	Mollic acid glucoside	634.84	3.91	6	9	156.91	8	0.11
16	Guggulsterone Z	312.45	4.03	0	2	34.14	0	0.55
17	7-O-galloyl-Dsedoheptulose	362.29	-2.09	8	11	205.21	9	0.17
18	Eremanthin	230.30	2.093	0	2	26.30	0	0.55
19	Costunolide	232.32	2.97	0	2	26.30	0	0.55
20	Trigonelline	137.14	-0.61	0	2	44.01	1	0.55
21	Nicotinic acid	123.11	0.32	1	3	50.19	1	0.85
22	Xanthorrhizol	281.33	4.31	1	1	20.23	4	0.55
23	Quercetin	302.24	1.23	5	7	131.36	1	0.55
24	Stigmasterol	412.69	6.97	1	1	20.23	5	0.55
25	Tiliroside	594.52	1.52	7	13	216.58	8	0.17
26	Embelin	294.39	3.68	2	4	74.60	10	0.85
27	Swertiamarin	432.38	-0.07	7	10	181.05	3	0.55
28	Isovitexin	432.38	0.05	7	10	181.05	3	0.55
29	Kolaviron	588.52	2.30	7	12	203.44	4	0.17
30	Isoorientin	448.38	-0.24	8	11	201.28	3	0.17
31	Globularin	492.47	-0.50	5	11	167.67	8	0.55
32	Licochalcone E	338.40	4.05	2	4	66.76	6	0.55
33	β-amyrin palmitate	665.13	12.42	0	2	26.30	16	0.17
34	Ferulic acid	194.18	1.36	2	4	66.76	3	0.85
35	Hydrangenol	256.25	2.40	2	4	66.76	1	0.55
36	Shikimic acid	174.15	-1.12	4	5	97.99	1	0.56
37	Butyl isobutyl phthalate	278.34	3.62	0	4	52.60	9	0.55
38	Marrubiin	332.43	3.43	1	4	59.67	3	0.55
39	Magnolol	266.33	4.25	2	2	40.46	5	0.55
40	Mangiferin	422.34	-0.81	8	11	201.28	2	0.17
41	Chlorogenic acid	354.31	-0.38	6	9	164.75	5	0.11
42	Rutin	610.52	-1.29	10	16	269.43	6	0.17
43	Quercetin 3-(6-malonylglucoside)	550.42	-0.67	8	15	253.88	8	0.11
44	Mahanine	347.45	5.20	2	2	45.25	3	0.55
45	Mahanimbine	331.45	5.62	1	1	25.02	3	0.55
47	Lupenone	424.70	7.31	0	1	17.07	1	0.55
48	Nymphayol	358.60	6.19	1	1	20.23	4	0.55
49	4-Hydroxypipecolic acid	145.16	-1.33	3	4	69.56	1	0.55
50	Morolic acid	456.70	6.13	2	3	57.53	1	0.85
51	Moronic acid	454.68	6.03	1	3	54.37	1	0.85
52	Phthalic acid	166.13	0.84	2	4	74.60	2	0.85

3.2. Prediction of ADMEt properties of selected compounds

Prediction of ADMEt parameters is a crucial step in drug design since drug substances should have acceptable parameters related to ADMEt. Screening of pharmacokinetic property and toxicological profile of drug compounds by conventional methods are costly, the time-consuming process requires a lot of resources hence in silico study is a beneficial and time-saving approach used widely nowadays (Tabeshpour et al., 2018). In this study pkCSM server was used to predict the various pharmacokinetic parameters of absorption, distribution, metabolism, excretion and toxicity of selected flavonoids, the result is tabulated in **Table 3**.

Table 3: ADMET prediction values of selected phytoconstituents

S.No	Phytoconstituents Name	Absorption Parameters			distribution Parameters			Metabolism Parameters		Excretion		Toxicity Profile	
		Caco-2 permeability	Intestinal absorption (human)	Skin Permeability	VDss (human)	BBB permeability	CNS permeability	Effect on cytochromes P450	Pg-P Substrate	Total Clearance	Renal OCT2 substrate	AMES toxicity	Hepatotoxicity
1	Acacetin	1.137	94.318	-2.737	0.346	-0.196	-2.159	CYP1A2, CYP2C9, CYP2C19 inhibitor	Yes Yes Yes	0.663	No	No	NO
2	Disometin	0.374	84.046	-3.5	-0.954	-0.907	-2.223	CYP1A2 inhibitor	Yes	0.597	No	Yes	No
3	Chikusetsu saponin IVa	-3.736	35.068	-2.737	-0.77	-1.901	-4.207	CYP3A4 substrate	Yes	-0.219	No	No	No
4	Delphinidin 3-sambubioside-5-glucoside	-1.09	0	-2.735	-1.457	-2.886	-5.935	CYP3A4 substrate	Yes	0.294	No	No	No
5	Eupatilin	0.388	87.069	-3.422	-1.044	-0.711	-2.913	CYP3A4 substrate	Yes	0.605	No	Yes	No
6	Astragaloside II	-0.372	51.27	-2.738	-0.589	-2.078	-4.628	CYP3A4 substrate	Yes	-0.089	No	No	No
7	Isoastragaloside I	-0.425	55.593	-2.737	-0.662	-2.317	-4.634	CYP3A4 substrate	Yes	-0.207	No	No	No
8	Bacosine	1.294	91.58	-20899	0.418	0.222	-1.138	CYP3A4 substrate	Yes	0.05	No	No	No
9	Bruceine D	-0.193	59.485	-3.226	-0.347	-0.998	-4.129	CYP3A4 substrate	Yes	0.654	No	No	No
10	Bruceine E	-0.208	54.201	-3.13	-0.313	-0.975	-4.2	CYP3A4 substrate	Yes	0.731	No	No	No
11	Bergenin	-0.437	57.713	-3.284	-0.943	-1.319	-4.006	CYP3A4 substrate	Yes	0.433	No	Yes	No
12	Rosmarinic acid	-0.434	45.214	-2.869	-1.446	-1.352	-3.459	CYP3A4 substrate	Yes	0.321	No	No	No

13	Caffeic acid	0.634	69.407	-2.722	-1.098	0.647	-2.608	-	-	0.508	No	No	No
14	Cinnamaldehyde	1.634	95.015	-2.355	0.266	0.436	-1.582	CYP1A2 inhibitor	Yes	0.203	No	No	No
15	Mollic acid glucoside	-0.039	54.198	-2.78	-0.328	-1.263	-3.497	CYP3A4 substrate	Yes	0.253	No	No	No
16	Guggulsterone Z	1.294	99.655	-2.465	0.158	0.141	-2.02	CYP3A4 substrate CYP3A4 inhibitor	Yes Yes	0.61	No	No	No
17	7-O-galloyl-Dsedoheptulose	-0.875	11.978	-2.735	0.326	-1914	-4.617	-	-	0.757	No	No	No
18	Eremanthin	1.553	68.917	-2.474	0.347	0.566	-2.168	CYP3A4 substrate CYP1A2inhibitor CYP2C19 inhibitor	Yes Yes Yes	0.693	Yes	No	No
19	Costunolide	1.6464	97.18	-2.423	0.31	0.512	-2.672	-	-	1.334	No	No	No
20	Trigonelline	1.124	96.44	-2.736	-0.758	-0.234	-2.739	-	-	0.378	No	No	No
21	Nicotinic acid	1.17	94.099	-2.735	-1.015	-0.323	-2.869			0.652	No	No	No
22	Xanthorrhizol	1.633	90.443	-1.617	0.885	0.423	-1.821	CYP3A4 substrate CYP1A2 inhibitor	Yes Yes	1.224	No	No	No
23	Quercetin	-0.229	77.207	-2.735	1.559	-1.098	-3.065	CYP1A2 inhibitor	Yes	0.407	No	No	No
24	Stigmasterol	1.213	94.97	-2.783	0.178	0.771	-1.652	CYP3A4 substrate	Yes	0.618	No	No	No
25	Tiliroside	-0.071	60.065	-2.735	0.655	-1.609	-4.263	-	-	-0.197	No	No	No
26	Embelin	0.503	89.155	-2.97	-0.03	-0.06	-2.625	CYP2D6 inhibitor	Yes	1.518	No	No	No
27	Swertiamarin	-0.956	46.695	-2.735	1.071	-1.449	-3.834	-	-	0.444	No	No	No
28	Isovitexin	-0.618	64.729	-2.735	1.239	-1.375	-3.754	-	-	0.442	No	No	No
29	Kolaviron	0.28	76.444	-2.735	-0.634	-1.259	-3.714	-	-	-0.359	Yes	No	No
30	Isoorientin	-0.912	61.768	-2.735	1.603	-1.564	-3.939	-	-	0.372	No	No	No
31	Globularin	0.319	25.86	-2.737	-0.098	-1.127	-4.137	-	-	0.968	No	No	No
32	LicochalconeE	0.61	89.022	-2.808	0.266	-0.105	-2.018	CYP3A4 substrate CYP1A2 inhibitor CYP2C19 inhibitor	Yes Yes Yes Yes Yes	0.45	No	No	No

								CYP2C9 inhibitor CYP3A4 inhibitor					
33	β-amyrin palmitate	1.259	91.405	-2.735	-0.668	0.898	-1.162	CYP3A4 substrate	Yes	0.08	No	No	No
34	Ferulic acid	0.176	93.685	-2.72	-1.367	-0.239	-2.612	-	-	0.623	No	No	No
35	Hydrangenol	1.3	93.858	-2.757	0.041	0.075	-2.136	CYP1A2 inhibitor CYP2C19 inhibitor	Yes Yes	0.485	No	No	No
36	Shikimic acid	-0.23	46.681	-2.74	-0.618	-0.683	-3.58	-	-	0.688	No	No	No
37	Butyl isobutyl phthalate	1.667	95.035	-2.65	-0.071	-0.025	-2.303	CYP3A4 substrate CYP1A2 inhibitor CYP2C19 inhibitor	Yes Yes Yes	0.877	No	No	No
38	Marrubiin	1.334	96.6	-2.787	0.483	0.034	-2.026	CYP3A4 substrate CYP2C19 inhibitor	Yes Yes	0.696	No	No	No
39	Magnolol	1.707	90.943	-2.758	0.378	-0.051	-1.649	CYP3A4 substrate CYP1A2 inhibitor CYP2C19 inhibitor CYP2C9 inhibitor CYP3A4 inhibitor	Yes Yes Yes Yes Yes	0.386	No	No	No
40	Mangiferin	-0.926	46.135	-2.735	1.364	-1.573	-4.211	-	-	0.347	No	No	No
41	Chlorogenic acid	-0.84	36.377	-2.735	0.581	-1.407	-3.856	-	-	0.307	No	No	No
42	Rutin	-0.949	23.446	-2.735	1.663	-1.899	-5.178	-	-	-0.369	No	No	No
43	Quercetin 3-(6-malonylglucoside)	-1.205	24.325	-2.735	1.717	-1.982	-4.377	-	-	0.531	No	No	No
44	Mahanine	1.185	88.259	-2.74	0.611	-0.036	-1.921	CYP3A4 substrate CYP1A2 inhibitor CYP2C19 inhibitor	Yes Yes Yes Yes Yes	0.249	No	Yes	No

								CYP2C9 inhibitor CYP3A4 inhibitor					
45	Mahanimbine	1.046	91.746	-2.488	1.063	0.28	-1.647	CYP3A4 substrate CYP1A2 inhibitor CYP2C19 inhibitor	Yes Yes Yes	0.556	No	Yes	Yes
47	Lupenone	1.448	98.467	-2.567	-0.216	0.751	-1.568	CYP3A4 substrate	Yes	0.102	No	No	No
48	Nymphayol	1.199	94.265	-2.873	0.547	0.722	-2.042	CYP3A4 substrate	Yes	0.721	No	No	No
49	4-Hydroxypipecolic acid	0.487	74.998	-2.735	-0.974	-0.604	-3.481	-	-	0.635	No	No	No
50	Morolic acid	1.17	100	-2.735	-1.023	-0.13	-1.178	CYP3A4 substrate	Yes	-0.064	No	No	Yes
51	Moronic acid	1.275	100	-2.735	-1.062	-0.08	-1.078	CYP3A4 substrate	Yes	-0.117	No	No	No
52	Phthalic acid	0.641	75.609	-2.735	-1.775	-0.038	-2.891	-	-	0.682	No	No	No

3.3. Molecular docking study

By molecular docking study of 52 natural compounds with PPAR-gamma protein, we investigated the interaction pattern and docking score which is shown in **Table 4.** A docking study of all selected compounds was performed successfully into the earlier defined constraint of PPAR-gamma protein. The affinity of all compounds as an antidiabetic agent by binding with PPAR-gamma was selected by MolDock score and Rerank score.

Table 4: Docking score of screened phytoconstituents and reference compounds in terms of MolDock score, Rerank score and H-Bond score.

Ligand Name	MolDock Score (Kcal/mol)	Rerank Score (Kcal/mol)	HBond (Kcal/mol)
Isoastragaloside I	-170.132	-89.9178	-8.89225
Astragaloside II	-166.006	-61.7018	-9.83755
Globularin	-153.114	-112.246	-1.45902
Tiliroside	-146.261	-87.0036	-5.81058
Rutin	-143.07	-80.8802	-9.04107
ß-amyrin palmitate	-141.736	-84.1726	0
Delphinidin 3- sambubioside-5-glucoside	-140.043	-111.263	-9.48489
Chikusetsu saponin IVa	-133.427	-100.115	-9.16053

Stigmasterol	-131.402	-77.1867	-0.836489
Quercetin 3-(6- malonylglucoside)	-129.803	-95.9664	-5.77304
Mahanimbine	-122.666	-94.3763	-0.436545
Licochalcone E	-121.215	-95.6881	-4.20965
Kolaviron	-120.611	-79.8201	-4.7036
Mahanine	-115.836	-87.5588	-2.10433
Rosmarinic acid	-114.267	-95.0208	-5.88829
Vitexin	-112.368	-101.74	-9.34526
Mangiferin	-110.248	-97.8653	-7.23287
Chlorogenic acid	-110.201	-94.7019	-2.6424
Nymphayol	-106.353	-42.7175	-2.29683
Lupenone	-104.848	-42.8137	0
Costunolide	-103.672	-79.2994	0
Aegle marmelos alkaloids C	-102.365	-81.7439	0
Bacosine	-101.072	-36.0551	-5.41382
Isoorientin	-100.408	-83.4443	-6.4283
Mollic acid glucoside	-99.2033	-77.0093	-4.92591
Isovitexin	-97.9774	-79.2274	-4.45319
Eupatilin	-97.0668	-85.7376	-1.70152
Guggulsterone Z	-97.0288	-75.975	0
Moronic acid	-95.8497	-29.2193	0
Embelin	-90.9725	-76.4663	-2.45008
Acacetin	-90.8811	-76.9982	-2.5
Morolic acid	-89.831	-49.713	0
Bruceine D	-88.6927	-72.1612	-7.75642
Magnolol	-88.6269	-66.5568	0
Xanthorrhizol	-88.1405	-71.0215	-2.42176
Butyl isobutyl phthalate	-87.8637	-75.2005	0
Swertiamarin	-83.9959	-75.8464	-7.33857
Marrubiin	-83.0518	-76.8836	0
Quercetin	-81.8566	-72.7857	-2.90159
Eremanthin	-80.6367	-57.7138	-0.837345
Hydrangenol	-79.4189	-69.1659	-1.35677
Ferulic acid	-79.0454	-67.224	-4.04763
Caffeic acid	-76.9418	-65.1476	-4.48416
Bergenin	-73.7356	-63.7453	-4.69299
7-O-galloyl-Dsedoheptulose	-72.9938	-73.0189	-10.9095
4-Hydroxypipecolic acid	-68.7043	-59.2834	-5.08652
Shikimic acid	-68.0743	-62.3771	-2.5
Phthalic acid	-67.8812	-55.9436	-1.2637
Bruceine E	-66.2626	-69.7613	-7.32022

Cinnamaldehyde	-63.4435	-52.8753	-1.15785
Trigonelline	-61.9575	-53.8864	-0.0863734
Nicotinic acid	-53.1442	-46.8856	-3.21699
Pioglitazone	-112.052	-73.7026	-0.337517
Rosiglitazone	-92.7265	-73.139	-0.783371

We found that 16 compounds having docking score better than Pioglitazone and 29 compounds than Rosiglitazone. However, Isoastragaloside I showed the highest binding affinity with PPAR-g in terms of MolDock (-170.132) and Rerank (-89.9178) score, the binding interaction is shown in **Fig. 3** and **Fig. 4**. Molecular docking study of Isoastragaloside I, Pioglitazone and Rosiglitazone shows hydrogen bond, Pi-Alkyl and Pi-cation interaction through the various residues represented in **Fig. 3**.

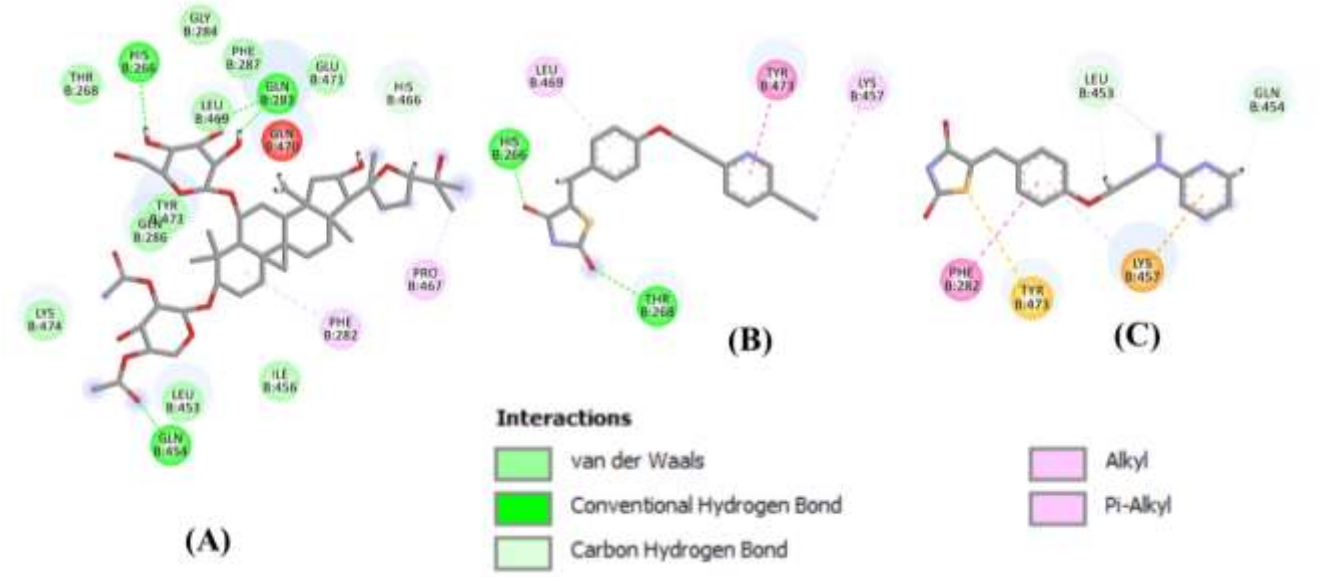


Fig. 3: 2D interaction diagram of (A) most potent compound i.e. Isoastragaloside I; (B) Pioglitazone; (C) Rosiglitazone

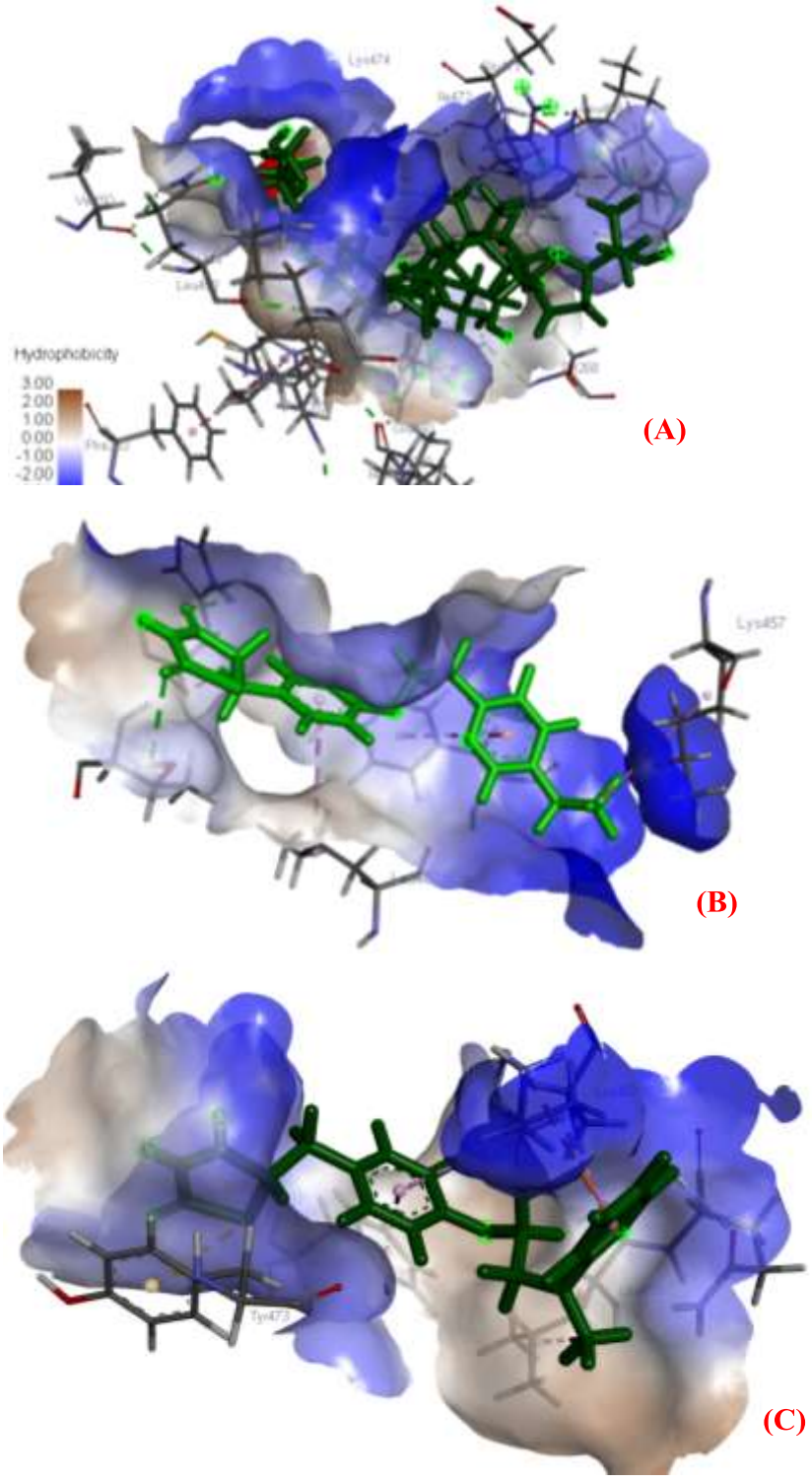


Fig. 4: 3D interaction diagram of (A) most potent compound i.e. Isoastragaloside I; (B) Pioglitazone; (C) Rosiglitazone

4. Conclusion

In conclusion, our molecular docking and physicochemical screening analysis discovered numerous natural compounds with the potential to be developed as novel anti-diabetic medicines. These compounds exhibit high affinity for PPAR-gamma and have drug-like physicochemical characteristics. Our research also underlines the potential of natural compounds as a source of novel therapeutic leads. Natural products have a long history of safe and successful usage in traditional medicine, and they are rapidly being recognized as a viable resource for the creation of novel medications for a range of disorders. Total 52 natural compounds were selected for *in silico* screening of physicochemical properties, bioactivity, ADMEt, and molecular docking studies against PPAR-gamma protein (PDB ID: 2XKW) as a major antidiabetic. This library was considered for tackling the rapidly increasing social and economic burden to the suffering population with type-2 diabeytes throughout the world. The present study of *in silico* screening of 52 compounds shows that only few compounds violated Lipinski's rule of five and out of these 52 compounds 8-10 compounds violated Veber's rule also. Selected compounds show the binding interaction through hydrogen bond and steric interaction with the colchicine binding pocket of PPAR-gamma proteins. This *in silico* study concluded that selected natural compounds could be explored further for better antidiabetic lead.

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Availability of data and materials

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

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