Research article

Molecular docking and dynamic studies of Indian blackberry in contrast to X-ray structure of human PPAR gamma

Aghil Soorya Aravindakshan[1], Suraj Katole[2], Sameer Sharma[2], Susha Dinesh[2], Manjula Shantaram[3], Raghavendra L. S. Hallur[4]

[1]Department of Botany, St. Teresa's College, Ernakulam, Kerala, India
[2]Department of Bioinformatics, BioNome, Hennur Cross Road, Bengaluru, 560 043, Karnataka, India
[3]A.J. Research Centre, A.J.Institute of Medical Sciences & Research Centre, Kuntikana, Mangalore, 575 004, Karnataka, India
[4]College of Biosciences and Technology, Pravara Institute of Medical Sciences (Deemed to be University), Loni, 413736, Rahata Taluk, Ahmednagar District, Maharashtra, India

(Received: December 2023 Revised: January 2024 Accepted: February 2024)

Corresponding author: **Raghavendra L.S. Hallur.** Email: raghavendra@pmtpims.org

ABSTRACT

Introduction and Aim: This study aims to elucidate the phytopharmacological and pharmacokinetic characteristics of Indian blackberries about human PPAR gamma receptors through *in silico* analyses. The protein receptor known as human PPAR gamma was retrieved, along with phytochemicals to perform molecular docking techniques to analyze the inhibitory effects of Indian blackberries.

Methods: The human PPAR gamma receptor was obtained from the Protein Data Bank, and a set of phytochemicals was sourced from the IMPPAT database, and subsequently retrieved through the PubChem database. Molecular docking studies were conducted to assess the inhibitory interactions between Indian blackberry constituents and human PPAR gamma.

Results: Among the various ligands investigated, ligand 12304985 exhibited the highest binding affinity towards the human PPAR gamma receptor. Subsequent analyses involved the assessment of ADMET profiling, secondary structure analysis, and Ramachandran plot analysis at the molecular level for human PPAR gamma.

Conclusion: Our findings suggest that Indian blackberry, particularly ligand 12304985, exhibits promising inhibitory interactions with human PPAR gamma receptors. This in silico investigation provides valuable insights into the potential phytopharmacological and pharmacokinetic properties of Indian blackberries, paving the way for further *in vitro* and *in vivo* studies to validate these findings.

Keywords: Indian blackberry; PPAR gamma; MD simulation; ADMET.

INTRODUCTION

Type 1 diabetes mellitus is a persistent autoimmune condition characterized by the immune system's assault on the insulin-producing beta cells located in the pancreas. This immune response results in the depletion of insulin, a pivotal hormone responsible for regulating blood sugar levels. Consequently, individuals with type 1 diabetes experience hyperglycemia, contributing to various complications. According to statistics from the International Diabetes Federation (IDF) (1-3), the global prevalence of diabetes reached an estimated 463 million adults in 2019, with projections indicating an escalation to 700 million by 2045. Type 1 diabetes constitutes approximately 10% of all diabetes cases, with a calculated global prevalence of 1.1%. In the specific context of India, the prevalence of type 1 diabetes remains relatively low, accounting for about 0.2% of the population. Nevertheless, there is a discernible upward trend in the incidence of Type 1 diabetes in India, characterized by an annual growth rate of 3.5%. Effective management of type 1 diabetes poses challenges arising from the intricate interplay of genetic, environmental, and lifestyle factors influencing the disease. Existing therapeutic strategies encompass insulin therapy, blood glucose monitoring, and lifestyle adjustments, such as dietary modifications and regular exercise. Despite their utility, these interventions are not without limitations, encompassing the potential for hypoglycemia, weight gain, and the necessity for frequent injections. Consequently, there exists a critical imperative for novel therapeutic modalities capable of enhancing glycemic control while mitigating the associated risks of complications (4-6).

One potential therapeutic target for managing type 1 diabetes is the peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear receptor integral to glucose and lipid metabolism (7). PPARγ finds expression in diverse tissues, including adipose tissue, liver, and skeletal muscle, playing pivotal roles in insulin sensitivity, adipogenesis, and inflammation. Clinical use of PPARγ agonists, exemplified by thiazolidinediones (TZDs), has been established in improving insulin sensitivity and glycemic control among individuals with type 2 diabetes. The mechanism of action involves TZDs binding to the nuclear PPARγ receptor, forming a heterodimer with the retinoid X receptor (RXR ; 8) and binding to specific peroxisome proliferator response elements

(PPREs) on target genes associated with carbohydrate and lipid metabolism. However, their application in type 1 diabetes is restricted due to concerns regarding weight gain, edema, and an elevated risk of fractures (9).

The endogenous ligands of PPARγ encompass long-chain unsaturated fatty acids and prostanoids. In the context of type 1 diabetes, the precise role of PPARγ is less elucidated compared to type 2 diabetes. Nevertheless, research indicates that PPARγ agonists exhibit the potential to enhance beta cell function, mitigate mitochondrial alterations, and prevent diabetes in animal models of type 1 diabetes. The involvement of PPARγ in insulin secretion remains incompletely understood. Some studies suggest that PPARγ activation or overexpression suppresses insulin secretion and proinsulin biosynthesis. For instance, the overexpression of PPARγ in INS-1E cells induces impairment in glucose-stimulated insulin secretion (GSIS). Conversely, other studies propose that PPARγ activation or overexpression potentiates GSIS in beta cells and isolated islets. The exact mechanism through which PPARγ regulates insulin secretion is not conclusively established, but it may encompass the modulation of genes related to glucose metabolism, such as glucokinase and glucose transporter 2. Additionally, PPARγ activation has demonstrated the capability to reduce islet endoplasmic reticulum (ER) stress in diabetic mouse models. ER stress is a crucial characteristic of beta cell dysfunction in type 1 diabetes and its reduction may contribute to the enhancement of beta cell function and survival (10).

The aim of this investigation is to scrutinize the impact of Indian blackberry on PPARγ for the management of type 1 diabetes. More precisely, this study seeks to assess the PPARγ agonist activity inherent in Indian blackberry extract, characterized by its richness in polyphenols and established anti-inflammatory, antioxidant, and hypoglycemic effects. The endeavor aspires to deepen comprehension regarding the intricacies of PPARγ signaling and its pivotal role in the management of diabetes. This research holds promise in potentially heralding the development of innovative therapeutic approaches for type 1 diabetes, capitalizing on the benefits of natural products such as Indian blackberry.

METHODOLOGY

Retrieval of the target protein

The 3-dimensional structure of the protein 8HUM of Human PPAR was downloaded from the RCSB PDB (https://www.rcsb.org/structure/8HUM) database.

Purification of target protein

The purification of this protein was done in Discovery Studios BIOVIA by removing ligands and water motifs, introducing hydrogen, and PyRx-optimized mutation sites. BIOVIA Discovery Studio Software is a structure visualization tool used to visualize the two-dimensional and three-dimensional structure of the docked ligand structure taken from PyRx in PDB format. In this step the 2D and 3D models were studied.

Ramachandran plot

The Ramachandran plot may also be used to validate protein structure. The plot may be used to detect amino acid residues in energetically unfavorable conformations, such as those in a section of the plot that is devoid of other residues. These residues may suggest protein structural defects, such as mismatched or erroneous residues. The Ramachandran plot may also be used to detect anomalies which are residues in particularly unfavorable conformations. The anomalies might be suggestive of protein structural defects, such as inaccurate amino acid assignments, or model refinement issues**.**

Hydropathy plot

The interpretation of a hydropathy plot can reveal information about the structure and function of a protein. Hydrophobic areas may suggest the existence of domains in the transmembrane or protein sections buried within the membrane. The structure of a protein, hydrophilic areas may suggest the existence of solvent-exposed loops or other protein surface-exposed regions. Researchers can acquire a more complete knowledge of a protein's structure and function by integrating material obtained via a hydropathy plot alongside additional experimental or computational data.

Secondary structure prediction

A protein's secondary structure pertains to the local spatial organization of a protein's framework without considering side-chain interactions. The most prevalent secondary structures are alpha-helices and beta-sheets, which are held together by hydrogen bonds between the atoms in the backbone. The secondary crystal structure of a protein must be determined to understand its function and support, as well as to anticipate its tertiary structure. Secondary structure data may also be utilized to discover possible binding sites and to develop medications that target specific parts of the protein.

Retrieval of ligands

The secondary metabolites of the Indian Black Berry were retrieved from IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics) (https://cb.imsc.res.in/imppat/home) database.

Pharmacological studies

Protein physicochemical qualities include a complicated combination of size, shape, charge, hydrophobicity, flexibility, and optical properties. Understanding these features is essential for

comprehending the structure and function of proteins, as well as creating new medications and treatments that target specific proteins.

Bioactivity prediction

These predictions might help to comprehend the molecular principles behind the compounds' biological action and forecast potential adverse effects or cross-reactivity.

Molecular docking

Molecular docking is an important method in molecular biological science and computer-assisted drug creation. The goal of ligand-protein docking is to predict the most probable interaction alternative(s) for any ligand with a protein's known three- dimensional structure. The RCSB Protein Data Bank (https://www.rcsb.org/) was used to collect the target proteins' crystal structures, which were subsequently purified by eliminating the ligands and water motifs, inserting hydrogen, and PyRx-optimized mutation sites. The 3D molecular structure formulas of the key ingredients were obtained from PubChem. The pure protein served as the macromolecule, whereas phytocompounds served as the ligands. Following energy reduction, the filled ligands were translated to pdbqt format. The docking interactions between the ligand and the target protein were assessed using the binding affinity**.** In PyRx (10), ligands exhibit 9 distinct conformations, with the best docking confirmation exhibiting zero root mean square deviation RMSD as the optimal binding complex. The docked ligand structure's interactions are visualized in BIOVIA Discovery Studio.

Molecular visualization

The best-docked complexes were further visualized in DS Biovia Discovery Studio Visualizer for the molecular interaction at the binding pocket of the target proteins. BIOVIA Discovery Studio Software is a structure visualization tool used to visualize the two-dimensional and three-dimensional structure of the docked ligand structure taken from PyRx in pdb format. In this step, the 2D and 3D models were studied.

RESULTS

Secondary structure and Ramachandran plots

The distribution of the dihedral angles is critical in determining protein stability and folding. The Ramachandran plot (Fig.1) analysis provides valuable insights into the structural architecture, topology, and irregularities in the protein. Therefore, it is imperative to assess the protein structures before docking.

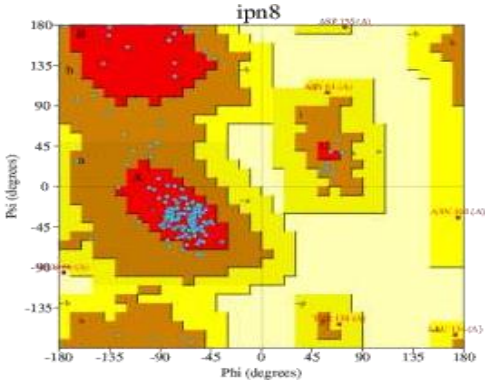

Fig. 1: Ramachandran plot

The purified structure of 2IL6 has 76.4% of its residues in the most favored regions, 19.7% in additionally allowed regions, 3.2% in generously allowed regions, and 0.6% in the disallowed regions (Fig. 2).

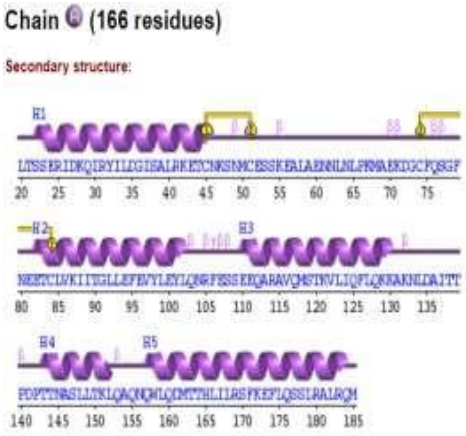

Fig. 2: Secondary structure

Hydropathy plot

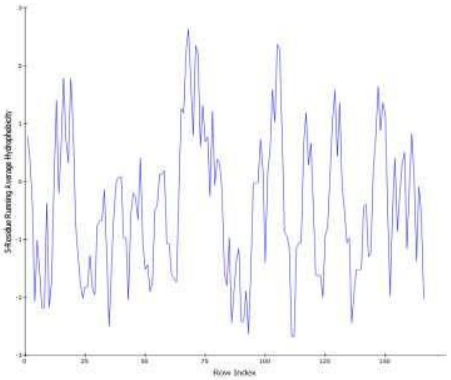

Fig. 3: Hydropathy plot

When identifying potential drug targets, it is crucial to assess the hydrophobic and hydrophilic nature of amino acids in the target proteins. The binding affinity of drugs is significantly influenced by the hydropathicity of the binding pocket, where hydrophobic drugs tend to bind to hydrophobic regions, and hydrophilic medications interact with hydrophilic areas. Excessive hydrophilicity in drugs may lead to inadequate attachment to the target protein, resulting in reduced efficacy. The hydropathicity of a protein not only affects its shape

and stability but also influences the binding efficiency of drugs. Some hydrophobic portions of a protein may be concealed within its structure, making them less accessible to medications. Hydropathy graphs typically range from -3 to +3, with positive values indicating hydrophobic regions and negative values indicating hydrophilic regions (Fig. 3). Peaks above the X-axis in the graph signify more hydrophobic regions. In the case of 2IL6, it is evident that there are more hydrophobic regions within the protein structure.

Retrieval of ligands

Berries are a group of fruits that are rich in a variety of phytochemicals, such as anthocyanins, flavonoids, and phenolic acids, which have been shown to have antioxidant and anti-inflammatory properties. These compounds have been linked to improved brain health and cognitive function. The ligands from strawberry, Kiwi berry, and Indian blackberry were retrieved from the IMPPAT database and the structures of the ligands which have exhibited better binding with 2IL6 are depicted.

Pharmacological studies

The therapeutic capabilities of phytocompounds found in strawberries, Kiwi berries, and Indian blackberries were explored by evaluating their ADMET properties, aiming for favorable drug-likeness characteristics. The top five compounds from each berry (Table 1), as identified through docking studies, underwent a comprehensive analysis of their physicochemical properties (Table 2), absorption, distribution, medicinal chemistry, metabolism, excretion (Table 3), toxicity (Table 4), and bioactivity. The phenological assessment revealed that all these compounds exhibited advantageous pharmacological properties and bioactivity.

Table 1: Top 5 ligands selected for Indian blackberry

Ligand	SMILE ID	PubChem ID
(-)-Globulol	CC1CCC2C1C3C(C3(C)C)CCC2(C)O	12304985
Oleanolic acid	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC	10494
Ledol	CC1CCC2C1C3C(C3(C)C)CCC2(C)O	92812
Delphinidin 3-gentiobioside	C1=C(C=C(C(=C1O)O)O)C2=[O+]C3=CC(=C	44256919
Petunidin 3-gentiobioside	COC1=CC(=CC(=C1O)O)C2=[O+]C3=CC(=C	44256956

Table 2: Physicochemical properties of Indian blackberry

PubChem ID	MW	TPSA	XLOGP3	Rot bonds
12304985	222.37	20.23	3.74	0
10494	456.7	57.53	7.49	1
92812	222.37	20.23	3.74	0
44256919	627.52	292.82	-2.36	7
44256956	641.55	281.82	-2.04	8

Table 3: Lipinski properties of Indian blackberry

PubChem ID	MLogP	Hydrogen Bond Acceptor	H-Bond Donor	Lipinski
12304985	3.81	1	1	0
10494	5.82	3	2	1
92812	3.81	1	1	0
44256919	-4.28	17	12	3
44256956	-4.08	17	11	0

Table 4: Toxicity prediction of Indian blackberry

PubChem ID	LD-50	Class
12304985	2000 mg/kg	4
10494	2000 mg/kg	4
92812	2000 mg/kg	4
44256919	5000 mg/kg	5
44256956	5000 mg/kg	5

Molecular docking

In PyRx docking, ligands are treated as flexible, while macromolecules are considered rigid. The evaluation of ligand effectiveness is based on binding affinity at zero RMSD (Root Mean Square Deviation). In the current study, given that the ligands exhibited superior binding with the 2IL6 targets, the top binding score was selected for further examination. It was found that beta-carotene [5280489] in Indian BlackBerry has a better binding with all the target 8HUM (Table 5) and Fig.4.

Table 5: Binding affinity against PPAR gamma

Ligand	Binding affinity (Kcal/mol)
12304985	-8.1
10494	-7.2
92812	-7.1
44256919	-6.9
44256956	-6.7

Molecular visualization

Information about the interactions, bond distances, amino acid residues were obtained by 3D and 2D visualization of the selected top 5 ligands using BIOVIA Discovery Studio. Indian blackberry: As we can see the 3D structure here in IBB the Arg [ARGININE] and Lys [LYSINE] are the most found amino acids after interaction with the respective ligands of the fruit.

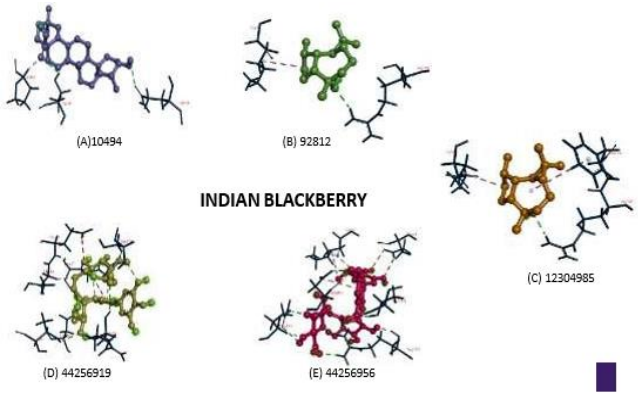

Fig. 4: Amino acids interactions of all 5 selected phytochemicals against PPAR-gamma

DISCUSSION

Type 1 diabetes stands as a persistent autoimmune malady afflicting millions globally. This condition arises from the autoimmune-induced degradation of pancreatic β-cells, culminating in insulin deficiency, hyperglycemia, and associated complications. Presently, the sole therapeutic recourse for type 1 diabetes remains insulin injection, notwithstanding its association with significant medical complexities. This underscores the imperative for alternative treatments, prompting exploration into the potential efficacy of natural products, notably berries, in the management of type 1 diabetes (11).

Berries stand out as a rich source of phytochemicals, a category of compounds showcasing noteworthy biological actions with clinical potential in the realm of type 1 diabetes prevention and treatment (12). These phytochemicals, encompassing anthocyanins, flavonoids, and tannins, exhibit pronounced anti-diabetic properties by influencing diverse pathways and mechanisms within the body. Notably, their effects extend to augmenting insulin secretion, enhancing cellular glucose uptake, and mitigating oxidative stress and inflammation (12, 13).

The application of in silico techniques emerges as a valuable strategy for screening phytocompounds concerning their potential in managing type 1 diabetes. Leveraging computer simulations, these computational methods enable the prediction of biological activity based on chemical structure, facilitating the identification of promising candidates for subsequent investigation. A specific natural candidate of interest is the Indian blackberry, colloquially known as jamun, subject to research evaluating its potential benefits in type 1 diabetes management (12). The secondary metabolites within Indian blackberry, comprising anthocyanins, flavonoids, and tannins, have demonstrated anti-diabetic properties as shown in table 3, further supporting their relevance in modulating diverse pathways and mechanisms associated with type 1 diabetes (13). Noteworthy effects include the augmentation of insulin secretion, improvement in cellular glucose uptake, and reduction of oxidative stress and inflammation (14).

This research endeavors to scrutinize the pharmacological properties of phytocompounds derived from Indian Blackberry, with a particular focus on identifying the top five compounds based on their binding affinity towards PPARγ. Our findings reveal that ligand 10494 (Oleanolic acid), 5280489 (beta-carotene), 12304985 (Globulal), 44256919 (Delphinidin), and 44256956 (Petunidin) exhibit notably favorable pharmacological characteristics in this context.

The present study focuses on utilizing PPARγ as a target for managing type 1 diabetes, emphasizing another crucial aspect of PPARγ involvement in the regulation of inflammation and immune responses. In the context of type 1 diabetes, immune-mediated destruction of pancreatic beta cells is a pivotal event leading to the loss of insulin production capacity. Activating PPARγ holds promise in modulating T cell survival, activation, and differentiation, presenting potential benefits for type 1 diabetes (15, 16). Despite its significance, this area remains underexplored, necessitating further research to elucidate the effects of PPAR activation on T cell function in type 1 diabetes.

Emerging evidence suggests the potential impact of oleanolic acid, a pentacyclic triterpene present in various plants, on PPARγ in diabetes. Oleanolic acid demonstrates a dual agonist action on PPARγ/α and GLUT4 translocation, correlating with its observed antihyperglycemic and anti-dyslipidemic effects. In a study involving type 2 diabetes (T2D) model mice, administration of oleanolic acid at 20 mg/kg ameliorated inflammatory conditions by downregulating circulating IL-1β, IL-6, and TNF-α, subsequently improving hepatic insulin sensitivity (17). Oleanolic acid also influenced the expression of PPARγ/α and their regulated genes, including adiponectin. In vitro studies further indicated that

oleanolic acid stimulated GLUT-4 translocation in C2C12 myoblasts, inhibited lipid accumulation in 3T3-L1 adipocytes, and affected PPARγ/α. These findings collectively suggest that oleanolic acid may exert an antihyperglycemic effect through its dual action on PPARγ/α and GLUT4 translocation (18).

Beta-carotene is correlated with enhancements in glucose metabolism and insulin sensitivity, factors intricately connected to PPARγ activity. Research findings indicate that beta-carotene possesses the capacity to mitigate insulin resistance, enhance insulin sensitivity, and reduce fasting blood glucose levels. Furthermore, there is evidence suggesting that beta-carotene can modulate PPARγ activity, consequently diminishing the lipid storage capacity of adipocytes (19).

Similarly, Delphinidin has been documented for its multifaceted properties, encompassing anti-inflammatory, antioxidant, anti-mutagenic, and anti-angiogenic activities (20). *In vivo*, research indicates that delphinidin exhibits preventive effects on endothelial cell function injuries associated with diabetes. Notably, the administration of 100 mg/kg delphinidin to diabetic mice demonstrated a reduction in HbA1c glycation and the rate of albumin, underscoring its potential therapeutic impact (20,21). The recognition of phytocompounds, including oleanolic acid, beta-carotene, and delphinidin, emphasizes their prospective role in targeting PPARγ and modulating critical pathways involved in insulin secretion, glucose uptake, and inflammation. These outcomes underscore the imperative for additional research and clinical inquiries to harness the therapeutic advantages offered by these natural compounds within the intricate domain of type 1 diabetes.

CONCLUSION

The exploration of natural compounds, particularly those derived from berries and Indian Blackberry, provides a compelling foundation for potential advancements in the management of type 1 diabetes. Noteworthy compounds, such as oleanolic acid, beta-carotene, and delphinidin, exhibit promising pharmacological attributes in targeting PPARγ and influencing pivotal pathways associated with diabetes. However, it is imperative to acknowledge the limitations inherent in this study. The reliance on in silico techniques and preclinical models necessitates cautious extrapolation to human conditions. Furthermore, the complex nature of type 1 diabetes warrants a comprehensive understanding, with the study primarily emphasizing PPARγ without fully elucidating the broader systemic effects. Future perspectives should encompass rigorous clinical trials to substantiate the efficacy of these compounds in human subjects, accounting for individual variations in response. Additionally, a holistic exploration of the interplay between PPARγ and immune responses, pivotal in type 1 diabetes, demands further investigation.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Gregory, G.A., Robinson, T.I.G., Linklater, S.E., Wang, F., Colagiuri, S., de Beaufort, C., *et al.*, Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. Lancet Diabetes Endocrinol. 2022;10(10):741-760.
2. Harding, J.L., Wander, P.L., Zhang, X., Li, X., Karuranga, S., Chen, H., *et al.*, The incidence of adult-onset type 1 diabetes: A systematic review from 32 countries and regions. Diabetes Care. 2022;45(4):994-1006.
3. Chhabra. M., Sharma, S. Potential role of peroxisome proliferator activated receptor gamma analogues in regulation of endothelial progenitor cells in diabetes mellitus: An overview. Diabetes Metab Syndr. 2019;13(2):1123-1129.
4. Soni, N.O., Pawar, S.V., Kale, S., Mane, U.A., Bhosale, P.U., Phandhare, K., *et al.*, Peroxisome proliferator-activated receptors and thiazolidinediones in diabetic nephropathy. Int J Basic Clin Pharmacol. 2019;8(10):2344.
5. Zhang, K., Yuan, Q., Xie, J., Yuan, L., Wang, Y. PPAR-γ activation increases insulin secretion independent of CASK in INS-1 cells. Acta Biochim Biophys Sin (Shanghai), 2019; 51(7):715-722.
6. Santini, E., Fallahi, P., Ferrari, S.M., Masoni, A., Antonelli, A., Ferrannini, E. Effect of PPAR-γ activation and inhibition on glucose-stimulated insulin release in INS-1e cells. Diabetes. 2004;53(suppl_3):S79-S83.
7. Evans-Molina, C. Peroxisome proliferator-activated receptor γ activation restores islet function in diabetic mice through reduction of endoplasmic reticulum stress and maintenance of euchromatin structure. Molecular and Cellular Biology. 2009; 29:2053-2067.
8. Yang, H., Tian, T., Wu, D., Guo, D., Lu, J. Prevention and treatment effects of edible berries for three deadly diseases: cardiovascular disease, cancer and diabetes. Crit Rev Food Sci Nutr. 2019;59(12):1903-1912.
9. Jayasurya, Swathy, Susha, Sharma, S. Molecular docking and investigation of *Boswellia serrata* phytocompounds as cancer therapeutics to target growth factor receptors: An *in-silico* approach. Int J Appl Pharm. 2023;15(4):173-183.
10. Galgale, S., Zainab, R., Kumar, A. P., Nithya, Susha, Sharma, S. Molecular docking and dynamic simulation-based screening identifies inhibitors of targeted SARS-CoV-2 3CLpro and human ACE2. Int J Appl Pharm. 2023;15(6):297-308.
11. DiMeglio, L.A., Evans-Molina, C., Oram, R.A. Type 1 diabetes. Lancet. 2018;391(10138):2449-2462.
12. Calvano, A., Izuora, K., Oh, E.C., Ebersole, J.L., Lyons, T.J., Basu, A. Dietary berries, insulin resistance and type 2 diabetes: An overview of human feeding trials. Food Funct. 2019; 10(10):6227-6243.
13. Aqil, F. Antioxidant and antiproliferative activities of anthocyanin/ellagitannin-enriched extracts from *Syzygium cumini* L.(Jamun, the Indian Blackberry). Nutrition and Cancer. 2012;64(3):428-438.
14. Sarkar, D., Orwat, J., Hurburt, T., Woods, F., Pitts, J.A., Shetty, K. Evaluation of phenolic bioactive-linked functionality of blackberry cultivars targeting dietary management of early stages type-2 diabetes using in vitro models. Sci Hortic (Amsterdam). 2016;212:193-202.
15. Jimenez-Garcia, S.N., Vazquez-Cruz, M.A., Garcia-Mier, L., Contreras-Medina, L.M., Guevara-González, R.G., Garcia-Trejo, J.F., *et al.*, Phytochemical and pharmacological properties of secondary metabolites in berries. In: Therapeutic Foods. Elsevier; 2018. p. 397-427.
16. Azofeifa, G., Quesada, S., Navarro, L., Hidalgo, O., Portet,

K., Pérez, A.M., *et al.,* Hypoglycaemic, hypolipidaemic and antioxidant effects of blackberry beverage consumption in streptozotocin-induced diabetic rats. J Funct Foods. 2016;26: 330-337.

17. Loza-Rodríguez, H., Estrada-Soto, S., Alarcón-Aguilar, F.J., Huang, F., Aquino-Jarquín, G., Fortis-Barrera, Á., *et al.*, Oleanolic acid induces a dual agonist action on PPARγ/α and GLUT4 translocation: A pentacyclic triterpene for dyslipidemia and type 2 diabetes. Eur J Pharmacol. 2020;883: 173252.
18. Sung, H.Y., Kang, S.W., Kim, J.L., Li, J., Lee, E.S., Gong, J.H., *et al.*, Oleanolic acid reduces markers of differentiation in 3T3-L1 adipocytes. Nutr Res. 2010;30(12):831-839.
19. Marcelino, G., Machate, D.J., Freitas, K., de C, Hiane, P.A., Maldonade, I.R., Pott, A., *et al.*, β-carotene: Preventive role for type 2 diabetes mellitus and obesity: A review. Molecules. 2020;25(24):5803.
20. Gharib, A., Faezizadeh, Z., Godarzee, M. Treatment of diabetes in the mouse model by delphinidin and cyanidin hydrochloride in free and liposomal forms. Planta Med. 2013; 79(17):1599-1604.
21. Vinayagam, R., Xu, B. Antidiabetic properties of dietary flavonoids: a cellular mechanism review. Nutr Metab (Lond). 2015;12(1):60.