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Bioactive Compounds from *Brachychiton diversifolius* Peel as Potential Inhibitors of Protein Tyrosine Phosphatase: Implications for Diabetes Therapy

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Abstract

The *Brachychiton diversifolius* is among the widely employed plants in conventional systems of medicine in numerous therapies, especially in the management of metabolic disorders such as diabetes. The tyrosine phosphorylation is important in glucose homeostasis and insulin signaling and the protein tyrosine phosphatase is an important target in the development of antidiabetic treatment. This investigation aimed at determining bioactive elements of the methanolic leaf extract of *B. diversifolius* and testing their inhibitory capacity against protein tyrosine phosphatase. Using the gas chromatography-mass spectrometry (GC-MS), eight individual phytocompounds were detected in the methanolic leaf extract of *B. diversifolius*. The drug-likeness and pharmacokinetics of each compound were analyzed using SwissADME web program. The findings were complete agreement with the Rule of Five of Lipinski, having good oral bioavailability and drug-like characteristics. Moreover, binding affinity against protein tyrosine phosphatase was determined by performing molecular docking with the PyRx virtual screening tool. Interaction analysis was done through the Discovery Studio Visualizer. The docking outcome showed that there are a few phytocompounds with robust binding affinities with the active site of protein tyrosine phosphatase. Acridin-9-yl (4-nitro-phenyl) was the compound with the highest binding affinity that showed good binding scores with the target protein followed by 3-Hydroxy-4-Methoxy-6-Methylflavone, 3 (3-Bromophenyl) propenone, and 1-Benzylindole. The results aid in

Vol. 4 No. 3 (2025)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

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proving the presence of bioactive compounds in the leaves of *Brachychiton diversifolius* that have significant inhibitory effects against protein tyrosine phosphatase, which validates the use of the leaves in the treatment of diabetes. Nevertheless, future *in vitro* and *in vivo* studies are recommended to establish the therapeutic efficacy of the identified compounds.

Introduction

Diabetes mellitus is one of the most acute global health issues of the twenty-first century. The evidence suggested by the World Health Organization shows that the number of diabetes cases has increased significantly across the globe, and in recent years, it is reported that more than 800 million adults currently have this chronic metabolic syndrome (Uzbekova *et al*.2026). The most common type of diabetes mellitus is type 2, which is insulin-resistant and progressive 8-cell dysfunction, resulting in chronic hyperglycemia and microvascular and macrovascular complications (Sriramavaratharajan *et al*., 2026). Plant-based medicines have a long history of providing invaluable bioactive compounds to drug discovery, making the search of safer and more effective therapeutic agents to rekindle interest in this field.

Reversible protein tyrosine phosphorylation is a molecular switch which critically regulates the insulin signaling pathway and controls the cellular responses to hormonal stimuli. Protein tyrosine phosphatase 1B (PTP1B) has become one of the leading negative regulators of insulin signal transduction, a non-transmembrane phosphatase that directly dephosphorylates insulin receptor and insulin receptor substrate protein products, which suppress downstream signaling cascades of glucose uptake and metabolism (Coronell-Tovar *et al*., 2024; Sun *et al*., 2024; Delibegović *et al*., 2024). According to recent developments, it was clarified that PTP1B is transposed on the cytoplasmic face of the endoplasmic reticulum and can reach the insulin receptor during endocytosis and biosynthesis making it a central keystone to insulin sensitivity (Jin *et al*., 2023). The overexpression and hyperactivity of PTP1B have been repeatedly reported in the insulin-resistant states and obese phenotypes, which makes this phosphatase a proven molecular target in the development of antidiabetic drugs (Haider *et al*., 2025; Delibegović *et al*., 2024). Therefore, selective inhibition of PTP1B is a logical therapeutic approach to improve insulin sensitivity and re-establish glycemic regulation, although the issues of PTP1B inhibitor selectivity and oral bioavailability are still in the state of research (Sun *et al*., 2024; Haider *et al*., 2025).

The Brachychiton is a genus of the family Malvaceae that contains about 30 species of tree endemic to Australia. One of them, *Brachychiton diversifolius* also referred to as the northern kurrajong, has been used in nutrition and as a source of medicine by Aboriginal people in Australia (Thabet *et al*., 2018). The inner bark which was fibrous was used to make ropes and nets, and the seeds which were rich in nutrients were eaten after being roasted. In medicine, wound infections, and diarrhea among other diseases have been treated with different parts of the plant (Salem *et al*., 2014).

Recent phytochemical studies on allied species of Brachychiton have identified the occurrence of various bioactive compounds, such as flavonoids, phenolic acids, and terpenoids, and have antioxidant, antibacterial, and antidiabetic activities (Yassinn *et al*., 2021). Nevertheless, there is a lack of evidence on the phytochemical makeup of the *B. diversifolius* leaves and their potential as a source of PTP1B inhibitors. The current study sought an in-depth description of the

Pakistan Journal of Medical & Cardiological Review
https://pakjmcr.com/index.php/1/about
Online ISSN 3007-2387
Print ISSN 3007-2379

phytochemical composition of the *Brachychiton diversifolius* methanol leaf extract using gas chromatography-mass spectrometry (GC-MS) and assessed its inhibitory effects on protein tyrosine phosphatase 1B by performing a molecular docking analysis. Moreover, computational tools were used to evaluate the pharmacokinetic aspects and the drug-likeness of the discovered compounds to establish their appropriateness as lead compounds to be used in antidiabetic treatment.

2. Materials and methods

2.1. Collection of plant material

Brachychiton diversifolius plants were collected and their leaves were thoroughly washed under running water and air-dried in the shade.

2.2. Preparation of methanolic extract of *Brachychiton diversified* leaves

The dried leaves of *B. diversifolius* were ground into a coarse powder with an electric grinder and then screened to remove big particles. We stored powder in a zip-lock container for future use. 50 grams of leaf powder were dissolved in 75% methanol at 1 gram per 10 milliliters in an orbital shaker at 24 °C for 72 hours. After filtration through Whatman filter paper no. 1, the mixture was dried at 40 °C using a rotary evaporator and then stored at 4 °C for later use (Nauroze *et al.*, 2023).

2.3. GC-MS analysis of *Brachychiton diversifolius*

Gas chromatography-mass spectrometry study of *Brachychiton diversifolius* methanolic extract identified different compounds. Agilent GC-MS systems have capillary standards and nonpolar columns DB 5MS. The column was 30 mm long, 0.25 mm wide, and 0.25 mm thick. Helium was chosen as the carrier gas and the mobile phase flowed one milliliter per minute. To reach 280 °C, the oven was heated to 10 °C per minute. The injection volume was 1 microliter. A 70eV electron ionization energy system was used for GC-MS analysis. Duration was 65 minutes. The extract was dissolved in methanol and analyzed for 10-850 m/z mass spectra. For identification, names of biologically active compounds were developed along with their molecular weight, structure, and molecular formula. Specific compounds were identified by comparing the fragmentation patterns of the mass spectra to the NIST mass spectral library (Saravanan *et al.*, 2022).

2.4. Pharmacokinetic profile

To investigate the pharmacokinetics of ligand molecules, SwissADME was utilized. It was used to enter the SMILES formula for each active component.

2.5. Ligand-Protein docking study

Chemical docking of *Brachychiton diversifies* phytocompounds against tyrosine phosphate protein was done during this investigation. Molecular docking studies using PyRx 0.8 revealed ligand-protein binding mechanisms. RCSB-PDB provided the receptor protein's 3D structure in PDB format. The PubChem database included SDF chemical structures of active phytoconstituents.

Vol. 4 No. 3 (2025)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

After eliminating bound ligands and water molecules, polar hydrogen atoms were added to construct the target protein's structure. Then used BIOVIA Discovery Studio's Visualizer program (v21.1.0.20298) to generate and display the phytocompounds' interactions and maps with the target protein (Arif *et al.*, 2024).

3. Results

3.1. Identification of bioactive compounds of *B. diversifolius* peel extract

Gas chromatography-mass spectrometry chromatogram reveals 8 peaks that show the presence of 8 bioactive compounds and their identification was based on their retention time (Rt) and peak area. Retention duration and mass spectra were compared to databases or standards to locate peaks. These compounds with their class name, peak area, retention time, and structure are given in Table (1). As shown in fig. (1), 1-Benzylindole shows the highest peak of 17.06% with RT of 8.542 minutes while Acridin-9-yl (4-nitro-phenyl) shows the lowest peak of 15.46% with RT of 18.137 minutes.

Vol. 4 No. 3 (2025)

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https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

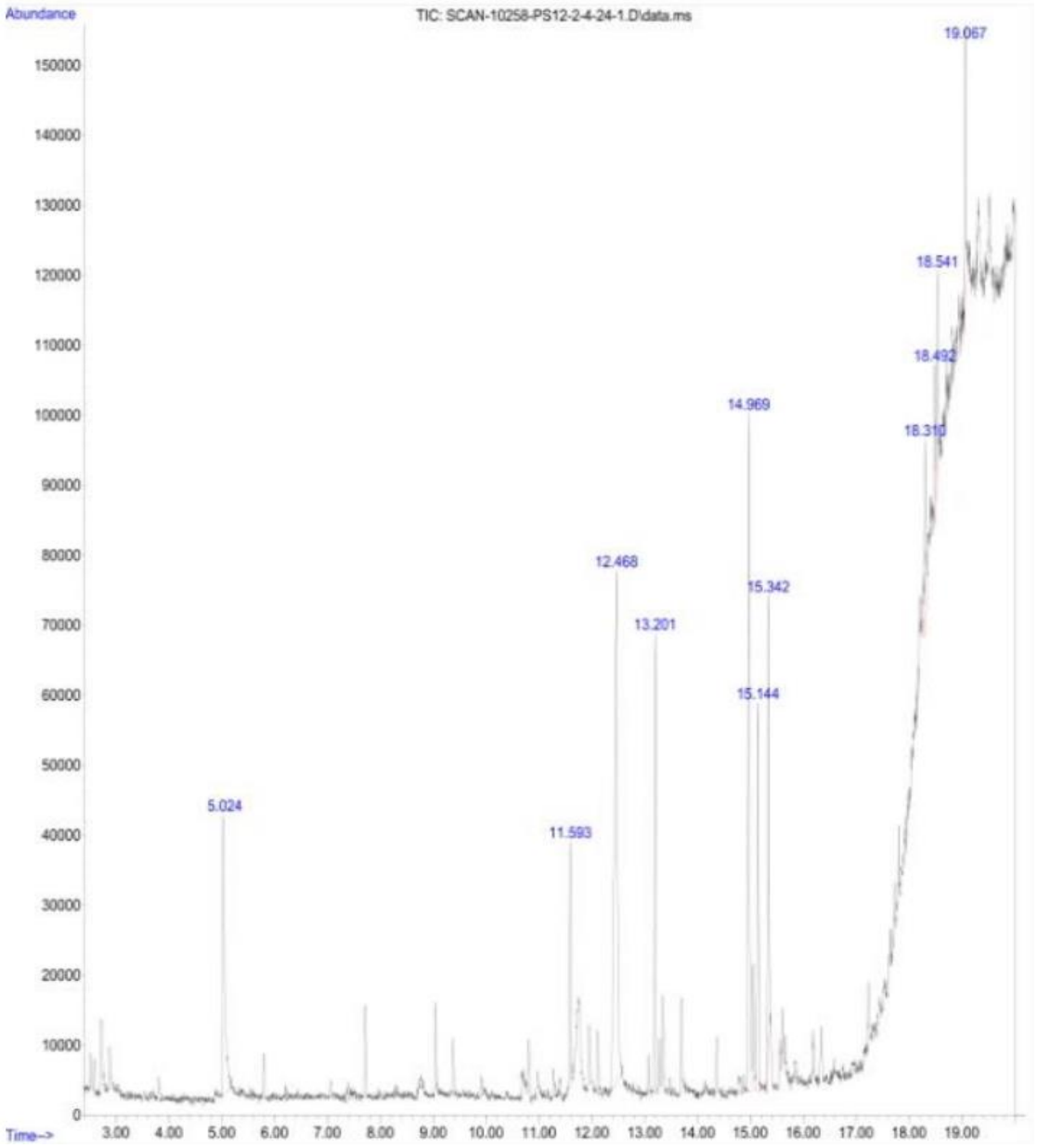

Figure 1: GC-MS chromatogram of methanolic leaf extract of *Brachychiton diversifolius*

Vol. 4 No. 3 (2025)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Table 1: Bioactive compounds derived from the methanolic leaves extract of *B. diversifolius* using GC-MS.

Sr. No.	Compound name	Class name	Peak area (%)	RT	Molecular formula
1	4_thiazolidinecarboxylic acid	D-alpha-amino acid	27.71	11.971	$C_4H_7NO_2S$
2	4-Methoxyphenyl Octanoate	Phenols ester	21.20	13.692	$C_{15}H_{22}O_3$
3	2-Ferrocenyl-3-(p-Tolyl) Thiazolidinone	Thiazolidinone	4.39	18.206	$C_{20}H_{19}FeNOS$
4	Acridin-9-yl (4-nitro-phenyl)	Acridine derivatives	15.46	18.137	$C_{19}H_{13}N_3O_2$
5	3-Hydroxy 4-Methoxy-6-Methylflavone	Flavonoids	8.42	18.309	$C_{17}H_{14}O_4$
6	3-(3-Bromophenyl) propenone	Chalcones	8.42	18.309	$C_{10}H_9BrO$
7	1-Benzyl-1,3,3-trimethylazetidine	Azetidinium salts	5.76	18.271	$C_{13}H_{19}N$
8	1-Benzylindole	Indoles	17.06	18.542	$C_{15}H_{13}N$

Pakistan Journal of Medical & Cardiological Review
https://pakjmcr.com/index.php/1/about
Online ISSN 3007-2387
Print ISSN 3007-2379

3.2. Pharmacokinetic profile

Pharmacokinetic analyses have been done through SwissADME by entering their SMILES formula to investigate the effects of these drugs or compounds within a biological system over time the results are shown in Table (2).

Table 2: Pharmacokinetic analyses of the identified compounds.

Sr. No.	Compounds name	Molecular mass (g/mol)	Acceptor H	Donor H	LogP	Lipinski
1.	4_thiazolidinecarboxylic acid	133.17	3	2	0.70	yes
2.	4-Methoxyphenyl Octanoate	250.33	3	0	3.15	yes
3.	2-Ferrocenyl-3-(p-Tolyl) Thiazolidinone	372.39	0	0	0.00	yes
4.	Acridin-9-yl (4-nitro-phenyl)	315.33	3	1	2.56	yes
5.	3-Hydroxy 4-Methoxy-6-Methylflavone	282.29	4	1	2.97	yes
6.	3-(3-Bromophenyl) propenone	213.07	1	0	2.05	yes
7.	1-Benzyl-1,3,3-trimethylazetidine	99.17	1	1	2.09	yes
8.	1-Benzylindole	207.27	0	0	2.52	yes

3.3. In silico-based screening of *Brachychiton diversifolius* to identify tyrosine phosphatase inhibitors:

Molecular docking of bioactive compounds found in the methanolic extract of *B. diversifolius* has been performed against tyrosine phosphatase inhibitors using the PyRx docking tool and the results are shown in table (3).

Table 3: Bioactive compounds with docking score and biological activities.

Sr. No.	Ligands	PubChem ID	Docking Score	Reported biological activity
1	4_thiazolidinecarboxylic acid	9934	-4	Antimicrobial, anticancer, anti-inflammatory, antioxidant (Vadabingi *et al.*, 2020).

Pakistan Journal of Medical & Cardiological Review
https://pakjmcr.com/index.php/1/about
Online ISSN 3007-2387
Print ISSN 3007-2379

2	4-Methoxyphenyl Octanoate	532450	-4.5	Antimicrobial, antioxidant anticancer, antiviral, and cytotoxic (Islam *et al*.,2014).
3	2-Ferrocenyl-3-(p-Tolyl) Thiazolidinone	15483480	-5.3	Anxiolytics, anticonvu lsant, sedative-hypnotic, and muscle-relaxant (Pejović *et al*.,2014)
4	Acridin-9-yl (4-nitro-phenyl)	47484	-7.3	Anticancer anti-malarial and anti-rheumatoid agents (Singh *et al*., 2015).
5	3-Hydroxy 4-Methoxy-6-Methylflavone	227443	-6.6	Antimicrobial, antiviral, antioxidant, and anti-inflammatory activities (Evidente *et al*., 2024).
6	3-(3-Bromophenyl) propanone	2734092	-6.3	Anticancer, antiplatelet, gastroprotective, antiallergic, antidiabetic, cytotoxic, hepatoprote ctive, and antiemetic (Rebai *et al*.,2023).
7	1-Benzyl-1,3,3-trimethylazetidine	21719780	-3.7	Antibacterial & antifungal (Baviskar *et al*., 2011).
8	1-Benzylindole	96913	-6.1	Anti-cancer anti-fungal and anti-bacterial (Al-Ghananeem.,2013).

3.4. Molecular docking of compounds

A total of 8 bioactive compounds is found in the Methanolic leaf extract of *Brachychiton diversifolius* were identified with good binding energy and then their attraction with the protein is shown in figures (2-4) below using the Discovery Studio tool. These are 1-Benzylindole with a binding score of –6.1 in fig (2), 3-Hydroxy 4-Methoxy-6- Methylflavone a binding score of -6.6 in fig (3), and Acridin-9-yl (4-nitro-phenyl) with a highest binding score of -7.3 in fig (4).

Vol. 4 No. 3 (2025)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

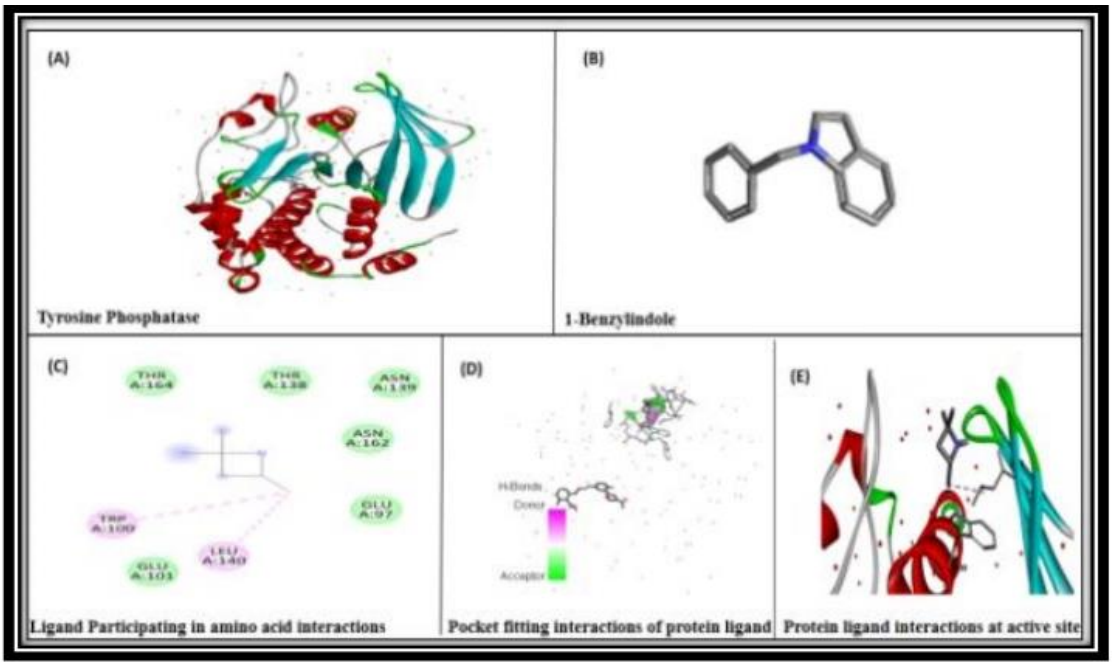

Figure 2: Protein tyrosine phosphatase interaction with 1-Benzylindole

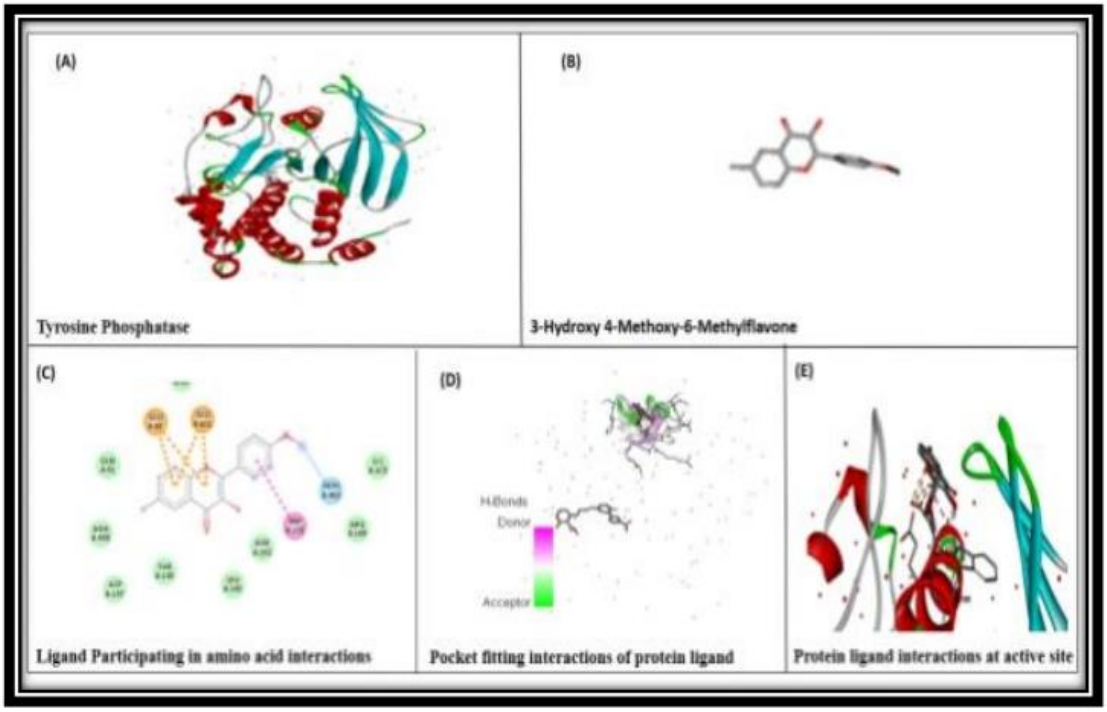

Figure 3: Protein tyrosine phosphatase interaction with 3-Hydroxy 4-Methoxy-6-Methylflavone

Vol. 4 No. 3 (2025)
Pakistan Journal of Medical & Cardiological Review
https://pakjmcr.com/index.php/1/about
Online ISSN 3007-2387
Print ISSN 3007-2379

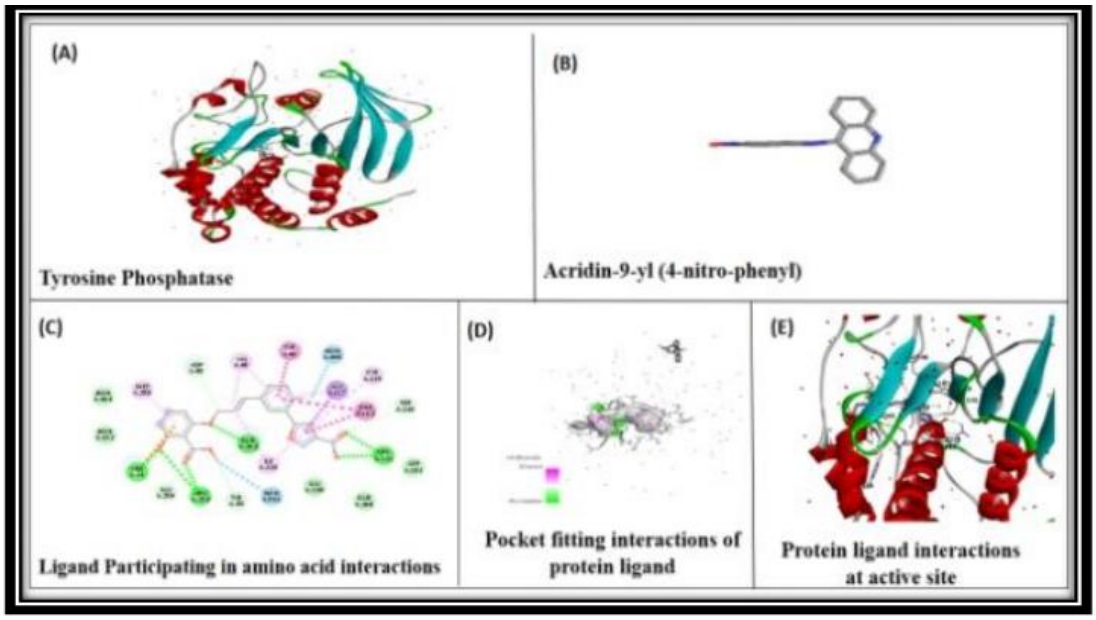

Figure 4: Protein tyrosine phosphatase interaction with Acridin-9-yl (4-nitro-phenyl)

5. Discussion

Current study was conducted for phytochemical profiling of *Brachychiton diversifolius* methanolic leaf extract and to identify its anti-diabetic effectiveness by inhibiting protein tyrosine phosphatase 1B (PTP1B), which is a confirmed therapeutic target. The presence of several bioactive compounds in the leaves of *B. diversifolius* justifies its use as traditional medicine and indicates that it could have a therapeutic use in metabolic diseases. A previous study by Kaur *et al.*, (2019); and Kamel *et al.*, (2025), examined the presence of several bioactive compounds in the leaves of *B. diversifolius* which justifies its use as traditional medicine and indicates that it could have a therapeutic use in metabolic diseases. The analysis of the extract was examined under gas chromatography-mass spectrometry (GC-MS) that showed different phytocompounds. These observations follow the earlier phytochemical studies of Brachychiton species that have reported the occurrence of flavonoids, terpenoids and phenolic compounds with varied biological activity (Kamel *et al.*, 2025). Similar inhibitory mechanisms could be possible due to the occurrence of structurally related compounds in the extract of *B. diversifolius.*

The variety of bioactive compounds found in the *B. diversifolius* extract can potentially act in a complementary effect on its antidiabetic activity and, thus, may increase the therapeutic effectiveness with reduced adverse effects of the therapies with single compounds. Pharmacokinetic screening based on the SwissADME web tool has shown that all the eight identified compounds showed good drug-like properties complete to the Rule of Five by Lipinski. Ahmed *et al.*, (2025), suggested that given pharmacological method is a particular benefit of plant-based treatments compared to the traditional synthetic drugs. Compliance with these basic requirements is a predictor of good oral bioavailability and absorption and allows the possibility to develop these compounds as therapeutic agents (Zeki *et al.*, 2024). More computational pharmacokinetic profiling studies have highlighted the value of early ADME profiling in natural product discovery and tools such as SwissADME have offered credible

Pakistan Journal of Medical & Cardiological Review
https://pakjmcr.com/index.php/1/about
Online ISSN 3007-2387
Print ISSN 3007-2379

information on drug-likeness and any issues with toxicity potential (Zeki *et al.*, 2024). Molecular docking experiments indicated that a number of phytocompounds had a high binding affinity to PTP1B. These binding interactions reveal that the ligand molecules form stable complexes with the PTP1B active site, which implies the possible competition with the enzyme activity. Kaur *et al.*, (2025), stated that overexpression and increased activity of this phosphatase have been consistently documented in insulin-resistant states and obese phenotypes, establishing it as a validated molecular target for therapeutic intervention. Recent structural investigations by Kumar *et al*., (2018), have been used to explain the molecular pathways of PTP1B inhibition, finding that the compounds presented to inhibit the catalytic active site and allosteric binding pockets may efficiently tune the enzyme activity and enhance selectivity profiles. The binding affinities in the current study are also favorable to those reported in the recent past about PTP1B inhibitors of natural origin.

Conclusion

This in silico study was able to identify eight bioactive phytochemicals of *Brachychiton diversifolius* methanolic extract of leaf, which are Acridin-9-yl (4-nitro-phenyl), 3-Hydroxy-4-Methoxy-6-Methylflavone, and 3- (3-Bromophenyl) propenone, as the most promising PTP1B inhibitors with docking scores -7.2 to -5.8 kcal/mol. All of them exhibited good drug-like behavior with complete adherence to Lipinski Rule of five, justifying their future presence as orally bioavailable lead molecules in antidiabetic therapy. The results give a scientific explanation of the traditional use of *B. diversifolius* in management of diabetes. The following researches should, however, consider in vitro enzyme inhibition experiments to confirm these projections, then move to molecular dynamics experiments, isolating the compounds and finally *in vivo* efficacy studies to propel these phytochemicals to clinical trials.

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