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Original Article

Synergistic antioxidant and antidiabetic activities of *Tinospora cordifolia* and *Azadirachta indica* extracts supported by *in-silico* molecular docking and ADMET evaluation

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ABSTRACT

Background: Type 2 Diabetes mellitus is a chronic metabolic disorder which worsens by increased oxidative stress resulting in other diabetes associated complications. *Tinospora cordifolia* (TC) and *Azadirachta indica* (AI) are traditional medicinal plants that has been used for diabetes since ancient times, but their combined potential as a formulation has not been evaluated for the management of diabetes. **Objectives:** This study aimed to profile the phytochemical composition of 70% hydroethanolic extracts of TC and AI, assess their antioxidant and antidiabetic activity individually and in combination, and further substantiate their efficacy through *in-silico* molecular docking and ADMET evaluation of their key bioactive compounds, berberine and nimbolide. **Methods:** Hydroethanolic extracts of TC and AI were subjected to preliminary phytochemical screening, quantification of primary and secondary metabolites, and HPTLC profiling. Five combinations of TC:AI (1:1, 1:2, 2:1, 1:3, 3:1) were prepared and evaluated for antioxidant activity using DPPH, ABTS, and FRAP assays, and for antidiabetic potential using α-amylase, α-glucosidase, and PTP1B inhibition assays. IC_{50} values were calculated. In-silico studies were performed for berberine and nimbolide using AutoDock 4.2.6 against PPARγ, GLUT4, and IRS1. Drug-likeness and ADMET properties were predicted using SwissADME and pkCSM. **Results:** Phytochemical profiling confirmed the presence of alkaloids, polyphenols, flavonoids, and tannins. TC demonstrated stronger inherent antioxidant and antidiabetic activity than AI. Among combinations, 1:1 and 3:1 formulations showed the highest potency with IC_{50} values comparable to standards. Molecular docking revealed strong binding affinities of berberine and nimbolide toward PPARγ, GLUT4, and IRS1, while ADMET prediction indicated acceptable pharmacokinetic behaviour and good drug-likeness. **Conclusion:** The TC:AI 1:1 formulation exhibited synergistic antioxidant and antidiabetic effects, supported by favorable in-silico interactions and pharmacokinetic profiles, highlighting its potential as a natural therapeutic option for T2DM management.

Keywords: Plants, *Tinospora cordifolia*, *Azadirachta indica*, health, antioxidants, α -amylase, α -glucosidase, PTP1B.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterised by elevated blood sugar owing to improper insulin production or action. T2DM is the most prevalent type of DM in the world and often presents with insulin resistance and insufficient insulin output[1]. The rapidly increasing incidence of T2DM that is exacerbated by sedentary lifestyle and unhealthy diet provides the rationale for improved long-term treatments[2]. Although the existing anti-diabetic drugs such as Metformin, Rosiglitazone, Glibenclamide, Glimepiride as well as insulin helps in lowering the blood glucose levels, long-term use of these drugs results in various side effects viz., weight gain, hypoglycemia, gastrointestinal problem, nausea and hepatotoxicity[3]. Owing to the reported side-effects, alternative medicines especially drugs sourced from traditional medicinal plants has gained attention among the public and researchers due to their potent anti-oxidant and anti-diabetic effects.

Medicinal plants have played a key role in traditional medicine system for the management of type 2 diabetes mellitus. These plants possess various beneficial compounds, including polyphenols, flavonoids, tannins, alkaloids, terpenoids, and glycosides. They help regulate carbohydrate metabolism and improve insulin secretion, which aids in glucose absorption by cells[4,5]. Among the medicinal plants studied, *Tinospora cordifolia* (Guduchi) and *Azadirachta indica* (Neem) have drawn significant interest because of their strong anti-diabetic and antioxidant qualities. Neem, widely known in Ayurveda, offers many health benefits, such as antioxidant, antimicrobial, anti-inflammatory, liver-protective, and anti-diabetic effects [6]. Research shows that different solvent extracts of *A. indica* lower plasma glucose levels by improving insulin sensitivity, boosting glucose absorption, and reducing oxidative stress[7-9]. The aqueous extract from *A. indica* leaves has been proven to significantly enhance blood sugar control, impact insulin signalling pathways, and restore normal glucose levels in diabetic models. Additionally, Neem extract has been shown to increase the expression of glucose transporter type 4 (GLUT4) proteins in skeletal muscles, which helps with glucose absorption[10-12].

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Tinospora cordifolia, also called Guduchi, is another medicinal plant with recognized anti-diabetic effects. It helps the body absorb glucose by increasing glucose transporters and affecting insulin receptor signaling[13, 14]. Studies in diabetic models have shown that *T. cordifolia* extracts can lower blood sugar by blocking the α-glucosidase enzyme and supporting pancreatic β-cell function[15,16]. This plant is also rich in polyphenols and flavonoids, which contribute to its antioxidant effects and help reduce oxidative stress, an important factor in diabetes complications[17].

Given their individual medicinal benefits, combining *A. indica* and *T. cordifolia* may produce stronger anti-diabetic effects. *A. indica* mainly improves insulin sensitivity and glucose metabolism, while *T. cordifolia* supports β-cell function and reduces oxidative damage. Together, these plants may offer a multi-targeted approach for effective diabetes management while minimizing adverse effects associated with synthetic antidiabetic agents. In addition to phytochemical profiling, antioxidant evaluation, and *in-vitro* anti-diabetic assays, the present study also explores the mechanistic basis of this synergistic activity through molecular docking and ADMET analysis of berberine and nimbolide, key bioactive constituents of *T. cordifolia* and *A. indica*, respectively. These computational evaluations provide insight into the interaction of these phytochemicals with crucial insulin-signalling targets and their drug-likeness and pharmacokinetic suitability. Overall, this research aims to establish a scientifically validated, natural, and multi-modal therapeutic strategy for type 2 diabetes mellitus and its complications, including diabetic nephropathy, neuropathy, and retinopathy, responding to the growing global interest in safe and sustainable plant-based interventions.

MATERIALS AND METHODS

Phytochemical Analysis - Qualitative Analysis

Phytochemical screening of the hydroethanolic extracts of *Tinospora cordifolia* and *Azadirachta indica* was carried out following established protocols. The presence of saponins, tannins, flavonoids, and steroids was determined according to the procedure described by Harborne[18]. In addition, alkaloids, terpenoids, anthraquinones, and phenolic compounds were identified using the methods outlined by Roghini and Vijayalakshmi (2018)[19].

Primary and Secondary Metabolites Quantification

The quantification of primary and secondary metabolites was carried out using standard protocols. Total protein content was estimated by the Folin–Ciocalteu method[20]. Carbohydrates were quantified by the Anthrone–Sulfuric acid method, which involved dehydration of sugars followed by colour development with phenol, and absorbance was measured at 620nm[21]. Lipids were estimated following the Bligh and Dyer extraction method[22]. Secondary metabolites were estimated by colorimetric assays. Total alkaloid content was determined using the bromocresol green (BCG) method[23]. Total phenolic content was quantified by the Folin–Ciocalteu assay[24]. Tannins were estimated using the polyvinylpolypyrrolidone (PVPP) precipitation method[25]. Total flavonoid content was measured by the aluminum chloride colorimetric method[26].

HPTLC Phyto profiling

70% hydroethanolic extracts of *Tinospora cordifolia* and *Azadirachta indica* were prepared (50 mg dissolved in 1.0 mL of 70% hydroethanol) and applied (2, 4, and 6 µL) as 6-mm bands onto pre-coated silica gel 60 F254 aluminum plates using a CAMAG Linomat V applicator[27,28]. Plates were developed in a twin-trough chamber using chloroform:methanol (9:1, v/v) as the mobile phase under standard pre-saturation conditions. After development, plates were air-dried and visualized under short-wave UV (254 nm) and long-wave UV (366 nm). Plates were then derivatized by spraying with anisaldehyde–sulfuric acid (ASA) reagent and documented under visible light at 650 nm. Densitometric scanning was performed at 254 nm, 366 nm, and 650 nm (post-derivatization), and the Rf values, band colour, and peak profiles were recorded for each sample.

In vitro free radical scavenging and antioxidant assays

The free radical scavenging potential of the *T. cordifolia* and *A. indica* extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay[29]. The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay was performed to determine the antioxidant capacity of the extracts, wherein the ability of the samples to quench $ABTS^{\cdot+}$ radicals, through electron donation, resulted in a decrease in absorbance[30]. In addition, the ferric reducing antioxidant power (FRAP) of the extracts was evaluated using the standard Fe^{3+} reducing assay, which measures the reduction of ferric ions to the ferrous form as an indicator of antioxidant activity[31].

In vitro diabetic enzymes inhibitory assays

The α-amylase inhibitory activity of the *T. cordifolia* and *A. indica* extracts was evaluated using the 3,5-dinitrosalicylic acid (DNSA) method [32]. The α-glucosidase inhibition was determined by employing the p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate-based assay[33]. Protein tyrosine phosphatase 1B (PTP1B) inhibition was assessed using the p-nitrophenyl phosphate (pNPP) method following established protocols[34].

Molecular docking

Crystal structures of PPARγ (PDB ID: 1PRG), GLUT4 (PDB ID: 7WSM), and IRS1 (PDB ID: 5U1M) were retrieved from the RCSB Protein Data Bank, while berberine (PubChem CID 2353) and nimbolide (PubChem CID 12313376) were obtained from PubChem, converted from SDF to PDB format, protonated at pH 7.4, and energy-minimised using the MMFF94 force field prior to generating PDBQT files. Protein structures were prepared in AutoDockTools by removing crystallographic water molecules, eliminating non-essential heteroatoms, repairing missing residues, assigning Kollman charges, adding polar hydrogens, and applying Gasteiger charges to create rigid receptor files. Docking grids were centred on the coordinates of each co-crystallised ligand and defined to fully encompass the active site (typically 60 × 60 × 60 points, 0.375 Å spacing). Molecular docking was performed using AutoDock 4.2.6 with the Lamarckian Genetic Algorithm (population size 150, maximum 2.5×10^6 evaluations, mutation rate 0.02, crossover rate 0.8), allowing full ligand flexibility while keeping receptors rigid. For each ligand–receptor pair, multiple docking runs were clustered at 2.0 Å RMSD, and the lowest-energy pose within the dominant cluster was selected for analysis. Docking reliability was confirmed by re-docking the native ligands (RMSD ≤ 2.0 Å), and key interactions—including hydrogen bonds, hydrophobic contacts, and π-interactions—were analysed and visualised using BIOVIA Discovery Studio Visualizer and Chimera[35].

Determination of ADMET, Lipinski criteria, pharmacokinetics, and drug-likeness

Drug-likeness and physicochemical properties of berberine and nimbolide were assessed using the SwissADME web tool (http://www.swissadme.ch), where their SMILES structures were submitted to evaluate compliance with major drug-likeness filters (Lipinski, Ghose, Veber, Egan, and Muegge rules), alongside calculated parameters such as molecular weight, consensus LogP, topological polar surface area (TPSA), hydrogen-bond donors and acceptors, rotatable bonds, solubility estimates, gastrointestinal absorption, blood–brain barrier

(BBB) permeability, P-glycoprotein interaction, bioavailability radar, and synthetic accessibility [36, 37]. Further pharmacokinetic and toxicity predictions were performed using pkCSM (https://biosig.lab.uq.edu.au/pkcsm), which provided computational estimates for absorption characteristics (water solubility, Caco-2 permeability, human intestinal absorption, skin permeability, and P-gp substrate/inhibitor status), distribution parameters (volume of distribution, fraction unbound, BBB and CNS permeability), metabolic behaviour (substrate status and inhibitory potential toward major CYP450 isoforms), excretion properties (total clearance and renal OCT2 affinity), and toxicity endpoints including AMES mutagenicity, maximum tolerated dose, hERG I/II inhibition, hepatotoxicity, oral LD_{50}, chronic LOAEL, and environmental toxicity indices. All predicted values were compiled and compared to interpret the pharmacokinetic suitability of both phytochemicals.

RESULTS

Preliminary phytochemical analysis

The aqueous extract from *T. cordifolia* produced a yield of 4.25% (w/w), while *A. indica* had a higher yield of 7.69% (Table 1). Initial qualitative tests indicated that both *T. cordifolia* and *A. indica* contained alkaloids, flavonoids, phenols, tannins, saponins, glycosides, and terpenoids (Table 2). *T. cordifolia* showed a carbohydrate content of 8.4±0.11% (w/w), protein at 3.36±0.02% (w/w), and lipids also at 3.36±0.04% (w/w). In comparison, *A. indica* had carbohydrate values of 1.83±0.01% (w/w), protein at 2.16±0.01% (w/w), and lipids at 5.12±0.11% (w/w) (Table 3). The total phenolic content (TPC) was measured at 7.8±0.11% (w/w) for *T. cordifolia* and a notable 20.5±0.16% (w/w) for *A. indica*. For *T. cordifolia*, the alkaloid, tannin, and flavonoid contents were 11.2±0.20% (w/w), 8.21±0.12% (w/w), and 3.32±0.11% (w/w), respectively. In contrast, *A. indica* possessed 10.62±0.11% (w/w) of alkaloids, 43.8±0.28% (w/w) of tannins, and 8.94±0.16% (w/w) of flavonoids (Table 4).

Table 1. Yield percentage of hydroethanolic extracts of *Tinospora cordifolia* and *Azadirachta* indica

Plant extracts	Colour of the extract	Yield % (w/w)
Tinospora cordifolia	Light yellow	4.25
Azadirachta indica	Dark green	7.69

Table 2. Comparison of phytoconstituents in hydroethanolic extracts of *Tinospora cordifolia* and *Azadirachta indica* - Preliminary Qualitative Phytochemical Analysis

Phytoconstituents	Tinospora cordifolia	Azadirachta indica
Proteins	+	+
Carbohydrates	+	+
Reducing sugars	+	-
Alkaloids	+	+
Phenols	+	+
Tannins	+	+
Flavones	+	+
Steroids	-	+
Saponins	+	+
Anthraquinones	-	-
Triterpenoids	+	+

Inference: + indicate presence and – indicate absence of phytoconstituents

Table 3. Quantification of Primary Metabolites in hydroethanolic extracts of *Tinospora cordifolia* and *Azadirachta indica*

Plant extracts	% (w/w)		
	Protein	Carbohydrate	Lipids
Tinospora cordifolia	3.36±0.02	8.4±0.11	3.36±0.04
Azadirachta indica	2.16±0.009	1.83±0.006	5.12±0.11

Results expressed as Mean±SEM (n=3)

Table 4. Quantification of Secondary Metabolites in hydroethanolic extracts of *Tinospora cordifolia* and *Azadirachta indica*

Plant extracts	% (w/w)			
	Alkaloids	Total Phenols	Tannins	Flavonoids
Tinospora cordifolia	11.2±0.20	7.8±0.11	8.21±0.12	3.32±0.11
Azadirachta indica	10.62±0.11	20.5±0.16	43.8±0.28	8.94±0.16

Results expressed as Mean±SEM (n=3)

HPLTC Profiling of aqueous extracts of *Tinospora cordifolia* and *Azadirachta indica*

HPTLC analysis of the aqueous extracts of *Tinospora cordifolia* and *Azadirachta indica* demonstrated distinct and well-resolved phytochemical fingerprints, confirming the presence of multiple bioactive constituents in both plants. Visualization under UV light at 254 nm and 366 nm, followed by post-derivatization under visible light, revealed multiple bands with varying Rf values, indicative of diverse phytochemical classes. T. cordifolia exhibited several prominent bands, particularly in the mid- to high-Rf regions (Rf ~0.28–0.65 and >0.80), reflecting the presence of alkaloids, flavonoids, and glycosides, consistent with its reported antioxidant and immunomodulatory profile (Figure 1). In comparison, A. indica displayed well-defined bands across the chromatogram, especially in the polar to mid-range Rf zones (Rf ~0.30–0.70), suggesting the presence of terpenoids, flavonoids, and phenolic compounds characteristic of neem (Figure 2). Densitometric scans supported these observations, showing multiple peaks with varying intensities for both extracts. Overall, T. cordifolia demonstrated slightly higher band density in the upper Rf region, whereas A. indica showed a broader distribution of polar constituents. These results confirm the rich phytochemical composition of both extracts and support their traditional medicinal relevance, while also suggesting potential complementary action when used together in polyherbal formulations.

DPPH free radical scavenging activity

The DPPH assay showed dose-dependent increase in radical scavenging activity for both *T. cordifolia* and *A. indica*, with *T. cordifolia* exhibiting higher antioxidant potential (IC_{50} = 33.08 µg/mL) than *A. indica* (IC_{50} = 63.71 µg/mL) (Table 5). At 200 µg/mL, *T. cordifolia* achieved 71.00% inhibition, compared to 67.72% for *A. indica*. Notably, combining the extracts enhanced activity further, with 1:1 and 3:1 ratios showing the highest inhibition (91.00% and 87.05%, respectively), approaching the effect of ascorbic acid (94.77%). The 3:1 ratio also recorded the lowest IC_{50} (27.27 µg/mL), indicating strong synergistic action. Overall, these results confirm that while *T. cordifolia* is inherently more potent, its combination with *A. indica* further amplifies antioxidant activity, supporting their polyherbal use against oxidative stress.

ABTS Radical Scavenging Activity

The ABTS radical scavenging assay results demonstrated a clear dose-dependent antioxidant effect of *T. cordifolia* and *A. indica* extracts (Table 6). At higher concentrations (100–200 µg/mL), both extracts showed significantly increased % inhibition compared to lower doses. Among combinations, the 1:1 ratio exhibited the highest scavenging activity (93.18% at 200 µg/mL) with the lowest IC_{50} (22.93 µg/mL), closely matching the standard ascorbic acid (Table 6). The 1:3 and 2:1 ratios also showed strong synergistic activity with low IC_{50} values. These findings clearly indicate that while both extracts individually possess significant antioxidant properties, their combinations, particularly in equal or TC-dominant proportions produce synergistic

Table 5. Effect of individual extracts of *T.cordifolia* and *A.indica* and its respective formulations on DPPH radical activity

Concentration (µg/mL)	TC	AI	TC:AI (1:1)	TC:AI (1:2)	TC:AI (2:1)	TC:AI (1:3)	TC:AI (3:1)	Ascorbic acid
	% inhibition of DPPH activity							
0	-2.25±1.87	-2.25±1.87	-2.25±1.87	-2.25±1.87	-2.25±1.87	-2.25±1.87	-2.25±1.87	-2.25±1.87
3.175	3.32±0.03	1.20±0.05	4.99±0.05	3.38±0.02	4.42±0.07	4.12±0.03	5.22±0.05	12.32±0.03
6.25	5.40±0.07	2.88±0.07	7.94±0.08	5.69±0.06	6.49±0.03	7.00±0.08	7.82±0.08	20.56±0.07
12.5	13.34±0.08	6.23±0.11	20.59±0.11	10.19±0.18	16.49±0.11	14.54±0.12	20.11±0.13	36.77±0.19
25	23.56±0.13	11.12±0.14	42.34±0.14	23.39±0.12	35.43±0.16	27.63±0.16	40.28±0.14	42.75±0.11
50	42.40±0.12	20.29±0.18	58.49±0.18	42.93±0.13	56.39±0.09	49.97±0.18	59.83±0.22	62.16±0.21
100	65.16±0.18	45.56±0.21	74.33±0.12	62.39±0.12	68.86±0.16	65.38±0.16	70.63±0.20	78.51±0.18
200	71.00±0.17	67.72±0.15	91.00±0.24	84.21±0.11	88.59±0.12	86.28±0.14	87.05±0.19	94.77±0.19
IC_{50} (µg/mL)	**33.08**	**63.71**	**28.43**	**43.88**	**32.34**	**37.68**	**27.27**	**21.87**

TC-*Tinospora cordifolia*, AI – *Azadirachta indica*; Results expressed as Mean±SEM (n=3). IC_{50} was calculated using linear regression analysis.

Table 6. Effect of individual extracts of *T.cordifolia* and *A.indica* and its respective formulations on ABTS radical activity

Concentration (µg/mL)	TC	AI	TC:AI (1:1)	TC:AI (1:2)	TC:AI (2:1)	TC:AI (1:3)	TC:AI (3:1)	Ascorbic acid
	% inhibition of ABTS activity							
0	-0.22±1.19	-0.22±1.19	-0.22±1.19	-0.22±1.19	-0.22±1.19	-0.22±1.19	-0.22±1.19	-0.22±1.19
3.175	4.12±0.07	2.83±0.02	8.02±0.05	4.09±0.07	5.32±0.04	5.98±0.04	7.59±0.05	8.88±0.06
6.25	7.92±0.04	5.33±0.05	15.72±0.03	7.48±0.05	11.28±0.03	9.34±0.06	14.59±0.03	16.67±0.07
12.5	12.89±0.10	10.27±0.08	33.18±0.11	13.34±0.12	22.82±0.09	17.38±0.04	24.93±0.12	34.56±0.04
25	22.92±0.16	19.29±0.11	48.34±0.14	24.06±0.09	32.29±0.11	27.38±0.08	36.69±0.10	51.23±0.21
50	34.23±0.15	29.72±0.07	63.29±0.18	41.24±0.14	50.18±0.05	43.04±0.11	53.45±0.16	65.69±0.18
100	43.09±0.18	41.66±0.18	77.92±0.12	53.56±0.08	62.39±0.11	59.59±0.09	72.34±0.09	81.11±0.13
200	74.32±0.14	67.72±0.15	93.18±0.24	76.22±0.12	79.11±0.12	77.4±0.10	90.10±0.14	95.34±0.12
IC_{50} (µg/mL)	**51.28**	**53.24**	**22.93**	**42.49**	**29.92**	**36.70**	**30.72**	**21.86**

TC-*Tinospora cordifolia*, AI – *Azadirachta indica*; Results expressed as Mean±SEM (n=3). IC_{50} was calculated using linear regression analysis.

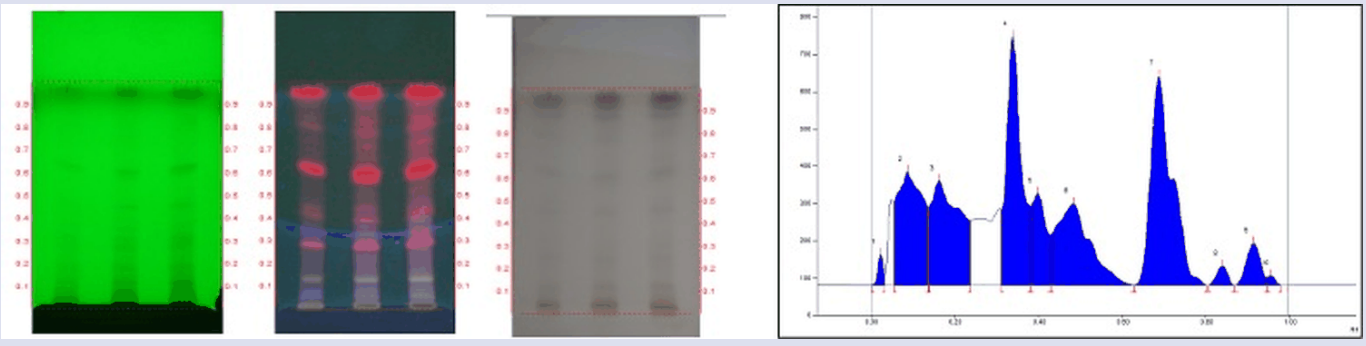

Figure 1. HPTLC chromatographic profile of *Azadirachta indica* aqueous extract.

Chromatographic bands observed under (A) UV light at 254 nm, (B) UV light at 366 nm, and (C) visible light after derivatization demonstrate the presence of multiple phytochemical constituents. Distinct bands at different Rf values indicate diverse bioactive components, including flavonoids, terpenoids, and phenolic compounds. (D) Densitometric scan showing corresponding peak intensities and Rf distribution.

interactions that substantially enhance free radical scavenging capacity. The performance of these combinations were comparable with that of Standard ascorbic acid.

Ferric Reducing/Antioxidant Power (FRAP) assay

The FRAP assay showed concentration-dependent ferric ion reducing activity for *T.cordifolia* and *A.indica* extracts, as well as their combinations (Table 7). Low concentration (3.175–12.5µg/mL) was characterized by weak reducing activity, with values below 8% in individual extracts and less than 22% in their mixtures. Activity nonetheless increased steadily with concentration. At 200µg/mL, *T. cordifolia* and *A. indica* single extracts showed 69.46% and 71.35% reduction, respectively. The IC_{50} of TC (55.25µg/mL) and AI (60.17µg/mL) indicate similar antioxidant capacity, although TC proved to be more active. On the other hand, the combinations showed significantly higher activity, reflecting synergism. The 1:1 and 3:1 ratios yielded the highest reducing capacity with 94.45% and 88.32% inhibition at 200µg/mL, respectively, and considerably lower IC_{50} values (35.74µg/mL and 34.95µg/mL) than the respective individual extracts. All the results were very close to standard ascorbic acid, which had 97.36% activity at 200µg/mL and lowest IC_{50} (21.47µg/mL). These observations suggest that whereas both extracts individually exhibit high ferric reducing

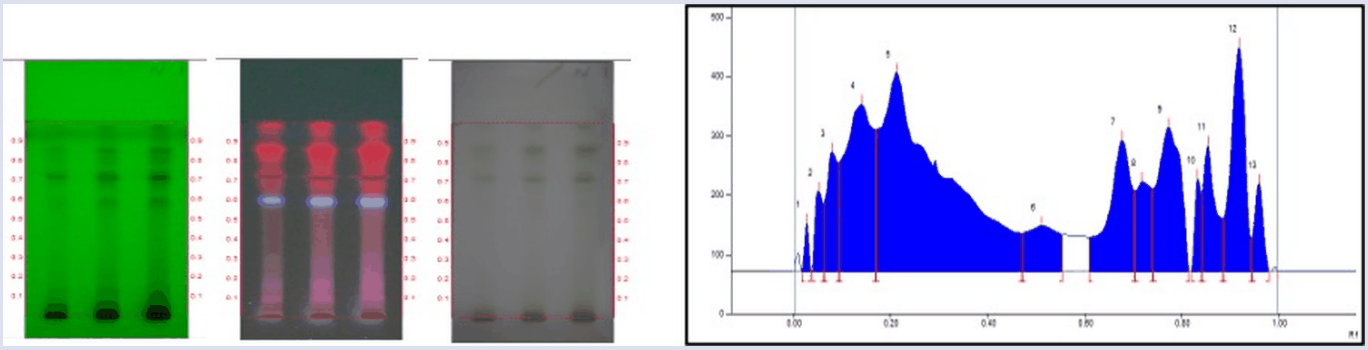

Figure 2. Representative HPTLC plate showing chromatographic separation of phytoconstituents under (A) UV light at 254 nm, (B) UV light at 366 nm, and (C) visible light after derivatization. Multiple well-defined bands at varying Rf values indicate the presence of diverse bioactive metabolites, including alkaloids, flavonoids, and glycosides characteristic of *T. cordifolia*. (D) Corresponding densitometric scan highlighting peak intensities and Rf distribution.

Table 7: Effect of individual extracts of *T.cordifolia* and *A.indica* and its respective formulations on Ferric reducing/Antioxidant power (FRAP)

Concentration (µg/mL)	TC	AI	TC:AI (1:1)	TC:AI (1:2)	TC:AI (2:1)	TC:AI (1:3)	TC:AI (3:1)	Ascorbic acid
	% Ferric Reducing/Antioxidant Power							
0	-0.42±0.98	-0.42±0.98	-0.42±0.98	-0.42±0.98	-0.42±0.98	-0.42±0.98	-0.42±0.98	-0.42±0.98
3.175	2.11±0.04	2.45±0.02	6.69±0.05	4.34±0.06	4.89±0.09	3.83±0.06	6.22±0.04	9.12±0.11
6.25	4.05±0.02	4.12±0.10	11.34±0.06	5.67±0.08	7.24±0.08	7.76±0.06	10.53±0.06	20.44±0.08
12.5	7.86±0.08	7.34±0.05	21.34±0.11	12.34±0.14	15.39±0.11	14.35±0.11	18.48±0.09	34.94±0.06
25	16.93±0.11	17.41±0.12	34.56±0.09	22.93±0.16	26.38±0.09	24.52±0.09	35.35±0.11	49.23±0.12
50	29.94±0.09	28.48±0.07	52.45±0.14	45.40±0.13	43.52±0.07	48.75±0.16	48.37±0.09	69.32±0.12
100	44.94±0.06	42.11±0.14	74.39±0.18	58.30±0.18	60.34±0.14	56.49±0.18	70.49±0.17	84.34±0.18
200	69.46±0.18	71.35±0.11	94.45±0.15	78.83±0.22	82.45±0.14	80.55±0.16	88.32±0.15	97.36±0.11
IC_{50} (µg/mL)	**55.25**	**60.17**	**35.74**	**41.49**	**41.60**	**40.04**	**34.95**	**21.47**

TC-*Tinospora cordifolia*, AI – *Azadirachta indica*; Results expressed as Mean±SEM (n=3). IC_{50} was calculated using linear regression analysis.

Table 8: Effect of individual extracts of *T.cordifolia* and *A.indica* and its respective formulations on α-amylase inhibitory activity

Concentration (µg/mL)	TC	AI	TC:AI (1:1)	TC:AI (1:2)	TC:AI (2:1)	TC:AI (1:3)	TC:AI (3:1)	Acarbose
	% inhibition of amylase activity							
0	-1.56±0.54	-1.56±0.54	-1.56±0.54	-1.56±0.54	-1.56±0.54	-1.56±0.54	-1.56±0.54	-1.56±0.54
3.175	2.45±0.12	1.63±0.08	5.39±0.05	3.23±0.04	4.10±0.03	3.78±0.07	4.47±0.06	6.68±0.06
6.25	4.65±0.33	3.59±0.05	11.38±0.11	7.12±0.07	8.48±0.08	5.39±0.08	7.84±0.08	11.99±0.09
12.5	7.08±0.19	7.14±0.11	22.57±0.21	12.89±0.14	15.38±0.16	13.25±0.15	14.08±0.12	22.89±0.18
25	15.75±0.14	14.01±0.12	43.35±0.34	23.89±0.32	28.39±0.29	22.49±0.29	27.85±0.85	42.09±0.24
50	27.28±0.25	25.76±0.26	58.38±0.85	34.57±0.44	43.28±0.48	38.56±0.65	46.50±0.59	67.86±0.17
100	51.89±0.54	42.56±0.41	71.39±0.77	50.75±0.82	57.51±0.59	52.32±0.34	58.59±0.63	84.38±0.28
200	69.98±0.86	63.24±0.21	90.37±0.91	70.36±0.77	77.44±0.45	72.55±0.58	78.59±0.92	95.08±0.58
IC_{50} (µg/mL)	53.16	54.45	27.71	40.97	36.32	40.94	36.20	26.16

TC-*Tinospora cordifolia*, AI – *Azadirachta indica*; Results expressed as Mean±SEM (n=3). IC_{50} was calculated using linear regression analysis

power, their mixtures, especially the 1:1 and 3:1 (TC:AI) ratios, exert synergism effects that significantly increase antioxidant capacity. This is in agreement with the DPPH and ABTS results and adds strength to the therapeutic significance of *T.cordifolia* and *A. indica* and for maximum antioxidant activity.

α-amylase inhibitory activity

The hydroethanolic extracts of *T.cordifolia* and *A.indica* showed considerable inhibition against α-amylase enzyme activity in a dose-dependent manner (Table 8). Inhibition was low, 1.6–7% for AI and 2.5–7% for TC, at lower concentrations (3.175–12.5µg/mL). The activity was found to be dose-dependent, increasing up to 69.98% inhibition for TC and 63.24% inhibition for AI at 200µg/mL. From the results of the individual extracts, it was revealed that TC was found to be more effective with IC_{50} of 53.16µg/mL and AI showed an IC_{50} of 54.45µg/mL. Nevertheless, the combination of TC and AI was found to be more potent than the individual extracts. The most effective combination was 1:1 ratio, with 90.37% inhibition at 200 µg/mL with lowest IC_{50} value (27.71µg/mL), which was remarkably close to the standard acarbose (26.16µg/mL). Other ratios like 1:2 and 3:1 also showed strong inhibitory activity with IC_{50} values (36.32µg/mL and 36.20µg/mL, respectively) that were considerably lower than individual extracts. Together, the findings substantiate that although the individual extracts of TC and AI has inhibitory effect on α-amylase, their

combinations particularly 1:1 ratio or combinations that possess TC enriched proportions have synergistic effects on the enzyme inhibition.

α-glucosidase inhibitory activity

The α-glucosidase inhibition assay showed a dose-dependent enhancement of the inhibitory activity for *T.cordifolia* and *A.indica* extracts, as well as for their combinations (Table 9). At the lower concentration levels of 3.175–12.5µg/mL, inhibition was moderate, ranging from 1.9–11.1% for AI and 3.1–11.1% for TC. At higher concentrations, however, TC showed 71.23% inhibition at 200µg/mL (IC_{50} = 44.99µg/mL) and AI showed 67.78% inhibition (IC_{50} = 55.20µg/mL). The combinations showed significantly greater inhibitory activities than the single extracts. The 1:1 ratio was most potent which exhibited 91.15% inhibition at 200µg/mL with the lowest IC_{50} (27.99 µg/mL), being very close to the standard acarbose (94.49% inhibition, IC_{50} = 23.65µg/mL). Other combinations, like 3:1 and 1:2, also showed significant inhibition, with IC_{50} values of 32.52µg/mL and 38.85µg/mL, respectively, revealing synergism between the extracts. Altogether, these findings indicate that although TC and AI on their own have moderate α-glucosidase inhibitory activity, their combinations specifically the 1:1 and 3:1 ratio display synergistic augmentation that significantly enhances potency.

PTP1B inhibitory activity

Table 10 demonstrates the PTP1B inhibition assay which showed both *T.cordifolia* and *A.indica* at lower concentrations of 3.175–12.5 µg/mL showed minimal inhibition ranging from 0.8-7.4% for AI and 1.2-7.3% for TC. The inhibition increased gradually as the concentration increased, with TC showing **73.69% inhibition at 200µg/mL** (IC_{50} = **53.13µg/mL**) and AI showing **64.32% inhibition** (IC_{50} = **62.63 µg/mL**). On the other hand, the combinations of the two extracts displayed markedly enhanced activity compared to the single extracts. Amongst the five combinations, the 1:1 ratio exhibited stronger inhibitory effect with the maximum concentration **200µg/mL** reaching **93.19% inhibition** as well as recording the lowest IC_{50} (**31.28µg/mL**). Other ratios such as 3:1 (IC_{50} = **36.72µg/mL**) and 1:2 (IC_{50} = **40.42µg/mL**) also showed effective inhibition, closely approaching the activity of the standard reference inhibitor sodium orthovanadate (IC_{50} = **26.01µg/mL**). Altogether, these findings suggest that while TC and AI individually exhibit moderate PTP1B inhibitory effects whereas their combinations particularly 1:1 and 3:1 ratios—demonstrate synergistic enhancement.

Molecular docking of Berberine with potential insulin signalling markers

Berberine showed strong binding affinity to all three insulin signalling targets—PPARγ, GLUT4, and IRS1—with the strongest interaction seen with PPARγ (ΔG = −8.81 kcal·mol^{-1}), followed by GLUT4 (−7.57 kcal·mol^{-1}) and IRS1 (−7.35 kcal·mol^{-1}). The binding was mainly stabilized by van der Waals and hydrogen bond interactions, with minimal electrostatic contributions (Figure 3). PPARγ binding involved π–π stacking and hydrogen bonds with key residues like Phe, His, Ile, and Arg, while GLUT4 interaction was dominated by hydrophobic and aromatic contacts (Trp and Phe). IRS1 binding was supported by hydrogen bonds with Arg and hydrophobic interactions with Met, Leu, and Ile. The low torsional and internal energies indicate stable complex formation. Overall, PPARγ emerged as the primary target for berberine, with additional interactions at GLUT4 and IRS1 that may enhance its insulin-sensitizing effects (Table 11).

Molecular docking of Nimbolide with potential insulin signaling markers

The molecular docking demonstrated that Nimbolide showed strong binding affinity to three insulin signaling targets, PPARγ, GLUT4, and IRS1—with the highest affinity for PPARγ (ΔG = −9.17 kcal·mol^{-1}), followed by GLUT4 (−8.02 kcal·mol^{-1}) and IRS1 (−7.25 kcal·mol^{-1}). The binding was mainly driven by van der Waals and hydrogen bond interactions, with minimal electrostatic contributions (Figure 4). For PPARγ, nimbolide formed multiple hydrogen bonds and hydrophobic contacts, ensuring stable binding in the ligand pocket. In GLUT4, the interaction involved strong hydrophobic and π–π contacts, suggesting an allosteric modulatory role, while for IRS1, hydrogen bonding and π–alkyl interactions provided moderate but stable binding. The consistent torsional energy and low internal strain indicate stable complex formation across all targets. Overall, PPARγ emerged as the primary target, with additional supportive interactions at GLUT4 and IRS1, highlighting nimbolide's potential to enhance insulin sensitivity through multi-target modulation (Table 12).

Drug likeliness and Pharmacokinetics by Swissadme

Estimation of drug-likeliness is a critical factor for determining the virtual assessment of potential drugs[36, 37]. According to Lipinski's drug rule of 5, a compound is evaluated for its drug-likeliness based on criteria viz., molecular weight, Lipophilicity, hydrogen bond donor, hydrogen bond acceptor etc., The present study results showed that both berberine and nimbolide satisfied all key parameters of Lipinski's Rule of Five, indicating favourable drug-likeness and good oral absorption potential. Berberine, with a molecular weight of 336.36 g/mol, four hydrogen-bond acceptors, no hydrogen-bond donors and a moderate LogP of 2.53, showed no violations and demonstrated characteristics supportive of membrane permeability and oral bioavailability. Similarly, nimbolide exhibited a molecular weight of 466.52 g/mol, seven hydrogen-bond acceptors, no donors and a balanced LogP of 3.04, also without any Lipinski violations. Overall, the compliance of both phytochemicals with all Lipinski criteria suggests strong oral drug-likeness and justifies their selection for further docking and pharmacokinetic evaluation within the study (Table 13).

Absorption, Distribution, Metabolism and Excretion properties evaluated by pkCSM

Both berberine and nimbolide demonstrated acceptable ADMET characteristics, though with distinct pharmacokinetic behaviours (Table 14). Berberine showed comparatively higher water solubility (log S −3.97 vs. −5.17) and better Caco-2 permeability, while both compounds exhibited excellent predicted intestinal absorption (97–100 percent). Skin permeability was low for both, indicating minimal transdermal diffusion. In terms of distribution, berberine showed a higher volume of distribution (VDss log L/kg 0.58), suggesting broader tissue penetration, and a positive logBB value indicative of moderate BBB permeability, whereas nimbolide (logBB −0.675) is predicted to have limited brain access. Metabolic predictions revealed that berberine inhibits CYP1A2 and CYP2D6, while nimbolide does not inhibit these isoenzymes, suggesting a lower likelihood of metabolic drug–drug interactions for nimbolide. Clearance rates indicated faster systemic elimination of berberine (1.27 mL/min/kg) compared with the slower clearance of nimbolide. Both molecules were predicted to be non-substrates of renal OCT2 transporters. Toxicity assessment showed that berberine has a slightly higher maximum tolerated dose, but it is AMES-positive, suggesting mutagenicity risk, whereas nimbolide is AMES-negative. Rat acute and chronic toxicity predictions were comparable, with nimbolide showing marginally lower LD50 and LOAEL values.

DISCUSSION AND CONCLUSION

Tinospora cordifolia and *Azadirachta indica* hold promising place in Ayurveda due to their diverse phytochemicals and wide pharmacological activities. In the current research, the phytochemical composition

Table 9: Effect of individual extracts of *T.cordifolia* and *A.indica* and its respective formulations on α-glucosidase inhibitory activity

Concentration (µg/mL)	TC	AI	TC:AI (1:1)	TC:AI (1:2)	TC:AI (2:1)	TC:AI (1:3)	TC:AI (3:1)	Acarbose
	% inhibition of α-glucosidase activity							
0	-3.12±1.56	-3.12±1.56	-3.12±1.56	-3.12±1.56	-3.12±1.56	-3.12±1.56	-3.12±1.56	-3.12±1.56
3.175	3.12±0.09	1.90±0.04	5.52±0.05	2.43±0.03	4.09±0.04	3.87±0.02	5.02±0.06	7.23±0.03
6.25	5.34±0.13	3.45±0.05	10.89±0.18	5.35±0.12	7.48±0.07	6.23±0.08	9.23±0.11	14.76±0.09
12.5	11.12±0.22	5.94±0.12	21.97±0.09	9.24±0.07	13.34±0.06	11.99±0.08	17.67±0.09	29.12±0.10
25	19.24±0.25	15.50±0.11	42.75±0.14	17.40±0.08	24.06±0.09	23.14±0.12	33.11±0.11	45.54±0.08
50	32.23±0.13	24.34±0.16	56.86±0.08	31.34±0.06	41.24±0.14	40.23±0.08	49.34±0.15	62.38±0.16
100	50.34±0.15	45.35±0.15	70.84±0.06	44.67±0.07	53.56±0.11	51.46±0.16	62.44±0.12	80.64±0.18
200	71.23±0.26	67.78±0.18	91.15±0.14	73.45±0.12	76.22±0.14	75.12±0.14	83.82±0.18	94.49±0.14
IC_{50} (µg/mL)	**44.99**	**55.20**	**27.99**	**52.48**	**38.85**	**40.47**	**32.52**	**23.65**

TC-*Tinospora cordifolia*, AI – *Azadirachta indica*; Results expressed as Mean±SEM (n=3). IC_{50} was calculated using linear regression analysis.

Table 10: Effect of individual extracts of *T.cordifolia* and *A.indica* and its respective formulations on PTP1B inhibitory activity

Concentration (µg/mL)	TC	AI	TC:AI (1:1)	TC:AI (1:2)	TC:AI (2:1)	TC:AI (1:3)	TC:AI (3:1)	Sodium Orthovanadate
	% inhibition of PTP1B activity							
0	-0.94±2.26	-0.94±2.26	-0.94±2.26	-0.94±2.26	-0.94±2.26	-0.94±2.26	-0.94±2.26	-0.94±2.26
3.175	1.25±0.04	0.78±0.02	5.12±0.09	2.19±0.07	3.96±0.08	3.12±0.02	4.62±0.04	7.85±0.05
6.25	3.29±0.07	1.42±0.09	9.95±0.08	4.56±0.05	7.11±0.14	5.25±0.08	8.11±0.06	15.22±0.02
12.5	7.38±0.07	4.37±0.08	19.11±0.12	10.23±0.10	14.78±0.06	11.23±0.09	16.84±0.14	29.89±0.08
25	16.38±0.12	10.27±0.11	39.74±0.10	18.38±0.09	26.92±0.08	20.23±0.12	30.62±0.18	43.83±0.18
50	34.55±0.16	23.82±0.09	59.12±0.16	37.29±0.18	48.84±0.09	39.78±0.08	52.38±0.07	60.77±0.16
100	48.75±0.18	42.14±0.12	74.56±0.18	55.39±0.22	63.39±0.16	57.11±0.12	69.77±0.22	75.67±0.12
200	73.69±0.16	64.32±0.16	93.19±0.12	78.84±0.14	86.48±0.08	81.13±0.27	89.39±0.18	94.12±0.11
IC_{50} (µg/mL)	**53.13**	**62.63**	**31.28**	**50.16**	**40.42**	**48.23**	**36.72**	**26.01**

TC-*Tinospora cordifolia*, AI – *Azadirachta indica*; Results expressed as Mean±SEM (n=3). IC_{50} was calculated using linear regression analysis.

Table 11: Docking energy parameters of berberine with PPARγ, GLUT4, and IRS1

Ligands	Target	Binding Energy (kcal/mol)	No. of H-Bonds	Amino Acid Residues (H-Bond donors/acceptors with length Å)	Hydrophobic Interactions (Residues & Type)
Berberine	PPAR-γ	−8.81	1	Ser289 (2.91 Å)	Phe264 (π-π), His266 (π-π), Ile281 (π-alkyl), Arg280 (C-H), Cys285 (π-alkyl), Met348 (π-alkyl), Val277, Leu340 (vdW)
Berberine	GLUT-4	−7.57	2	Ser415 (3.23 Å), Gly154 (3.18 Å)	Trp404 (π-π), Phe428 (π-π), Leu209/Leu215 (π-alkyl), Pro211 (π-alkyl), Asn427 (C-H), Ala421 (vdW)
Berberine	IRS-1	−7.35	3	Arg312 (2.06 Å), Arg227 (1.85 Å), Ser228 (3.71 Å)	Ile211, Leu208, Met209 (π-alkyl), Gly226 (C-H), Asn210 (vdW)

Table 12: Docking energy parameters of nimbolide with PPARγ, GLUT4, and IRS1

Ligands	Target	Binding Energy (kcal/mol)	No. of H-Bonds	Amino Acid Residues (H-Bond donors/acceptors with length Å)	Hydrophobic Interactions (Residues & Type)
Nimbolide	PPAR-γ	−9.17	1	Arg288 (2.96 Å)	Ile281, Leu255, Leu340 (alkyl/π-alkyl), Phe264 (π-alkyl), His266 (π-sigma), Lys265 (vdW)
Nimbolide	GLUT-4	−8.02	0	—	Leu209, Leu215, Ile186, Pro212 (π-alkyl), Tyr406, Gln437 (vdW)
Nimbolide	IRS-1	−7.25	3	Arg312 (2.36 Å), Arg227 (2.94 Å), Ser228 (3.15 Å)	Asn210 (C-H), Ala229, Leu208 (vdW), Ile211 (π-alkyl)

Table 13: Lipinski Rule, Pharmacokinetics and drug-likeliness of Berberine and Nimbolide

Ligand	Molecular weight (g/mol)	H-donors	H-acceptors	LogP	Lipinski Rule	
					Result	Violation
Berberine	336.36	0	4	2.53	Yes	0
Nimbolide	466.52	0	7	3.04	Yes	0

Table 14: ADME properties of Berberine and Nimbolide evaluated by pkCSM

Ligand Name	Properties	Berberine	Nimbolide
Absorption	Water Solubility (log mol/L)	-3.973	-5.166
	Caco2 Permeability X10-^{6} cm/s	1.734	0.92
	Intestinal absorption (% Absorbed)	97.147	100
	Skin Permeability (log Kp)	-2.576	-3.599
Distribution	VDss (human) (log L/kg)	0.58	0.028
	BBB Permeability (log BB)	0.198	-0.675
Metabolism	CYP1A2 inhibitor	Yes	No
	CYP2D6 inhibitor	Yes	No
Excretion	Total Clearance (ml/min/kg)	1.27	0.249
	Renal OCT2 substrate	No	No
Toxicity	Max. tolerated the dose (log mg/kg/day)	0.144	-0.476
	AMES toxicity	Yes	No
	Oral Rat Acute Toxicity (LD50) (mol/kg)	2.571	2.374
	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	1.89	1.554

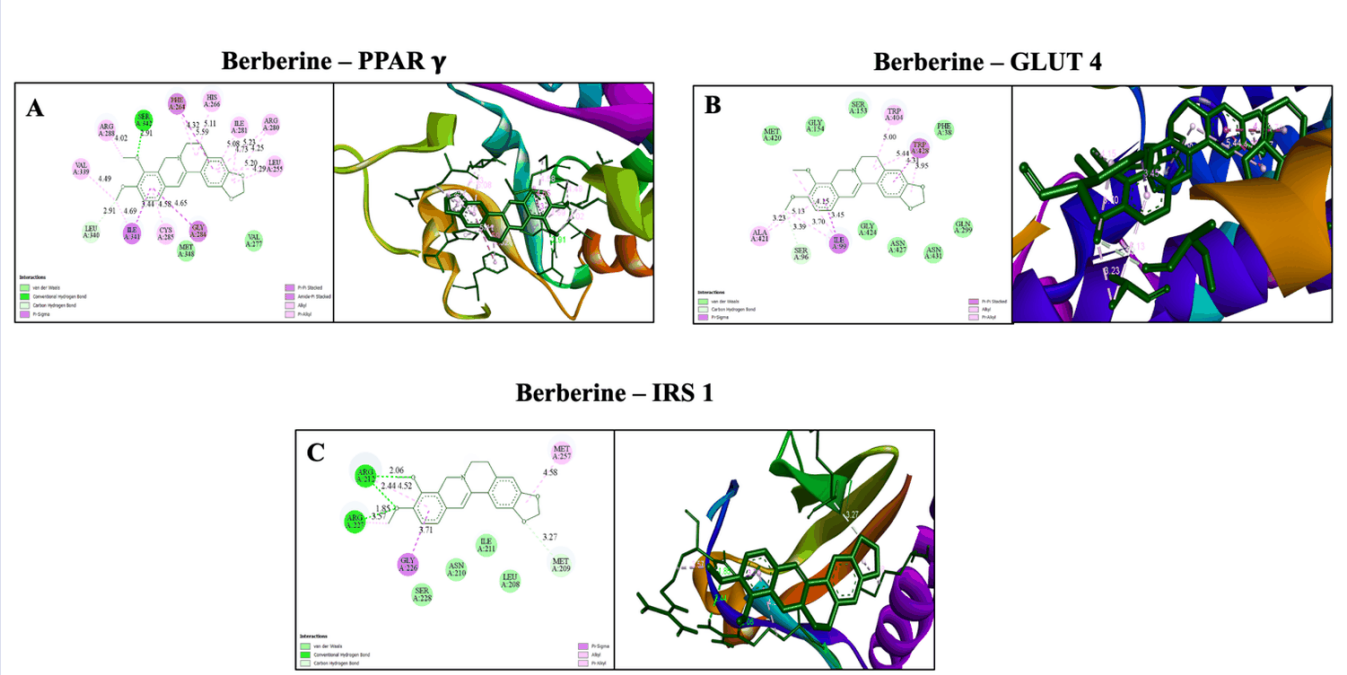

Figure 3. Molecular docking interaction profile of berberine with insulin signalling targets. Left panels represent 2D interaction maps indicating the nature and distance of key intermolecular contacts, while right panels depict 3D docking poses of berberine within the respective binding sites. Colour codes: green – van der Waals and hydrogen bonds; pink – π–π stacking/π–alkyl interactions.

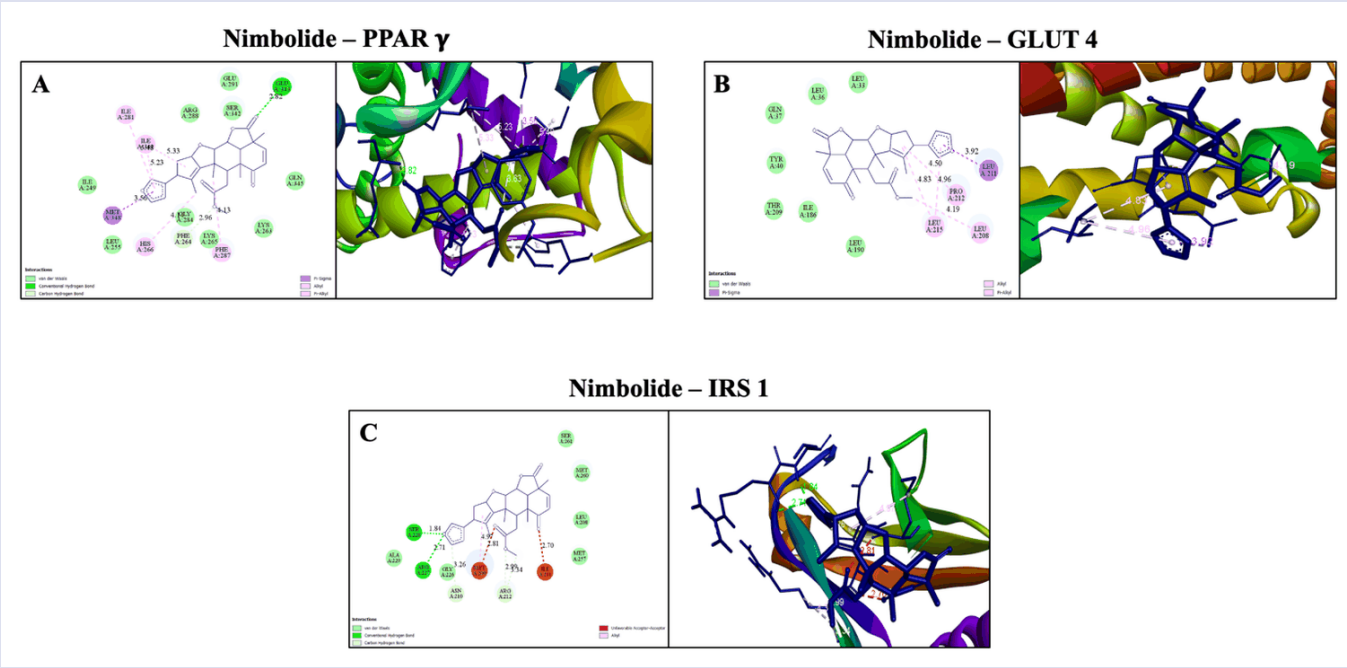

Figure 4. Molecular docking interaction analysis of Nimbolide with key insulin-signalling targets. For each target, the left panel shows the 2D interaction map displaying hydrogen bonds, van der Waals forces, and hydrophobic interactions, while the right panel shows the 3D binding pose of nimbolide within the protein pocket.

along with antioxidant and antidiabetic potential of TC and AI were comprehensively studied individually as well as in combination. The extracts showed high phenolic and flavonoid concentration, known for its free radical scavenging and enzyme-modulatory activities. Importantly, the combined formulations, particularly at 1:1 and 3:1 ratios, displayed enhanced efficacy compared to individual extracts, highlighting the therapeutic advantage of polyherbal approaches.

Antioxidant activity is essential for evaluating the therapeutic value of medicinal plants. This can be assessed using in vitro assays such as DPPH and ABTS, which measure the extracts' ability to neutralize free radicals. The antioxidant capacity of TC and AI was confirmed through DPPH, ABTS, and FRAP assays, each reflecting different mechanisms of radical neutralization. TC consistently demonstrated stronger antioxidant activity than AI, as shown by lower IC_{50} values, supporting previous findings that attribute this to its diterpenoid lactones, alkaloids, and polyphenols[38]. AI also showed significant antioxidant activity, which might be attributed to its flavonoids, limonoids, and tannins[39]. The combined extracts exhibited synergistic effects, with the 1:1 mixture nearly matching ascorbic acid in ABTS and FRAP assays. These results support the Ayurvedic concept of *yogavahi*, where combining herbs enhances therapeutic efficacy, and are consistent with studies showing improved antioxidant effects in polyherbal formulations[40,41].

α-amylase and α-glucosidase are key enzymes responsible for the digestion of complex carbohydrates like starch and the spike in blood glucose levels. Suppressing the levels of these enzymes significantly contributes in the regulation of postprandial blood glucose, thereby turning them out to be key targets for the management of type 2 diabetes mellitus[42]. The present study findings are in agreement with this exhibiting the dose-dependent inhibition of these enzymes by both TC and AI as individual extracts as well as combinations with 1:1 (TC:AI) being more potent. Earlier studies have shown that the carbohydrate hydrolysing enzyme inhibition might be attributed to the presence of phytoconstituents such as flavonoids, lactones, alkaloids and terpenoids in TC and AI[43,44]. Amongst the individual extracts and the different tested combinations, the 1:1 ratio showed lowest IC_{50} value which is comparable with that of standard acarbose indicating potent synergistic effect. The multiple binding sites of the various phytochemicals present in the crude hydroethanolic extract may be the contributing factor for the synergistic effect which could reduce the enzyme catalytic efficiency more effectively than individual compounds[44]. This suggests that TC:AI formulations may serve as effective natural alternative to synthetic α-glucosidase inhibitors, which are often limited by gastrointestinal side effects.

Besides carbohydrate digestion, another enzyme that plays a critical role in the regulation of insulin signalling and glucose homeostasis is the protein tyrosine phosphatase 1B (PTP1B) enzyme. PTP1B dephosphorylates the tyrosine residues thereby negatively regulating the insulin receptor signalling resulting to insulin resistance condition. As a result, PTP1B inhibition has emerged as a promising therapeutic approach for type 2 diabetes management[45]. Previously many plant-derived compounds have been reported to exhibit PTP1B inhibitory activity suggesting their potential in improving insulin sensitivity[46]. The present study results also demonstrated moderate inhibitory activity against PTP1B, with TC slightly outperforming AI, which is in agreement with earlier results of alkaloids, terpenoids, and flavonoids acting on PTP1B[47,48]. The combinations, particularly 1:1 and 3:1 ratios, showed markedly improved activity, with IC_{50} values close to sodium orthovanadate. Similar results have been reported for other medicinal plants, where polyphenolic mixtures exert synergistic inhibition of PTP1B[49,50].

To substantiate these biological findings, in-silico molecular docking was performed using berberine (from TC) and nimbolide (from AI), two prominent phytochemicals repeatedly reported for antidiabetic activity. Berberine demonstrated strong binding affinity toward all three insulin-signalling targets—PPARγ, GLUT4, and IRS1—with PPARγ showing the most favourable interaction (ΔG = −8.81 kcal/mol). These results are consistent with earlier docking studies that

identified berberine as a potent PPARγ modulator through π–π stacking and hydrophobic interactions with key residues such as Phe, His, and Ile. This is in agreement with earlier published findings that has demonstrated the anti-diabetic potential of Berberine by improving the regulation of glucose and lipid metabolism in the body. Berberine can inhibit mitochondrial function and activate the AMPK pathway[51–53]. Berberine's interactions with GLUT4 and IRS1 were stabilized by hydrophobic and hydrogen-bonding networks, supporting its reported insulin-sensitizing and glucose-uptake-enhancing actions[54].

Similarly, nimbolide exhibited strong binding to all three targets, with PPARγ again emerging as the primary site of interaction (ΔG = −9.17 kcal/mol). This is consistent with previous computational analyses showing strong PPARγ and IRS1 affinity for nimbolide and related limonoids through hydrogen bonding and van der Waals interactions[55,56]. The absence of hydrogen bonds in the GLUT4 complex, yet favourable binding energy, suggests a predominantly hydrophobic or allosteric mode of engagement. Together, these findings indicate that berberine and nimbolide may act through complementary, multi-target mechanisms that enhance insulin sensitivity, glucose transport, and downstream signalling—supporting the strong antidiabetic performance observed *in vitro*.

Drug-likeness evaluation using SwissADME revealed that both berberine and nimbolide comply with all major parameters of Lipinski's Rule of Five, indicating favourable oral drug-like characteristics. Berberine possessed optimal molecular weight, logP, and hydrogen-bonding parameters, while nimbolide similarly met all criteria despite its slightly higher molecular weight. Compliance with drug-likeness criteria has been reported for several bioactive alkaloids and limonoids, supporting their potential as orally active phytopharmaceutical leads[35,57].

Pharmacokinetic predictions using pkCSM demonstrated acceptable ADMET profiles for both compounds, albeit with notable differences. Both exhibited excellent predicted intestinal absorption; however, berberine showed greater water solubility and higher Caco-2 permeability. Berberine also displayed broader tissue distribution (VDss) and moderate BBB permeability, whereas nimbolide was predicted to have limited brain penetration. In terms of metabolism, berberine showed potential CYP1A2 and CYP2D6 inhibition, while nimbolide did not inhibit these isoenzymes, suggesting a more favourable metabolic interaction profile. Although both compounds exhibited acceptable toxicity profiles, berberine's AMES positivity indicated a mutagenicity concern, whereas nimbolide tested negative. These outcomes align with earlier ADMET reports of berberine's metabolic complexity and nimbolide's relatively safer profile[58].

Taken together, the results of the phytochemical analysis, antioxidant and enzyme inhibition assays, molecular docking, drug-likeness assessment, and ADMET prediction demonstrate a strong, multi-faceted antidiabetic potential for the T. cordifolia–A. indica combination. The enhanced efficacy of the 1:1 and 3:1 ratios, supported by potent molecular interactions and favourable pharmacokinetic predictions of their key phytoconstituents, suggests that these polyherbal formulations may serve as promising natural therapeutic candidates for managing type 2 diabetes mellitus and its associated complications. Moreover, the observed synergism reflects the Ayurvedic principle of combining herbs (*yogavahi*) to enhance efficacy. While these *in vitro* findings are promising, future investigations are warranted that includes *in vivo* validation and mechanistic studies to establish the translational potential.

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