Trop J Phytochem Pharm Sci, July 2026; 5(4): 550 - 560 **ISSN 2955-1226 (Print)**
ISSN 2955-123(Electronic)

Tropical Journal of Phytochemistry & Pharmaceutical Sciences

Available online at https://www.tjpps.org

Original Research Article

Integrated Phytochemical Screening, LC–MS Analysis, and Molecular Docking Approach to Evaluate the Therapeutic Potential of *Mentha arvensis* Leaves

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ABSTRACT

Mentha arvensis L. (Japanese mint) is an aromatic medicinal plant widely recognized for its diverse therapeutic applications. The present study aimed to evaluate the phytochemical composition, LC-MS, antioxidant, antidiabetic, and *in-silico* potential of *M. arvensis* leaf extracts. Solvent extract of leaves was analysed qualitatively, which indicated the presence of alkaloids, flavonoids, tannins, phenolics, terpenoids, glycosides, and saponins. The extract exhibited anti-inflammatory activity. The antidiabetic activity of the extract was evaluated against pancreatic alpha amylase using the DNS method. The extract showed dose dependent activity with an IC_{50} value of 171.77 µg/mL, indicating inhibition of the amylase enzyme. At higher concentration, the extract reduced enzyme activity by 75%. LC–MS analysis identified bioactive compounds like caffeic, p-coumaric, rosmarinic acids, isoorientin, luteolin, hesperidin and carnosic acid. The interaction of these with 4W93 (Human pancreatic alpha-amylase) and 1B2Y (Human pancreatic alpha-amylase) was investigated using *in silico* molecular docking. The ligands luteolin and rosmarinic acid showed the highest binding energy of -7.5 Kcal/mol as compared to other ligands like isoorientin, p-coumaric acid, hesperidin and carnosic acid, which exhibited a binding affinity of −7.01 kcal/mol to -5.63 kcal /mol against two enzymes. ADMET analysis also indicated favourable pharmacokinetic properties and low toxicity of luteolin. This suggests that *Mentha arvensis* could serve as a promising natural resource for the pharmaceutical industry in developing eco-friendly, plant-based antidiabetic formulations.

Keywords: Phytochemical analysis, LC-MS, docking, ADME, antidiabetic activity, *Mentha arvensis.*

Received 13 May 2026
Revised 26 July 2026
Accepted 28 July 2026
Published online: 04 August 2026

Introduction

Medicinal plants have long been recognized as a fundamental component of traditional healthcare systems and continue to play a crucial role in modern drug discovery. As per WHO, almost eighty percent of the world population depends on plant-based medicine.[1] Moreover, the contribution of this natural-based medicine is approximately 25-30% in the pharmaceutical drug market, which indicates their importance as bioactive molecules with pharma applications.[2] Due to the occurrence of chronic disease and restrictions associated with the synthetic drugs, researchers are exploring medicinal plants to look for safer and more effective therapeutic agents. Among the medicinally important plants, *Mentha arvensis* L., commonly known as field mint or corn mint, is an important herb that is distributed among their temperate and subtropical regions. The production of mint oil in India is over 75%.[3] The plant is used in ancient medicine systems due to its aroma, therapeutic value, and availability.

The medicinal value of *M. arvensis* is largely attributed to its diverse array of bioactive secondary metabolites. The medicinal value of the plant is due to the presence of a wide variety of secondary metabolites like flavonoids, phenolic acids, terpenoids, glycosides, and other bioactive constituents. These compounds are known to exert wide range of pharmacological activities like antioxidant, anti-inflammatory, antimicrobial, and metabolic regulatory effects by modulating key biochemical pathways.[4]

Traditionally, *M. arvensis* has been used for the management of simple diseases and disorders.[5] However, in recent years, the use of plant metabolite for the management of metabolic disorders has been highlighted, including diabetes mellitus. Diabetes is a major global health challenge, with around 537 million adults living with diabetes worldwide.[6] One of the known ways to control this is to inhibit the enzyme alpha amylase, which is responsible for carbohydrate metabolism. An increase in its activity will lead to rapid release of glucose, oxidative stress, and inflammation, linking metabolic imbalance with chronic inflammatory conditions.[7] It has been demonstrated that the plant derived metabolites have the ability to inhibit amylase and also exhibit anti-inflammatory effect, making them potential for antidiabetic therapy. Also, the use of advanced techniques and computational approaches is required for correlating the metabolites with their associated activity.[8]

The present study was undertaken to evaluate the medicinal potential of *Mentha arvensis.* The study involved phytochemical analysis, LC-MS profiling of extract and evaluating its anti- inflammatory and antidiabetic potential. Also, *in-silico* docking was performed to study the interaction between the metabolites and enzyme. This integrated approach helps to provide validation for the medicinal use of *M. arvensis* and to support its potential application in the development of plant-based therapeutics for inflammation and diabetes management.

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Citation: Sankannavar BS, Dhani L, Desai SS. Integrated Phytochemical Screening, LC–MS Analysis, and Molecular Docking Approach to Evaluate the Therapeutic Potential of *Mentha arvensis* Leaves. Trop J Phytochem Pharm Sci, 2026, 5(4); 550 - 560
http://www.doi.org/10.26538/tjpps/v5i4.5

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Material and Methods

Plant collection and Preparation of leaf extracts

Plant leaves were collected from the farmer's field, Madalgeri, during July 2025 (Fig.1a) (Gadag, Karnataka, India). The plant was authenticated with voucher number PCJ/BOT/HUBI/17/2024-25.

ISSN 2955-1226 (Print)
ISSN 2955-123(Electronic)

Fresh leaves of *Mentha arvensis* were washed, shade-dried, powdered, and extracted using Soxhlet apparatus with petroleum ether as solvent. About 50 g of powdered leaf sample was extracted with 300 mL solvent for 6–8 hours to obtain phytochemicals.[9-13] The extract was filtered, concentrated using a rotary evaporator at 40–45°C, air- dried, weighed for yield, and stored at 4°C for further analysis (Fig.1b).[14]

Figure 1: a) *Mentha arvensis* plant b) Extraction of phytochemicals using solvents

Phytochemical analysis

Phytochemical analysis of leaf extracts was conducted as per the standard procedure to determine the presence of tannins, saponins, terpenoids, carbohydrates, flavonoids, alkaloids, glycosides, steroids, phenols, carbohydrates, proteins and amino acids.[10]

Liquid Chromatography -Mass Spectrum Analysis

LC–MS analysis was performed using a Waters Alliance HPLC system coupled with a Micromass ZQ mass spectrometer equipped with an electrospray ionization (ESI) source at the Sophisticated Analytical Instrument Facility (SAIF). Extracts were filtered through 0.22 µm syringe filters, diluted with HPLC grade methanol: water (1:1, v/v), and stored at 4°C prior to analysis. C18 reverse-phase column with a solvent systems of water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B), a flow rate @ 1.0 mL/min, and a gradient elution from 5% to 95% B for over 40 minutes was used for separation. The temperature of 30°C was maintained in column and sample volume injected was 10–20 µL. Data were obtained in the mass range of m/z 100–1000 in full scan mode and processed using Mass Lynx software. The detected peaks were identified by comparison of retention times and m/z values with reported literature and databases, enabling characterization of bioactive compounds in the extracts.[8]

Anti-inflammatory activity

The protein denaturation method was used for evaluation of anti-inflammatory activity of the petroleum ether extract of *Mentha arvensis* leaves. A phosphate-buffered saline (PBS, pH 6.4) was used to prepare 2% egg albumin (Protein) solution. The sample was dissolved in water at a concentration of 1 mg/mL and serially diluted, while ibuprofen (0.2 mg/mL) was used as the standard reference drug, and distilled water served as the negative control. For the assay, 4.05 mL of PBS, 0.45 mL of egg albumin, and 0.5 mL of test sample, standard, or control were mixed gently. The mixtures were kept in incubator with temperature of 37°C for a period of 15–20 minutes. Later, it was heated at 70°C for 5 minutes, then and cooled to room temperature for 15 minutes. The absorbance was measured at 660nm before and after denaturation using a colorimeter[15]. Each test was carried out in triplicates and the percentage inhibition of protein denaturation was calculated relative to the control using the formula:

% Inhibition = $(A_c\text{-}A_s)/A_c \times 100$,

Where, A_c = Absorbance of control; A_s = Absorbance of sample.

α-amylase inhibitory Assay

Human pancreatic α-amylase is a key digestive enzyme that breaks down complex carbohydrates into simple sugars, thereby playing a crucial role in regulating postprandial blood glucose levels. Inhibition of the enzyme is important for the management of type 2 diabetes and obesity, as it helps reduce rapid glucose absorption and control blood sugar spikes after meals. The alpha-amylase inhibition assay was carried out as per the protocol: in an Eppendorf tube, a known volume of PBS solution was mixed with various concentrations (50, 100, 150, 200, and 250 µg/ml) of samples and a known volume of enzyme α-amylase was added. Then starch was added and incubated for ten minutes. Starch with enzyme and without enzyme was used as a control. After 10 minutes, DNS was added to stop the reaction and kept in the boiling water bath. After cooling, the absorbance was recorded at 540 nm. Metformin was used as standard drug. [16] The percent of amylase enzyme inhibition was calculated using the following formula:

$$\% \text{ Enzyme Inhibition} = \frac{(\text{Abosrbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} * 100$$

In-silico docking

Luteolin, a major bioactive component of the petroleum ether extract of *M. arvensis*, through LCMS analysis, was selected for molecular docking studies to evaluate its interaction with alpha amylase. Along with this, rosmarinic acid, isoorientin and metformin (standard drug) were used as ligands for docking (Figure 2a). The 3-D structure of the ligand was retrieved from the PubChem database, (https://pubchem.ncbi.nlm.nih.gov/), while the crystal structures of amylase were downloaded from the Protein Data Bank (PDB) (https://www.rcsb.org/). The protein targets included 4W93 (human pancreatic alpha-amylase complexed with montbretin A) and 1B2Y (human pancreatic alpha-amylase complexed acarbose) (Figure 2b). Protein -ligand docking was done using Swiss Auto Dock Vina and the results were analysed using PyMOL and Discovery Studio Visualizer.[8]

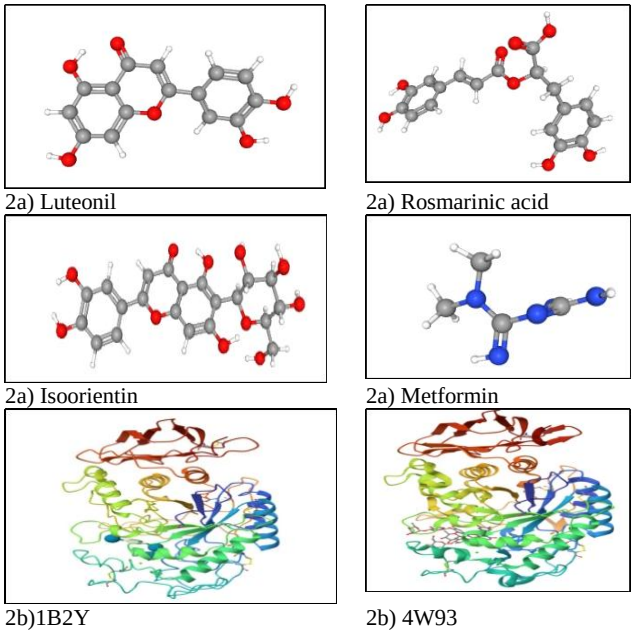


Figure 2a): 2D Structure of ligands selected for the study
2b): 3D Structure of enzymes used for analysis.

ADME and Drug-likeness Prediction of Active Molecule

Swiss ADME, an online tool commonly used in the early stages of drug research, was applied to evaluate the drug-like nature and pharmacokinetic properties of Luteolin and other metabolites. The assessment was mainly based on Lipinski's Rule of Five, where in a compound is more likely to be well absorbed if its lipophilicity (LogP) is not greater than 5, its molecular weight is below 500 Da, and it has no more than 5 hydrogen bond donors and 10 hydrogen bond acceptors. In addition, the topological polar surface area (TPSA) should ideally be 140 Å² or less to ensure good permeability across biological membranes. This rule helps to predict if the molecule is effective to be taken orally. [17]

ISSN 2955-1226 (Print)
ISSN 2955-123(Electronic)

Statistical Analysis

Data analysis and graph plotting were performed using Microsoft Excel 2019 (Microsoft Corp., USA). Experiments were conducted in triplicates (n = 3), and values are expressed as mean ± standard deviation (SD).[18]

Results and Discussion

The results of the experiments are explained in this section.

Phytochemical screening of Mentha arvensis leaf extract

The phytochemical analysis of petroleum ether extract of leaves of *Mentha arvensis* indicates the presence of phytochemicals like alkaloids, flavonoids, phytosterols, tannins, steroids, terpenoids, phenolics, and strong presence of saponins (Table 1). Carbohydrates and proteins/amino acids were absent. This may be due to the use of non-polar solvents which extracted lipophilic compounds and also metabolites which have partial solubility or due to amphiphilic nature.[19-21] This suggest that petroleum ether could very well extract metabolites with non-polar nature having potential pharma importance.

Table 1: Phytochemical analysis of petroleum ether extract of *Mentha arvensis* leaves.

Sl.No.	Metabolites	Test	Pt. ether
01	Alkaloid Test	Mayer's Test	+
02	Flavonoids Test	Alkaline reagent Test	+
03	Glycosides Test	Keller-Kiliani Test	+
04	Phytosterol Test	Salkowski's Test	+
05	Carbohydrate Test	Fehling's Test	-
06	Tannin Test	Lead Acetate Test	+
07	Steroid Test	L-B Test	+
08	Terpenoid Test	Chloroform Test	+
09	Phenolic Test	Fecl3 Test	+
10	Saponin Test	Foam test	++

+ : Present , - : Absent

Liquid Chromatography -Mass Spectrum Analysis (LC-MS)

The LC chromatogram displayed five major peaks at retention times 2.60, 2.75, 2.92, 3.35 and 5.22 min. The dominant peak was observed at 2.92 min, contributing 67.71% of total area. Electrospray ionization in both positive and negative modes was employed. In ES− mode, a prominent ion at m/z 359.3244 corresponded to the deprotonated molecule $[M-H]^-$ of rosmarinic acid (theoretical m/z 359.077), suggesting it as the major phenolic compound. In ES+ mode, a significant ion at m/z 449.5500 was assigned to isoorientin ($[M+H]^+$ theoretical m/z 449.108). Also, an ion at m/z 287.3815 was given to luteolin (Figure 3). Further ions in the 609–610 m/z range suggested the presence of hesperidin. Minor phenolic acids such as p-coumaric acid and caffeic acid were inferred from fragment ions in the low mass region. All assignments were made based on mass comparison and literature reports of phytochemicals commonly present in *Mentha* species (Table 2, Figure 3a,3b).

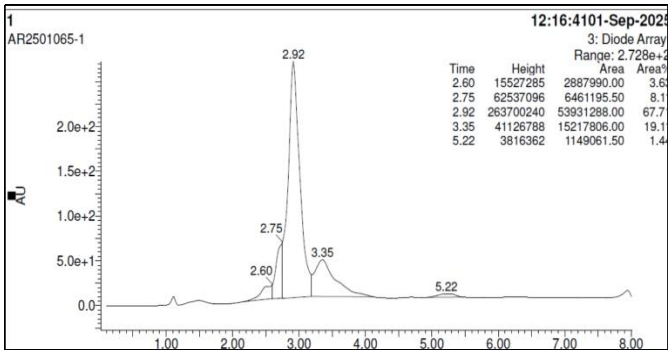
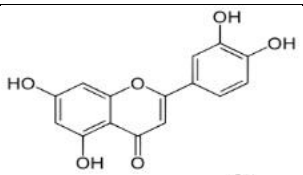
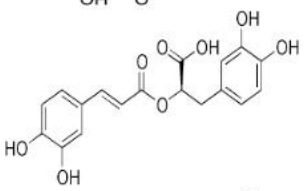
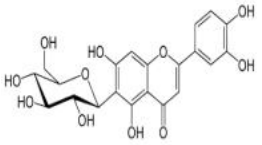
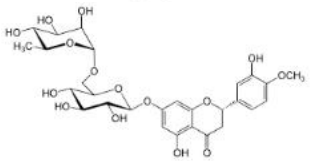
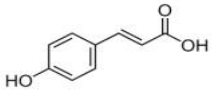


Figure 3 a:LC-MS Profiling of Petroleum ether extract of *Mentha arvensis* leaves.

Table 2: LC-MS report of petroleum ether extract of *Mentha arvensis* leaves.

Observed m/z	Compound	Structure	Reference
287 (ES+)	Luteolin		[8]
359 (ES−)	Rosmarinic acid		[8,37]
449 (ES+)	Isoorientin		[8]
609–610	Hesperidin		[25]
163	p-Coumaric acid		[25]

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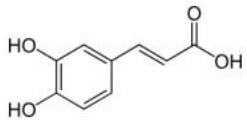
181	Caffeic acid		[25]

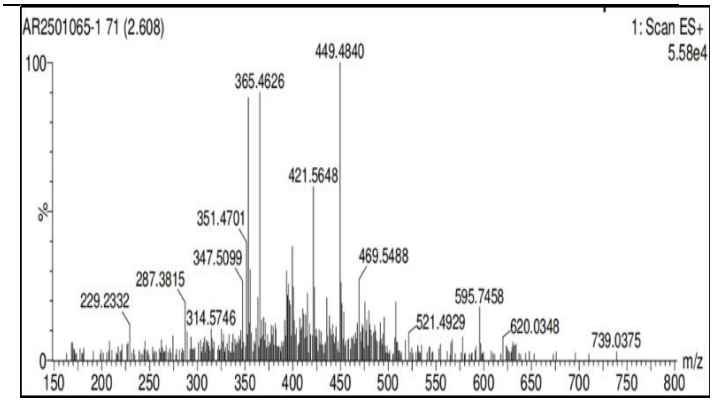


Figure 3b: LC-MS Profiling of Petroleum ether extract of *Mentha arvensis* leaves.

The LC-MS profile of *Mentha arvensis* confirmed the presence of phenols and flavonoids. Rosmarinic acid was identified as a major peak at signal m/z 359, which is in consistent with previous reports. The presence of isoorientin and luteolin further indicates the presence of flavonoids in the extract. These compounds exhibit antioxidant and anti-inflammatory properties, which contribute to the medicinal properties of the plant.

The results of LC-MS indicated the presence of various phytochemicals, which were identified based on their m/z value and literature comparison. The peak at m/z 287 corresponds to luteolin- a flavonoid, 359 corresponds to rosmarinic acid–phenolic compound;[8] 449 corresponds to flavonoid glucoside, other metabolite includes hesperidin,[23-24] p-coumaric acid and caffeic acid.[8,25] These compounds are known for their antioxidant and therapeutic properties.

Anti-inflammatory activity

The anti-inflammatory activity of *Mentha arvensis* extract was evaluated using a protein denaturation assay where aspirin was used as a reference drug. It was observed that as the concentration of the extract increased, the inhibition of protein denaturation also increased. At low concentration, the inhibition was low. As the concentration increased, the extract showed significant inhibitory activity of approximately 91%, which is quite close to the standard drug aspirin (93%) (Table 3, Figure 4). These findings indicate that *Mentha arvensis* possesses significant anti-inflammatory potential, which can be harnessed for other biological activities.

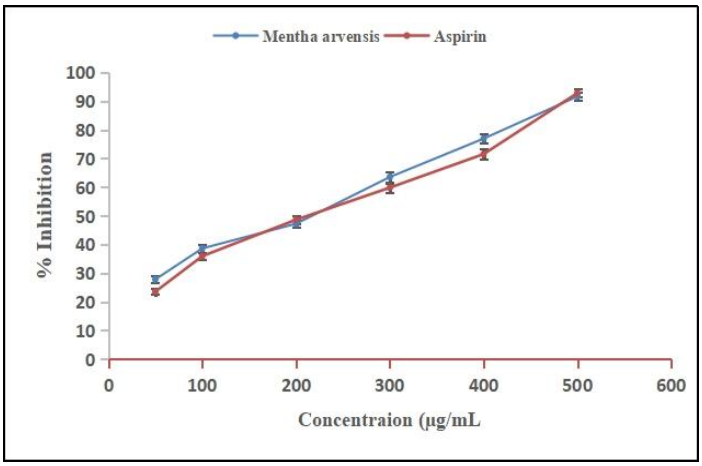


Figure 4: Anti-inflammatory Activity of *Mentha arvensis* by Protein Denaturation Assay

One of the reasons for inflammation is protein denaturation because denatured protein can act as an autoantigen and trigger an inflammatory reaction. Hence, the ability of plant extract to prevent protein denaturation is considered a reliable indicator of anti-inflammatory activity (Mizushima and Kobayashi).[26] The observed dose-dependent increase in % inhibition suggests that the bioactive compounds present in *Mentha arvensis* effectively stabilize protein structure under stress conditions. At higher concentrations, the extract exhibited activity comparable to aspirin. This may be due to its rich phytochemical composition, particularly flavonoids, phenolic compounds, and essential oils such as menthol, which are known to possess anti-inflammatory and antioxidant properties.[27]

Table 3: Anti-inflammatory activity of petroleum ether leaf extract of *Mentha arvensis* and standard.

Concentration (µg/mL)	Petroleum ether extract	Standard (Aspirin)
50	27.8 ± 1.2	23.5 ± 1.0
100	38.6 ± 1.5	35.9 ± 1.3
200	47.2 ± 1.3	48.6 ± 1.4
300	63.5 ± 1.8	59.8 ± 1.6
400	76.9 ± 1.6	71.5 ± 1.7
500	91.6 ± 1.4	92.8 ± 1.5

Results are expressed as Mean ± Standard deviation (n=3)

Alpha-Amylase (pancreatic) inhibition assay by DNS method

The α-amylase inhibition assay demonstrated dose dependent inhibition of pancreatic α- amylase activity for plant extract and also a standard reference drug. Plant extract exhibited 75% inhibition at 250 µg/ml with an IC_{50} value of 171.77, while the standard drug exhibited 86.58% inhibition at 250 µg/ml, with an IC_{50} value of 66.64 µg/ml (Figure 5). Regression analysis indicated good linearity ($R^2 > 0.98$), supporting the concentration-dependent nature of inhibition. These results suggest that the plant extract possesses α-amylase inhibitory activity, highlighting its potential role as a natural antidiabetic agent. However, its activity is lower than the standard drug metformin, as indicated by the IC_{50} value. The metabolites present in the extract contribute to amylase inhibition. These compounds may either bind to the active site of enzyme or bind to other sites causing a change in the confirmation which prevent hydrolysis of starch. [28-28] Earlier reports also confirmed the presence of metabolites like phenols and flavonoids that contributed to the enzyme inhibition.[8,30] This interaction suggests the potential use of extract as a natural antidiabetic agent, esp. in managing postprandial hyperglycemia.

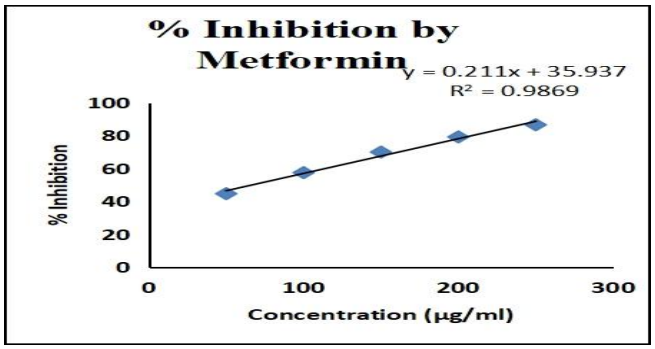
Docking studies

The docking studies were carried out using ligands and receptors as described earlier.

ISSN 2955-1226 (Print)
ISSN 2955-123(Electronic)

Structural Validation of 1B2Y and 4W93

Prior to molecular docking, the stereochemical quality of both proteins was evaluated (Figure 6). Ramachandran plot analysis of 1B2Y revealed that maximum residues were located in the most favoured region, and only a little was in the disallowed region. The overall G-factor of −0.04 further supports acceptable stereochemical quality. [31-32] Similarly, Ramachandran plot analysis of 4W93 demonstrated that the majority of residues (>90%) were located within the most favoured regions, with only a minimal percentage in additionally allowed regions and negligible residues in disallowed regions. The absence of significant steric clashes further validates the reliability of 4W93 for downstream molecular docking and binding-site interaction studies. Collectively, these validation metrics confirm that both protein structures are suitable for computational docking and interaction analysis.



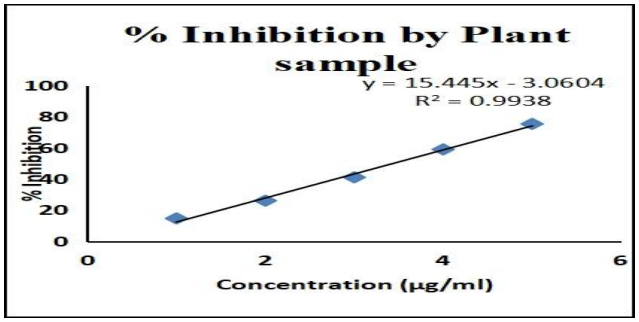
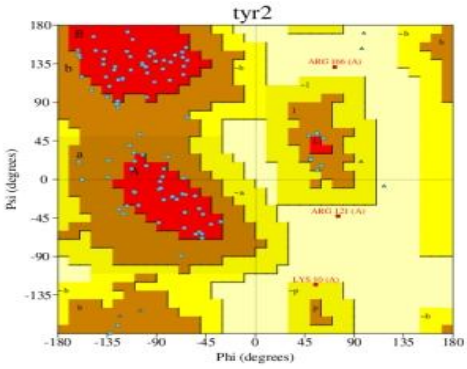
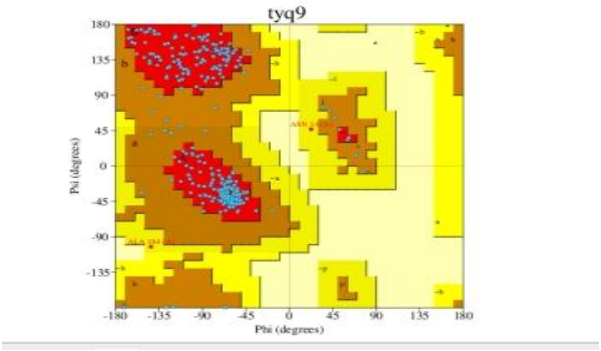


Figure 5: Dose response curve of Standard Metformin and petroleum ether leaves extract (Plant sample).



a



b

Figure 6: Ramachandran plot for a) 1B2Y and b) 4W93.

The stereochemical quality assessment of protein structures 1B2Y and 4W93 indicated that both models possess acceptable structural integrity and stability. The Ramachandran plot is a widely used tool for validating protein structures by analysing the φ (phi) and ψ (psi) dihedral angles of amino acid residues, where a high percentage of residues in the most favoured regions reflects good stereochemical quality.[33-34] In the present analysis, the majority of residues for both 1B2Y and 4W93 were found within favoured and allowed regions, suggesting minimal steric hindrance. The results confirm that both protein structures are stereochemically sound and suitable for further *in silico* analysis.[35]

The in-silico molecular docking analysis of phytochemicals against target proteins 1B2Y and 4W93 demonstrated favourable binding interactions when compared with the standard drug metformin. Docking studies are widely used to predict ligand–protein interactions based on binding affinity and molecular interactions such as hydrogen bonding and hydrophobic interactions. Hydrogen bonds play a critical role in ligand specificity and binding stability by contributing enthalpically favourable interactions.[36] Hydrophobic interactions contribute significantly to binding affinity by promoting favourable desolvation and van der Waals stabilization.[36] Previous studies have reported that luteolin and rosmarinic acid exhibit strong binding affinities with target proteins through multiple hydrogen bond interactions, contributing to stable ligand–protein complexes.[37-38] Furthermore, flavonoids like luteolin and glycosides such as isoorientin are known for their structural compatibility with protein binding pockets due to the presence of hydroxyl groups, which enhance hydrogen bonding and binding stability. Rosmarinic acid, in particular, has been shown to form multiple hydrogen bonds with amino acid residues, resulting in strong binding energies and potential biological activity.[38] The docking studies of these compounds indicate that the extract of *Mentha arvensis* contains phytochemicals that have significant therapeutic potential against proteins 1b2y and 4W93. However, further in-vitro and in-vivo validation is required for drug development. Among various ligands, luteolin demonstrated superior affinity toward the target protein receptor. Considering the pharmacokinetic properties and low toxicity, luteolin was identified as the most promising lead compound. Hence, luteolin can be used as a natural drug for the treatment of diabetes.

Molecular Docking and Binding Affinity Assessment

Molecular docking was carried out using AutoDock Vina, which predicts protein-ligand interaction. It also integrates hydrogen bonding, hydrophobic constituents. Reports binding affinity, where more negative values correspond to stronger predicted binding. [39,40] Among the various phytochemicals identified in *Mentha arvensis*, luteolin demonstrated the strongest binding affinity toward both α-amylase crystal structures (PDB IDs: 1B2Y and 4W93); next is rosmarinic acid and isoorientin.

Alpha amylase acts on substrate starch by hydrolyzing α-1,4-glycosidic linkages, liberating maltose, limit dextrin, and glucose, which contributes to postprandial hyperglycemia. These ligands bind to the enzyme in such a way as to inhibit the enzyme activity and hence exerting antidiabetic effect. This is through antioxidant

ISSN 2955-1226 (Print)
ISSN 2955-123(Electronic)

protection of pancreatic β-cells. Chronic hyperglycemia induces excessive reactive oxygen species (ROS) production, leading to oxidative stress-mediated β-cell dysfunction and apoptosis. Due to its polyphenolic structure and conjugated double bond system, luteolin efficiently scavenges free radicals and chelates pro-oxidant metal ions. Luteolin may preserve insulin secretion capacity and improve glucose homeostasis by reducing oxidative stress. The antidiabetic activity may be either due to competitive inhibition or by antioxidant mediated protection of pancreatic cells. However, further studies are required for validation of these findings.

Interaction Profiling
a) Luteolin –1B2Y Complex

The docked structure of the ligand luteolin with the protein 1B2Y complex is shown in Figure 7. The ligand interacts primarily with residues Gln7, Gly9, Arg10, and Thr11, which are not the part of catalytic triad of human pancreatic α-amylase. Since the interacting residues are different from the catalytic residue, this suggests that the interaction may be on the other side, indicating a non-competitive mode of inhibition. The Ligplot analysis indicates the presence of both hydrogen bonding and hydrophobic interaction. Luteolin forms multiple hydrogen bonds with residues like Gln7, Gly9, Arg10, Thr11, and Pro332, with bond distances that vary in the range of ~2.7–3.1. Hydrophobic interaction with residues like Asp402, Gly334, Ser289, Ser290, and Pro4, and Van der Waals interaction contribute to the overall stability of the ligand. This could be a non-competitive mode of inhibition.

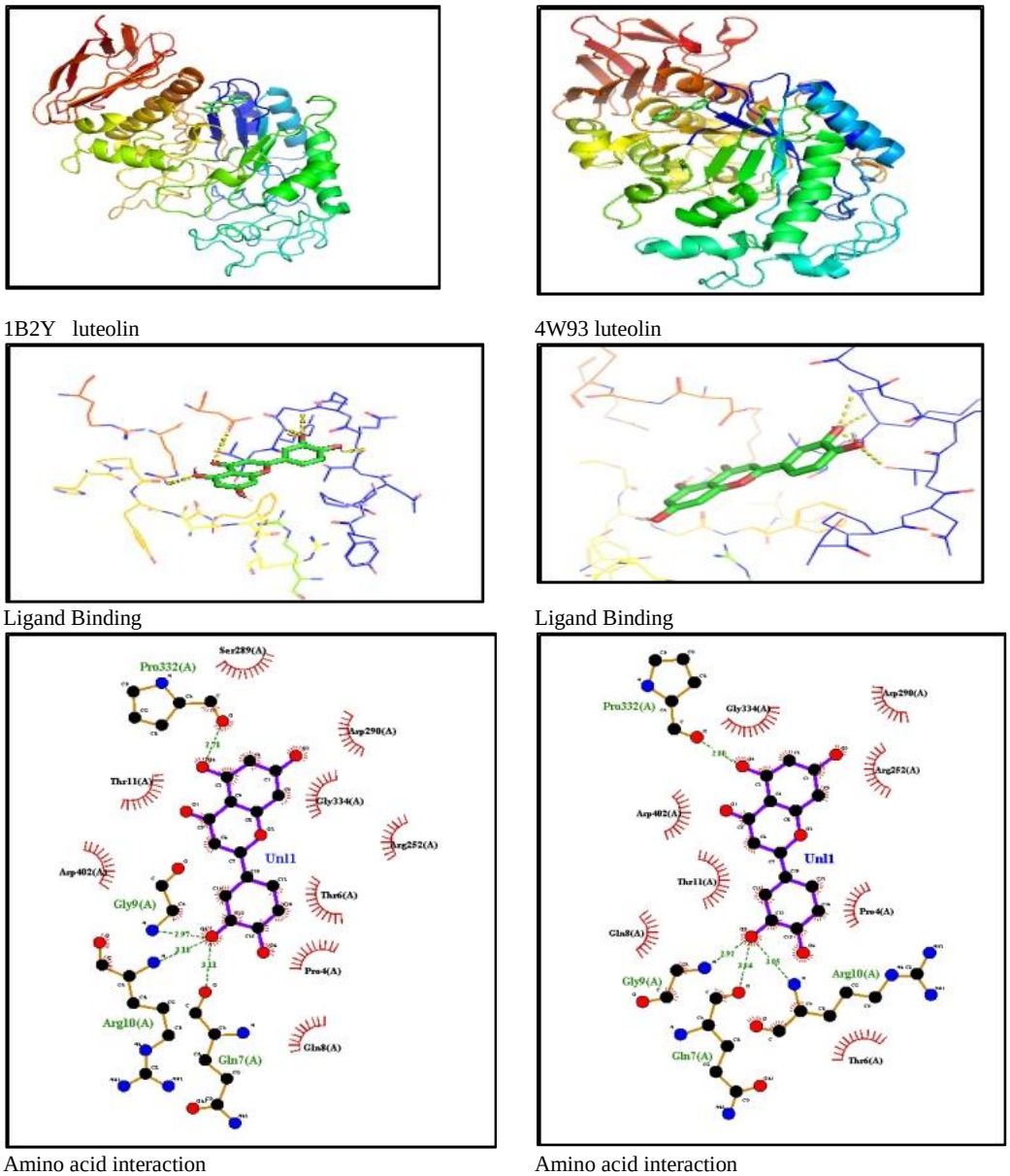


Figure 7: Docking of ligand Luteolin with 1B2Y and 4W93 and their interaction shown in two columns respectively.

b) Luteolin–4W93 Complex

The docked structure of the ligand luteolin and 4W93 complex is shown in Figure 7. LigPlot analysis of the ligand luteolin with protein 4W93 indicates the involvement of hydrogen bonds and hydrophobic bonds, which stabilizes it. The interaction of this ligand with the protein is not in the active site of the enzyme. Ligands form hydrogen bonds with the amino acid residues like Pro332, Gly9, Gln7, and Arg10, with bond distances around ~2.7–3.1 Å. A hydrophobic environment is created by residues like Asp402, Gly334, Asp290, Arg252, Thr11, Pro4, Glu8, and Thr6 which stabilizes the ligand.

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Since the interaction is not within the active site, the ligand binds to other sites or allosteric sites which may alter the confirmation or accessibility of the active site. This could be a non-competitive or allosteric mode of inhibition.

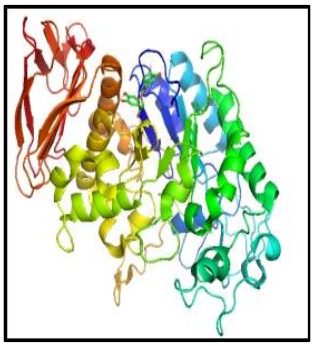
c) Rosmarinic acid-1B2Y Complex

The LigPlot analysis of rosmarinic acid with 1B2Y (human pancreatic α-amylase) is depicted in figure 8. It supports the presence of many hydrogen bonds and hydrophobic interactions. Hydrogen bonds with amino acid residues like Gln7, Gly9, Arg10, and Pro332, having bond distances of ~2.7–3.2 Å, indicate stable binding. Also, the ligand interacts with other residues which further helps to anchor the ligand. Hydrophobic interactions with residues like Asp402, Phe335, Arg252, Asp300, Glu8, Gly334, and Thr11 enhance the stability of the complex through van der Waals interactions. Though Asp 300 is a part of the active site residue, the ligand is not positioned in a way to block catalytic activity, indicating that it is not a competitive inhibitor. The inhibition could be non-competitive or allosteric inhibition, wherein the binding of the ligand changes the conformation of the enzyme and affects the activity.

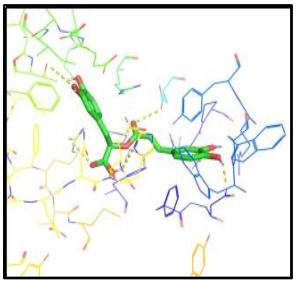
d) Rosmarinic acid – 4W93 Complex

The LigPlot analysis of rosmarinic acid with 4W93 (α-amylase) is shown in Figure 8. It also supports the presence of various hydrogen bonds and hydrophobic interactions. Ligand forms hydrogen bonds with amino acid residues like Pro332, Gly9, Arg10, Gln7, and Thr6, with bond distances that ranges from ~2.7 to 3.2 Å. This indicates strong binding affinity and proper orientation. Further interaction with amino acids like Ser289 and Arg252 stabilizes the ligand. Hydrophobic interaction with residues like Asp402, Phe335, Gly334, Asp290, Glu8, Thr11, and Arg398 creates a supportive environment that enhances ligand retention through van der Waals forces. Since the interaction does not involve the amino acids of catalytic site, the ligand interaction is suggested to be a non-competitive or allosteric model wherein binding of the ligand brings about the confirmational change, and hence its enzyme activity is affected.

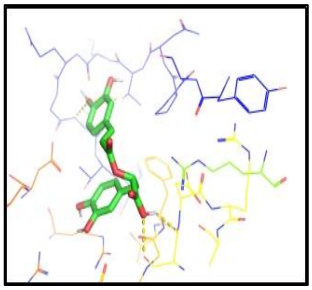
1B2Y rosmarinic acid



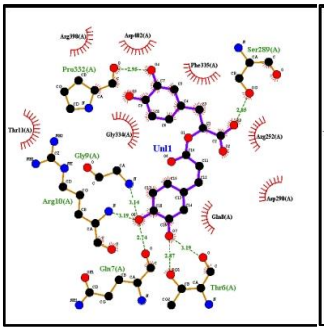
4W93 rosmarinic acid



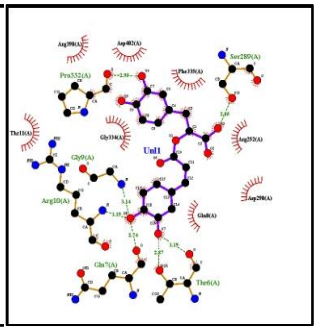
Ligand interaction



Ligand interaction



Amino acid interaction

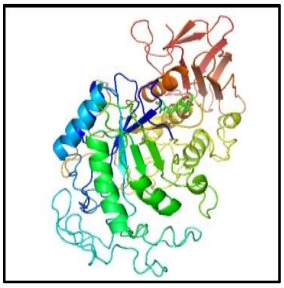


Amino acid interaction

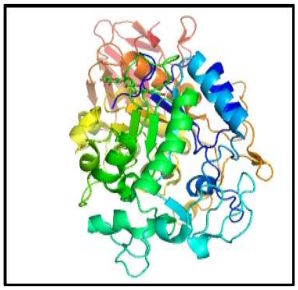
Figure 8: Docking of ligand rosmarinic acid with 1B2Y and 4W93 and their interactions shown in two columns respectively.

e) Isoorientin–1B2Y Complex

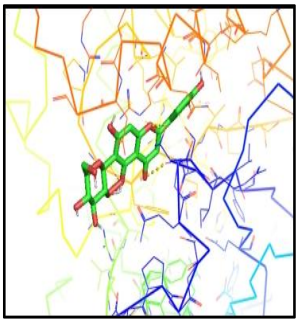
The LigPlot analysis of isoorientin with 1B2Y is shown in figure 9. The interaction has multiple hydrogen bonds and hydrophobic interactions. Hydrogen bonds with amino acid residues like Gly334, Thr11, Thr330, Ser12, Asp402, and Arg398, with bond distances typically around ~2.6–3.1 Å, indicates strong and favourable interaction Further, it also interacts with amino acid like Val163 and other residues enhancing ligand positioning. Hydrophobic interaction with residues like Phe335, Leu353, Val163, and other residues contributes to the overall stability of the complex through van der Waals forces. This interaction too, suggests that it is not within the catalytic triad. This supports that the interaction is non-competitive or mixed or allosteric inhibition, wherein binding of the ligand brings about conformational change in the structure of the protein.



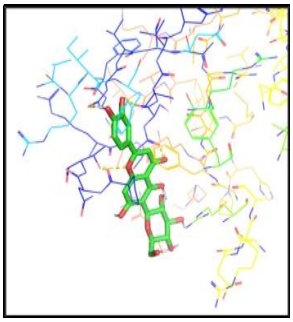
1B2Y Isoorientin



4w93 Isoorientin

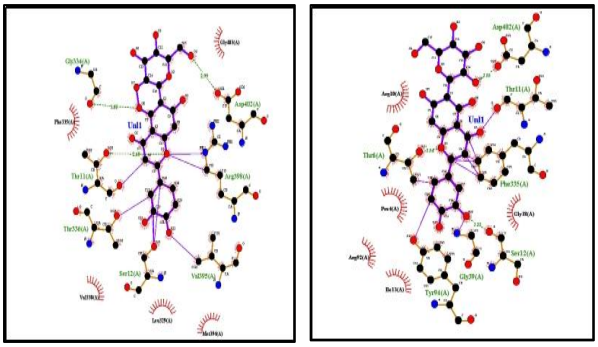


Ligand binding-1B2Y Isoorientin



Ligand Binding -4W93 Isoorientin

ISSN 2955-1226 (Print)
ISSN 2955-123(Electronic)



Amino acid interaction Amino acid interaction

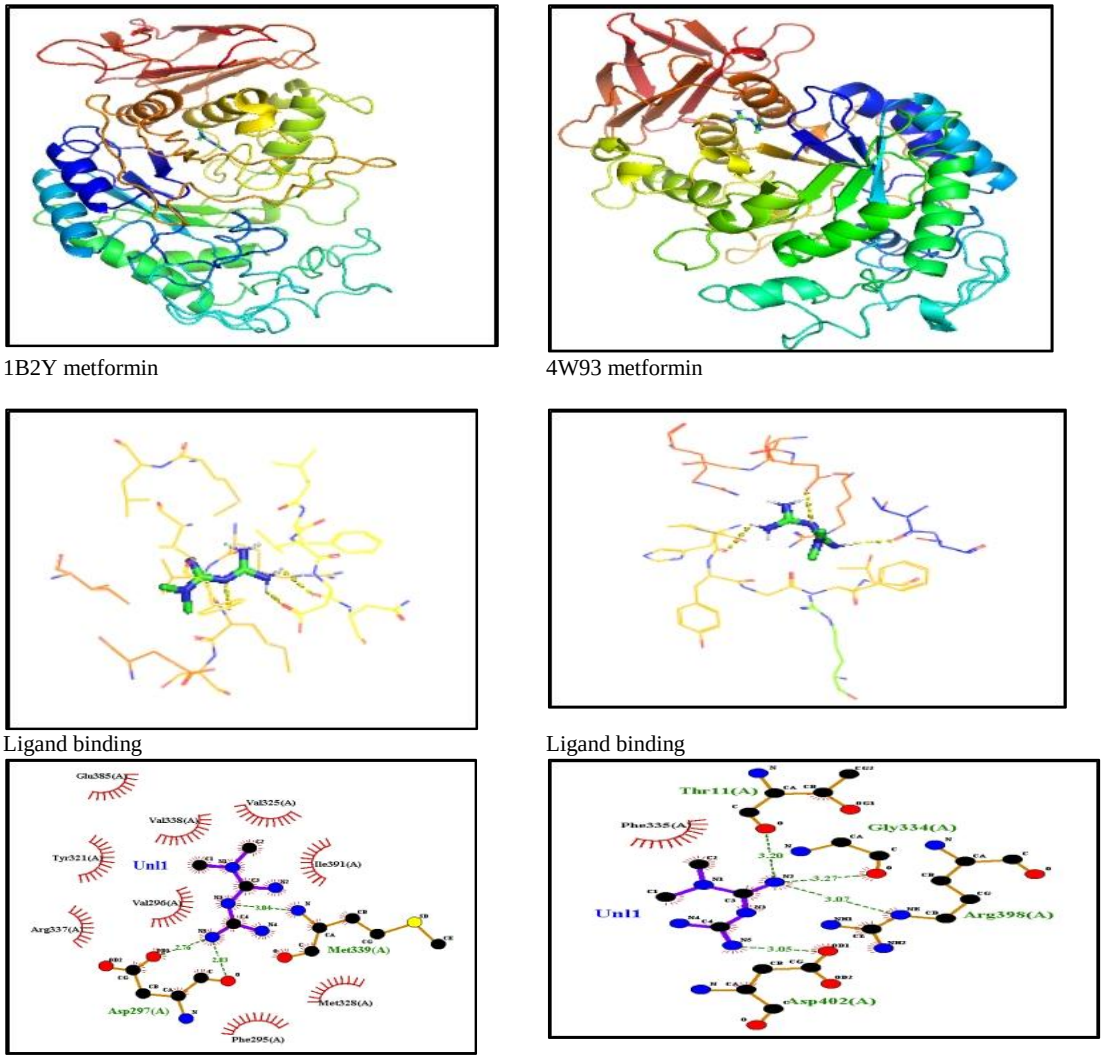
Figure 9: Docking of ligand Isoorietin with 1B2Y and 4W93 and its interactions shown in two columns respectively.

f) Isoorientin–4W93 Complex

Detailed interaction analysis using LigPlot revealed that isoorientin establishes an extensive hydrogen-bonding network within the active site: Six hydrogen bonds were observed involving: Thr 6 (1.87 Å), Ser 12 (1.51 Å), Gly 38 (1.96 Å), Val 40 (3.46 Å), Arg 92 (3.30 Å), Thr 336 (2.76 Å) (Figure 9). Other amino acids involved in interaction, like Thr6 and Thr336 suggest multi-point stabilization. Hydrophobic interaction with residues like Pro 4, Thr6, Thr11, Ile13, Tyr94 and Phe335 is observed. Notably, Phe335 exhibited strong hydrophobic contact (2.27 Å), indicating aromatic stabilization. The combined presence of hydrogen bonding and hydrophobic interactions indicates strong geometric complementarity between isoorientin and the 4W93 active site.

g) Metformin -1B2Y Complex

Docking analysis showed that metformin binds to the human pancreatic alpha-amylase through hydrogen bonding and other interactions (Figure 10). Two hydrogen bonds were observed with the residue Asp297, with bond distances of approximately 2.76 Å and 2.83 Å. One more hydrogen bond with residue Met 339 with a bond distance of 3.04 Å. Asp297 is an important catalytic residue that is involved in starch hydrolysis. Interaction of metformin at this amino acid indicates it interferes with catalytic activity and hence acts as an inhibitor. Also, several hydrophobic amino acids like Ile391, Val325, Val296, Tyr321, and Phe295 further help stabilize the ligand within this pocket.



1B2Y metformin 4W93 metformin

Ligand binding Ligand binding

Amino acid interaction Amino acid interaction

Figure 10: Docking of ligand metformin with 1B2Y and 4W93 and its interactions shown in two columns respectively.

ISSN 2955-1226 (Print)
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g) Metformin -4W93 Complex

The docking and Ligplot analysis of the interaction between metformin and 4w93 indicate hydrogen bond interactions with important amino acid residues such as Thr11, Gly334, Arg398, and Asp402 (Figure 10). Among these, Asp402 and Arg398 play significant roles in stabilizing the ligand within the enzyme binding region, while Gly334 contributes to close molecular association through hydrogen bonding. The presence of multiple hydrogen bonds indicates strong binding affinity and suggests that metformin may interfere with substrate binding or catalytic activity of α-amylase. Also, hydrophobic interaction with amino acid residues like Phe335 further enhances the stability of the ligand–protein complex. These interactions together support the potential inhibitory effect of metformin against α-amylase, which may help reduce carbohydrate metabolism and release of glucose.

ADME and Drug-likeness Prediction of Active Molecule

The pharmacokinetic and toxicity profile of the ligands compound was evaluated using *in silico* ADMET prediction tools to understand its drug-likeness and safety characteristics (Table 4 and 5). The compounds exhibited moderate water solubility (log mol/L −2.9) and acceptable human intestinal absorption (61.76%), suggesting reasonable oral bioavailability. Although the Caco-2 permeability value (−0.912 log Papp) indicates moderate permeability, the compound was predicted to be a P-glycoprotein substrate, which may influence its absorption and efflux. Skin permeability (log Kp −2.735) suggests limited transdermal penetration. In terms of distribution, the predicted volume of distribution (log VDss 1.603 L/kg) indicates good tissue distribution, while the fraction unbound (0.219) shows that a moderate portion remains free in circulation. The low BBB permeability (log BB −1.564) and CNS permeability (log PS −3.939) suggest minimal penetration into the brain, indicating reduced risk of CNS-related side effects. Metabolic prediction revealed that the compound has a lower probability of drug-drug interaction. Also, the clearance value indicates moderate elimination. Additionally, toxicity assessment showed that the compound is not toxic. Among the analysed ligands, rosmarinic acid and luteolin doesn't violate the Lipinski rule, among others. Also, absorption and BBB permeation are high for luteolin and also exhibit good drug likeness properties. Moreover, it's not-toxic nature makes it a suitable candidate. Overall, the in-silico result indicates that the compound luteolin has promising pharmacokinetic properties, supporting it to be a potential candidate for further experimental validation.

Table 4: Drug likeliness and safety characteristics

Ligand	MW (g/mol)	Lipinski Violations	GI Absorption	BBB Permeation	Bioavailability Score	CYP Inhibition (General Trend)	Drug-likeness
Rosmarinic acid	360.31	0	Low–Moderate	No	0.55	Weak	Moderate
Isoorientin	448.38	2	Low	No	0.17	Minimal	Poor
Luteolin	286.24	0	High	Possible	0.55	Moderate CYP inhibition	Good
Hesperidin	610.56	3	Low	No	0.17	Minimal	Poor
Carnosic acid	332.43	0	High	Likely	0.55	Possible CYP inhibition	Very Good
p-Coumaric acid	164.16	0	High	Yes	0.85	Very Low	Good (small molecule)
Caffeic acid	180.16	0	High	Yes (limited)	0.85	Very Low	Good

Table 5: Toxicity of the metabolites

Ligand	Predicted LD50 (Oral, Rat)	Toxicity Class	Hepatotoxicity	Carcinogenicity	Mutagenicity (AMES)	Overall Toxic Risk
Rosmarinic acid	>2000 mg/kg	Low (Class 5)	No	No	Negative	Low
Isoorientin	>2000 mg/kg	Low (Class 5)	No	No	Negative	Low
Luteolin	~3919 mg/kg	Low (Class 5)	No	No evidence	Possible weak positive	Low–Moderate
Hesperidin	>2000 mg/kg	Low (Class 5)	No	No	Negative	Low
Carnosic acid	~2000 mg/kg	Class 4–5	Possible mild hepatotoxicity	No	Negative	Moderate
p-Coumaric acid	>2000 mg/kg	Low	No	No	Negative	Very Low

Conclusion

The present investigation suggests that the leaf extract of *Mentha arvensis* has significant pharmaceutical potential due to the presence of various metabolites like phenols, alkaloids, flavonoids. The metabolites were identified by LC-MS. The extract exhibited anti-inflammatory and anti-diabetic properties, suggesting its therapeutic relevance in the management of diabetes. Furthermore, the *in-silico* studies revealed strong binding affinity between key metabolites and the receptor or protein involved in diabetic pathway. The integration of phytochemical analysis, biological activity and *in silico* analysis provided scientific validation for the use of plants, highlighting the importance having therapeutic value of the compounds. However, *in vivo* experimentation and clinical studies are required to validate its efficacy and use in pharma industries.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to acknowledge Alumni Association for the seed money grant number PCJ/AASM/25-26/SP/3 to carry out project. The authors would also like to acknowledge Principal and Management, KLE'S P.C. Jabin Science College, Hubballi, Karnataka, India for their constant support and encouragement.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by us.

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