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Research Article

Terminalia Chebula for Anti-Diabetic Activity

Isolation and Characterization of Chemical Compounds from *Terminalia Chebula* for Anti-Diabetic Evaluation Through *In-Silico* Approach

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Abstract: Diabetes is a chronic metabolic disorder affecting millions of people and needs to be addressed. Alternative strategies are required for better diabetes management, as evidenced by the high costs of modern medicines. The field of herbal medicine research has been gaining significant importance in the last few decades, and the demand to use natural products in the treatment of diabetes is increasing across the globe. Traditional plant medicines are used worldwide for a range of diabetic complications. *Terminalia chebula* is one of the widely used traditional medicine by diabetic patients. In this present work, T*erminalia chebula* fruits were used for the potential of anti-diabetic activity. The phytochemical investigation revealed that its fruit contains high amounts of tannins, alkaloids, flavonoids, terpenoids, and glycosides. The percolation method was followed by using *T. chebula* fruit for the extraction, further fractionating with different solvents, *n*-Hexane, Ethyl acetate, *n*- Butanol, and water. When ethyl acetate extract of *T. chebula* was tested, it was found to have significant effects on blood glucose levels. It suggests that it can be used as an antidiabetic agent. Gallic acid and quercetin were compounds identified by NMR, MS, and UPLC analysis. Molecular docking studies of designed compounds were carried out through Auto Dock Vina [1.1.2] to investigate the interaction with PPAR-γ associated with diabetes, and pkCSM software was used for ADMET analysis. Thus, the result of the study proved that the ethyl acetate extract of *T. chebula* had good potential as an antidiabetic, and this plant can be used to discover natural bioactive compounds that may serve for the advances of novel pharmaceutical development. Moreover, the compounds found in the extract could be used to develop new pharmaceuticals for diabetes treatment.

Key Words: Diabetes mellitus; Molecular docking; blood glucose; *Terminalia chebula*; herb medicine; *in silico*;

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1. INTRODUCTION

Herbal medicines are most widely used among all the authorized health institutions worldwide and have been considered affordable and reliable sources of various bioactive constituents. World Health Organization (WHO) estimated that almost 80% of the population depends on herbal medicine for primary health care and treatment. It is because herbal medicines have a natural origin, no side effects, and are economical [1]. *T. chebula is a common* medicinal plant used as folk medicine and belongs to the family Combretaceae. It is widely spread throughout southern Asia and used in various home remedies [2]. *T. chebula* is also known as chebulic myrobalan, black myrobalan, and ink tree. It is a key component in the well-known Ayurvedic remedy Triphala, which treats liver and kidney disease. Additionally, the plant is very useful in the treatment of conditions like gout, arthritis, ulcers, cancer, paralysis, and cardiovascular diseases [3]. The plant *T. chebula* is termed the "King of Medicine" in Tibet and is indigenous to India. The dried ripe fruits of *T. chebula* are valued as excellent herbs often employed in our traditional healthcare system for their laxative, homeostatic, diuretic, cardiotonic, and antitussive properties [4,5]. The observed health benefits may be credited to phytochemicals like polyphenols, flavonoids, terpenes, alkaloids, glycosides, and anthocyanins. In this research, unripe fruits have been exploited to isolate and characterize bioactive compounds for their potential antidiabetic properties, along with the *in-silico* studies of the isolated phytoconstituents.

1.1. Botanical Description

- *T. chebula* is a large evergreen tree with crowded branches and an umbrella-shaped crown, growing to a height of 25 m. It usually grows in clayey and shaded soils between 1500 and 2000m altitude.
- The leaves of *T. chebula* are 10-20 cm long, simple, sub-opposite, exstipulate; petiolate; rarely oval; broad elliptic to elliptic-oblong laminae with acute or acuminate tips.
- The flowers vary in color from white to yellow, are mono-tonous autoicous, and have a strong, pungent smell, born in short prickles or terminating panicles.
- Fruit: The fruits are elongated, ovate drupes, color varies from yellow to orange-brown, and are glabrous.
- Seed: The plant seeds are ellipsoid, single, rough, and 1.0–2.0 cm by 0.2–0.7 cm in size without ridges.

T. chebula has three different stages, each with a different phase of fruit development
(a) large Myrobalan - completely developed stage of fruit
(b) small Myrobalan - immature fruit;
(c) yellow Myrobalan - after seed growth, the fruit's mature stage.

1.2. Traditional uses

T. chebula is the most widely used plant in the typical remedies in India and other countries. It is a key component in the Ayurvedic remedy Triphala, which treats liver and kidney disease.

- The dried ripe fruits of *Terminalia chebula* are valued as excellent herbs often employed in our traditional healthcare system for their laxative, homeostatic, diuretic, cardiotonic, and antitussive properties.
- Fruit is often used to treat conjunctivitis due to its anti–inflammatory properties.
- The Haritaki fruits are used both externally and internally for medical purposes. Externally, its water-mixed paste has proven to be analgesic and anti-inflammatory, and healing wounds and ulcers.
- Used to treat various infectious disorders, including pneumonia, tuberculosis, fever, and cough.
- Useful as a digestive aid to increase the appetite.
- The Haritaki oil helps in the faster healing of wounds, especially burns.
- Used as anti-astringent.
- Used as an herbal laxative and colon cleaner.
- It reduces the chances of acquiring typhoid fever.
- It has been found to improve mental abilities and is used as an anti-aging agent.
- The plant functions as an adrenergic and helps in stress relief.

Also, it has a wide variety of pharmacological and biological uses, including antiviral, antifungal, antibacterial, adaptogenic, anti-anaphylactic properties, hypolipidemic, anti–ulcerogenic, and gastrointestinal motility-improving, antidiabetic and renoprotective, hepatoprotective, cardioprotective, purgative, wound healing, antispasmodic, chemopreventive and immunomodulatory.

1.3. Chemical constituents

T. chebula contains several constituents, including phenolics, hydrolyzable tannins, anthraquinone, flavanol, carbohydrates, glucose, and sorbitol. Tannic acid is present in its fruit. The major constituents are chebulagic acid, chebulic acid, gallic acid, and corilagin. The pyrogallol type of tannic acid present in *T. chebula* is hydrolyzable. Regional differences affect the tannins concentration of *T. chebula*. Tri-terpenoids, coumarins conjugated with gallic acid, and flavonol glycosides, known as chebulin also identified [7,8]. Some ellagitannins have been associated with the plant, including cerulenin, neochebulinic acid, terchebulin, casurarinin, and punicalagin, and others like chebulinic acid, chebulagic acid, and chebulic acid.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Analytical grade solvents and reagents used in this current study were compatible with every required analytical standard in the CSIR-IHBT Chemical Technology lab.

2.2 Collection of plant material

The unripe fruits of *T. chebula* were collected from District Hamirpur (H.P.) and were identified by the Department of Herbarium at CSIR- IHBT Palampur.

2.3 Extraction

Extraction is a common and simple technique used to isolate bioactive compounds. The powdered plant material (1.13 kg) of *T. chebula* was extracted by percolation with ethanol: water (80:20 v/v, 3×24 h). The filtered extract was dried

under reduced pressure using a rotary evaporator to yield 74.6 g of crude extract.

2.4 Partitioning

The crude extract of *T. chebula* was partitioned with *n*-hexane, ethyl acetate, *n*-butanol, and water to yield 4.0g, 17.38g, 9.0g & 44.22g, respectively.

2.5 Preliminary tests of crude extract of T. chebula[16-21]

2.5.1 Test for Phenols (Ferric chloride test)

The crude extract was added to 3-4 drops of $FeCl_3$ for the formation of a blue-black color, which indicates the presence of phenol, and this test is considered a ferric chloride test.

2.5.2 Test for Terpenoids (Salkowski test)

Extract (5ml) mixed with chloroform (2ml) & conc. Sulphuric acid (3ml) was added to form a layer. A reddish-brown coloration of the interface was formed, showing the presence of terpenoids.

2.5.3 Test for Alkaloids (Dragendroff's test)

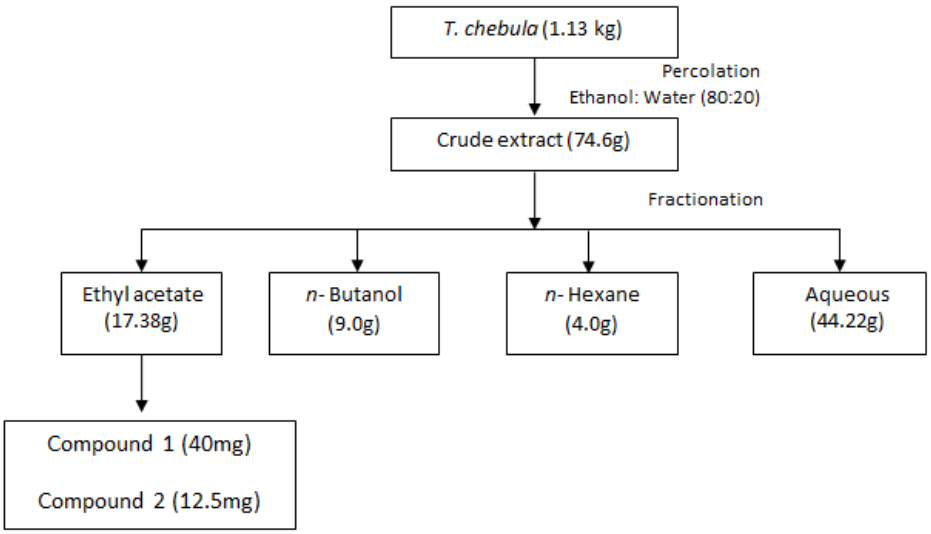
By adding 1ml of Dragendroff's reagent to 2ml of extract, an orange-red precipitate formed, indicating the presence of alkaloids.

2.5.4 Test for Flavonoids (Alkaline reagent test)

A few drops of NaOH solution were added to the crude extract to form an intense yellow color that became colorless, and 10 drops of 1% hydrochloric acid showed the presence of flavonoids.

2.5.5 Test for Saponins

0.25g of crude plant extract mixed with 20 ml distilled water and shaken in a graduate cylinder for 15 mins to form a foam with a 1cm layer indicating the presence of saponin.



Flow chart 1: Extraction & Isolation process

3. EVALUATION OF ANTIDIABETIC ACTIVITY THROUGH *IN-SILICO* STUDIES [40]

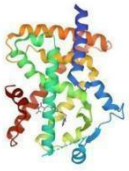
3.1 Computational tools and database source (ligand and protein)

This study was carried out using the Auto Dock Vina (version 1.1.2). Suitable ligands were retrieved from the PDB database. These molecules were converted to 3D structures in the Auto Dock Vina. Protein molecules of DJ-1/RS were retrieved from the protein data bank.

3.2 Protein Preparation

The protein structure for docking was prepared using the PDB database. The protein molecule PPAR-γ (4EMA), Peroxisome proliferator-activated receptor gamma, was docked with the two phytocompounds (quercetin and gallic acid) and two synthetic ligands, metformin and troglitazone. Peroxisome proliferator-activated receptors (PPARs) are transcription factors with a key role in glucose and lipid metabolism. PPAR-γ is known to increase the glucose-sensing ability of pancreatic β-cells. The 3D structure of PPAR-γ (4EMA) is presented in Table 1, having resolution 2.54 A, R-value Free 0.225.

Table 1. PDB Database

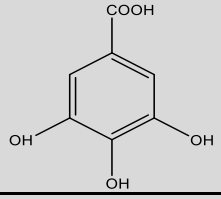
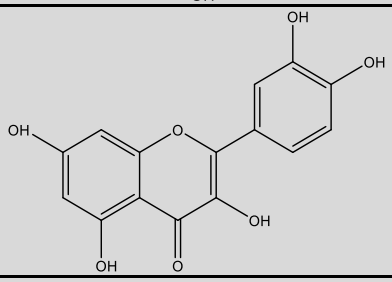
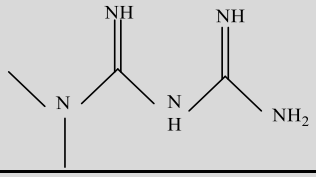
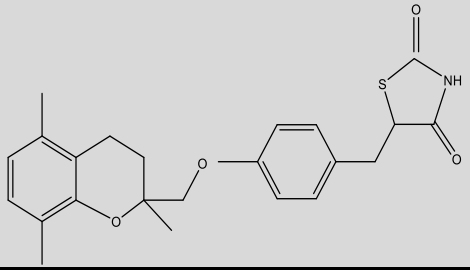
Name of target	PDB ID	Structure	Reference
PPAR-γ	4EMA		15

PPAR-γ is a nuclear receptor that plays a role in regulating fat storage and glucose metabolism. It involves many diseases, including type 2 diabetes, obesity, and cardiovascular disease. Activation of PPAR-γ by specific chemical compounds can be used to treat these conditions.

3.3 Ligand Preparation

The ligand preparation was performed using the PubChem database. The drawn ligands were converted to SDF format in the Chem draw Professionals 16.0 to combine tools to generate structures from 1D (Smiles) and 2D (SDF) representations.

Table 2: PubChem Database

Name of ligand	CID	Structure	Smile format
Gallic acid	370		C1=C(C=C(C(=C1O)O)O)C(=O)O
Quercetin	5280343		C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O) O)O)O
Metformin	4091		CN(C)C(=N)N=C(N)N
Troglitazone	5591		CC1=C(C2=C(CCC(O2)(C)COC3=CC=C(C=C3)CC4C(=O)NC(=O)S4)C(=C1O)C)C

Gallic acid structure is a naturally occurring phenolic acid. It has three hydroxyl groups and a carboxylic acid group making it an organic acid. Quercetin is a type of flavonoid found in many fruits and vegetables. It is a powerful antioxidant with anti-inflammatory properties and can help protect the body from various diseases. It is also used in supplements to help boost immunity. Metformin is an anti-diabetic drug used to treat type 2 diabetes. It works by reducing the amount of sugar the liver produces and improving the body's sensitivity to insulin. Troglitazone is a drug that is used to treat type 2 diabetes. It works by increasing the body's insulin sensitivity and helping control glucose levels. It is effective in treating the condition.

3.4 Molecular Docking & Binding Evaluation[41]

The molecular docking studies were carried out through Auto Dock Vina [1.1.2]. Through its automated work system, the ligand was allowed to dock in the active sites of the target proteins. The docking study was performed with a flexible ligand into a rigid enzyme active site. The docking process is initiated by adding an energy-minimized target ligand. The molecule's structure was prepared through Chem draw Software and procured in. mol file. The procured molecule was imported into the software, and the software minimized its energy. The molecule with stable conformation was allowed to dock in the active site. Biovia Discovery Studio Visualizer 2021 was used to visualize the molecular docking results, and thus, through the docking score observation, the antidiabetic potential of the compound can be studied. The final docking results were represented in kcal/mol. The complex formed by the docked ligands gallic acid & quercetin, along with the selected targets, was interpreted using the ligand's H- bonding or hydrophobic interaction with the amino acid residues of the active site. Moreover, pkCSM software was used for ADMET analysis (Table 5 & Table 6).

Table 3. Molecular Property

Descriptor	Gallic acid (Value)	Quercetin (Value)
Molecular weight	170.12	302.238
LogP	0.5016	1.988
Rotatable bonds	1	1
Acceptors	4	7
Donors	4	5
Surface Area	67.135	122.108

Lipinski's rule of five is essential for rational drug design, and it has been suggested that the low permeability or poor absorption for a given compound results when it violates one of Lipinski's five rules. Molecular weight, LogP, and several hydrogen bond acceptors (NHBAs) of both molecules are within the recognized values of less than 500, 3, and 10, respectively [16]. Gallic acid, 3, 4, 5-trihydroxy benzoic acid, C_6H_2 $(OH)^3$ COOH. It is classified as a phenolic acid, belongs to a group of hydrolyzable tannins, and has a molecular weight of 170.12 g/mol, having a log P value <5, which predicts the drug likeliness of the compound for good absorption. It has good solubility in water and other organic solvents. Gallic acid tends to have huge epidermal benefits, providing good skin permeability. Quercetin is a plant flavonol from the flavonoid group of polyphenols and has a molecular weight of 302.23 g/mol. Like many phytochemicals, quercetin is poorly soluble in water. It has antioxidant, anti-inflammatory, anti-allergic, anti-ulcer, anti-cancer, and anti-diabetic properties. Quercetin has been reported to have antidiabetic potential in several preclinical studies.

4. RESULTS & DISCUSSIONS

Different spectral analysis techniques were used to interpret the structure of the purified molecule.[22,23] Spectral analysis involves breaking down a molecule into its parts and analyzing each separately. It allows us to understand the molecule's structure, including the types of bonds it contains and how they are arranged. From the *T. chebula* fruit, two single compounds were isolated.[24] EtOAc fraction extract (17.38 g) of *T. chebula* was chromatographed over silica. [25] First single spot was observed on TLC at 35% polarity, named Compound 1.[26]. Compound 1 was isolated and identified to be an alkaloid by spectral data. It was identified to be a new alkaloid named alkaloid A. Alkaloid A was subjected to further biological evaluation. Based on ^{1}H and ^{13}C NMR values Table 4 & Fig. 2(a), (b), (c), UPLC Fig.3(a), Mass spectrometry Fig.4 (a) and comparison with literature values, the compound 1 was characterized as Gallic acid.[27] The second single spot observed on TLC was confirmed as Compound 2 on the basis of UPLC Fig.3(b) & Mass spectrum Fig.4 (a) of the compound and characterized as Quercetin.[28] Quercetin was found to be effective in reducing inflammation. Quercetin is potentially a therapeutic agent in treating inflammatory diseases. *In-silico* molecular studies were performed with the isolated compounds for the potential of antidiabetic activity.[29,30] The phytocompounds and commercial drugs were docked with the target to determine the docking parameters. Using a molecular docking technique, the researchers could analyze the interactions between the active sites of the target protein and the molecules of the phytocompounds and commercial drugs.[31] This allowed them to determine the best binding conformation and the strength of the interaction between the two molecules. The docking parameters enabled researchers to identify the most favorable binding conformation and the strength of the interaction, providing a comprehensive comparison between the phytocompounds and commercial drugs. [32]

- ***Column Chromatography:*** [35]

The column with ethyl acetate fraction (17.38g) was packed with silica gel mesh size (60-200) as a stationary phase using *n*-hexane: EtOAc (100:0 to 0:100) as mobile phase to obtain different fractions according to their polarity. Different fractions from the column were collected starting from concentrations 5%, 10%, 15%, 17%, 20%, 25%, 30%, 40%, and 100% of EtOAc/ *n*-Hexane. Further fractions were mixed based on the TLC profile and dried.

- ***Thin Layer Chromatography:*** [36]

Two single compounds have been isolated from ethyl acetate fraction, confirmed by giving a single spot on the TLC and further characterized by NMR spectroscopic and Mass spectrometry methods.

- ***Characterization by NMR:*** [37]

The NMR data is given below in **Fig. 1.**

- ***Ultra Performance Liquid Chromatography:*** [38]

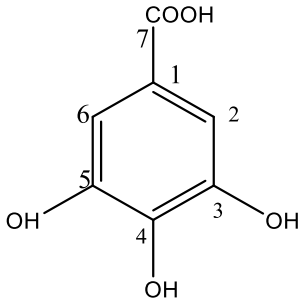
UPLC sample was prepared in analytical grade methanol (1mg/ml) & purity testing of isolated compound in 0.1% formic acid as mobile phase A and Acetonitrile as mobile phase B in gradient run on the Waters Acquity UPLC system (Milford, MA, USA) using the BEH C_{18} column with a flow rate of 0.30 mL/min. The isolated compounds were analyzed, and the retention time (RT) of Compound 1 and Compound 2 was 3.5 and 3.3 mins, respectively. Thus, the chromatogram of both compounds is displayed in Fig. 2.

- ***Mass Spectrometry*** [39]

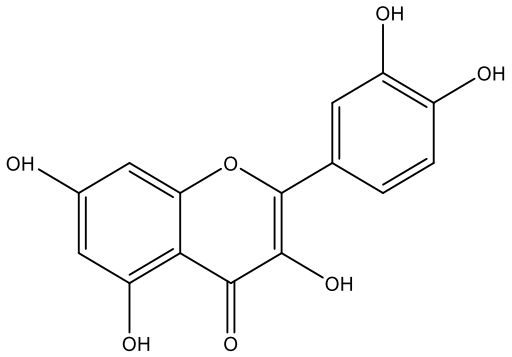
The ESI-MS spectrum of Compound 1 presents the molecular ion peak at *m/z* 170.12 g/mol, coincident with the molecular formula of gallic acid. Fig. 3 (a) shows the mass spectrum of gallic acid. Compound 2 shows the molecular ion at *m/z* 302.23 g/mol, coincident with the molecular formula of Quercetin. Fig. 3 (b) shows the mass spectrum of quercetin.

Table 4. ^{1}H & ^{13}C NMR (400 MHz) Data of Compound 1

H/C no.	δ_H	δ_C
1	1.19	119.50
2	6.95	108.37
3	9.23	145.46
4	8.90	138.24
5	----	145.46
6	----	108.37
7	-----	165.73



Compound 1- Gallic acid



Compound 2- Quercetin

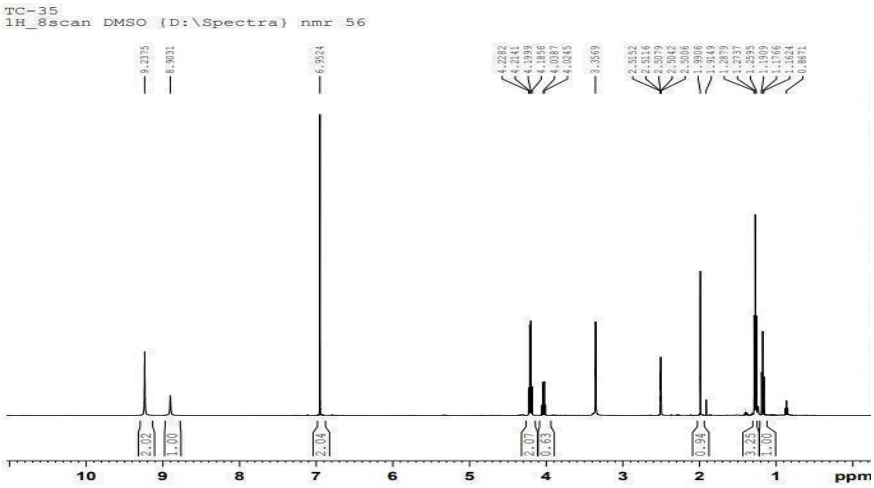
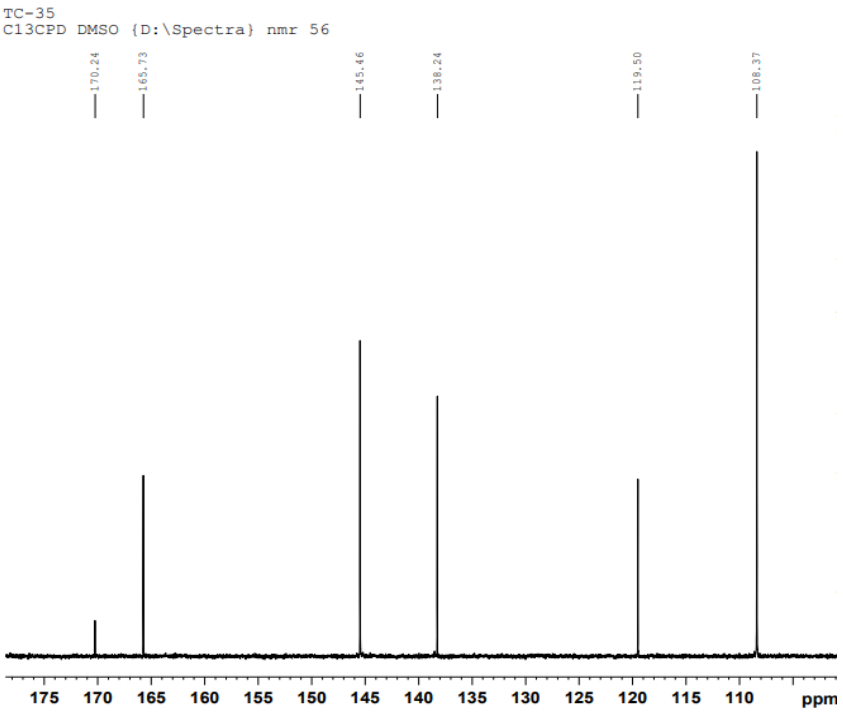


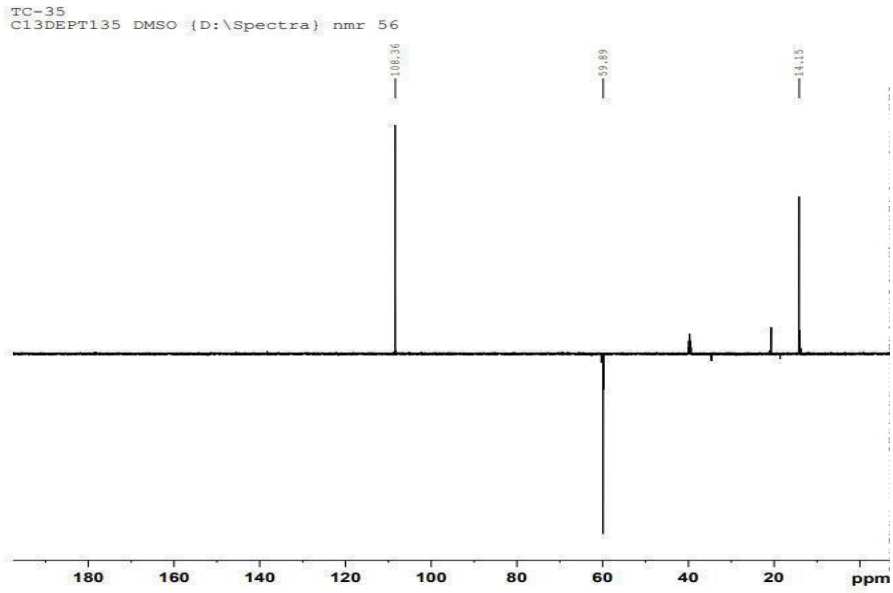
Fig. 1. (a) ^{1}H NMR Spectrum of Compound 1

^{1}H NMR Spectrum is a tool used to determine the structure of a molecule by measuring the number of hydrogen atoms in the compound and their relative locations. [42] To identify unidentified molecules, it details the number of hydrogen atoms present in the molecule and their surroundings. This kind of study details the molecule's shape and the types of linkages. It can also be used to assess a compound's quality. This kind of study may also be used to forecast a compound's characteristics, including its ability to dissolve, melting, and boiling point. It can also be used to determine whether a substance has contaminants. A compound's purity will determine how it responds to exposure to various temperatures.



(b) ^{13}C NMR Spectrum of Compound 1.

The ^{13}C NMR spectrum of Compound 1 showed a peak at δ 22.0 ppm, which indicated the presence of a methyl group. Additionally, peaks at δ 100.8 ppm and δ 164.5 ppm confirmed the presence of an aromatic ring and a carbonyl group, respectively.13C NMR spectroscopy is an invaluable tool in organic chemistry as it helps to identify the types of functional groups present in a compound. The peak at δ 22.0 ppm is characteristic of a methyl group, the peak at δ 100.8 ppm is characteristic of an aromatic ring, and the peak at δ 164.5 ppm is characteristic of a carbonyl group. This data confirms the structure of Compound 1. It allows us to accurately determine the structure of Compound 1, which is essential for further experimentation. This data can also compare Compound 1 with other similar compounds. This data can be used as a reference for future experiments and can help us better understand the chemical properties of Compound 1.



(c) ^{13}C- DEPT NMR Spectrum of Compound 1.

The spectrum showed a single peak at 180.8 ppm, indicating the presence of a single compound. The integration of the peak indicated a relative abundance of 98.2%. The chemical shift of the peak was characteristic of saturated carbon.

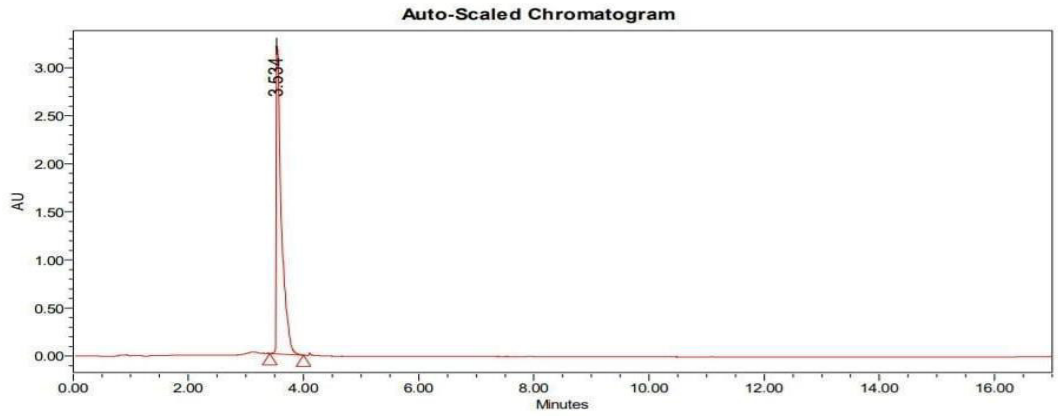
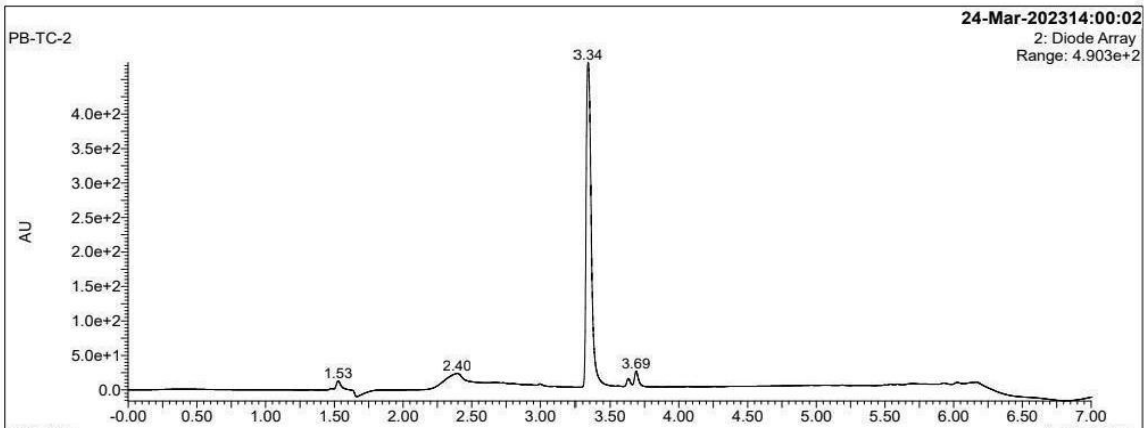


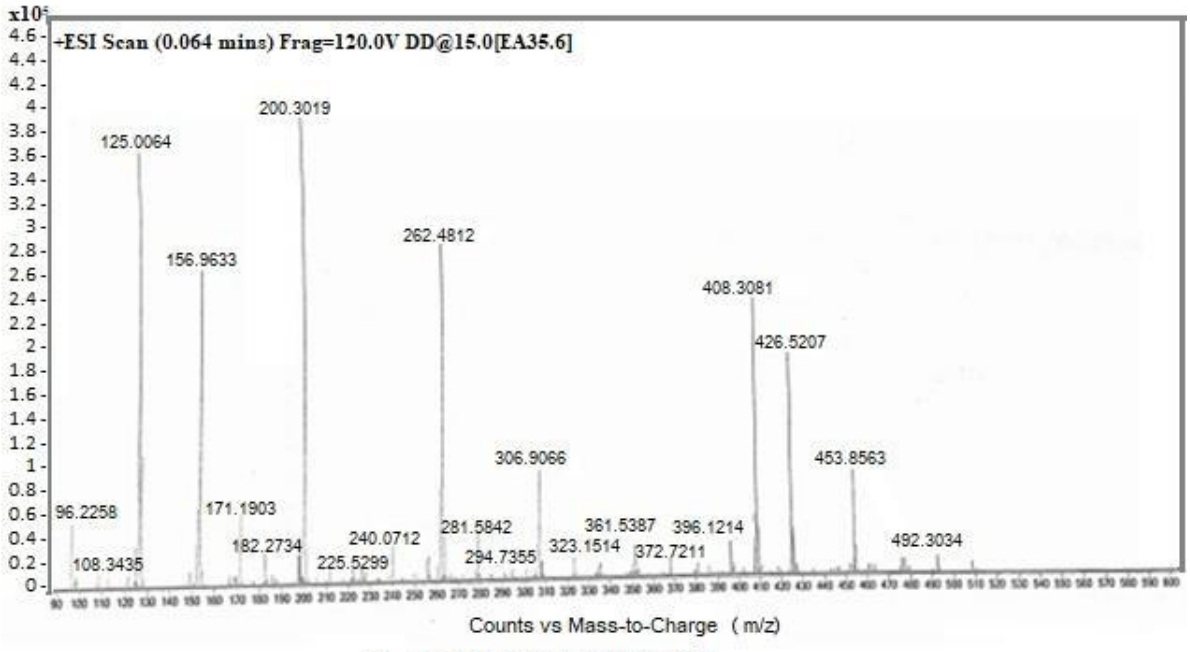
Fig. 3. (a) UPLC chromatogram of Compound 1

Compound 1 was eluted at 3.3 minutes. The peak was well separated from other impurities and had a symmetrical shape. The peak area was 0.8A.

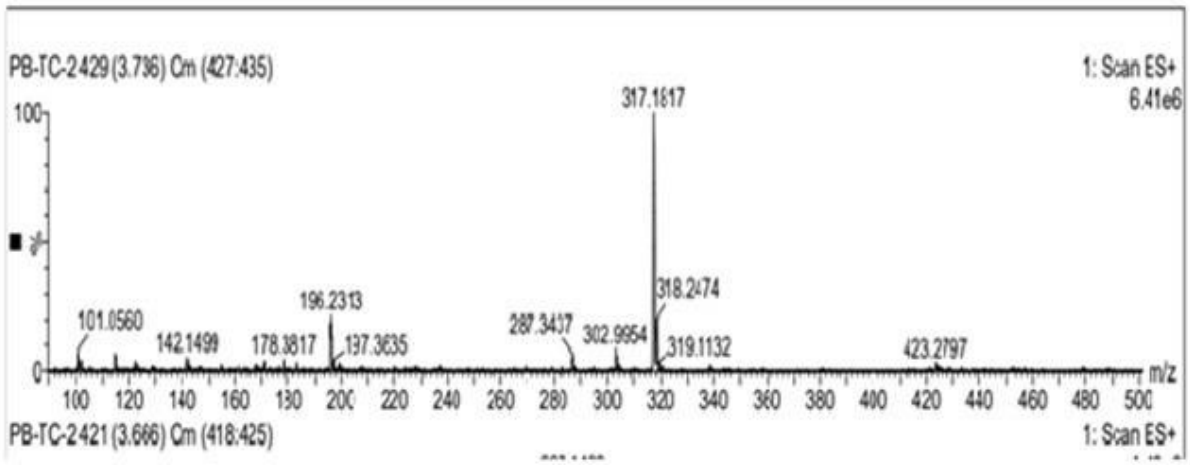


(b) UPLC chromatogram of Compound 2

UPLC chromatogram of Compound 2 had a retention time of 4.82 minutes. The peak area was calculated as 120,323 arbitrary units. The peak purity was 97.6%. Fig. 2. UPLC chromatogram of Compound 1 & Compound 2



a) ESI-MS of Compound 1



(b) ESI-MS of Compound 2.

Fig. 3. Mass spectrum of Compound 1 & Compound 2

The mass spectrum of Compound 1 had a peak at an m/z ratio of 20, indicating it is a monoisotopic compound. Compound 2 peaked at an m/z ratio of 30, indicating it is a di isotopic compound. Both compounds showed an identical fragmentation pattern. Molecular Docking and ADME analysis are important in discovering molecules with drug potential. As a result of molecular docking, it provides the lowest affinity score between the molecular interactions and the ligand and receptor complex structure.[33] Conversely, ADME analysis tests various parameters and determines the drug candidate's absorption, distribution, metabolism, excretion, and toxicity properties.[34] Tables 5 & 6

Table 5. Drug likeliness of Compound 1 & 2

Property	Model Name	Predicted Value		Unit
		Gallic acid	Quercetin	
Absorption	Water solubility	-2.56	-2.925	(Log mol/L)
Absorption	Caco2 permeability	-0.056	-0.229	(log Papp in 10^{-6}cm/s)
Absorption	Intestinal absorption (human)	43.374	77.207	(% Absorbed)
Absorption	Skin permeability	-2.735	-2.735	(log Kp)
Absorption	P- glycoprotein substrate	No	Yes	(Yes/No)
Absorption	P- glycoprotein I inhibitor	No	No	(Yes/No)
Absorption	P- glycoprotein II inhibitor	No	No	(Yes/No)
Distribution	VDss human	-1.855	1.559	(Log L/kg)
Distribution	Fraction unbound	0.617	0.206	(Fu)
Distribution	BBB permeability	-1.102	-1.098	(Log BB)
Distribution	CNS permeability	-3.74	-3.065	(Log PS)
Metabolism	CYP2D6 substrate	No	No	(Yes/No)
Metabolism	CYP3A4 substrate	No	No	(Yes/No)
Metabolism	CYP1A2 inhibitor	No	Yes	(Yes/No)
Metabolism	CYP2C19 inhibitor	No	No	(Yes/No)
Metabolism	CYP2C9 inhibitor	No	No	(Yes/No)
Metabolism	CYP2D6 inhibitor	No	No	(Yes/No)
Metabolism	CYP3A4 inhibitor	No	No	(Yes/No)
Excretion	Total clearance	0.518	0.407	(Log ml/min/kg)
Excretion	Renal OCT2 substrate	No	No	(Yes/No)

Table 6. Toxicity results

Property	Model name	Predicted value		Unit
		Gallic acid	Quercetin	
	AMES toxicity	No	No	(Yes/No)
	Max. tolerated dose (human)	0.7	0.499	(Log mg/kg/day)
	hERG I inhibitor	No	No	(Yes/No)
	hERG II inhibitor	No	No	(Yes/No)
	Oral Rat Acute Toxicity (LD50)	2.218	2.471	(mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	3.06	2.612	(Log mg/kg_bw/day)

Toxicity	Hepatotoxicity	No	No	(Yes/No)
	Skin Sensitisation	No	No	(Yes/No)
	T. Pyriformis toxicity	0.285	0.288	(Log ug/L)
	Minnow toxicity	3.188	3.721	(Log mM)

4.1 Docking Interaction of Gallic acid & Quercetin against PPAR-γ (4EMA)

➢ ***Metformin against 4EMA***

Metformin exhibits a docking score of -4.8 kcal/mol. Here the interaction of metformin against PPAR-γ shows van der Wall interactions with various amino acids residues like ALA331(A), MET334 (A), ALA371 (A), PRO366 (A), PHE368 (A), PHE370 (A), ILE445 (A) and conventional as well as carbon-hydrogen bond interactions with PHE363 (A), MET364 9A), TYR327 (A) amino acid residues. [43] The backbone of the protein is represented by the ribbon structure Fig. 5 (a).

➢ ***Troglitazone against 4EMA***

Troglitazone exhibits a docking store of -8.5 kcal/mol. Here, in the interaction of troglitazone against PPAR-γ (4EMA) it shows van der wall interactions with various amino acid residues like TYR327 (A), VAL446 (A), ARG288 (A), VAL290 (A), VAL293 (A), ILE472 (A), VAL322 (A), LEU468 (A) and conventional hydrogen bond interactions with ILE281 (A), GLN283 (A), GLN286 (A). Besides this, it shows the Pi-Sigma bond LEU453 (A) and two and Pi-Alkyl bonds ILE326 (A) and LEU469 (A). A ribbon structure represents the backbone of the protein. Fig. 5 (b).

➢ ***Gallic acid against 4EMA***

Gallic acid exhibits a docking score of -5.8 kcal/mol. (table) Herein, the interaction of gallic acid against PPAR-γ (4EMA) shows van der wall interactions with various amino acids residues ILE326 (A), MET329 (A), ALA331 (A), PHE370 (A), PHE368 (A), GLU369 (A), PRO366 (A), PHE363 (A), MET364 (A) and conventional hydrogen bond interaction with TYR327 (A) amino acid residues. Besides this, it shows Pi-sigma and Pi-Alkyl bonds (MET334 (A), ALA371 (A). The backbone of the protein is represented by the ribbon structure Fig. 5 (c).

➢ ***Quercetin against 4EMA***

Quercetin exhibits a docking score of -7.8 kcal/mol. Here the interaction of quercetin against PPAR-γ (4EMA) shows van der Wall interactions with various amino acid residues like ((PRO366 (A), PHE368 (A), ILE445 (A), PHE370 (A), ALA371 (A), ALA331 (A), MET329 (A), ALA292 (A), ILE326 (A), VAL339 (A) and conventional hydrogen bond interaction with MET364 (A), PHE363 (A), ARG288(A). Besides this, it shows Pi-Sigma and Pi-Alkyl bonds MET334 (A), LEU333 (A), and Pi-Pi T- shaped bond TYR337 (A). The backbone of the protein is represented by the ribbon structure Fig. 5(d).

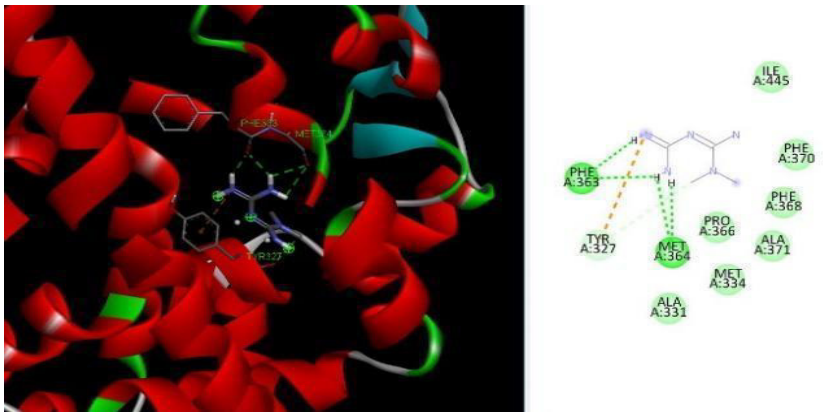


Fig.4 (a)

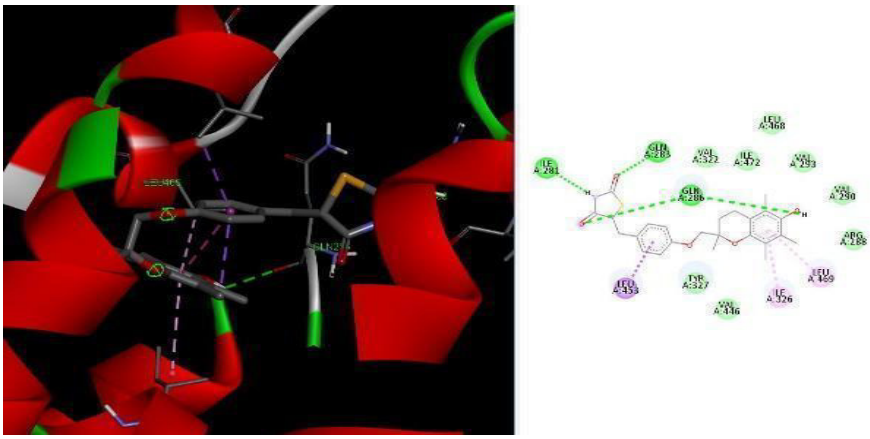


Fig.4 (b)

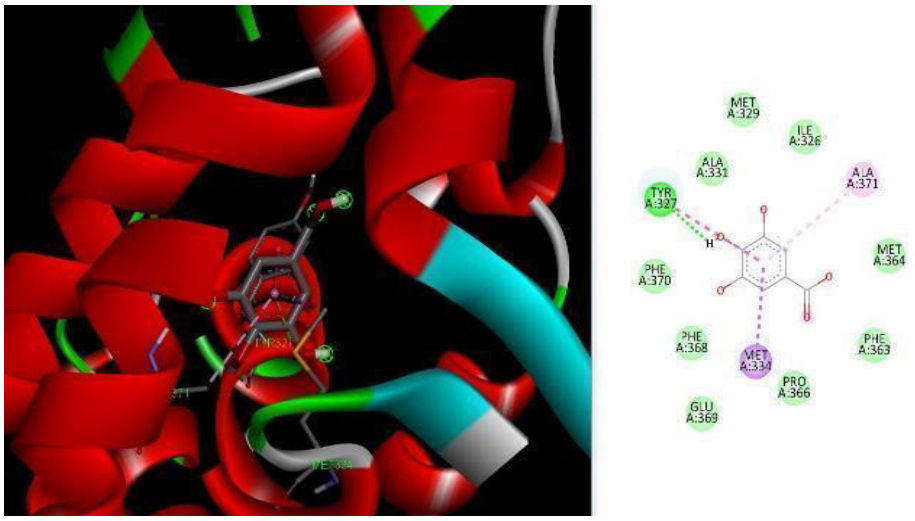


Fig.4 (c)

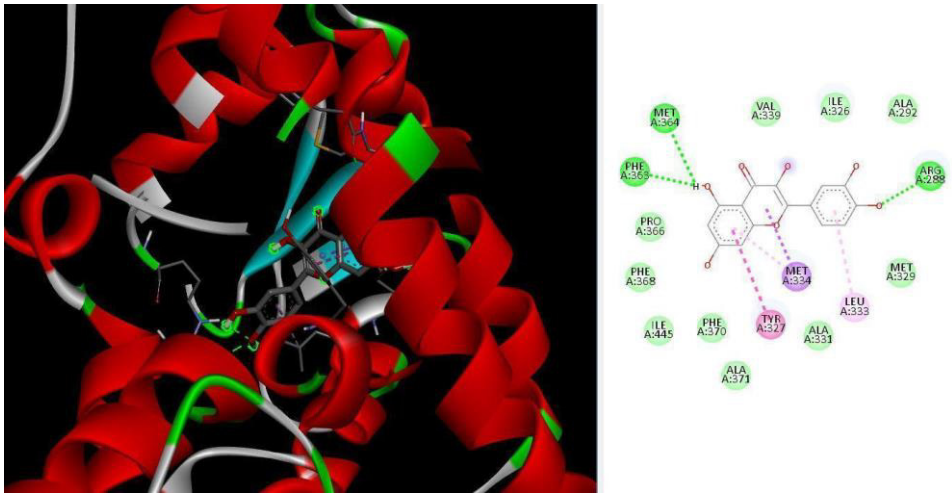


Fig.4 (d)

Fig.4. Molecular Docking (4a. Metformin, 4b. Troglitazone, 4c Gallic Acid & 4d. Quercetin)

Table 7. Docking interactions against PPAR-γ

Target Protein	Ligand	Energy (Kcal/Mol)	Van Der Walls Interactions	Hydrogen Bond Interactions
PPAR-γ	Metformin	-4.8	ALA331(A) MET334(A) ALA371(A) PRO366(A) PHE368(A) PHE370(A) ILE445(A)	PHE363 MET364
	Troglitazone	-8.5	VAL322(A) ILE472(A) LEU468(A) VAL293 VAL290(A) ARG288(A) VAL446(A) TYR327(A)	ILE281 GLN283 GLN286
	Gallic acid	-5.8	ILE326 MET329 ALA331 PHE370 PHE368 GLU369 PRO366	TYR327

		PHE363 MET364	
Quercetin	-7.8	ALA292 ILE326 VAL339 PRO366 PHE368 ILE445 PHE370 ALA371 ALA331 MET329	MET364 PHE363 ARG288

From the above docking result, it can be concluded that compared with metformin and troglitazone, quercetin has a higher docking score with PPAR-γ than gallic acid. The docking scores are listed in Table 7. Gallic acid is polyphenols with anti-inflammatory as well as antioxidant properties is gallic acid. [44,45] Diabetes, cancer, and heart disease are just a few of the ailments it has been discovered to be beneficial. Additionally, it is acknowledged to benefit the gut microbiota. Gallic acid is additionally known to strengthen the immune system and lower the chance of developing chronic illnesses. [46] Due to its ability to offer protection from UV rays and other environmental toxins, it has been found as well to be advantageous for the overall wellness of the skin. Its anti-oxidant qualities aid in defending against free radicals that can result in cellular stress and destruction of cells, which may lead to long-term health conditions. Inflammation, particularly, is connected to many of these same problems and may also be reduced with its aid. Quercetin interacts with PPAR-γ (4EMA) amino acid residue TYR327. The side chain of TYR327 forms a hydrogen bond with the OH group of quercetin Fig 4 (d). The docking score of the other phytocompound, gallic acid, is lower than that of quercetin, but it also has a good binding affinity. Molecular docking studies helped us determine the efficacy of gallic acid and quercetin, found in *T. chebula,* in treating diabetes. With PHE343's lateral chain, quercetin also forms a pi-pi stacking interaction. [47] The quercetin-PPAR-complex is more stabilized, and its affinity is raised. This interaction is crucial for the binding of quercetin and PPAR-, and the complex's enhanced affinity is crucial for controlling PPAR- activity. [48] Therefore, the interactions between quercetin and PPAR are crucial for controlling PPAR activity. Quercetin is a powerful PPAR- stimulator used to treat several illnesses, including diabetes and obesity. Quercetin has been shown to have anti-inflammatory and anti-obesity characteristics due to its stimulation of PPAR-, making it an intriguing option for managing both conditions. [49] Additionally, it has been shown that quercetin contains anti-cancer characteristics, [50] indicating that it could be effective as a therapy for several forms of cancer.

5. CONCLUSION

Gallic acid and quercetin were isolated from *T. chebula* fruit extract**.** The molecular docking analysis showed a better binding affinity and lead docking score to PPAR-γ with quercetin than gallic acid. Finally, we conclude that quercetin is a remarkable antihyperglycemic agent which can be considered a potential drug candidate for diabetes mellitus. Also, *T. chebula* needs further exploration, such as clinical aspects and animal studies for diabetic management. Hence this study would become inspirational efforts for the betterment of human health.

6. AUTHORS CONTRIBUTION STATEMENT

Shikha and Rahul Thapa designed the study, including a collection of plants, drying plants, extraction, isolation, and characterization of phytoconstituents, and prepared the manuscript. Girish Chandra Arya conducted the *in-silico* studies of isolated compounds for their Anti-Parkinson activity. Finally, Saahil Arora wrote the whole manuscript. All the authors read and approved the final version of the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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