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Characterization, Antioxidant Potential, and *In Silico* Study of *Ruellia Tuberosa* Hydroethanol Extract of Leaf

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ABSTRACT

This study was conducted to evaluate the phytochemical profile, alpha-amylase inhibitory potential, and *in vitro* antioxidant activity of the hydro-ethanolic [70:30] extract from Ruellia tuberosa leaves, along with molecular docking with catalase (7VD9), PPARγ (2P4Y), and AChE (4PQE) enzymes. Fresh leaves were collected, purified, and air-dried prior to extraction with hydroethanol to obtain crude extracts that were rich in secondary metabolites. Qualitative phytochemical screening has revealed the presence of flavonoids, phenolic acids, tannins, and other beneficial compounds. LC-MS analysis provided a comprehensive profile of these compounds, identifying numerous peaks corresponding to established anti-diabetic medications and antioxidants. The antioxidant capacity of the extract was evaluated by DPPH, ABTS, nitric oxide radical scavenging, and hydroxyl free radical assays with IC_{50} values at the concentration of 70, 305, 350, and 155 µg/ml, respectively, demonstrating a substantial dose-dependent capacity for free radical scavenging. The hydroethanol extract demonstrated significant alpha amylase inhibitory activity (IC_{50} 44±0.18 µg/mL), indicating its potential to regulate glucose metabolism and aid in the management of postprandial hyperglycemia. The interactions among the identified phytochemicals may explain the observed anti-diabetic and antioxidant effects. These findings validate the traditional application of R. tuberosa in addressing metabolic diseases and oxidative stress, while highlighting the potential of the plant as a source of natural therapeutic agents. The molecular docking study of the RTL extract revealed the strong interactions of its key phytochemicals, catechin, amyrin, and kaempferol, with the target proteins catalase, PPARγ, and AChE. These compounds exhibited significant binding affinities, suggesting potential therapeutic activities. These *in silico* findings support the bioactivity observed in in in-vitro assays and provide a basis for further pharmacological research. Additional studies, including isolation of specific compounds and *in vivo* testing, are necessary to elucidate the mechanisms of action and inform the development of novel pharmacological and nutraceutical applications.

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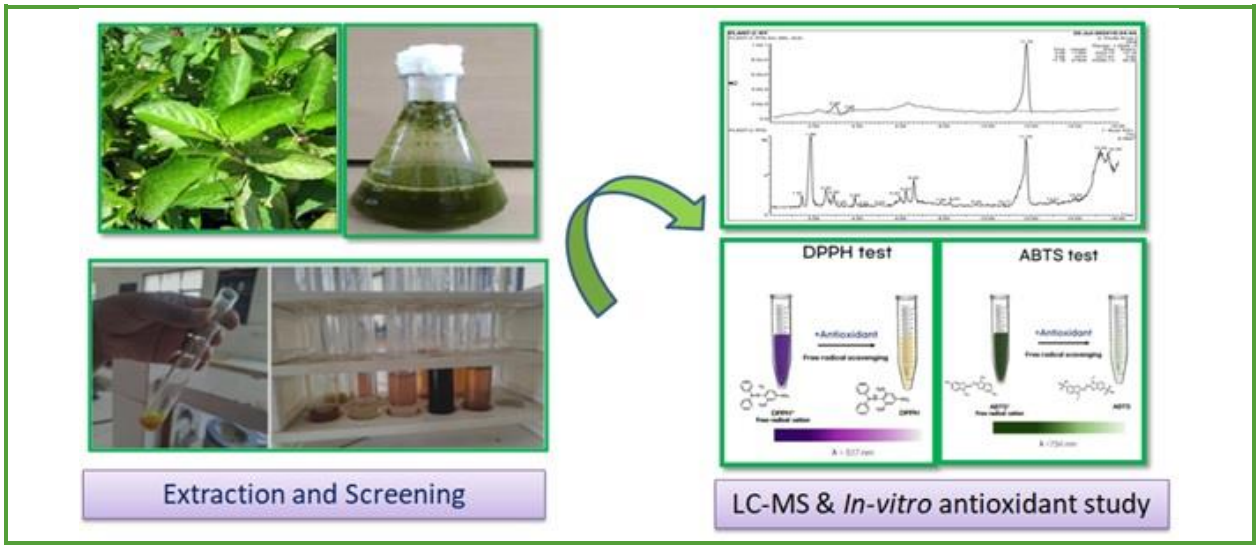
Graphical Abstract



Introduction

The field of pharmacognosy focuses on research and development of new medications by studying their physical, chemical, biological, and biochemical properties. Pharmaceutical analysis assists in the screening of commercial versions, substitutes, adulterants, and other issues related to medicine quality control. The physical and biological properties of crude drugs can be determined using this user-friendly and reliable tool [1-4].

Alternative medicine has been slow to gain traction in affluent countries due to rigid quality control regulations and a lack of evidence supporting its use. Therefore, it is essential to conduct research on traditional medicines. Given this drawback, it is of utmost importance to ensure that medicinal plants and their components are standardized uniformly. Unbalanced redox activity in human tissue cells produces free radicals, also known as reactive oxygen species (ROS), including superoxide, peroxide, hydroxyl radicals, nitric oxide, and singlet oxygen. Lifestyle choices and dietary habits may also contribute to ROS overproduction. High levels of ROS in the body can oxidize biomolecules, leading to a wide range of endogenous diseases. A healthy metabolic process can be supported by antioxidants, which prevent the ROS detoxification chains. Most cells have a basic system to remove ROS generated by metabolism to prevent damage caused by oxygen free radicals [5-7].

Superoxide dismutase (SOD), an enzyme naturally found in the body, plays a crucial role in these processes by controlling the amount of ROS that harm cells. The enzyme converts superoxide ($O_2^{\bullet}$) to hydrogen peroxide (H_2O_2), which is then converted to harmless water (H_2O). *Ruellia* is the collective name of 250 species of flowering plants that comprise the genus. This genus is also found in tropical countries. Several traditional medicinal plants have been used to treat gonorrhea, fever, asthma, and bronchitis. Among these, *Ruellia tuberosa* L. It is believed to be native to South America. This plant grows throughout Indonesia, but primarily in damp areas. The biological activities and medicinal qualities of the plant, such as antibacterial, antioxidant, anti-hyperlipidemic, anti-nociceptive, anti-inflammatory, anti-anthelmintic, and anti-

carcinogenic properties, are well known. Studies of the phytochemical components of plants have revealed the presence of phenolic compounds, alkaloids, triterpenoids, flavonoids, sterols, and saponins. Some isolated flavonoids have been identified in this plant [8-10].

The maceration technique was used to extract the leaves, and the extracted components were subsequently identified and explained. Since bioactive substances such terpenoids, flavonoids, alkaloids, saponins, and tannins are secondary metabolites and typically dissolve in polar solvents, ethanol and water were utilized in this work to extract *Ruellia tuberose* (RT). The phytochemical screening approach was used to characterize hydroethanolic extracts and to identify the secondary metabolites of the extracts. The components were also characterized using LC-MS. Because of the biological roles of *R. tuberosa L*. in the past, a free radical scavenging assay was employed to assess the potential antioxidant activity of hydroethanolic extracts.

Experimental

Materials

All of the analytical-grade chemicals and solvents came from HiMedia Chemicals, based in Mumbai, India, From Sigma Chemicals in the United States, we bought 2,2-azinobis (3-ethylbenzo-thiazoline-6-sulphonate) (ABTS). The other chemicals employed were 1,1-diphenyl, 2-picrylhydrazyl (DPPH), sodium nitroprusside, sulfanilamide, o-phosphoric acid, naphthyl ethylene diamine dihydrochloride, ferrous sulfate ($FeSO_4$), thiobarbituric acid (TBA), trichloroacetic acid (TCA), nitroblue tetrazolium (NBT), and ethylenediaminetetraacetic acid (EDTA). The plant component of *Ruellia tuberosa* leaves (RTL) were collected from several Yadadri bhuvanagiri and Telangana habitats during December 2023. Dr. Madhava Chetty, professor at Sri Venkateswara University, Tirupati, A.P., certifies them.

Methodology

Plant extract preparation

Approximately 1500 g of RTL was pulverized, shade-dried, and completely extracted using cold maceration and 95% ethanol. The residue was concentrated after filtering until it became stable. The extract was stored in a desiccator until required. Sometimes referred to as menstruum, the liquid used to extract medicinal compounds, solvent choice is affected by the type of plant, the specific section of the plant being extracted, the characteristics of the bioactive components, and the availability of the solvent [11].

While nonpolar compounds are extracted using nonpolar solvents such as hexane and ethyl acetate, polar molecules are typically extracted using polar solvents such as methanol, ethanol, and water. Moreover, the polarity of the extraction solvents is noted; n-hexane is the least polar, while water is the most polar.

Phytochemical analysis

Preliminary screening of phytochemicals, including alkaloids, carbohydrates, glycosides, saponins, proteins, phytosterols, terpenoids, fixed oils, phenolic compounds, flavonoids, and tannins, was performed using preliminary qualitative analysis of the stem extract [12,13].

In vitro study

α-amylase activity

The *in vitro* evaluation of alpha-amylase activity involves enzyme-mediated hydrolysis of starch. This method can be assessed using the

DNSA (3,5-dinitrosalicylic acid) reagent, which produces a red coloration upon reacting with maltose, a reducing sugar derived from starch hydrolysis. The intensity of the red hue indicates the extent to which starch is hydrolyzed into maltose by enzymes. An extract demonstrating alpha-amylase inhibition reduced the intensity of redness. The intensity of the red hue is inversely related to the inhibitory action of alpha-amylase [14].

Spectrophotometric studies

DPPH radical scavenging activity

DPPH scavenging activity was measured using a spectrophotometer. Test chemicals dissolved in ethanol (0.05 ml) were added to DPPH (200µM) methanolic solutions at various dosages (100–500 µg/ml). The control group was administered the same volume of ethanol. Quenching of the DPPH free radicals caused the absorbance of the test mixture to decrease at 517 nm after 20 min; the formula was applied to calculate the inhibition percentage [14].

$$inhibition(\%) = \frac{\text{Control} - \text{Test}}{\text{test}} * 100$$

ABTS radical cation decolorisation assay

In this improved variant, the ABTS. -, persulfate oxidation of 2,2´, -azinobis (3-ethylbenzothiazoline-6-sulfonic acid) generates an oxidant. ABTS radical cations (ABTS. +) with 2.45 mM ammonium persulfate were added to ABTS solution (7 mM). The mixture was allowed to sit in the dark at room temperature for 12-16 h before use. The final quantity was 1 ml, made by filling up with ethanol. For the study, 0.3 ml of ABTS solution was combined with 0.5 ml of ethanolic extract at different concentrations (100-500 µg/ml). The absorbance was recorded at 745 nm, and the % inhibition was calculated [15].

Scavenging of nitric oxide radical

The Griess reaction 18, 19 was used to quantify sodium nitroprusside converted to nitric oxide. Sodium nitroprusside (5 mM) in a standard phosphate buffer solution was incubated with ethanol extract diluted in phosphate buffer (0.025 M, pH 7.4) at various doses (100-500 µg/ml) for five hours at 25 °C. The control tests were conducted in the same manner, but without the test substances, similar quantities of buffer were used. Five hours later, the incubation solution (0.5 mL) was removed and combined with Griess reagent (0.1% naphthylethylene diamine dihydrochloride, 2% o-phosphoric acid, and 1% sulfanilamide). The absorbance of the chromophore produced when nitrite was diazotized with sulfanilamide and subsequently coupled with naphthylethylenediamine was measured at 546 nm. The experiment was performed three times [16,17].

Scavenging of hydroxyl radical

The hydroxyl radical scavenging activity was evaluated using the Kunch and Y and Rao methods by examining the competition between deoxyribose and the test extract for the hydroxyl radicals produced by Fenton's reaction. The final volume of 1.0 ml, The reaction mixture included deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbate (0.1 mM), KH_2PO_4-KOH buffer (20 mM, pH 7.4), and various amounts of the sample extracts. One hour at 37 °C was spent incubating the reaction mixture. Thiobarbituric acid reactive substances (TBARS) were used to assess deoxyribose degradation, and the percentage of inhibition was then computed [18].

LCMS analysis of hydroalcoholic extracts of ruellia tuberosa leaves

HERT LCMS was obtained from a Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Powai, Mumbai. Chemical fingerprints of HERT were generated using Agilent's high-resolution liquid chromatography and mass spectrometry system. Their unique mass fragmentation patterns and mass spectra were used to identify the chemicals [19].

Molecular docking study

The crystal structure of the cholinesterase enzyme was obtained from RCSB PDB (rcsb.org) Catalase, PPRγ, and AChE. Swiss PDB Viewer and PyMOL were used to prepare the proteins. Based on the LC-MS study, six RTL compounds were subjected to a molecular docking investigation. The structures were drawn using the Chem3D and ChemDraw software. The geometry of all structures was optimized using Gaussian 09's semi-empirical PM6 method. The optimized structures obtained in the PDB format were used for molecular docking analysis. Using the 'PyRx Virtual Screening Tool (version 0.8), all molecules were docked to AChE and BChE. The docking process was performed three times for each ligand, and the average binding affinities are reported. Our docking method was validated by docking the optimized protein with the recovered ligand from the crystal structure using the same technique. The docked ligand-protein complexes were examined in BIOVIA Discovery Studio (Version 4.5) to identify non-covalent interactions [20-23].

Results

The phytochemical study of the leaf extract with several solvents revealed in Table 1. It indicates that the hexane extract contains phytosterols and terpenoids, while chloroform contains only phytosterols, ethyl acetate glycosides, and terpenoids. The maximum phytochemical components, such as carbohydrates, glycosides, saponins, terpenoids, fixed oils, flavonoids, and tannins, are found in fixed oil phenolic compounds, flavonoids, and the methanol extract.

The percentage yields of leaf extracts in petroleum ether, chloroform, ethyl acetate, ethanol, and hydroalcohol were 1.8**%,** 1.6, 1.9, 1.6%, and 2.5%, respectively (Table 2).

In vitro study

Alpha amylase activity of various extracts of Ruellia tuberosa leaves

The percent inhibition of amylase activity was found to be higher in the hydroalcoholic extract than in petroleum ether, chloroform, ethyl acetate, and ethanol extracts. The IC_{50} value of hydroalcoholic extract were 44±0.18 µg/mL, while the values for petroleum ether, chloroform, ethanol, and ethyl acetate are 62±0.88, 69±2.1, 72±2.1, and 53±1.4 µg/mL respectively, as indicated in Table 3.

LC-MS of Hydroalcoholicleaves extract of RuelliaTuberosa

Twenty metabolite compounds were identified from the RT extract using LC-MS (Figure 1 and Table 4) based on mass accuracy and fragmentation patterns. These compounds belong to the phenolic classes of flavonoids, diterpenoids, sesquiterpenes, and triterpenes. Table 2 presents the retention times, masses, and names of the isolated compounds. Flavonoids exhibit potential preventive activity against oxidative cell damage as well as the ability to inhibit tumor induction, stimulation, and progression [24].

Table 1. Preliminary phytochemical analysis of *Ruellia Tuberosa* leaves

Chemicaltest	PetroleumetherExtract	ChloroformExtract	Ethyl acetateExtract	EthanolicExtract	HydroalcoholicExtract
Alkaloids					
Mayer's test	-	+	-	+	+
Dragandorff'stest	-	+	-	+	+
Hager'stest	-	+	-	+	+
Wagner'stest	-	+	-	+	+
Carbohydrates					
Molisch'stest	-	-	-	+	+
Glycosides					
Anthronetest	-	-	-	+	+
Borntrager'stest	-	-	-	+	+
Legal'stest	-	-	_	+	+
Proteins					
Biuret TestMillon'test	-	-	-	+	+
Xanthoproteictest	-	-	-	+	+
	-	-	-	+	+
Aminoacids					
Ninhydrintest	-	-	_	+	++
Saponins					
Foamtest	-	-	-	_	++
Flavanoids					
Shinodatest	-	-	++	++	++
Phenoliccompounds					
Ferricchloridetest	-	+	++	++	++
Leadacetate solutiontest	-	+	++	++	++
Tannins					
Ferric chloride test	-	-	++	++	++
Lead acetate test	-	-	++	++	++
Gelatinsolutiontest	-	-	++	++	++
Terpenoids					
Noller'sSalkowskiitest	+	+	+	+	+
Oils andfats					
Spottest	-	+	-	+	+
Steroids					
Liebermann'sBurchad test	+	+	+	-	+
Salkowskiitest	+	+	+	-	+

Table 2. Percentage yield of *RTL*

Sr./No.	Fractions	Yield of extract (gm)	Yield of extract (%w/w)
1	Petroleum ether	27	1.8
2	Chloroform	24	1.6
3	Ethyl acetate	28.5	1.9
4	Ethanol	24	1.6
5	Hydroalcohol	37.5	2.5

Table 3. Percent inhibition of α-amylase activity of RTL

Extract	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml	IC_{50} (µg/ml)
Petroleum ether	62±0.12	71±0.18	83±0.21	116±0.21	127±0.12	62±0.88
Chloroform	61±0.18	69±0.62	82±0.42	1104±0.30	125±0.46	69±2.1
Ethyl acetate	64±0.29	73±0.35	85±0.30	117±0.38	129±0.58	51±1.4
Ethanol	74±0.29	80±0.35	85±0.30	121±0.38	131±0.58	72±2.1
Hydroalcohol	65±0.15	75±0.23	86±0.54	119±0.088	132±0.12	44±0.18

Additionally, a group of terpenoids, known as andrographolide and boswellic acid, suppresses NO and iNOS production, along with pro-inflammatory mediators [25]. Hesperidin demonstrated anti-inflammatory effects in lipopolysaccharide-stimulated mouse macrophages (RAW264.7) in an *in vitro* edema model, inhibiting the protein expression of nitric oxide synthase and COX-2 [26].

Antioxidant study

Various doses between 100 and 500 µg/ml of the distinct extracts were evaluated for their antioxidant activities in multiple *in vitro* models. The test chemicals scavenged free radicals in a concentration-dependent manner in all models. Based on Tables 1-3, it was deduced that the RTL extracts exhibited maximal percentage inhibition of 88.77%, 90.05%, 72.60%, and 85.88% in the DPPH, ABTS, nitric oxide, and hydroxyl radical assays, respectively, at a concentration of 500 µg/ml. The IC50 values for DPPH, ABTS, nitric oxide, and hydroxyl radicals were 70, 305, 350, and 155 µg/ml, respectively, as provided in Table 5.

Molecular docking

Recent structure-based drug design has been successful in *in silico* molecular docking. LCMS research of leaf extract revealed that of the seven compounds chosen for screening based on the literature review, these molecules are less reported for the selected three enzymes, namely catalase, PPAR**γ**, and AChE. To study the antioxidant, anti-diabetic, and neuroprotective actions of these molecules in *in vivo*, molecular docking was performed with these three enzymes. Kaempferol, amyrin, and catechin exhibited the most potent inhibitory effects. Molecular docking revealed strong binding affinities between the seven selected compounds and catalase protein, PPAR gamma, and AChE. Table 6 shows the dock scores and Table 7 presents the 2D and 3D structures of AChE, catalase, and PPAR gamma. -7 to -9.9 and -7 to -8.4, respectively.

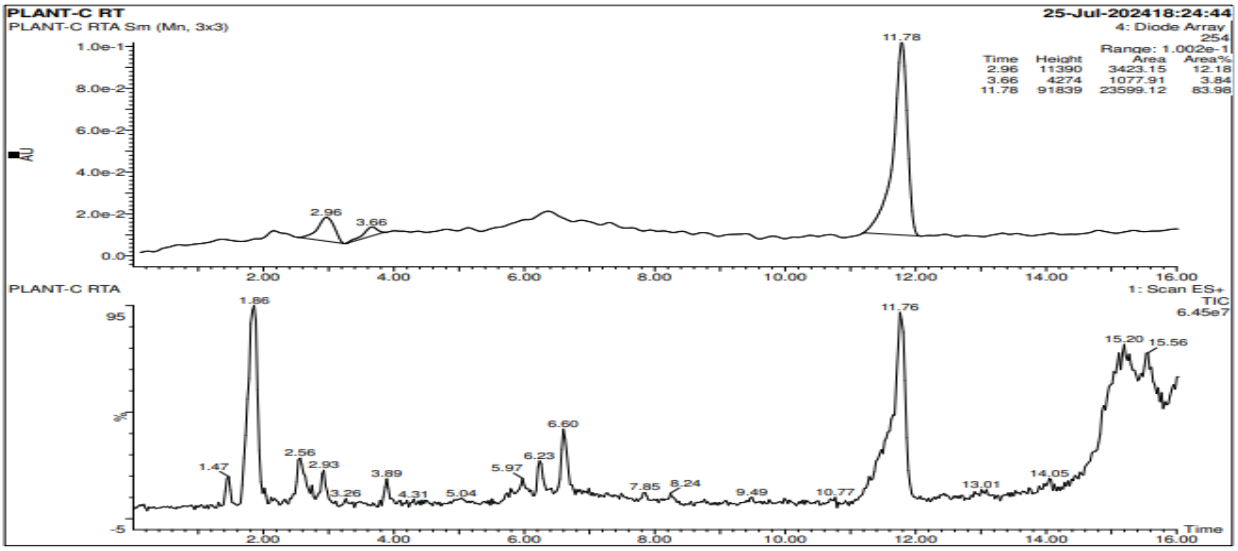


Figure 1. LC-MS of hydroalcoholic leaves extract of *Ruellia Tuberosa*

Table 4. LC-MS phytochemical screening of hydroalcoholic leaf extract of *RuelliaTuberosa*

RT (min)	Chemical Formula	Molecular Weight	Identified Compound	Nature of Compound
1.47	$C_{11}H_{13}O_6$	257.06	Piscidic acid	monocarboxylic acid
1.86	$C_8H_6O_4$	194	Phthalic acid	aromatic dicarboxylic acid
2.56	$C_{16}H_{18}O_9$	354.31	Isochloregenic acid	Polyphenolic acid
2.93	$C_{30}H_{50}O_2$	442.38	Betulin	Pentacyclictriterpenoid
3.26	$C_{21}H_{44}O$	312.58	1-hencicosonol	Fatty alcohol
3.89	$C_9H_{13}NO$	151.09	Cathine	MonoAminoalkaloid
4.31	$C_{30}H_{50}O$	426.72	Amyrin	Triterpenoid
5.04	$C_{30}H_{50}O$	426.72	Lupeol	Triterpenoid
5.97	$C_{10}H_{14}O_2$	166.22	4-(3-hydroxy butyl)phenol	Phenolic compound
6.23	$C_7H_6O_2$	126	1,2,3-benzoic acid	Phenolic compound
6.60	$C_5H_{12}O_3$	46.06	Ethanone,1-(3-ethyl oxiranyl)	volatile compound
7.85	$C_{28}H_{34}O_{15}$	610.56	Hesperidin	Flavanoid
8.24	$C_{10}H_{22}$	142.29	Decane	Saturated hydrocarbon
9.49	$C_{17}H_{32}O_2$	74	Hexadecanoic acid methyl ester	fatty acid
10.77	$C_{21}H_{36}O_2$	320.51	11,14,17-eicosatrienoic acid,methyl ester	polyunsaturated long-chain fatty acid
11.76	$C_{15}H_{10}O_6$	286.04	Kaempferol	Flavanoid
13.01	$C_{30}H_5O$	410.73	Squalene	Triterpene
14.5	$C_{18}H_{34}O$	323.20	13-octadecenol(z)	fatty acid
15.20	$C_{16}H_{34}$	226.44	2,6,10-trimethyl tridecane	sesquiterpenoid
15.26	$C_{14}H_{30}$	198.39	Tetradecane	fatty acid

Table 5. Effect of hydro-alcoholic extract of RTL on different antioxidant models

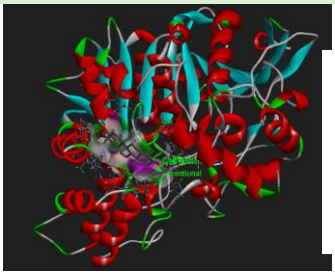
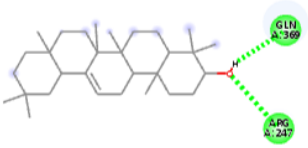
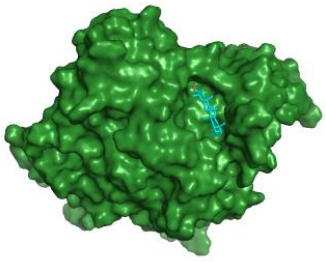
Conc. (µg/ml) Inhibition (%)	DPPH	ABTS	NitricOxide	Hydroxylradical
100	72.72	4.48	32.65	44.05
200	76.23	27.36	39.02	54.53
300	80.74	49.25	43.45	63.73
400	83.89	74.00	56.31	69.80
500	88.77	90.05	72.60	85.88
IC50(µg/ml)	70	305	350	155

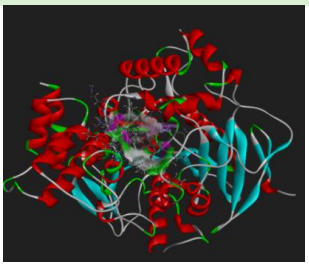
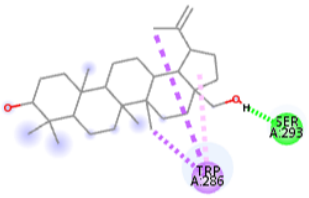
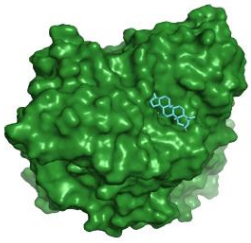
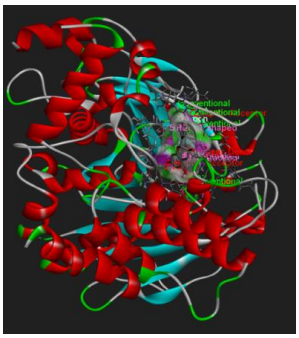
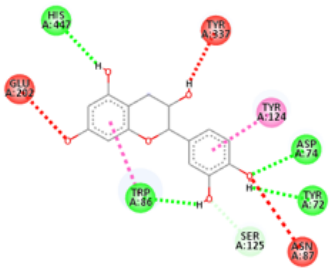
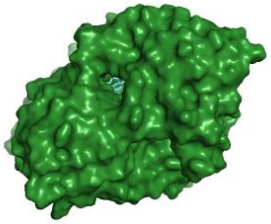
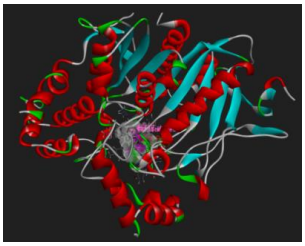
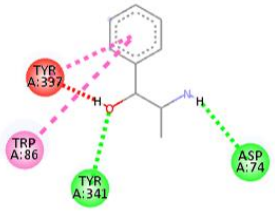
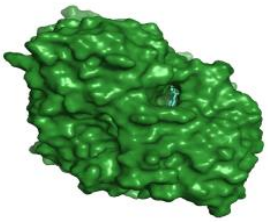
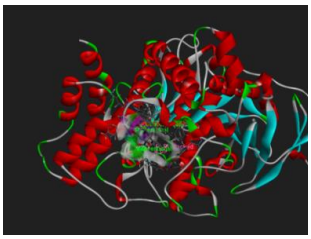
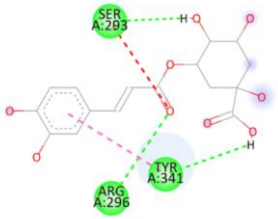
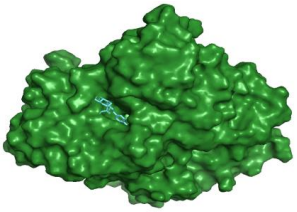
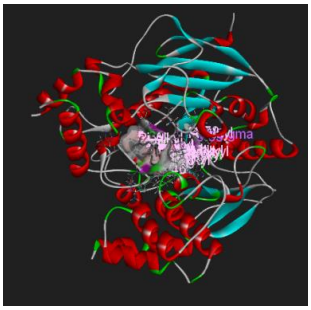
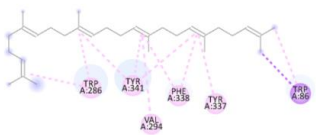
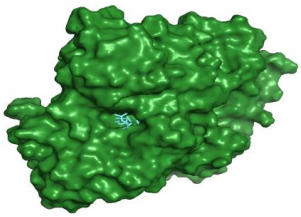
Values are the means of three replicates.

Table 6. Result of virtual screening by Swiss-similarity server

Phytomolecule	Dock Score[kcal/mol]					
	Catalase (7VD9)		PPARγ (2P4Y)		AChE (4PQE)	
	RTL	Native ligand and score	RTL	Native ligand and score	RTL	Native ligand and score
Catechin	-9.1	HEM group No specific score reported[1]	-7.4	Rosiglitazone -7.4 [2]	-9.9	Galantamine -7.8 [3]
Betulin	-8.8		-8.7		-8.8	
Cathine	-6.8		-5.7		-6.9	
Isochlorgenic acid	-7.3		-7.7		-8.4	
Amyrin	-9.9		-8.5		-9.4	
Kaempherol	-9.9		-7.6		-9.3	
Squalene	-7		-7		-9	

Table 7. 3D and 2D structures of AChE, catalase, and PPARγ

Compound Names	3D Structure	2D Structure	Enzyme and ligand interaction
	AChE		
Amyrin			

Compound Names	3D Structure	2D Structure	Enzyme and ligand interaction
	AChE		
Betuline			
Catechin			
Cathin			
Isochorogenic acid			
Squalene			

Compound Names	3D Structure	2D Structure	Enzyme and ligand interaction
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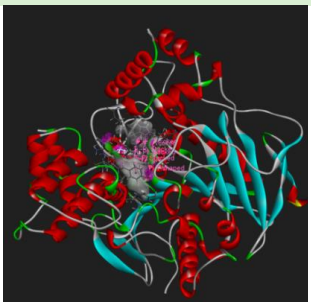
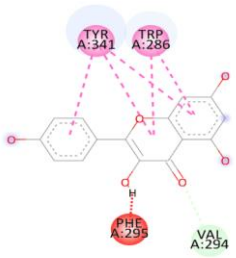
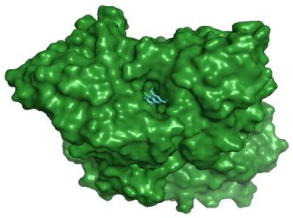
Kaempferol			
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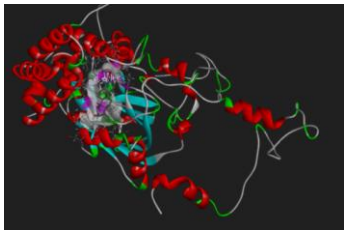
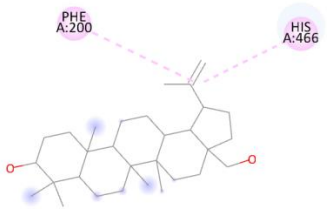
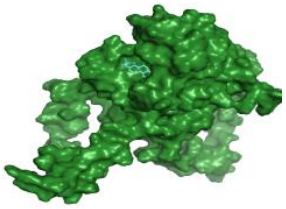
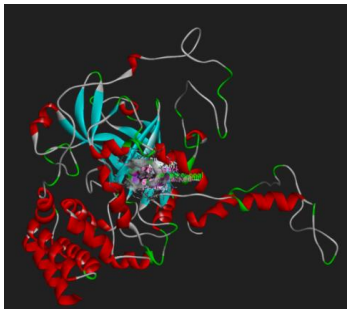
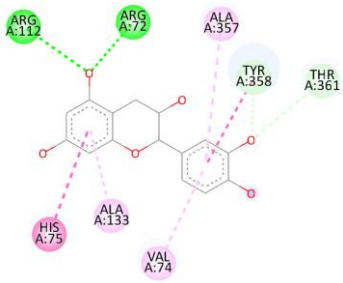
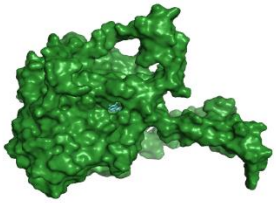
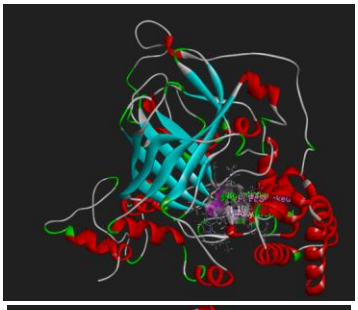
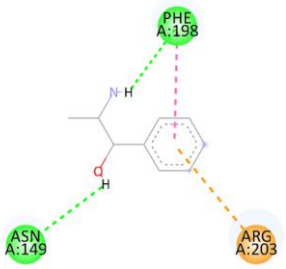
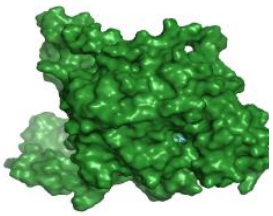
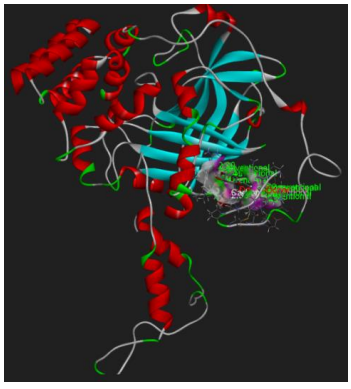
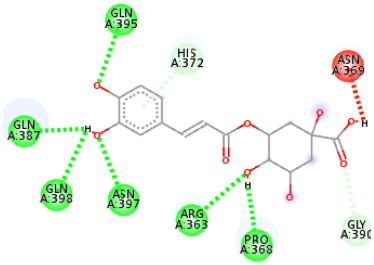
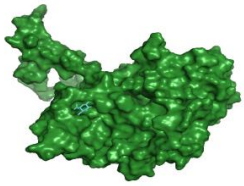
Betuline			
Catechin			
Cathin			
Isochoroge nic acid			

Compound Names	3D Structure	2D Structure	Enzyme and ligand interaction
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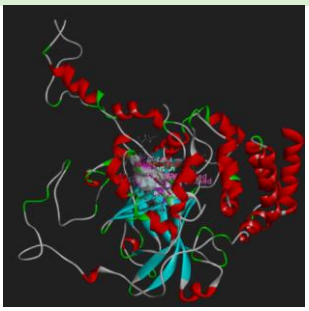
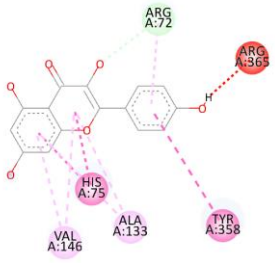
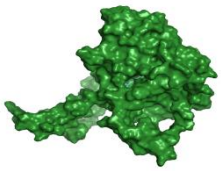
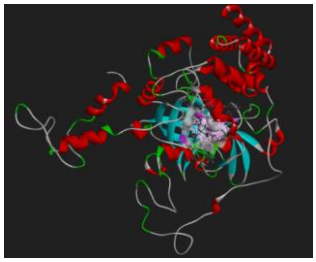
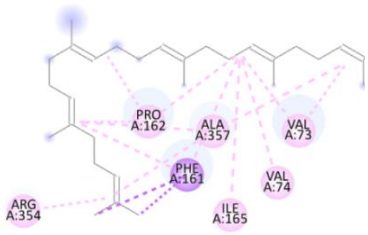
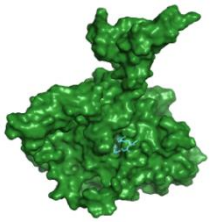
Kaempferol			
Squalene			
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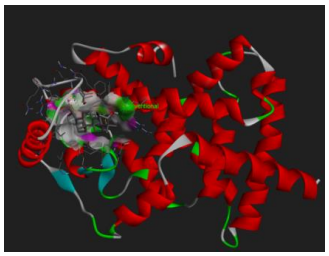
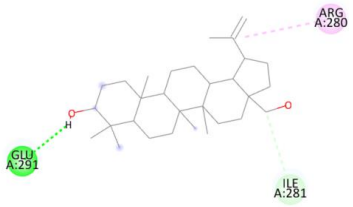
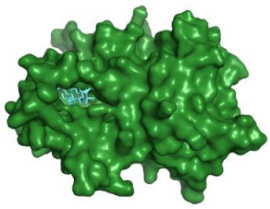
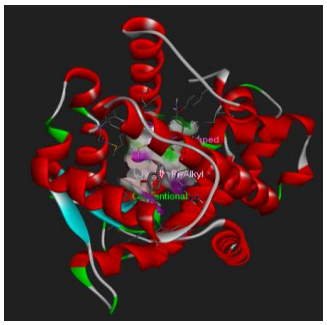
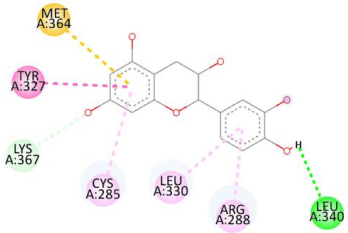
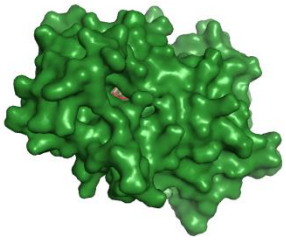
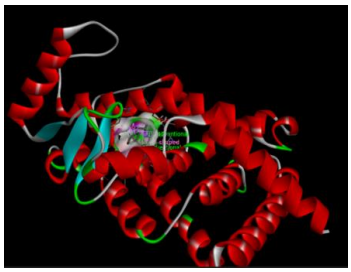
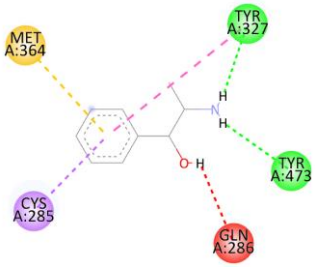
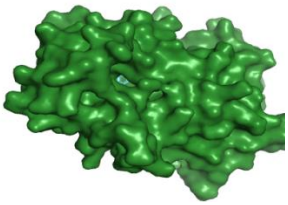
Betuline			
Catechin			
Cathin			

Compound Names	3D Structure	2D Structure	Enzyme and ligand interaction
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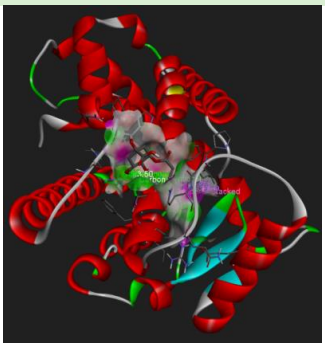
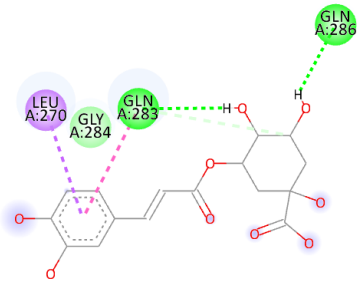
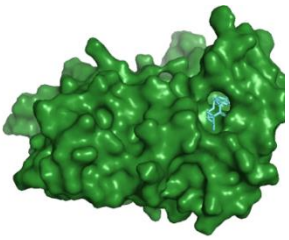
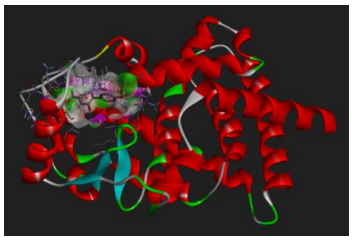
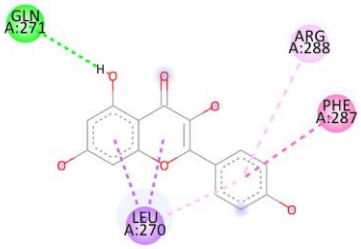
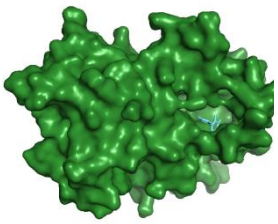
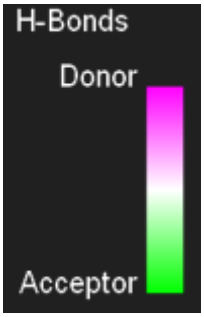
Isochorogenic acid			
Kaempferol			
			

Discussion

The findings of the alpha-amylase inhibitory tests revealed that numerous extracts from RT leaves had notable inhibitory potentials, with hydroalcohol showing the highest value among them. These alpha-amylase inhibitors are also called starch blockers, because they primarily prevent the hydrolysis of 1,4-glycosidic bonds between starch and other oligosaccharides into maltose, maltriose, and other simple sugars. It is beneficial to conduct further studies and isolate the pure active compounds, since polar molecules are more likely to be the source of the α-amylase inhibitory action in the hydroalcohol extract. The peroxidation of membrane lipids by oxygen radicals may harm cells. Ferrous sulfate initiates a series of oxidative processes that cause lipid peroxidation via the ferryl-perferryl complex or Fenton's reaction radical. Inhibition of the growth of the ferryl-perferryl complex may explain the findings of the current study. Methods such as scavenging, chelating iron, altering the Fe^{3+}/Fe^{2+} ratio, and decelerating the rate of ferrous to ferric transformations. Superoxide and OH radicals are examples of these [27].

Chemicals known as free radicals can exist alone with one or more unpaired electrons. The

generation of free radicals can lead to major tissue damage. Free radicals harm the body's DNA, lipids, and proteins. By removing harmful free radicals from the body, antioxidants help to defend against oxidative stress. Using the '1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical as a model system, researchers have investigated the scavenging qualities of numerous natural compounds, including phenolic acids and anthocyanins, as well as crude mixtures, including plant ethanol extracts. By adding a proton to create a lower DPPH concentration, antioxidants can scavenge DPPH radicals. A decline in absorbance at 517 nm indicates a color change from purple to yellow after reduction [28].

The percentage of free radical inhibition and radical scavenging activity were directly correlated. These findings imply that each DPPH extract was effective [20]. Because it has a unique absorption spectrum at long wavelengths, the ABTS test relies on the inhibition of absorption by the radical cation $ABTS^{\cdot+}$. These results suggest that each extract inhibits or scavenges $ABTS^{+}$ radicals as antioxidants have been demonstrated to possess both scavenging and inhibitory effects on these radicals [29].

Endothelial cells, macrophages, neurons, and other body cells generate free radical nitric oxide. It also regulates several physiological functions. Many diseases are linked to excessive NO levels. Excess nitric oxide forms peroxynitrite anions and nitrite by reacting with oxygen, creating free radicals. The hydroalcoholic extract of RTL outperformed oxygen in competitive interactions with nitric oxide, thereby reducing the production of anions. First, by considering the rivalry between deoxyribose and the extract for hydrogen radicals generated from Fe^{3+}/ascorbate/EDTA/H_2O_2 systems, the percentage of hydroxyl radicals prevented in Fenton's reaction mixture can be determined. This defines the scavenging ability of hydroxyl radicals. The attack of hydroxyl radicals on deoxyribose generates TBARS. According to the findings above [20-24], the % inhibition indicates that the hydroxyl radical scavenging function of each extract is superior. The findings showed that every extract has a notable phenolic concentration linked to total polyphenols (TPCs) and the anti-inflammatory and antioxidant qualities demonstrated by spectrophotometric studies. Isolated from RTL extracts, these phenolic compounds could not only clarify how phenolic compounds could function as antioxidants in living organisms, but they may also be excellent candidates for molecular docking research; seven molecules have notably high docking scores, which strongly suggest their binding and resulting antioxidant action. Moreover, an intriguing finding connected to the docking pose analysis revealed that all catechin, amyrin, and kaempferol exhibit antioxidant activity and AChE binding ability, which could prolong the acetylcholine half-life and support neuroprotective action [29-32].

Molecular docking studies of *Ruellia tuberosa* hydroethanolic extracts revealed promising interactions with various biological targets. Bioactive molecules, such as flavonoids and phenolic acids, exhibit strong binding affinities with key enzymes such as α-glucosidase and α-amylase, supporting their anti-diabetic potential. Docking results showed stable interactions through hydrogen bonds, hydrophobic contacts, and π-π stacking, indicating good compatibility with the active sites. These interactions help to explain the pharmacological efficacy observed in experimental studies. Overall, docking provides insight into the mechanistic basis of *Ruellia tuberosa's* therapeutic activities of Ruellia tuberosa and guides future drug development.

kcal/mol, respectively. The molecules have good docking score compared to native ligands; however,, three molecules catechin, amyrin, and kaemferol showed better results than all other molecules three enzymes (Catalase- 9.1 kcal/mol, 9.9 kcal/mol, and 9.9 kcal/mol; PPAR γ-7.4 kcal/mol, 8.5 kcal/mol, and 7.6 kcal/mol, and AChE- 9.4 kcal/mol, 9.9 kcal/mol, and 9.3 kcal/mol).

Conclusion

This study found that different RTL extracts were inhibited, with hydroalcohol being the most potent. Starch blocker extracts prevent hydrolysis of 1,4-glycosidic linkages between starch and other oligosaccharides into maltose, maltotriose, and other simple sugars. The inhibition of the ferryl-perferryl complex, which induces lipid peroxidation, may explain these results. Antioxidants prevent oxidative stress by eliminating harmful free radicals. The RTL hydroalcoholic extract surpassed oxygen in competitive interactions with nitric oxide to decrease anion production. All extracts exhibited exceptional hydroxyl radical-scavenging activity, as indicated by % inhibition. All extracts contained high levels of phenolics, which are associated with total polyphenols (TPCs) and antioxidant and anti-inflammatory properties. These RTL extract phenolic compounds may elucidate how they function as antioxidants in living organisms, and are excellent candidates for molecular docking research. Molecular docking results revealed strong binding affinities of catechin, amyrin, and kaempferol to key target proteins, indicating their potential biological activity. These findings support their role as lead compounds in further drug development. The in vitro and *in silico* investigations of RTL extract provide avenues for the identification and isolation of bioactive components. Target interactions can be predicted and chemical efficacy can be maximized with the assistance of computational modeling. Additional *in vitro* studies can explore dose-dependent effects and confirm these hypotheses. These results could lead to the development of potent medications. This study establishes a foundation for further *in vivo* investigations and potential therapeutic applications.

Abbreviations

DPPH: 1, 1-diphenyl-2-picryl hydrazyl
RTL*: Ruellia tuberosa* leaves
ROS: Reactive oxygen species
TPCs: Total polyphenols
PPAR: Peroxisome proliferator-activated receptors
AChE: Acetylcholinesterase
ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
SOD: Superoxide Dismutase
H_2O_2: Hydrogen Peroxide
O_{2^-}: Superoxide
$FeSO_4$: Ferrous sulphate,
TBA; Thiobarbituric acid ,
TCA: Trichloroacetic acid,
NBT: Nitroblue tetrazolium and
EDTA: Ethylene diamine tetra acetic acid
DNSA: 3,5-dinitrosalicylic acid
TBARS; Thiabarturic acid reactive chemicals
RCSB PDB: Research Collaboratory for Structural Bioinformatics Protein Data Bank
BChE : Butyrylcholinesterase
IC_{50} : Half-maximal inhibitory concentration
NO: Nitric Oxide
Inos: Inhaled Nitric Oxide
PPAR γ: Peroxisome proliferator-activated receptor gamma

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All authors contributed to data analysis, drafting, and revising of the article and agreed to be responsible for all the aspects of this work.

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