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Original article

Chemical Profiling, *in-vitro* biological evaluation and molecular docking studies of *Ruellia tweediana*: An unexplored plant

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ABSTRACT

Many *Ruellia* species have been utilized in traditional medicine and despite the prevalent use of *Ruellia tweediana* in folk medicine, its antioxidant potential and polyphenol content have not been investigated. Therefore, the present study aimed to explore the medicinal value of *R. tweediana* by evaluating its total phenolic (TPC) and flavonoid contents (TFC), GC–MS analysis, antioxidant, antibacterial, and enzyme inhibition activities. The TPC and TFC of the extract/fractions were assessed using the Folin-Ciocalteu and aluminum trichloride methods, respectively. To determine the antioxidant capacity, five different assays were used: DPPH, ABTS, CUPRAC, FRAP, and metal chelating assays. The inhibition activity against α-glucosidase, α-amylase, cholinesterases, and lipoxygenase enzymes was also analyzed. Furthermore, GC–MS was performed for chemical screening of non-polar fraction. The methanol extract showed the maximum TPC (167.34 ± 2.23 mg GAE/g) and TFC (120.43 ± 1.71 mg RE/g) values among all the tested samples. GC–MS screening of the *n*-hexane fraction showed the presence of 40 different phytoconstituents. The results demonstrated the highest scavenging potential of the methanol extract against DPPH (167.79 ± 2.75 mg TE/g) and ABTS (255.32 ± 2.91 mg TE/g) radicals, as well as the metal-reducing capacity measured by CUPRAC (321.34 ± 3.09 mg TE/g), FRAP (311.32 ± 2.91 mg TE/g), and metal chelating assay (246.78 ± 10.34 mg EDTAE/g). Notably, the *n*-hexane fraction revealed the highest α-glucosidase and α-amylase inhibition activity (186.8 ± 2.84 and 179.7 ± 4.32 mg ACAE/g, respectively) while methanol extract showed highest acetylcholinesterase and butyrylcholinesterase inhibition activity (198.6 ± 3.31 and 184.3 ± 2.92 mg GALE/g, respectively). The GC–MS identified Lupeol showed best binding affinity with all docked enzymes as compared to standard compounds. The presence of bioactive phytoconstituents showed by GC–MS underscores the medicinal importance of *R. tweediana*, making it a promising candidate for natural medicine.

1. Introduction

Traditional herbal medicines have a crucial role in treating and preventing various infections and ailments (Xiong et al., 2020). Natural compounds derived from traditional Chinese medicines show significant effect in combating multidrug resistance with low toxicity and also herbal medicines are known for their non-addictive nature (Tejaputri et al., 2020). While the use of synthetic medicines has serious apprehensions for human health. As a result, a lot of research has been performed to explore plants for their phytochemical analysis and investigate their biological activities, including anti-inflammatory, antioxidant, and antidiabetic effects (Grochowski et al., 2019). In the developed world, nearly a quarter of all pharmaceuticals and medicines are formulated with ingredients derived from medicinal plants (Güler et al., 2021).

Phytochemicals are natural bioactive constituents found in plants, and one major subclass among them is polyphenols. Polyphenols are characterized by their diverse chemical structures and can be found

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plentifully in fruits, vegetables, whole grains, and numerous plant-based foods. One of the most notable activities of polyphenols is their potent antioxidant activity (Rizvi et al., 2023). By neutralizing these free radicals, polyphenols play a key role in reducing the risk of diseases like cancer, neurodegenerative disorders, and inflammation-related conditions (Ghalloo et al., 2022). Additionally, their anti-inflammatory effects contribute to the body's healing process. Harnessing the antioxidant and anti-inflammatory activity of polyphenols in medicinal plants offers significant therapeutic applications, complementing modern medical treatments and promoting all-inclusive healthcare approaches. Furthermore, resistance against various infectious diseases is increasing day by day as microbes are producing mutations in their structures. So, the antimicrobial potential of *R. tweediana* was also determined as a contribution to overcoming this problem (Rizvi et al., 2023).

In recent times, there has been a growing interest in multi-target drugs as potential treatments for numerous complex diseases such as skin pigmentation issues, neurodegenerative disorders, metabolic conditions, and anti-ulcer effects. Additionally, these drugs offer promising solutions for health problems associated with drug resistance. Enzymes, playing crucial roles in numerous physiological processes, remain a primary focus for drug development in human diseases. (Meziant et al., 2021). Controlling enzyme activity can produce rapid and specific effects, making them highly required targets for therapeutic interventions (Ramsay and Tipton, 2017). As a noteworthy global public health issue, diabetes mellitus continues to pose a foremost challenge. Among the various classes of oral antidiabetic drugs, α-Amylase and α-Glucosidase inhibitors are generally utilized (Bhagyawant et al., 2019). The management of certain neurodegenerative disorders like dementia and Alzheimer's disease includes targeting and inhibiting cholinesterase enzymes, which are crucial in neural functioning through cholinergic pathways (Ghalloo et al., 2022). Lipoxygenase is an enzyme that plays a noteworthy role in inflammation by catalyzing the conversion of polyunsaturated fatty acids into effective pro-inflammatory mediators known as leukotrienes. Inhibition of lipoxygenase activity is a potential therapeutic methodology to alleviate inflammation and its associated conditions (Chakraborty et al., 2019).

Ruellia tweediana Griseb belongs to the genus *Ruellia* and the family *Acanthaceae.* (Xu and Chang, 2017). The genus *Ruellia* is also known as *Dipteracanthus* and comprises about 150 species (Samy et al., 2015a). Most of them are shrubs and are found in temperate areas and others are distributed in Africa, Pakistan, Brazil, Indonesia, Central America, and Malaysia. Traditionally, this genus is claimed for the treatment of eczema, fever, flu, asthma, hypertension, bronchitis, and diabetes (Afzal et al., 2015). *R. tuberosa* is reported for antihypertensive, diuretic, antipyretic, thirst-quenching, analgesic, and antidiabetic activities. The genus *Ruellia* is also used to treat hypercholesteremia (Samy et al., 2015b). Many species have tremendous medicinal potential against ulcers, pains, inflammations, and diabetes. Some are antioxidant and antispasmodic (Khan et al., 2017).

The plant *R. tweediana* may have some incredible medicinal potential like other species of this genus hence, was selected for current research work. The present research work aimed to explore the biological potential of this unexplored plant, *R. tweediana,* including qualitative and quantitative phytochemicals evaluation, antioxidant, antibacterial, and enzyme inhibition capability with a major focus on its antidiabetic, anti-inflammatory and neurodegenerative properties along with their molecular docking studies.

2. Materials and methods

2.1. Materials

$FeCl_3$, Lead acetate, Amyl alcohol, Dragendroff's reagent, Wagner's reagent, Mayer's reagent, Borntrager's reagent, Salkowski's reagent, and Acetic acid were procured from Merck, Germany. In addition, enzymes including α-glucosidase, α-amylase, Urease, Tyrosinase, Lipoxygenase, Butyrylcholinesterase, and Acetylcholinesterase were purchased from Sigma Aldrich, USA.

2.2. Preparation of extracts and fractions

2.2.1. Collection, Drying, and extraction of R. Tweediana

The *Ruellia tweediana* Griseb (whole plant) was collected in the spring from the Islamia University of Bahawalpur (Latitude: 29.378 and Longitude: 71.7593), District Bahawalpur Province, Punjab, Pakistan. The plant identification was performed by the Department of Botany, IUB and specimen was submitted with a voucher number of 483/Botany. After drying and grinding, soaking was performed in 80 % hydro-alcoholic solution (80 % Methanol) for 7 days with continuous agitation and mixing daily. The marc was saved after the filtration with the muslin cloth. The filtered extract was dried by a rotary evaporator at 40 °C. Semisolid extract collected from the rotary evaporator was further dried at room temperature in trays with a large surface area to evaporate the last content of the hydroalcoholic solution used for the extraction.

2.2.2. Fractionation

For the fractionation, the aqueous fraction of crude extract was made by adding 1L of distilled deionized water to the methanol extract. Three solvents, *n*-Hexane, Chloroform, and *n*-Butanol were used and three fractions of these solvents were prepared. The fractionating flask was used for the fractionation. First, the *n*-hexane fraction was prepared by pouring and mixing the aqueous fraction with *n*-hexane (50:50) in the fractionating flask in vigorous agitation. After that, the fractionating flask was adjusted to stand to separate the *n*-hexane layer from the aqueous layer. The upper *n*-hexane layer was removed from the aqueous layer. The process was repeated until the transparent layer of *n*-hexane was obtained by mixing the *n*-hexane with the aqueous layer. The same procedure was adopted to obtain chloroform and *n*-butanol fractions using the same aqueous fraction.

2.3. Polyphenolic contents and chemical composition

2.3.1. Qualitative analysis of Phytochemicals

The various extracts and fractions of *Ruellia tweediana* were subjected to analysis for screening of primary and secondary metabolites. This investigation aimed to confirm the presence of phytochemicals such as alkaloids, tannins, phenols, flavonoids, saponins, steroids, glycosides, and resins. The methods employed for this analysis were based on those detailed in relevant literature (Dilshad et al., 2022a).

2.3.2. Quantification of total phenolic content (TPC)

TPC was performed with the F.C. reagent method. Equal volumes (1 mL each) of sample extract and F.C. reagent was mixed. Then 2.8 mL of $NaHCO_3$ was added in the reaction solution. This whole reaction mixture was incubated for 30 min. Then absorbance was measured at 725 nm. Gallic acid was used as standard and methanol was used as negative control. The outcome values for TPC were written as GAE/g of extract (gallic acid equivalent per gram) and the average of three readings was taken for each sample (Shahzad et al., 2022).

2.3.3. Quantification of total flavonoid contents (TFC)

TFC in all sample solutions were quantified by the $AlCl_3$ method. In this method, TFC was quantified as rutin equivalent by establishing its calibration curve. 1 mL of standard (rutin) or test solution was combined with same volume of 2 % $AlCl_3$. UV absorbance was measured at 415 nm. Methanol was used as a negative control or blank. Based on the absorbance, TFC were quantified from the calibration equation, and the TFC were presented in terms of mg of RE/g (milligrams of rutin equivalent per gram) (Basit et al., 2022a).

2.3.4. Chemical composition with GC–MS analysis

The contents of non-polar (*n*-hexane) fractions of *Ruellia tweediana*

were screened by GC–MS. The Agilent 7890B GC–MS with mass hunter acquisition software was used. The column dimensions were 30 m × s 0.25 mm × 0.25 μm. The oven temperature was maintained from 50 °C (after 2 min) to 240 °C for 10 min. The temperature of injector and detector were programmed at 200 °C and 240 °C and hydrogen was used as a carrier gas. 0.5 μL of test sample was injected through injector to GC and peaks were recorded. The phytoconstituents of NHRT were tentatively identified based on the comparison with their retention indices with the reference mass spectra, the NIST Mass Spectral Library (Ghalloo et al., 2022).

2.4. Antioxidant potential

The methanol extract and all its fractions were analyzed for antioxidant activity by DPPH, ABTS, FRAP, and CUPRAC and metal chelating methods.

2.4.1. DPPH assay

To measure the antioxidant potential by DPPH method, 0.1 mL of sample solution and 3.9 mL of DPPH reagent was mixed. The absorbance of resulting solution was performed at 517 nm. Trolox and methanol were used as standard antioxidant and as negative control, respectively. The results were written as mg TE/g (mg trolox equivalent/gram), indicating the antioxidant capacity of the samples (Basit et al., 2022b).

2.4.2. ABTS assay

The antioxidant potential of *R. tweediana* was also measured using the ABTS method, following a previously described method. A 1 mL sample was combined with 2 mL of $ABTS^{+}$ and left at 25 °C for 30 min. The absorbance was then taken at 734 nm, and the results were written as mg TE/g (mg of Trolox equivalent/gram). As a positive control, Trolox was employed, and methanol served as the negative control (Dilshad et al., 2022b).

2.4.3. FRAP assay

The FRAP method was employed according to the protocol described in the literature to assess the antioxidant activity of the sample solutions, which measures their capacity to reduce Fe^{3+} to Fe^{2+}. To carry out the assay, 1 mL of each sample was combined separately with 2.5 mL of 1 % potassium ferricyanide and 2.5 mL of phosphate-buffered saline. The absorbance was taken at 700 nm. Trolox and methanol were used as standard and negative control, respectively. The results were written as mg TE/g (mg of Trolox equivalent/gram) (Dilshad et al., 2022).

2.4.4. CUPRAC assay

The CUPRAC assay was done following a method from the literature. Initially, 1 mL of distil·H_2O was added with 0.1 mL of each sample solution separately. Then, a solution containing CuCl, neocuproine, and ammonium acetate buffer solution with pH of 7 in a 1:1:1 ratio was combined to the mixture. The resulting solution was incubated at 25 °C for 30 min, and its absorbance was taken at 450 nm. Trolox and methanol served as the positive control and negative control, respectively. The results were expressed as mg TE/g (mg of Trolox equivalent/gram) (Dilshad et al., 2022a).

2.4.5. Metal chelating assay

The quantification of ferrous ions (Fe^{2+}) in the extracts was made as follows: 0.5 mL of sample at varying concentrations was mixed with 1.6 mL of distil·H_2O, and then 0.05 mL of $FeCl_2$ (2 mM) was mixed. Subsequently, 0.1 mL of Ferrozine (5 mM) was poured to the mixture, which was then incubated for 10 min at 25 °C. The formation of the ferrous ions (Fe^{2+}) was measured at 562 nm. EDTA and methanol were utilized as the standard metal chelating agent, and negative control, respectively. The results were written in terms of μmol of EDTAE/g (μmoles of EDTA equivalents/gram) (Sabraoui et al., 2020).

2.5. Enzyme inhibition potential

The enzyme inhibition activity for α-glucosidase, α-amylase, tyrosinase, urease, acetylcholinesterase, butyrylcholinesterase, and lipoxygenase was analyzed.

2.5.1. In-vitro antidiabetic activity

- α -Glucosidase Enzyme Inhibition Assay

The α-glucosidase inhibition potential was measured by incubating 50 mL of the sample along with 50 mL of glutathione with concentration of 0.5 mg/mL, 50 mL of α-glucosidase (0.2 U/mL) in phosphate buffer having pH 6.8, and 50 mL of *para*-nitrophenyl-α-D-glucopyranoside (pNPG; 10 mM) at 37 °C for 15 min. To stop the reaction, 50 mL of 0.2 M sodium carbonate was mixed. A blank sample was also used without the extract following the same procedure. After an additional 15 min of incubation at 37 °C, the absorbance was taken at 400 nm. The results were written as mg ACAE/g (mg of acarbose equivalents/gram) (Grochowski et al., 2019).

- α- Amylase Enzyme Inhibition Assay

To assess the α-amylase inhibition activity, a 25 mL solution was mixed with 50 mL of α-amylase (10 U/mL) in buffer with pH 6.9 adjusted with sodium chloride and incubated at 37 °C for 15 min. Then, 50 mL of 0.05 % starch was combined to the mixture. The reaction was stopped by adding 25 mL of 1 M HCl, followed by the mixing of 100 mL of Iodine-potassium iodide. A blank solution was also made using the same procedure but without the addition of the sample. After further incubation at 37 °C for 10 min, the absorbance was noted at 630 nm. The results were written as mg ACAE/g (mg of acarbose equivalents/gram), representing the α-amylase inhibitory potential of the samples (Grochowski et al., 2019).

2.5.2. In-vitro enzyme activity against neurological disorders

To analyze the activity of plants for neurological disorders, the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition assay was utilized. The reaction mixture comprised 50 mL of extract/fraction solution, 125 mL of 3 mM 5,5′dithiobis nitrobenzoic acid (DTNB), and 25 mL of enzyme solution in Tris-HCl buffer (pH 8.0). After incubating at 37 °C for 15 min, 25 mL of substrates (15 mM-acetylthiocholineiodide (ACTI) or 15-mM butyrylthiocholinechloride (BTCl)) were mixed. In the same way, a blank sample was prepared without the extract. After another 15 min, the UV-absorbance was measured at 405 nm. The results were expressed as mg GALAE/g (mg of galantamine equivalents/gram), indicating the potential of the samples as compared to galantamine in inhibiting AChE and BChE enzymes (Grochowski et al., 2019).

2.5.3. In-vitro anti-inflammatory activity

The anti-inflammatory activity of *R. tweediana* was measured using the Lipoxygenase (LOX) enzyme inhibition assay. To analyze the anti-inflammatory property, each sample extract (10 μL) was added with lipoxygenase solution (90 μL) and incubated at 37 °C for 5 min. After incubation, arachidonic acid was mixed to reaction mixture and shaken for 15 min. Subsequently, 100 μL of chromogen was added, and the mixture was shaken for an additional 5 min. The UV absorbance of mixture was measured at 500 nm. The negative control used was buffer, while indomethacin served as the standard. The results were expressed as mg IndoE/g (mg of indomethacin equivalents/gram), representing the anti-inflammatory activity of the samples (Olech et al., 2020).

2.6. Antibacterial assay

The antibacterial activity was made in a 96-well microplate in

aseptic environment. This method's principle is that the medium's absorbance is proportional to the number of microbial cells. The study was performed on *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 9027), and *S. typhi* (ATCC 19426), *B. subtilis* (ATCC 1692) *and S. aureus* (ATCC 6538). The wells were filled with samples (20 µg/well). A 180 µL of bacterial culture was added to each well, followed by initial absorbance at 540 nm. Keeping the volume of 200 µL in each well, the microplate was incubated at 37 °C for 24 hr, followed by the absorbance at 540 nm. The difference in both absorbances was taken as an index of bacterial growth. Ciprofloxacin was used as a standard antibacterial agent. The percentage inhibition was calculated using the following formula given below:

$$Inhibition(\%) = \left(\frac{Abs(c) - Abs(s)}{Abs(s)}\right) \times 100$$

Abs (c) = Absorbance of control; Abs (s) = Absorbance of a test sample.

2.7. In-silico molecular docking studies (MD)

The MD Study is a valued tool in "molecular biology and computer-aided drug designing". For this purpose, AutoDock Vina Software, Babel, Discovery Studio, and PyRx were utilized. From PDB, these enzymes; α-Glucosidase, α-Amylase, Butyrycholinestrase, Acetylcholinesterase, and Lipoxygenase were downloaded and further prepared by using Discovery Studio 2021 Client. Ligands and Standard compounds were downloaded in sdf from PubChem. Babel was used to preparing small molecules (compounds). The designed small molecules and macromolecules were uploaded in Vina which is embedded in PyRx and docking was performed (Yousuf et al., 2022).

2.8. Statistical analysis

The data obtained from the aforementioned experiments were subjected to statistical analysis using the SPSS version 20. Each experiment was performed three times. The collected parameters were examined using Analysis of Variance (ANOVA), and the results were presented as mean ± standard deviation (SD). The significance level was set at $P < 0.05$.

3. Results

3.1. Extracts yield

The 10 kg powdered plant material of *R. tweediana* (whole plant) was subjected to extraction with 80 % hydroalcoholic solution (80 % methanol). The filtrates were condensed on a rotary evaporator. The three different fractions were made from methanolic extract by the process of fractionation by using *n*-Hexane, Chloroform and *n*-Butanol in increasing order of polarity. The maximum yield was observed for Chloroform fraction (110 g). The findings are given in Table 1.

Table 1
Extraction yield of *Ruellia tweediana.*

Name of extract	Solvent used	Weight of extract
MHRT	Hydroalcoholic (80 % Methanol)	500 g
NHRT	*n*-Hexane	65 g
CHRT	Chloroform	110 g
NBRT	*n*-Butanol	60 g

MHRT: methanol extract, NHRT: *n*-hexane fraction, CHRT: chloroform fraction, NBRT: *n*-butanol fraction

3.2. Qualitative phytochemical analysis

Phytochemicals are bioactive constituents of plants and chemically these constituents are tannins, terpenoids, glycosides, flavonoids, alkaloids, carotenoids, and steroids. These secondary metabolites exhibit different pharmacological activities e.g. antioxidant, antiaging, antidiabetic, antimicrobial, anti-inflammatory, hemolytic, etc. The presence of these secondary metabolites was explored by performing qualitative phytochemical screening (Table 2) on plant powder by using the methods mentioned in the literature (Santhi and Sengottuvel, 2016, Mani et al., 2020).

3.3. Quantitative screening of phenolic and flavonoid contents

The plant samples were screened for quantitative analysis of total phenols and flavonoids contents. In methanol extract, highest total phenolic and total flavonoid contents were observed as related to other plant samples (Table 3).

3.4. Chemical composition with gas Chromatography-Mass Spectrometry (GC–MS) analysis

Chemical composition of NHRT from *R. tweediana* was investigated by GC–MS analysis. Total 40 phytoconstituents were identified as shown in Fig. 1 and Table 4. These phytoconstituents belong to various chemical classes. These chemical classes include Ketones, Aldehydes, Esters, Pyrroles, Phenol, Fatty acids and Fatty acid esters.

3.5. Antioxidant assay

The crude methanolic extract (MHRT) and its three fractions, e.g. *n*-Hexane (NHRT), Chloroform (CHRT), and *n*-Butanol (NBRT) of *Ruellia tweediana* were subjected to access antioxidant potential by DPPH, FRAP, CUPRAC, and Metal Chelating assays (Table 5). The methanol extract showed highest antioxidant potential as compared to *n*-butanol and chloroform fraction, while the antioxidant potential of *n*-hexane fraction was comparable with the methanol extract.

3.6. Antibacterial activity

As resistance against various infectious diseases is increasing day by day. The plant *R. tweediana* was subjected to an antibacterial assay to evaluate its antibacterial potential against two gram-positive and three gram-negative bacteria, as shown in Table 6. The maximum activity was exhibited by *n*-hexane fraction.

Table 2
Qualitative Phytochemical Analysis of *Ruellia tweediana.*

Chemical constituent	Test name	Methanol extract	*n*-Hexane fraction	Chloroform fraction	n-Butanol fraction
Phenol	$FeCl_3$	+	+	+	+
Tannins	Lead acetate	+	-	+	+
Flavonoids	Amyl alcohol	+	+	+	+
Saponin	Frothing test	+	+	+	+
Alkaloids	Wagner's test	-	-	-	-
	Mayer's test	-	-	-	-
Glycosides	Borntrager's	+	-	+	+
Steroids & Terpenes	Salkowski's	+	+	-	-
Resins	Acetic acid	+	+	+	+

−: Absent and +: Present.

Table 3
Quantitative Estimation of Total Phenolics and Total Flavonoids Contents in different extract/fractions of *R. tweediana.*

Extracts	TPC (mg GAE/g D.E)	TFC (mg RE/g D.E)
MHRT	167.34 ± 2.23[a]	120.43 ± 1.71[a]
NHRT	130.79 ± 2.31[c]	105.51 ± 0.82[b]
CHRT	146.5 ± 3.46[b]	96.59 ± 0.75[c]
NBRT	167.34 ± 2.23[a]	120.43 ± 1.71[a]

MHRT: methanol extract, NHRT: *n*-hexane fraction, CHRT: chloroform fraction, NBRT: *n*-butanol fraction. The superscripts a, b, c, and d represent the significant difference ($P \leq 0.05$).

3.7. Enzyme inhibition potential Ruellia tweediana

The enzyme inhibition activities of all the sample extracts were analyzed against α-glucosidase, α-amylase, acetylcholinesterase, butyrylcholinesterase, and lipoxygenase as shown in Tables 7,8, and 9.

3.7.1. In-vitro antidiabetic activity

Diabetes is a clinical condition characterized by increase in blood sugar, caused by a deficiency of insulin either absolute or relative. Over the past few decades, there has been a significant rise in the prevalence of diabetes mellitus. One therapeutic approach to treating diabetes involves reducing the production and absorption of glucose in the gastrointestinal tract by inhibiting enzymes responsible for carbohydrate digestion, such as α-amylase and α-glucosidase. By inhibiting these enzymes, the digestion of carbohydrates can be slowed down, leading to a significant decrease in the rise of postprandial blood glucose levels after consuming a mixed carbohydrate diet. This strategy plays a crucial role in managing blood glucose levels (Narkhede et al., 2011). The objective of the current study was to assess the antidiabetic activity of the extracts derived from the *R. tweediana*. Our assay results indicated that *n*-Hexane extract (NHRT) demonstrated maximum inhibitory activity against α-glucosidase enzymes (186.8 ± 2.84 mg ACAE/g) and α-amylase (179.7 ± 4.32 mg ACAE/g) as given in Table 7. Acarbose was used as the standard drug for comparison.

3.7.2. In-vitro acetylcholinesterase and butyrylcholinesterase inhibition activity for the treatment of neurological disorders

It has been reported that plants may possess properties as potential inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), offering an alternative treatment option for Alzheimer's disorder (AD). Throughout traditional practices, a variety of plants have been utilized to address cognitive disorders, such as neurodegenerative disorders (Shoaib et al., 2015).

The inhibition of AChE and BChE, enzymes responsible for breaking down acetylcholine and butyrylcholine respectively, is considered an

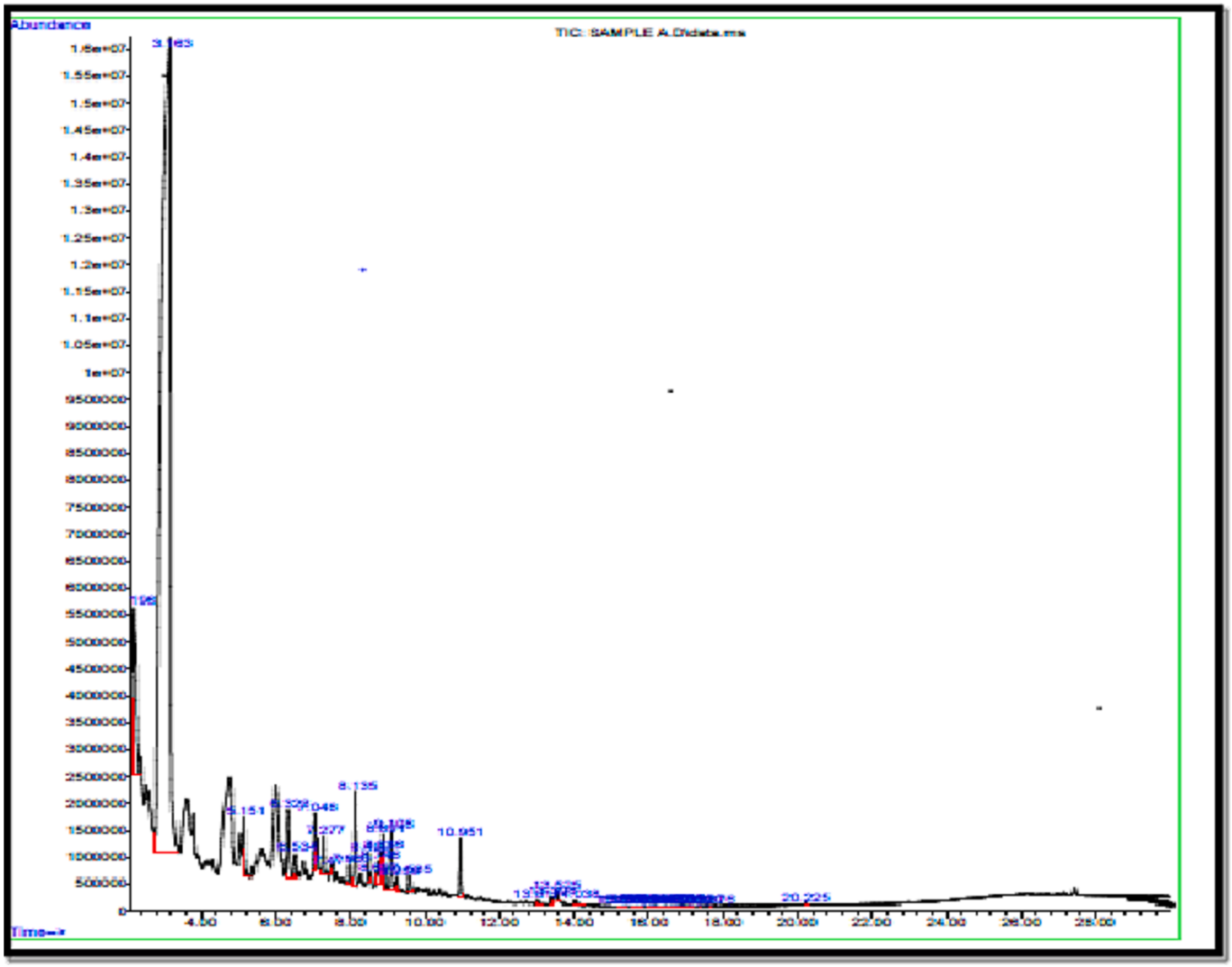

Fig. 1. GC–MS chromatogram of NHRT.

Table 4
Chemical constituents tentatively identified in GC–MS analysis of NHRT.

Peak. No.	Name of compound	Retention time (Minutes)	Molecular Formula	Molecular weight (gram/mole)	Chemical class	Peak percent area
1	2,3-dihydro-3,5-dihydroxy-6-methyl, 4H-Pyran-4-one	2.196	$C_6H_8O_4$	144.12	Ketone	5.74
2	5-Hydroxymethylfurfural	3.163	$C_6H_6O_3$	126.11	Aldehyde	71.65
3	2,4-Octadienoic acid, 3-methyl-, methyl ester	5.151	$C_9H_{14}O_2$	154.21	Ester	1.04
4	1-(2-Furylmethyl)-1H-pyrrole	6.323	C_9H_9NO	147.17	Pyrroles	1.60
5	Sulfurous acid, 2-propyl tetradecyl ester	6.534	$C_{17}H_{36}O_3S$	320.50	Ester	0.59
6	Phenol, 3,4,5-trimethoxy	7.048	$_9H_{12}O_4$	184.19	Phenol	1.14
7	Bicyclo heptane, 3-methyl-7- pentyl	7.277	$C_{15}H_{28}$	208.38	Cyclo Alkane	0.82
8	7-Butyl-6,6-dimethylbicyclo oct-7-en-2-one	7.471	$C_{15}H_{28}$	208.38	Ketone	0.73
9	D-Tyrosine, 3-hydroxy-	7.960	$C_{24}H_{28}O_2$	348.5	Ester	0.99
10	4-(3-hydroxybutyl)-3,5,5-trimethyl, 2-Cyclohexen-1-one	8.135	$C_{13}H_{22}O_2$	210.31	Ketone	1.51
11	2,5-Dimethylhex-5-en-3-yn-2-ol	8.492	$C_8H_{12}O$	124.18	Alcohol	0.96
12	3-(4-hydroxyphenyl)-, methyl ester, 2-Propenoic acid	8.697	$C_{10}H_{10}O_3$	178.18	Ester	0.83
13	2,5-Dimethyl-2,4-hexadienedioic acid	8.746	$C_8H_{10}O_4$.	170.16	Acid	0.89
14	4-hydroxy-3,5-dimethoxy-, methyl ester Benzoic acid	8.836	$C_{10}H_{12}O_5$	212.20	Ester	0.94
15	Loliolide	8.891	$_{11}H_{16}O_3$	196.24	Benzofurans	1.10
16	Mequinol	9.1	C_7H_8O	124.14	Alcohol	1.00
17	1-(1-Butyny) cyclopentanol	9.14	$C_9H_{18}O$	142.24	Alcohol	1.00
18	3-[N-Aziridyl] butyraldehyde hydrazone	9.259	$C_6H_{13}N_3$	127.19	Hydrazone	0.85
19	3-(4-hydroxy-3-methoxyphenyl)-, methyl ester, 2-Propenoic acid	9.585	$C_{11}H_{12}O_4$	208.21	Ester	0.78
20	n-Hexadecanoic acid	10.951	$C_{16}H_{32}O_2$	256.4	Fatty Acid	0.92
21	Phytol	12.017	$C_{20}H_{40}O$	296.53	Diterpene Alcohol	1.29
22	Nonacosane	12.52	$C_{29}H_{60}$	408.60	Straight-Chain Hydrocarbon	1.02
23	Docosane	12. 89	$C_{22}H_{46}$	310.60	Straight-Chain Hydrocarbon	1.01
24	Lupeol	13.01	$C_{30}H_{50}O$	426.72	Triterpenoid	2.55
25	Linoelaidic acid	13.398	$C_{18}H_{32}O_2$	280.45	Omega-6 fatty acid	0.96
26	Methyl 8,11,14-heptadecatrienoate	13.5	$C_{18}H_{30}O_2$	278.40	Ester	0.82
27	Octadecanoic acid	14.0	$C_{18}H_{34}O_2$	284.48	Ester	0.88
28	Pterin-6-carboxylic acid	15.31	$C_7H_5N_5O_3$	207.15	Carboxylic Acid	0.72
29	2-Amino-1-(o-methoxyphenyl)propane	15.38	$C_{10}H_{15}NO$	165.23	Alkane	0.81
30	Adrenalone	15.591	$C_9H_{11}NO_3$	181.19	Ketone	1.01
31	Selenocystamine	15.627	$C_4H_{12}N_2Se_2$	246.09	Amine	0.96
32	Ethanamine, 2-phenoxy	16.04	$C_8H_{11}NO$	137.18	Amine	0.79
33	Phenylephrine	16.044	$C_9H_{13}NO_2$	167.21	Amine	0.94
34	Ledol	16.165	$C_{15}H_{26}O$	222.37	Sesquiterpenoid	0.82
35	Neoclovene oxide	16.902	$C_{15}H_{24}O$	220.35	Sesquiterpene	0.69
36	Isobutyramide, N-methyl-N-octyl	17.059	$C_{17}H_{37}N$	255.48	Amine	0.91
37	Carbamic acid, N-methyl, hexyl ester	17.059	$C_8H_{17}NO_2$	159.23	Ester	0.87
38	Thunbergol	7.090	$C_{20}H_{34}O$	290.5	Alcohol	0.61
39	Sesquicineole	17.676	$C_{15}H_{26}O$	222.37	Oxanes.	0.80
40	Mono(2-ethylhexyl) phthalate	20.225	$C_{16}H_{21}O_4$	277.33	Ester	0.58

Table 5
Estimation of Antioxidant potential of *Ruellia tweediana* by DPPH, ABTS, FRAP, CUPRAC, and Metal-chelating assays.

Extracts	DPPH radical (mg TE/g)	ABTS (mg TE/g)	FRAP (mg TE/g)	CUPRAC (mg TE/g)	Metal-chelating (mg EDTAE/g)
MHRT	167.79 ± 2.75^a	255.32 ± 2.91^a	311.32 ± 2.91^a	321.34 ± 3.09^a	246.78 ± 10.34^a
NHRT	164.46 ± 2.87^a	245.17 ± 3.02^a	301.17 ± 3.02^a	296.43 ± 2.92^b	240.96 ± 8.57^a
CHRT	129.96 ± 3.06^c	193.59 ± 3.01^c	186.59 ± 4.05^c	228.67 ± 2.01^d	193.74 ± 33.57^c
NBRT	134.3 ± 2.29^b	201.21 ± 2.31^b	214.32 ± 2.74^b	250.56 ± 3.3^c	201.76 ± 5.01^b

MHRT: methanol extract, NHRT: *n*-hexane fraction, CHRT: chloroform fraction, NBRT: *n*-butanol fraction. TE: Trolox equivalent and EDTAE: EDTA equivalent; The superscripts a, b, c, and d represent the significant difference ($P \leq 0.05$).

approach in the treatment of AD (Saleem et al., 2016). To investigate the potential of *R. tweediana* as an anti-Alzheimer's agent, extracts obtained from the plant were tested for their AChE and BChE inhibition activity. The results revealed that the Methanol extract (MHRT) revealed highest inhibition potential for both AChE and BChE (198.6 ± 3.31 and 184.3 ± 2.92 mg GALE/g, respectively), as compared to other fractions (Table 8).

3.7.3. *In-vitro anti-inflammatory activity*

In response to various factors like infections, harmful chemicals, and thermal injuries, cells exhibit inflammation. This process is typically marked by redness, swelling, heat, and pain, which can ultimately result in exudation and loss of function. The body's potent inflammatory mediators include prostaglandins, leukotrienes, prostacyclins, lymphokines, and chemokines. While inflammation can serve a protective role, it can also have detrimental effects in conditions such as asthma, rheumatoid arthritis, vasculitis, and glomerulonephritis. Lipoxygenase (LOX) is an enzyme that contains metal and plays a role in the oxidation process of unsaturated fatty acids. Additionally, LOX is involved in the metabolism of arachidonic acid, leading to the production of

Table 6
Estimation of Antibacterial Activity (% Inhibition) of Crude Extract and Fractions of *Ruellia tweediana.*

	Salmonella typhi (-)	*Escherichia coli (-)*	*Bacillus subtilis (+)*	*Staphylococcus aureus(+)*	*Pseudomonas aeruginosa (-)*
MHRT	32.30 ± 2.86^{c}	32.75 ± 2.9^{c}	72.50 ± 2.10^{b}	70.10 ± 2.12^{c}	34.50 ± 1.36^{b}
NHRT	46.30 ± 2.25^{b}	49.75 ± 1.7^{b}	69.50 ± 1.10^{c}	75.10 ± 1.15^{b}	50.50 ± 2.35^{c}
CHRT	25.30 ± 2.69^{d}	24.20 ± 2.2^{e}	58.13 ± 2.97^{d}	63.50 ± 1.90^{d}	25.50 ± 2.95^{e}
NBRT	20.50 ± 2.78^{e}	28.00 ± 1.6^{d}	50.00 ± 2.30^{e}	57.25 ± 3.05^{e}	21.90 ± 2.55^{d}
Ciprofloxacin	61.60 ± 3.00^{a}	57.50 ± 2.8^{a}	76.80 ± 1.95^{a}	79.80 ± 2.40^{a}	66.60 ± 2.40^{a}

MHRT: methanol extract, NHRT: *n*-hexane fraction, CHRT: chloroform fraction, NBRT: *n*-butanol fraction. (-) = Gram negative, (+) = Gram positive and the superscripts a, b, c, and d represent the significant difference ($P \leq 0.05$).

Table 7
In-vitro Antidiabetic Potential of *Ruellia tweediana.*

Extracts	α-Glucosidase (mg ACAE/g)	α-Amylase (mg ACAE/g)
MHRT	116.6 ± 3.03^{b}	147.5 ± 3.56^{b}
NHRT	186.8 ± 2.84^{a}	179.7 ± 4.32^{a}
CHRT	83.89 ± 1.31^{c}	101.89 ± 2.7^{d}
NBRT	72.8 ± 1.12^{d}	118.6 ± 2.94^{c}

MHRT: methanol extract, NHRT: *n*-hexane fraction, CHRT: chloroform fraction, NBRT: *n*-butanol fraction. The superscripts a, b, c, and d significantly represent the difference ($P \leq 0.05$), ACAE: acarbose equivalent.

Table 8
In-vitro Acetylcholinesterase and Butyrylcholinesterase Inhibition Potential of *R. tweediana.*

Extracts	Acetylcholinesterase (mg GALE/g)	Butyrylcholinesterase (mg GALE/g)
MHRT	198.6 ± 3.31^{a}	184.3 ± 2.92^{a}
NHRT	122.7 ± 2.33^{d}	114.94 ± 2.10^{c}
CHRT	134.45 ± 3.45^{c}	138.71 ± 2.01^{b}
NBRT	153.03 ± 2.86^{b}	140.99 ± 2.24^{b}

MHRT: methanol extract, NHRT: *n*-hexane fraction, CHRT: chloroform fraction, NBRT: *n*-butanol fraction. The superscripts a, b, and c significantly represent the difference ($P \leq 0.05$), GALE: galantamine equivalent.

leukotrienes, which are potent inflammatory mediators. Due to its involvement in these processes, LOX is considered a potential target for drug therapy in various disorders. Inflammatory pathways greatly rely on the activity of lipoxygenase (LOX), making it a crucial enzyme in these pathways (Wisastra and Dekker, 2014).

MHRT, NBRT, CHRT and NHRT were tested to evaluate their inhibitory effects on lipoxygenase activity (Table 9). Among all extracts, NHRT showed maximum inhibition (225.94 ± 2.89 mg IndE/g). Indomethacin was used as standard. Outcomes were written as mg equivalent of Indomethacin/gram of dried extract.

3.8. *In-silico molecular docking studies*

The molecular docking interactions of all compounds identified with GC–MS analysis of NHRT were studied against α- glucosidase, α-amylase, urease, acetylcholinesterase, butyrylcholinesterase, tyrosinase, and lipoxygenase and the best binding energy of top 14 docked compounds are shown in Table 10. Lupeol showed best binding energy against all docked enzymes and even better than the standard compounds used in the inhibition assays. The interaction of Lupeol with each docked enzyme along with the standard compounds are shown in the figures (Figs. 2-6).

Table 9
In-vitro Lipoxygenase Inhibition of *R. tweediana.*

Extracts	Lipoxygenase (mg IndE/g)
MHRT	189.91 ± 1.98^{b}
NHRT	245.94 ± 2.89^{a}
CHRT	164.01 ± 2.11^{c}
NBRT	161.79 ± 2.62^{c}

MHRT: methanol extract, NHRT: *n*-hexane fraction, CHRT: chloroform fraction, NBRT: *n*-butanol fraction. The superscripts a, b and c significantly represent the difference ($P \leq 0.05$) and IndE: indomethacin equivalent.

4. Discussion

Phytochemicals refer to bioactive constituents naturally produced by plants, possessing numerous biological effects. Plants provide the main source to treat different microbial infections and chronic degenerative ailments like cancers and diabetes (Mendoza and Silva, 2018). For this purpose, the extracts of *R. tweediana* (whole plant) were prepared in 80 % hydroalcoholic solution by maceration. Primarily, the phytochemical analysis was performed using the protocols stated in the literature (Zheljazkov et al., 2021). We obtained positive results for phenols, tannins, flavonoids, saponins, glycosides, steroids, and terpenes (Table 2). Our findings agree with the previously reported studies performed on the other species of the genus *Ruellia* (Afzal et al., 2015). Major phytoconstituents i.e. flavonoids and tannins are antioxidant and antibacterial moities and saponins contribute to the treatment of diabetes, cancers, inflammations, and bacterial infections (Urzúa et al., 2008). The confirmation of these constituents in the extracts of *R. tweediana* might contribute to its therapeutic potential. Flavonoids

Table 10
Binding energy (Kcal/mol) of ligands with different enzymes.

Sr. No	Ligand	α-Gluco.	α-Amyl.	AChE	BChE	LOX
1	5-Hydroxymethylfurfural	−6.5	−4.6	−7.9	−6.7	−4.9
2	1H-Pyrrole, 1-(2-furanylmethyl)	−5.8	−5.1	−6.0	−5.0	−5.1
3	Bicyclo[4.1.0]heptane, 3-methyl-7- pentyl	−5.3	−6.2	−7.2	−6.2	−6.6
4	7-Butyl-6,6-dimethylbicyclo oct-7-en-2-one	−6.2	−6.2	−7.9	−6.4	−6.1
5	D-Tyrosine, 3-hydroxy-	−7.1	6.4	−7.1	−6.3	−6.3
6	Loliolide	−5.5	−4.6	−7.9	−6.7	−6.9
7	3-(4-hydroxy-3-methoxyphenyl)-, methyl ester, 2-Propenoic acid	−6.6	−6.6	−7.5	−6.6	−6.7
8	Pterin-6-carboxylic acid	−7.7	−6.9	−8.1	−7.3	−7.4
9	Ethanamine, 2-phenoxy	−6.9	−6.7	−7.4	−7.3	−7
10	Ledol	−6.9	−7.2	−8.6	−7.7	−7.2
11	Neoclovene oxide	−6.5	−7	−8.3	−7.4	−7.8
12	Thunbergol	−6.5	7.9	−9.3	−8.6	−7.2
13	Sesquicineole	−6.3	−6.8	−8	−7	−7.1
14	Lupeol	−7.9	−8.8	−11.9	−10.6	−8
15	Standard	−7.1^{a}	−7.7^{a}	−9.4^{b}	−9.2^{b}	−8.9^{c}

α-Gluco.: α-Glucosidase, α- Amyl.: α- Amylase, AChE.: Acetylcholinesterase, BChE.: Butyrylcholinesterase, LOX.: Lipoxygenase, a: acarbose, b: galantamine, and c: indomethacin

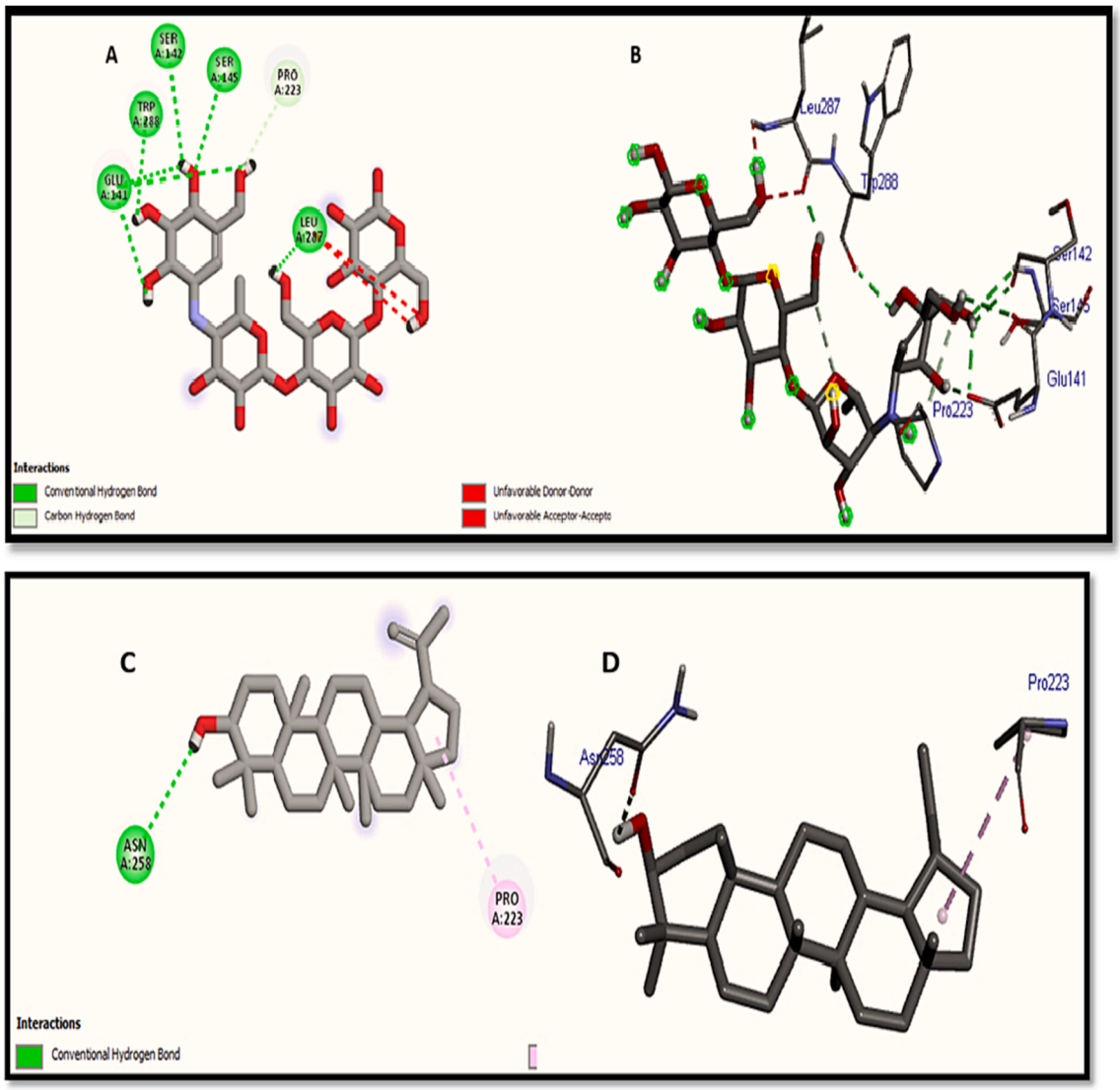

Fig. 2. 2D and 3D Interactions of α-glucosidase with Acarbose (A and B) and Lupeol (C and D).

and phenolic are active secondary metabolites having potential benefits (Fukalova Fukalova et al., 2022). In the current analysis, the maximum TPC value was observed in MHRT (167.34 ± 2.23 mg GAE/g). TFC results were also highest in MHRT (120.43 ± 1.71 mg QE/g) as compared to the fractions of *R. tweediana* as shown in Table 3. The findings of the study showed the similar trend as shown in a previously reported study in which phytochemical investigation of *Ruellia brittoniana* proved that it contained flavonoids, alkaloids, tannins, glycosides and triterpenes (Tejaputri et al., 2019). Currently, research is being conducted on all these endogenous metabolites because of their eminent pharmacological activities and medicinal values (El-Gawad et al., 2019).

Antioxidants are constituents that slow down the oxidation process caused by atmospheric oxygen. They play a crucial role in the prevention of pathogenesis caused by free radicals (Aziz et al., 2022). In this study, for the first time, four different extract/fractions (MHRT, NHRT, CHRT, and NBRT)) of *R. tweediana* were examined for their antioxidant potential to support its uses in aging and other disorders. The extracts of *R. tweediana* were evaluated to have significant antioxidant potency through DPPH, ABTS, CUPRAC, FRAP, and metal chelating assays.

In the current situation, MHRT and NHRT showed good antioxidant potential in all types of assays, i.e. DPPH, ABTS, FRAP, CUPRAC, and metal chelating activity. Minimum antioxidant potential was observed in NBRT (Table 5). The antioxidant potential determined by DPPH, ABTS, FRAP, CUPRAC, and metal chelating was highest in MHRT due to the occurrence of a maximum concentration of phenols and flavonoids, and this study is supported by literature in which ethyl acetate fraction of *R. patula* exhibited antioxidant activity with IC_{50} 25.5 ± 2.29 µg/mL in comparison with standard Trolox 16.7 ± 1.86 µg/mL while Ethyl acetate and chloroform extracts of *R. tuberosa* have significant antioxidant activity (Samy et al., 2015, Liu et al., 2019). Historically, diverse species belonging to the *Ruellia* genus have been extensively utilized for their antioxidant properties in Ayurvedic medicines. In a study reported earlier, the antioxidant activity of *Ruellia brittoniana* was investigated. The flowers of *Ruellia brittoniana* exhibited significant antioxidant potential, as evidenced by an IC_{50} value of 68.42 ppm (Tejaputri et al., 2019). The *R. tweediana* can be considered as a candidate for the additional antidiabetic agent.

In the present investigation, the antibacterial potential of all sample extracts was assessed at the concentration of 20 µg/well. The results revealed that NHRT was more efficient in suppressing the growth of all bacterial strains with variable potency as stated in Table 6. Previously, *R. tuberosa* is reported with potent antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli Proteus mirablis, Klebsiella pneumoniae, Bacillus subtilis,* thus, supporting the antibacterial potential of current research work (Arirudran et al., 2014, Samy et al., 2015). *Pseudomonas aeruginosa* is the main reason for infection in patients with

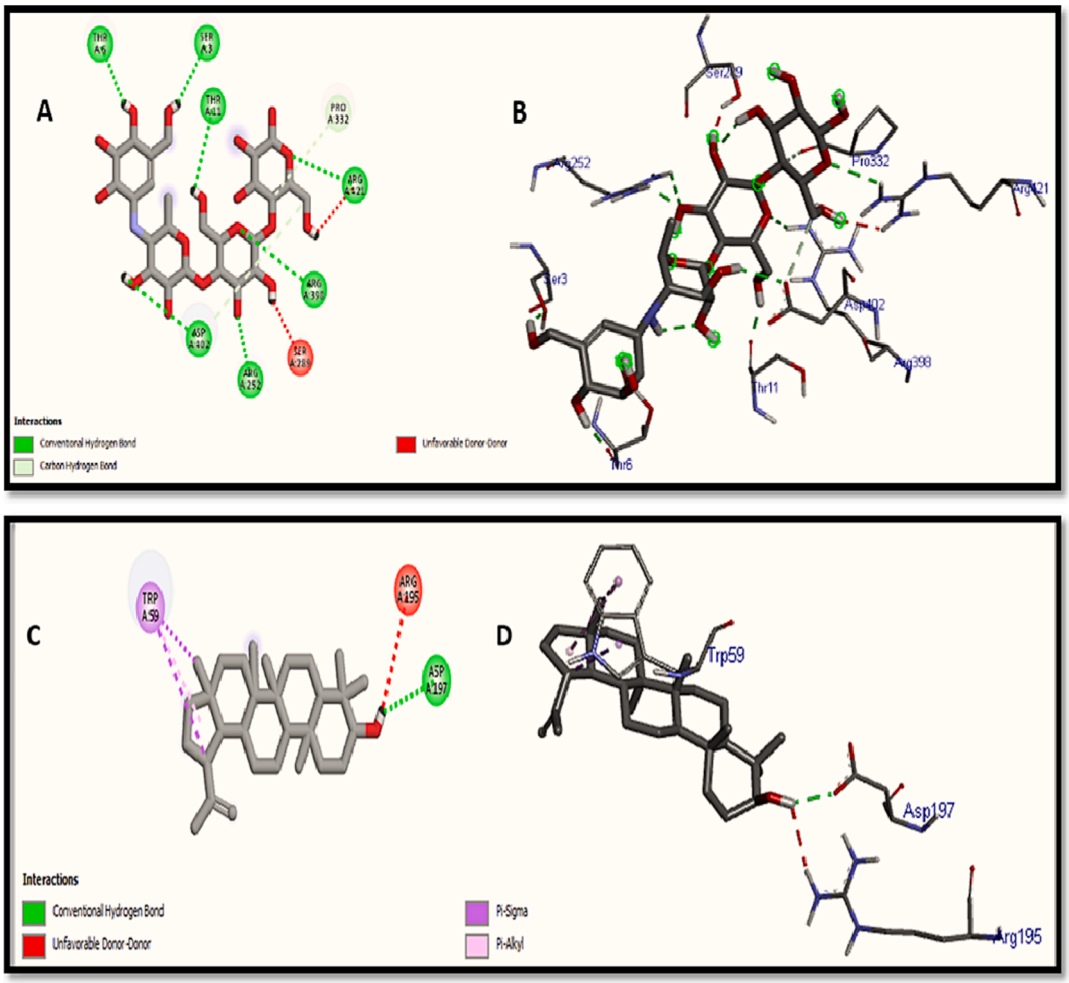

Fig. 3. 2D and 3D Interactions of α-amylase with Acarbose (A and B) and Lupeol (C and D).

compromised defense mechanisms and is the most known pathogen isolated from hospitalized patients with a longer stay and is a major source of nosocomial infections. *P. aeruginosa* infections are sometimes life-threatening and complicated, while *P. aeruginosa* typically causes blood, airways, and urinary tract infections (del Barrio-Tofiño et al., 2020, Murgia et al., 2020). *E. coli* is the most causative bacteria for various diseases, e.g. gastroenteritis, urinary tract infections, and bloodstream infections (de Lastours et al., 2020, Zalewska-Piątek and Piątek, 2020). Thus, NHRT can serve to provide a lead antibacterial drug, and this study is supported by the antibacterial potential of other species of the genus *Ruellia* (Samy et al., 2015). The presence of flavonoids might be responsible for the antibacterial potential of NHRT as flavonoids have gained significant attention due to their well-known ability to fight various pathogenic microorganisms as effective antibacterial moieties. Given the escalating incidence of untreatable infections caused by antibiotic-resistant bacteria, these compounds have arisen as promising alternatives to antibiotics. Their ability as substitutes for conventional antibiotics has sparked extensive interest in the scientific community (Prabuseenivasan et al., 2006, Xie et al., 2015).

Diabetes mellitus is one of the most complicated problem of modern societies. Diabetes mellitus causes high blood sugar and it is associated with long-term damage and failure of many vital organs (Ota and Ulrih, 2017). As there is no definite treatment for diabetes, the use of natural and traditional medicine is suitable solution to manage it (Bahmani et al., 2014). For this purpose, the extracts MHRT, NHRT, CHRT, and NBRT of *R. tweediana* were screened for enzyme inhibition activity against α-glucosidase and α-amylase for the first time. NHRT exhibited highest inhibition against α-glucosidase (186.8 ± 2.84 mmol of ACAE/g) and α-amylase (179.7 ± 4.32 mmol of ACAE/g) as compared to MHRT, CHRT, and NBRT (Table 7). The inhibition of α-glucosidase and α-amylase was assessed by using acarbose as standard. This investigation may be supported by the presence of terpenes, flavonoids, and polyphenols as discussed in a previously reported study (Pavlić et al., 2021).

MHRT, NHRT, CHRT, and NBRT of *R. tweediana* under analysis showed notable effects on acetylcholinesterase, butyrylcholinesterase, and lipoxygenase inhibition activities (Table 8and 9), suggesting its potential therapeutic relevance in managing neurodegenerative disorders and inflammatory conditions. Exploring antioxidants as potential therapies for neurodegenerative disorders and anti-inflammatory diseases is gaining significant attention in current research. Studies indicate that fractions rich in phenolics and flavonoids exhibit robust cholinesterase inhibition activity, suggesting a promising avenue for addressing cognitive impairment associated with neurodegenerative illnesses (Dilshad et al., 2022b). Additionally, the extracts having antioxidant potential, also showc potent anti-inflammatory properties, potentially influencing pathways relevant to inflammatory diseases (Rodríguez-Yoldi, 2021). The inhibition of acetylcholinesterase and butyrylcholinesterase is particularly significant, as these enzymes are associated with the regulation of neurotransmitter levels, and their dysregulation is implicated in cognitive decline observed in conditions like Alzheimer's disease (Khan et al., 2023). The observed inhibition of lipoxygenase, an enzyme involved in the inflammatory pathway, further highlights the extract's anti-inflammatory properties (Dilshad et al.,

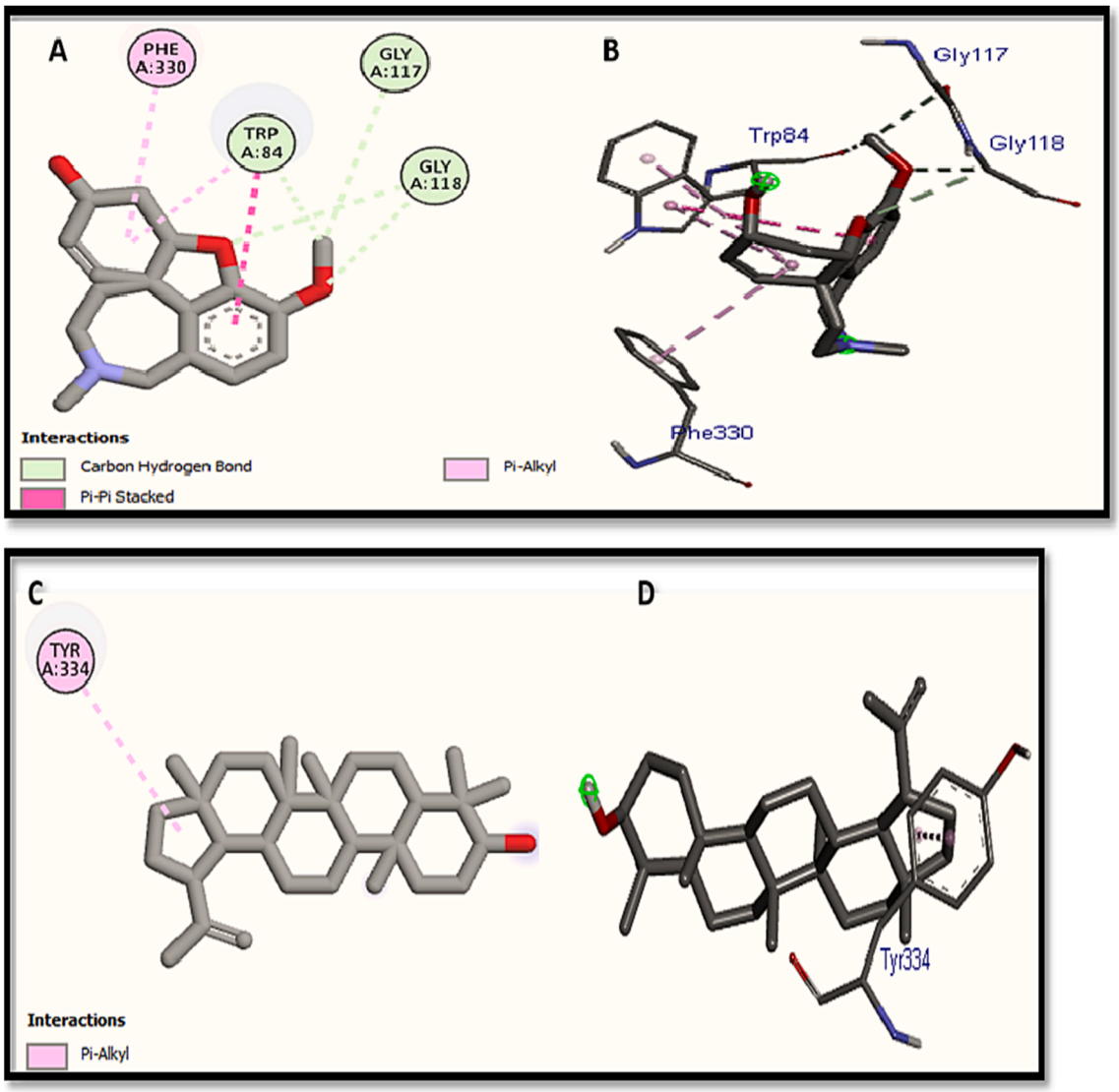

Fig. 4. 2D and 3D Interactions of acetylcholinesterase with Galantamine (A and B) and Lupeol (C and D).

2023). These findings underscore the multifaceted pharmacological potential of the plant extract, positioning it as a promising candidate for further exploration in the development of novel therapeutic interventions for neurodegenerative diseases and inflammatory disorders.

Gas Chromatography-Mass Spectrometry (GC–MS) is a valuable analytical technique used for the identification of chemical compounds in complex mixtures, such as the *n*-hexane fraction of a plant extract. The *n*-hexane fraction is particularly crucial in phytochemical analysis due to its capability to selectively extract non-polar compounds from the plant material. By subjecting the *n*-hexane fraction to GC–MS, a wide range of volatile and semi-volatile compounds can be identified, providing comprehensive insights into the plant's chemical composition. This information is critical for understanding the possible bioactive compounds present in the plant, which could have significant implications for numerous applications in pharmaceuticals and natural products research (Ghalloo et al., 2022). Additionally, the identification of these compounds through GC–MS allows researchers to explore the plant's ecological effects, interactions with other organisms, and potential benefits for human health and well-being. The *n*-hexane fraction of *Ruellia tweediana* (NHRT) exhibited better biological activities, including enzyme inhibition, and antioxidant and antibacterial activity, The NHRT fraction was subjected to GC–MS and 40 bioactive compounds were identified (Table 4). These compounds belong to several chemical classes. These bioactive compounds may be responsible for the biological potential of the plant. Lupeol decreases the serum glucose level with a related increase in insulin level (Gupta et al., 2012). Lupeol decreases the oxygen-reactive species hence, can be used in aging and pigmentation. In a previous study, it is reported with antioxidant, anti-inflammatory, antimicrobial, and anticancer potential, thus supporting its biological activities. (Liu et al., 2021). Lupeol has good antioxidative, anti-inflammatory, and antinociceptive potential and may be responsible for the maximum enzyme inhibition potential of *n*-hexane extract. It also possesses a crucial role in the wound healing and contributes to the normalization of a lipid profile to protect against hypercholesterolemia, mostly associated with renal and liver damage (Laghari et al., 2011). Phytol has antioxidant, anti-inflammatory, anti-allergic, and antinociceptive activities. Phytol has also significant antibacterial activity against *S. aureus* and *M. tuberculosis*. Studies have revealed excellent immunostimulant effects of phytol to activate innate and acquired immunity (De Moraes et al., 2014).

In the process of drug development, the computational approach of molecular docking is worth going technique. It has the advantage of identifying the binding interactions between ligands and macromolecule (Chandak et al., 2014). In this analysis, the *in-silico* molecular docking interactions were studied on the phytocomponents identified with GC–MS of NHRT. The best binding energy (Kcal/mol) was observed

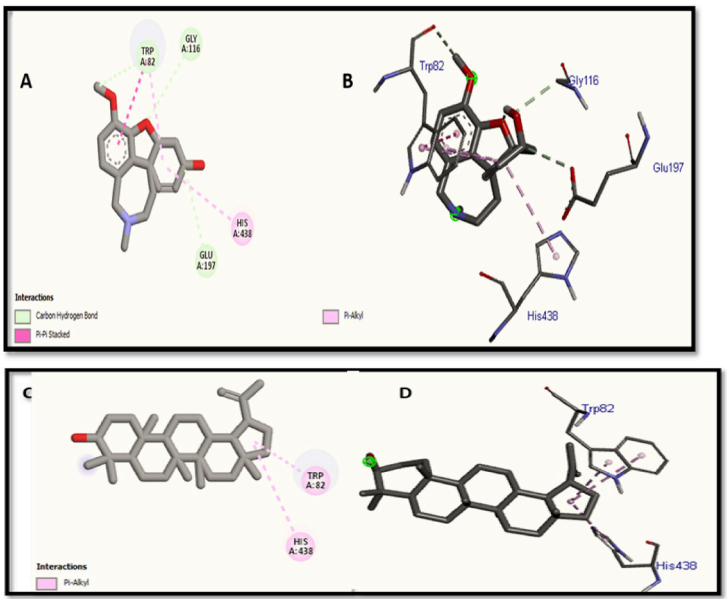

Fig. 5. 2D and 3D Interactions of butyrylcholinesterase with Galantamine (A and B) and Lupeol (C and D).

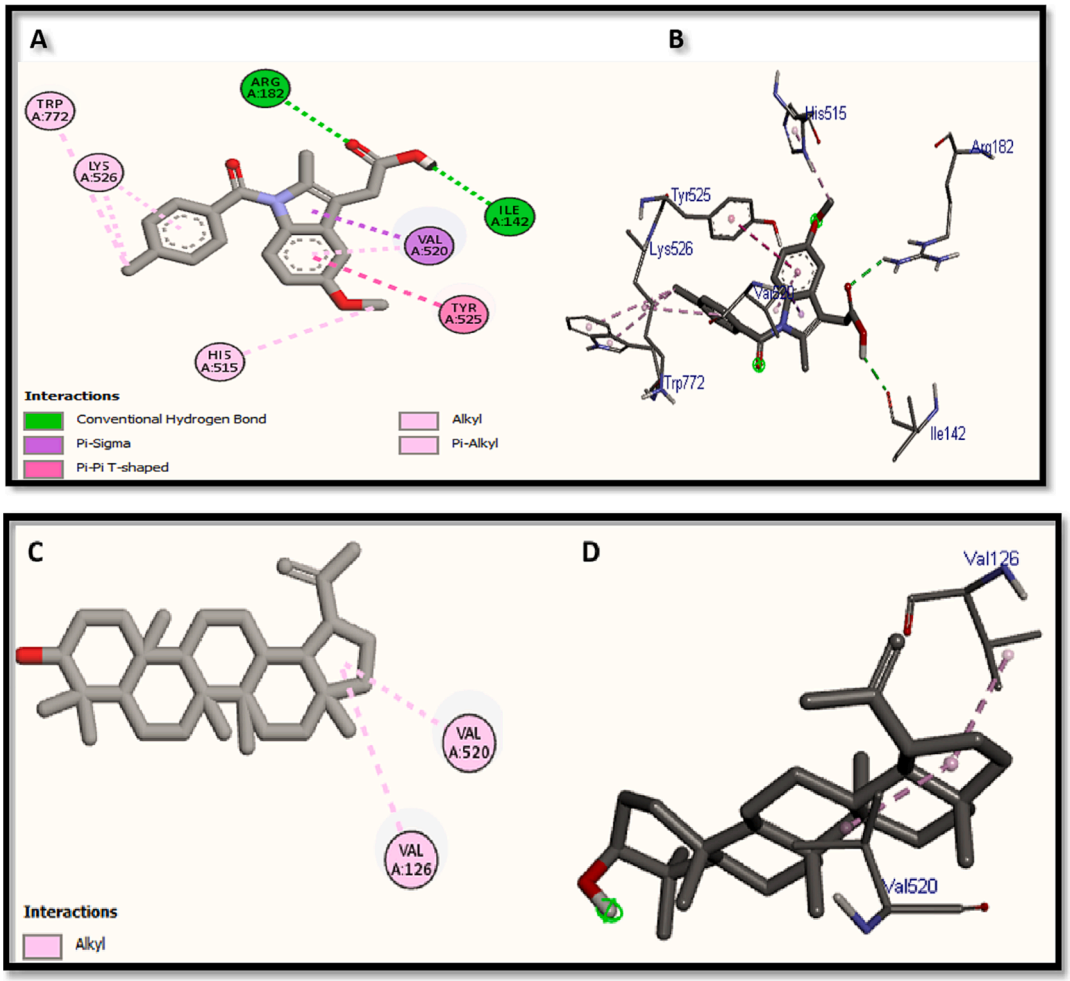

Fig. 6. 2D and 3D Interactions of lipoxygenase with Indomethacin (A and B) and Lupeol (C and D).

for Lupeol against α-glucosidase −7.9, α-amylase −8.8, tyrosinase −8.4, urease −9.4, acetylcholinesterase −11.9, butyrylcholinesterase −10.6 and lipoxygenase −8.0. The binding energies of the compounds tentatively identified showed that all have good potential in preventing and treating diseases related to these enzymes but Lupeol is a more efficient candidate in treating neurological disorders and as anti-ulcer.

So, it is concluded that further work is required on this plant for purification and characterization studies of compounds responsible for its significant enzyme inhibition, and antioxidant and antibacterial activities to discover new drug leads.

5. Conclusions

The study offers comprehensive insights into the phytochemical composition and bioactivity of *Ruellia tweedina*, an unexplored plant, shedding light on its potential applications in numerous fields. The evaluation of polyphenolic contents (TPC and TFC) revealed the presence of abundant polyphenolic contents, which are known for their antioxidant properties. The GC–MS screening further unveiled a diverse array of bio active compounds in the *n*-hexane fraction, which could contribute to the observed bioactivities. The findings of antibacterial activity showed that *Ruellia tweediana* is an excellent candidate for treating bacterial infections. Remarkably, all the tested extracts and fractions revealed significant antioxidant and enzyme inhibition activities, highlighting the plant's potential as a valued source of bioactive phytoconstituents. Notably, *Ruellia tweedina* showed maximum activity against lipoxygenase and α-glucosidase, signifying their possible use in anti-inflammatory and anti-diabetic therapies. The binding energies of GC–MS identified phytocnstituents showed that most of these compounds have good potential in preventing and treating diseases related to these enzymes used in molecular docking. So, it is concluded that further work is required on this plant for isolation and characterization studies of compounds responsible for its significant antioxidant, anti-diabetic, anti-inflammatory, and antibacterial activities to discover new drug lead.

CRediT authorship contribution statement

Shamsa Kanwal: Writing – original draft. **Saeed Ahmad:** Supervision, Project administration. **M. Yasmin Begum:** Conceptualization, Funding acquisition. **Ayesha Siddiqua:** Conceptualization, Funding acquisition. **Huma Rao:** Validation, Investigation. **Bilal Ahmad Ghalloo:** Methodology, Writing – original draft, Writing – review & editing. **Muhammad Nadeem Shahzad:** Validation, Writing – review & editing. **Imtiaz Ahmad:** Software. **Kashif-ur-Rehman Khan:** Investigation.

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