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Unlocking the therapeutic potential of *Catharanthus roseus* leaves via in-vitro, in-vivo, and in-silico study

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Catharanthus roseus **(L.) (CR), is a perennial flowering herb, has traditionally been used by local populations in South Asia, Africa, China and Malaysia to treat diabetes, cancer and microbial infection. The principal objective of this study was to investigate the antidiabetic, antilipidemic and antiarthritic effects of methanol (MCR) and ethanol (ECR) extracts and dichloromethane fraction (DCR) of *Catharanthus roseus* leaves by in-vitro, in-vivo, and in-silico model. The in-vivo antihyperglycemic activity of *C. roseus* was explored in streptozotocin (STZ)-induced Type- 1 diabetic mice model. MCR, ECR and DCR exhibited a significant ability to lower fasting blood glucose levels and normalized the altered body weight when treatment was carried out for 20 days in a dose-dependent manner. Among the experimental samples, ECR at 200 mg/kg (4.95 ± 0.31 mmol/L) showed greater fasting blood glucose lowering activity than the standard drugs metformin (6.8 ± 0.34 mmol/L), and glibenclamide (5.20 ± 0.29 mmol/L). MCR at 200 mg/kg, likewise significantly ($p < 0.001$) reduced the blood glucose (5.70 ± 0.12 mmol/L) compared to the control at day 20. The lipid profile (LDL, HDL, TG, and TC) of diabetic mice was significantly reduced by ECR in a biochemical study compared to the control group. The in-vitro antidiabetic study revealed a dose-dependent hypoglycemic potential of the extracts and fraction; where ECR showed the highest α-amylase and α-glucosidase inhibitory effects with the IC_{50} value of 0.62 ± 0.02 mg/ml and 0.64 ± 0.01 mg/ml respectively. The antiarthritic activity was assessed by using bovine serum albumin denaturation inhibition assay and the denaturation was found to be inhibited by all extracts and fraction. The MCR and DCR fractions inhibited the denaturation by 70.20% and 62.09%, respectively, at 1600 µg/mL compared to the standard aspirin (81.57%). Moreover, the Gas chromatography-Mass spectrometric (GC-MS) investigation of the extract revealed several bioactive constituents and the in-silico docking study suggested that Ergost- 5-en- 3-ol, (3.beta.)-; Stigmasterol; 9,19-Cyclolanostan- 3-ol, acetate, (3.beta.)-;.gamma.-Sitosterol, and 24-Norursa-3,12-diene would be the probable lead compounds for hypoglycemic and antiarthritic effect in many potential targets, however, further mechanistic and experimental elucidation is needed to solidify our findings.**

Keywords *Catharanthus roseus*, GC-MS study, Antidiabetic activity, Streptozotocin, Antilipidemic, Antiarthritic, Molecular docking

The origins of certain elements of contemporary medicine can be traced back to the ancient utilization of plants for therapeutic purposes, a practice that predates the existence of human civilization[1]. Approximately 75–80% of the global population residing in undeveloped nations predominantly relies on herbal medicine as their primary treatment modality[2]. This can mainly be attributed to the widely held belief that herbal medications are cost-effective, readily accessible, and devoid of adverse effects[3]. According to the World Health Organization (WHO), the consumption of herbal drugs is two to three times greater than that of pharmaceutical medications[4].

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Diabetes mellitus is a pathological condition characterized by a persistent increase in blood glucose levels. Hyperglycemia is a manifestation of diabetes mellitus (DM), also known as diabetes. Diabetes is a long-lasting illness characterized by intricate physiological processes that might manifest in diverse manners as time progresses[5].Presently, the existing antidiabetic drugs are not devoid of significant adverse effects, and their effectiveness is also constrained by their high cost when administered over an extended period. The search for antidiabetic phytochemicals is a significant focus of medicinal plant research.

Rheumatoid arthritis (RA) is a persistent, widespread, and inflammatory autoimmune disease that affects the joints[6]. Systemic autoimmune disease impacts several organs beyond the joints, including the skin, eyes, lungs, heart, kidneys, salivary glands, nerve tissue, bone marrow, and blood vessels[7]. Nevertheless, rheumatoid arthritis primarily targets the joints. This prevalent immunological disorder impairs the mobility and functionality of the joints, significantly impacting the physical health and overall quality of life of affected individuals. Based on epidemiological data, the global prevalence of RA is about 1%, which signifies that around 700 million individuals worldwide have RA, where more than 80% of RA patients are women[8,9].

Patients with RA are obligated to modify their lifestyle[10]. Treatment for RA mostly consists of non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoid drugs, and disease-modifying anti-rheumatic therapies[11]. Recent preclinical investigations have shown that natural plant extracts (NPE) have a substantial effect in reducing the symptoms of RA[9,12]. Considering the multifaceted nature of NPE drugs used to treat RA, investigating the therapeutic potential of NPEs for RA therapy might be advantageous for individuals with RA. Therefore, in this study, evaluation of antiarthritic potential of *Catharanthus roseus* was one of our focused research fields.

C. roseus is a perennial subshrub or herbaceous plant with a maximum height of 1 m and is a member of the family Apocynaceae[13]. The leaves exhibit an alternate arrangement and vary in shape from oval to oblong[14]. Scientists investigating the medical qualities of the substance found that it contained a group of alkaloids that, despite their high toxicity, could be beneficial in cancer therapy[15–18]. The ethanolic extracts of *C. roseus* leaves demonstrated a dose-dependent decrease in blood sugar[19].Leaf juice had a notable effect on decreasing the total cholesterol, triglyceride, LDL-c, and VLDL-c levels in the blood serum[20]. Additionally, *C. roseus* evidenced to possess various medicinal properties including antimicrobial[21,22], antiviral[23], antifungal[24], anticancer[25,26], wound healing[27], antihypertensive[14], anti-inflammatory[28],antidiuretic[14], antimalerial[29], cardiotonic, and cytotoxic activity[14]. This study aimed to analyze the phytochemicals and bioactive components of leaf extracts and fraction from the *Catharanthus roseus* plant using GC–MS. Additionally, the study investigated the potential in-vivo, in-vitro, and in-silico antidiabetic, antilipidemic, and antiarthritic activity of these extracts, which have been traditionally utilized in medicine. Numerous researchers have proved the antidiabetic action of *C. roseus* in their studies. In one study, the leaf juice of *C. roseus* showed dose-dependent reduction in blood glucose of both normal and diabetic rabbits that is comparable to the standard drug, glibenclamide[30]. Ethanolic extract of *Catharanthus roseus* exhibited significant anti-hyperlipidemic & anti-diabetic effects ($p < 0.05$, $p < 0.01$) when compared with control[31]. Singh *et al.,* (2001) investigated with dichloromethane: methanol extract (1:1) of leaves and twigs of *Catharanthus roseus* and obtained considerable reduction of fasting blood glucose in streptozotocin (STZ) induced diabetic rat model at 500 mg/kg oral dose[32]. As an evidence from previous studies, the ethanolic extract of *Catharanthus roseus* exhibited significant anti-arthritic activity in cotton pellet granuloma method and Freund's adjuvant induced arthritis models[33]. In spite of a plethora of studies on *C. roseus*, no work has been documented on comparative study of *C. roseus* using different solvent systems. So we aimed to compare among two extracts and one fraction of *C. roseus* leaves in respect to their hypoglycemic, antihyperlipidemic and in vitro anti-arthritic potential.

Materials and methods

Materials Methanol, sodium chloride, and Twin 80. Streptozotocin was purchased from Lova, India. Metformin hydrochloride and glibenclamide, which are active pharmaceutical ingredients (APIs), were obtained from Square Pharmaceuticals Limited and Opsonin Pharma, respectively.

Plant collection and identification For the present study, *Catharanthus roseus* was collected from the Savar area, Dhaka, Bangladesh, and was distinguished from the Jahangirnagar University Herbarium, Dhaka, where the accession number was JUH 10,122. The botanical identity of this plant was detected and authenticated from the literature available in the Department of Botany, Jahangirnagar University, Dhaka. **Mohammad Abdur Rahim**, Principal Experimental Officer, Department of Botany, Jahangirnagar University was responsible for this identification.

Approximately 5 kg of the whole plant was collected and cleaned with tap water. The collected leaves were dried under sunlight for two weeks before being subsequently ground into a coarse powder using a suitable grinder. After that, the powder was sieved, and the granular section was regenerated. The powder was then measured using an analytical balance after the ground part was sieved once more. The powder was then placed in a waterproof container and kept cool, dull, and dry until the research began.

Extraction of plant materials In a clean, round-bottomed, amber-colored extraction vial, 500 g of powdered leaf material was immersed in 1.5 L of methanol and ethanol separately. The containers and their contents were wrapped in foil and maintained for 15 days, shaking and stirring occasionally. After that, the entire mixture was filtered through a new cotton plug and then through Whatman's No.1 filter paper. Under a rotary vacuum evaporator, the filtrate was evaporated. This resulted in a blackish-green sticky concentration. The sticky concentrate was labeled as a crude extract. The methanol crude extract was subsequently fractionated with dichloromethane to obtain another sample of the plant[34].

Study animals In-vivo experiments were performed using swiss-albino mice. Mice were collected from the Pharmacology Laboratory at Jahangirnagar University, Savar, Dhaka, Bangladesh. After the specific study period, ketamine (500 mg/kg intraperitoneally) was used to euthanize the study mice before further investigation. All activities involving swiss-albino mice were undergone according the approved protocol of institutional ethical review committee (**Biosafety**, **Biosecurity & Ethical Committee**, **Faculty of Biological Sciences**, **Jahangirnagar University**, **Savar**, **Dhaka**, **Bangladesh**).

Ethics declarations All these animal related procedures were conducted in accordance with the United Kingdom Animal (Scientific Procedures) Act 1986, approved by institutional ethical review committee *(***Biosafety**, **Biosecurity & Ethical Committee**, **Faculty of Biological Sciences**, **Jahangirnagar University**, **Savar**, **Dhaka**, **Bangladesh**) and conducted under the authority of the Project Licence (*Approval Ref No*: ***BBEC***, ***JU/M 2024/01 (79)***).

Use of experimental animals As we worked with live vertebrates, **Biosafety**, **Biosecurity & Ethical Committee** of Jahangirnagar University approved our overall experiments, like collection, acclimatization, further study and sacrifice, blood collection and disposal of the mice as well. Proper guidelines and scrutiny in all experiments were ensured by this authorizing committee.

In-vitro antidiabetic assay

α-amylase inhibition assay α-Amylase inhibition was carried out using the starch-iodine method. For the experiment, labeled test containers were used. In each container, 1 ml of either experimental sample (at concentrations of 2, 1, or 0.5 mg/ml) or standard (at concentrations of 1, 0.5, or 0.25 mg/ml) was added, together with 20 µl of α-amylase. Following a 10-minute incubation period at 37 °C, a 200 µl aliquot of 1% starch solution was introduced into every test tube. The mixture was incubated again for one hour at 37 °C. Subsequently, 200 µl of 1% iodine solution was carefully added to each test tube, and then 10 ml of distilled water was gently poured in. The absorbance of the mixture was measured at a wavelength of 565 nm. Substrate, sample, and α-amylase blank were all tested using identical conditions. Each experiment was conducted in triplicate to ensure accuracy[35].

Calculation

$$\% \text{ of } \alpha - \text{amylase inhibition } = 1 - \frac{(\boldsymbol{SA} - \boldsymbol{SBB}) - \boldsymbol{SMB}}{\boldsymbol{AAB}} \times \ 100$$

SA = Sample/Standard Absorbance, SBB = Substrate Control, SMB = Sample control AAB = α-amylase control.

The median inhibitory concentration (IC_{50}) values were calculated using MSExcel (Office 10) software.The full graph was created using Graph Pad Prism Software version 8.0.

α-Glucosidase Inhibition Assay Using a p-nitro-phenyl—D glucopyranoside (p-NPG) substrate solution, we determined whether the extract inhibited glucosidase activity. In addition, alpha-glucosidase was diluted to 0.1 units/ml in the same potassium phosphate buffer. Dimethyl sulfoxide was used to dissolve the solution. Forty milliliters of the substrate (p-NPG) solution was added to 20 milliliters of sample solution and stirred. An 80-milliliter dose of sodium carbonate solution (0.2 M) was added following a 40-minute incubation at 70 °C.Finally, the absorbance at 405 nm was measured using a double-beam UV spectrophotometer[36].

To compute the inhibition percentage, we used the following formula:

$$\% \ \mathbf{inhibition} = (\mathbf{A_C} - \mathbf{A_S}) / \mathbf{A_C} \times 100$$

where, A_C = absorbance of the control and A_S= absorbance of the sample.

Finally, the IC_{50} was determined by comparing the percentages of inhibition achieved by each extract with that of the reference medication. The median inhibitory concentration (IC_{50}) values were calculated using MSExcel (Office 10) software.The full graph was created using Graph Pad Prism Software version 8.0.

In-vitro antiarthritic activity

Protein denaturation assay using bovine serum albumin (BSA) The experimental setup involved a reaction mixture with a total volume of 0.5 ml. The mixture consisted of 0.45 ml of a 5% aqueous solution of bovine serum albumin (BSA) and 0.05 ml of different concentrations (12.5, 25, 50, 100, 200, 400, 800, and 1600 µg/ml) of *C. roseus* crude extract, fraction, and aspirin (used as a reference drug), individually. The pH of each solution was adjusted to 6.3 using 1 N HCl. Following a 20-minute incubation at 37 °C, the samples underwent a subsequent 30-minute heating period at 57 °C. Following that, a phosphate buffer of 2.5 ml was introduced, and the absorbance was measured at 660 nm using a spectrophotometer. The test and product controls were both prepared, with the previous ratiocontaining 0.05 ml of distilled water instead of extract and the product control without BSA[37].

The following formula was used to determine the percentage inhibition of protein denaturation:

$$\mathbf{Percentage\ inhibition} \ = \ 100 - \left[\frac{\mathbf{Abs\ Test\ Solution} - \mathbf{Abs\ Product\ Control}}{\mathbf{Abs\ Test\ Control}}\right] \times \ 100$$

Abs = Absorbance.

The median inhibitory concentration (IC_{50}) values were calculated using MSExcel (Office 10) software. The full graph was created using Graph Pad Prism Software version 8.0.

Acute toxicity study Plant extracts of *A. indianum* were administered orally in a dose of 50, 100, 200, 400, 800, 1600 mg/kg to groups of mice (n= 6) and % of mortality was noted 24 h later[38].

In-vivo anti-diabetic study

As this work involved experimental animals, all the experimental procedures were performed according the **ARRIVE** guidelines as follows.

Study design In-vivo anti-diabetic assay was performed with 60 swiss-albino mice divided in 10 groups consisting 6 mice in each group. The groups were numbered from I to X. Different groups were treated differently e.g. with streptozotocin, plant extracts/fraction, standard drugs. Plasma samples of mice were collected and analyzed on different days.

Sample size 6 mice were kept in each group and treated as follows: Group I – untreated (control), Group II – Streptozotocin-treated (diabetic), Group III– Streptozotocin-induced diabetic mice were treated with 100 mg/kg/day metformin (standard)[39], Group IV– Streptozotocin-induced diabetic mice were treated with 10 mg/kg/day glibenclamide (standard), Group V– Streptozotocin-induced diabetic mice treated with 100 mg/kg p.o. methanolic extract of the leaves of *Catharanthus roseus* (MCR) for 20 days (treated), Group VI– Streptozotocin-induced diabetic mice treated with 200 mg/kg p.o. methanolic extract of *Catharanthus roseus* (MCR) leaves for 20 days, Group VII- Streptozotocin-induced diabetic mice were treated with 100 mg/kg ethanolic extract of the leaves of *Catharanthus roseus* (ECR) p.o. for 20 days, Group VIII- Streptozotocin-induced diabetic mice were treated with the ethanolic extract of the leaves of *Catharanthus roseus* (ECR) at 200 mg/kg p.o. for 20 days (treated), Group IX- Streptozotocin-induced diabetic mice were treated with 100 mg/kg P.O. dichloromethane fraction from the leaves of *Catharanthus roseus* (DCR) for 20 days, Group X- Streptozotocin-induced diabetic mice were treated with 200 mg/kg P.O. dichloromethane fraction from the leaves of *Catharanthus roseus* (DCR) for 20 days.

Inclusion and exclusion criteria Among the selected 60 mice, 54 streptozotocin induced diabetic mice showing fasting blood glucose level of 15 mmol/l were included in this study and grouped from II-X. 6 mice were not injected with streptozotocin and kept in group I as non-diabetic normal control group.

Randomisation 60 swiss-albino mice were collected from the Pharmacology Laboratory at Jahangirnagar University. Then, the animals were acclimated in a typical environment for one week (at 24.0 °C temperature, 55–65% relative humidity, and a 12-hour light/12-hour dark cycle) and given food and water ad libitum.

All the mice were distributed in 10 groups randomly keeping 6 mice in each group. All ten groups were kept in separate cages. Tail of each mouse was stained with marker to monitor them effectively throughout the study period. The identification markings were renewed on a regular basis to ensure clear monitoring.

Blinding For each animal, three different investigators were involved as follows: a first investigator administered the treatment based on the initial randomization. This investigator was the only person aware of the treatment group allocation. A second investigator (SC) was responsible for the anaesthetic procedure, whereas a third investigator performed the surgical procedure.

Outcome measures Body weight and fasting blood glucose level of mice were measured on day 1, 5, 10, 15 and day 20. Glucose oxidase-peroxidase reactive strips and a glucometer were used to test blood glucose and blood glucose levels on days 1, 5, 10, 15, and 20 of the study. On the 20 th day after the animals were decapitated, the effects of the methanolic and ethanolic extracts and dichloromethane fraction of *Catharanthus roseus* on diabetic mice were assessed. In diabetic mice treated with extracts and fraction, fasting blood glucose levels were measured and compared to those of diabetic control and normal untreated animals[40].

On day 20, Blood samples were collected using fine capillary heparinized tubes from each mouse (overnight fasting) after anesthesia by intraperitoneal injection of ketamine (500 mg/kg) followed by puncturing the retro-orbital plexus. Collected blood was stored in a heparinized tube that was centrifuged at 3000 rpm to get the serum. It was used to estimate lipid profile (TC, TG, LDL, and HDL) following the procedures provided with the respective kit. A semi-automated analyzer (Humalyzer 3000) was used to estimate biochemical parameters[41]. After blood collection, mice of all study groups were sacrificed and disposed properly according to the guidelines of United Kingdom Animal (Scientific Procedures) Act 1986.

Statistical methods The data is displayed as the average of several individual experiments (n= 6) with the standard error of the mean (SEM). The software GraphPad Prism (Version 8) is utilized for conducting an ANOVA followed by a Dunnett's test. The statistical significance levels of $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ were utilized to assess the significance of the results in comparison to the control group or between groups. The full graph was created using Graph Pad Prism Software version 8.0.

Experimental animals The experiment used 60 white furred young swiss-albino mice of either sex aged 6–8 weeks and weighing 25–30 g.

Treatment groups	Body weight (gm)				
	Day 01	Day 5	Day 10	Day 15	Day 20
Normal control	29.75 ± 3.15	30.87 ± 2.34	33.26 ± 2.22	35.06 ± 1.87	36.43 ± 1.70
Diabetic Control	28.79 ± 2.5	29.49 ± 1.65[a]	32.06 ± 2.18 [a]	26.54 ± 1.24 [a]	24.14 ± 2.69 [a]
MET	32.75 ± 2.64	29.87 ± 0.94	26.34 ± 2.32	23.21 ± 2.36	22.89 ± 2.58
GBN	30.75 ± 1.88	29.05 ± 2.42	31.65 ± 3.02	33.67 ± 2.23*	34.87 ± 3.65**
MCR 100	32.45 ± 2.93	31.21 ± 2.56	32.34 ± 3.19	33.67 ± 2.43*	33.01 ± 2.59*
MCR 200	29.23 ± 2.67	28.65 ± 2.45	31.89 ± 3.67	32.47 ± 3.69	34.58 ± 4.26*
ECR 100	31.08 ± 3.51	29.67 ± 4.96	31.08 ± 3.09	33.36 ± 3.78*	34.92 ± 2.38**
ECR 200	29.98 ± 2.87	30.1 ± 1.76	33.78 ± 4.62	34.95 ± 2.36*	36.01 ± 3.61**
DCR 100	30.58 ± 3.18	28.85 ± 4.19	29.78 ± 5.56	31.75 ± 3.49	32.00 ± 2.65
DCR 200	29.86 ± 2.68	30.27 ± 2.89	31.98 ± 1.48	33.97 ± 1.91	34.23 ± 2.03*

Table 1. Effect of *C. roseus* on the body weight of diabetes model mice. MET = Metformin hydrochloride, GBN = Glibenclamide, MCR = Methanol extract of *C. roseus*, ECR = Ethanol extract of *C. roseus*, DCR = Dichloromethane fraction of *C. roseus*. [Each value represents the mean ± SEM ($n = 6$ animals per group)]. [a]Represents statistical significance vs. normal control ($p < 0.05$). *Represents statistical significance vs. diabetic control ($p < 0.05$). **Represents statistical significance vs. diabetic control ($p < 0.01$).

Treatment groups	Fasting Blood Glucose Concentration (mmol/l)				
	Day 01	Day 5	Day 10	Day 15	Day 20
Normal control	5.62 ± 0.21	5.28 ± 0.18	6.01 ± 0.22	7.01 ± 0.09	7.10 ± 0.11
Diabetic Control	18.83 ± 0.96	19.79 ± 1.04	19.32 ± 0.83	20.58 ± 1.28	20.98 ± 1.06
MET	18.55 ± 0.64	13.30 ± 0.94	10.36 ± 0.54*	8.19 ± 0.36**	6.79 ± 0.34***
GBN	18.41 ± 0.74	10.29 ± 0.42*	9.38 ± 0.43*	6.73 ± 0.27***	5.20 ± 0.29***
MCR 100	19.00 ± 1.08	13.75 ± 0.85	11.43 ± 0.51*	8.48 ± 0.28*	7.30 ± 0.26**
MCR 200	20.04 ± 0.66	12.88 ± 0.24	8.93 ± 0.34*	6.53 ± 0.42**	5.70 ± 0.12***
ECR 100	19.15 ± 1.36	13.53 ± 0.31	8.65 ± 0.53*	6.78 ± 0.14**	6.40 ± 0.26**
ECR 200	19.58 ± 1.15	11.98 ± 0.38*	7.84 ± 0.28*	6.00 ± 0.52**	4.95 ± 0.31***
DCR 100	20.35 ± 0.62	15.47 ± 0.21	12.65 ± 0.39	9.80 ± 0.18*	9.40 ± 0.25*
DCR 200	19.38 ± 0.91	13.13 ± 0.39	10.48 ± 0.31*	8.58 ± 0.56*	7.30 ± 0.13**

Table 2. Effect of *C. roseus* leaf extracts and fraction on blood sugar levels in diabetes-induced mice. MET = Metformin hydrochloride, GBN = Glibenclamide, MCR = Methanol extract of *C. roseus*, ECR = Ethanol extract of *C. roseus*, DCR = Dichloromethane fraction of *C. roseus*. [Each value represents the mean ± SEM ($n = 6$ animals per group)]. *Represents statistical significance vs. diabetic control ($p < 0.05$). **Represents statistical significance vs. diabetic control ($p < 0.01$). ***Represents statistical significance vs. diabetic control ($p < 0.001$)].

Experimental procedures In mice, diabetes was induced by intraperitoneally (i.p.) injecting 60 mg/kg body weight of streptozotocin (STZ) dissolved in 0.1 M cold citrate buffer (pH = 4.5). On the third day after STZ treatment, the presence of diabetes was established by measuring fasting blood glucose levels[42,43].

Results Body weight, fasting blood glucose level and lipid profile of study mice were presented in Tables 1, 2 and 3 respectively.

Gas chromatography-mass spectrometry (GC–MS) analysis

GC–MS analysis was carried out on a Shimadzu-TQ8040 model, where a gas chromatograph was combined with a mass spectrophotometer. Rxi- 5 ms, 30 m, 0.25 mm ID, 0.25 µm df column was used. Helium gas was used as the carrier gas, and the flow rate was adjusted to a flow rate of 1.0 ml/min. The other GC–MS conditions used were a column oven temperature of 50 °C and an injection temperature of 250 °C.The injection mode was split less, and 53.5 KPa pressure was applied. The retention time and peak area % were estimated with the compound names.The peak area indicates the proportion of extracted components, and the compounds were identified by contrasting the acquired spectrum configurations with those recorded in the mass spectral database of the National Institute of Standards and Technology (NIST).

In-silico study

Ligand selection and preparation Sixteen major compounds were selected for docking analysis from the chromatogram based on the dominating peak (≥ 3% area) and Lipinski's rule of five[44] among the GC-MS identification of phytochemicals from DCR, MCR, and ECR leaf extracts. The 3D (SDF) structures of major bioactive chemicals, together with reference standards, were obtained from the NCBI PubChem online database

Extracts/Drugs	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
NC	100.45 ± 6.45	115.50 ± 2.79	55.26 ± 2.5	34.4 ± 4.56
DC	188.56 ± 10.56	211.75 ± 5.92	27.67 ± 2.45	165.07 ± 11.34
GLI	109.2 ± 4.20	118.52 ± 4.60	49.34 ± 1.45	55.27 ± 4.65
MET	94.56 ± 2.85	105.82 ± 3.11	45.34 ± 3.66	41.31 ± 3.87
MCR 100	128.88 ± 8.22	155.70 ± 8.14	36.45 ± 6.57	96.84 ± 5.23
MCR 200	119.78 ± 7.25	137.11 ± 7.20	35.23 ± 2.69	77.92 ± 5.98
ECR 100	120.56 ± 4.62	131.29 ± 2.44	38.66 ± 5.8	69.88 ± 3.54
ECR 200	108.67 ± 4.55	111.89 ± 2.96	44.34 ± 3.24	44.43 ± 5.9
DCR 100	155.67 ± 8.76	202.52 ± 4.23	32.34 ± 4.35	138.95 ± 10.58
DCR 200	150.45 ± 7.20	188.63 ± 5.84	30.44 ± 6.76	128.10 ± 9.67

Table 3. Effect of *C. roseus* leaf extracts, fraction on lipid parameters in diabetes model mice. MET = Metformin hydrochloride, GBN = Glibenclamide, MCR = Methanol extract of *C. roseus*, ECR = Ethanol extract of *C. roseus*, DCR = Dichloromethane fraction of *C. roseus*. [Each value represents the mean ± SEM (n = 6 animals per group)].

(https://pubchem.ncbi.nlm.nih.gov/). A few 2D structures of the ligands were drawn inChemDraw 3D16.0. Before docking, the compounds were prepared by assigning nonpolar hydrogen atoms, a Gasteiger charge was added, and the structure was minimized using the AMBER ff99SB standard residue at UCSF Chimera[45]. Furthermore, the structure was optimized for the best binding by minimizing energy using the MM2 force field in ChemDraw 3D16.0 and saved in pdb format.

Protein preparation The 3D crystal structure of human pancreatic Alpha-Amylase in complex with nitrate and acarbose (PDB ID: 3BAJ)[46], human lysosomal acid-alpha-glucosidase in complex with acarbose (PDB ID: 5 NN8)[47], and the crystal structure of Aspirin acetylated human Cyclooxygenase- 2 (Cox- 2) (PDB ID: 5 F19)[48] were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/). The protein PDB structures were refi ned by removing water molecules, heteroatoms, and existing ligands that were already bound to the protein and keeping the desired chain fold in the BIOVIA Discovery Studio Visualizer[49]. Then, the structures was optimized for docking by energy minimization using the GROMACS 96 43B1 algorithm in the SWISS-PDB viewer[50].

Molecular docking analysis Molecular docking is a significant approach to structural biology and is a technique for predicting the optimal binding mode of drug molecules to receptor macromolecules[51]. We thereby incorporated molecular docking analysis into our study to better understand the binding affinity of the peptides with reference standards and selected 16 major GC–MS-identified compounds against the targeted proteins. We used the PyRx virtual screening tool AutoDockfor molecular docking. This study utilized the AutoDockVina Wizard's default configuration settings, a virtual screening tool built by PyRx. Before docking, earlier optimized proteins and ligands were incorporated into the PyRx application, and the ligand energy was minimized and subsequently converted into the autodockpdbqt format. Eventually, an effective receptor grid box was generated to conduct the docking work[52]. The docking result with the highest binding affinity (kcal/mol) and a negative sign was chosen for further assessment. Finally, the protein-ligand interaction complexes were visualized to identify their strong binding pose with active site residues by using the BIOVIA Discovery Studio Visualizer.

In-silico ADME The ADME (absorption, distribution, metabolism, excretion) characterization of GC–MS-identified major phytochemicals from DCR, MCR, and ECR extracts was assessed by employing the Swiss-ADME (http://www.swissadme.ch/) web tool to predict their drug-likeness and select the most promising phytocompo unds with the least risk of drug attrition in further studies. The major compounds with the most reliable ADME and drug-likeness properties were taken into consideration for docking studies[53].

Statistical analysis

The data is displayed as the average of several individual experiments (n = 3, n = 6) with the standard error of the mean (SEM). The software GraphPad Prism (Version 8) is utilized for conducting an ANOVA followed by a Dunnett's test. The statistical significance levels of $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ were utilized to assess the significance of the results in comparison to the control group or between groups. The median inhibitory concentration (IC_{50}) values were calculated using MSExcel (Office 10) software.The full graph was created using Graph Pad Prism Software version 8.0.

Results

In-vitro Antidiabetic activity

α-amylase inhibitory effect All the extracts and the standard agent acarbose had an α-amylase inhibitory effect in a concentration-dependent fashion (Supplementary Table 1). Acarbose had the highest percentage of inhibition, with an IC_{50} value of 0.30 ± 0.01 mg/ml. Among the three extracts, ECR and MCR inhibited the α-amylase enzyme more effectively than DCR, with IC_{50} values of 0.62 ± 0.02 mg/ml, 0.74 ± 0.02 mg/ml, and 1.12 ± 0.01 mg/ml, respectively. A comparison of the percentages of inhibition among the three extracts was

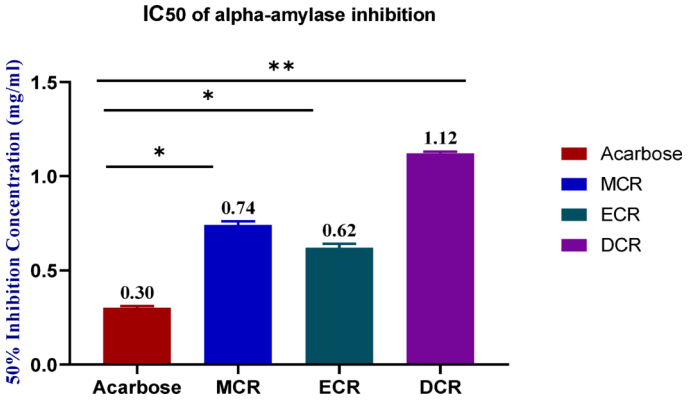

Fig. 1. Alpha-amylase inhibitory effect of *C. roseus* leaf extracts, fraction and Acarbose.

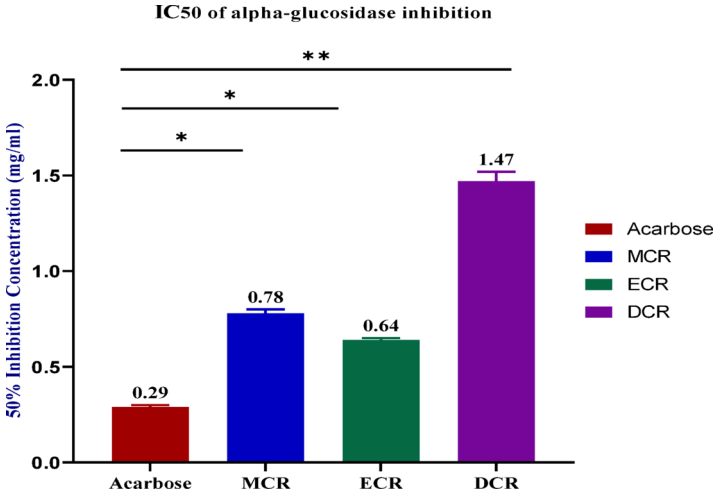

Fig. 2. α-Glucosidase inhibitory effect of *C. roseus* leaf extracts, fraction and acarbose.

performed (Fig. 1). The sequence of the highest inhibitory activities was as follows: Acarbose (Standard) > ECR > MCR > DCR.

α-glucosidase inhibitory effect Figure 2 and Supplementary Table 2 depict the α-glucosidase enzyme inhibitory effects of standard acarbose and three leaf extracts (MCR, ECR, and DCR) at various concentrations. All the extracts and the standard agent acarbose had dose-dependent α-glucosidase inhibitory effects. Acarbose had the highest percentage of inhibition, with an IC_{50}value of 0.29 ± 0.01 mg/ml. Among the three extracts, ECR had the highest potential to inhibit α-Glucosidase, with an IC_{50} value of 0.64 ± 0.01 mg/ml.

Acute toxicity study

C. roseus was found to be safe at all tested doses and did not cause noxious symptoms, such as sedation, convulsions, diarrhea, or irritation, in the mice. There was no mortality found during the 24-hour examination.

In-vivo antidiabetic effects

Effect on the body weight of the experimental mice The weight of the individuals in the normal control group increased gradually in a regular pattern throughout the whole study. In the case of the diabetic control group, the increase in body weight did not follow any regular pattern, and body weight even decreased from 28.79 ± 2.5 g (day 1) to 24.14 ± 2.69 g (day 20) for 20 days with increase in blood sugar levels. In the case of the standard Met groups, the improvement in body weight was not significant. However, treatment with the GBN standard and extracts improved the body weight to match the body weight of normal mice (Table 1). The normal biological appearance also improved, as did a greater intake of water and food.

Fasting blood glucose level The antidiabetic effects of standard and *C. roseus* leaf extracts and fraction (100 mg and 200 mg/kg) on the FBG levels of streptozotocin-induced diabetic mice are shown in Table 2. Intraperitoneal administration of STZ induced diabetes, as indicated by an increase in the blood sugar level to almost 20.35 ± 0.62 mmol/L. The hypoglycemic effects of the extracts increased in a dose-dependent manner compared with

those of the standard drugs (MET and GBN), the normal control, and the diabetic control. Among the three extracts (MCR, ECR, and DCR), the ethanolic extract had the most potent effect on reducing blood glucose levels.

Lipid profile of diabetic mice The induction of diabetes in animals causes changes in lipid profiles, such as LDL, HDL, TG, and TC. The mice in the normal control group had normal biochemical parameters, while there were many changes in the diabetic control mice. However, treatment of mice with metformin, glibencamide, or extracts causes these marker levels to shift toward normal levels. Among the three test samples, ECR had a more potent effect (Table 3).

Antiarthritic activity

In-vitro protein denaturation assay The ethanolic crude extract of *C. roseus* significantly inhibited bovine serum ALB denaturation (81.57%) at 1600 µg/ml. These results were comparable to those of the standard drug aspirin, which was 89.63% at 1600 µg/ml. However, the methanolic and dichloromethane fractions inhibited bovine serum ALB denaturation by 70.20% and 62.09%, respectively, at 1600 µg/mL compared to that of aspirin (Supplementary Table 3). The IC_{50} values are also depicted in Fig. 3.

Identification of GC–MS-Based phytochemicals

The GC–MS chromatograms of the methanol, ethanol extracts, and dichloromethane fraction of *Catharanthus roseus* exhibited a total of 60, 80, and 55 peaks, respectively, corresponding to the bioactive compounds that were recognized by relating their peak retention time and peak area (%). The list of all the phytochemicals identified from the DCR fraction, ECR and MCR extracts, their retention times, peak areas (%), and chromatograms are provided in Supplementary file (Supplementary Table 4-6). The major sixteen compounds from the chromatogram based on the dominating peak (≥ 3% area) were subjected to molecular docking analysis in association with in vivo and in vitro analysis. The major compounds with percentages of area, molecular formula, molecular weight, and PubChem CID are listed in Table 4.

In-silico study

Molecular docking analysis We performed docking simulation analysis in this study to correlate the in-vitro, in-vivo, and in-silico antidiabetic and antiarthriticactivities of DCR fraction, MCR and ECR leaf extracts of *Catharanthus roseus* to explore the possible lead compounds identified as major bioactive chemicals from the GC-MS analysis of these three samples. PyRxAutoDockVina wizard was utilized to perform molecular docking of 16 major identified compounds (indicated as C1 to C16) (Table 5) against alpha-amylase and alpha-glucosidase for antidiabetic activity by considering acarbose as a standard and against the cyclooxygenase- 2 (Cox- 2) enzyme for antiarthritic activity by taking aspirin as a standard (Fig. 4).

Table 6 shows the docking results of 16 major compounds and reference molecules against the alpha-amylase, alpha-glucosidase, and Cox- 2 proteins. The binding affinities of phytochemicals have demonstrated a distributed range between −4.4 and −9.9 kcal/mol. Based on the highest binding affinities with negative signs, binding poses, and interactions with active sites compared to those of the standard, the top six ranked compounds were selected for interactive analysis; these compounds exhibited better results for each enzyme than those of the other standards (Table 7).

The top six docked bioactive compounds against alpha-amylase were ergost- 5-en- 3-ol (3.beta.)- (C4), stigmasterol (C5), 9,19-cyclolanostan- 3-ol, acetate, (3.beta.)- (C8), gamma-sitosterol (C6), 24-norursa-3,12-diene (C9), 5 H- 3,5a-epoxynaphth[2,1-c]oxepin, dodecahydro- 3,8,8,11a-tetramethyl-, [3 S-(3.alpha.,5a.alpha.,7a.alpha.,11a.beta.,11b.alpha.)]- (C7) (Fig. 5). This six compounds showed binding affinities of – 9.9 Kcal/mol (C4), – 9.7 Kcal/mol (C5), – 9.7 Kcal/mol (C8), – 9.6 Kcal/mol (C6), – 8.9 Kcal/mol (C9), and – 8.6 Kcal/mol (C7), compared to the standard inhibitor acarbose (– 8 Kcal/mol). 24-Norursa- 3,12-diene (Compound number 9) displayed the highest affinity for alpha-glucosidase, at – 7.8 kcal/mol, whereas the standard acarbose showed the same affinity at – 7.8 kcal/mol. The binding affinities of C4, C5, C6, C7, and C8 against alpha-

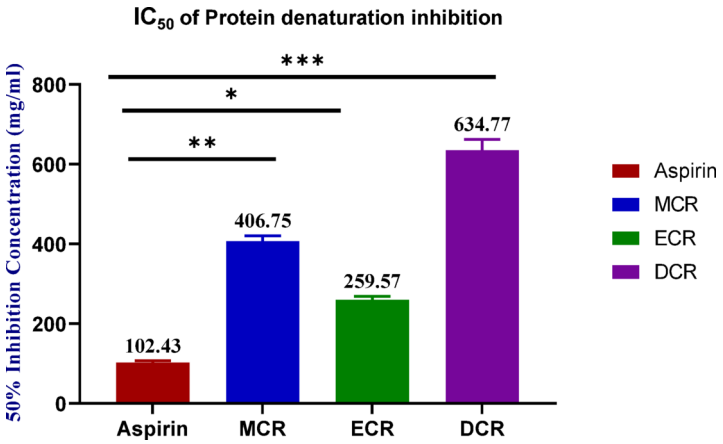

Fig. 3. IC_{50} values of three test samples and aspirin (standard) for the inhibition of BSA denaturation.

Solvent	C. No	%Area	Compound name	MW	Formula	PubChem CID
DCM/MeOH	C1	4.15	8,11,14-Docosatrienoic acid, methyl ester	348.6	$C_{23}H_{40}O_2$	5,364,473
DCM	C2	14.27	13-Docosenamide, (Z)-	337.6	$C_{22}H_{43}NO$	5,365,371
DCM/MeOH	C3	3.58	2-Methylhexacosane	380.7	$C_{27}H_{56}$	150,931
DCM	C4	6.41	Ergost- 5-en- 3-ol, (3.beta.)-	400.7	$C_{28}H_{48}O$	173,183
DCM/Me/EtOH	C5	4.23	Stigmasterol	412.7	$C_{29}H_{48}O$	5,280,794
DCM/Me/EtOH	C6	13.14	.gamma.-Sitosterol	414.7	$C_{29}H_{50}O$	457,801
DCM	C7	3.15	5 H- 3,5a-Epoxynaphth[2,1-c]oxepin, dodecahydro- 3,8,8,11a-tetramethyl-, [3 S-(3.alpha.,5a.alpha.,7a.alpha.,11a.beta.,11b.alpha.)]-	278.4	$C_{18}H_{30}O_2$	93,639
DCM	C8	10.28	9,19-Cyclolanostan- 3-ol, acetate, (3.beta.)-	470.8	$C_{32}H_{54}O_2$	537,304
DCM/Me/EtOH	C9	7.93	24-Norursa- 3,12-diene	394.7	$C_{29}H_{46}$	91,735,342
DCM/Me/EtOH	C10	3.62	Azulene	128.17	$C_{10}H_8$	9231
Me/EtOH	C11	15.69	3-O-Methyl-d-glucose	194.18	$C_7H_{14}O_6$	8973
Me/EtOH	C12	26.34	9-Octadecenamide, (Z)-	281.5	$C_{18}H_{35}NO$	5,283,387
Me/EtOH	C13	3.32	Benzene, 1-(1,1-dimethylethyl)– 4-[(2-methyl- 2-propenyl)oxy]-	204.31	$C_{14}H_{20}O$	345,922
EtOH	C14	3.64	Naphthalene	128.17	$C_{10}H_8$	931
EtOH	C15	3.14	1 H-Indene, 1-methylene-	128.17	$C_{10}H_8$	75,581
EtOH	C16	3.07	6-Octadecenoic acid, methyl ester, (Z)-	296.5	$C_{19}H_{36}O_2$	5,362,717

Table 4. Major phytochemicals identified by GC–MS analysis of *Catharanthus roseus*.

C. No	Compounds name	Binding affinity (Kcal/mol)		
		Alpha amylase (3BAJ)	Alpha glucosidase (5 NN8)	Cox-2 (7 F19)
C1	8,11,14-Docosatrienoic acid, methyl ester	– 5.00	– 4.94	– 5.13
C2	13-Docosenamide, (Z)-	– 5.07	– 4.45	– 5.56
C3	2-Methylhexacosane	– 4.93	– 4.83	– 6.17
C4	Ergost- 5-en- 3-ol, (3.beta.)-	– 9.94	– 7.68	– 9.85
C5	Stigmasterol	– 9.71	– 7.43	– 8.33
C6	gamma.-Sitosterol	– 9.64	– 7.36	– 7.62
C7	5 H- 3,5a-Epoxynaphth[2,1-c]oxepin, dodecahydro- 3,8,8,11a-tetramethyl-, [3 S-(3.alpha.,5a.alpha.,7a.alpha.,11a.beta.,11b.alpha.)]-	– 8.62	– 7.06	– 8.00
C8	9,19-Cyclolanostan- 3-ol, acetate, (3.beta.)-	– 9.73	– 7.27	– 9.53
C9	24-Norursa- 3,12-diene	– 8.91	– 7.83	– 8.72
C10	Azulene	– 5.74	– 5.46	– 6.98
C11	3-O-Methyl-d-glucose	– 5.12	– 5.45	– 5.39
C12	9-Octadecenamide, (Z)-	– 5.14	– 4.61	– 4.55
C13	Benzene, 1-(1,1-dimethylethyl)– 4-[(2-methyl- 2-propenyl)oxy]-	– 6.12	– 5.94	– 7.34
C14	Naphthalene	– 5.92	– 5.92	– 6.52
C15	1 H-Indene, 1-methylene-	– 5.50	– 5.34	– 6.91
C16	6-Octadecenoic acid, methyl ester, (Z)-	– 4.82	– 4.73	– 5.26
D1	Acarbose	– 8.00	– 7.81	
D2	Aspirin			– 6.00

Table 5. Molecular Docking scores of selected major compounds from GC–MS chromatograms and standards against alpha-amylase, alpha-glucosidase, and Cox- 2 protein.

glucosidase were – 7.6 kcal/mol, – 7.4 kcal/mol, – 7.3 kcal/mol, – 7 kcal/mol, and – 7.2 kcal/mol, respectively. For Cox- 2, the top-ranked compounds with the highest binding affinity were ergost- 5-en- 3-ol (3.beta.)- (C4), – 9.8 Kcal/mol. The compounds C8, C9, C5, C7, and C6 also exhibited higher binding affinities than did the standard inhibitors, with binding affinities of – 9.5 kcal/mol, – 8.7 kcal/mol, – 8 kcal/mol, – 8 kcal/mol, and – 7.6 kcal/mol, respectively, compared to those of aspirin (standard), which was – 6 kcal/mol. The docking results showed that these six compounds had multiple binding sites.

Protein-ligand interaction analysis The nonbonding interactions of the protein-ligand complexes of the top six compounds were visualized to predict the interaction type, bonding category, and bonding distances of the respective ligands with specific amino acid (AA) residues of the target receptor macromolecule. The interactions

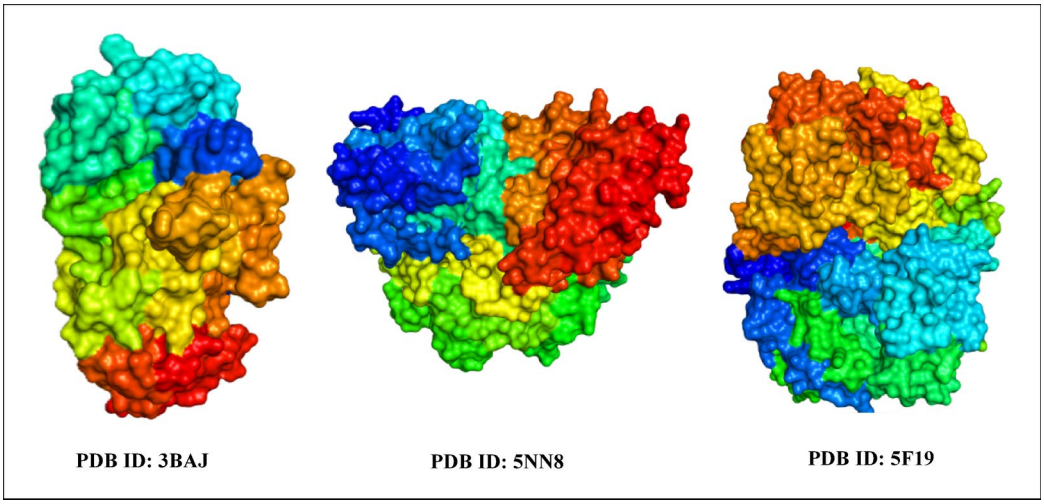

Fig. 4. 3D structure of selected target proteins.

C. No	Compounds	Binding Affinity (Kcal/mol)			Pharmacological activity
		Alpha amylase	Alpha-glucosidase	Cox- 2	
C4	Ergost- 5-en- 3-ol, (3.beta.)-	– 9.94	– 7.68	– 9.85	Cholesterol lowering and anticarcinogenic effects[54]
C5	Stigmasterol	– 9.71	– 7.43	– 8.33	Anti-diabetic, anti-neoplastic[55]
C8	9,19-Cyclolanostan- 3-ol, acetate, (3.beta.)-	– 9.73	– 7.27	– 9.53	Antidiabetic, antibacterial, and antioxidant activities[56]
C6	.gamma.-Sitosterol	– 9.64	– 7.36	– 7.62	Anticancer, cytotoxic, antihyperlipidemic, antidiabetic[57]
C9	24-Norursa- 3,12-diene	– 8.91	– 7.83	– 8.72	Gynecological ailments, bacterial infections and heal wounds[58]
C7	5 H- 3,5a-Epoxynaphth[2,1-c]oxepin, dodecahydro- 3,8,8,11a-tetramethyl-, [3 S-(3.alpha.,5a.alpha.,7a.alpha.,11a.beta.,11b.alpha.)]-	– 8.62	– 7.06	– 8.00	Not identified
D1	Acarbose (Standard)	– 8.00	– 7.81	-	
D2	Aspirin (Standard)	-	-	– 6.00	

Table 6. Molecular docking scores of the top six selected compounds with standards.

of the distinct ligand groups with the enzyme residues were mostly hydrophobic, with a few H-bonds. Ergost-5-en- 3-ol, (3.beta.)- (C4) provides solid binding interactions with alpha-amylase by forming one conventional H-bond with ASP300 (bonding distance of 2.06 Å), and eleven other hydrophobic bonds—two alkyl bonds with ALA106 and ILE51; two alkyl bonds with VAL107; four pi-alkyl bonds with TRP59 with different distances; and three pi-alkyl bonds with the amino acid residues HIS305, TRP58, and TYR62. However, the interaction of standard acarbose was stabilized by the formation of six conventional H-bonds with the TYR151, LYS200, GLU233, HIS305, HIS201, and THR163 amino acid residues and one carbon H-bond with GLN63, as well as two pi-alkyl bonds with the TRP58 and TYR62 AA residues. The C5 and C6 compounds did not form H-bonds like the standard acarbose, but they did interact with the active site residues at the hydrophobic gate of the alpha-amylase group; these H-bonds included LEU165, VAL107, ILE51, TRP59, and TYR62. The C7 and C8 compounds formed one conventional H-bond with GLN63 and one pi-sigma bond with TRP59. In addition, C7 formed one alkyl group with LEU165 and eight hydrophobic pi-alkyl bonds with the TRP59, TRP58, TYR62, and HIS305 catalytic residues. In addition, C8 formed seven hydrophobic alkyl and pi-alkyl bonds with LYS200, ILE235, HIS201, LEU162, TRP59, and LEU165. The C9 compound also stabilized interactions by forming eight alkyl and pi-alkyl hydrophobic bonds with the ALA198, TYR62, LEU165, LEU162, TRP58, ILE235, and TYR151 catalytic residues of alpha-amylase. The 2D and 3D nonbonding interactions of the selected top-docked compounds with alpha-amylase (3BAJ) are depicted in Fig. 6; Table 7.

The interaction between C5 compounds with alpha-glucosidase was stabilized by the formation of one conventional H-bond with ARG281 and one carbon H-bond with the ASP282 amino acid residue. In addition, it also interacted by forming hydrophobic alkyl and pi-alkyl bonds with the AA residues of the ALA555, LEU677, LEU678, LEU650, TRP376, TRP481, and PHE649 active sites. On the other hand, the standard acarbose formed six conventional H-bonds with ARG608, GLU866, LEU868, PRO361, MET363, and SER864 and one carbon H-bond with the HIS717 catalytic site residue of alpha-glucosidase. The C7 compounds created one conventional

C. No	Type of interaction		Interacting AA residues
	Bond category	Bond type	
C4	H-bond	Conventional	ASP300
	Hydrophobic bond	Alkyl	ALA106, VAL107,ILE51
		Pi-Alkyl	TRP58, TRP59, TYR62, HIS305
C5	Hydrophobic bond	Alkyl	LEU165, VAL107, ILE51
		Pi-Alkyl	TRP59, TYR62
C6	Hydrophobic bond	Alkyl	LEU165, ILE51, VAL107
		Pi-Alkyl	TRP59, TYR62
C7	H-bond	Conventional	GLN63
	Hydrophobic bond	Pi-Sigma	TRP59
		Alkyl	LEU165
		Pi-Alkyl	TRP58, TRP59, TYR62, HIS305
C8	H-bond	Conventional	GLN63
	Hydrophobic bond	Pi-Sigma	TRP59
		Alkyl	LEU162, LEU165, ALA198, LYS200, ILE235
		Pi-Alkyl	TRP59, HIS201
C9	Hydrophobic bond	Alkyl	ALA198, LEU162, LEU165, ILE235
		Pi-Alkyl	TRP58, TYR62, TYR151
D1-Acarbose	H-bond	Conventional	TYR151, LYS200, GLU233, HIS305, HIS201, THR163
		Carbon H-Bond	GLN63
	Hydrophobic bond	Pi-Alkyl	TRP58, TYR62

Table 7. Nonbonding interactions of the top six selected compounds and the standard with alpha-amylase (3BAJ).

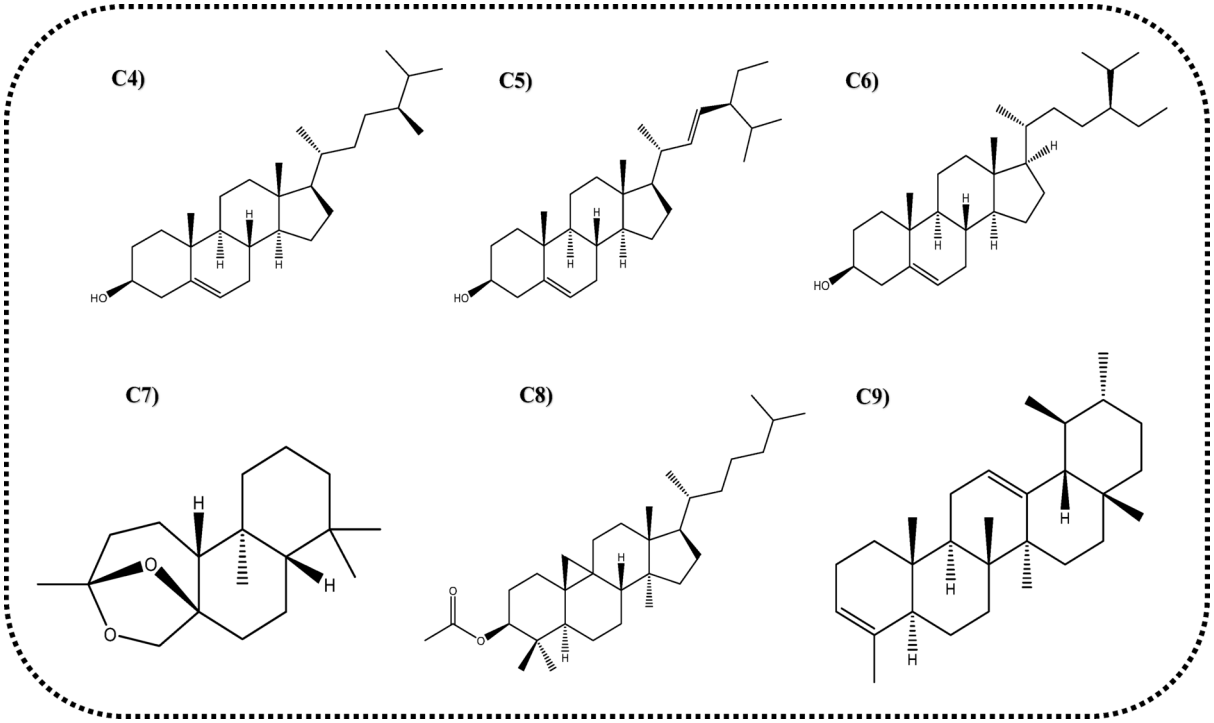

Fig. 5. Structures of the major bioactive compounds showing the highest binding affinities to *Catharanthus roseus*, where C4) is an Ergost- 5-en- 3-ol, (3.beta.)-, C5) Stigmasterol, C6).gamma.-Sitosterol, C7) 5 H-3,5a-Epoxynaphth[2,1-c]oxepin, dodecahydro- 3,8,8, 11a-tetramethyl-, [3 S-(3.alpha.,5a.alpha.,7a.alpha.,11a.beta.,11b.alpha.)]-, C8) 9,19-Cyclolanostan- 3-ol, acetate, (3.beta.)-, and C9) 24-Norursa- 3,12-diene.

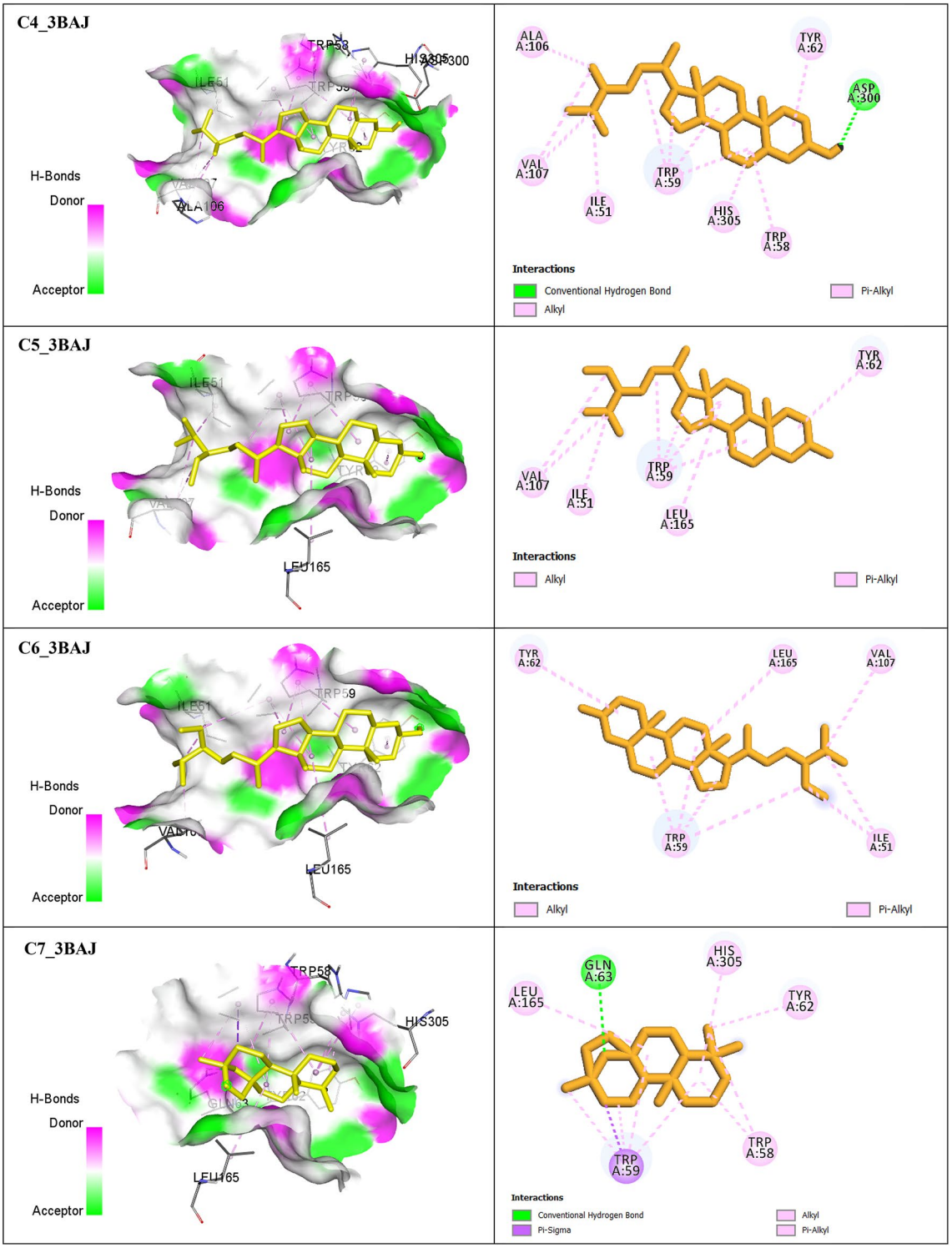

Fig. 6. Nonbonding 2 & 3D interactions of the top six selected compounds and standard with alpha-amylase (3BAJ).

H-bond with ARG608 and several hydrophobic alkyl and pi-alkyl bonds with the active site residues ARG608, ARG594, PRO595, LEU865, PHE362, HIS584, and HIS717. C6 formed one carbon H-bond with SER929 and hydrophobic bonds with PRO779, VAL934, LYS697, and ILE780. The C4, C8, and C9 compounds did not form any hydrogen bonds during their interaction but formed hydrophobic bonds with several AA residues, ARG585, VAL588, LEU865, ARG594, HIS584, HIS717, ALA93, ARG275, VAL321, TRP126, and TYR360, respectively. The 2D and 3D nonbonding interactions of the selected top-docked compounds with alpha-glucosidase are shown in Fig. 7; Table 8.

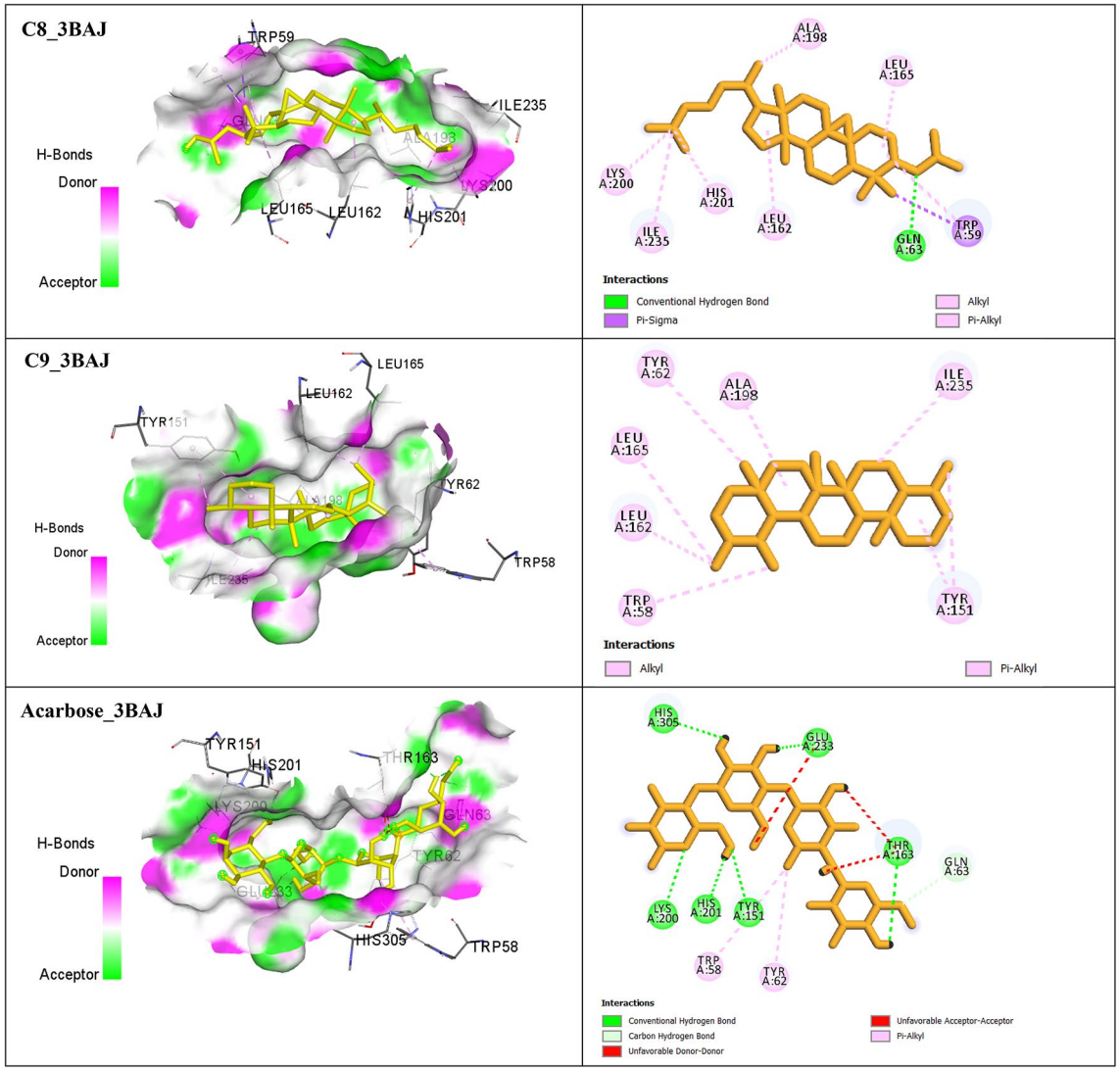

Fig. 6. (continued)

The nonbonding interaction between the C4 compound and Cox- 2 was established by the formation of one conventional H-bond with GLY225 and three pi-alkyl bonds with the TRP139 and PHE142 active site amino acid residues. However, the standard drug aspirin interacted through two conventional H-bonds with the CYS47 and HIS39 residues and four alkyl hydrophobic bonds with the ARG44, LEU152, ARG469, and PRO153 catalytic site residues of the Cox- 2 enzyme. The C5 and C7 compounds formed one H-bond with the THR62 and ASN375 residues. In addition, C5 formed one pi-alkyl bond with TYR373 along with five alkyl bonds with the PRO542, ALA543, ARG44, ARG61, and PRO542 amino acid residues. In addition, C7 formed one alkyl and pi-alkyl bond with the LEU145 and PHE142 residues. C6, C8, and 9 did not form any H-bonds with Cox- 2, but they interacted through alkyl, pi-alkyl, and pi-sigma hydrophobic bonds with the catalytic LEU145, LEU224, PHE142, PRO542, ALA543, ARG44, PHE371, TYR115, PHE99, VAL89, ILE92, LEU93, ILE112, PHE96, PHE99, TRP100, and TYR115 distinct amino acid residues of the Cox- 2 enzyme. The 2D and 3D nonbonding interactions of the selected top-docked compounds against Cox- 2 are shown in Fig. 8; Table 9.

In silico ADME prediction

In silico predictions of pharmacokinetic (ADME) and drug-likeness properties were calculated from the free web server Swiss-ADME tool. All the major compounds obeyed at least four of the "Lipinski rules" and possessed druggable ADME properties. Table 10 illustrates the drug-likeness, GI absorption, BBB permeability, and other ADME profiles.

Discussion

The incidence of diabetes mellitus is currently increasing at an alarming rate worldwide. It affects three-quarters of the world's population and is recognized as a leading cause of high economic loss, which can, in turn, impede the growth of nations. In addition, uncontrolled diabetes can result in a wide variety of long-term complications,

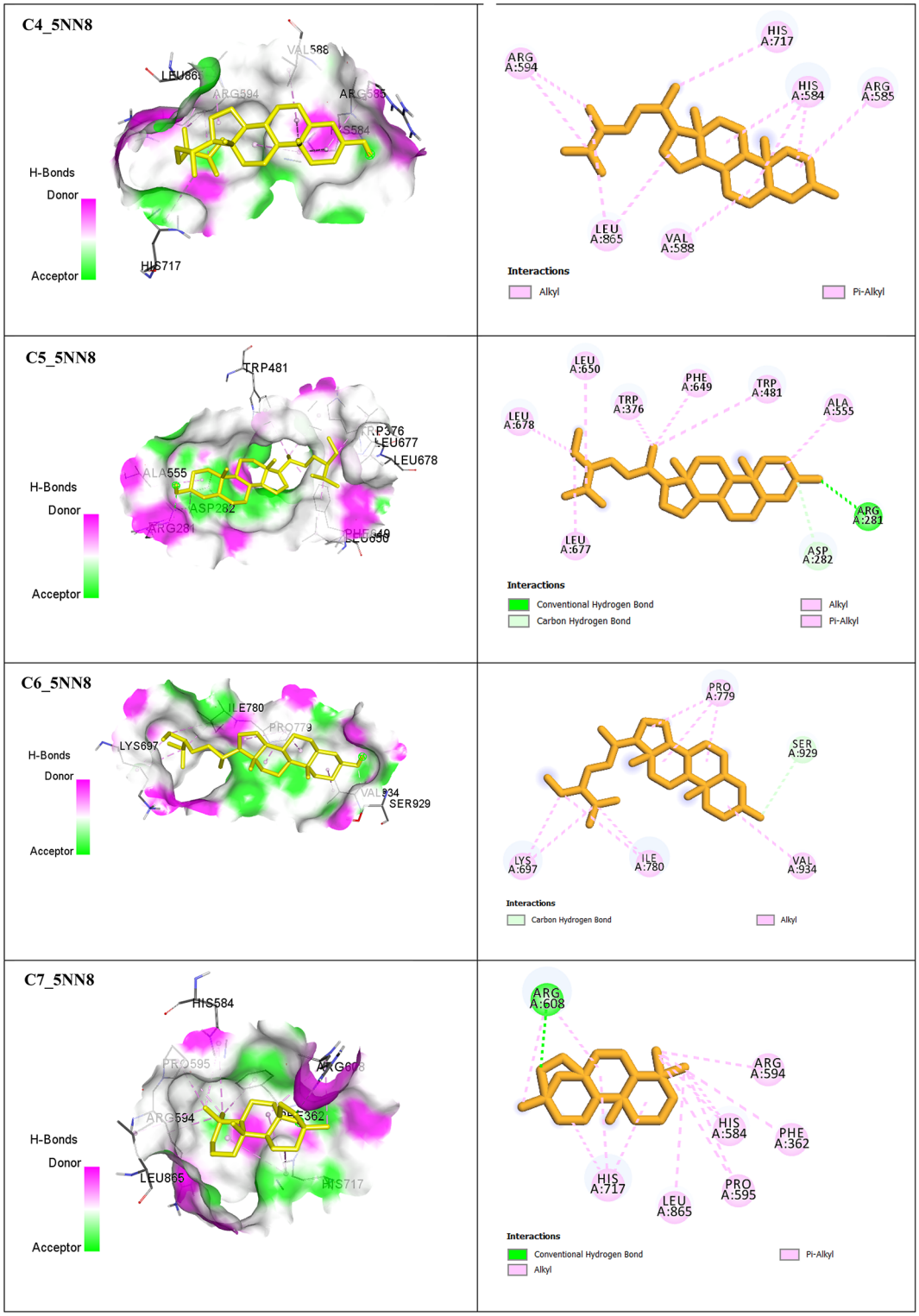

Fig. 7. Nonbonding 2D & 3D interactions of the top six selected compounds and standard with alpha-glucosidase (5 NN8).

including but not limited to renal failure, heart disease, and blindness. Because of this, treatments developed following the tenets of Western medicine (allopathy) frequently have a limited capacity for helping patients, can cause unwanted side effects, and are frequently unaffordable, particularly in the developing world. As a result, treating diabetes mellitus with plant-derived compounds, which are easily accessible and do not require laborious pharmaceutical synthesis, is an extremely appealing option[35]. Natural medicines made from medicinal

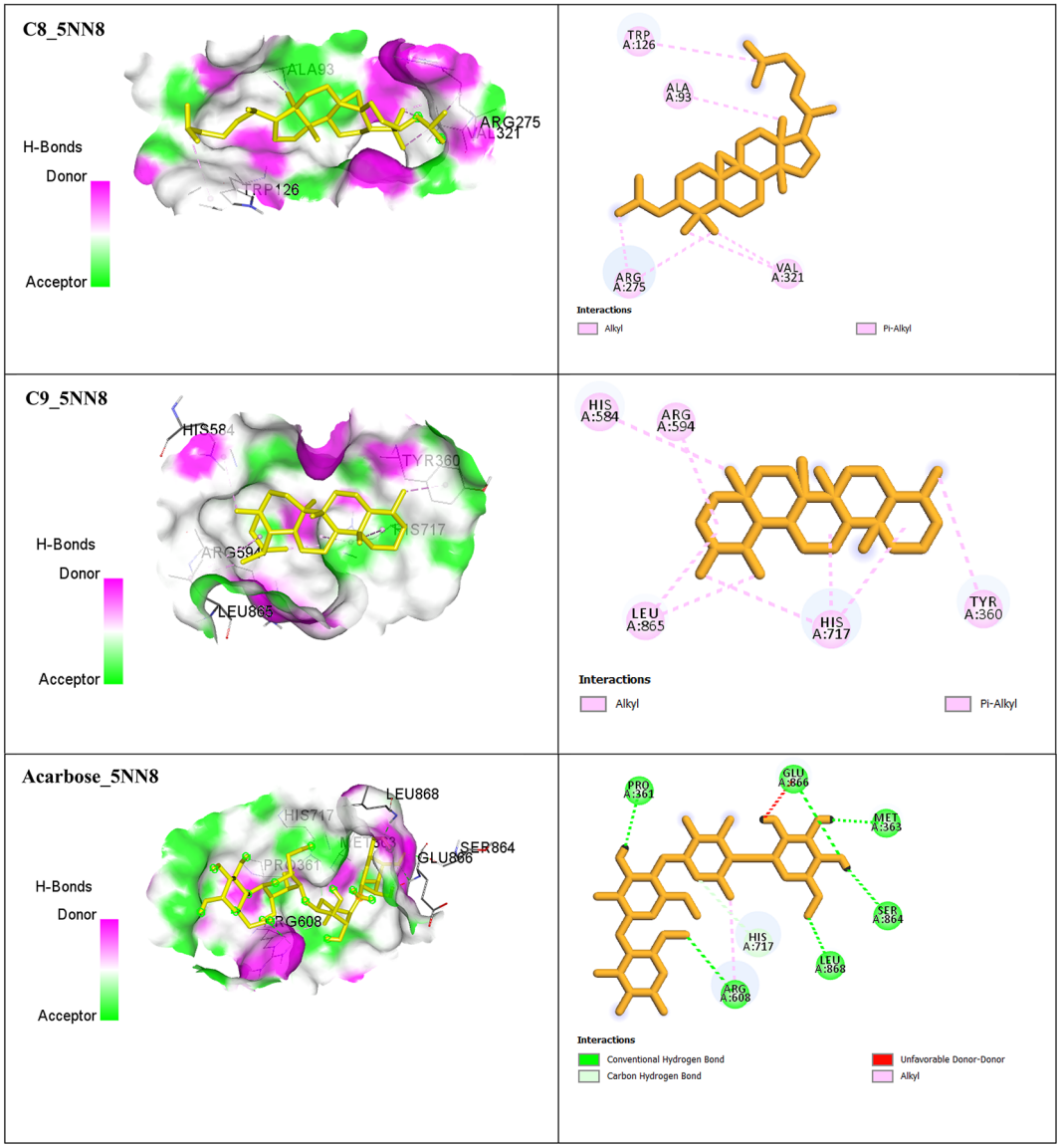

Fig. 7. (continued)

plants are widely used for self-medication, as the consensus is that they are risk-free and do not result in any adverse health effects[3].

In this study, we investigated the pharmacological effects of various *C. roseus* plant extracts on glucose-lowering potential, which has been demonstrated in various in-vitro and in vivo model studies. Our goal is to identify the cheapest source of key blood glucose regulatory molecules from natural origin as an alternative to the synthetic drugs currently in use. In the STZ-induced diabetic model, in the normoglycemic mice, and in the mice that were given oral glucose, the test substances significantly reduced the blood glucose level. Additionally, in vitro alpha-amylase and alpha-glucosidase inhibition activities were observed both in the crude extract and in the solvent fractions of the extract.

The enzyme pancreatic alpha-amylase is essential for the digestive process because it hydrolyzes starch into a mixture of smaller oligosaccharides. These oligosaccharides are then hydrolyzed even further by alpha-glucosidase to glucose. The glucose that is produced as a byproduct is absorbed into the bloodstream, which ultimately results in an elevated level of postmeal hyperglycemia (PPHG). Therefore, plant extracts that have the potential to inhibit the aforementioned two enzymes might be useful for lowering the incidence of PPHG-associated complications that are associated with type 2 diabetes[59]. Both types of enzymes were inhibited by crude extracts of methanol and ethanol, as well as fractionated extracts of DCM. Among the three extracts, ECR and MCR inhibited the α-amylase enzyme more effectively than DCR, with IC_{50} values of 0.62 ± 0.02 mg/ml, 0.74 ± 0.02 mg/ml, and 1.12 ± 0.01 mg/ml, respectively. The activity of the ethanol extract (ECR) was significantly lower than that of the other two extracts, and the activity of the standard drug acarbose was very close.

Similarly, the oral administration of *C. roseus* extracts and fraction (MCR, ECR, and DCR) at all tested doses (100 and 200 mg/kg), as well as the standard glibenclamide and metformin at a dose of 10 mg/kg, produced a

C. No	Type of interaction		Interacting AA residues
	Bond category	Bond type	
C4	Hydrophobic bond	Alkyl	ARG585, VAL588, LEU865, ARG594
		Pi-Alkyl	HIS584, HIS717
C5	H-bond	Conventional	ARG281
		Carbon H-Bond	ASP282
	Hydrophobic bond	Alkyl	ALA555, LEU677, LEU678, LEU650
		Pi-Alkyl	TRP376, TRP481, PHE649
C6	H-bond	Carbon H-Bond	SER929
	Hydrophobic bond	Alkyl	PRO779, VAL934, LYS697, ILE780
C7	H-bond	Conventional	ARG608
	Hydrophobic bond	Alkyl	ARG608, ARG594, PRO595, LEU865
		Pi-Alkyl	PHE362, HIS584, HIS717
C8	Hydrophobic bond	Alkyl	ALA93, ARG275, VAL321
		Pi-Alkyl	TRP126
C9	Hydrophobic bond	Alkyl	ARG594, LEU865
		Pi-Alkyl	TYR360, HIS584, HIS717
D1-Acarbose	H-bond	Conventional	ARG608, GLU866, LEU868, PRO361, MET363, SER864
		Carbon H-Bond	HIS717
	Hydrophobic bond	Alkyl	ARG608

Table 8. Nonbonding interactions of the top six selected compounds and the standard with alpha-glucosidase (5 NN8).

significant reduction in the blood glucose level of STZ-induced diabetic mice. The hypoglycemic effects of the extracts increased in a dose-dependent manner compared with those of the standard extracts (MET and GBN), the normal control, and the diabetic control. Among the three extracts, ECR potently reduced blood glucose levels. These results suggest that the crude extract may have the potential to exhibit hypoglycemic activity in normal mice, which is consistent with the findings of other studies conducted on similar genera. A significant decrease in blood glucose levels was observed in the diabetic mouse groups treated with the ethanolic extract of *Catharanthus roseus* at 200 mg/kg. These findings are consistent with those of Muralidharan (2015)[60], who reported that *Catharanthus roseus* has an antidiabetic impact on diabetic rats. These results are consistent with those who reported that rats were fed a suspension of *C. roseus* leaf powder in water for 60 days[61]. The suspension of *C. roseus* significantly reduced blood sugar levels. According to related research, long-term use of *C.roseus* prevents the body from becoming insulin-resistant[61]. *Singh et al.* (2001) found that Ethanolic extract of *Catharanthus roseus* has significant anti-hyperlipidemic & anti-diabetic effects ($p < 0.05$, $p < 0.01$), which truly aligned with our finding also where ethanolic extracts showed highest effect[32].

In the present study, the ethanolic *Catharanthus roseus* leaf extract (200 mg/kg) significantly reduced blood cholesterol levels in the hyperlipidemic groups. These results are consistent with those of Antia and Okokon (2005)[62], who noted that the leaf juice of *C.roseus* had an antihyperlipidemic effect on normal rats. The LDL, total TG (triglyceride), and total cholesterol levels decreased. At a high dose (200 mg/kg), there was a greater reduction in LDL cholesterol, triglycerides, and total cholesterol. This outcome is consistent with research by Patel *et al.* (2011)[20], who examined the antihyperlipidemic effects of *C. roseus* leaf juice in guinea pigs.

According to the findings of the present study, the ethanolic extract of *C. roseus* was shown to have significant antihyperglycemic activity in STZ-induced diabetic mice through the decrease of glucose in the blood. Additional pancreatic activity, such as stimulating peripheral glucose utilization or boosting glycolytic and glycogenic processes accompanied by decreases in glycogenolysis and gluconeogenesis, may account for some of these effects[63]. Based on these findings, it is hypothesized that the extract could be responsible for stimulating the release of insulin. In addition, the ability of the extract to lower blood glucose levels might also be attributable to increased utilization of glucose in peripheral tissues. STZ is responsible for the induction of diabetes, which in turn leads to a loss of body weight due to increased muscle waste and loss of tissue proteins[64]. After 20 days of treatment with all the extracts, a gain in the body weight of the diabetic mice was observed, and the results of ECR and MCR were comparable to those of the standard drug glibenclamide.

Although several classes of drugs are currently used to treat RA, there is a need for new and more potent natural agents due to the side effects and toxicities of these existing drugs. In contrast to those of synthetic compounds, the structural diversity of natural products and bioactive compounds derived from these materials is extremely wide. For this reason, we conducted this study to investigate the antiarthritic effect of the commonly used plant *C. roseus*. An in vitro study revealed a dose-dependent antiarthritic effect of *C. roseus* methanol, ethanol extracts, and the DCM fraction. Previous studies have suggested that the production of autoantigens in certain rheumatic diseases may be attributable to in vivo protein denaturation, which is one of the well-documented causes of inflammatory and arthritic diseases[65].

Protein denaturation occurs when a protein is exposed to extreme conditions, such as heat, an organic solvent, or a strong acid or base, which causes it to lose its secondary and tertiary structure[65]. Electrostatic,

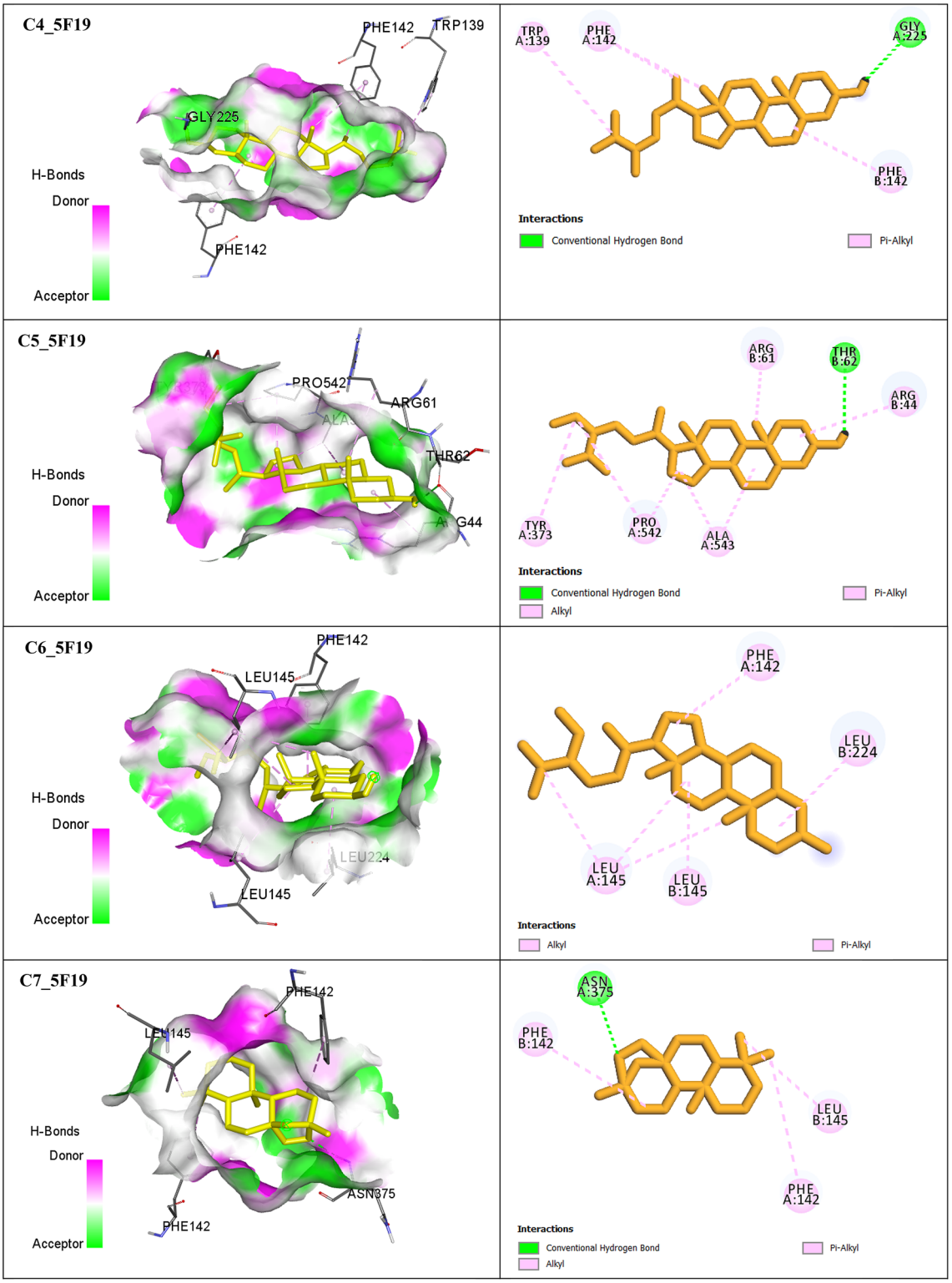

Fig. 8. Nonbonding 2D & 3D interactions of the top six selected compounds and standard with Cox- 2 (5 F19).

hydrogen, hydrophobic, and disulfide bonding all play a role in the denaturation mechanism[65]. In the present study, ECR had the greatest repressive effect on HELLP syndrome, and the plant extract achieved an inhibitory effect on protein denaturation that was comparable to that of aspirin which is concordant with the finding of Asija *et al.* (2022), who established that ethanolic extract of *Catharanthus roseus* exhibited significant anti-arthritic activity as compared to other extracts in rats[33]. *C. roseus*'s ability to reduce the thermal denaturation

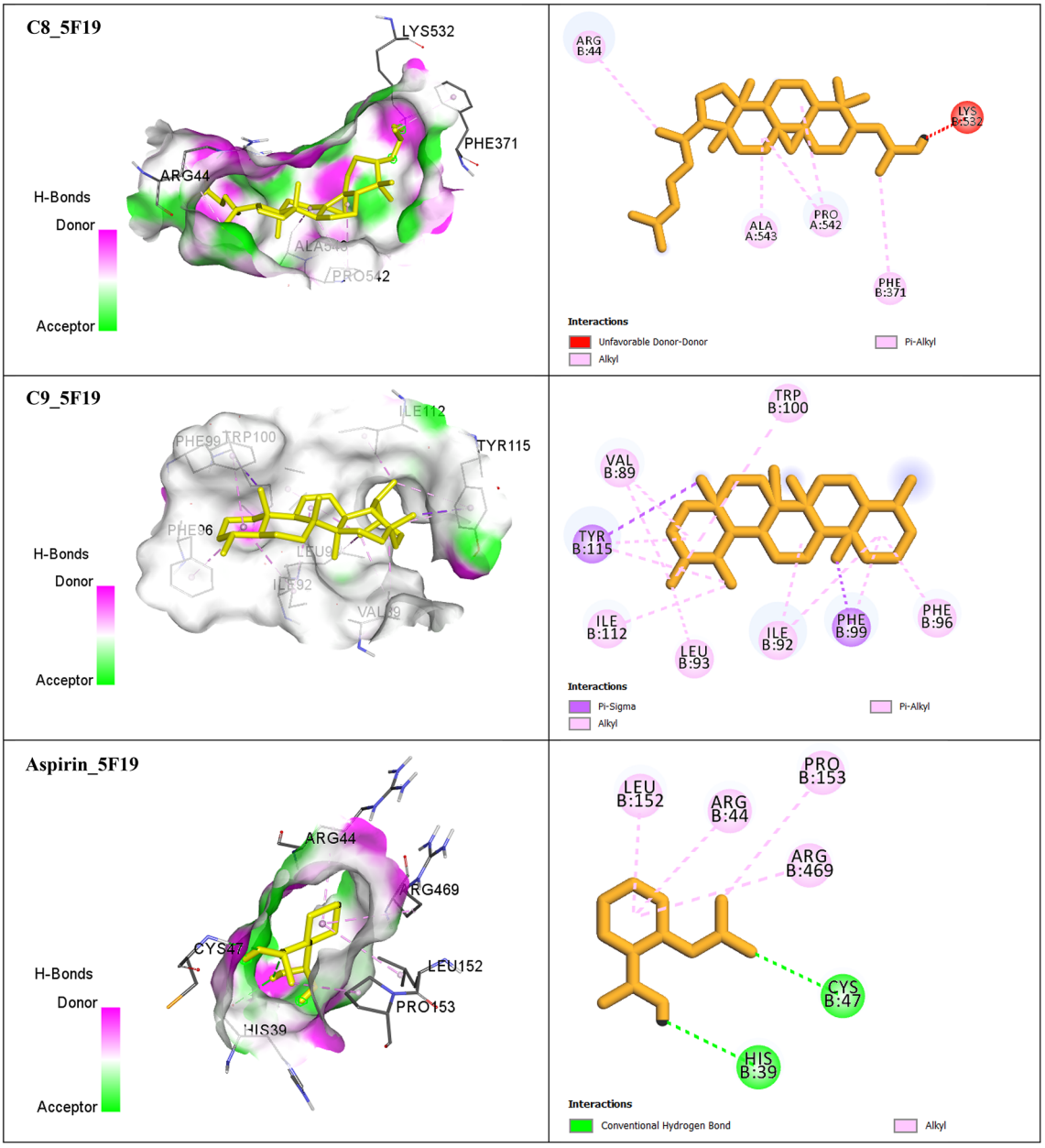

Fig. 8. (continued)

of proteins was demonstrated by increases in testsample absorbance compared to that of the control (albumin). Based on the available evidence, it seems likely that the antiarthritic effect of *C. roseus* is due to its ability to prevent protein denaturation.

A novel and inventive approach to medication development has been made possible by computer-aided study. Through the virtual docking of targeted proteins with ligands under investigation, we might obtain a better understanding of the many biological functions of compounds derived from nature by examining their binding affinities to receptors and binding processes[66]. To validate the results obtained from the antidiabetic and antiarthritic activity tests performed in vitro (test tube) and in vivo in rat models, molecular docking was carried out utilizing the major bioactive compounds identified from extracts by GC-MS. Compared with the standard FDA-approved drugs, six of the major compounds exhibited high binding affinities with alpha-amylase, alpha-glucosidase, and cox- 2 receptors ranging from −7 to − 9.9 kcal/mol). In vivo and in vitro studies revealed that *C. roseus* extracts have strong antidiabetic and antiarthritic activities, which corroborates the increased binding of these six compounds to the active sites of alpha-amylase, alpha-glucosidase, and the cox- 2 receptor, which are responsible for suppressing glycemic and prostaglandin levels via their inhibition. Hence, the aforementioned compounds might be responsible for the extracts' antiarthritic and antihyperglycemic activities.

C. No	Type of interaction		Interacting AA residues
	Bond category	Bond type	
C4	H-bond	Conventional	GLY225
	Hydrophobic bond	Pi-Alkyl	TRP139, PHE142
C5	H-bond	Conventional	THR62
	Hydrophobic bond	Alkyl	PRO542, ALA543, ARG44, ARG61, PRO542
		Pi-Alkyl	TYR373
C6	Hydrophobic bond	Alkyl	LEU145, LEU224
		Pi-Alkyl	PHE142
C7	H-bond	Conventional	ASN375
	Hydrophobic bond	Alkyl	LEU145
		Pi-Alkyl	PHE142
C8	Hydrophobic bond	Alkyl	PRO542, ALA543, ARG44
		Pi-Alkyl	PHE371
C9	Hydrophobic bond	Pi-Sigma	TYR115, PHE99
		Alkyl	VAL89, ILE92, LEU93, ILE112
		Pi-Alkyl	PHE96, PHE99, TRP100, TYR115
D2-Aspirin	H-bond	Conventional	CYS47, HIS39
	Hydrophobic bond	Alkyl	ARG44, LEU152, ARG469, PRO153

Table 9. Nonbonding interactions of the top six selected compounds and the standard with Cox- 2 (5 F19).

C. No	MW	HBA	HBD	TPSA (Å^2)	Log S (ESOL)	Consensus Log Po/w	GI absorption	BBB permeant	Drug Likeness (Lipinski Rules)	
									Result	Violation
C4	400.7	1	1	20.23	− 7.54	6.90	Low	No	Yes	1
C5	412.7	1	1	20.23	− 7.46	6.97	Low	No	Yes	1
C6	414.7	1	1	20.23	− 7.90	7.19	Low	No	Yes	1
C7	278.4	2	0	18.46	− 4.51	4.20	High	Yes	Yes	0
C8	470.8	2	0	26.30	− 9.10	8.14	Low	No	Yes	1
C9	394.7	0	0	0.00	− 8.02	7.41	Low	No	Yes	1

Table 10. Evaluation of the ADME and drug-likeness properties of the selected top six compounds. MW-molecular weight, HBA-hydrogen bond acceptor, HBD-hydrogen bond donor, TPSA-topological polar surface area, GI-gastrointestinal, BBB-blood brain barrier.

Constraints and further research opportunity

The findings suggest that the compounds being studied have properties that make them promising candidates for medications that address a variety of medical conditions, including the treatment of dyslipidaemia, diabetes, and arthritis. Even though these findings are promising, additional preclinical research is required to thoroughly examine the efficacy and safety of these medicines, including long-term animal testing and clinical studies including human participants. These further investigations are required to validate the potential medicinal applications of these compounds and pave the way for the development of potent medications for a variety of ailments.

Limitations of the study

Histopathological studies of pancreas could have been performed after sacrifing the mice to observe any significant changes. Besides, instead of using di-chloromethane fraction, this solvent could have been used to directly extract the leaf part of the plant. Moreover, molecular docking study if performed, could give an idea about the specific mechanism of the obtained therapeutical potential of this plant leaves on the mice models. Since, it was a self-funded research project, so we have limited financial support. For this reason, we could not conduct in vivo analysis for antiarthitic test. Again, we could not carry forward our work to compound isolation through sophisticated analytical techniques.

Conclusion

In the present study, the methanolic, ethanolic extracts, and dichloromethane fraction of *C. roseus* leaf significantly reduced fasting blood glucose levels in streptozotocin-induced diabetic mice in a 20-day study period in a dose-dependent manner. The reduction in FBG by the 100 and 200 mg/kg doses was close to normal. Correspondingly, all the test samples also improved the lipid profiles of diabetic mice. Moreover, during this study, the extracts also improved different biological features, such as body weight and food and water intake,

more significantly. GC-MS profiling revealed all of these pharmacological benefits by revealing the presence of important phytochemicals in these extracts. The molecular docking approach revealed that six major potential compounds from the GC-MS chromatogram of *C. roseus* extracts exhibit strong binding affinities against alpha-amylase, alpha-glucosidase, and cox- 2 receptors and might be responsible for suppressing glycemic and prostaglandin control by inhibiting them. A more thorough and long-term study of this topic may be initiated in the future. These plant extracts could be excellent choices for the treatment of diabetes, arthritis, hyperlipidemia, and oxidative damage during diabetes treatment. However, further investigations are required to determine the specific mechanism of action of these extracts in treating diabetes and arthritis at the cellular level.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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References

1. Evans, M. *A Guide to Herbal Remedies*Orient Paperbacks,. (1994).
2. Kamboj, V. P. Herbal medicine. *Curr. Sci.* **78**, 35–39 (2000).
3. Gupta, L. M. & Raina, R. Side effects of some medicinal plants. *Curr. Sci.* **75**, 897–900 (1998).
4. Pal, S. K. & Shukla, Y. Herbal medicine: current status and the future. *Asian Pac. J. cancer Prev.* **4**, 281–288 (2003).
5. Banday, M. Z., Sameer, A. S. & Nissar, S. Pathophysiology of diabetes: an overview. *Avicenna J. Med.* **10**, 174–188 (2020).
6. Scott, D. L., Wolfe, F. & Huizinga, T. W. Artritis reumatoide. *Lancet* **376**, 1094–1108 (2010).
7. McInnes, I. B. & Schett, G. The pathogenesis of rheumatoid arthritis. *N Engl. J. Med.* **365**, 2205–2219 (2011).
8. Petsch, C. et al. Prevalence of monosodium urate deposits in a population of rheumatoid arthritis patients with hyperuricemia. *In Seminars in arthritis and rheumatism* **45**(6), 663–668. https://doi.org/10.1016/j.semarthrit.2015.11.014 (2016).
9. Zhao, X. et al. Natural Plant Extracts and Compounds for Rheumatoid Arthritis Therapy. *Medicina* vol. 57 at (2021). https://doi.org/10.3390/medicina57030266
10. Lau, C. S. et al. 2018 Update of the APLAR recommendations for treatment of rheumatoid arthritis. *Int. J. Rheum. Dis.* **22**, 357–375 (2019).
11. Ferro, F., Elefante, E., Luciano, N., Talarico, R. & Todoerti, M. One year in review 2017: novelties in the treatment of rheumatoid arthritis. *Clin. Exp. Rheumatol.* **35**, 721–734 (2017).
12. Xu, T. et al. A study on the effective substance of the Wu-tou formula based on the metabonomic method using UPLC-Q-TOF-HDMS. *Mol. Biosyst.* **11**, 3081–3091 (2015).
13. Mishra, J. N. & Verma, N. K. A brief study on catharanthus roseus: A review. *Int. J. Res. Pharm. Pharm. Sci.* **2**, 2455–2698 (2017).
14. Aslam, J. et al. Catharanthus roseus (L.) G. Don. An important drug: it's applications and production. *Int. J. Compr. Pharm.* **1**, 1–16 (2010).
15. Article, R. International Journal of Pharma and Bio Sciences ISSN PHARMACOLOGICAL ACTIVITIES OF CATHARANTHUS ROSEUS: A PERSPECTIVE REVIEW. 4, 431–439 (2013).
16. Singh, V. P. & Jagdev, R. S. D. Ajmalicine (raubacine); A medicinally important alkaloid from catharanthus roseus (Vinca rosea). *Suppl. Cultiv Util. Med. Plants Jammu-Tawi Reg. Res. Lab. India* 199–206 (1996).
17. Jaleel, C. A. & Panneerselvam, R. Variations in antioxidant and indole alkaloid status in different parts of two varieties of Catharanthus roseus. *Chin. J. Pharmacol. Toxicol.* **21**(6), 487–494 (2007).
18. Magnotta, M., Murata, J., Chen, J. & De Luca, V. Identification of a low Vindoline accumulating cultivar of catharanthus roseus (L.) G. Don by alkaloid and enzymatic profiling. *Phytochemistry* **67**, 1758–1764 (2006).
19. Rasineni, K., Bellamkonda, R., Singareddy, S. R. & Desireddy, S. Antihyperglycemic activity of catharanthus roseus leaf powder in streptozotocin-induced diabetic rats. *Pharmacognosy Res.* **2**, 195–201 (2010).
20. Patel, Y., Vadgama, V., Baxi, S. & Tripathi, C. B. Evaluation of hypolipidemic activity of leaf juice of catharanthus roseus (Linn.) G. Donn. In Guinea pigs. *Acta Pol. Pharm.* **68**, 927–935 (2011).
21. Carew, D. P. & Patterson, B. D. The effect of antibiotics on the growth of Catharanthus roseus tissue cultures. (1970).
22. Rajas, M. C. N. & Cuellar, M. C. A. Comparative Microbiological studies of the alkaloids of catharanthus roseus and other related compounds. *Rev. Cuba Farm.* **15**, 131–138 (1981).
23. Farnsworth, N. R., Svoboda, G. H. & Blomster, R. N. Antiviral activity of selected catharanthus alkaloids. *J. Pharm. Sci.* **57**, 2174–2175 (1968).
24. Jaleel, C. A. et al. Induction of drought stress tolerance by ketoconazole in catharanthus roseus is mediated by enhanced antioxidant potentials and secondary metabolite accumulation. *Colloids Surf. B Biointerfaces*. **60**, 201–206 (2007).
25. Ram, V. J. & Kumari, S. Natural products of plant origin as anticancer agents. *Drug News Perspect.* **14**, 465–482 (2001).
26. El-Sayed, A. & Cordell, G. A. Catharanthus alkaloids. XXXIV. Catharanthamine, a new antitumor bisindole alkaloid from catharanthus roseus. *J. Nat. Prod.* **44**, 289–293 (1981).
27. Nayak, B. S. & Pinto Pereira, L. M. Catharanthus roseus flower extract has wound-healing activity in Sprague Dawley rats. *BMC Complement. Altern. Med.* **6**, 1–6 (2006).
28. Chattopadhyay, R. R., Banerjee, R. N., Sarkar, S. K., Ganguly, S. & Basu, T. Antiinflammatory and acute toxicity studies with the leaves of Vinca rosea Linn in experimental animals. *Indian J. Physiol. Pharmacol.* **36**(4), 291–292 (1992).
29. Spencer, E. & Al C. F. Survey of plants for antimalarial activity. (1947).
30. Nammi, S., Boini, M. K., Lodagala, S. D. & Behara, R. B. The juice of fresh leaves of catharanthus roseus Linn. Reduces blood glucose in normal and Alloxan diabetic rabbits. *BMC Complement. Altern. Med.* **3**, 1–4 (2003).
31. Azam, K., Rasheed, M. A., Omer, M. O., Altaf, I. & Akhlaq, A. Anti-hyperlipidemic and anti-diabetic evaluation of ethanolic leaf extract of catharanthus roseus alone and in combination therapy. *Brazilian J. Pharm. Sci.* **58**, e18672 (2022).
32. Singh, S. N. et al. Effect of an antidiabetic extract of catharanthus roseus on enzymic activities in streptozotocin induced diabetic rats. *J. Ethnopharmacol.* **76**, 269–277 (2001).
33. Asija, R., Samaria, S., Khanijau, R. & Verma, T. Evaluation of anti-arthritic activity of the ethanolic extract of catharanthus roseus in Wistar rats. *Chem. Res. J.* **7**, 116–127 (2022).
34. Hossain, M. T. Antioxidant, cytotoxicity, membrane stabilization and anthelmintic activity of ethanolic extract of sarcochlamys pulcherrima leaves. *Int. J. Green. Herb. Chem.* **4**, 274–283 (2015).
35. Hossain, S. J. et al. Evaluation of antioxidant, antidiabetic and antibacterial activities of the fruit of sonneratia Apetala (Buch.-Ham). *Orient. Pharm. Exp. Med.* **13**, 95–102 (2013).
36. Bari, M. W. et al. Antidiabetic effect of Wedelia chinensis leaf extract in Alloxan induced Swiss albino diabetic mice. *Clin. Phytoscience*. **6**, 1–8 (2020).

37. Alamgeer, Uttra, A. M. & Hasan, U. H. Anti-arthritic activity of aqueous-methanolic extract and various fractions of berberis orthobotrys Bien ex Aitch. *BMC Complement. Altern. Med.* **17**, 1–16 (2017).
38. Sharma, B., Salunke, R., Balomajumder, C., Daniel, S. & Roy, P. Anti-diabetic potential of alkaloid rich fraction from capparis decidua on diabetic mice. *J. Ethnopharmacol.* **127**, 457–462 (2010).
39. Lorke, D. A new approach to practical acute toxicity testing. *Arch. Toxicol.* **54**, 275–287 (1983).
40. Gupta, R. K., Kumar, D., Chaudhary, A. K., Maithani, M. & Singh, R. Antidiabetic activity of passiflora Incarnata Linn. In streptozotocin-induced diabetes In mice. *J. Ethnopharmacol.* **139**, 801–806 (2012).
41. Bamidele, O., Arokoyo, D. S., Akinnuga, A. M. & Oluwarole, A. O. Antidiabetic effect of aqueous extract of Basella alba leaves and metformin in alloxan-induced diabetic albino rats. *African J. Biotechnol.* **13**, (2014).
42. Ghasemi A, Jeddi S. Streptozotocin as a tool for induction of rat models of diabetes: a practical guide. EXCLI J. 2023 Feb 21;22:274-294. https://doi.org/10.17179/excli2022-5720. PMID: 36998708; PMCID: PMC10043433.
43. Salim, N. S., Abdel-Alim, M., Said, H. E. M. & Foda, M. F. Phenolic profiles, antihyperglycemic, anti-Diabetic, and antioxidant properties of Egyptian Sonchus oleraceus leaves extract: an in vivo study. *Molecules* **28**, 6389 (2023).
44. Walters, W. P. Going further than Lipinski's rule in drug design. *Expert Opin. Drug Discov.* **7**, 99–107 (2012).
45. Pettersen, E. F. et al. UCSF Chimera—a visualization system for exploratory research and analysis. *J. Comput. Chem.* **25**, 1605–1612 (2004).
46. Maurus, R. et al. Alternative catalytic anions differentially modulate human α-amylase activity and specificity. *Biochemistry* **47**, 3332–3344 (2008).
47. Roig-Zamboni, V. et al. Structure of human lysosomal acid α-glucosidase–a guide for the treatment of Pompe disease. *Nat. Commun.* **8**, 1111 (2017).
48. Lucido, M. J., Orlando, B. J., Vecchio, A. J. & Malkowski, M. G. Crystal structure of aspirin-acetylated human cyclooxygenase-2: insight into the formation of products with reversed stereochemistry. *Biochemistry* **55**, 1226–1238 (2016).
49. BIOVA, D. S. Enhancing Rational Ligand Design Pharmacophore and Ligand-Based Design With Biovia Discovery Studio ® Datasheet. (2023).
50. Kaplan, W. Software review Swiss-PDB viewer (Deep View). *Brief. Bioinform.* **2**, 195–197 (2001).
51. Ferreira, L. G., Santos, D., Oliva, R. N., Andricopulo, A. D. & G. & Molecular Docking and structure-based drug design strategies. *Molecules* **20**, 13384–13421 (2015).
52. Yuliana, D., Bahtiar, F. I. & Najib, A. Silico screening of chemical compounds from roselle (Hibiscus Sabdariffa) as Angiotensin-I converting enzyme inhibitor used pyrx program. *J. Sci. Technol.* **3**, 1158–1160 (2013).
53. Daina, A., Michielin, O. & Zoete, V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.* **7**, 42717 (2017).
54. Odewo, S. A., Ajani, B. A., Osiyemi, O. A., Adeniji, K. A. & Ugbogu, O. A. Phytochemical profiling of leaf of glinus Lotoides (Molluginеceae) using GC-MS. *Niger J. Chem. Res.* **28**, 1–11 (2023).
55. Hadi, M. Y., Mohammed, G. J. & Hameed, I. H. Analysis of bioactive chemical compounds of Nigella sativa using gas chromatography-mass spectrometry. *J. Pharmacogn Phyther.* **8**, 8–24 (2016).
56. Akash, S. et al. Antioxidant, Antidiabetic, Analgesic, and Antibacterial Properties of Chrysopogon zizanioides Leaf Extract: An In Vivo, In Vitro, and In Silico Evaluation. (2024).
57. Sonia, S. & Singh, S. Phytoconstituents of Ziziphus nummularia (Burm. f.) Wight and Arn. Leaves extracts using GC-MS spectroscopy. *Res. Rev. J. Life Sci.* **9**, 109–118 (2019).
58. Athiappan, M. Antibiofilm Efficacy of Mangifera indica Kernel Methanol Extract against Staphylococcus aureus.
59. Zinjarde, S. S., Bhargava, S. Y. & Kumar, A. R. Potent α-amylase inhibitory activity of Indian ayurvedic medicinal plants. *BMC Complement. Altern. Med.* **11**, 1–10 (2011).
60. Muralidharan, L. Hypoglycemic and biochemical remedies of Cathanthus roseus (Linn) on Alloxan–induced diabetic rat and its antioxidant status in rat lenses. *Int. J. Med. Res. Pharm. Sci.* **2**, 1–6 (2015).
61. Karuna, R. & Saralakumari, D. Preventive effect of Catharanthus roseus (Linn.) against high-fructose diet-induced insulin resistance and oxida-tive stress in male Wistar rats. *J. diabetes Mellit.* (2011). (2011).
62. Antia, B. S. & Okokon, J. E. Effect of leaf juice of catharanthus roseus Linn on cholesterol, triglyceride and lipoproteins levels in normal rats. *Indian J. Pharmacol.* **37**, 401–402 (2005).
63. Porchezhian, E., Ansari, S. H. & Shreedharan, N. K. K. Antihyperglycemic activity of Euphrasia officinale leaves. *Fitoterapia* **71**, 522–526 (2000).
64. Swanston-Flatt, S. K., Day, C., Bailey, C. J. & Flatt, P. R. Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. *Diabetologia* **33**, 462–464 (1990).
65. Hasan, U. H., Uttra, A. M. & Rasool, S. Evaluation of in vitro and in vivo anti-arthritic potential of berberis calliobotrys. ||| *Bangladesh J. Pharmacol.* **10**, 807–819 (2015).
66. Medha, M. M., Devnath, H. S., Biswas, B., Bokshi, B. & Sadhu, S. K. In Silico profiling of analgesic and antihyperglycemic effects of ethanolic leaves extract of amischotolype mollissima: evidence from in vivo studies. *Saudi J. Biol. Sci.* **29**, 103312 (2022).

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Author contributions

BKS performed review & editing, Conceptualization, Methodology, Project administration; MJI and JIP were involved in Writing – review & editing, Conceptualization, Methodology; MAH, NJ, MSH done Writing – original draft, Conceptualization, Methodology, figure generation; SKK, BK completed the Writing – review & editing, Formal Analysis.

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Declarations

Competing interests

The authors declare no competing interests.

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