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Research Article

Biochemical Analysis and Human Aldose Reductase Inhibition Activity of Selected Medicinal Plants of Nepal

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Aldose reductase has received extensive research as a key enzyme in the development of long-term problems linked to diabetes mellitus. Overexpression of this enzyme or with exceeded glucose concentration in the blood increases sorbitol on the retina leading to retinopathy, which is the adverse effect of type II diabetes. Approximately 100 million people are suffering from diabetic retinopathy globally. This research is focused on studying the total phenolic content (TPC), total flavonoid content (TFC), antioxidant potential, and aldose reductase inhibiting properties of selected medicinal plants such as *Anacyclus pyrethrum, Bergenia ciliata, Rhododendron arboreum,* and *Swertia chirayita.* In addition, ADMET analysis and molecular docking of seven previously identified compounds from the chosen medicinal plants were carried out against human aldose reductase (PDB ID: 4JIR). The ethanol extract of *S. chirayita* exhibited the highest TPC (4.63 ± 0.16 mg GAE/g) and TFC (0.90 ± 0.06 mg QE/g). Analysis of 2,2-diphenyl-1-picrylhydrazyl (DPPH)-based antioxidant assay showed that IC_{50} of the ethanolic extract of *B. cilata* and *R. arboreum* showed a significant antioxidant activity with IC_{50} value of 0.05 mg/mL. The percentage inhibition of AR by extract of *B. ciliata* ($94.74 \pm 0.01\%$) was higher than other plant extracts. A molecular docking study showed that morin isolated from *B. ciliata* showed a good binding interaction with AR. This study showed that the extracts of *A. pyrethrum, B. ciliata,* and *R. arboreum* could be potential sources of inhibitors against AR to treat retinopathy.

1. Introduction

Aldose reductase (AR) is an NADPH-dependent oxidoreductase that metabolizes the transformation of glucose to sorbitol in the polyol pathway [1]. In hyperglycemic states, an increased flux of glucose through the polyol pathway has been thought to harm tissues via different processes, including sorbitol accumulation leading to an osmotic imbalance [2] and pyridine nucleotide redox state dysregulation decreasing cellular antioxidant capacity [3], as well as a rise in advanced glycated end products [4, 5]. In diabetes mellitus, the polyol pathway produces more sorbitol than normal, which does not easily diffuse across the cell membranes, and the intracellular sorbitol accumulation has also been linked to the development of chronic complications of diabetes, including cataracts, neuropathy, and retinopathy (Figure 1) [6–8]. A complication of diabetes called diabetic retinopathy (DR) is brought on by high blood sugar levels harming the retina. If not identified and treated early, it might result in blindness. In adults aged 20 to 74, DR is the most common factor in new instances of blindness [9] and is a leading cause of preventable blindness among the working population of adults. Approximately 100 million adults worldwide suffer from DR and that number is expected to rise to 160.5 million by 2045 [10]. According to the Global Burden of Disease Study, DR is the fifth leading cause of blindness and vision impairment among

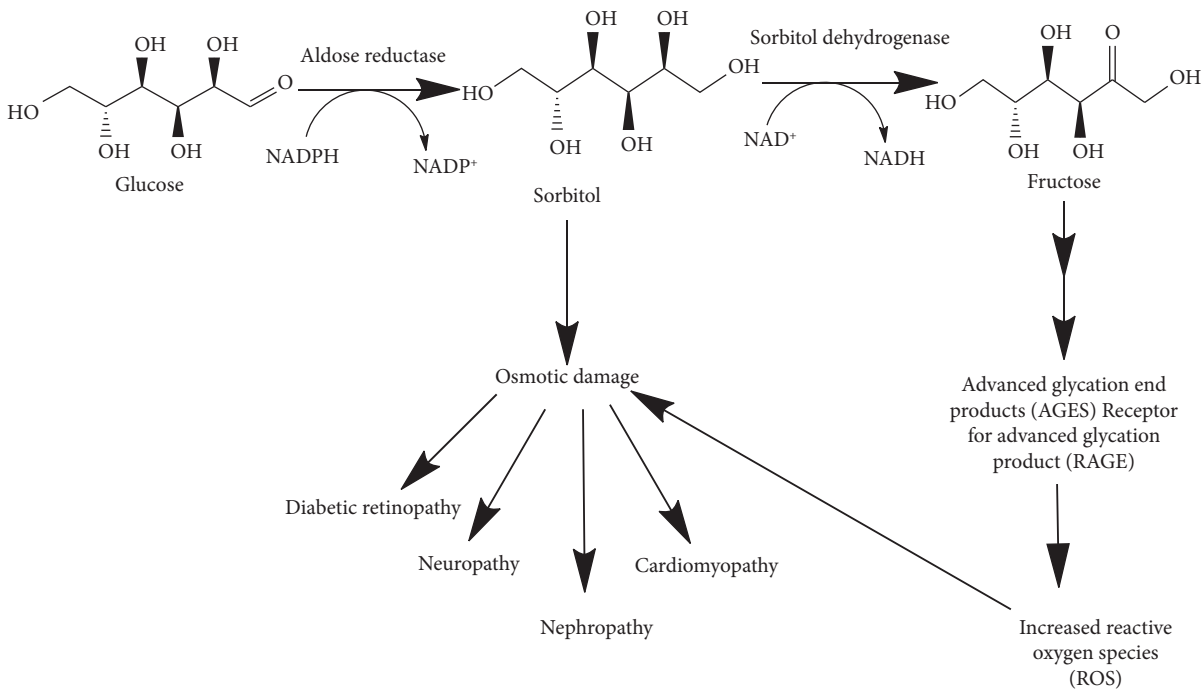

Figure 1: Role of aldose reductase in diabetic complications.

adults aged 50 and older. Between 1990 and 2020, the age-standardized global prevalence of blindness caused by diabetic eye disease increased from 14.9% to 18.5% [9–11].

Inhibitors of AR, including epalrestat, alrestatin, zenarestat, ponalrestat, lidorestat, tolrestat, sorbinil, minalrestat, and fidarestat, were synthesized [12, 13]. The AR inhibitors come from two distinct chemical subgroups; sorbinil, Dilantin, and minalrestat are examples of hydantoin derivatives. Similarly, epalrestat, alrestatin, and tolrestat are examples of carboxylic acid derivatives [14]. The only commercially accessible synthetic aldose reductase inhibitor is epalrestat [15]. The adverse effects of epalrestat have caused it to be taken off the market in some countries, and all other inhibitors have failed in clinical studies due to poor pharmacokinetic qualities [16]. Therefore, it is necessary to investigate the natural sources to find safer therapeutic chemicals. Naturally occurring compounds and medicinal plant extracts exhibit AR inhibitory effects, and their preclinical ability to treat diabetes problems is promising [12]. For instance, flowers of the *Rhododendron arboreum* are of very high medicinal value. It is well known for its versatility and efficacy in treating various disorders, including eczema, diarrhea, menstrual problems, choleretic, diuretic, antispasmodic, and anti-inflammatory disorder, and as it acts as an antioxidant [17]. Different pharmacological activities of *Swertia chirayita* include anthelmintic activities [18], hypoglycemic and antipyretic properties [19], antiviral activities [20], anticancer activities [21], and hypoglycemic and anti-inflammatory activities [22]. The medicinal plant *Bergenia ciliata* treats a variety of disorders, including diabetes, cancer, respiratory problems, diarrhea, fever, cough, vomiting, and is also used for wound healing [23]. The roots of *Anacyclus pyrethrum* are advocated for use in folk medicine to treat a variety of ailments, including angina, digestive issues, female infertility, lethargy, and even paralysis of the tongue and limbs [24]. *A. pyrethrum* roots also exhibit sialagogue [25], aphrodisiac [26], immunostimulant [27], anti-inflammatory [28], anticonvulsant, antioxidant [29], antidiabetic, and memory-improving effects [30]. Some computational analyses have been developed to support the *in vitro* assays investigating the potential binding mechanisms of compounds. Molecular docking helps in the field of *in silico* drug design by identifying the molecules that can bind to a protein's active site. It illustrates how a promising therapeutic candidate (ligand) interacts with the target receptor's binding site and inhibits the target receptor's biological and catalytic activities [31]. Likewise, before chemical synthesis and biological testing, the prediction of biological activity for substances (PASS) can be used to estimate the biological activity profiles of compounds [32]. In this study, we focused on some medicinal plants, which contain important bioactive compounds that could inhibit the catalytic activity of an enzyme aldose reductase (AR) thereby preventing diabetic complications through *in vitro* and *in silico* studies.

2. Materials and Methods

2.1. Chemicals. Most of the chemicals and solvents were of the analytical grade. Gallic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Molychem and Hi-Media (India), respectively.

Quercetin, dimethyl sulfoxide (DMSO), ethanol, acetone, and other solvents were purchased from Fisher Scientific (India), E. Merck, and Qualigens. Sorbinil and DL-glyceraldehyde were purchased from Sigma-Aldrich and NADPH from Calbiochem.

2.2. Collection of Plant Materials. Different parts (leaves, rhizomes, flowers, and fruits) of plants were collected from various parts of Nepal based on the ethnomedicinal and traditional medicinal practices, as shown in Table 1. *Rhododendron arboreum* was collected from Kathmandu while *Anacyclus pyrethrum*, *Bergenia ciliata,* and *Swertia chirayita* were collected from the Kaski district of Nepal. The plant samples got identified at the National Herbarium and Plant Laboratories at Godawari, Lalitpur, Nepal.

2.3. Preparation of Plant Extract. The plant materials were grounded into a fine powder after being air-dried for a month at room temperature in a shady place. The maceration method was applied for preparing a crude extract of medicinal plants. For this, 160 mL of 95% ethanol and 60 g of fine powder were mixed at room temperature for three days. The dissolved components were filtered daily and kept in a glass bottle. The final collection of the dissolved parts was then evaporated at a reduced pressure at 50°C using a rotary evaporator to yield the crude extracts.

$$\text{Percentage yield} = \frac{\text{Dry weight of extract}}{\text{Dry weight of sample}} \times 100\%. \quad (1)$$

2.4. Phytochemical Screening. Phytochemicals are a variety of primary and secondary metabolites responsible for antihyperglycemic, anti-inflammatory, antidiabetic, and antimicrobial action along with other recognized biological activities which are abundant in medicinal plants and herbs [36]. Major phytoconstituents present in the crude extracts of *A. pyrethrum*, *B. ciliata, S. chirayita*, and *R. arboreum* were screened by using standard qualitative methods as presented in [37, 38] and [39]. Assays were carried out to ascertain flavonoids, alkaloids, phenolic compounds, steroids, carbohydrates, saponins, tannins, and terpenoids. Flavonoids were detected based on the Shinoda test and alkaline test. Braemer's test, Salkowski' test, and foam test were performed for the detection of tannins, terpenoids, and saponins, respectively. Phenolic compounds and alkaloids were determined by the ferric chloride test and Mayer's test, respectively. Fehling's test and steroid test were performed to detect carbohydrates and steroids.

2.5. Determination of the Total Phenolic Content and Total Flavonoid Content. The naturally occurring phenolic and flavonoid components have an antioxidant ability, which in turn prevents the chain reaction of reactive oxygen species. Reactive oxygen species have the potential to induce oxidative stress [40]. The total phenolic content of the extracts was determined by using the Folin–Ciocalteu colorimetric method [41, 42]. Initially, 0.5 mL of 95% ethanol extract was mixed with 5 mL of 10% Folin–Ciocalteau reagent, and 4 mL of 1M Na_2CO_3 solution was added. The mixture was subsequently left to stand for 15 minutes at room temperature. The absorbance of the reaction mixture was measured at 765 nm by using a spectrophotometer.

Similarly, the total flavonoid content was determined according to the colorimetric method [43, 44]. For this, 0.5 mL of the extract (5 mg/mL) was mixed with 1.5 mL of 95% ethanol and 0.1 mL of aluminum trichloride ($AlCl_3$, 10%). Subsequently, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water were added to each bottle, and the reaction mixture was allowed to stand for 30 minutes. The UV-visible spectrophotometer was used to measure the absorbance at 415 nm. The standard curve for quercetin (10–50 μg/mL) was utilized for TFC and standard gallic acid (10–80 μg/mL) was used for TPC. The amount of all polyphenolic and flavonoid components in the extracts was represented as milligrams of gallic acid and quercetin equivalent per gram of dry weight, respectively.

2.6. Determination of Antioxidant Activity. Antioxidants interact with free radicals and prevent the oxidative stress induced by excess free radicals. One of the stable free radicals is 2,2-diphenyl-1-picrylhydrazyl (DPPH), which shows a strong absorption band at 517 nm, and the absorption decreases when reduced by an oxidant [45]. Based on the radical scavenging properties of DPPH, the antioxidant activity of the extracts and the standard (ascorbic acid) were evaluated following the protocol of Alabri et al. [46]. Various concentrations of the plant extract (sample solutions) and ascorbic acid (reference samples) were prepared in ethanol (1000, 500, 100, and 50 μg/mL). To the various concentrations of the sample plant extracts and ascorbic acid solutions, 4 mL of a 0.1 mM DPPH solution in ethanol was mixed. The mixture was left in the dark for 30 minutes. Similarly, 1 mL of ethanol (solvent) was added to 4 mL of 0.1 mM DPPH as a control, and the mixture was left in the dark for 30 minutes. The absorbance was measured at 517 nm after 30 minutes. The following equation is used to determine the ability to scavenge the DPPH radical [47]:

$$\text{Percentage scavenging} = \frac{A_o - A_t}{A_o} \times 100\,\%, \quad (2)$$

where A_o = absorbance of DPPH solution (control, without samples) and A_t = absorbance of solution mixture of the test or reference sample and DPPH.

The percentage scavenging was then plotted against concentration and a regression equation was obtained from which IC_{50} values were calculated for each plant extract by the formula given in Microsoft Excel 2007 software.

2.7. Inhibition of Aldose Reductase. The aldose reductase inhibition activity of the selected plant extract was accessed spectrophotometrically, by using glyceraldehyde as a substrate and NADPH as a cofactor following the protocol of Nakano and Petrash [48]. Initially, 100 μL of potassium phosphate buffer (pH 7.0), 755 μL of double distilled water, 20 μL of 1 mM DL-glyceraldehyde, 100 μL of 1.5 mM NADPH, and 20 μL of plant extract were added to the cuvette. Similarly, the blank solution contains 20 μL of double distilled water instead of plant extract. After that, 5 μL of RHAR was added and sorbinil was used as a positive

Table 1: Description of the selected medicinal plants.

Scientific name	Family	Parts used	Traditional usage	References
Anacyclus pyrethrum	Asteraceae	Rhizome	Used to treat toothaches, salivary secretions, and digestive problems	[33]
Bergenia ciliata	Saxifragaceae	Rhizome	Used to treat fever and cough	[34]
Rhododendron arboreum	Ericaceae	Flower	Used to cure fever, stomach ache, and blood dysentery	[17]
Swertia chirayita	Gentianaceae	Whole plant	Used to treat liver disorders, malaria, and diabetes	[35]

control for the inhibitor study. The optical density (OD) of the reaction was monitored at 340 nm for 5 minutes at the interval of 30 seconds and the percentage inhibition was calculated. A decrease of OD/min represents the inhibition activity.

$$\text{Percentage inhibition} = \frac{\text{OD of blank solution} - \text{OD of plant extract}}{\text{OD of blank solution}} \times 100\,\%. \quad (3)$$

2.8. Statistical Analysis. All the experiments were performed in triplicates and data were presented in mean ± standard error from three independent assays. Microsoft Excel 2007 software was used to calculate TPC, TFC, and antioxidant assay. All the chemical structures were prepared by using ChemDraw Ultra 12.0 software. Principle component analysis (PCA) and correlation analysis were performed by using R (version 4.2.1) and R Studio (version 2022.07.1).

2.9. Molecular Docking Studies and Pharmacokinetic Analysis

2.9.1. Determination of Ligands and Receptors. A list of isolated compounds from the different parts (i.e., rhizome, flower, and whole plant) of the selected medicinal plants was prepared through a literature review. The bioactive compounds were chosen based on earlier *in vitro* research. The selected compounds were morin, catechin, coumaric acid, arbutin, ursolic acid, kaempferol, and swertiamarin which are shown in Table S1 with their IC_{50} or percentage inhibition and their chemical structures are shown in Figure 2.

2.9.2. Receptor and Ligand Preparation. The crystal structure of the protein was retrieved from Protein Data Bank (https://www.rcsb.org/) and prepared using BIOVIA Discovery Studio Visualizer 2020. The water, ligands, and heteroatoms were deleted, while polar hydrogens and Kollman's charges were added. Before removing the complex ligands, their attributes were observed and noted. The PubChem database was searched for the ligands. They were optimized for docking and were saved in .pdbqt format by using the AutoDock tool.

2.9.3. Molecular Docking. Molecular docking was carried out using AutoDock Vina software version 1.5.7. The characteristics, including the position and size of the grid box, were defined in a configuration file. The obtained grid box had the following dimensions: $x = -8.4388$, $y = 8.820$, and $z = 18.683$; size $X = 40$, $Y = 40$, and $Z = 40$; and exhaustiveness = 8. The cocrystalized ligand was removed from the cocrystal structure and redocked with the same receptor (i.e., 4JIR) to validate the docking structure. The docking studies were carried out for the selected ligands along with standard epalrestat. After docking, the pose with the lowest binding energy (kcal/mol) and most H-bonds was determined to be the best. The binding interaction between the ligand and receptor was examined using BIOVIA Discovery Visualizer.

2.9.4. Pharmacokinetic and ADMET Profile. To reduce side effects, ADMET and drug-likeness of potential hit compounds are essential for the pharmaceutical industry [49]. The pharmacokinetic parameters were predicted using the web-based program SwissADME (https://www.swissadme.ch) [50]. The rule of five, commonly known as Lipinski's rule, is used to determine drug-likeness [51]. Another web server, ProTox-II, was used for the toxicity analysis [52].

2.10. Estimation of Biological Activity. The selected phytochemical's biological activity was predicted by using the main Way2Drug server. It predicts a wide range of biological activity based on the structure of molecules instantaneously. The activity is determined using the variables Pa (probable activity) and Pi (probable inactivity). For a specific pharmacological activity, only substances with a Pa greater than Pi were examined [32]. As a result, it is possible to forecast whether ligands are potentially active or inactive based on their Pa and Pi values.

The detailed experimental procedure of this research work is shown in Figure 3.

3. Results

3.1. Phytochemical Analysis. The extract prepared from dissolving 60 g of dry weight in ethanol showed variation in the percentage yield from 18% to 42%. Out of the four extracts, *R. arboreum* showed the highest percentage yield with 42% followed by *A. pyrethrum* (37%), *B. ciliata* (30%), and *S. chirayita* (18%) as shown in Table 2. Similarly, the phytochemical analysis of plant crude extracts displayed the presence of flavonoids, glycosides, tannins, and terpenoids in all plants. However, proteins, amino acids, and saponins

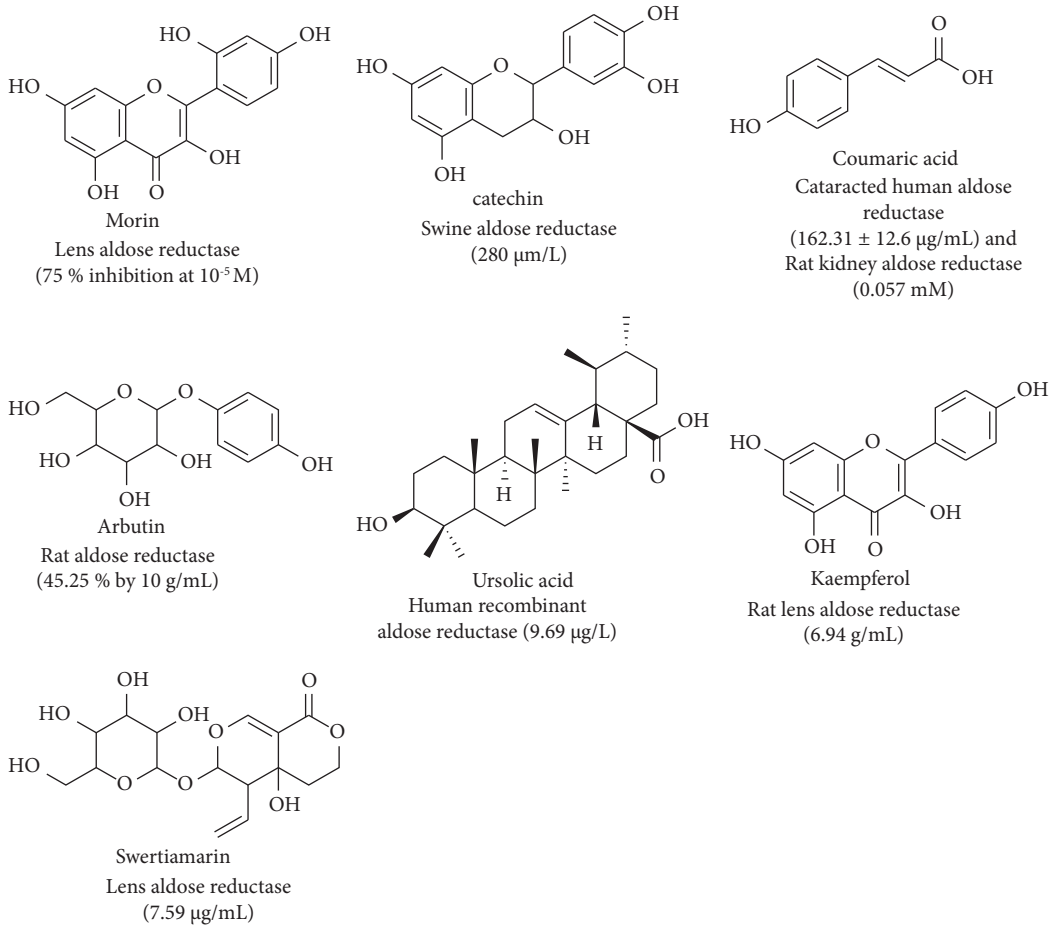

Figure 2: Chemical structures of phytoconstituents of the selected medicinal plants, which have shown inhibitory effects with various aldose reductases.

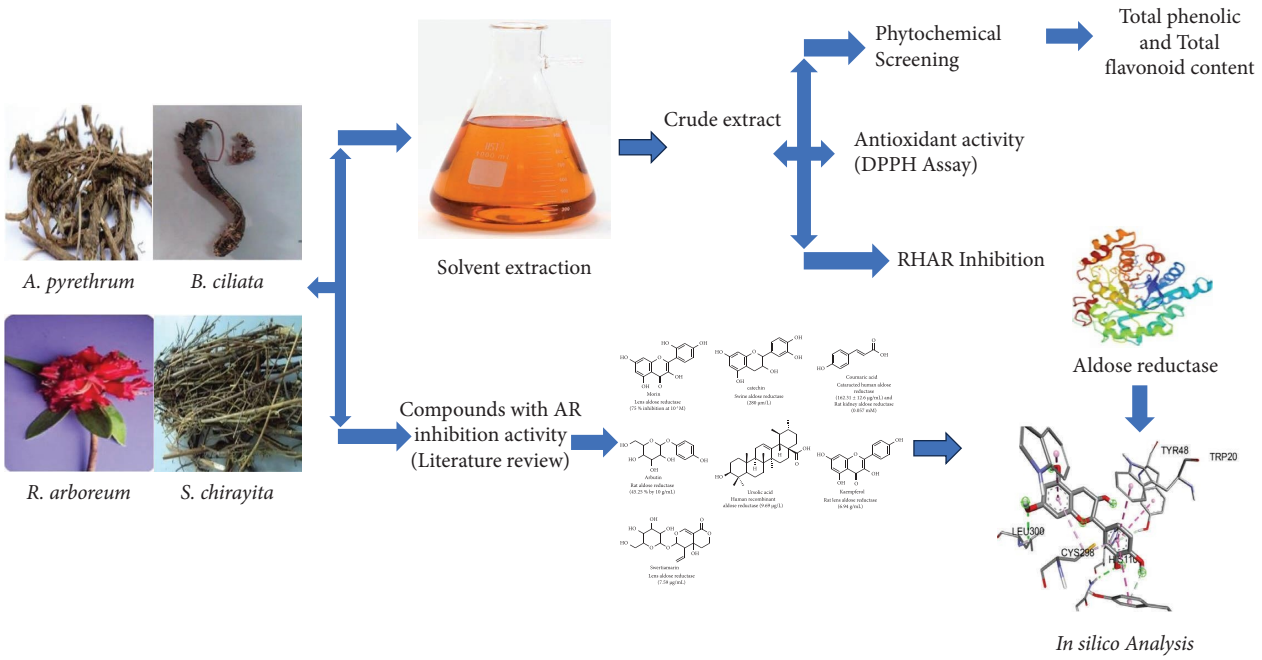

Figure 3: Graphical representation of the experimental procedure.

Table 2: Physical characteristics, percentage yield, TPC, and TFC of plants in the ethanol extract.

Plants	Color of crude extract	Percentage yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)
S. chirayita	Dark brown	18	4.63 ± 0.16	0.90 ± 0.06
R. arboreum	Red	42	4.56 ± 0.06	0.45 ± 0.05
A. pyrethrum	Dark brown	37	3.07 ± 0.07	0.62 ± 0.01
B. ciliata	Dark brown	30	2.86 ± 0.21	0.48 ± 0.08

were found in all plants except *R. arboreum*. All these findings of plant crude extracts are shown in Table S2.

3.2. Determination of TPC and TFC. The total phenolic content (TPC) of the extract is expressed in terms of gallic acid equivalent (mg GAE/g dry weight of extract) with a calibration curve of gallic acid (($y = 0.001x + 0.001$, $R^2 = 0.989$) and ($y = 0.014x$, $R^2 = 0.988$)). Similarly, the total flavonoid content (TFC) of the different plants in ethanol extract is expressed in terms of quercetin equivalent (mg QE/g) with a calibration curve of quercetin ($y = 0.012x - 0.007$, $R^2 = 0.997$).

The ethanol extract of *S. chirayita* showed the highest TPC (4.63 ± 0.16 mg GAE/g) and TFC content (0.90 ± 0.06 mg QE/g) followed by *R. arboreum and A. pyrethrum,* respectively (Table 2).

3.3. DPPH Free Radical Scavenging Activity. Free radical scavenging was performed to evaluate the antioxidant activity of plant extracts, and the result was expressed as IC_{50} (half inhibitory concentration). Stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was used to measure the free radical scavenging activity. The results were compared with the standard ascorbic acid with an IC_{50} value of 0.015 mg/mL. The ethanolic extract of *B. ciliata* and *R. arboreum* showed significant antioxidant activity with an IC_{50} value of 0.05 mg/mL, while *A. pyrethrum* and *S. chirayita* extract exhibited a poor antioxidant activity with IC_{50} of 0.28 mg/mL and 0.54 mg/ml, respectively.

3.4. Aldose Reductase Inhibitory Activity. The crude ethanol extracts of all four plants were found potent towards RHAR. The ethanolic extract of *B. ciliata* showed a greater inhibitory effect on RHAR (94.74 ± 0.01%) followed by *A. pyrethrum, R. arboreum,* and *S. chirayita* (89.47 ± 0.01%, 63.64 ± 0.01%, and 56.25 ± 0.01%), respectively.

3.5. Statistical Analyses

3.5.1. Correlation. The Shapiro–Wilk test has indicated that all data are normally distributed (having a p value greater than 0.05), as shown in Table 3.

Since data are normally distributed, the Karl–Pearson correlation coefficient was evaluated (Table 4).

The Karl–Pearson coefficient demonstrates a strong positive correlation between TPC and IC_{50} and a strong negative correlation between TPC and RHAR. However, TPC and TFC are weakly correlated and RHAR shows a strong negative correlation with all three components, i.e., TPC, TFC, and IC_{50}.

Table 3: Shapiro–Wilk test for normality.

	TPC	TFC	RHAR	IC_{50}
W	0.79965	0.87305	0.87918	0.8607
P value	0.1016	0.3098	0.3351	0.2627

Table 4: Karl–Pearson correlation coefficient.

	TPC	TFC	RHAR	IC_{50}
TPC	1	0.4018469	−0.991292	0.9002571
TFC	0.4018469	1	−0.5184795	0.7193974
RHAR	−0.991292	−0.5184795	1	−0.9458361
IC_{50}	0.9002571	0.7193974	- 0.9458361	1

3.5.2. Principal Component Analysis. The scree plot of principal component analysis is shown in Figure 4, and it shows that only two components have an eigenvalue greater or equal to 1. So, only principal components 1 and 2 were used for further analysis as they account for the majority of variance in data. Tables S3 and S4 show that all the variables except RHAR are negatively correlated to PC1. In addition, only TPC is negatively correlated to PC2.

3.6. Molecular Docking Analysis. Table 5 shows the ligand's binding affinities (docking scores) and H-bonding catalytic residues for human aldose reductase (PDB ID: 4JIR). The results of the docking analysis suggested that morin (−9.2 kcal/mol), ursolic acid (−8.9 kcal/mol), and kaempferol (−8.2 kcal/mol) were the potential candidates that could inhibit the target protein. The active sites of the receptor are shown in bold in Table 5. Morin was observed to form five conventional hydrogen bonds at LYS-21, TYR-48, GLN-183, SER-214, and CYS-298, whereas two pi-pi stacked bonds formed at TRP-20 and TYR-289. Similarly, the control drug epalrestat was stabilized by one conventional hydrogen bond at TRP-111. Along with H-bonding, additional interactions between the epalrestat and morin with the receptor included pi-pi, alkyl, and pi-alkyl, as shown in Figure 5. Similarly, the 2D and 3D interactions of other compounds are shown in Figure S1.

3.7. Pharmacokinetic and ADMET Properties. The pharmacokinetics and drug-like characteristics of the chosen compounds are shown in Table S5. Ursolic acid breaks one out of the five rules, but all other compounds were found

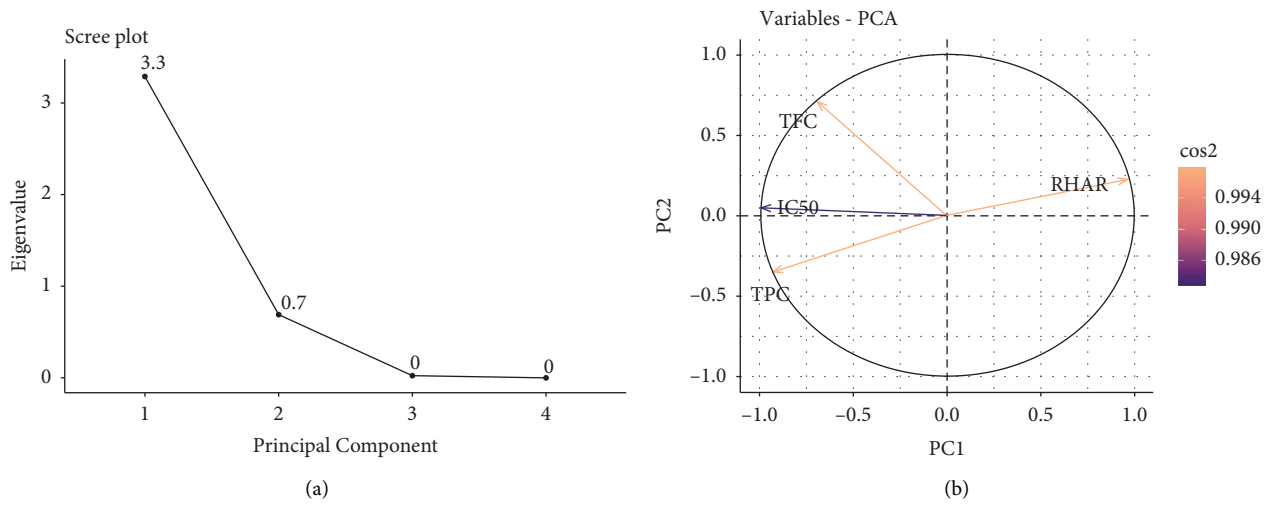

Figure 4: (a) Scree plot of principal component analysis and (b) principal component plot.

Table 5: Docking score results for human aldose reductase (PDB ID: 4JIR) receptor and selected metabolites.

Compound	Docking score (kcal/mol)	Interacting residue	Interaction type	Bond separation (Å)
Epalrestat	−8.1	TRP-111	Conventional hydrogen bond	2.67
		PHE-122	Pi-pi stacked	4.96
		TRP-219	Pi-sulfur	5.83
			Pi-pi T-shaped	4.97
		ALA-299	Pi-alkyl	5.36
		LEU-301	Pi-alkyl	4.56
Morin	−9.2	GLY-18	Carbon H-bond	3.66
		TRP-20	Pi-pi stacked	5.61
		LYS-21	Conventional hydrogen bond	1.96
		TYR-48	Conventional hydrogen bond	2.53
		GLN-183	Conventional hydrogen bond	2.44
		TYR-209	Pi-pi T-shaped	3.97
		SER-210	Carbon hydrogen bond	2.88
			Pi-sigma	3.97
		PRO-211	Pi-alkyl	5.33
		LEU-212	Pi-alkyl	5.49
		SER-214	Conventional hydrogen bond	1.96
		PRO-215	Pi-donor hydrogen bond	3.35
		CYS-262	Carbon hydrogen bond	3.08
		CYS-298	Conventional hydrogen bond	3.35
Catechin	−8.4	HIS-110	Conventional hydrogen bond	2.21
		TRP-111	Conventional hydrogen bond	2.0
			Pi-pi T-shaped	2.31
		PHE-122	Pi-pi stacked	4.17
		CYS-298	Pi-alkyl	4.76
		SER-302	Conventional hydrogen bond	2.30
Coumaric acid	−7.4	HIS-110	Conventional hydrogen bond	1.90
		TYR-209	Pi-sigma	3.78
		SER-210	Pi-donor hydrogen bond	3.04
Arbutin	−6.6	TRP-111	Conventional hydrogen bond	2.20
			Pi-pi T-shaped	5.43
		PHE-122	Pi-donor hydrogen bond	2.96
		CYS-298	Pi-alkyl	4.98
		ALA-299	Conventional hydrogen bond	2.30
		LEU-300	Conventional hydrogen bond	2.87

Table 5: Continued.

Compound	Docking score (kcal/mol)	Interacting residue	Interaction type	Bond separation (Å)
Kaempferol	−8.2	TRP-20	Pi-pi stacked	5.68
		TYR-48	Conventional hydrogen bond	1.82
		TRP-111	Pi-pi T-shaped	5.61
		TRP-219	Pi-pi T-shaped	5.36
			Pi-pi stacked	5.86
		LEU-301	Conventional hydrogen bond	2.99
		SER-302	Conventional hydrogen bond	2.28
Ursolic acid	−8.9	TRP-20	Pi-sigma	3.97
		HIS-110	Conventional hydrogen bond	2.92
Swertiamarin	−6.8	TRP-111	Conventional hydrogen bond	2.45
		TRP-219	Pi-alkyl bond	5.15
		ALA-299	Alkyl bond	4.45
		LEU-300	Carbon hydrogen bond	3.55
			Pi-sigma bond	3.87
		LEU-301	Alkyl bond	4.40
		SER-302	Conventional hydrogen bond	2.36
Lidorestat	−8.5	TRP-20	Conventional hydrogen bond	2.24
			Pi-pi stacked	7.29
			Pi-pi T-shaped	6.10
		HIS-110	Pi-cation	4.58
		TRP-111	Pi-pi T-shaped	5.43
		TRP-219	Pi-pi stacked	4.58
			Pi-pi T-shaped	5.46
		VAL-297	Halogen (fluorine)	2.02
		CYS-298	Pi-alkyl	4.93
		ALA-299	Conventional hydrogen bond	2.02
		LEU-300	Conventional hydrogen bond	2.54
		LEU-301	Pi-alkyl	5.39
Sorbinil	−8.1	TRP-20	Pi-pi T-shaped bond	5.49
			Pi-alkyl bond	6.03
		HIS-110	Pi-cation bond	4.27
		TRP-111	Conventional hydrogen bond	2.81
		CYS-298	Alkyl bond	4.54, 5.49
		ALA-299	Conventional hydrogen bond	2.91
		LEU-300	Conventional hydrogen bond	2.08
RG7774	−7.6	PHE-122	Pi-alkyl bond	5.33
		TRP-219	Pi-pi stacked	4.56, 4.85
			Pi-alkyl	3.93, 4.47
		ALA-299	Conventional hydrogen bond	1.92
			Pi-alkyl	4.82, 5.00
		LEU-300	Pi-alkyl	5.10
		LEU-301	Conventional hydrogen bond	2.56
			Pi-alkyl	4.55
			Pi-sigma	3.60

with no violations, indicating greater drug-like characteristics. The standard compound, epalrestat, also showed zero violation of the Lipinski's rule but fell under toxicity class two. Table S6 shows the results of ADMET and toxicity analysis.

3.8. PASS Activity Analysis. The prediction of activity spectra for substances (PASS) was analyzed with selected phytochemicals as shown in Table S7. Pa and Pi are the two parameters that played a role in PASS prediction, and their values ranged from 0 to 1. Table S3 shows that all our phytocompounds except ursolic acid showed activity of aldose reductase inhibitor. Among the phytocompounds, morin had the highest value of Pa 0.456.

4. Discussion

Many people worldwide are attempting to find effective drugs to treat diabetes and related illnesses as a result of the growing attention given to the global challenges of diabetes and related disorders over the past few decades. The development of new drugs is greatly aided by medicinal plants. The use of medicinal plants in the treatment of diabetes and their effectiveness in reducing its secondary consequences are topics that have attracted a lot of research in the recent

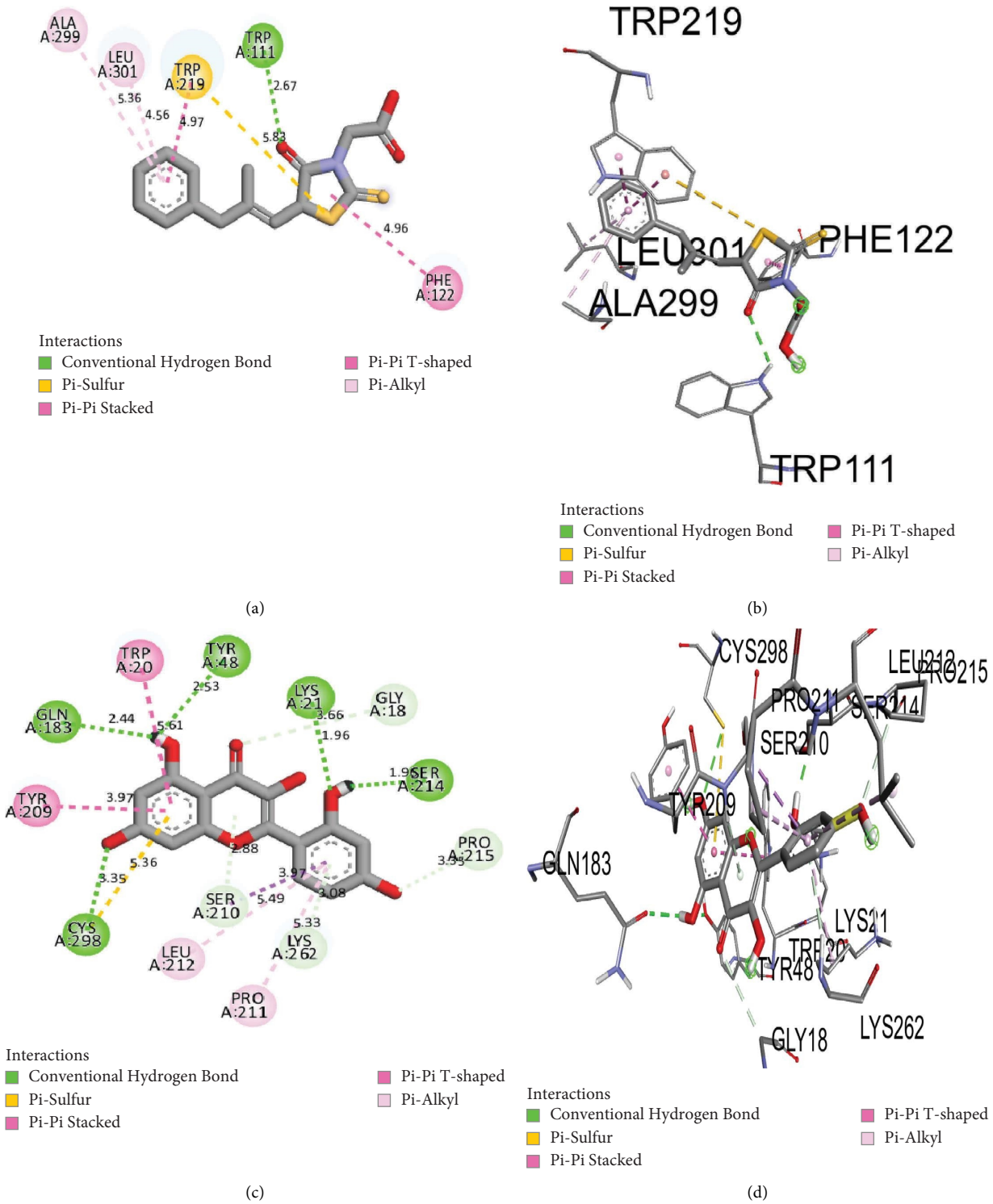

Figure 5: (a–d) The 2D and 3D interactions of epalrestat and morin with 4JIR, respectively.

years [53]. For more than 40 years, extensive research has been conducted on the significant enzyme known as AR. It has repeatedly been linked to the etiology of diabetic problems and acts as a rate-limiting enzyme in the polyol pathway. The reduction of glucose to sorbitol is catalyzed by this enzyme [54]. In the present study, we have selected four different medicinal plants which are evaluated for the inhibition of AR. Results of a study showing inhibition of RHAR by ethanolic extracts of *B. ciliata*, *R. arboreum*, *A. pyrethrum*, and *S. chirayita* indicated significant outputs. The ethanolic extracts of *B. ciliata* exhibited greater ($94.74 \pm 0.01\%$) inhibition of RHAR followed by *A. pyrethrum*, *R. arboreum,* and *S. chirayita* ($89.47 \pm 0.01\%$, $63.64 \pm 0.01\%$, and $56.25 \pm 0.01\%$), respectively. But

Table 6: Comparative study of TPC, TFC, and antioxidant activity between the previous study and current findings.

Plants	Present study			Previous study			References
	TPC (mg GAE/g)	TFC (mg QE/g)	Antioxidant activity (mg/mL)	TPC (mg GAE/g)	TFC (mg QE/g)	Antioxidant activity	
A. pyrethrum	3.07 ± 0.07	0.62 ± 0.01	0.28	25.96	0.88	0.18 mg/mL	[60]
B. ciliata	2.86 ± 0.21	0.48 ± 0.08	0.05	63.49	72.70	122 μg/mL	[23]
R. arboreum	4.56 ± 0.06	0.45 ± 0.05	0.05	123.6	—	102.06 μg/mL	[61]
S. chirayita	4.63 ± 0.16	0.90 ± 0.06	0.54	243.02 ± 4.70	4.98 ± 0.40 mg rutin equivalent/g	267.80 μg/mL	[62]

comparatively, the solvent used to dissolve the extracts of dimethyl sulfoxide (DMSO) showed a low (33.33 ± 0.01%) inhibition of RHAR. Besides the significant AR inhibition, all plants demonstrated the presence of a considerable number of diversified components. The essential phytochemicals and several secondary plant metabolites found in the medicinal plants are crucial for their antibacterial, anti-inflammatory, and other known biological effects [55]. This investigation reported reliable amounts of phenols, flavonoids, tannins, and alkaloids detected from the ethanolic extracts of plants. The presence of secondary metabolites is usually associated with potential biological effects [56]. The vast variety of physiologically active substances make up the main secondary metabolites known as phenolic compounds which function as reducing and antioxidant agents because of their redox characteristics [57]. In addition, flavonoids are also recognized as significant biological compounds with a variety of biological effects, including antioxidant, anticancer, anti-inflammatory, antiangiogenic, and antiallergic properties [58]. Moreover, in the plant system, antioxidants are responsible for detoxifying reactive oxygen intermediates. Commonly, DPPH is used to screen antioxidants. The extract's ability to scavenge free radicals is evidenced by the DPPH solution's discoloration [59]. Table 6 compares the antioxidant, TPC, and TFC activity of the selected plants with data from earlier studies and current findings.

On the other hand, docking analysis showed that out of seven selected phytocompounds, morin and ursolic acid showed significant interaction ($\Delta G < -8.0$ kcal/mol) with the receptor. Morin had the best docking score ($\Delta G = -9.2$ kcal/mol), forming five H-bonds, two with active residues (TYR-48 and CYS-298) and three with nonactive residues (LYS-21, GLN-183, and SER-214). Arbutin showed a good affinity ($\Delta G = -6.6$ kcal/mol) for forming H-bonds with TRP-111, ALA-299, and LEU-300. The coumaric acid and ursolic acid showed only one H-bond (HIS-110) with a receptor. In our study among the docked compounds, morin had the highest binding affinity (−9.2 kcal/mol), outperforming the clinically tested ARIs lidorestat (−8.5 kcal/mol), sorbinil (−8.1 kcal/mol), and RG7774 (−7.6 kcal/mol), as well as the commercial drug epalrestat (−8.1 kcal/mol). The catalytic residue contains H-bonding with TRP-20, TYR-48, HIS-110, TRP-111, CYS-298, ALA-299, LEU-300, and SER-302, which resembles the previously described active residues [63]. TYR-48 and HIS-110 are positioned adjacent to the C4 of the nicotinamide ring in structural models of human aldose reductase complexed with NADPH, suggesting that one of these residues may serve as the proton donor in the reaction process. A hydrogen-bonding network that comprises LYS-77 and ASP-43 also includes TYR-48. So, ASP-43, LYS-77, and HIS-110 are important active sites for aldose reductase inhibition [64]. While ADMET analysis showed that all compounds get absorbed readily in the intestine, Cytochrome CYP450 (1A2, 2C9, 2C19, 2D6, and 3A4), which is primarily in-charge of the biotransformation of more than 90% of the drugs in phase-1 metabolism, has a considerable impact on drug metabolism [65]. Morin showed CYP1A2, CYP2D6, and CYP3A4 inhibitions, and kaempferol showed CYP2D6 and CYP3A4 inhibitions. Coumaric acid readily crossed the BBB while other compounds were not found to cross the BBB. Morin and ursolic acid showed immune toxicity, while only ursolic acid showed hepatotoxicity. None of the compounds showed cytotoxicity. From the toxicity class and LD_{50} analysis, it can be concluded that catechin (having LD_{50} 10,000) was safer to use. Besides this, from PASS analysis, all phytochemicals showed antidiabetic activity.

Several *in vitro* studies were also performed on the selected phytochemicals for AR inhibition. For example, morin showed 75% inhibition at 10^{-5} M against lens AR [66]. Catechin showed inhibition against porcine AR with an IC_{50} value of 280 μm/L [67]. Coumaric acid showed inhibition against cataracted human eye lens AR and rat kidney AR with IC_{50} values of 162.31 ± 12.6 µg/mL and 0.057 mM, respectively [68, 69]. Arbutin showed 45.25% inhibition by 10 gm/mL against rat AR [70]. Ursolic acid showed inhibition against RHAR with an IC_{50} value of 9.69 μM [71]. Kaempferol and swertiamarin showed inhibition with IC_{50} of 6.94 g/mL and 7.59 μg/mL, respectively, against rat lens AR [70, 72].

Furthermore, the correlation among TPC, TFC, antioxidant activity, and RHAR inhibition property was determined by principal component analysis (PCA). PCA was carried out to break the dataset for the reduction of dimensionality. Though principal component analysis was carried out for four components, only two components showed an eigenvalue greater than 1. So, only two components were chosen for further analysis. These two components were responsible for 99.8% of the variance of data. From, the factor loading score, it can be concluded that PC1 is primarily a measure of RHAR, and PC2 is primarily a measure of TFC, as only these variables have a positive factor loading greater than 0.5.

5. Conclusion

Since antiquity, several diseases have been treated with traditional medicinal plants. About 90% of the Nepalese population residing in rural areas depends on traditional medicine as they lack governmental healthcare facilities. This study focused on the evaluation of phytochemicals and biological activity of four different plants collected from different places in Nepal. From *in vitro* analysis, we found that the ethanolic extract of the selected plant has significant aldose reductase inhibition. This was confirmed by a molecular docking analysis of the human aldose reductase protein. The ethanolic extract of *B. ciliata*, *A. pyrethrum,* and *R. arboreum* exhibits a significant antioxidant activity with a high TPC and exhibited greater inhibition of RHAR. The previously isolated morin from *B. ciliata* showed a high binding affinity with a greater number of H-bonding towards catalytic residues by docking analysis. Although *in vitro* results of this study may have limited implications, these findings provide the direction for exploring medicinal plants taken under study to avert or delay the onset of diabetic complications. Further studies on animal models and the isolation of pure compounds are required to support these findings [73].

Abbreviations

AR: Aldose reductase
BBB: Blood-brain barrier
DMSO: Dimethyl sulfoxide
DPPH: 2,2-Diphenyl-1-picrylhydrazyl-hydrate
DR: Diabetic retinopathy
FCR: Folin-Ciocalteu reagent
GAE/g: Gallic acid equivalent per gram
HAR: Human aldose reductase
IC_{50}: Inhibitory concentration 50%
OD: Optical density
Pa: Probable activity
PASS: Prediction of activity spectra for substances
PCA: Principal component analysis
Pi: Probable inactivity
QE/g: Quercetin equivalent per gram
RHAR: Recombinant human aldose reductase
TFC: Total flavonoid content
TPC: Total phenolic content.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

N.P. designed the research; L.B.M. and K.K. performed the research; S.R.U. and R.T. wrote the manuscript and generated molecular docking and did statistical analysis; and S.J., B.P.M., and N.P. edited the manuscript. Siddha Raj Upadhyaya and Lila Bahadur Magar contributed equally to this work.

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Supplementary Materials

Table S1: reported IC_{50} or percentage inhibition and enzyme type of selected natural products; Table S2: phytochemical screening of crude extract of plants; Table S3: principal component (standard deviation, proportion of variance, and cumulative proportion) analysis; Table S4: factor loading data of variables with PC1 and PC2; Table S5: pharmacokinetic and drug-likeliness properties of selected compounds; Table S6: ADMET profiles of selected compounds; Table S7: prediction of activity spectra for substances for selected compounds by the main Way2Drug server; Figure S1: 2D and 3D interactions of (A) catechin, (B) coumaric acid, (C) arbutin, (D) kaempferol, (E) ursolic acid, (F) swertiamarin, (G) lidorestat, (H) sorbinil, and (I) RG7774 with 4JIR. (*Supplementary Materials*)

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