Avicenna Journal of Phytomedicine (AJP)

Original Research Article

Comparison *of Hibiscus sabdariffa L.* extract and hydrochlorothiazide as adjuncts to Valsartan in managing hypertension in type 2 diabetic nephropathy: A randomized clinical trial

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Article history:
Received: Mar 05, 2025
Received in revised form: May 26, 2025
Accepted: May 28, 2025
Epub ahead of print

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Keywords:
Hibiscus sabdariffa L.
Diabetes mellitus
Molecular docking

Abstract

Objective: The effects of *Hibiscus sabdariffa* L. extract (HSE) as a supplemental therapy with valsartan for type 2 diabetic patients with nephropathy and its underlying biological functions were investigated in this study.

Materials and Methods: This clinical trial took place in Gorgan, Iran with 70 diabetic nephropathy patients who had hypertension. The participants were divided into two groups: The HSE group which received 500 mg HSE along with valsartan 40 mg twice daily over three months (n=35) and the control group which received valsartan plus hydrochlorothiazide 12.5 mg (n=35). The study measured blood pressure levels with additional assessments of microalbuminuria, proteinuria, and glomerular filtration rate (GFR) alongside lipid profile analysis, and hemoglobin A1c (HbA1c), fasting blood glucose (FBS), and electrolyte tests. Molecular docking simulations tested HSE compounds as potential inhibitors of sodium-glucose cotransporter-2 (SGLT2), dipeptidyl peptidase-4 (DPP-4), glucagon-like peptide-1 (GLP-1), and Receptor for advanced glycation end products (RAGE).

Results: Both groups showed improvements, but HSE had a greater impact on kidney function and lipid levels, while control group was more effective in lowering blood pressure and improving glucose metabolism. Docking analysis revealed that cyanidin 3-O-beta-D-sambubioside and tiliroside strongly interacted with targets, suggesting a role in blood glucose regulation and insulin secretion.

Conclusion: HSE demonstrates the potential to help type 2 diabetic nephropathy patients by modifying renin-angiotensin-aldosterone system activity carbohydrate digestion processes and lipid metabolism which reduces diabetic complications risks.

Please cite this paper as:
Kiani M, Mirzaei H, Enayati A, Ghorbani S, Zengin G, Amirkhanlou S. Comparison of Hibiscus sabdariffa L. extract and hydrochlorothiazide as adjuncts to Valsartan in managing hypertension in type 2 diabetic nephropathy: A randomized clinical trial. Avicenna J Phytomed, 2025. Epub ahead of print.

Introduction

The chronic metabolic condition diabetes mellitus creates hyperglycemia and insulin deficiency together with resistance across 400 million people worldwide despite raising their chances of heart disease renal ailments and eye complications(Pecoits-Filho et al. 2016; Xu et al. 2023). Type 2 diabetes patients manage albuminuria indicators of kidney damage with antihypertensive medications that include angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), and diuretics(Georgianos et al. 2023). Antihypertensive medications including ACEIs, ARBs, and diuretics, are used to manage albuminuria. However, established risks associated with these medications such as metabolic disorders, hypotension, and hyperkalemia, necessitate the exploration of safer treatment options(Agarwal et al. 2023). The plant *Hibiscus sabdariffa* L. (HSE) has antihypertensive and antidiabetic effects together with nephroprotective actions because it contains anthocyanins as well as phenolic compounds and hydroxy citric acid (Al Majid et al. 2023). HSE reveals its positive effect on blood pressure and glucose and lipid metabolism according to research conducted on diabetic and hypertensive models(El Midaoui et al. 2019; García-Muñoz et al. 2023). Type 2 diabetes mellitus with nephropathy (T2DM) impacts multiple mechanisms, including SGLT2 (renal glucose reabsorption), DPP-4 (incretin degradation), GLP-1 (glucose regulation), and RAGE (AGE-mediated complications) (Li et al. 2023; Palmer et al. 2021). The combination of AGE-RAGE receptor activation causes diabetic nephropathy along with cardiovascular complications yet GLP-1 agonists work to increase insulin production and appetite regulation (Nauck et al. 2021; Yue et al. 2022). However, research fails to explain how HSE works as a therapeutic tool.

The present study investigates the effectiveness of HSE tablets as medication to reduce albuminuria together with hypertension and metabolic distress in T2DM patients with nephropathy who take valsartan. The molecular docking technique identified HSE bond patterns with SGLT2, DPP-4, GLP-1, and RAGE components to understand diabetic pathophysiological processes.

Materials and Methods

Study design

This randomized clinical trial was conducted on T2DM nephropathy patients from November to December 2022 at Sayyad Medical and Educational Center, Gorgan, Golestan University of Medical Sciences, Iran.

Study population

Eligible participants for this study were individuals aged between 40 and 70 years who had been diagnosed with T2DM. Furthermore, they were required to have a diagnosis of diabetic nephropathy which was defined by the presence of microalbuminuria or proteinuria as assessed during the screening process. Participants also needed to present with hypertension, characterized by a systolic blood pressure of 130 mmHg or higher, or a diastolic blood pressure of 80 mmHg or higher. Finally, all included patients provided written informed consent to participate in the trial.

Exclusion criteria included normal or severe hypertension (stage 2+), renal failure (stage III or V), cardiovascular or metabolic diseases, psychiatric illness, pregnancy, lactation, herbal medicine use, or HSE sensitivity.

The recruitment and randomization of participants are detailed in the Consolidated Standards of Reporting Trials (CONSORT) flowchart (Figure 1). Eighty-two individuals were assessed for eligibility. Twelve were excluded: seven did not meet inclusion criteria, four refused to consent, and one was excluded for other reasons. Seventy participants were randomized into two groups (n=35 each).

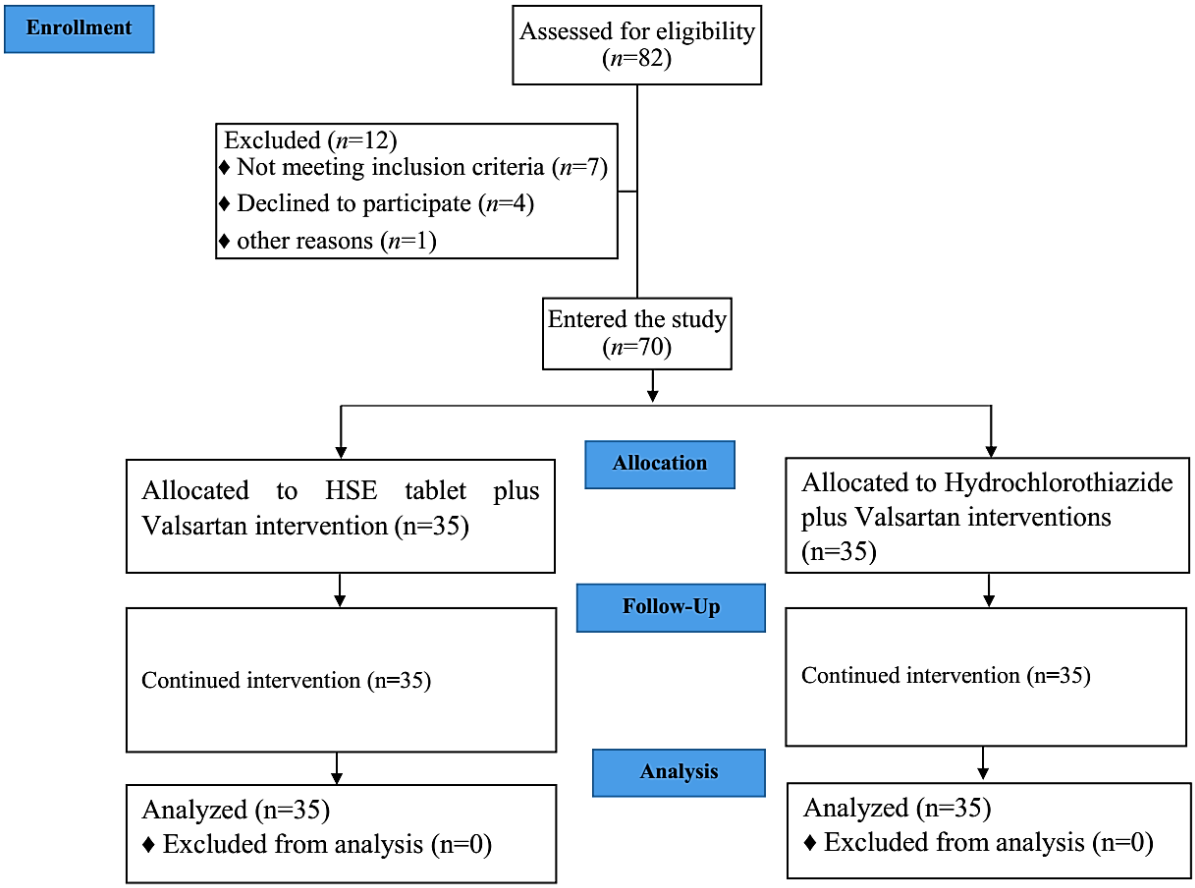

Figure 1. CONSORT flowchart of the study

Ethics

The trial was approved by the Ethics Committee of Golestan University of Medical Sciences (IR.GOUMS.REC.1401.264) and registered at the Iranian Registry of Clinical Trials (IRCT20200522047538N1) on September 26, 2022. All patients filled out and signed the written informed consent to participate in the trial.

Preparation and formulation of HSE tablet

The roselle tablets were formulated as 500 mg tablets, following the preparation and formulation process described previously (Dehkhoda et al. 2024).

The plant was identified and registered at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, under the herbarium number PMP-2381.

Clinical evaluation

Sample size

To assess microalbuminuria and blood pressure with 90% power (α=0.05) and account for a 10% follow-up loss, 35 patients per group were required. Eligible patients with controlled diabetes were randomized into intervention and control groups using block randomization (size four). Randomized numbers determined block sequences, and participants were assigned accordingly. Each participant received a unique code linked to their medication pack, ensuring blinding. Care providers were also blinded to group assignments (Dehkhoda et al. 2024).

Intervention

Both groups (HSE as the intervention and a control group) received 40 mg of valsartan every 12 hr. In addition, participants received either 500 mg of HSE

tablets or 12.5 mg of hydrochlorothiazide every 12 hr (twice daily) for a duration of 90 days, as follows:

Intervention group: Valsartan (40 mg) + HSE (500 mg), every 12 hr for 90 days

Control group: Valsartan (40 mg) + Hydrochlorothiazide (12.5 mg), every 12 hr for 90 days

Participants were followed up regularly throughout the study period.

Monitoring and safety assessments

Participants were monitored throughout the three-month study period for adherence to the treatment protocol and any potential adverse events through scheduled follow-up visits at baseline, 1.5 months, and 3 months. At each visit, blood pressure was measured, and inquiries were made about adverse events. The key efficacy and safety parameters, as detailed in the "Measurements" section, were assessed at baseline and at the end of the three-month intervention period. Potential anticipated adverse effects included gastrointestinal discomfort for HSE; electrolyte imbalances (e.g. hypokalemia, and hyponatremia), dizziness, or changes in glucose/uric acid levels for hydrochlorothiazide; and dizziness or hyperkalemia for valsartan. Participants were instructed to report any adverse events to the study investigators.

Measurements

The primary outcome was a reduction in hypertension and microalbuminuria in T2DM nephropathy patients after three months. Blood pressure was measured using the Riester Nova Mercury barometer, with changes from baseline recorded.

- Secondary outcomes included proteinuria, estimated glomerular filtration rate (eGFR), blood urea nitrogen (BUN), serum electrolytes, creatinine, HbA1C, fasting blood sugar (FBS), and lipid profile. These were assessed at baseline and after the intervention.

Molecular docking

AutoDock Vina was used to predict the binding mode and affinity of compounds to receptors based on their 3D structures. It employs an iterative local search to optimize ligand conformation and orientation in the protein binding site, using an empirical free energy scoring function that considers entropic and solvation effects (Santos-Martins et al. 2019).

Preparation of the receptor and ligand files

Structures for four protein targets (SGLT2, DPP-4, GLP-1, and RAGE) were retrieved from the Protein Data Bank (PDB IDs: 7VSI, 1X70, 5VEW, and 3O3U), while ligand structures were obtained from PubChem. Both receptors and ligands were converted to PDBQT format using AutoDock Tools, adding polar hydrogens and assigning Gasteiger charges. Docking parameters in AutoDock Vina generated predicted binding modes and affinities, selecting the best mode based on the lowest binding energy. Binding interactions were then analyzed using Maestro and Discovery Studio to illustrate hydrogen bonds, hydrophobic contacts, and key residues (Keighobadi et al. 2019).

Statistical analysis

Data are shown as medians with interquartile ranges for non-normal variables and as means ± standard deviations for normally distributed ones. The Student's t-test and Mann–Whitney U-test compared groups, with p-values <0.05 considered significant, using SPSS version 22.0.3.

Results

Baseline characteristics

All 70 completed their interventions. Participants were aged 40-65 years; 31.4% in the HSE group and 45.7% in the control group were male (Table 1).

Table 1. General characteristics of the T2DM patients with nephropathy and hypertension.

	HSE group	Control group	p-value
Age (years) (mean ± SD)	54.286 ± 8.642	52.457 ± 9.889	0.413
Sex N (%)	11 (31.4) Male 24 (68.6) Female	16 (45.7) Male 19 (54.3) Female	0.220

SD= Standard deviation.

Efficacy of the treatment

Primary outcomes

The primary endpoint, blood pressure, significantly decreased against baseline in both groups ($p<0.001$) (Table 2). In addition, the SBP reduction from baseline by the end of the study was 10 mmHg (control group) and 5 mmHg (HSE group). In the 3-month study, both groups showed significant decreases in systolic blood pressure (SBP) and diastolic blood pressure (DBP) compared to baseline ($p<0.001$ or SBP and $p<0.01$ or DBP) and the changes in both groups were similar in magnitude (Table 2). In addition, both groups showed a statistically significant reduction in urinary albumin levels compared to baseline at the end of the intervention period, although the reduction in the control group was significantly higher than in the HSE group ($p<0.05$) (Table 2).

Table 2. The variables of nephropathic T2DM patients in both groups.

Variables	**Group**	**N**	**Baseline**	**After 3 months intervention**	**p value***	**Changes****	**p value*****
SBP (mmHg)	Control	35	140 [135,140]	130 [125, 130]	< 0.001	10 [10, 15]	< 0.001
	HSE	35	135 [130, 140]	130 [120, 135]	< 0.001	5 [0, 10]	
DBP (mmHg)	Control	35	85 [80, 90]	80 [75, 80]	< 0.001	5 [5, 10]	0.010
	HSE	35	80 [80, 90]	80 [75, 80]	< 0.001	5 [0, 5]	
GFR	Control	35	89.26 [80.70, 92.34]	82.44 [79.15, 90.42]	0.036	3.919 ± 10.208	0.003
(ml/min/1.73m²)	HSE	35	90.05 [80.37, 94.55]	90.65 [84.59, 96.85]	0.030	-2.467 ± 6.897	
Upr24hr	Control	35	163 [123, 200]	102.5 [75, 128[	< 0.001	49.666 ± 68.129	0.410
(mg/24hr)	HSE	35	310 [130, 555]	216 [150, 325]	0.007	71.086 ± 136.426	
Ualb24hr	Control	35	33 [24.6, 42.8]	25 [15, 25.6]	< 0.001	10.447 ± 14.040	0.413
(mg/24hr)	HSE	35	52 [26, 111]	47 [30, 65]	0.003	14.669 ± 26.786	
BUN (mg/dl)	Control	35	17 [13, 20]	17 [14, 22]	0.066	-7 [-3, 1]	0.010
	HSE	35	21 [16, 23]	18 [16, 21]	0.066	2 [-2, 4]	
Cr (mg/dl)	Control	35	0.8 [0.7, 0.9]	0.8 [0.75, 0.9]	0.039	-0.02 [-0.1, 0.0]	0.001
	HSE	35	0.9 [0.8, 0.98]	0.83 [0.74, 0.93]	0.014	0.03 [0.0, 0.1]	
Na (mEq/l)	Control	35	141.200 ± 2.576	140 [138, 142]	0.020	0.800 ± 1.907	0.008
	HSE	35	139.580 ± 1.901	140 [139, 142]	0.151	-0.506 ± 2.114	
K (mEq/l)	Control	35	4.3 [4, 4.6]	4.1 [4, 4.4]	0.002	0.1 [0, 0.2]	0.104
	HSE	35	4.1 [4, 4.3]	4 [3.9,4.2]	0.370	0 [-0.1, 0.2]	
Cholesterol	Control	35	145 [133, 163]	144.114 ± 30.928	0.005	8 [2, 19]	0.242
(mg/dl)	HSE	35	191 [158, 225]	179.943 ± 38.225	< 0.001	15 [-4, 30]	
TG (mg/dl)	Control	35	164.886 ± 83.660	139.571 ± 54.359	0.027	9 [-2, 31]	0.318
	HSE	35	217.857 ± 81.524	193.314 ± 67.153	< 0.001	21 [5, 45]	
LDL(mg/dl)	Control	35	80.834 ± 27.829	74.717 ± 24.458	0.038	7 [-4, 16]	0.920
	HSE	35	112.729 ± 36.714	104.066 ± 31.316	0.010	5 [-2,17]	
HDL (mg/dl)	Control	35	38 [33, 45]	42.914 ± 9.675	0.253	-1.57 ± 8.85	0.091
	HSE	35	46 [39, 54]	44.606 ± 8.142	0.215	1.78 ± 7.45	
HBA1C (%)	Control	35	7.528 ± 0.899	7.203 ± 0.530	0.025	0.3 [-0.2, 1]	0.720
	HSE	35	7.271 ± 0.780	6.971 ± .489	0.006	0.2 [-0.2, 0.6]	
FBS (mg/dl)	Control	35	139 [117, 167]	123 [104, 145]	0.006	17 [-10, 44]	0.153
	HSE	35	138 [127, 152]	131 [124, 140]	0.002	8 [-4, 15]	

Group 1= Control and group 2 =HSE; *: Mann-Whitney U test; **: Changes from baseline to month 3; ***: T-test or Mann-Whitney U test. SBP: Systolic blood pressure; DBP: diastolic blood pressure; GFR: glomerular filtration rate; Upr24hr= 24 hr urine protein; UAlb24hr: 24 hr urinary albumin; BUN: Blood urea nitrogen; Cr: Creatinine; Na: sodium; K: Potassium; TG: triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; HBA1C: hemoglobin A1C; FBS: fasting blood glucose; Median [interquartile range] for non-normally distributed variables, and means ± standard deviations for normally distributed continuous variables.

Secondary outcomes

Both groups showed significant changes in urine protein and blood urea nitrogen (BUN), and creatinine (Cr) compared to baseline at three months. The control group had a greater urine protein reduction ($p<0.001$) than the HSE group ($p=0.007$) without significant intergroup differences. However, Cr and BUN changes were higher in the HSE group ($p=0.001$ and $p=0.010$, respectively) (Table 2). After 90 days, HSE significantly reduced cholesterol ($p<0.001$), Low-Density Lipoprotein (LDL ($p=0.010$), and TG ($p<0.001$) levels more than control (Table 2). Differences in high-density lipoprotein (HDL) levels between the HSE group and the control group were not statistically significant ($p=0.091$, Table 2). Both HSE and hydrochlorothiazide lowered FBS and HBA1C, while the control group showed significant Potassium (K) and Sodium (Na) changes. These results suggest HSE may replace hydrochlorothiazide, but larger randomized controlled trials (RCTs) are needed.

Safety of the treatment

There were no observed drawbacks or adverse events regarding HSE tablet consumption during the study. In addition, laboratory results indicated that lipid profile and kidney markers (BUN, creatinine, and electrolytes) were not harmfully changed within the 3-month consumption period.

Molecular docking

This study explored the inhibitory potentials of HSE-derived ingredients on sodium-glucose cotransporter-2 (SGLT2), Dipeptidyl peptidase 4 (DPP-4), glucagon-like peptide-1 receptor (GLP1) and receptor for advanced glycation end products (RAGE) via molecular docking (Table S1).

SGLT2 (PDB ID: 7VSI) target

Cyanidin 3-O-beta-D-sambubioside showed selective inhibition on SGLT2 with a binding energy of -11.7 kcal/mol (Table S1). Tiliroside also exhibited high inhibitory activity. Compared to empagliflozin, these ingredients demonstrated the highest binding affinity. Cyanidin 3-O-beta-D-sambubioside interacted with active sites (Ala102, Tyr290, Trp291, Trp289, Phe98, Val95, Val286, Leu283, Phe453, Leu84, Ile456, Ile76, Val157, Ile397) via hydrophobic interactions and with Ser393, Ser74, Asn75, Ser78, His80, Thr153, Ser460, Gln457, Ser287, Glu99, Lys321, Asp158, Lys154 via polar contacts (Table S2). It formed eight hydrogen bonds at distances from 1.56 to 2.54 Å (Figure S1-A).

DPP-4 (PDB ID:1X70) target

Tiliroside interacted with a hydrophobic pocket formed by residues such as Tyr585, Phe357, Cys551, Pro550, Tyr547, Val546, Trp627, Trp629, Tyr662, and Tyr666, and established polar contacts with additional residues. Twelve hydrogen bonds (1.62–2.84 Å) were noted (Figure S1-B, Table S2), with a dock score of -9.3 kcal/mol.

GLP-1 (PDB ID: 5VEW) target

Tiliroside bound effectively through six hydrogen bonds, hydrophobic, and polar interactions, indicating key residues in the binding mode (Table S2 and Figure S1-C).

Receptor for advanced glycation end products (RAGE) (PDB ID:3O3U)

Tiliroside displayed a low dock score (-10.3 kcal/mol) compared to pioglitazone (Table S1), forming both hydrophobic and polar interactions with several residues and seven hydrogen bonds (Table S2 and Figure S1-D)

Discussion

The main objective of this study was to compare the effects of HSE tablets on blood pressure and renal function in patients with T2DM, hypertension, and chronic kidney disease compared to hydrochlorothiazide. Based on the previous study(Dehkhoda et al. 2024), the hypothesis was that HSE would be as effective as

hydrochlorothiazide as its safe alternative, reducing hypertension, improving the lipid profile in patients with T2DM nephropathy, while reducing the side effects of hydrochlorothiazide.

HSE and blood pressure

This clinical trial demonstrated that Roselle (HSE) may lower blood pressure in individuals with T2DM. Clinical and experimental studies confirm that Roselle is safe and effective in reducing free radicals and releasing nitric oxide from the vascular endothelium, contributing to its antihypertensive effects (Carvajal-Zarrabal et al. 2012; Herrera-Arellano et al. 2007; McKay et al. 2010) However, the optimal dose and treatment duration remain unclear due to varied protocols and inconsistent findings (McKay et al. 2010).

Several clinical trials have compared Roselle with placebo or other antihypertensive drugs in adults with mild to moderate hypertension. Some studies observed significant reductions in systolic and/or diastolic blood pressure, while others reported only marginal or no significant differences (Asgary et al. 2016; Mojiminiyi et al. 2007). These mixed outcomes may be explained by differences in participant characteristics—such as age, gender, comorbidities, and concurrent medications—as well as variations in the quality and quantity of Roselle used, intervention duration, frequency, and blood pressure measurement methods.

Research in patients with diabetes and/or chronic kidney disease, who face higher cardiovascular risks, suggests that Roselle may also benefit renal function. Observations include reductions in proteinuria, microalbuminuria, and serum creatinine, along with improvements in glomerular filtration rate (GFR) (Herrera-Arellano et al. 2007; Mohammadi et al. 2021). However, these findings are limited by small sample sizes, short follow-up periods, and lack of control groups, necessitating more rigorous trials.

Animal studies reveal that in hypertensive rat models, Roselle extracts induced vasodilation through both endothelium-dependent and -independent pathways (Carvajal-Zarrabal et al. 2012), with higher doses correlating with greater blood pressure reductions (Inuwa et al. 2012; Mojiminiyi et al. 2007). Proposed mechanisms include aldosterone antagonism, angiotensin-converting enzyme (ACE) inhibition, muscarinic receptor activation with nitric oxide release, and inhibition of calcium ion influx (Dehkhoda et al. 2024; Ojeda et al. 2010).

HSE and lipid profile

This study demonstrated that both hydrochlorothiazide and the HSE tablet improved the lipid profile in patients with hypertension and chronic kidney disease by significantly reducing triglycerides, LDL, and total cholesterol. These findings align with previous work showing HSE's lipid-lowering effect, which may help prevent hyperlipidemia and cardiovascular diseases (Dehkhoda et al. 2024).

Roselle extract has been shown to reduce LDL-c, triacylglycerol (TAG), total cholesterol, and lipid peroxidation while increasing HDL-c levels in animal models, with effects varying by dose and solvent (Da-Costa-Rocha et al. 2014). In rodents on high-fat or high-cholesterol diets, roselle extract lowered body weight, total cholesterol, LDL-c, and TAG, and raised HDL-c in a dose-dependent manner (Hainida et al. 2008; Melchert et al. 2016). It has also been linked to vasodilation and blood pressure reduction in hypertensive rats, indirectly benefiting lipid metabolism (Carvajal-Zarrabal et al. 2012; Inuwa et al. 2012).

Clinical trials report that roselle significantly reduces serum triglycerides, total cholesterol, and LDL-c while increasing HDL-c in patients with metabolic syndrome, hypertension, or diabetes compared to placebo or other treatments (Asgary et al. 2016; Gosain et al. 2010). However, some studies noted only

marginal or no significant effects, possibly due to differences in participant characteristics, HSE quality, intervention duration, or lipid measurement methods (Mohagheghi et al. 2011; Onyenekwe et al. 2010).

Roselle extract, derived from the plant's dry calyx or leaves, is rich in polyphenolic compounds such as anthocyanins, protocatechuic acid, organic acids, and flavonoids. These constituents inhibit triglyceride synthesis and enzymes like pancreatic alpha-amylase, potentially aiding type IV and V hyperlipidemia treatment (Ademiluyi and Oboh 2013; Zulfiqar et al. 2022).

HSE and glucose metabolism

Our study showed that HSE tablets reduced fasting blood glucose and HbA1c levels, though less than hydrochlorothiazide. Research on HSE effects on glucose metabolism is mixed. Some studies report a slight increase in fasting blood sugar in patients with hypertension, metabolic syndrome, or mice on a high-fat diet (García-Muñoz et al. 2023). Others show reduced blood glucose and insulin resistance in mice at 200 mg/kg (García-Muñoz et al. 2023). Roselle extract may control postprandial hyperglycemia by inhibiting alpha-glucosidase and alpha-amylase, which digest carbohydrates (Gondokesumo et al. 2017).

HSE and kidney function

Our study found that HSE improved renal function versus hydrochlorothiazide tablets, as evidenced by lower creatinine, BUN, proteinuria, microalbuminuria, and higher GFR. HSE modestly increased blood sodium and decreased potassium, whereas hydrochlorothiazide significantly reduced both electrolytes. Similar results appeared in rats on a hibiscus diet, which showed higher serum sodium, chloride, creatinine clearance, and lower urea than controls (Melchert et al. 2016). HSE also lowered BUN, urine albumin, and blood and urine creatinine in diabetic nephropathy patients (Mohammadi et al. 2021). Conversely, some studies noted no significant impact on kidney function or electrolyte balance in hypertensive patients or healthy volunteers (Da-Costa-Rocha et al. 2014; Mohammadi et al. 2021). Animal studies confirm that HSE tea is safe and preserves water and electrolyte homeostasis (Gosain et al. 2010). Two studies reported nephroprotective effects of HSE extract in diabetic nephropathy models (García-Muñoz et al. 2023; Gosain et al. 2010). These findings underscore HSE's potential renoprotective benefits.

Molecular docking

This research investigated how compounds extracted from HSE inhibited diabetes-related protein targets including SGLT2, DPP-4, GLP-1, and RAGE. Using molecular docking, the study identified cyanidin 3-O-beta-D-sambubioside and tiliroside as top candidate compounds. Cyanidin 3-O-beta-D-sambubioside demonstrated the strongest binding to SGLT2, a kidney protein regulating glucose reuptake, functioning as an SGLT2 inhibitor to enhance glucose excretion more effectively than empagliflozin. It formed eight hydrogen bonds along with multiple hydrophobic and polar interactions, establishing a stable, effective bond.

Tiliroside exhibited exceptional binding capabilities toward DPP-4, GLP-1, and RAGE. It inhibited DPP-4 activity via twelve hydrogen bonds and additional interactions, outperforming sitagliptin (Gilbert and Pratley 2020). Its affinity for the GLP-1 receptor, mediated by six hydrogen bonds, is comparable to liraglutide, promoting enhanced insulin release (Santos-Martins et al. 2019). Moreover, tiliroside formed nine hydrogen bonds with RAGE, effectively blocking AGE interactions better than pioglitazone (Biswas et al. 2022). Overall, these compounds exhibit complex glucose reduction capabilities with enhanced insulin production and fewer side effects.

Further research is needed to validate their clinical efficacy and safety.

Acknowledgment

We thank the Giah Essence Phytopharmaceutical Dr. Soleimani Company for preparing HSE tablet. Moreover, we would like to acknowledge the Sayyad Medical and Educational Center for cooperation during the study

Conflicts of interest

The authors declare no conflicts of interest.

Funding

This trial has been supported by Golestan University of Medical Sciences, Gorgan, Iran (IR.GOUMS.REC.1401.264).

Ethical considerations

The study protocol was approved by the Ethics Committee of Golestan University of Medical Sciences, Gorgan, Iran (IR.GOUMS.REC.1401.264). All participants provided informed consent before participating in the trial. Approval date of Registry and the Registration No. of the study/trial: The date of approval is September 26, 2022. The approval number is IRCT20200522047538N1.

Code of Ethics

IR.GOUMS.REC.1401.264

Authors' Contributions

The study's conceptualization and design were conducted by Ayesheh Enayati.

The analysis and interpretation of the results were performed by Maryam Kiani and Somayeh Ghorbani. Hassan Mirzaei and Gokhan Zengin was responsible for drafting the manuscript, while Aysheh Enayati and Hassan Mirzaei and Saeid Amirkhanlou contributed to its critical revision for significant intellectual content. All authors participated in reviewing the manuscript.

Consent to publish

Not applicable to this study.

Data availability

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

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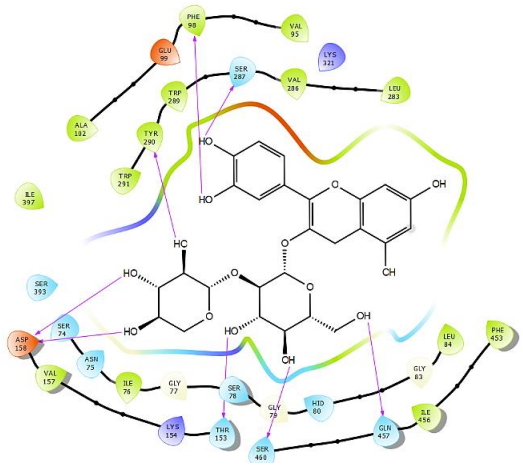
Supplementary

A)



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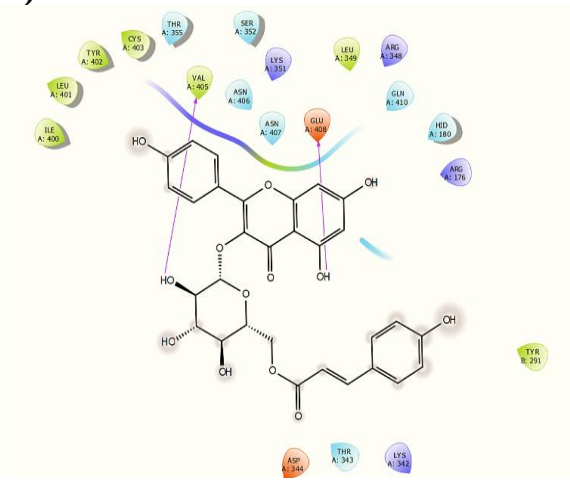

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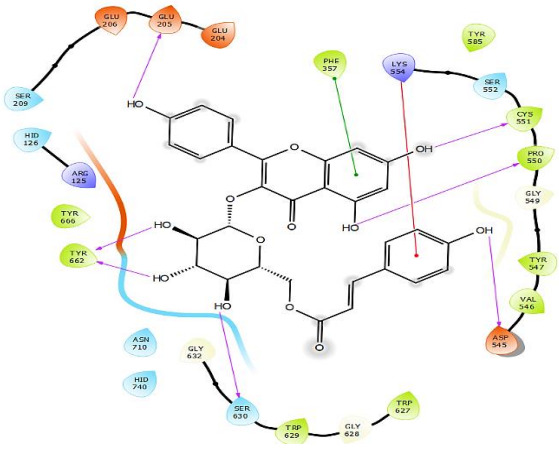

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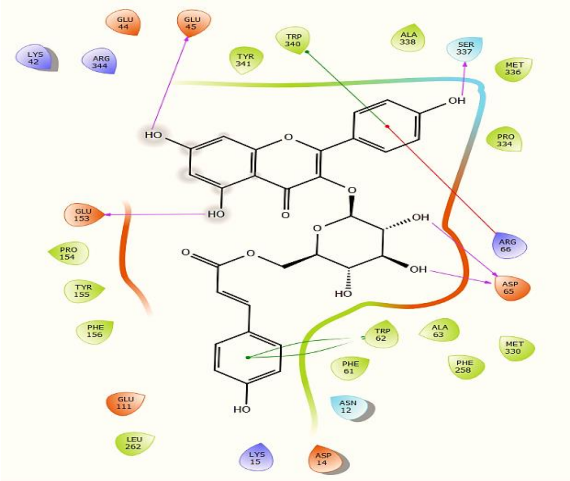

Supplementary Figure 1. Presentation of 2D model of interactions between A) Cyanidin 3-O-beta-D-sambubioside and PDB ID: 7VSI, B) Tiliroside and PDB ID: 1X70, C) Tiliroside and PDB ID: 5VEW, D) Tiliroside and PDB ID: 3O3U.

Supplementary Table 1. Molecular docking simulation results for the HSE compounds and receptors.

Compounds		**Binding Energy (kcal/mol)** **PDB ID:**			
	CID	**7VSI**	**1X70**	**5VEW**	**3O3U**
Tiliroside	5320686	-11.6	-9.3	-7.7	-10.2
Delphinidin-3-sambubioside	74977035	-11.5	-8.2	-7.0	-9.7
Cyanidin 3-O-beta-D-sambubioside	6602304	-12.2	-7.6	-7.1	-9.6
Delphinidin-3-glucoside	443650	-9.9	-7.1	-6.4	-8.4
Cyanidin 3-galactoside	4481259	-8.6	-6.5	-6.0	-8.1
Galloyl ester	85207469	-8.2	-6.9	-6.2	-7.4
Hibiscitrin	15559736	-8.5	-6.6	-5.4	-8.0
Quercetin	5280343	-6.6	-5.4	-6.1	-6.0
Luteolin	5280445	-6.7	-5.8	-5.7	-5.8
Rutin	5280805	-11.4	-7.6	-6.1	-9.3
Kaempferol	5280863	-6.2	-5.3	-5.9	-6.0
Eugenol	3314	-4.7	-4.7	-5.4	-4.7
Protocatechuic acid	528594	-9.6	-7.1	-6.6	-9.4
Hydroxycitric acid	123908	-4.4	-3.7	-4.4	-4.1
Citric acid	311	-4.8	-4.2	-4.5	-4.3
Malic acid	444266	-3.9	-3.8	-3.9	-3.9
Tartaric acid	875	-3.7	-3.7	-4.1	-3.4
Ascorbic acid	54670067	-4.6	-4.7	-4.8	-4.2
Coumaroylquinic acid	14158103	-7.5	-5.8	-6.7	-7.1
Chlorogenic	1794427	-8.1	-6.6	-5.3	-6.9
Gallic acid	24721416	-4.4	-4.4	-4.9	-4.3
β-Sitosterol	222284	-9.1	-7.6	-5.9	-7.8
Ergosterol	444679	-8.1	0.0	-5.4	-7.2
Empagliflozin	11949646	-10.0	-7.5	-6.7	-8.4
Sitagliptin	4369359	-8.7	-7.6	-7.5	-8.5
Pioglitazone	4829	-8.5	-7.5	-5.8	-8.6

Supplementary Table 2. Key amino acid residues between Cyanidin 3-O-beta-D-sambubioside and 7VSI target or Tiliroside and the active sites of 1X70, 5VEW and 3O3U targets.

Bonding type / Targets	**7VSI**	**1X70**	**5VEW**	**3O3U**
Hydrophilic	Ala102, tyr290, Trp291, trp289, Phe98, Val95, Val286, Leu283, Phe453, Leu84, Ile456, Ile76, Val157, Ile397	Tyr585, Phe357, Cys551, Pro550, Tyr547, Val546, Trp627, Trp629, Tyr662, Tyr666	Ile400, Leu401, Tyr402, Cys403, Val405, Leu349, Tyr291	Tyr341, trp340, Ala338, Met336, pro334, Ala63, Trp62, Phe61, Phe258, leu262, Phe156, tyr155, pro154
Polar	Serr393, Ser74, Asn75, Ser78, His80, Thr153, Ser460, Gln457, Ser287, Glu99, Lys321, Asp158, Lys154	Ser209, His126, Arg125, Glu206, Glu205, Glu204, Asn710, His740, Ser630, Asp545, Ser552, Lys554	Thr355, Ser352, Asn406, Asn407, Arg348, Gln410, His180, Arg176, Lys342, Asp344, The343	Lys42, Arg344, Glu44, Glu45, Ser337, Arg66, Asn12, Lys15, Asp14, Glu111, Glu153
Hydrogenous	Asp158, Thr153, Thr290, Ser460, Gln457, Phe98, ser287	Asp545, Ser630, tyr547, His740, Asn710, Tyr662, Pro550, Tyr585, Cys551	Glu408, Asn407, Thr343, Glu408, Val405, lys351	Glu45, Glu153, Ser337, Trp62, Ala63, asp65