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In Silico Evaluation of Bioactive Compounds from *Gokshura* (*Tribulus terrestris* L.) and *Punarnava* (*Boerhavia diffusa* L.) for their Nephroprotective Activity in Chronic Kidney Disease and Related Complications

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Abstract

Kidney diseases are one of the leading causes of mortality worldwide. Diabetes and hypertension are the main causes of kidney failure resulting in 3 out of 4 new cases. Most of the conventional drugs used in various disease conditions are reported for their nephrotoxic actions and their continuous use can also damage the kidneys. Ayurveda recommends certain herbal drugs like *Gokshura* (*Tribulus terrestris* L.) and *Punarnava* (*Boerhavia diffusa* L.) which can control end-stage kidney disease and its complications through the rejuvenation of the kidneys. The present study is an effort to show the nephroprotective potential of bioactive compounds present in *Tribulus terrestris* L. and *Boerhavia diffusa* L. against critical nephroprotective targets carbonic anhydrase II, renin, HIF propyl hydroxylase 2/ EGLN1, angiotensin-converting enzyme II, vasopressin receptor 2 against their respective standard drugs through *in silico* technique and to verify the probable efficacy of these herbs in chronic kidney disease against modern medication. Discovery Studio (DS Visualizer 2016) and Auto Dock tool (ADT Tools-1.5.6) were used for molecular docking. Among the major bioactive compounds screened, chlorogenin, hecogenin, diosgenin, neotigogenin and beta-sitosterol from *Tribulus terrestris* L., Beta-sitosterol, boerhavisterol, liriodenine, boerhadiffusene and ursolic acid from *Boerhavia diffusa* L. observed to exhibit significantly higher binding energy (BE) and inhibition constant (IC_{50}) towards CA II, Renin, EGLN1, ACE II and V2R than their respective standard drugs. The study has demonstrated the nephroprotective activity of *Tribulus terrestris* L. and *Boerhavia diffusa* L. by inhibiting receptor activity against standard drug molecules.

Keywords: *Boerhavia diffusa*, *In-silico*, Nephroprotective, Molecular Docking, *Tribulus terrestris*

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1. Introduction

Chronic Kidney Disease (CKD) refers to an irreversible deterioration in renal function that usually develops over years. CKD is an umbrella term for renal diseases with different aetiologies, diagnosed based on clinical and histopathological features but commonly associated with longstanding diabetes and hypertension[1]. There has been a significant increase in the occurrence of Acute Kidney Injury (AKI) in the past two decades[2,3]. In 2017, the global prevalence of CKD was 9.1%, which is roughly 700 million cases. Large variations in death due to CKD were noted in between-region and between-country; for example, in central and Andean Latin America, the second and fifth-ranked cause of CKD death in 2017 respectively. Kidney disease is a complex disease with several different aetiologies and underlying associated pathophysiology. The kidney modulates various complex physiological processes viz., endocrine functions, the regulation of blood pressure and intra-glomerular hemodynamics, solute and water transport, acid-base balance and excretion of drug metabolites which are accomplished by intricate mechanisms of renal response. Renal impairment caused by modern medications involves many classes of drugs which include Non-Steroidal Anti-Inflammatory Drugs ($NSAID_s$), angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, antimicrobial, antiviral, antifungal, chemotherapeutic agents[4]. Drug-induced nephrotoxicity has been reported as a major cause of the high incidence and prevalence of AKI[5]. Recent advances in molecular biology and genetics have enhanced our knowledge about a few of the here familial disorders. In tropical countries acute renal involvement and acute renal failure could be manifestations of an array of tropical disorders like malaria, filariasis or leptospirosis. CKD increases mortality and morbidity due to its vascular complications resulting in cardiovascular-cerebrovascular events and its progression leads to end-stage kidney failure[6]. These complications can be prevented by end-stage CKD and delayed with appropriate access to proper preventive as well as curative drugs of herbal origin. Two drugs of herbal origin, namely fruits of *Gokshura*, botanically identified as *Tribulus terrestris* L. (TT) and roots of *Punarnava* botanically identified as *Boerhavia diffusa* L. (BD) are widely used in clinical practice in Ayurveda for broad-spectrum kidney disorders. Both TT and BD are one of the most effective nephroprotective drugs in Ayurveda and are used for rejuvenation therapy in various disorders. Molecular docking is one of the most used high-throughput screening techniques to study 3D ligand-target interactions. It plays an important role in predicting the binding modes and binding abilities of small molecules towards the targeted proteins, which is crucial in designing potential drugs[7]. The *in silico* approach offers a platform for the evaluation of the efficacy of potential therapeutic agents against molecular targets, thereby facilitating the identification of those with the greatest potential for further experimentation. Drugs exert their function through binding to one or more protein targets. This study may help to scientifically validate their nephroprotective activity by identifying the new potent biomolecules and selection of target-based potent drugs among these two through an *in silico* docking study. TT has been reported to have anti-inflammatory, antioxidant, diuretic, anti-hypertensive anti-urolithiasis efficacy[8-10]. BD has several medicinal properties such as analgesic, anti-inflammatory, diuretic, hepatoprotective, immunomodulatory and nephroprotective, antiulcer and antihistaminic activity[11-15]. These plants contain phytochemicals like alkaloids, flavonoids, and phytosterols which are responsible for various pharmacological properties[16].

2.1 Ayurvedic Pharmacological Profile of TT and BD

According to the classical text of Ayurveda, *Gokshura* possesses *Madhura Rasa* (sweet taste), *Guru-Snigdha Guna* (heavy and unctuous quality), *Shita Veerya* (cold potency) and *Madhura Vipaka* (sweet taste after biotransformation)[17]. *Punarnava* has dominant *Katu-Tikta-Kashaya Rasa* (pungent, bitter and astringent taste), *Laghu-Ruksha Guna* (light and dry quality), *Ushna Veerya* (hot potency), *Katu Vipaka* (pungent taste after biotransformation)[18]. Both drugs are used for *Rasayana* (rejuvenation), *Mutravirechana* (diuretic and nephroprotective), *Shothahara* (anti-inflammatory), *Vajikarana* and *Vrushya* (aphrodisiac), *Ashmarighna* (antiurolithic), *Pramehahara* (antidiabetic), *Vedanahara* (analgesic), *Arbudanashaka* (anticancer), *Ojovardhaka* (immunomodulatory)[19].

2. Materials and Methods

2.1 Preparation of Protein

The crystal structures of carbonic anhydrase (CAII -3HS4), renin (REN-2REN), HIF propyl hydroxylase 2/PHD2 (EGLN1-5V1B), angiotensin-converting enzyme II (ACE

II- 1O86), vasopressin 2 receptor (V2R -7BB6) obtained from Protein Data Bank (PDB) with their PDB ID and crystal resolution for the molecular docking studies. Initially, for the preparation of protein AutoDock Tools (ADT) was used for the removal of all HOH molecules, adding Kollman charges and polar hydrogen atoms and assigning hydrogen polarities. Further, Gasteiger charges were applied to the prepared protein and protein structures file converted into PDBQT. In BIOVIA Discovery Studio, a complex of all five proteins was prepared by removing all the water molecules, ligands and hetero-atom from the complex[20].

2.2 Ligand Preparation

In the present study, important ligands from TT and BD were selected from IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics)[21] and confirmed by LC-MS analysis. Agilent 6538 Q-TOF Data acquisition parameters were used for the LC-MS study. The samples were loaded onto InfinityLab Poroshell 120 SB-C18 column of 2.7µm particle size and dimensions 3.0 x 100 mm (Agilent Technologies) maintained at 40°C. Standard/control drugs acetazolamide, alsikerin, roxadustat, captopril and tolvaptan were selected for docking against the above targets respectively. The three-dimensional structures of ligands were retrieved from the PubChem database and energy minimization was done. All the ligand structures were saved in pdbqt file format. Ligand protein was deleted from the side panel of ADT Tools- 1.5.6[20].

2.3 Grid Preparation

The structures of CAII (A:5,64.92,94,121,131,135,198,199,209), REN (A:34,76,77,218,219,220,289) EGLN1 (A:287,297,299,311,313,327,360,369), ACE II (A:353,354,380,411,511,513,518,523), V2R (Q-96,W-99,F-105,K-116,F-307) were sketched with the help of ChewDraw 15. Further, they were converted into three-dimensional (3D) structures through energy minimization using the MMFF94s force field. These mol2 files were converted to pdbqt using Autodock tools 1.5.6. Autogrid-4.0 was used to calculate grid maps of interaction energies among the various types of atoms present (A, C, HD, NA, N, OA, S). The grid box size was set to 40×40×40 with a grid spacing of 0.375 Å. The docking was performed by Autodock-4.2.6 using the Lamarckian Genetic Algorithm with 10 runs, 150 population size, 2,500,000 maximum number of energy evaluations and 27,000 maximum number of generations[22].

2.4 Bioactive Compounds (Ligands) from TT and BD

Tribulusamide-B, diosgenin, stigmasterol, campesterol, chlorogenin, kaempferol, terrestriammide, quercetin, disogluside, tataramide-B, tiliroside, gitogenin, moupinamide, nicotiflorin, tigogenin, hecogenin, neotigogenin, astragalin, beta-sitosterol and rutin were major phytoconstituents found in TT. Punarnavoside, boeravinone-D, boeravione-E, boeravinone-C, liriodenine, liriodendrin, eupalitin, 1-triacontanol, betuletrin, hentriacontane, boeravinone-B, Ursolic acid, 20-Hydroxyecdysone, boeravinone-F, boerhavisterol, boerhadiffusene, diffusarotenoid, myristic acid, beta-sitosterol and methyl behenate were major bioactive compounds found in BD. Among all compounds tribulusamide B, tiliroside, nicotiflorin, astragalin, rutin, Punarnavoide, liriodendrin and betuletrin do not follow Lipinski rule.

2.5 ADME and Toxicity Prediction

For prediction of the drug-likeness properties of compounds from both drugs, *in silico* absorption, distribution, metabolism, excretion and toxicity prediction were carried out. The ADMET properties of all compounds were explored by using PreADMET. The parameters explored are Lipinski rule, A Log P, Molecular weight, no. of atoms, BBB, CYP_2C19, CYP_2C9, CYP_2D6, CYP_3A4, HIA, MDCK, Caco-2, Pgp inhibition, Plasma protein binding, Skin permeability, Ames test, Daphnia at.

2.6 Molecular Docking

Selected molecules from TT and BD were docked with nephroprotective targets on an individual basis. The selected compounds with different conformations were docked with the macromolecules under rigid conditions. After docking, 2D and 3D structures were visualized in Discovery Studio Visualizer software[23].

3. Results

3.1 LC-MS Study

3.2 Molecular Docking Analysis

According to *in silico* study findings, when the standard drug acetazolamide was docked with target at the active site

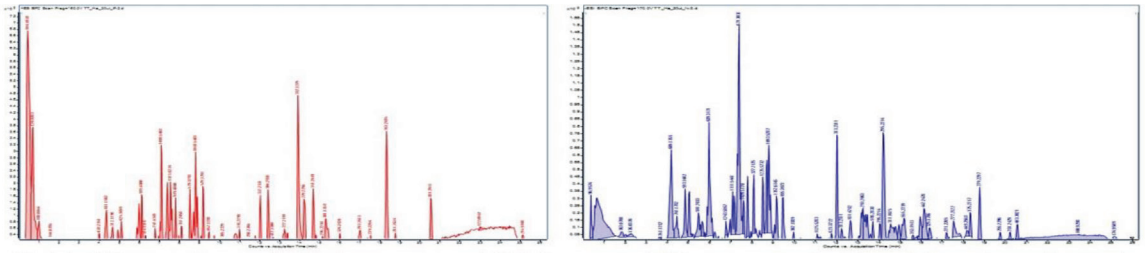


Figure 1. **(a, b)** Base peak count (BPC) chromatogram of *Tribulus terrestris* HA extract acquired in positive and negitive mode.

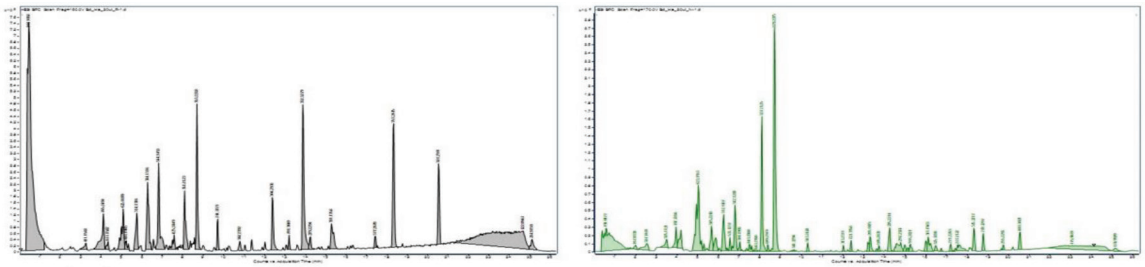


Figure 2. **(a, b)** Base peak count (BPC) chromatogram of *Boerhavia diffusa* HA extract acquired in positive and negitive mode.

Table 1. LC-MS analysis: *Tribulus terrestris* L.

Sr. No.	Name	Formula	Mass	Abundance	RT
1	Rutin	$C_{27}H_{30}O_{16}$	610.52	78356	4.398
2	Diosgenin	$C_{27}H_{42}O_{3}$	414.63	19434	11.079
3	Stigmasterol	$C_{29}H_{48}O$	412.69	19434	11.079
4	Chlorogenin	$C_{27}H_{44}O_{4}$	432.65	20676	12.038
5	Moupinamide	$C_{18}H_{19}NO_{4}$	313.35	17262	14.642
6	Kaempferol	$C_{15}H_{10}O_{6}$	286.24	56291	17.013
7	Terrestriamide	$C_{18}H_{17}NO_{5}$	327.34	14505	16.069
8	Disogluside	$C_{33}H_{52}O_{8}$	576.77	135713	13.324
9	Astragalin	$C_{21}H_{20}O_{11}$	448.38	44496	18.286
10	Tiliroside	$C_{30}H_{26}O_{13}$	594.53	55919	13.681
11	Campesterol	$C_{28}H_{48}O$	400.68	50228	20.609
12	Moupinamide	$C_{18}H_{19}NO_{4}$	313.35	82266	14.528

of CA II, the Binding Energy (BE) was -5.84 kcal and the inhibition constant (IC_{50}) was 52.58 µm which is compatibly less stable than compounds present in TT and BD. Bioactive compounds showed maximum stability towards the CA II target than standard drugs as it has the least energy and stable interaction with the target. Among all bioactive compounds screened from TT, chlorogenin, hecogenin, diosgenin, tigogenin, and neotigogenin showed maximum affinity towards CA II (Figure 3 (a)). The side chain residues VAL A-242 and VAL A-243 formed two H-bonds with chlorogenin. At the active site of CA II, chlorogenin revealed strong interaction with a docking score of -9.9 kcal and an inhibition constant of 0.05563 µm (Figure 3 (b), (c)). Beta-sitosterol, boerhadiffusene, diffusarotenoid, boerhavisterol and liriodenine from BD showed maximum affinity towards CA II (Figure 4 (a)). The side chain residues GLU A: 299 formed one H-bond with beta-sitosterol. At the active site of CA II, beta-sitosterol revealed strong interaction with a docking score of -9.53 and an inhibition constant of 0.10299 µm (Figure 4 (b), (c)).

The direct renin inhibitor alsikerin was docked with target at the active site of renin, where the binding energy

Table 2. LC-MS analysis: *Boerhavia diffusa* L.

Sr. No.	Name	Formula	Mass	Abundance	RT
1	Boeravinone C	$C_{18}H_{16}O_7$	344.32	36387	8.681
2	Eupalitin	$C_{17}H_{14}O_7$	330.29	31268	18.257
3	Boeravinone B	$C_{17}H_{12}O_6$	312.28	56257	18.284
4	Boeravinone F	$C_{17}H_{10}O_7$	326.26	41638	14.12
5	Methyl behenate	$C_{23}H_{46}O_2$	354.61	198108	8.665
6	Boeravinone-E	$C_{17}H_{12}O_7$	328.28	232509	8.64
7	Diffusarotenoid	$C_{22}H_{20}O_7$	396.4	69021	2.46
8	Ursolic acid	$C_{30}H_{48}O_3$	456.71	42522	8.16
9	Boeravinone-D	$C_{18}H_{14}O_7$	342.3	45510	17.856
10	Myrstic acid	$C_{24}H_{28}O_2$	228.37	51090	8.694

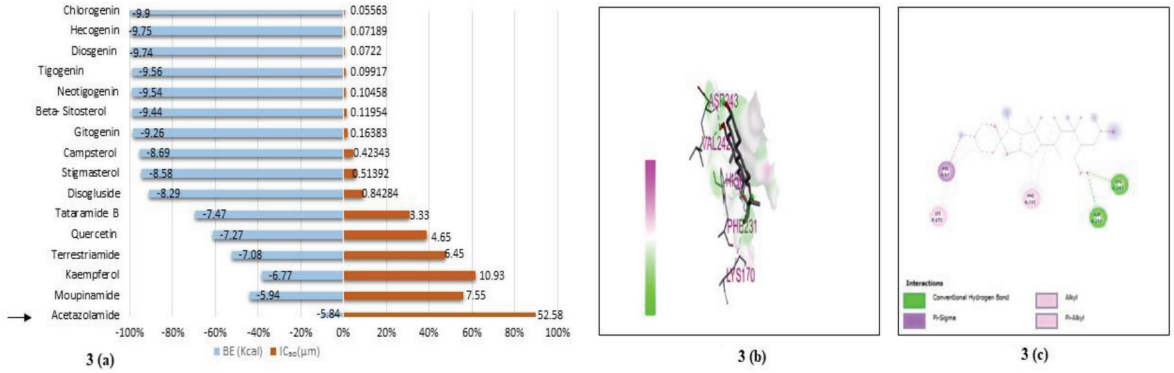


Figure 3. **(a)** Molecular docking score of *Tribulus terrestris* compounds and Acetazolamide with CA II **(b)** Molecular interaction of chlorogenin with CA II (3D). **(c)** 2D interaction of docked CA II with chlorogenin.

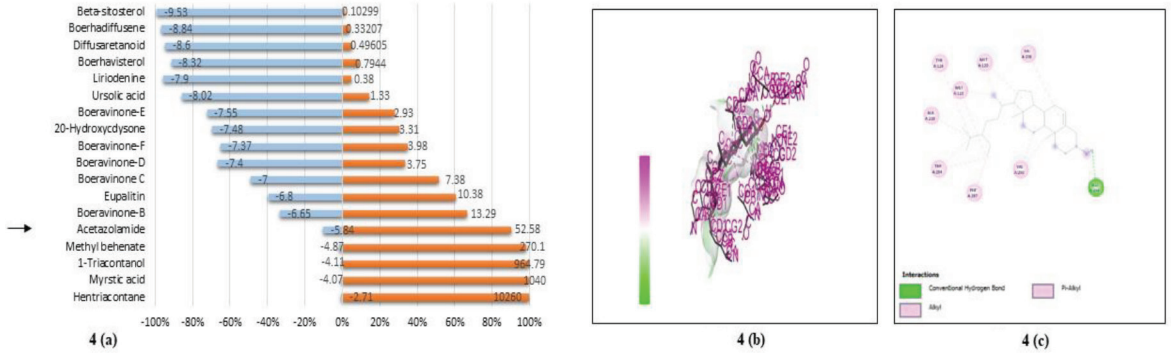


Figure 4. **(a)** Molecular docking score of *Boerhavia diffusa* compounds and Acetazolamide with CA II. **(b)** Molecular interaction of beta-sitosterol with CA II (3D). **(c)** 2D interaction of docked CA II with beta-sitosterol.

was -9.15 kcal and the inhibition constant was 0.19588 µm which is significant than bioactives present in TT and BD when docked with renin. Disogluside, campesterol, neotigogenin, chlorogenin and hecogenin from TT showed maximum affinity towards renin than alsikerin (Figure 5 (a)). The side chain residues TYR C: 220 formed one H-bond with disogluside. At the active site of renin, disogluside revealed strong interaction with a docking score of -11.3 kcal and an inhibition constant of 0.0052 µm (Figure 5 (b), (c)). Beta-sitosterol, boerhadiffusene,

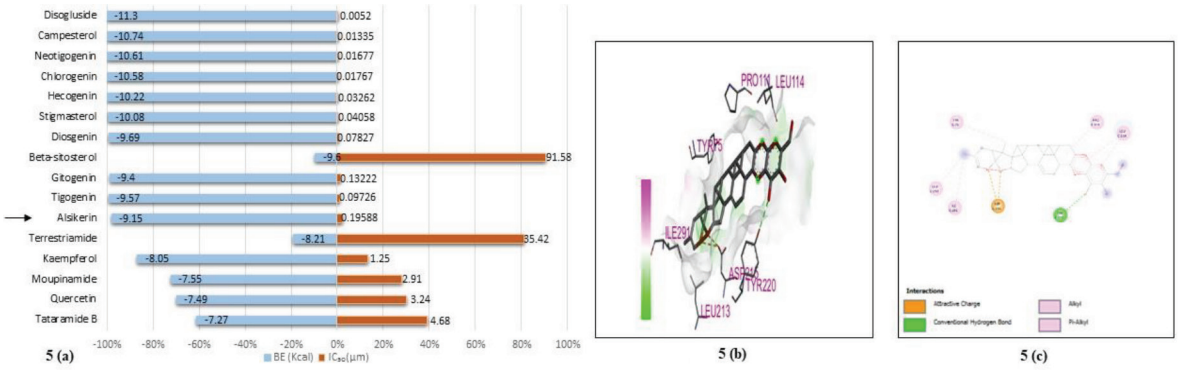


Figure 5. **(a)** Molecular docking score of Tribulus terrestris compounds and Alsikerin with Renin. **(b)** Molecular interaction of disogluside with Renin (3D). **(c)** 2D interaction of docked renin with disogluside.

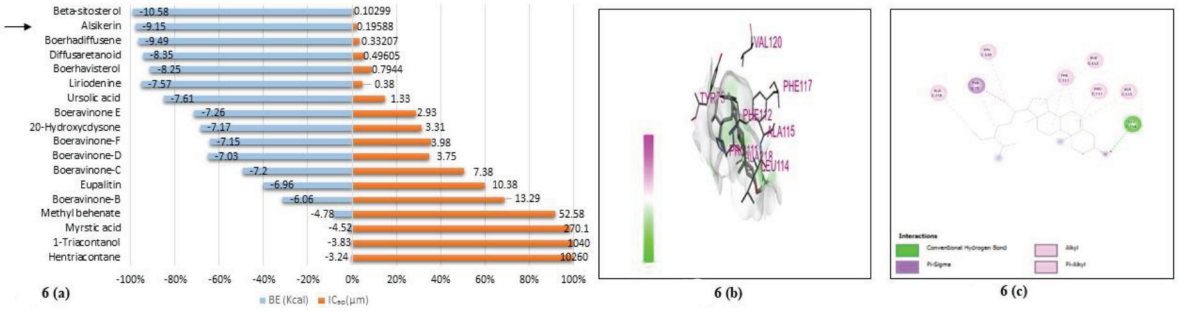


Figure 6. **(a)** Molecular docking score of *Boerhavia diffusa* compounds and Alsikerin with Renin. **(b)** Molecular interaction of beta-sitosterol with renin (3D). **(c)** 2D interaction of docked renin with beta-sitosterol.

20-hydroxyecdysone, diffusarotenoid and boerhavisterol from BD showed maximum affinity (Figure 6 (a)). The side chain residues LEU C: 114 formed one H-bond with beta-sitosterol. At the active site of renin, beta-sitosterol revealed strong interaction with a docking score of -10.58 kcal and an inhibition constant of 0.10299 µm (Figure 6 (b), (c)).

The HIF propyl-hydroxylase inhibitor roxadustat was docked with the target at the active site of EGLN1, the binding energy was -9.09 kcal and the inhibition constant was 0.21694 µm which is significant than that of bioactives present in TT and BD when docked with EGLN1. Neotigogenin, disogluside, hecogenin, tigogenin and gitogenin showed maximum affinity towards EGLN1 than roxadustat (Figure 7 (a)). The side chain residues GLY A: 325 formed one H-bond with neotigogenin. At the active site of EGLN1, neotigogenin revealed strong interaction with a docking score of -12.01kcal and an inhibition constant of 0.00158 µm (Figure 7 (b), (c)). Ursolic acid, boerhavisterol, beta-sitosterol, liriodenine and boeravinone-F showed maximum affinity (Figure 8 (a)). The side chain residues ASP C: 299 formed one H-bond with ursolic acid. At the active site of EGLN1, ursolic acid revealed strong interaction with a docking score of -9.91kcal and an inhibition constant of 0.05465 µm (Figure 8 (b), (c)).

ACE II antagonist, captopril was docked with the target at the active site of ACE II, the binding energy was -5.95 kcal and the inhibition constant was 43.68 µm which is higher than compounds present in TT and BD when docked with ACE II. Tataramide B, diosgenin, disogluside, neotigogenin and hecogenin showed maximum affinity towards ACE II (Figure 9 (a)). The side chain residues GLU A: 403, GLU A: 384 and HIS A: 387 formed three H-bonds with tataramide B. At the active site of ACE II, tataramide B revealed strong interaction with a docking score of -10.78 kcal and an inhibition constant of 12.46 µm (Figure 9 (b), (c)). Boerhavisterol, beta-sitosterol, boerhadiffusene, diffusarotenoid and liriodenine showed maximum affinity. The side chain residues HIS A: 353 formed one H-bond with boerhavisterol (Figure 10 (a)). At the active site of ACE II, boerhavisterol revealed strong interaction with a docking score of -9.55 kcal and an inhibition constant of 0.10039 µm (Figure 10 (b), (c)).

The vasopressin receptor II antagonist tolvaptan was docked with the target at the active site of V2R, the

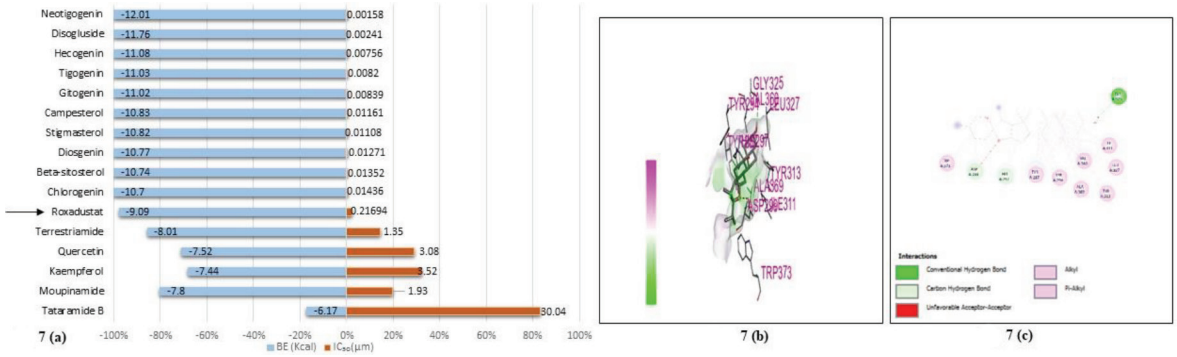


Figure 7. **(a)** Molecular docking score of *Tribulus terrestris* compounds and Roxadustat with EGLN1 **(b)** Molecular interaction of neotigogenin with EGLN1 (3D). **(c)** 2D interaction of docked EGLN1 with neotigogenin.

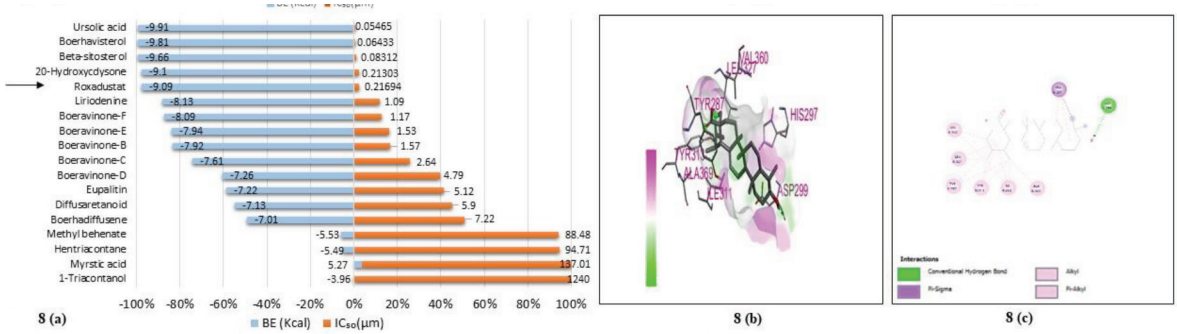


Figure 8. **(a)** Molecular docking score of *Boerhavia diffusa* compounds and Roxadustat with EGLN1. **(b)** Molecular interaction of ursolic acid with EGLN1 (3D). **(c)** 2D interaction of docked EGLN1 with ursolic acid.

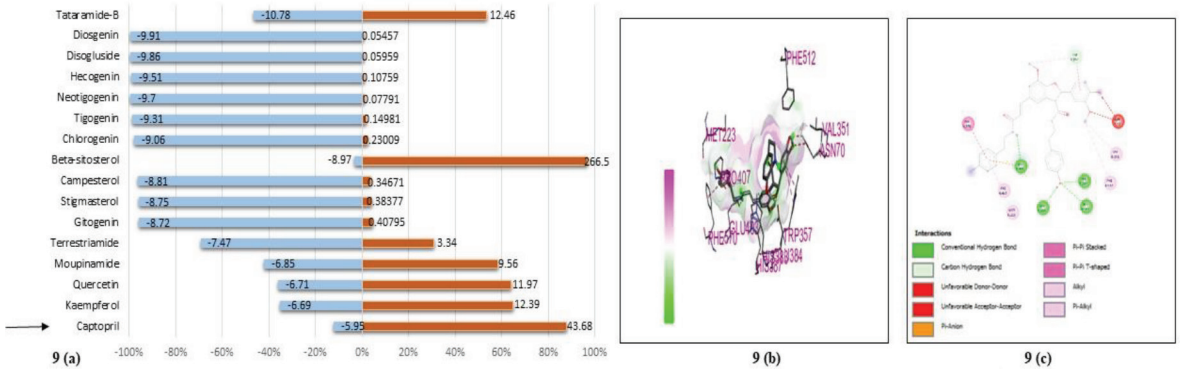


Figure 9. **(a)** Molecular docking score of *Tribulus terrestris* compounds and Captopril with ACE II **(b)** Molecular interaction of tataramide B with ACE II (3D). **(c)** 2D interaction of docked ACE II with tataramide B.

binding energy was -8.52 kcal and the inhibition constant was 0.57104 µm which is higher than compounds present in TT and BD when docked with V2R. Disogluside, gitogenin, neotigogenin, hecogenin and beta-sitosterol showed maximum affinity towards V2R (Figure 11 (a)). At the active site of V2R, disogluside revealed strong interaction with a docking score of -10.26 kcal and an inhibition constant of 0.0304 µm (Figure 11 (b), (c)). Ursolic acid, beta-sitosterol, boerhavisterol, boerhadiffusene and 20-hydroxyecdysone showed maximum affinity. (Figure 12 (a)) The side chain residues ASP A: 191, and LYS A: 116 formed two H-bonds with ursolic acid. At the active site of V2R, ursolic acid revealed strong interaction with the docking of score -9.36 kcal and an inhibition constant of 0.13842 µm (Figure 12 (b), (c)).

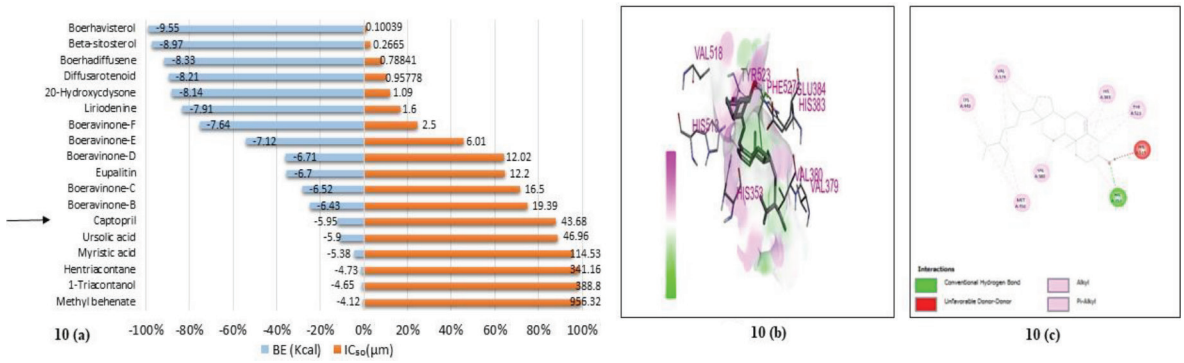


Figure 10. **(a)** Molecular docking score of *Boerhavia diffusa* compounds and captopril with ACE II **(b)** Molecular interaction of boerhavisterol with ACE II (3D). **(c)** 2D interaction of docked ACE II with boerhavisterol.

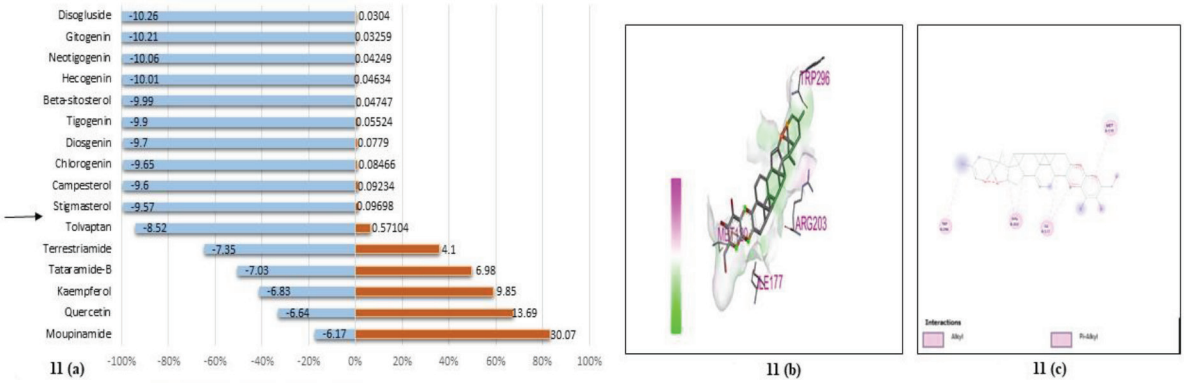


Figure 11. **(a)** Molecular docking score of *Tribulus terrestris* compounds and Tolvaptan with V2R. **(b)** Molecular interaction of disogluside with V2R (3D). **(c)** 2D interaction of docked V2R with disogluside.

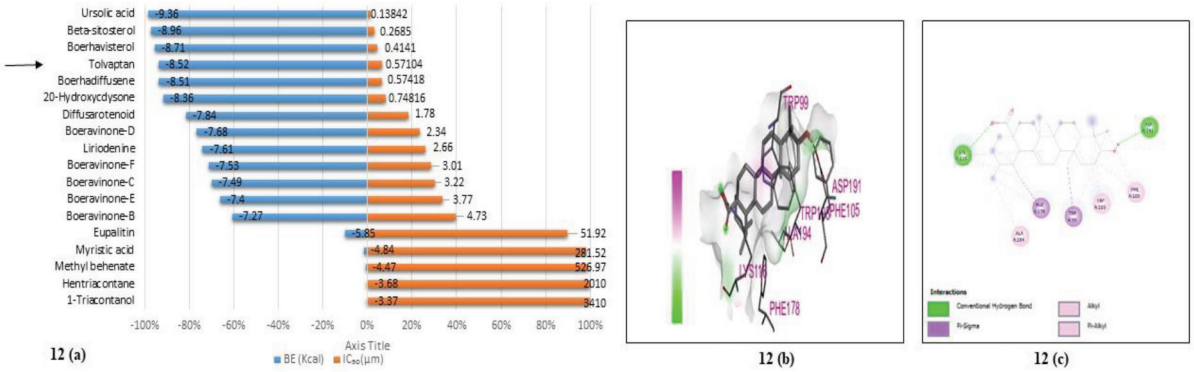


Figure 12. **(a)** Molecular docking score of *Boerhavia diffusa* compounds and Tolvaptan with V2R. **(b)** Molecular interaction of ursolic acid with V2R (3D). **(c)** 2D interaction of docked V2R with ursolic acid.

4. Discussion

Chronic kidney disease is a prevalent condition, with a limited number of effective treatments available for kidney disorders. The frequently recommended treatments include lifestyle modifications, including dietary changes, diuretics, β-blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers[24]. The utilization of Ayurvedic herbal drugs in the treatment of kidney disease have demonstrated several benefits compared to the use of a single conventional drug. The advantageous impact of medicinal plants on CKD often

stems from their capacity to enhance normal kidney functions with minimal or no adverse outcomes[25].

Tribulus terrestris L. and *Boerhavia diffusa* L. possess various well-documented properties, such as diuretic, vasodilatory, ACE-inhibitory, and effects on kidney-related complications[26,27]. *Tribulus terrestris* L. has been found to exhibit a preventive effect against damage caused by Reactive Oxygen Species (ROS) in the renal endothelium. Additionally, it plays a crucial role in mitigating vascular endothelial dysfunction and impaired enzyme activity[28]. *Boerhavia diffusa* L. can effectively reduce kidney cell damage and oxidative trauma induced by urinary stones[29].

We have evaluated the safety and pharmacological effects of both plants through the utilization of virtual computer screening techniques, drug-likeness analysis, ADMET prediction and the exclusion of compounds that do not follow the Lipinski rule. The results obtained from ADME/T analysis of bioactive compounds present in *Tribulus terrestris* and *Boerhavia diffusa* are shown to be toxic individually at particular doses. However, the use of the drug as a whole would not have shown toxicity as the concentration of the bioactive compound in the whole drug would be less and some of the fractions other than major constituents are responsible for balancing the toxic effects of the drug. These findings have already been proven in various research studies of *Tribulus terrstris* L. and *Boerhavia diffusa* L., which do not show any toxicity or carcinogenicity and are considered safe for use[30,31].

Based on the molecular docking results, the compounds chlorogenin, disogluside, neotigogenin, tataramide-B, and disogluside from TT exhibited the highest stability when docked with carbonic anhydrase II, renin, HIF propyl hydroxylase 2/EGLN1, angiotensin-converting enzyme II, and vasopressin receptor 2, respectively (Figure 3(a), 5(a), 7(a), 9(a), 11(a)). On the other hand, Beta-sitosterol from BD demonstrated the most stable interaction with carbonic anhydrase II and renin (Figure 4(a) and 6(a)). Boerhavisterol exhibited the strongest affinity towards angiotensin-converting enzyme II (Figure 10(a)). Ursolic acid displayed significant stability when docked with HIF propyl hydroxylase 2 and vasopressin receptor 2 (Figure 8(a) and 12(a)). Potential nephroprotective compounds from *Tribulus terrestris* were found to be chlorogenin, hecogenin, diosgenin, neotigogenin, beta-sitosterol, gitogenin, campesterol and disogluside. Whereas, beta-sitosterol, boerhavisterol, liriodenine, boerhadiffusene, ursolic acid, diffusaretenoid, boeravinone-E and boeravinone-F in *Boerhavia diffusa* showed maximum potential for all selected targets analogously against renal toxicity. Previous research studies showed the nephroprotective role of diosgenin in gentamicin-induced renal toxicity[32]. Gitogenin is a steroidal saponin that is widely distributed in medicinal plants and has potential applications in cardiovascular disorders[33].

Tribulus terrestris and *Boerhavia diffusa* are used as diuretics in Ayurveda which are known to inhibit the action of the carbonic anhydrase enzyme and thus can be used in the prevention of high-dose methotrexate nephrotoxicity and contrast-induced nephrotoxicity as bioactive compounds in both drugs mimic the action of acetazolamide[34,35]. The results obtained from the docking study show to have, potential renin inhibitory activity of TT and BD. These plants would have beneficial effects for renal protection, hypertension and the prevention of CVD in patients with CKD as renin is a critical regulator of blood volume, electrolyte balance and systemic vascular resistance[36,37]. The reported compound inhibits the EGLN1 target and has a similar effect as roxadustat thus treating renal anaemia and being effective in both non-dialysis-dependent and dialysis-dependent CKD patients[38]. Phytoconstituents in both plants inhibit the ACE II enzyme; hence, they lower blood pressure, improve renal injury and suppress renal inflammation[39]. Bioactive compounds in TT and BD showed significant stability towards the V2R receptor than tolvaptan, which is effective in the treatment of autosomal dominant polycystic kidney disease[40]. Thus, these herbs have potential phytochemicals that can be further explored through *in-vitro*, *in-vivo* and clinical research to determine their effectiveness in treating CKD and its associated complications.

5. Conclusion

Based on molecular docking and ADMET analysis the study revealed that among the selected individual compounds present in *Tribulus terrestris*, chlorogenin, hecogenin, diosgenin, neotigogenin, beta-sitosterol, gitogenin, campesterol and disogluside showed maximum nephroprotective activity for all selected critical targets. Compounds present in *Boerhavia diffusa*, beta-sitosterol, boerhavisterol, liriodenine, boerhadiffusene, ursolic acid, diffusaretenoid, boeravinone and boeravinone-F showed maximum nephroprotective activity for all selected critical targets. The bioactive compounds present in *Tribulus terrestris* were significantly more potent than *Boerhavia diffusa* based on their binding energy and inhibition constant. The novelty

of this study is the application of modern computational tools like molecular docking to provide better leads to investigate Ayurvedic plants for their therapeutic effects by various mechanisms in kidney diseases. It will provide an approach to maintain the inter-relationship between complex kidney disease and selected drugs; it might be validated through further experimental and clinical studies.

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7. References

1. Lee JW, Chou CL, Knepper MA. Deep sequencing in microdissected renal tubules identifies nephron segment-specific transcriptomes. J Am Soc Nephrol. 2015; 26(11):2669-77. PMid: 25817355; PMcid: PMC4625681. https://doi.org/10.1681/ASN.2014111067
2. Siew ED, Davenport A. The growth of acute kidney injury: A rising tide or just closer attention to detail? Kidney Int. 2015; 87(1):46-61. PMid: 25229340; PMcid: PMC4281297. https://doi.org/10.1038/ki.2014.293
3. Eswarappa M, Gireesh MS, Ravi V, Kumar D, Dev G. Spectrum of acute kidney injury in critically ill patients: A single center study from South India. Indian J Nephrol. 2014; 24(5):280-5. PMid: 25249716; PMcid: PMC4165051. https://doi.org/10.4103/0971-4065.132991
4. Pazhayattil GS, Shirali AC. Drug-induced impairment of renal function. Int J Nephrol Renovasc Dis. 2014; 7:457-68. PMid: 25540591; PMcid: PMC4270362. https://doi.org/10.2147/IJNRD.S39747
5. Vaidya VS, Ozer JS, Dieterle F, Collings FB, Ramirez V, Troth S, *et al*. Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. Nat Biotechnol. 2010; 28(5):478-85. PMid: 20458318; PMcid: PMC2885849. https://doi.org/10.1038/nbt.1623
6. Siddharth N, Shah MPA. Nephrology. In: Aspi R. Billimoria SAK, editors. Textbook of API Medicine, Volume 1, 8th ed. New Delhi: Jaypee Brothers Medical; 2009 p.732-3.
7. Yaraguppi DA, Udapudi BB, Patil LR, Hombalimath VS, Shet AR. *In silico* analysis for predicting protein ligand interaction for snake venom protein. Journal of Advanced Bioinformatics Applications and Research. 2012; 3(3): 345-56.
8. Lee HH, Ahn EK, Hong SS, Oh JS. Anti-inflammatory effect of tribulusamide D isolated from *Tribulus terrestris* in lipopolysaccharide-stimulated RAW264.7 macrophages. Mol Med Rep. 2017; 16(4):4421-8. PMid: 28849109; PMcid: PMC5647001. https://doi.org/10.3892/mmr.2017.7208
9. Hammoda HM, Ghazy NM, Harraz FM, Radwan MM, ElSohly MA, Abdallah II. Chemical constituents from *Tribulus terrestris* and screening of their antioxidant-activity. Phytochemistry. 2013; 92:153-9. PMid: 23642392. https://doi.org/10.1016/j.phytochem.2013.04.005
10. Kaushik J, Tandon S, Gupta V, Nayyar J, Singla SK, Tandon C. Response surface methodology based extraction of *Tribulus terrestris* leads to an upsurge of antilithiatic potential by inhibition of calcium oxalate crystallization processes. PloS One. 2017; 12(8). PMid: 28846699; PMcid: PMC5573133. https://doi.org/10.1371/journal.pone.0183218
11. Mudgal V. Studies on medicinal properties of *Convolvulus pluricaulis* and *Boerhaavia diffusa*. Planta Med. 1975; 28(1):62-8. PMid: 1178789. https://doi.org/10.1055/s-0028-1097830
12. Hiruma-Lima CA, Gracioso JS, Bighetti EJ, Robineou LG, Brito ARS. The juice of fresh leaves of *Boerhavia diffusa* L. (Nyctaginaceae) markedly reduces pain in mice. J of Ethnopharmacol. 2000; 71(1-2):267-74. PMid: 10904173. https://doi.org/10.1016/s0378-8741(00)00178-1
13. Mishra S, Aeri V, Gaur PK, Jachak SM. Phytochemical, therapeutic, and ethnopharmacological overview for a traditionally important herb: *Boerhavia diffusa* Linn. Biomed Res Int. 2014; 2014. PMid: 24949473; PMcid: PMC4053255. https://doi.org/10.1155/2014/808302
14. Mungantiwar AA, Nair AM, Kamal KK, Saraf MN. Adaptogenic activity of aqueous extract of the roots of *Boerhaavia diffusa* Linn. Indian Drugs. 1997; 34(4):184-9
15. Rawat AK, Mehrotra S, Tripathi SC, Shome U. Hepatoprotective activity of *Boerhaavia diffusa* L. roots - A popular Indian ethnomedicine. J Ethnopharmacol. 1997; 56(1):61-6. PMid: 9147255. https://doi.org/10.1016/s0378-8741(96)01507-3
16. Mishra S, Aeri V, Gaur PK, Jachak SM. Phytochemical, therapeutic, and ethnopharmacological overview for a traditionally important herb: *Boerhavia diffusa* Linn. BioMed Res Int. 2014. PMid: 24949473; PMcid: PMC4053255. https://doi.org/10.1155/2014/808302
17. Bhavamishra KC. Chunekar. Bhavaprakash Nighantu. Pandey GS, editor. Guduchyadi Varga, Varanasi: Chaukhamba Bharati Academy; 2013. p. 279.
18. Bhavamishra KC. Chunekar. Bhavaprakash Nighantu. Pandey GS, editor. Guduchyadi Varga, Varanasi: Chaukhamba Bharati Academy; 2013. p. 406.
19. Sastry JLN, Illustrated Dravyaguna Vijyana, Vol.-2, Chaukhambha Orientalia, Varanasi, India. 2010. p. 100.
20. Alam Q, Ganeshpurkar A, Singh SK, Krishnamurthy S. Novel gastroprotective and thermostable cocrystal of

dimethyl fumarate: Its preparation, characterization, and *in vitro* and *in vivo* evaluation. ACS Omega. 2023. https://doi.org/10.1021/acsomega.3c02463
21. IMPPAT: Indian Medicinal Plants, Phytochemistry And Therapeutics. By Anon cb.imsc.res.in. https://cb.imsc.res.in/imppat/phytochemical/Boerhavia%20diffusa and https://cb.imsc.res.in/imppat/phytochemical/Tribulus%20terrestris (last accessed on. 3.2.2024)
22. Alam Q, Ganeshpurkar A, Singh SK, Krishnamurthy S. Preparation, characterization, *in-vitro* and *in-vivo* pharmacokinetic evaluation of thermostable dimethyl fumarate cocrystals. J Pharm Sci. 2024; 113(3):647-58. PMid: 37595751. https://doi.org/10.1016/j.xphs.2023.07.001
23. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, *et al*. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J of Comput Chem. 2009; 30(16):2785-91. PMid: 19399780; PMcid: PMC2760638. https://doi.org/10.1002/jcc.21256
24. Shlipak MG, Tummalapalli SL, Boulware LE, Grams ME, Ix JH, Jha V, *et al*. The case for early identification and intervention of chronic kidney disease: Conclusions from a kidney disease: Improving Global Outcomes (KDIGO) controversies conference. Kidney Int. 2021; 99(1):34-47. PMid: 33127436. https://doi.org/10.1016/j.kint.2020.10.012
25. Khan MA, Kassianos AJ, Hoy WE, Alam AK, Healy HG, Gobe GC. Promoting plant-based therapies for chronic kidney disease. J Evid Based Integr Med. 2022; 27. PMid: 35243916; PMcid: PMC8902019. https://doi.org/10.1177/2515690X221079688
26. Mishra S, Aeri V, Gaur PK, Jachak SM. Phytochemical, therapeutic, and ethnopharmacological overview for a traditionally important herb: *Boerhavia diffusa* Linn. BioMed Res Int. 2014. PMid: 24949473; PMcid: PMC4053255. https://doi.org/10.1155/2014/808302
27. Yadav HN, Sharma US, Singh S, Gupta YK. Effect of *Tribulus terrestris* in mercuric chloride-induced renal accumulation of mercury and nephrotoxicity in rat. J Adv Pharm Techno Res. 2019; 10(3):132-7. PMid: 31334096; PMcid: PMC6621347. https://doi.org/10.4103/japtr.JAPTR_386_18
28. Jiang YH, Yang CH, Li W, Wu S, Meng XQ, Li DN. Aqueous extracts of *Tribulus terrestris* protects against oxidized low-density lipoprotein-induced endothelial dysfunction. Chin J Integr Med. 2016; 22:193-200. https://doi.org/10.1007/s11655-015-2321-0
29. Mishra S, Aeri V, Gaur PK, Jachak SM. Phytochemical, therapeutic, and ethnopharmacological overview for a traditionally important herb: *Boerhavia diffusa* Linn. BioMed Res Int. 2014. PMid: 24949473; PMcid: PMC4053255. https://doi.org/10.1155/2014/808302
30. Hemalatha S, Hari R. Acute and subacute toxicity studies of the saponin rich butanol extracts of *Tribulus terrestris* fruits in wistar rats. Int J Pharm Sci Rev Res. 2014; 27. 307-13.
31. Orish E, Orisakwe O, Johnson O, Adaora M, Obi E, Dioka C. Sub-chronic toxicity studies of the aqueous extract of *Boerhavia diffusa* Leaves. J Health Sci. 2003; 49. 444-7. https://doi.org/10.1248/jhs.49.444
32. Mishra P, Mandlik D, Arulmozhi S, Mahadik K. Nephroprotective role of diosgenin in gentamicin-induced renal toxicity: Biochemical, antioxidant, immunological and histopathological approach. Futur J Pharm Sci. 2021; 7:169. https://doi.org/10.1186/s43094-021-00318-z
33. Ul Haq I, Khan T, Ahmad T, Shah AJ. Insight into the cardiovascular mechanisms of blood pressure lowering effect of gitogenin: A steroidal saponin. Clin Exp Hypertens. 2021; 43(8):723-9. PMid: 34396877. https://doi.org/10.1080/10641963.2021.1950748
34. Ku M, Bazargan A, Tam C. Addition of low dose acetazolamide as an adjunct in patients undergoing high dose methotrexate is safe and beneficial. Intern Med J. 2020; 50(3), 357-62. PMid: 31403755. https://doi.org/10.1111/imj.14468
35. Cheng CM, Salazar A, Amato MG, Lambert BL, Volk LA, Schiff GD. Using drug knowledge base information to distinguish between look-alike-sound-alike drugs. J Am Med Inform Assoc. 2018; 2(7):872-84. PMid: 29800453; PMcid: PMC7647046. https://doi.org/10.1093/jamia/ocy043
36. Morishita Y, Kusano E. Direct Renin inhibitor: Aliskiren in chronic kidney disease. Nephro-Urol Mon. 2013; 5(1):668-72. PMid: 23577328; PMcid: PMC3614331. https://doi.org/10.5812/numonthly.3679
37. Fountain JH, Kaur J, Lappin SL. Physiology, renin angiotensin system. In StatPearls. StatPearls Publishing; 2023. https://www.ncbi.nlm.nih.gov/books/NBK470410/.
38. Liu J, Yang F, Waheed Y, Li S, Liu K, Zhou X. The role of roxadustat in chronic kidney disease patients complicated with anemia. Korean J Intern Med. 2023; 38(2):147-56. PMid: 36588451; PMcid: PMC9993099. https://doi.org/10.3904/kjim.2022.318
39. Gan Z, Huang D, Jiang J, Li Y, Li H, Ke Y. Captopril alleviates hypertension-induced renal damage, inflammation, and NF-κB activation. Braz J Med Biol Res. 2018; 51(11). PMid: 30183974; PMcid: PMC6125835. https://doi.org/10.1590/1414-431X20187338
40. Sans-Atxer L, Joly D. Tolvaptan in the treatment of autosomal dominant polycystic kidney disease: Patient selection and special considerations. Int J Nephrol Renovasc Dis. 2018; 11:41-51. PMid: 29430193; PMcid: PMC5797468. https://doi.org/10.2147/IJNRD.S125942