Evaluation of Diuretic Activity of *Ampelocissus Latifolia* Methanolic Root Extract by *In vivo and in silico* Studies

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Abstract

The present study was designed to investigate the phytochemical constituents and evaluate the diuretic activity of the methanolic extract of *Ampelocissus latifolia* roots through in vivo and in silico approaches. Preliminary phytochemical analysis revealed the presence of various secondary metabolites, including flavonoids, alkaloids, tannins, glycosides, saponins, and phenolic compounds, which are known to contribute to diuretic and renal-modulating effects. The diuretic activity was assessed in experimental animal models, using standard protocols to monitor urine output, electrolyte excretion, and key indices related to renal function. The extract demonstrated a significant increase in diuresis compared to the control group, suggesting dose-dependent activity. Furthermore, in silico molecular docking studies were conducted to predict the interaction of selected phytoconstituents with diuretic targets such as the carbonic anhydrase enzyme. The docking results supported the in vivo findings, indicating strong binding affinities and potential inhibition mechanisms. The combined data from experimental and computational analyses suggest that *Ampelocissus latifolia* root extract exhibits promising diuretic properties and could serve as a potential source for the development of natural diuretic agents. In silico molecular docking was performed against the receptor [PDB ID : 5OW8] using Furosemide , Campesterol, Hexadecanoic acid , Squalene and 5 - Hydroxymethylfurfural.

Keywords: *Ampelocissus latifolia,* roots, Diuretic Activity, methanolic extract

INTRODUCTION

Medicinal plants are an important source of therapeutic agents and have been used for centuries in traditional medicine systems around the world. Nearly 80% of the population in developing countries depends on plant-based medicines for primary healthcare needs, and about 25% of modern prescribed drugs are derived from natural sources.[1] However, due to overharvesting and habitat destruction, many medicinal plants are now endangered, highlighting the need for their conservation and sustainable use. [2] Diuretics are substances that promote the excretion of water and electrolytes from the body by increasing urine production. They are widely used in the treatment of hypertension, edema, and kidney disorders. [3]Based on their site and mechanism of action, diuretics are classified into five main categories: carbonic anhydrase inhibitors, osmotic diuretics, loop diuretics, thiazide diuretics, and potassium-sparing diuretics.[4] Traditionally, several plants such as parsley, juniper, and digitalis were known for their diuretic properties long before synthetic diuretics were developed. [5]The nephron, the functional unit of the kidney, plays a crucial role in regulating water and electrolyte balance, and understanding its physiology helps explain how diuretics exert their effects. The diuretic potential of plants is mainly due to their bioactive phytoconstituents like alkaloids, flavonoids, glycosides, tannins, triterpenoids, and saponins.[6]

In the present study, the methanolic extract of *Ampelocissus latifolia* roots was prepared using Soxhlet extraction with different solvents to isolate active compounds. The extract was evaluated for diuretic activity in animal models, using various doses and compared with the standard drug furosemide. Urine samples were analyzed for sodium, potassium, and chloride content to determine the effect on electrolyte excretion. This study aims to scientifically validate the traditional use of *Ampelocissus latifolia* as a natural diuretic and explore its potential as a safe and effective herbal alternative to synthetic diuretics.

MATERIALS AND METHODS

Extraction

The prcocess of extracting a substance from a matrix is known as extraction in chemistry.Plant active principles have been isolated or acquired as pure compounds with precisely known stability and

potency in recent years .The plant has several chemical compounds that can be used to cure a variety of ailments."marc" refers to the undissolved residue that is left over after the process,while "Menustrum" is the name of the extraction solvent.

Selection of solvents

In order to determine extractive values, *Ampelocissus latifolia* was extracted using seven different solvents based on their polarity: Petroleum ether, Toluene, Chloroform, Ethyl acetate, Acetone, Methanol, and water. The main solvent was either alcohol and aqueous extraction. methanol is a semipolar solvent that can extract arrange of phytoconstituents whereas water is a polar solvent that is cheap and safe.

Procedure

Within a porous bag or'' thimble'' composed of durable filter paper ,200 grams of dried powdered Ampelocissus latifolia root was carefully weighed and placed in the thimble chamber of the Soxhlet apparatus. The extraction solvent is heated in a round bottom flask using a heating mantle. The heating temperature is determined by the extraction solvent. The heat causes the solvent in the bottom of the flask to vaporise into the condenser before dripping back to the sample thimble. When the liquid content reaches the syphon arm and is again poured into the bottom flask, the process is complete, as indicated by the clear solution in the syphon tube [7]. The filtrate is transferred to a flat -bottomed plate that ha been previously weighed and then evaporated on a water bath to dry it. To fully dry the ingredients, the dish is next put in the oven for six hours .The food is immediately weighed after cooling in a desicator for 30 minutes .After the filter paper has dried fully gathered ,dual mass is still visible on it. This technique is performed with each of the seven solvents. Milligrammes per gramme of air -dried material is the unit of measurement for the extractable matter content.

Phytochemical investigation

Preliminary tests were carried out on methanolic and water extract for the presence/absence of phytoconstituents such as alkaloids, flavonoids, tannins, resins, carbohydrates, protein and saponins [9].

Treatment groups for diuretic activity

Group 1	Negative control (distilled water)
Group 2	Ampelocissus latifolia Extract 250mg/kg
Group 3	Ampelocissus latifolia Extract 375mg/kg
Group 4	Ampelocissus latifolia Extract 500mg/kg
Group 5	Standard drug (Furosemide-13mg/kg)

The animals spent a day acclimating to methanolic cages before to the experiment. They were then allowed unlimited access to water and left to starve all night. Six, twelve, and twenty- four hours following the last treatment, urine samples were taken. For electrolyte analysis, the urine samples were filtered and kept at -20^{0}C.

Lipschitz Method

The diuretic effectiveness of *Ampelocissus latifolia* was evaluated in Wistar rats using the Lipschitz method five groups of six animals are formed group 2,3,4 received 250,375,and 500 mg/ kg of *A.latifolia*,extracts respectively, group 5 received the standard diuretic drug furosemide (13 mg /kg).As a negative control , distilled water was admistered to group 1.all treatments was given orally followed by an oral route of normal saline (25 ml / kg) to ensure constant hydration The rats were subsequently joined in different metabolic cages and urine was collected from full day the measured urine volume was used to calculate the diuretic index and urinary excretion percentage.urinary electrolytes (Na,K,Cl) were measured using tritrimetric and flame photometric methods, and indices such as saluretic index, natriuretic index and carbonic anhydrase inhibition ratio were produced. All outcomes were statistically analysed using Dunnett's test and one-way ANOVA, and the results were expressed using mean ± SEM. [8] This method provided a reliable assessment of

Statistical evaluation

All values were represented as mean values ± SEM (standard error of the mean), and the data was analyzed using one way ANOVA followed by a Dunnett’s t-test using GraphPad Prism. The results were considered statistically significant if $p < 0.05$, $p < 0.01$, or $p < 0.001$

IN -SILICO STUDIES:.

Molecular docking The binding interactions of 5-hydroxymethyl furfural, furosemide, Campesterol, and Hexadecenoic acid with the COX receptor were investigated using molecular docking with AutoDock software (version 1.5.7) [PDB ID:5OW8]. UCSF Chimaera (version 1.18) and Biovia Discovery Studio were used for molecular visualisation and analysis. Ligand Preparation: Chimaera was used to generate 3D structures and minimise energy, after which the structures were saved as PDB files. Furosemide, Campesterol, Hexadecanoic acid, and 5-hydroxymethylfurfural were all shown to have two-dimensional structures (e.g., PubChem). [Figure 2] Preparing the Receptor: The 5OW8 protein structure was obtained from the protein data bank (PDB). Chimaera removed unnecessary chains and water molecules. AutoDock Tools were used to assign AD4 atom types, hydrogen, and Kolman charges For the receptor, a PDBQT file was stored.Grind generation: A grid box was drawn around the receptor's active region (5OW8) to incorporate potential ligand binding sites. Docking Simulation: AutoDock was used to perform docking simulations, calculating binding energies and identifying optimal binding locations using genetic algorithms and simulated annealing. Analysis: Docking results (binding energy, inhibition constant) were analysed in AutoDock and shown in Discovery Studio in order to evaluate ligand-receptor interactions such as Vander wall forces, carbon hydrogen bonds and pi-stacking interactions [3] among the tested compounds. in comparison to Furosemide, Campesterol, Hexadecanoic acid, and 5-Hydroxymethylfurfural, squalene has the highest binding affinity (-8.59 kcal/mol). Molecular Features and Drug Likeness Prediction Motivated by Molinspiration Using cheminformatics, molecular parameters such as molecular weight volume, TPSA, log p and the number of rotatable bonds were computed, while molinspiration and molsoft were used for evaluation. Lipinskins rule of 5 (molecular mass <500DA ,log p < 5 ,< 5 HBD, < 10 HBA, PSA < 140 Å²) was used to assess drug-likeness. Swiss ADME was used to predict pharmacokinetic profiles, physiochemical properties, drug-likeness, and ADME (absorption, distribution, metabolism, and excretion) parameters, such as blood brain barrier penetration (BBB) and human gastrointestinal absorption (HIA) physicochemical as and, using the 'Boiled Egg' model [11–15].

Biochemical Estimation:

Biochemical estimation is performed for sodium, colour development, Potassium, chloride, Saluretic, natriuretic and carbonic anhydrase by assays.

RESULT & DISCUSSION

Diuretic Activity of Extracts:

The pharmacological evaluation of the diuretic action of Ampelocissus latifolia extract is presented in this study, presuming that extract was admistered at the dose 200 mg / kg b.w the By assessing variables and the concentration of electrolytes like sodium, potassium, chloride are expressed mean ± SEM (n = 6 animals per group) data analysed ANOVA Dunnett's test significantly ($p<0.001$) and significantly ($p<0.01$)chloride in the urine, the diuretic potential of each group was assessed. Comparing urine volume in methanol and aqueous extract to control, there was a substantial increase ($p<0.001$). The methanol extract's sodium content was significantly ($p<0.001$) greater than the control's. The methanol extract and aqueous extract had greater potassium contents than the control. Comparing the level of chloride to the control, there was a little increase. Urine volume, sodium, potassium, and chloride ion levels of furosemide were all highly significant ($p<0.001$).

Preparation of sodium and serum proteins

All values are expressed by using. Mean ± SEM (n = 6 animals per group) The data can be analysed by using Anova and dunnets test (or another post-hoc test) to see if they differ significantly from the control.

Estimation of Urinary Potassium:

A. latifolia treatment causes a dose-dependent increase in potassium excretion in the urine. At the highest dosage (500 mg/kg), potassium excretion is similar to that of furosemide. This suggests that higher dosages may have saluretic and diuretic effects.

Estimation of Urinary Chloride:

Ampelocissus latifolia extract dramatically and dose-dependently raised urinary chloride excretion. When using the popular diuretic furosemide, the chloride excretion is similar to that of the group receiving the highest dose (500 mg/kg). These findings confirm the extract's saluretic potential, which is the enhanced excretion of ions, especially Cl^-.

Estimation of Saluretic Index, Natriuretic Index, CAI Ratio:

The increase in saluretic activity with dosage indicates that A. latifolia enhances electrolyte excretion.

The natriuretic indices for all groups remained over 2, indicating a preference for sodium excretion and

very little potassium loss. The CAI ratio slightly dropped as the dosage rose, suggesting a mild inhibitory effect of carbonic anhydrase.

Estimation of Urine Volume, Diuretic Index, Urinary Excretion:

When compared to the control, urine volume increased significantly with all A. latifolia dosages. The diuretic index increased in a dose-dependent manner, confirming effective diuretic activity. The fact that the maximal dosage (500 mg/kg) was nearly equivalent to the effects of ordinary furosemide demonstrated strong diuretic potential.

DISSCUSSION

The present study demonstrates that the methanolic extract of *Ampelocissus latifolia* possesses significant diuretic activity, as evidenced by increased urine output and elevated excretion of sodium (Na^+) and potassium (K^+) ions when compared to the control group. Since urine volume is primarily influenced by the glomerular filtration rate (GFR) and the extent of tubular reabsorption, the enhanced diuresis observed in this study may be attributed to mechanisms that improve renal blood flow, thereby increasing GFR.

The extract showed strong diuretic potential, with effects comparable to the standard diuretic furosemide, particularly in promoting the excretion of Na^+ and K^+ ions. This suggests that the plant extract may interfere with tubular handling of electrolytes, leading to reduced reabsorption and increased urinary loss.

Pharmacognostic and phytochemical investigations confirmed the presence of active phytoconstituents in the methanolic extract, which may be responsible for the observed biological activity. However, the specific compound(s) mediating this effect remains unknown.

Although the results strongly indicate that Ampelocissus latifolia extract exerts a potent diuretic effect in experimental animals, further research is essential. Additional studies should include:

Isolation and identification of the active phytochemical components.
Evaluation of long-term efficacy and safety, especially considering chronic use.
Mechanistic studies to understand the precise pathway of diuresis.
Toxicological profiling to ensure safe therapeutic application.

Given its promising diuretic potential, the extract may have future relevance in managing conditions like hypertension and fluid retention, but confirmation through extensive pharmacological and clinical investigations is necessary.

CONCLUSION:

The methanolic extracts of *Ampelocissus latifolia* significantly increased the excretion of potassium and sodium electrolyte concentrations as well as urine output as compared to the control. There are two factors that affect urine volume. One is the glomerular filtration rate (GFR), and the other is the extent of tubular re-absorption. The observed effect could be caused by mechanisms like increased renal blood flow and the ensuing increase in GFR. 68 According to the current study, urine output was significantly increased by Ampelocissus latifolia extract in methanol. While methanol extracts significantly increased sodium and potassium ions, methanolic extracts only modestly increased potassium ions. The primary findings are as follows: The pharmacognostic evaluation of Ampelocissus latifolia was determined by extraction and fractionation, as well as by preliminary phytochemical study of methanolic extracts. The findings demonstrated that the plant was strong, with an intensity similar to that of furosemide, and that it was linked to significant increases in urine K+ and Na+ levels. However, more research is encouraged to isolate the active phytochemical

ingredient in order to discover the exact mechanism of diuresis. It is logical to believe that Ampelocissus latifolia methanolic extract has a strong diuretic impact on rats based on the study's findings. Further research is needed to determine the diuretic's long-term efficacy and safety profile as well as to extract, purify ,structurally clarify and propose the many mechanism of action Given the possibility that these findings could aid in the treatment of hypertension, more investigation is required.

Acknowledgement

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Ethics statement

The experimental groups were allocated the samples utilizing a random distribution technique. By utilizing this methodology, the initial bodyweights can be approximately equalized, and participants can be randomly assigned to experimental groups bearing the CPCSEA REG NO: CCSEA/IAEC/JLS/22/23/24/047.

MATH FORMULAE

$$Sodium\ in\ mmole/L = \frac{Abs\ of\ B - Abs\ of\ T}{Abs\ of\ B - Abs\ of\ T} \text{ X100}$$

$$Potassium\ in\ mmol/L = \frac{Abs\ of\ T}{Abs\ of\ S} \text{ x 5.0}$$

$$Chloride\ mmol/L = \frac{\Delta A_{sam}\ C}{\Delta A_{St}}$$

$$Diuretic\ action = \frac{\text{Urinary excretion volume of the test group}}{\text{Urinary excretion volume of the control group}}$$

$$Diuretic\ activity = \frac{Diuretic\ action\ of\ test}{\text{Diuretic action of standard}}$$

$$Salureticindex = \frac{\text{Urinary excretion of electrolytes of the test group}}{\text{Urinary excretion of electrolytes of the standard}}$$

$$\text{Natriuretic index} = \frac{\text{Urinary excretion of } Na^{+}}{\text{Urinary excretion of } K^{+}}$$

$$\text{Carbonic anhydrase inhibition} = \frac{\text{Urinary excretion of } Cl^{-}}{\text{Urinary excretion of } Na^{+}\ K^{+}}$$

TABLES

Table No 1:

Sl. No.	Solvent	Colour & Consistency	Plant material taken (g)	Weight of extract (g)	% Yield w/w
1.	Methanol	Reddish brown, semisolid viscous	200	26	13

TABLE 2:

Sl. No.	Phytoconstituents	Test result
1	Alkaloid	+ve
2	Glycosides	+ve
3	Carbohydrate	+ve
4	Protein	+ve
5	Amino acid	+ve
6	Steroids	+ve
7	Flavonoids	+ve
8	Terpenoids	+ve
9	Phenols	+ve
10	Saponins	+ve
11	Tannin	+ve

+ present ; - Absent

Table 3:

Group	Treatment	Urinary Sodium (mEq/L)	Serum Proteins (mg/dL)
I	Negative Control (Distilled Water)	45.12 ± 2.31	6.52 ± 0.28
II	*A. latifolia* Extract 250 mg/kg	58.37 ± 2.85	5.94 ± 0.22
III	*A. latifolia* Extract 375 mg/kg	68.45 ± 3.12	5.41 ± 0.30
IV	*A. latifolia* Extract 500 mg/kg	75.68 ± 3.48	5.02 ± 0.26
V	Standard (Furosemide 13 mg/kg)	82.91 ± 3.76	4.85 ± 0.19

Table 4:

Group	Treatment	Urinary Potassium (mEq/L)
I	Negative Control (Distilled Water)	18.26 ± 1.02
II	*A. latifolia* Extract 250 mg/kg	23.47 ± 1.15
III	*A. latifolia* Extract 375 mg/kg	26.89 ± 1.32
IV	*A. latifolia* Extract 500 mg/kg	30.24 ± 1.48
V	Standard Drug (Furosemide 13 mg/kg)	35.61 ± 1.76

Table 5:

Group	Treatment	Urinary Chloride (mEq/L)
I	Negative Control (Distilled Water)	40.78 ± 2.05
II	*A. latifolia* Extract 250 mg/kg	52.13 ± 2.42
III	*A. latifolia* Extract 375 mg/kg	60.97 ± 2.86
IV	*A. latifolia* Extract 500 mg/kg	68.44 ± 3.12
V	Standard Drug (Furosemide 13 mg/kg)	74.85 ± 3.38

Table 6:

Group	Treatment	Saluretic Index	Natriuretic Index (Na^+/K^+)	CAI Ratio ($Cl^-/(Na^+ + K^+)$)
I	Negative Control	1.00 ± 0.00	2.47 ± 0.12	0.53 ± 0.03
II	*A. latifolia* 250 mg/kg	1.38 ± 0.06	2.48 ± 0.10	0.50 ± 0.02
III	*A. latifolia* 375 mg/kg	1.59 ± 0.08	2.54 ± 0.11	0.49 ± 0.02
IV	*A. latifolia* 500 mg/kg	1.78 ± 0.09	2.50 ± 0.09	0.47 ± 0.02
V	Furosemide 13 mg/kg	1.96 ± 0.10	2.33 ± 0.08	0.45 ± 0.01

Table 7:

Group	Treatment	Urine Volume (mL/100g/24h)	Diuretic Index	Urinary Excretion (%)
I	Negative Control (Distilled Water)	4.25 ± 0.22	1	34.28 ± 1.65
II	*A. latifolia* Extract 250 mg/kg	6.32 ± 0.28	1.49	48.65 ± 2.18
III	*A. latifolia* Extract 375 mg/kg	7.45 ± 0.31	1.75	56.87 ± 2.44
IV	*A. latifolia* Extract 500 mg/kg	8.61 ± 0.36	2.02	64.52 ± 2.62
V	Standard (Furosemide 13 mg/kg)	9.25 ± 0.38	2.17	69.83 ± 2.71

DOCKING PROPERTIES:

Table 8:

Properties/Compounds	Furosemide	Campesterol	Hexadecanoic acid	Squalene	5-Hydroxymethylfurfural
Binding energy	-7.85	-8.87	-8.61	-8.93	-4.45
Ligand efficiency	-0.37	-0.31	-0.3	-0.31	-0.49
Inhibition constant	1.76	315.1	492.3	284.1	551.13

FIGURES

Figure 1

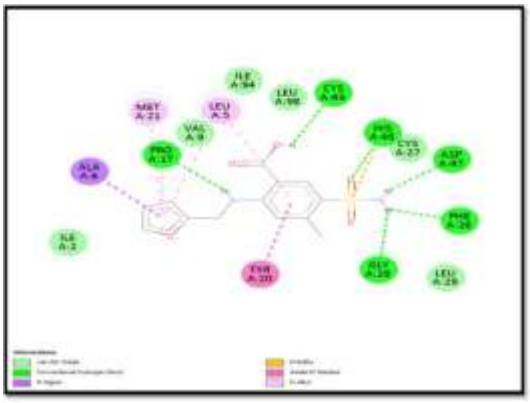


Figure 2

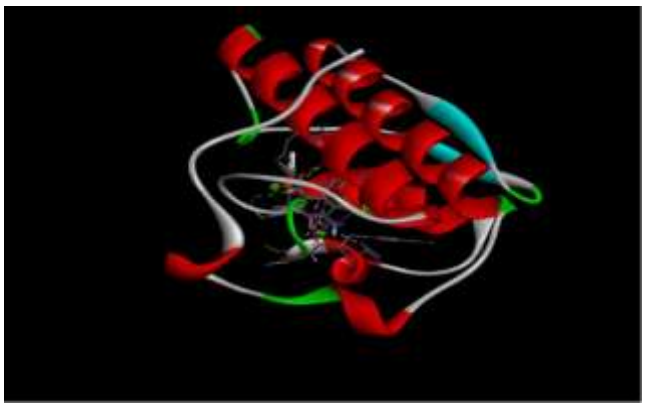
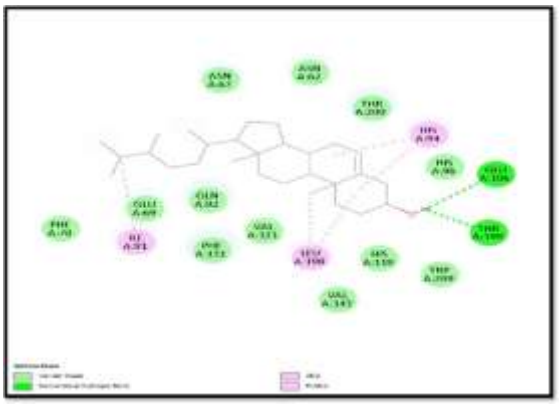


Figure 3



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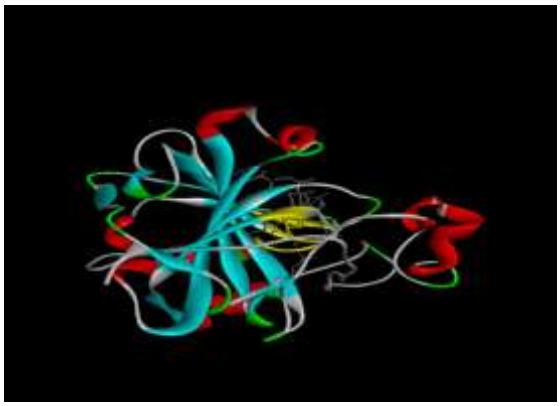

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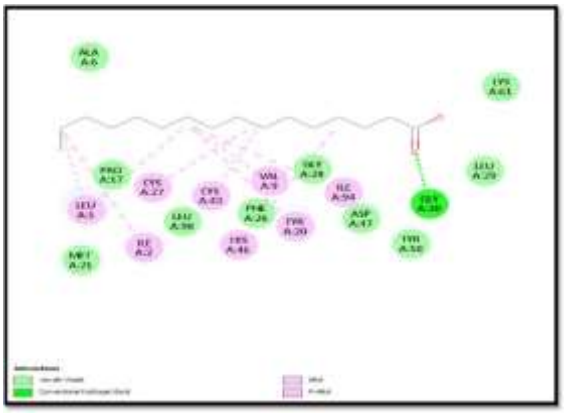

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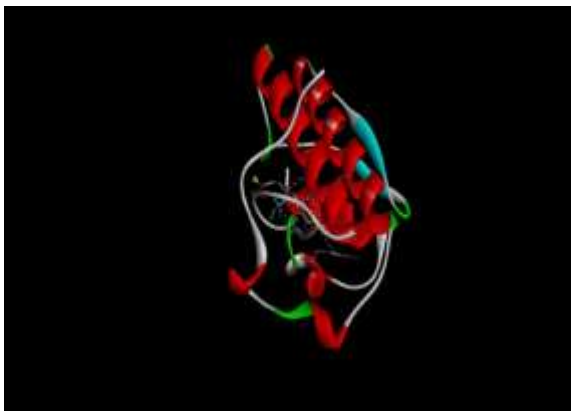

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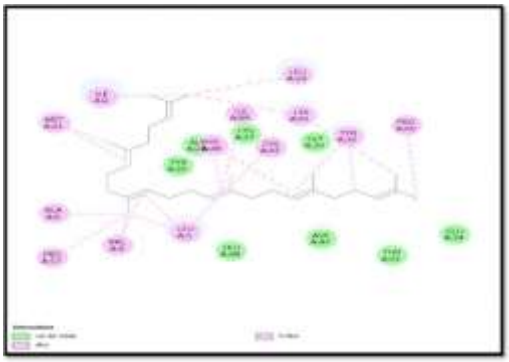
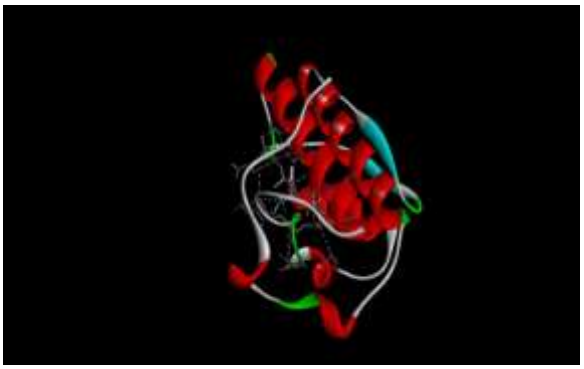


Figure 8



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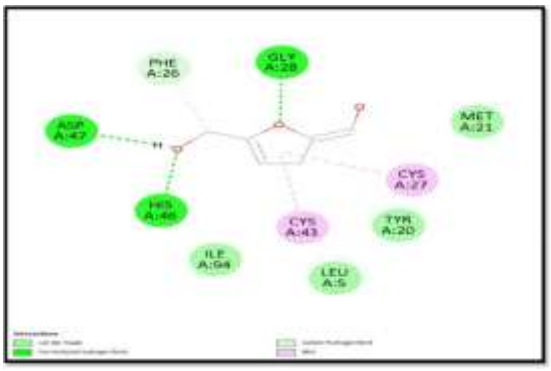

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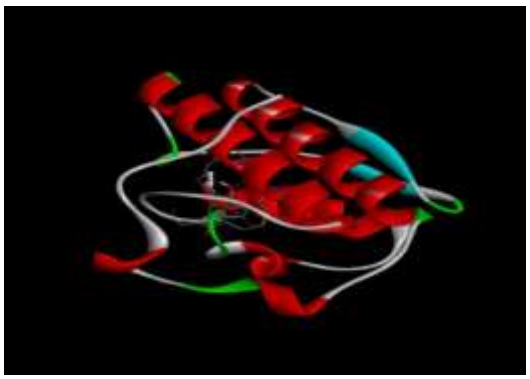

Table No 1 : Characteristics of different extract of *Ampelocissus latifolia*

TABLE 2 : Phytochemical investigation

Table 3: Precipitation of Sodium and Serum Proteins

Table 4: Estimation of Urinary Potassium

Table 5: Estimation of Urinary Chloride

Table 6: Estimation of Saluretic Index, Natriuretic Index, CAI Ratio

Table 7: Estimation of Urine Volume, Diuretic Index, Urinary Excretion

FIGURES

LIGAND INTERACTIONS WITH RECEPTOR 5OW8

- **FUROSEMIDE**
  Figure 1 : 2D IMAGE. Figure 2 : 3D IMAGE
- **CAMPESTEROL**
  Figure 3 : 2D IMAGE Figure 4 : 3D IMAGE
- **HEXADECANOIC ACID:**
  Figure 5 :2D IMAGE Figure 6: 3D IMAGE
- **SQUALENE:**
  Figure 7: 2D IMAGE Figure 8: 3D IMAGE
- **5-HYDROXYMETHYLFURFURAL:**
  Figure 9: 2D IMAGE Figure 10: 3D IMAGE

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