International Journal of Botany Studies
ISSN: 2455-541X
Impact Factor: RJIF 5.12
www.botanyjournals.com
Volume 3; Issue 1; January 2018; Page No. 25-28

In silico analysis of the aqueous bark extract *Crateva magna* Lour. (DC.) for docking analysis of the compound 14-hydroxy-12-abietene-7-one

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Abstract
Molecular docking is a bioinformatics tool used to study and analyse ligand receptor interactions. This helps in identifying the receptors (molecular targets) for different ligands. Using these technologies, compound isolation and drug discovery from herbals is achieved. Herbs are widely used in treatment of various ailments from time immemorial. Phytochemists and drug developers are now interestingly working in developing new molecules that can act effectively than conventional drugs. As they are developing it mostly from herbs they are found to be effective and safer drugs and quantity to be used become minimum. *Crateva magna* Lour. (DC.) is a plant distributed widely in India and used for urinary disorders. The plant bark was extracted and studied for its active compounds that possess antiurolithiatic activity. After performing various preliminary phytochemical studies and applying chromatographic methods, molecular docking was carried out with isolated bioactive compound and Tamm–Horsfall protein (THP). By docking analysis the bioactive compound 14-hydroxy-12-abietene-7-one interacted with THP and it may inhibit calcium oxalate crystallization.

Keywords: docking analysis, *Crateva magna* Lour. (DC.) antiurolithiatic activity, tamm–horsfall protein

Introduction
Docking is a method of molecular modeling, which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Molecular docking can be defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest and is used to predict the structure of the intermolecular complex formed between two or more molecules. The most interesting case is the protein ligand interaction, because of its applications in medicines. Ligand is a small molecule, which interacts with protein's binding sites. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes. In modern drug designing, molecular docking is routinely used for understanding drug information about drug receptor interactions and is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule [1]. In order to identify antagonist for urinary stones entry level receptor namely Tamm-Horsfall protein, molecular docking was carried out.

Tamm-Horsfall protein (THP) is one of the main components of urinary proteins. It is a glycoprotein produced and secreted by the thick ascendant limb of the loop of Henle, being the most abundant protein in normal human urine, excreted in quantities of 20 to 200 mg/24 h [2, 3]. THP of normal subjects inhibits the aggregation but has little effect on nucleation and growth of CaOx crystals. However, THP activity is influenced by its own concentration, urinary pH and ionic strength, playing a dual role such as inhibitor as wells as promoter in crystal formation depending on the environmental conditions [4]. Moreover, THP isolated from the urine of recurrent stone formers sometimes becomes a promoter of CaOx aggregation due to a tendency to self-aggregation, which removes it from effective interaction with CaOx monohydrate crystals [5].

Medicinal plants are advantageous in the field of drug discovery as they are utilized by humans for centuries. The bioactive compounds found in the plants are having many properties that are applied in the treatment of diseases. Molecular docking was used to study the interaction of withanolides with DNA binding site of NF-kB through docking analysis [6].

Since ancient times Indian medicine "Ayurveda'' recommends several medicinal plants for the successful treatment of urolithiasis [7]. They are effective with fewer side effects and are also inexpensive. Hence, the Indian plants are constantly being evaluated for possible antilithiatic effects in a systematic manner [8]. On the other hand, traditional system of Indian medicine Ayurveda recommends *Crateva magna* to be antilithiatic, but scientific data supporting this statement is still lacking. *Crateva magna* belongs to the family Capparaceae that represent about 33 genera and 700 Species. The plant is nontoxic, available in rural areas, culturally acceptable, and found to be effective for urinary disease and disorders. Hence the present study was undertaken to assess antiurolithiatic activity of *Crateva magna*.

The bark and root extracts have been used to cure cough, obesity, blood disorders, rheumatoid arthritis and heart diseases. The external application of *C. magna* leaf paste and the Oral consumption of leaf juice is used in the treatment of piles [9]. The whole plant is used for the medical purpose such as diuretic, laxative, thonotriptic, antirehumatic, antiperiodic,

bitter tonic, rubifacient and counterirritant [10, 11]. The plant used as an antidote in snake bite and the bark used for kidney and bladder stones, contraceptive and cytotoxic, fever, vomiting and gastric infections [12] Fruit juice, leaves and bark are useful to cure snakebite, infected wound and cuts. It increases appetite and controls other skin diseases [14]. *Crateva magna* is a potent medicinal plant in the Indian systems of medicine. Traditionally used for inflammation, fever, arthritis, bronchitis, urinary calculi and cough.

It is also useful in disorders of urinary organs, urinary tract infections, pain, intermittent fever, asthma, bronchitis, renal and vesicle calculi. Bark yields triterpenoids (α and β- amyrin, ceryl alcohol, lupeol, friedelin, betulinic acid, 4-taraxasterol, lupenone), flavonoids (rutin, catechin, quercetin) and alkaloids [15, 16]. The present paper deals with the utility of compound isolated from *Crateva magna* Lour. (DC.) aqueous root extract by molecular docking to assess its antiurolithiatic property with THP.

Methodology

Molecular docking of bioactive compound isolated from *C.magna* aqueous bark extract was carried out with Tamm–Horsfall protein (THP) using automated docking software.

LigPrep

LigPrep is a robust collection of tools designed to prepare high quality, all-atom 3D structures for large number of drug-like molecules, starting with 2D or 3D structures in sdf or Maestro format. The resulting structures can be saved in either sdf or Maestro format. The simplest use of LigPrep produces a single, low energy, 3D structure with correct chiralities for each input structure with various ionization states, tautomers, stereochemistries, ring conformations and eliminate molecules using various criteria including molecular weight or specified numbers and types of functional groups present. The LigPrep script provides an efficient way to use a set of tools for ligand preparation collectively and consistently.

Protein Preparation

Since, 3D structure of Tamm-Horsfall protein was not available in the protein structural data base, a homology model of the THP with a sequencing number P07911 was generated using modBase automodel based on the template 3qw9A. The active site of the target protein was identified using Schrodinger module. Glide calculations use an all-atom force field for accurate energy evaluation. Thus, Glide requires bond orders and ionization states to be properly assigned and performs better, when side chains are reoriented when necessary and steric clashes are relieved. The entire procedure can be performed in the Protein Preparation Wizard panel, from the Workflows menu on the main toolbar.

Receptor grid generation

Glide searches for favourable interactions between one or more ligand molecules and a receptor molecule, usually a protein. 14-hydroxy-12-abietane 7-one ligand binds with relatively hydrophobic amino acids includes GLY 584, GLY 590.The binding pockets are lined by the remaining amino acids namely ASP 525, ARG 586, ARG 526, SER 583, SER 589, CYS 527, THR 585, TYR 569. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. The options in each tab of the receptor Grid Generation Panel allows to define the receptor structure by excluding any co crystallized ligand that may be present, determine the position and size of the active site as it will be represented by receptor grids, and set up Glide constraints.

Ligand docking jobs cannot be performed until the receptor grids have been generated. Receptor grid generation requires a "prepared" structure: an all atom structure with appropriate bond orders and formal charges. The Receptor Grid Generation panel has three tabs which is used to specify settings for the receptor grid generation job:

- Receptor
- Site
- Constraints

Glide 5.9 (Glide-based Ligand Docking with Energetics)

Glide offers the full spectrum of speed and accuracy from high throughput virtual screening of millions of compounds to extremely accurate binding mode predictions, providing consistently high enrichment at every level. Glide searches for favourable interactions between one or more ligand molecules and a receptor molecule, usually a protein. Each ligand must be a single molecule, while the receptor may include more than one molecule, e.g., a protein and a cofactor. Glide can be run in rigid or flexible docking modes; the latter automatically generates conformations for each input ligand. The combination of position and orientation of a ligand relative to the receptor, along with its conformation in flexible docking, is referred to as a ligand pose. The ligand poses that Glide generated were passed through a series of hierarchial filters that evaluate ligands interaction with the receptor. The initial filter test the spatial fit of the ligand to the defined active site, and examines the complementary of the ligand-receptor interactions using a grid-based method patterened after empirical ChemScore function.

Poses that pass these initial screens enter the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS- AA non-bonded ligand-receptor interaction energy. Finally, the minimized poses are re-scored using Schrodinger's proprietary GlideScore scoring function. GlideScore is based on ChemScore, but includes a steric-clash term and adds buried polar terms devised by Schrodinger to penalize electrostatic mismatches, and has modifications to other terms.

GScore = 0.065*vdW + 0.130*Coul + Lipo + Hbond + Metal + BuryP + RotB + Site

Where

vdW	- Vander walls energy
Coul	- Coulomb energy
Lipo	- Favourable hydrophobic interaction
Hbond	- Hydrogen bonding term
BuryP	- Penalty for buried polar groups
Rot B	- Penalty for freezing rotatable bonds
Site	- Polar interaction in active site

Ligand Docking

Glide ligand docking jobs require a set of previously

calculated receptor glides and one or more ligand structures. The force field used for docking is the OPLS_2001 force field. Typically, Glide standard-precision docking is used to find probable good binders in a large set; the top-scoring 10% to 30% can then be investigated more intensively using Glide extra-precision (XP) docking or other methods available from Schrodinger.

Ligplot

It is a program for automatically plotting protein-ligand interactions. It automatically generates diagrams of protein-ligand interactions for a given PDB file. The interactions shown are those mediated by hydrogen bonds and by hydrophobic contacts. Hydrogen bonds are indicated by dashed lines between the atoms involved.

Results and Discussion

The histopathological analysis of rat models have shown reduced calcium oxalate depositions and other abnormalities in *C. magna* bark treatment that shows the utility in treatment of urolithiasis. It is mainly considered with the dissolution of existing stones and preventing recurrence of stones. The bioactive compound diterpenoid was isolated from the aqueous bark extract of *C. magna* and it was characterised by employing different chromatographic and spectral techniques. Performance of docking analysis with the compound 14-hydroxy-12-abietene-7-one from *C.magna* with Tamm–Horsfall protein exhibited the efficiency of interaction. The interaction brings an idea that the compound may inhibit calcium oxalate crystallization in urolithiatic condition (Figs. 1, 2).

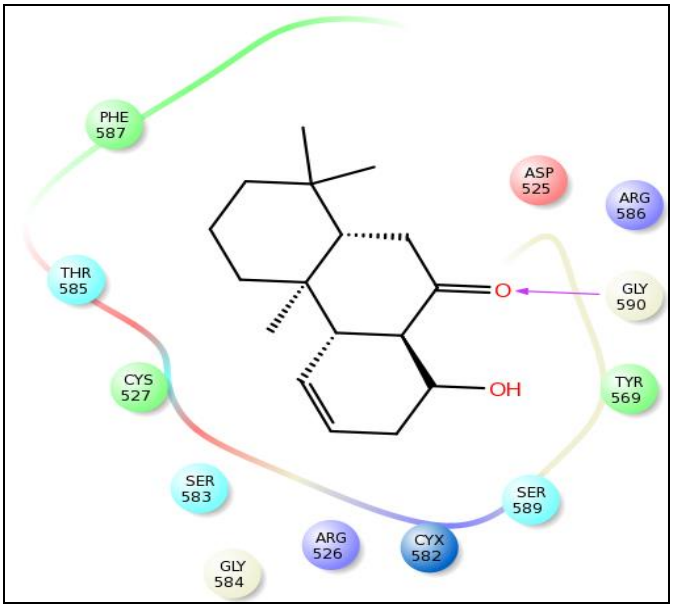

Fig 1: Ligplot view of the docked structure

Molecular Docking

Molecular docking is an efficient technique to predict the predominant binding modes of the ligand with the protein of known three-dimensional structure. Studies on binding modes are essential to elucidate key structural characteristics interaction and they provide helpful data for designing effective inhibitors.

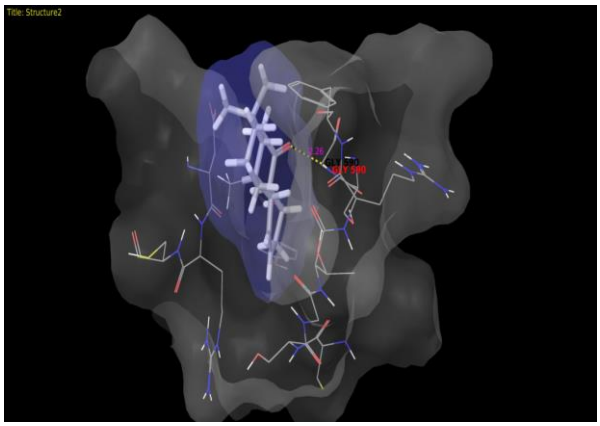

Fig 2: Molecular docking of Tamm – Horsfall protein with 14-hydroxy-12-abietene-7-one and its inhibition

Table 1: Hydrogen bond interactions and Glide score between Tamm Horsfall and the ligand.

Glide score	**-5.202**
Glide energy	38.622
Number of Hydrogen bonds	1
Interacted aminoacids	Glycine 590
Hydrogen bond length	2.26
Molecular weight	262.39

The effect of Tamm-Horsfall protein isolated from urine of healthy subjects on calcium oxalate precipitation was studied in model systems of precipitation by Benkovic *et al*., 1995. Tamm-Horsfall protein was found to inhibit the growth of calcium oxalate monohydrate crystals and stimulate their aggregation in the given experimental conditions. Both effects were enhanced by increase in the concentrations of Tamm-Horsfall protein and were most pronounced at the concentration of Tamm- Horsfall protein of 10 mg/1.

The role of Tamm-Horsfall protein and uromodulin in calcium oxalate crystallization *in vitro*. Results indicate a different effect of Tamm-Horsfall protein and uromodulin. This dual behaviour suggests different functions. Tamm-Horsfall protein may act on nucleation and inhibit crystal aggregation, while uromodulin may promote aggregation of calcium oxalate crystals. The *In silico* analysis performed by [16] isolated a triterpenoid compound namely 3-O-Acetyl-11-Keto-β-Boswellic acid from the root of *Rotula aquatica* Lour. The compound interacted with Tamm-Horsfall protein and showed a least glide score of -2.35.

Conclusion

The treatment of urolithiasis is mainly considered with the dissolution of existing stones and preventing recurrence of stones. In the present study a diterpenoid compound 14-hydroxy-12-abietene-7-one was obtained from aqueous bark extract of *C.magna*. It was characterized by different techniques like IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. Comparison of the obtained melting point of the isloated compound with the previously reported data for the diterpenoid led to the conclusion that the compound isolated

from aqueous root extract of *C.magna*. Further by docking analysis the compound 14-hydroxy-12-abietene-7-one interacted with Tamm–Horsfall protein. The interaction results exhibits the ability of the compound towards inhibition of calcium oxalate crystallization. The study encourages the utility of the compound for future drug discovery through advanced techniques. This can help the patients with urolithiatic difficulties without undergoing complex chemical based treatments. The herbal extracted compound can provide a healthy approach without any side effects as it is obtained from the nature and also reduced the burden on exploitation of herbs for treatments.

Financial support and sponsorship: Nil.

Conflict of interests: There are no conflicts of interest

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