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 herbas 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. *Crakeva 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, *Crakeva magna* Lour. (DC.), Antiurolithiatic activity and 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\textsuperscript{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 ascending 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\textsuperscript{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 well as promoter in crystal formation depending on the environmental conditions\textsuperscript{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\textsuperscript{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 with anolides with DNA binding site of NF-kB through docking analysis\textsuperscript{6}. Since ancient times Indian medicine “Ayurveda” recommends several medicinal plants for the successful treatment of urolithiasis\textsuperscript{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\textsuperscript{8}. On the other hand, traditional system of Indian medicine Ayurveda recommends \textit{Crateva magna} to be antilithiatic, but scientific data supporting this statement is still lacking. \textit{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 \textit{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 \textit{C. magna} leaf paste and the Oral consumption of leaf juice is used in the treatment of piles\textsuperscript{9}. The whole plant is used for the medical purpose such as diuretic, laxative, thonotriptic, antireumatic, antiperiodic, bitter tonic, rubificient and counterirritant\textsuperscript{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\textsuperscript{12}. Fruit juice, leaves and bark is useful to cure snakebite, infected wound and cuts. It increases appetite and controls other skin diseases\textsuperscript{14}. \textit{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 (\textit{a} and \textit{b}- amyrin, ceryl alcohol, lupeol, friedelin, betulinic acid, 4-taraxasterol, lupenone), flavonoids (rutin, catechin, quercetin) and alkaloids\textsuperscript{15,16}. The present paper deals with the utility of compound isolated from \textit{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.

**Lig Prep**
Lig Prep 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 sd for Maestro format. The simplest use of Lig Prep 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 Lig Prep 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 mod Baseauto model 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. 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 patterned after empirical Chem Score 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 Glide Score scoring function. Glide Score is based on Chem Score, but includes a steric-clash term and adds buried polar terms devised by Schrodinger to penalize electrostatic mismatches, and has modifications to other terms.

\[
G\text{Score} = 0.065 \times \text{vdW} + 0.130 \times \text{Coul} + \text{Lipo} + \text{Hbond} + \text{Metal} + \text{BuryP} + \text{RotB} + \text{Site}
\]

Where

- \text{vdW} - Vander walls energy
- \text{Coul} - Coulomb energy
- \text{Lipo} - Favourable hydrophobic interaction
- \text{Hbond} - Hydrogen bonding term
- \text{Bury P} - Penalty for buried polar groups
- \text{Rot B} - Penalty for freezing rotatable bonds
- \text{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-abiitene-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 urolithic condition (Figure No.1, 2).

**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.

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/l.

Carvalho *et al.* (2002) screened 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\textsuperscript{17} isolated a triterpenoid compound namely 3-O-Acetyl-11-Keto-β-Boswellic acid from the root of *Rotula* *aqua* *tica* Lour. The compound interacted with Tamm-Horsfall protein and showed a least glide score of -2.35.

<table>
<thead>
<tr>
<th>Table No.1: Hydrogen bond interactions and Glide score between Tamm Horsfall and the ligand</th>
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<tr>
<td>Glide score</td>
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<td>Glide energy</td>
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<td>Number of Hydrogen bonds</td>
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<td>Interacted aminoacids</td>
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<td>Hydrogen bond length</td>
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<td>Molecular weight</td>
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Figure No.1: Ligplot view of the docked structure

Figure No.2: Molecular docking of Tamm - Horsfall protein with 14-hydroxy-12-abietene-7-one and its inhibition
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-abietaene-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 isolated 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-abietaene-7-oneinteracted 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.

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