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Original Research Article

In-silico Investigation of Anti-urolithiatic activity of selected Medicinal Plants of Pakistan

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Abstract

One of the major ailments of very common occurrence amongst the people of Northern Pakistan is the diseases of urinary tract including kidney with the formation of calculi or stones in majority of the cases also known as urolithiasis. It is caused by missense mutations in Adenine phosphoribosyltransferase (APRT) enzyme, encoded by APRT gene. In this project, Ethnobotanical data was collected through informed consent semi-structured interviews. Nine plant species viz: *Bryophyllum calycinum, Centella asiatica, Tribulus terristris*, *Berberislycium, Solanum nigrum, Phylanthus nirruri, Trianthema portulacastrum, Aerva lanata and Achyranthes aspera* were identified to cure urolithiasis. The effective anti-urolitiatic chemical constituents of plants were downloaded and docked with APRT. Interactions of chemical constituents with APRT were analyzed. In each docked complex the common interacting amino acid residues were Threonine25, Proline85, Proline77, Leucine28, Leucine62, Isoleucine24, Leucine84, Histadine105, Alanine86 and phenylalanine75. Results showed that each chemical constituent best fit in the pocket of enzyme, interacted with each amino acid residue of mutated APRT enzyme's pocket and does not leave the complex that demonstrates its stability and soundness. On the basis of docking results it is suggested that all these chemical constituents of plant can be used to treat Urolithiasis.

Keywords: Abbottabad, APRT, Docking, Interactions, Kidney stone, Medicinal plants, Pakistan.

INTRODUCTION

The kidneys are one of the most vital parts of our body (Reilly, 2005).Kidney acts as a filter for blood, removes waste products from the body thus; help to regulate the levels of chemicals which are important for body functions. The urine drains from the kidney into the bladder through a narrow tube called the ureter. When the bladder fills and there is an urge to urinate, the bladder empties through the urethra, a much wider tube than the ureter. Urolithiasis (nephrolithiasis) or kidney stone is formation of urinary calculi at any level of urinary tract. Normally, urine contains chemicals that prevent or inhibit the crystals from urinary tract. These crystals remain tiny enough; they will travel through the urinary tract and pass out of the body in the urine without being noticed. When the stone sits in the kidney, it rarely causes problems, but when it falls into the ureter, it acts like a dam. Kidney continues to function and make urine, which backs up behind the stone, stretching the kidney. A kidney stone is actually a hard mass developed from crystals that separate from the urine and build up on the inner surfaces of the kidney.

An enzyme known as Adenine phosphoribosyl-transferase (APRT) that takes part in purine salvage pathway performs the catalysis of Adenosine monophosphate and 5-phosphoribosyl-lpyrophosphate. (APRTase) is an enzyme encoded by the *APRT* gene, found in humans on chromosome 16(Valaperta *et al.,* 2014).It is part of the Type I APRTase family and is involved in the nucleotide salvage pathway, which provides an alternative to nucleotide biosynthesis de novo in humans and most other animals In the absence of APRT, xanthine dehydrogenase (XDH) converts the

adenine into 2,8-dihydroxyadenine (2,8-DHA) that is highly insoluble in urine. Deficiency of APRT results in a disorder called 2,8-dihydroxyadenine (2,8-DHA) urolithiasis and formation of recurrent kidney stones (Sahota *et al.,* 1994; Furrow *et al.,* 2013). When APRTase has reduced or nonexistent activity, adenine accumulates from other pathways. It is degraded by xanthine dehydrogenase to 2,8-dihydroxyadenine (DHA). Although DHA is protein-bound in plasma, it has poor solubility in urine and gradually precipitates in kidney tubules, leading to the formation of kidney stones (urolithiasis). If left untreated, the condition can eventually produce kidney failure (Shi *et al.,* 2001).Natural products have been used in folk medicine for thousands of years. Drug lead screening has been an active area of research for many years. Due to the tedious and expensive nature of experimental screening procedures, computational compound screening has been pursued extensively in recent years. Receptors are macromolecules involved in chemical signaling between and within cells (Taha, 2012).

They may be located on the cell surface membrane or within the cytoplasm. Activated receptors directly or indirectly regulate cellular biochemical processes e.g. ion conductance, protein phosphorylation, DNA transcription, enzymatic activity. Molecules such as drugs, hormones and neurotransmitters which bind to a receptor are called ligands. A ligand may activate or inactivate a receptor; activation may either increase or decrease a particular cell function. A high throughput virtual screening by molecular docking can be used nowadays in screening millions of compounds rapidly, reliably and cost effectively. Bioinformatics and systems biology approaches are becoming increasingly important along with chemo informatics methods to study the therapeutic potential of medicinal plants. They are used to select targets for docking and to identify relationships between the revealed actions of phytochemical on targets and the known therapeutic effects of medicinal plants In the present study the some compounds from *Bergeniaciliata, Centella asiatica, Tribulusterristris, Berberis lycium, Solanum nigrum, Phylanthus nirruri, Trianthema protulacastrum, Aerva lanata* and *Achyranthusaspera* were *in silico* tools for their utilization in new drug discovery based on expanding the use of folk medicinal plants through the exploration of phytochemical diversity evaluated by *docking* with Adeninephosphoribosyltransferase (APRT) to find out the antiurolithic activity.

MATERIAL AND METHODS

Ethical approval

Ethical approval was taken from competent authority of Hazara University, Mansehra Pakistan and COMSATS Institute of Information Technology, Abbottabad-Pakistan before starting the field survey, while informed permission was obtained from all the participants prior to the administration of the questionnaire.

Ethnomedicinal data collection

Ethnomedicinaldata on antiurolithic plants was collected through informed consent semi-structured interviews during spring and summer 2014-2015 form 15 different sites of Lesser Himalayas. Approximately 95 informants (15-20 informants in each site) between that age of 25-75 years with a sound traditional knowledge of useful wild plants, mostly either native born or had been living in the region for more than 30 years, were interviewed. Questions addressed to the informants about medicinal uses were mainly focused on local name of plant, habitats, place of collection, season of collection, parts used, use categories, the manner of drug preparation and administration and diseases cured. Plants were mostly collected in flowering and fruiting conditions and confirmed by the local inhabitants to ensure that the proper plants have been collected. Plants were identified with the help of available literature (Parker, 1973; Ali and Nasir, 1977-2002; Ali and Qaisar, 1986; Polunin and Staintan, 1986; Nasir and Rafique, 1996). Specimens were dried, pressed, poisoned and mounted on herbarium sheets and deposited in the Herbarium of Botany Department Hazara University Mansehra Pakistan (HUP) for future studies.

Identification of plants constituents and APRT Gene

The effective chemical constituents of plants were identified through literature and downloaded from Pubchem database (Wang *et al.,* 2009). The mutated enzyme structure of APRT was downloaded from Research Collaboratory for structural bioinformatics protein database (RCSB Pdb) (Berman, 2008). The three dimensional (3D) structure of APRT is shown in figure 1

Interaction of Plants chemical constituents with APRT enzyme

All the downloaded chemical constituents of plants were docked with APRT enzyme with the help of online docking server one by one to analyze their interactions with amino acid residues of APRT, and to determine their stability as drug compounds. The docked results were analyzed in *Biovia* discovery studio software and interactions were identified with the help of LeView software.

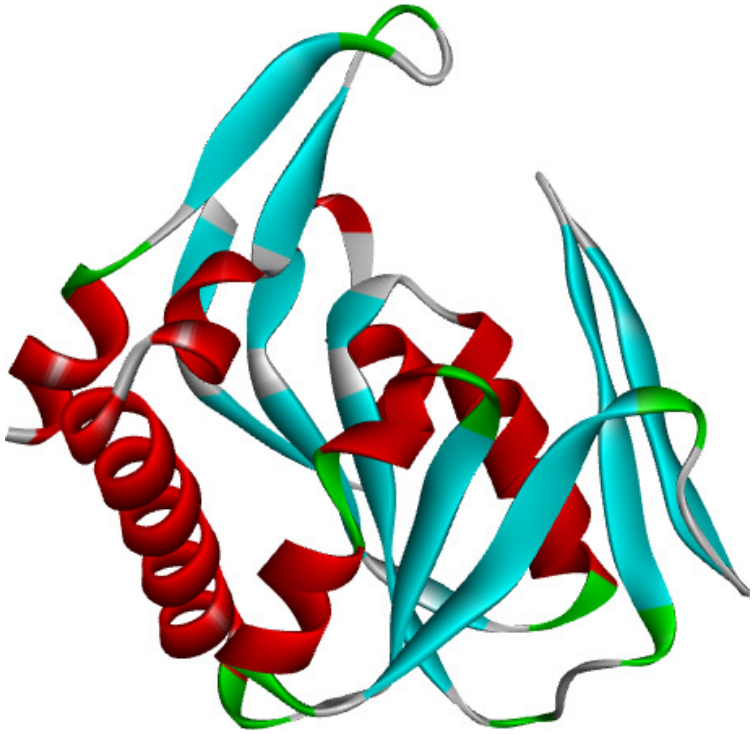

Figure 1. The three dimensional structure of mutated APRT enzyme, the alpha helices are represented by red color while the beta strands are represented by blue color, the grey color represents coil and green color represents turns between strands and coils.

Table 1. Chemical constituents of plants that were docked with APRT enzyme to determine interactions among them

Constituents	Antiurolithic plants species
Afzelchin, Bergenin, Bryophillin A	*Bryophyllum calycinum*
Asiatcoside, Centilloside	*Centella asiatica*
Kaempherol, quercetin	*Tribulus terristris*
Berberine	*Berberis lycium*
Pinoresinol, syrinagaresinol, medioresinol and scopoletin	*Solanum nigrum*
Corilagin	*Phylanthus nirruri*
Trianthemine	*Trianthema portulacastrum*
Campesterol	*Aerva lanata*
Achyranthine	*Achyranthes aspera*

RESULTS AND DISCUSSIONS

About 16 plants were identified as anti-urolitiatic, their effective chemical constituents were identified. Most of the chemical constituents are from leaf, root and stem part of plant. The effective chemical constituents of plants identified through literature are shown in table 1.

Docking allows every scientist to explore databases of large chemical compounds and design effective inhibitors to cure diseases in light of interaction score (Munir *et al.,* 2015). In each docked complex it was observed that every chemical constituent interact with similar amino acid residues of mutated APRT enzyme. The interactions among each complex are shown in figure 2 – 5. In case of structure-based drug designing, the molecular docking is the most well-known strategy which has been generally utilized as far back as the mid of 1980s. The molecular docking methodology can be utilized to display the association between a chemical compound and a protein, which permit us to characterize the conduct of chemical compounds in the binding site of target proteins and additionally to explain crucial biochemical procedures (Meng *et al.,* 2011).

Here our goal to perform docking of chemical constitu-

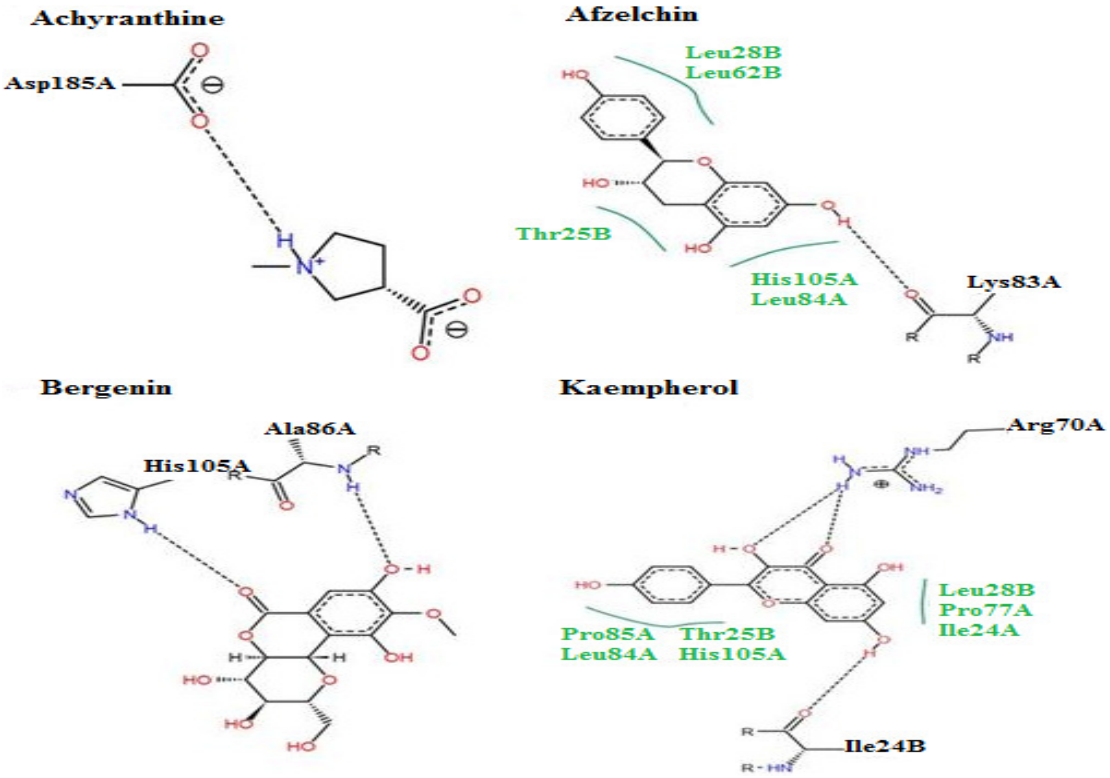

Figure 2. Interactions of compounds with APRT enzyme a) Achyrantine interactions with APRT, b) Afzelchin interactions with APRT, c) Bergenin interactions with APRT, d) Kaempherol interactions with APRT

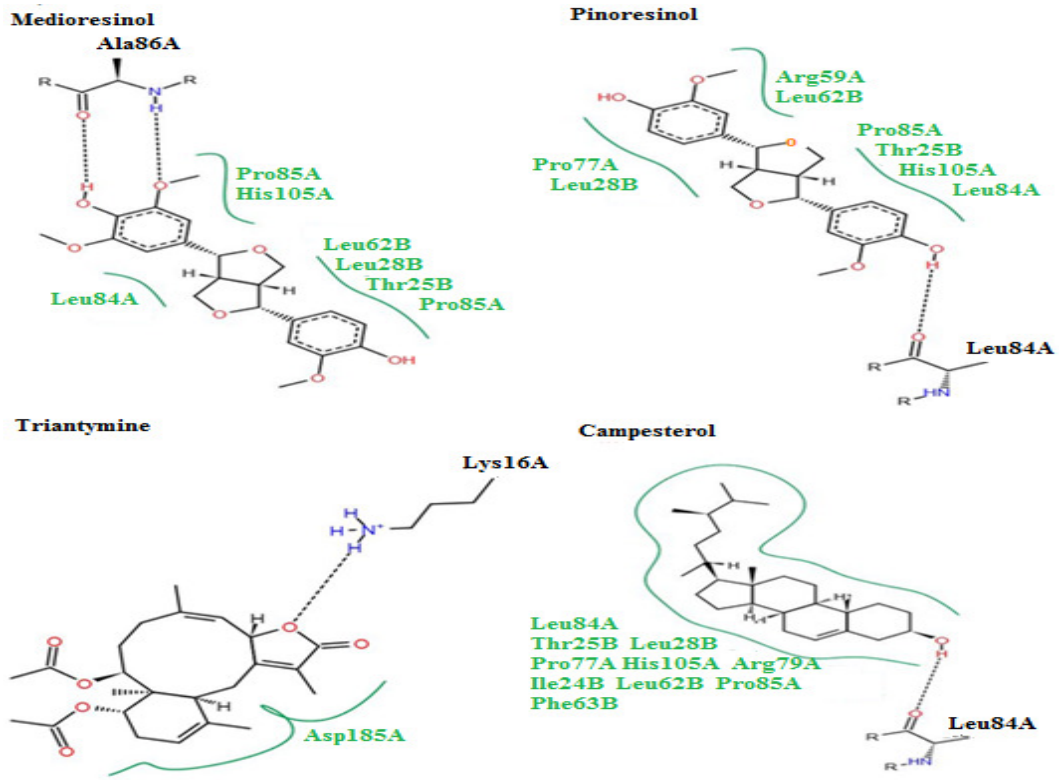

Figure 3. Interactions of compounds with APRT enzyme a) Medoresinol interactions with APRT, b) Pinoresionl interactions with APRT, c) Triantyine interactions with APRT, d) Campesterol interactions with APRT

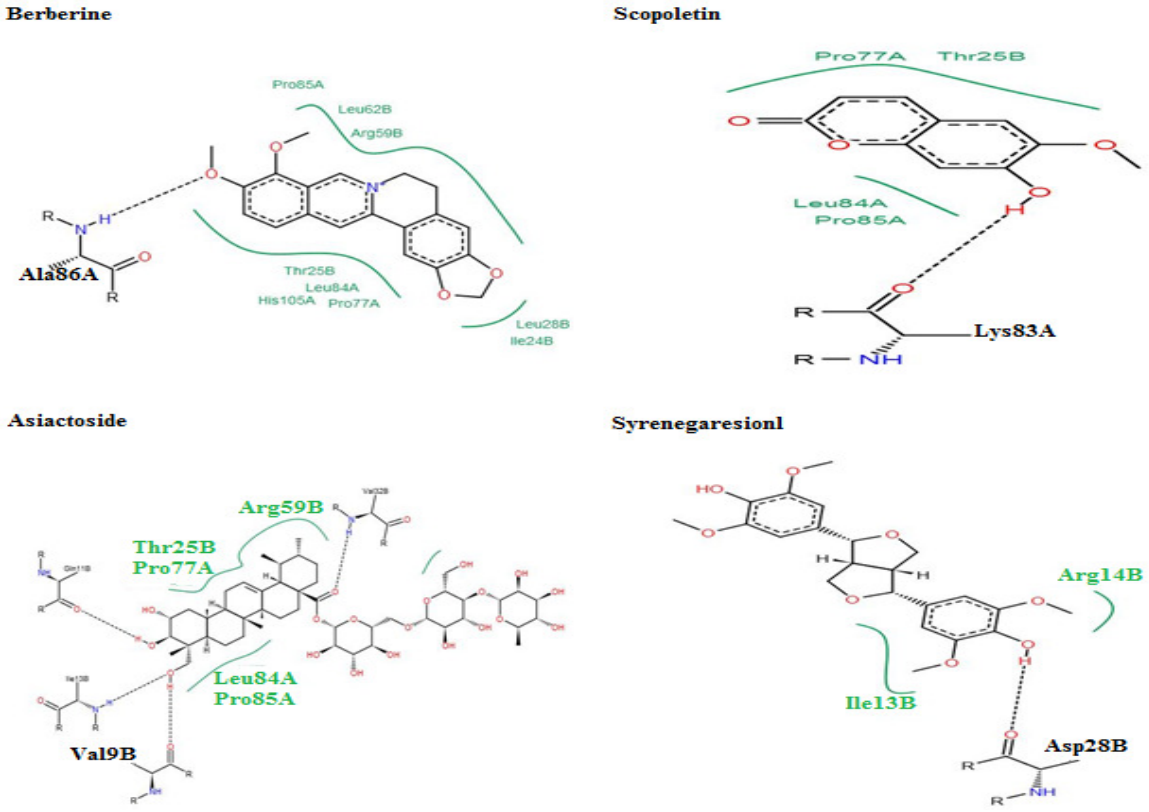

Figure 4. Interactions of compounds with APRT enzyme a) Berberine interactions with APRT, b) Scopoletin interactions with APRT, c) Asiactoside interactions with APRT, d) Syrenegaresinol interactions with APRT

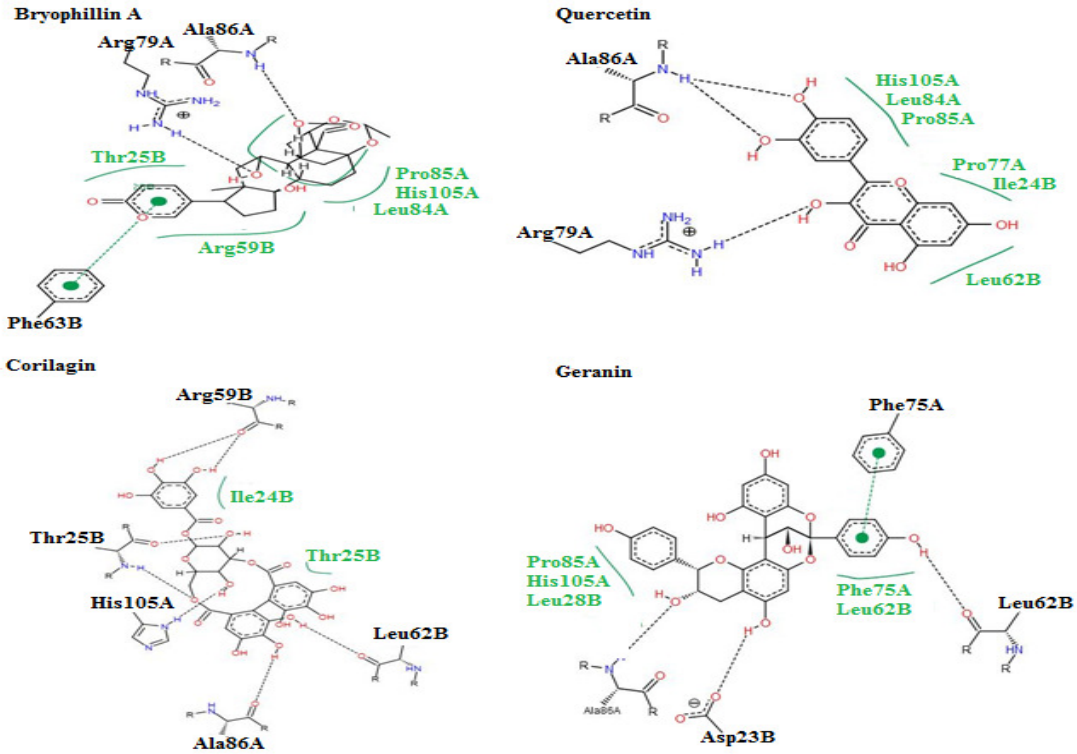

Figure 5. Interactions of compounds with APRT enzyme a) Bryophillin A interactions with APRT, b) Quercetin interactions with APRT, c) Corilagin interactions with APRT, d) Geranin interactions with APRT

ents with APRT was to predict the binding interactions of constituents with APRT. The more the interactions produced by the chemical constituents more will be stable to use in the treatment of urolithiasis. (Figure 3)

When Ligand molecules are docked with proteins several atoms of ligand compounds interact with the amino acid residues of mutated pocket and make several bonds, the inhibitors of high activity have suchcharacteristics that restrainthe protein molecule (Chen, 2012; Chouhan *et al.,* 2014)

In each docked complex it was observed that chemical constituents represented a lot of interactions with the amino acid residues of mutated pocket. Though, the molecular docking is a crucial tool in structural molecular biology and computer-aided drug design.The setting up of the input structures for the docking and analyzing the results of docking is just as important as the docking itself (Lengauer and Rarry 1996; Hartenfeller, 2004). (Figure 5)

In each docked complex the common interacting amino acid residues were Threonine25, Proline85, Proline77, Leucine28, Leucine62, Isoleucine24, Leucine84, Histadine105, Alanine86 and phenylalanine75. Results showed that each chemical constituent best fit in the pocket of enzyme, interacted with each amino acid residue of mutated APRT enzyme's pocket and doesnot leave the complex that demonstrates its stability and soundness. On the basis of docking results it is suggested that all these chemical constituents of plant can be used to treat Urolithiasis.

CONCLUSION

There are 16 species used to expel kidney stones. Chemical constituents of plants were obtained through literature and downloaded. The chemical structures were docked with mutated APRT enzyme; Urolithiasis causing agent, In each docked complex the common interacting amino acid residues were Threonine25, Proline85, Proline77, Leucine28, Leucine62, Isoleucine24, Leucine84, Histadine105, Alanine86 and phenylalanine75. On the basis of docking results it is suggested that all these chemical constituents of plant can be used to treat Urolithiasis. In future this work can be further used as a part of clinical trials to check its appropriateness and suitability.

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We are thankful to the local communities of District Abbottabad who contributed their knowledge on *Antiurolithiaticethnomedicinal plants.*

Conflict of Interests

None of the author has any challenging conflict of interest. This Research work is unique and has not been submitted in any journal yet.

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