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IN-SILICO STUDY OF ANTI-UROLITHIATIC ACTIVITY FROM NIGELLA SATIVA AGAINST UROLITHIATIC TARGETS

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Abstract:

Black cumin (Nigella sativa) traditionally known as Antiurolithiatic legume in India, a representation of the family Ranunculaceae, is widely available. It is used as treat bronchitis, diarrhoea, rheumatism, asthma and skin disorder, digestive problems, tight parasitic infection, increase milk production in nursing mother, stops vomiting. Black cumin can be used as a comprehensive treatment in case of urolithiasis. Till date the molecular mechanism of Antiurolithiatic activity of black cumin is not clearly known. From the protein data bank the crystals structure of Adenine phosphoriboxyl transferase (PDB ID: 1L1Q), Glycolate oxidase (PDB ID: 2YVS), Oxalate oxidase (PDB ID: 2ETE) were acquired. Molecular docking of active component (Rutin, Quercine, Kaempferol) of Nigella sativa with Urolithiatic including enzyme shown significal reduction and change in preparation of urolithiatic activity. This investigation proved that Nigella sativa has potential to cure kidney stone. This present investigation will be important information in the field of urolithiatic. This is first hand report on in-silico analysis of antiurolithiatic activity of Nigella sativa. The outcome of this research have tremendous scope asindividual insights and including contribution to research inputs for researchers working on the field of in-vivo and in-vitro studies.

Keywords: *Nigella sativa, Adenine phosphoriboxyl transferase, Glycolate oxidase, Oxalate oxidase*

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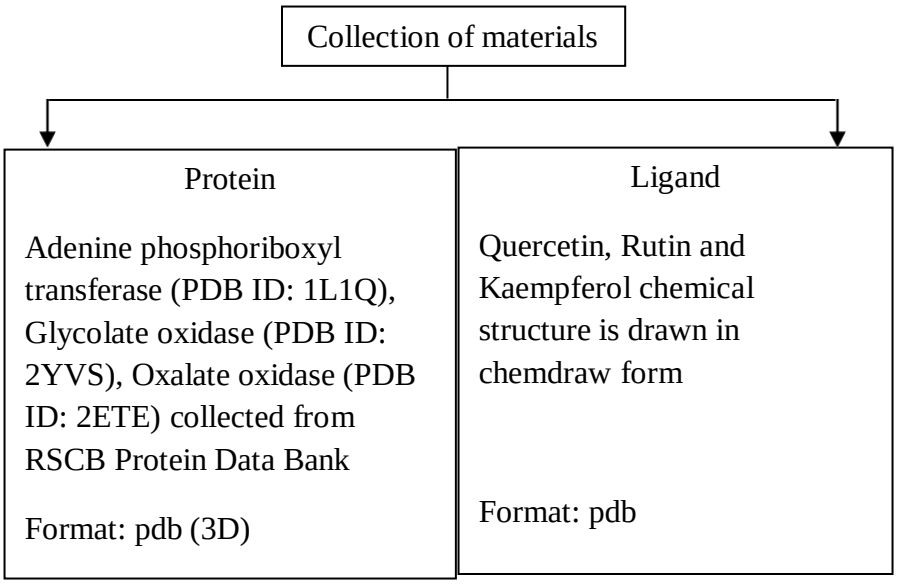
INTRODUCTION:

In the total world population approximately 85% use the Herbal or traditional medicine in health related problems. In the recent years therefore, high development in research area which has been focused on evaluation of herbal medicines from plants scientifically. This eraof research on traditional drugs has created the belief of safe and no side effects motivated humans to return to natural remedies when compared to synthetic drugs which have lots of side effects [1] Urolithiasisis a process of stone forming in the kidney, bladder, and/or Urethra(urinary tract) [2]. In urinary system, urolithiasis (greek-ouron, "urine" and lithos, "stone") is the condition where urinary stones are formed (or) located. The Urinary stones are formed, dense particles that produced; it may block the urine flow results in agonizing pain [3]. Patient with urinary stones with continuous medical care have common recurrent stone formation. Some synthetic drugs itself results in urinary stones [4]. There are various types of stones present in urinary stones with composition of calcium, phosphate, sodium, magnesium etc.80-90% of calcium stones occurs in most of cases followed by structure stones 5-10% & least with 1% of cystine stone are seen [5] *Nigella sativa* commonly known as black cumin belongs to *Ranaculacar* family which is widely distributed in Syria, Lebanon, Israel, Bangladesh, Turkey. In India it is particularly abundant Punjab, Bihar, Himachel Pradesh, Bengal, Assam and Maharashtra. It is used for treat bronchitis, diarrhoea, rheumatism, asthma & skin disorders digestive problems [6] [7]. To assess the effect *Nigella sativa* against urolithiasis, molecular docking is one of major computer assisted drug design for determining the affinity of newly discovered drugs with the targeted enzymes that is ligand - protein docking. By review of literature, the structure of ligands can be docked with protein interact in pathway of urolithiasis [8]. The aim of the current study was to investigate the *in-silico* activity of *Nigella sativa*.

In-silico docking studies:



The each ligand structure was docked with protein separately using PyRx and the energy bindingvalues were reported.

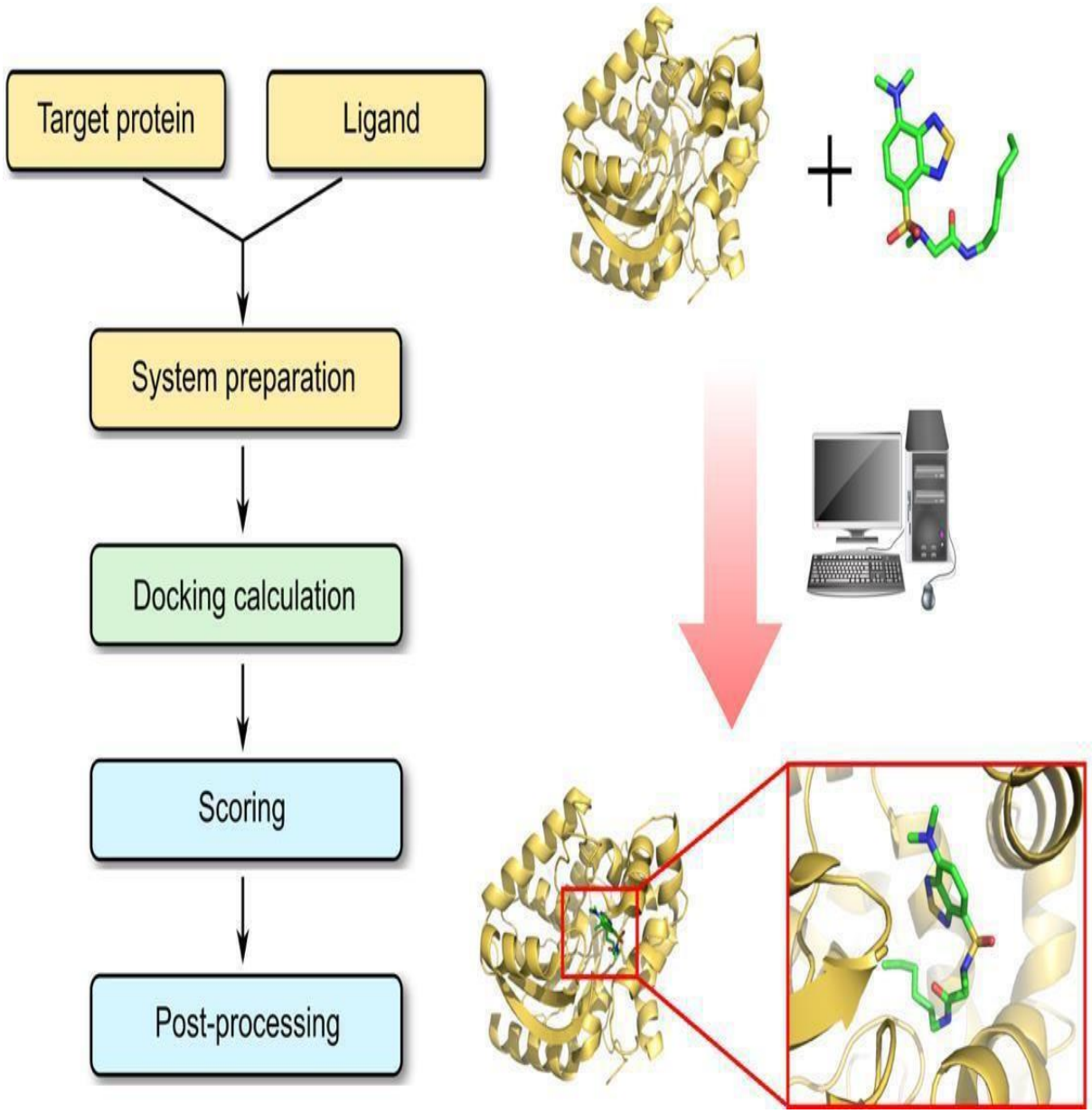


Fig 1: Representation of protein - ligand docking

The compound chosen for the docking studies is based upon the review of literature and based upon the activity of compounds the ligands were selected with this chemical structure and those were used to bind with targets to determine the binding affinity for the docking studies [8]. Identified compounds were studied by using docking software (PyRx). The protein sequence of Adenine phosphoriboxyl transferae, Glycolate oxidase, Oxalate oxidase with three molecules Quercetin, Rutin and Kaempferol [9].

Plant profile:

Botanical name	Nigella sativa
Synonyms	Nigella
Family	Ranunculaceae
English name	Nutmeg flower
Phylum	Magnoliophyte
Class	Magnoliopsida
Order	Ranunculales
Species	Nigella sativa

Fig 2: ***Nigella Sativa***

MATERIALS AND METHODS:

Materials:

For our present study we used biological databases like PDB (Protein Data Bank), PubChem and software's like PyRx, BIOVIA Discovery studio visualize 2.0.

PDB (Protein Data Bank):

The PDB (Protein Data Bank), founded in Brookhaven National Labs (BNL) in 1971, is the only global repository for structural information on biological macromolecules.

PubChem:

PubChem is a public database containing details on chemical substances and their biological functions (https://pubchem.ncbi.nlm.nih.gov).

Since its debut in 2004 as a part of the Molecular Libraries Roadmap Initiatives of the US National Institutes of Health (NIH), Pub Chem has quickly developed into a significant source of chemical information that supports scientific communities in a variety of fields, including cheminformatics, chemical biology, medicinal chemistry, and drug discovery.

Over the past 11 years, PubChem has developed into a substantial infrastructure that provides the scientific research community with access to

chemical information. Substance, Compound, and Bio Assay are three interconnected databases that make up PubChem.

Individual data contributors to PubChem have contributed chemical data to the Substance database, and the Compound database has extracted specific chemical structures from the Substance database. The Bio Assay database contains information on the biological activity of chemical compounds that have been examined in assay tests.

The PubChem Substance and Compound databases are described in general terms in this document, along with their data sources, contents, organization, standardization of chemical structures, web-based interfaces for text and non-text searches, and programmatic access.

Also, it provides a brief explanation of PubChem3D, a resource created from theoretically accurate three-dimensional models of molecules in PubChem, as well as PubChem RDF, a formatted version of PubChem data for use in data sharing, analysis, and integration with data from other databases.

PyRx:
PyRx is virtual screening software for computational drug discovery that can be used to screen libraries of compounds against potential drug targets. PyRx enables medicinal chemists to run virtual screening from any platform and helps users in every step of this process, from data preparation to job submission and analysis of the results. While it is that there are no magical buttons that would help to discover new drugs, PyRx wizard feature easy to use user interface and chemical spreads heat like functionality that makes it valuable tools for Rational Drug Design.

PyRx app will be installed under application.

BIOVIA Discovery studio visualize 2.0:
Built on BIOVIA Pipeline Pilot, BIOVIA Discovery Studio is a vast collection of proven science applications. The programme offers a special combination of open, scalable, collaborative research tools created for the needs of contemporary life sciences discovery research.

METHODOLOGY:

Docking procedure:

Step1: Preparation of receptor protein:
A 3D crystallographic structure of protein Adenine phosphoriboxyl transferase (PDB ID: 1L1Q), Glycolate oxidase (PDB ID: 2YVS), Oxalate oxidase (PDB ID: 2ETE) was obtained from protein data bank (PDB). The 3D structure of protein was retrieved. Read molecule from file.

- Molegro Molecular Viewer → Import file → Export molecule.
- Protein is only applied → Export → Save as pdb file.
- Save as prepared protein (PDB form).

Step2: Preparation of ligand:
Quercetin, Rutin, Kampferol is taken as ligand molecule. They are downloaded from pubchem and then converted into MDL MOL format.

- Molegro Molecular Viewer → Import → File → Export molecule.
- Save as (MDL MOL form)

Step3: Docking:

PyRx:

- File → Load molecule → Select the protein structure.
- Right click on protein → "AutoDock" → Make macromolecule.
- File → Load molecule → Select the ligand.
- Right click on ligand → "AutoDock" → Make ligand.
- Select vina wizard → Start → Select the protein and ligand.
- Click forward → Adjust the grid box → again click forward.
- It will start docking and will display the processing.
- After docking is finished, their binding affinities are displayed.

Finally 2D interactions are predicted from BIOVIA discovery studio 2.0.

RESULT AND DISCUSSION:

Docking result:
The 3D structure of Adenine phosphoriboxyl transferase (PDB ID: 1L1Q), Glycolate oxidase (PDB ID: 2YVS), Oxalate oxidase (PDB ID: 2ETE) with a resolution of 1.75Å, 2.0Å and 1.85Å respectively is obtained from the protein data bank (RCSB-PDB). Protein was prepared by removing the ligand groups, nucleic acid groups, heteroatom's, water molecules and then adding polar hydrogen's. Prepared protein was saved in .pdb (protein data bank) format. The structure of the ligand was drawn using ACD/ChemSketch FREEWARE and saved in .mol format. The energy minimization of the ligands was performed using PyRx. Ink and converted to .pdbqt format. PyRx is used to estimate the affinities and

interactions of Targets and Ligands.

TABLE 1: Docking result.

PHYTOCHEMICAL CONSTITUENT	ADENINE PHOSPHORIBOXYL TRANSFERASE (PDB ID: 1L1Q)	OXALATE OXIDASE (PDB ID: 2ETE)	GLYCOLATE OXIDASE (PDB ID: 2YVS)
Quercetin	-7.2 kcal/mol	-7.7 kcal/mol	-8.8 kcal/mol
Rutin	-7.5 kcal/mol	-7.2 kcal/mol	-10.3 kcal/mol
Kaempferol 3-glucosyl galactosyl glucoside	-7.8 kcal/mol	-6.7 kcal/mol	-9.4 kcal/mol

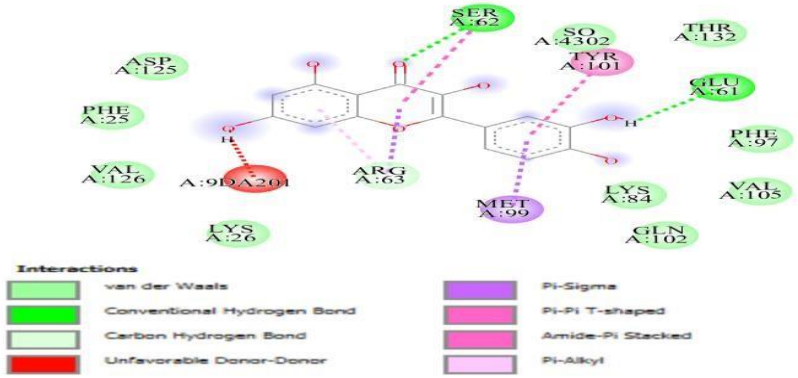


Fig 3: 2D Structure of Quercetin ligand (CID: 5280343) with Adenine phosphoriboxyltransferase protein (PDB id: 1L1Q).

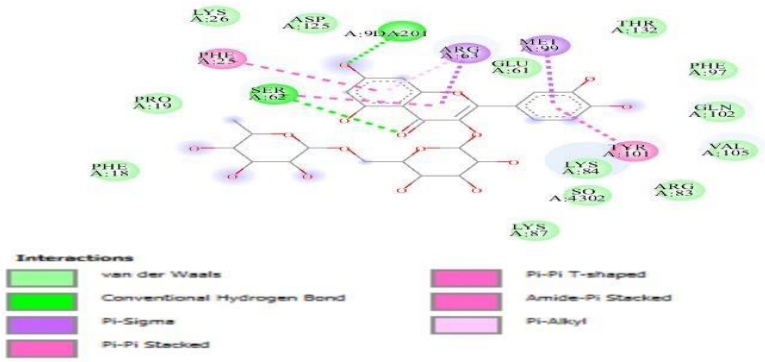


Fig 4: 2D Structure of Rutin ligand (CID: 5280805) with Adenine phosphoriboxyltransferase protein (PDB id: 1L1Q).

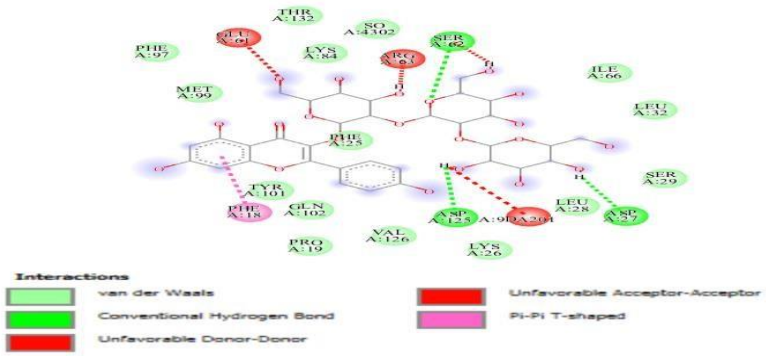


Fig 5: 2D Structure of Kaempferol 3-glucosyl galactosyl glucoside ligand (CID: 44258988)with Adenine phosphoriboxyl transferase protein (PDB id: 1L1Q).

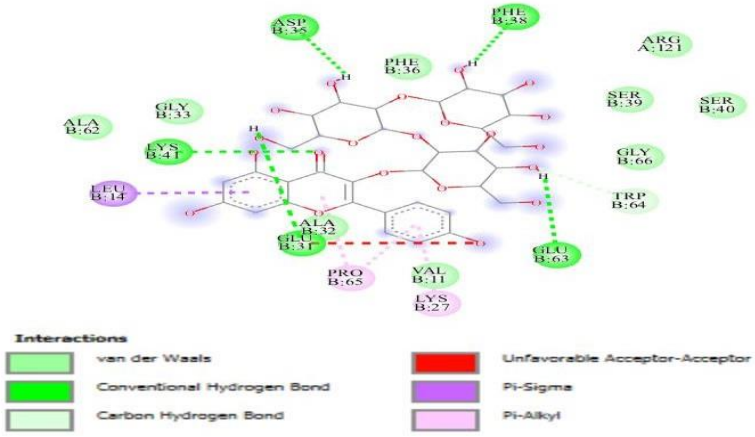


Fig 6: 2D Structure of Kaempferol 3-glucosyl galactosyl glucoside ligand (CID: 44258988)with Oxalate oxidase (PDB id: 2ETE).

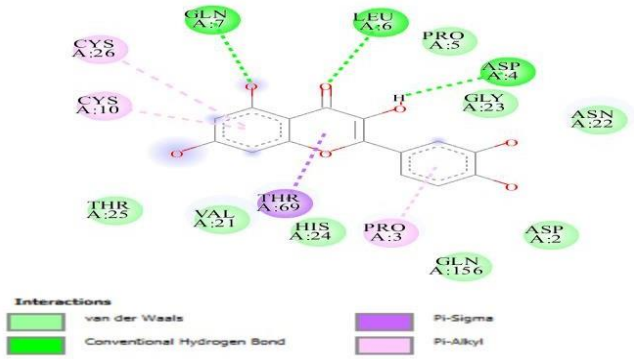


Fig 7: 2D Structure of Quercetin ligand (CID: 5280343) with Oxalate oxidase (PDB id:2ETE).

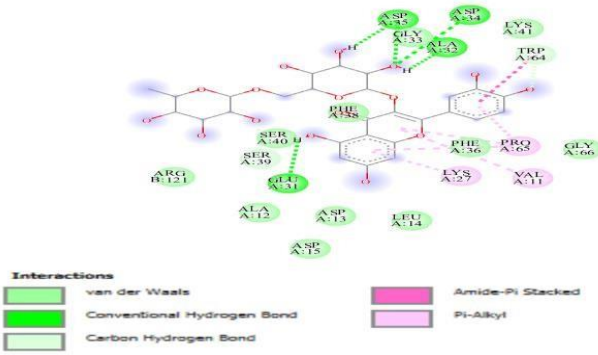


Fig 8: 2D Structure of Rutin ligand (CID: 5280805) with Oxalate oxidase (PDB id: 2ETE).

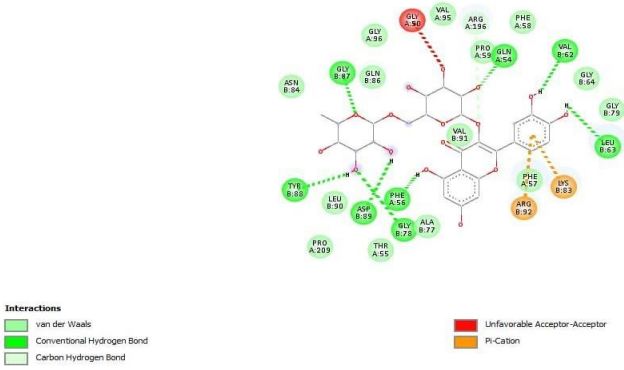


Fig 9: 2D Structure of Rutin ligand (CID: 5280805) with Glycolate oxidase (PDB id:2YVS).

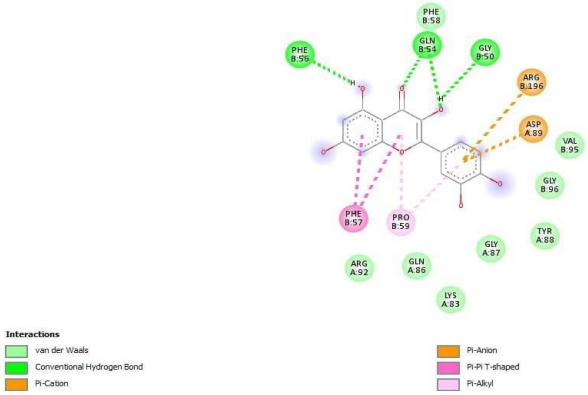


Fig 10: 2D Structure of Quercetin ligand (CID: 5280343) with Glycolate oxidase(PDB id: 2YVS).

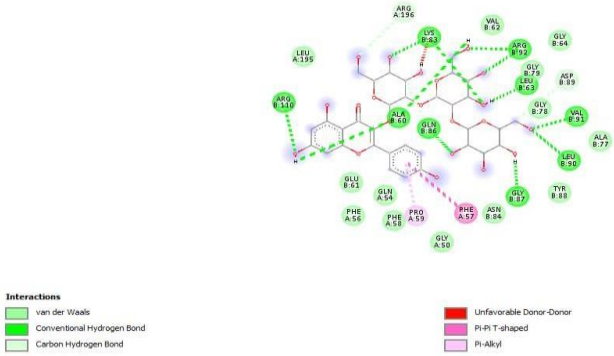


Fig 11: 2D Structure of Kaempferol 3-glucosyl galactosyl glucoside ligand (CID: 44258988)with Glycolate oxidase (PDB id: 2YVS).

DISCUSSION:

According to Logeshwari (2020) molecular docking study and phytochemical analysis of hybanthus ennaespermus exhibits that high quantity of flavonoids, which could effectively inhibit the formation of calcium oxalate & other urinary stones. Flavonoids are rich in rutin and quercetin, rutin prevents stone formation and inhibits calcium oxalate in urolithiasis whereas quercetin possess hypo-uricemic, exhibits inhibitory effects on deposition of calcium oxalate crystals etc..

According to Merfot et.al.., (1997) flavonoids also include kampferol 3-glucosyl galactosyl glucoside were it is one of the active constituents which was used in our docking studies.

According to Jason tom Abraham , (2022) this study shows in silico molecular docking approach against enzymes using PyRx and bio via visualizer studio 2.0 software**.**

In this present study, PyRx software was used to dock proteins adenine phosphoriboxyl transferase (PDB: 1L1Q), glycolate oxidase (PDB: 2YVS), and oxalate oxidase (PDB: 2ETE) with rutin, quercetin and kampferol.

The binding scores of ligands with Urolithiatic proteins were -7.2 kcal/mol ,-7.7 kcal/mol,-8.8 kcal/mol, -7.5 kcal/mol, -7.2 kcal/mol, -10.3 kcal/mol, -7.8 kcal/mol, -6.7 kcal/mol, -9.4 kcal/mol.

Therefore least negative docking score indicates stronger binding affinity between protein and ligand, the binding energy of kampferol with oxalate oxidase was recorded -6.7 kcal/mol .

CONCLUSION:

The result obtained from *in-silico* studies showed an effective inference on utilization of the compounds showed higher binding energy and

affinity towards enzymes and used as treating aids for urolithiasis.

In this present study, active constituents (Rutin, Querciten, and Kaempferol) were docked successfully with target protein adenine phosphoriboxyl transferase, glycolate oxidase, and oxalate oxidase. The binding energy of kaempferol was record -6.7 kcal/mol. Therefore, it can be potential medication for Anti-urolithiatic activity because the least negative binding score indicates stronger binding affinity between protein and ligand.

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