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Potential anti-osteoporosis drugs from Phyto constituents: Virtual screening approach

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Abstract
Cissus quadrangularis L. is a succulent plant of family Vitaceae commonly found in tropical and subtropical region. It is a fleshy, cactus like widely used as a common food item in India. The plant is outlined in the ancient Ayurvedic literature as a magical tonic and analgesic, with specific bone fracture healing properties. The plant is believed to be useful in various folk claims for cure of various diseases, efforts have been made by researchers to verify the efficacy of the plant through scientific biological screening. Even though the plant as a whole has been used for osteoporosis the active principle responsible is yet to be identified. The present study deals with the *in silico* approach to identify the active principle which fight against osteoporosis.

Keywords: Potential anti-osteoporosis drugs, Phyto constituents, virtual screening approach

Introduction
Cissus quadrangularis (Linn) is a common perennial climber, which belongs to the family Vitacea. It is also known as *Vitis quadrangularis* Wall. This plant is commonly known as Asthisamhari. All parts of the plant is used in Sidha, Unani and Ayurveda medical practices. It is not only used as a medicinal plant but also serve in healthy diet. This makes Cissus quadrangularis a magical plant [1-10]. Osteoporosis, a silent epidemic, has become a major health hazard in the recent years afflicting over 2000million people worldwide. It is a chronic, progressive condition associated with micro-architectural deterioration of bone tissue that results in low bone mass. The leading cause of osteoporosis is the lack of certain hormones, particularly estrogen in women and androgen in men as well as imbalance in the activities of osteoblasts and osteoclasts cells lead to osteoporosis in post menopausal women [11]. In osteoporosis the bones begin to deteriorate due to calcium deficiency. In menopause, the decrease in hormones affects the body's ability to maintain calcium levels resulting in an increased loss of minerals from the bone [12-13].

Cathepsin K
Cathepsin K (Cat K) is a lysosomal cysteine protease [14]. Cat K has high collagenase activity. Its activity is preffered at the acidic pH that is required to dissolve the calcium hydroxyapatite component of bone. Emerging evidences suggest that Cat K is the primary enzyme involved in osteoclastic bone resorption and have made it an important target for the treatment of osteoporosis. For our studies, X-ray crystal structure of Cathepsin K (Figure 1) (PDB: 1TU6) was taken, having resolution of 1.76 A°
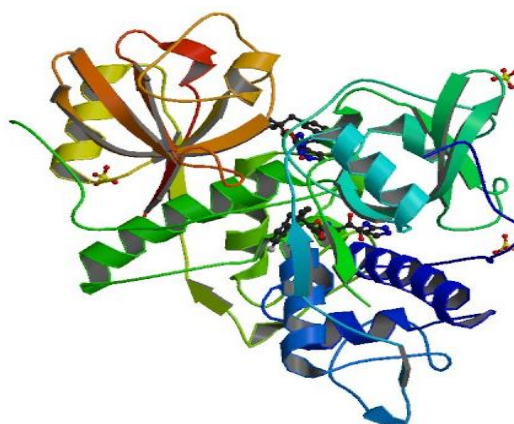
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Fig 1

Active site Identification

Active site of BRCA1, COX-2 and Cat K were identified using CASTp server, a new program, which can be used for locating protein pockets and cavities. It is based on precise computational geometry methods, including alpha shape and discrete flow theory.

Softwares Required

Python 2.7 - language was downloaded from www.python.com, Cygwin and Python 2.5 were also downloaded from www.cygwin.com, Molecular graphics laboratory (MGL) tools and Auto Dock 4.2 was downloaded from www.scripps.edu, Discovery studio visualizer 2.5.5 was downloaded from www.accelerys.com, Molecular orbital package (MOPAC), Chem Sketch was downloaded from www.acdlabs.com.

Docking study was performed for the compounds obtained after separation through column chromatography followed by spectral analysis. The structures of the ligands were drawn using Chem Sketch 12.01 in order to obtain the MOL format. The MOL files were converted to PDB format using Open Babel tool. The receptor taken for docking were chosen from RSCB. PDB structure of the receptor was obtained through Protein Data Bank (PDB) database. The bacterial life cycle determines the receptor. In the beginning, heteroatoms were removed from the receptor. Then hydrogen bonds and charges were added to the receptor using Autodock 4.2. The docking was carried out by increasing the grid box size so that the whole protein was accommodated. PDBQT files of target and ligand, gpf and dpf files were retrieved using Autodock 4.2. Molecular docking was done using software known as Cygwin. The final docked complex are formed by cygwin. Results with 10 different conformations were obtained. The conformation with a minimum binding energy was selected. The docked complex was analyzed with Discovery studio visualizer. The ligand binding patterns were very clear by this tool [15-19].

Results and Discussion

Lipinski rule of five analysis

The drug-likeness is necessary to be evaluated at the primary stage as this reduces the chances of selecting the false positive results. Various basic physicochemical properties such as log P, H-bond acceptor, H-bond donor, molecular weight ad molar refractivity were calculated to evaluate a molecule to act as drug. The value of logP should be ≤ 5; this is the distribution coefficient important for finding the solubility of the drug that is lipophilicity. Lipinski rule stick on molecular weight of the compound that must be below 500Da as most of the drugs are small molecules. The physical properties of the ligand molecules and their drug likeness score is tabulated in Table-1.

Table-1

Ligand/ Drug	M. Wt	HBA < than 10	HBD < than 5	LogP< than 5	LogS moles/L	PSA	Drug likeness score
VitC	176.03	6	4	-2.40	0.44	85.73A^2	0.84
Phytol	296.31	1	1	8.28	-5.27	17.17A^2	-0.87
Quercetin	302.04	7	5	2.11	-3.87	102.61A^2	0.93
Luteolin	286.05	6	4	2.68	-4.07	89.05A^2	0.86

In the case of molecules mentioned above Phytol does not obey the Lipinski rule of five in which Log P values exceeds the limit. And hence it cannot be considered as an active drug for further docking studies.

Docking

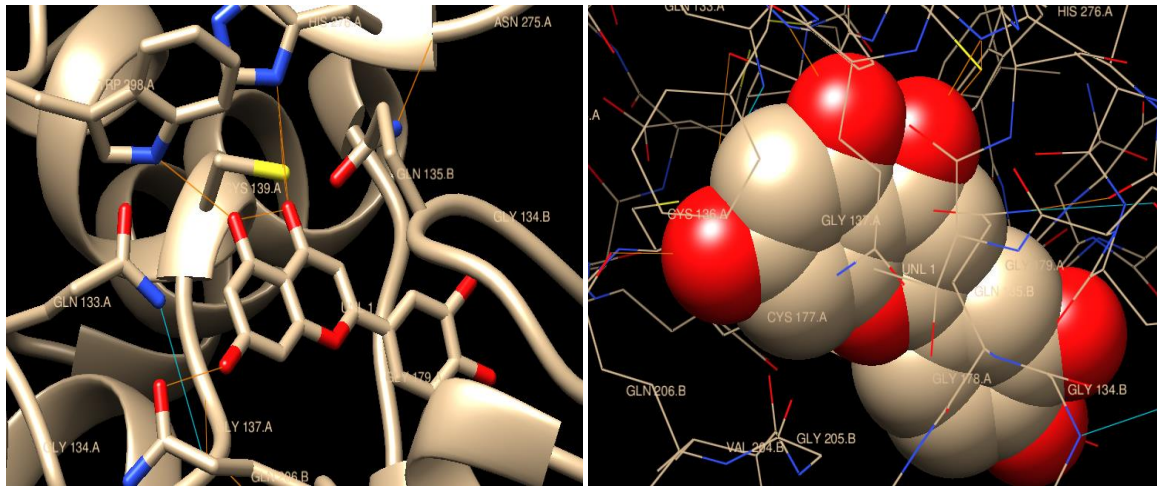Docking study explained that the ligands bind to the receptor with a good binding energy. The hydrogen bonds formed refers to the strength of binding between the ligand and the receptor. Table 2 shows the binding energy of ligand with the receptor in Kcal/mol, number of hydrogen bonds formed between ligand and the amino acid. Compounds exhibiting higher negative binding energy contribute to the maximum activity. The docking of four among the isolated compounds Ascorbic acid, Luteolin, Quercetin and Phytol is given in the Table-2

Table-2

Compound	Binding energy(KJ/mol)
Luteolin vs Cat K	-7.73
Ascorbic Acid vs Cat K	-5.49
Quercetin vs Cat K	-6.36

It is evident from Table 1 that Ascorbic acid has a lower Log P value than others which indicates that Ascorbic acid has higher hydrophobic activity than others. The best ligand for docking studies is determined by evaluating the interaction energy for the specific ligand-receptor complex under study. It recognize from the above result that Luteolin is a more promising drug against Osteoporosis because of its lower interaction energy (-7.94 KJ). However, none of the ligands has violated Lipinski rule. The binding energy values uphold Luteolin a natural remedy for Osteoporosis.

Ligand –Enzyme interactions are shown in the figures. It is clear from the images the ligand binds well with the enzymes at their active site with lower (more negative) binding energy. More negative binding energy scores supports strong binding between enzymes and ligands. The hydrogen bonds shown by yellow lines. The blue lines also are significant hydrogen bonds. The number of H-interactions increases the activity of the ligand increases as well.

Cat K -LUTEOLIN
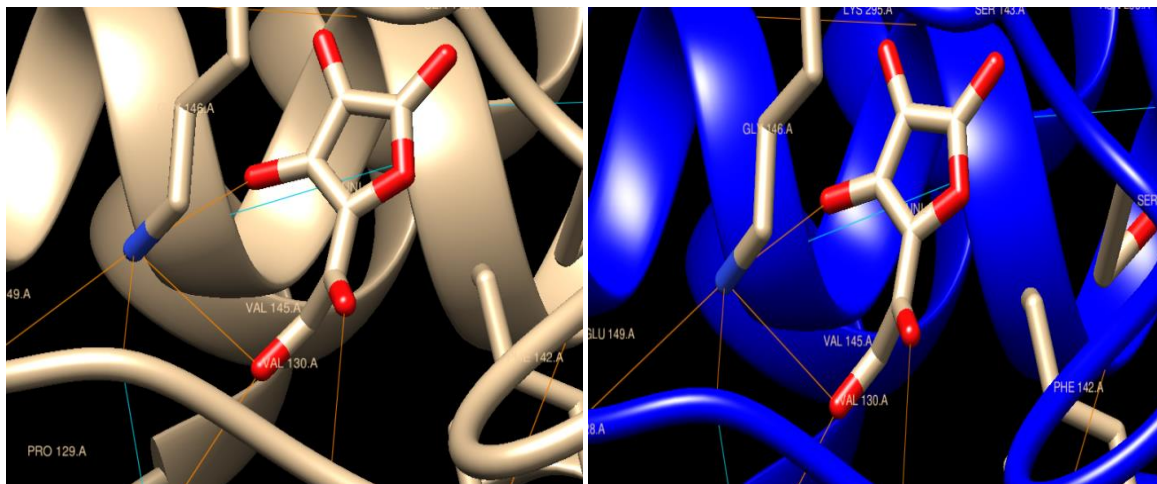
Cat K -ASCORBIC ACID
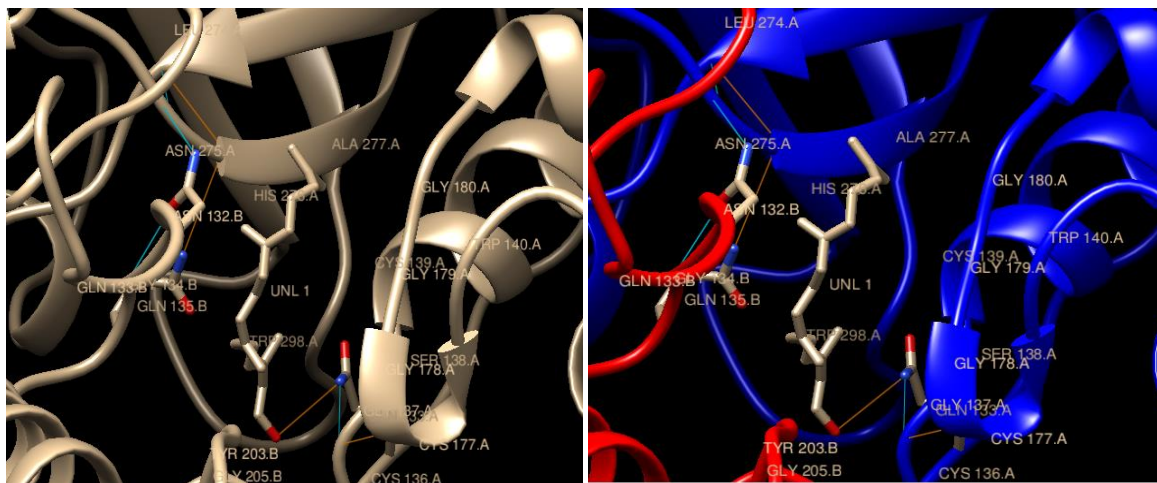
Cat K-QUERCETIN

Conclusion

The molecular docking helps in drug design and provide a good understanding of the mechanism of interaction of the drug and target protein. From this study, we conclude that Luteolin and Quercetin can be subjected to further analysis and preceding preclinical trials. Through docking study, high negative binding energy was obtained on binding of the ligand with the receptor. *In vivo* studies can also be performed to evaluate the efficacy of the plant for treating diseases in animals and human beings. Docking study explained that the ligands bind to the receptor with a good binding energy. The hydrogen bonds formed refers to the strength of binding between the ligand and the receptor. Compounds exhibiting higher negative binding energy contribute to the maximum activity.

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