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IN SILICO MOLECULAR DOCKING STUDIES OF SOME ISOLATED COMPOUNDS FROM *NAUCLEA LATIFOLIA* FOR A-AMYLASE INHIBITORY ACTIVITY AND ADME/T PROPERTY ANALYSIS OF THE COMPOUNDS.

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ABSTRACT

The aim of the study to find the mechanism of action of the isolated compounds from *Nauclea latifolia* was explored the α-amylase inhibitory activity by molecular docking analysis used for five phytoconstituents namely beta-sitosterol, daucosterol, quinovic acid, rotundic acid and ursolic aldehyde isolated from *N. latifolia*, to identify whether these compounds interact with the responsible protein (α-amylase enzyme). And also ADME/T properties of the phytoconstituents were analyzed using Qikprop 3.2 module. A wide range of docking score found during molecular docking by CPI server. beta-sitosterol, daucosterol, quinovic acid, rotundic acid and ursolic aldehyde isolated showed the docking score - 3.211, -3.526, -3.569, -4.199, -4.262, respectively. Among all the compounds, rotundic acid and ursolic aldehyde showed well docking score, glide emodel and glide energy. Predicted properties of beta-sitosterol, daucosterol, quinovic acid were not within the range for satisfying all the Lipinski's rule of five to be considered as drug like potential. But rotundic acid was satisfying all the Lipinski's rule of five to be considered as drug like potential. Ursolic aldehyde was satisfying all the Lipinski's rule of five except in lipophilicity (LogP) property. So, among all

the compounds rotundic acid was highly considered as safe drug for human. Further *in vivo* investigation need to identify whether isolated compounds from *N. latifolia* have α-amylase inhibitory activity or not.

KEYWORDS: *Nauclea latifolia* α-amylase, molecular docking, ADME/T properties,.

INTRODUCTION

Alpha amylases (endo-1, 4-α-D-glucan glucanohydrolase EC 3.2.1.1) are extracellular endoenzymes that indiscriminately cleave α-1,4 linkages between adjacent glucose units in the linear amylose chain and ultimately generate glucose, maltose, and maltotriose units. This category of industrial enzymes constitutes approximately 25% of the enzyme market. Conversion of starch into sugar syrups such as glucose, maltose, maltotriose, dextrins sugar, or fructose syrups, etc. are the major part of the starch process trade.[1,2] The spectrum of amylase application has widened in many different fields, like that clinical, medicinal and analytical chemistry; additionally as their widespread application in starch saccharification and within the textile, food, paper and pharmaceutical industries.[3-7] However, enzymes from fungal and bacterial sources have dominated applications in industrial sectors, attributable to advantages like cost effectiveness, less time and space requirement and ease of process modification and improvement.[8,9]

In silico is an expression used to mean "performed on computer or via computer simulation". In silico methods can help in identifying drug targets via bioinformatics tools. They can also be used to analyze the target structures for potential binding/active sites, generate candidate molecules, check for their drug likeness, dock these molecules with the target, rank them according to their binding affinities, further optimize the molecules to improve binding characteristics The utilization of computers and computational methods permeates all aspects of drug discovery nowadays and forms the core of structure-based drug design.[10]

Nauclea latifolia Smith (family: Rubiaceae) is a straggling, evergreen, multistemmed shrub or small tree native to tropical Africa and Asia. It normally produces interesting flowers, edible, but not appealing, large red ball fruits with long projecting stamens.[11] Commonly used parts of *Nauclea latifolia* include the leaves, roots, stem, and fruits. Nauclea latifolia roots decoction is one of such herbal preparations that have been used traditionally for treating different disease conditions. Medicinal uses vary from one traditional setting to another, its traditional uses include: fever, pain, dental caries, septic mouth, malaria,

dysentery, diarrhea, and diseases of the central nervous system such as epilepsy.[12][13-15] The fruits serve as key source of food for the baboons, livestock, reptiles, birds, and man. Phytochemicals majorly found in *Nauclea latifolia* include indole-quinolizidine, alkaloids (glycoalkaloids), and saponins.[16] Nkafamiya et al.[17] also reported that the fruits of *Nauclea latifolia* contain copper, iron, cobalt, calcium, magnesium, zinc, phosphorus, and vitamins (A, B1, B2, C, and E).

The aim of the study to find the mechanism of action of the isolated compounds from *Nauclea latifolia* was explored the α-amylase inhibitory activity by molecular docking analysis and ADME/T property studies used to measure the safety of the compounds as drug.

MATERIALS AND METHODS

In silico analysis

Molecular docking analysis of isolated compounds from *Nauclea latifolia*[18]

Preparation of protein structure

The 3D coordinates of crystal structure of α-amylase (PDB: 1PPI) was downloaded from the RCSB protein data bank (http://www.rcsb.org/pdb) set up at Brookhaven National Laboratory in 1971. It is a worldwide repository of information about the 3D structures of large biological molecules, including proteins and nucleic acids. Water molecules were removed from the protein 1PPI before the instigation of molecular docking. The protein structure was corrected by the utilization of alternate conformations and valence monitor options as some crystallographic disorders as well as some unfilled valance atoms were present in the protein file. The resultant protein file was subjected to energy minimization by applying Chemistry at HARvard Macromolecular Mechanics (CHARMm) force fields. CHARm is a program which provides a large suite of computational tools that encompass numerous conformational and path sampling methods, free energy estimates, molecular minimization, dynamics, and analysis techniques, and mode l- building capabilities (http://www.charmm.org/). After the energy minimization the protein fie was subjected to define and edit binding site option available on tools panel to explore the plausible binding site within the protein (1PPI).

Preparation of ligand

The structures of compounds beta-sitosterol, daucosterol, quinovic acid, rotundic acid and ursolic aldehyde were drawn using ChemBioDraw software. ChemBioDraw™ is a software from PerkinElmer for development of chemical structures of bioactive compounds. The

prepared ligand was then subjected to add the hydrogen bonds and the energy has been minimized using CHARm force field.

Docking analysis

To find out the accurate binding model for the active site of α-amylase enzyme, molecular docking analysis was performed using ligand fit of GLIDE software from Schrodinger (http://www.schrodinger.com /) . Molecular docking analysis was performed using crystal structure of α-amylase (PDB: 1PPI). The structure of crystal structure of α-amylase enzyme (PDB: 1PPI) were obtained from Protein Data Bank (http://www.rcsb.org). The mechanism of ligand position is based on the fitting points. Fitting points are incorporated into the hydrogen bonding groups on the ligand and the proteins. The ligand fit module[19] from GLIDE software was utilized to execute the molecular docking analysis, based on shape-based searching and Monte Carlo methods. At the time of docking, variable trials Monte Carlo conformation was applied where the number of steps depends on the n umber of rotatable bonds present in the compounds/ligands. By default the torsion number is 2, the maximum minimizations steps is 300 and maximum successive failure is 110. During the docking process the top ten conformations were engendered for each o f the compound after the minimization of the energy.[20]

ADME/T property analysis

Ligand based ADME/Toxicity prediction

The QikProp module of Schrodinger (Maestro, version 10.1) is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion (ADME) prediction program design to produce certain descriptors related to ADME. It predicts both physicochemical significant descriptors and pharmacokinetically relevant properties. ADME properties determine drug-like activity of ligand molecules based on Lipinski's rule of five. ADME/T properties of the compound (DIM) was analyzed using Qikprop 3.2 module.[21]

RESULTS AND DISCUSSIONS

***In silico* analysis**

Molecular docking analysis

In this study, the binding mode of α-amylase enzyme was investigated by doing computational analysis, glide docking. Both glide standard (SP) and extra precision (XP) mode had been introduced, where extra precision mode used for cross validation purpose. The results of docking analysis were described in Table 2 and the docking figure showed in

Figure 1. Among all the compounds, rotundic acid and ursolic aldehyde showed well docking score, glide emodel and glide energy.

Table 1: Docking results with beta-sitosterol, daucosterol, quinovic acid, rotundic acid and ursolic aldehyde in the α-amylase enzyme (PDB: 1PPI).

Compound Name	Docking Score	Glide emodel	Glide Energy
beta-sitosterol	-3.211	-24.99	-23.43
daucosterol	-3.526	-24.26	-23.80
quinovic acid	-3.569	-39.50	-32.34
rotundic acid	-4.199	-39.97	-32.99
ursolic aldehyde	-4.262	-41.82	-33.014

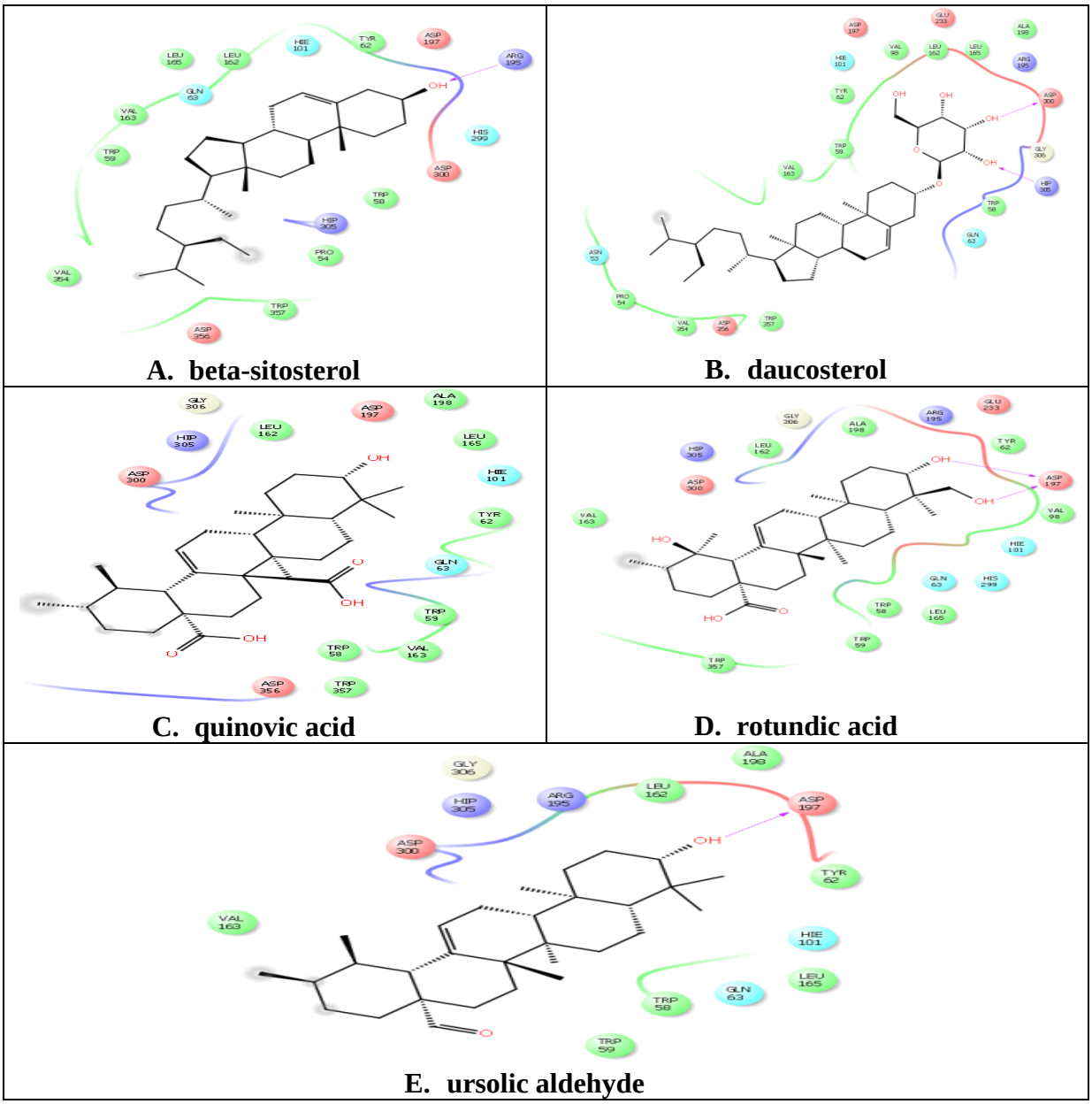


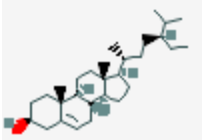
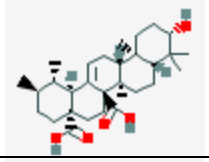
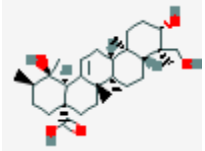
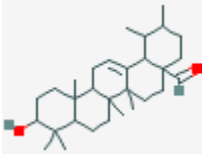
Figure 1: Molecular docking analysis of A. beta-sitosterol, B. daucosterol, C. quinovic acid, D. rotundic acid and E. ursolic aldehyde with α-amylase enzyme (PDB: 1PPI) receptor complex obtained from Glide docking.

ADME and Toxicity analysis

Ligand based ADME/Toxicity prediction

The drug-like activity of the ligand molecule was categorized using ADME properties by QikProp module of Schrodinger. The ADME properties of the beta-sitosterol, daucosterol, quinovic acid, rotundic acid and ursolic aldehyde were evaluated with QikProp module of Schrodinger, shown in Table 2. The selected properties are known to influence metabolism, cell permeation, and bioavailability. Predicted properties of beta-sitosterol, daucosterol, quinovic acid were not within the range for satisfying all the Lipinski's rule of five to be considered as drug like potential. But rotundic acid was satisfying all the Lipinski's rule of five to be considered as drug like potential. Ursolic aldehyde was satisfying all the Lipinski's rule of five except in lipophilicity (LogP) property. So, among all the compounds rotundic acid was highly considered as safe drug.

Table 2: ADME/T properties of beta-sitosterol, daucosterol, quinovic acid, rotundic acid and ursolic aldehyde by QikProp.

Name of Molecules	PubChem CID	Structure	MW[α]	HB donor[β]	HB acceptor[€]	LogP[¥]	Molar Refractivity[µ]
beta-sitosterol	222284		414.71	1	1	8.34	129.21
daucosterol	5742590		576.85	5	6	7.15	162.93
quinovic acid	120678		486.68	3	5	5.49	135.17
rotundic acid	12315075		488.69	4	5	4.67	136.62
ursolic aldehyde	14423519		440.70	2	2	7.36	132.21

[α]Molecular weight (acceptable range: <500).

[β]Hydrogen bond donor (acceptable range: ≤5).

[€]Hydrogen bond acceptor (acceptable range: ≤10).

[¥]High lipophilicity (expressed as LogP, acceptable range: <5).

[µ]Molar refractivity should be between 40-130.

CONCLUSION

From the study it was found that, *Nauclea latifolia* could be great source of new α-amylase inhibitor. *In silico* model support that all the isolated compounds from *N. latifolia* might be α-amylase inhibitor. Among all the compounds, rotundic acid and ursolic aldehyde showed well docking score, glide emodel and glide energy. Predicted properties of beta-sitosterol, daucosterol, quinovic acid were not within the range for satisfying all the Lipinski's rule of five to be considered as drug like potential. But rotundic acid was satisfying all the Lipinski's rule of five to be considered as drug like potential. Ursolic aldehyde was satisfying all the Lipinski's rule of five except in lipophilicity (LogP) property. So, among all the compounds rotundic acid was highly considered as safe drug for human. Further *in vivo* investigation need to identify whether isolated compounds from *N. latifolia* have α-amylase inhibitory activity or not.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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