https://doi.org/10.48047/AFJBS.6.Si4.2024.503-510

African Journal of Biological Sciences

IN SILICO MOLECULAR DOCKING STUDIES ON THE PHYTOCONSTITUENTS FROM PLANT OF LEAVES AZADIRACHTA INDICA

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Article History

Volume 6, Issue Si4, 2024
Received: 15 May 2024
Accepted: 05 June 2024
doi:
10.48047/AFJBS.6.Si4.2024.503-510

ABSTRACT:
Neem, or Azadirachta indica, is an evergreen tree in the Meliaceae family. It grows all over the world and is native to the Indian subcontinent. Because of its many medicinal and pharmacological qualities, it is often referred to as the "village pharmacy" in India. With the purpose of creating an in-silico library of the compounds found in A. indica, this study also suggested analysing these compounds computationally for potential anti-hyperglycemic effects. utilising the Chemsketch software, 2D chemical structures were created from Simplified Molecular Input Line Entry Specification (SMILES) notation utilising phytochemical compounds that were obtained from the Pubchem database. Using the Protein Data Bank, the three-dimensional structure of LXRα was obtained. Swiss - PDB Viewer was used to view the structure in order to gain a better knowledge of the molecule and maybe use it as a target for medication. Nimbolide has the highest GOLD score of any of the twelve phytochemicals, at 29.21. It is clear from the insilico docking results that the phytochemicals found in A. indica leaves have a significant deal of potential to prevent atherosclerosis. They may also function as better leads, which would further prevent atherosclerosis. By employing the ADMET structure-activity relationship database, ADME and toxicity were predicted.
KEYWORDS: Azadirachta indica, molecular docking, In-silico, CADD

1. INTRODUCTION

A key component of the contemporary drug discovery process is now in-silico screening, often known as computer-aided drug design, or CADD. It significantly cuts down on the time and resources required in the conventional lab-bench-based drug delivery process by using a range of bioinformatics software and algorithms to effectively screen for possible drug candidates (1). Furthermore, it is possible to forecast the pharmacological characteristics and interactions of compounds using the previously discussed methods. One kind of CADD that forecasts the protein-ligand interaction between the drug target and the drug candidate is called molecular docking. It simulates using computer programmes how various 3D postures of ligands, or possible drug candidates, might interact with drug targets, or proteins, and calculates how favourable those interactions would be in terms of binding energy. As a result, these forecasts may be applied as the initial stage of drug candidate screening, eliminating improbable candidates with comparatively little time and effort. (2) Nuclear hormone receptors known as liver X receptors (LXRs) α and β control several genes related to reverse cholesterol transport (RCT) and are possible targets for atherosclerosis drugs. While LXR β is found everywhere, LXRα is substantially abundant in the liver and at lower levels in the gut, macrophages, kidney, and other organs. (3, 4) Natural goods, such as plants, animals, and minerals, have long been employed in folklore and ancient cultures to heal a wide range of ailments. (5) Medicinal plants continue to be a valuable source of compounds with therapeutic potential and have demonstrated their worth historically. They are currently a major source of new medication leads. Traditional medicine is one of the most significant health care systems in many countries. (6, 7) Growing interest in natural goods, growing worries about the adverse effects of traditional treatment, and the enhanced potency of new therapies derived from plants have all contributed to a rise in scientific interest in phytomedicine. (8) Neem, or Azadirachta indica A. Juss, is a plant that belongs to the Meliaceae family. It is native to South and Southeast Asia and is currently grown in Australia, America, and other tropical and subtropical countries (9).With a broad spectrum of pharmacological activity, including antioxidant, anti-inflammatory, antidiabetic, anticancer,

antimalarial, antifungal, antibacterial, antiviral, hepatoprotective, neuroprotective, and wound healing impact activities, it is one of the most adaptable medicinal plants (10). Among the bioactive substances of A. indica that are known to possess the amazing capacity to reorganise numerous biological pathways both in vivo and in vitro are gedunine, nimbolide, and azadirachtin (11).

Molecular docking is widely utilised in modern drug development to anticipate the binding orientation of small molecule drug candidates to their protein targets in order to estimate the affinity and activity of the small molecule. It also provides valuable information regarding drug receptor interactions. (12) In the process of choosing and promoting new drugs, further computer prediction of pharmacokinetic parameters such as Absorption, Distribution, Metabolism, and Excretion (ADME) & toxicity studies has become crucial. These computational predictions are also useful for early screening of possible drug candidates. (13) Thus, the goal of the current work was to use GOLD to insilico dock and examine the molecular interaction between the phytochemicals in the methanolic leaf extract of A. indica and the LXRα for the prevention of atherosclerosis. Phytochemical ADMET profiles are insilico screened utilising the ADMET structure-activity relationship database (admetSAR).

2. MATERIALS AND METHODS

Liver X Alpha Receptor (LXRα) retrieval: Using Protein Data Bank, the 3-dimensional structure of LXRα (Protein Date Bank [PDB] ID – 3IPQ) was obtained, which might serve as a target molecule for molecular docking. Swiss - PDB Viewer was used to view the structure in order to gain a better knowledge of the molecule and maybe use it as a target for medication. (14)

Building of herbal compounds:A. indica leaf extract methanolic extract yielded twelve phytochemicals, which were tested against LXRα. Table 1 displays the list of detected phytoconstituents. The phytochemical compounds were obtained from the Pubchem database, and the Chemsketch software was utilised to construct the 2-D chemical structures using SMILES notation (Simplified Molecular Input Line Entry Specification). The structures were then transformed into three dimensions, their geometries optimised, and they were saved using the Open Babel server in the "MDL mol file" format. (14, 15)

Active site prediction: The target protein's active site was predicted using PDB sum, a programme that describes the total number of active sites together with details on their amino acid sequence, cavity locations, and average cavity volume. PDB sum requires a PDB file as input. (16)

Screening of docked complex: Twelve molecules that were generated from A. indica were molecularly docked with the LXRα. GOLD conducted screenings of several docked complexes based on energy as a key stability restriction. As part of the GOLD Suite, a collection of tools for structure visualisation and manipulation (Hermes), protein-ligand docking (GOLD), post-processing (GoldMine), and docking result visualisation, GOLD is a programme for determining the docking modes of small molecules in protein binding sites. The optimal drug was determined to be the ligand with the highest binding affinity to the receptor molecule. (17)

ADMET Prediction: AdmetSAR offers the most up-to-date and thorough hand curated data for a variety of compounds linked to established ADMET profiles. This database contains five highly predictive quantitative regression models and twenty two qualitative classifications that are used to evaluate the features of mammalian ADMET for new compounds. For various model types, the admetSAR server predicts the ADMET-associated features of the active chemicals, and all of the predictions are favourable. (18–20) The in silico screening of ADMET profiles for the active compounds obtained from the methanolic extract of A. indica leaves was done using the admetSAR programme.

3. RESULTS AND DISCUSSION

Using the GOLD software, the molecular docking analysis of the twelve compounds obtained from the methanolic extract of A. indica leaves with the LXRα was performed. The optimal chemical that interacts with the receptor was found by using the GOLD programme. The docked energy in kcal/mol, or binding compatibility, was used to assess the results (fitness). The amount of hydrogen bonds that were established and the bond distance between the inhibitor's and active site's atomic coordinates were used to evaluate the final docked conformations that were produced for various compounds. Table 1 displays the GOLD docking scores for the phytochemicals.

Table 1: Gold Docking Score Of Phytoconstituents Present In A. indica Leaves

S. No	Name of the compound	No. of H-bonds	H-bond distance	Gold score
1	Odoratone	1	3.069	4.21
2	Salimuzzalin	2	1.922	3.18
3	Limocinin	1	3.1532	21.98
4	Nimbocinone	1	2.584	26.86
5	Naheedin	1	1.964	21.32
6	Meliacinol	2	2.865	16.01
7	Nimbisonol	2	2.012	-6.42
8	Nimbilin	1	1.754	22.78
9	Nimbolide	2	3.172	29.21
10	Nimbolide B	1	2.654	11.89
11	Isonimbinolide	2	1.976	23.54
12	Azadirone	1	2.043	25.65

Based on the GOLD score of 29.21, the chemical Nimbolide has the maximum affinity to bind with LXRα. Nimbocinone, Azadirone, Isonimbinolide, Nimbilin, Limocinin, and Meliacinol exhibit a comparatively strong binding affinity, as indicated by their respective GOLD scores of 26.86, 25.65, 23.54, 22.78, 21.98, and 21.32. Comparatively speaking, Meliacinol, Odoratone, and Nimbolide B have lower binding affinities, with GOLD values of 16.01, 15.34, and 11.89, respectively. The least affinities are shown by odoratone and nimbidosonol, with GOLD ratings of 4.21 and -6.42, respectively. Fig. 1 displays the docking of Nimbolide with LXRα. Figs. 2 show the various Docking schematic patterns for Nimbolide with LXRα. It is clear from the insilico docking results that chemicals derived from A. indica possess significant potential in preventing atherosclerosis.

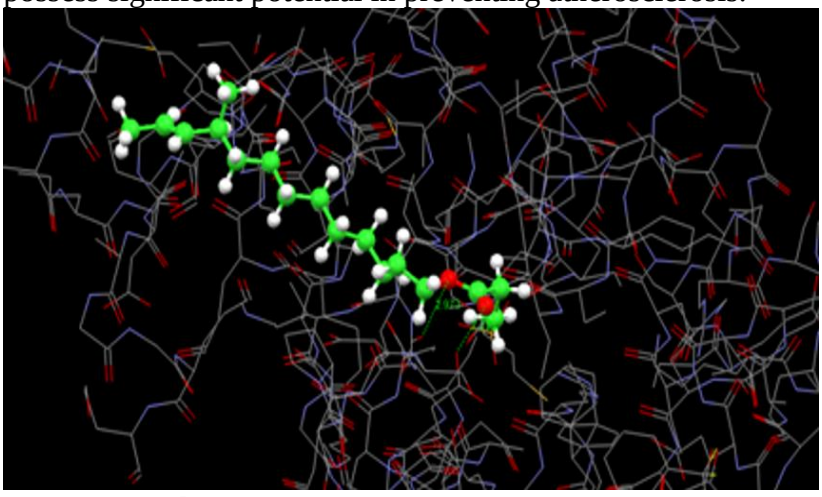

Figure 1: Docking of NimbolideWith Liver X Alpha Receptor

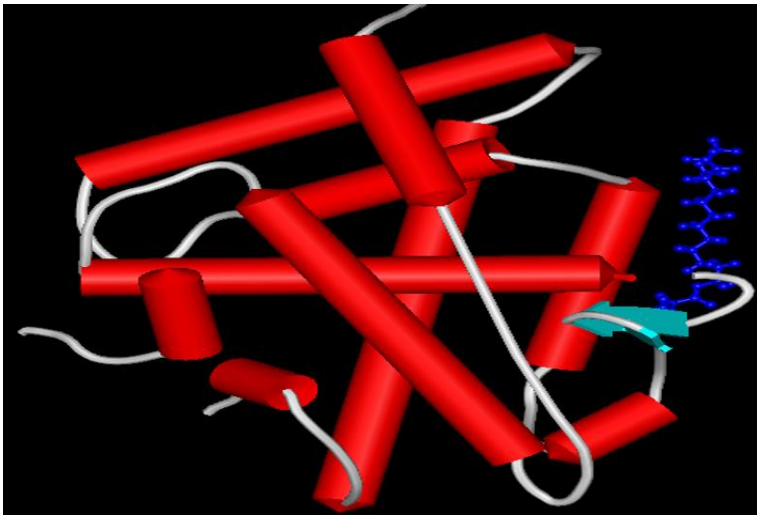

Figure 2: Docking Schematic Pattern 2: Protein - Schematic, Ligand - Ball And Stick

Drug design is producing more effective compounds as a result of the application of ADMET profiling of drug candidates in conjunction with biological efficacy and safety optimisation, which has significantly decreased pharmacokinetic failures in clinical trials. The ADMET properties, obtained from the admetSAR server, indicate that when it comes to absorption, the active phytochemicals demonstrated favourable outcomes for various models, including BBB penetration, P-glycoprotein substrate, renal organic cation transporter, human intestinal absorption, and CaCO2 permeability. These findings reaffirm the phytochemicals' potential as therapeutic agents. Table 2 provides the absorption prediction profile for the active chemicals found in Azadirachta indica.

Table 2: Absorption Prediction Profile For Active Compound From A. indica

Parameter	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Blood-Brain Barrier	+	+	+	+	+	+	+	+	+	+	+	+
Human Intestinal Absorption	+	+	+	+	+	+	+	+	+	+	+	+
Caco-2 Permeability	+	+	+	+	+	+	+	+	+	+	+	+
P-glycoprotein Inhibitor	NI	NI	S	NI	NI	NS	NI	NI	S	NI	NS	NI
P-glycoprotein Substrate	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S
Renal Organic Cation Transporter	NI	NI	NS	NI	NI	NI	NS	NI	S	NI	NI	S

+: positive, -: negative, NS: Non-substrate, S: Substrate, NI: Non-Inhibitor

A class of isozymes known as cytochrome P450 (CYP) is involved in the metabolism of bile acids, steroids, fatty acids, medicines, and carcinogens. Nearly 75% of phase I drug metabolism is dependent on CYP enzyme association. Several CYP substrate and inhibitor models are developed in the context of metabolism, and the results demonstrate that these active phytochemicals are neither substrates nor inhibitors of CYP enzymes. Nimbolide B on its own has been shown to function as the CYP450 3A4 Substrate's substrate. Table 3 provides the metabolism prediction profile for the active chemicals derived from A. indica. It has been discovered that none of the phytochemicals are harmful. Table 4 provides the toxicity prediction profile for the active chemicals derived from A. indica.

Table 3: Metabolism Prediction Profile For Active Compound From A indica

Parameter	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
CYP450 2C9 Substrate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CYP450 2D6 Substrate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CYP450 3A4 Substrate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CYP450 1A2 Inhibitor	NI	I	I	NI	NI	NI	I	I	NI	NI	NI	NI
CYP450 2C9 Inhibitor	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
CYP450 2D6 Inhibitor	NI	NI	NI	NS	NI	NI	NI	NI	NI	NI	NI	NI
CYP450 2C19 Inhibitor	NI	NI	NI	NI	NI	NI	NI	NS	NI	NI	NI	NI
CYP450 3A4 Inhibitor	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

NS: Non-substrate; NI: Non-Inhibitor; I: Inhibitors; S: Substrate

Table 4: Toxicity Prediction Profile For Active Compound From A. indica

Parameter	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
AMES Toxicity	T	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Acute Oral Toxicity	III	III	III	III	III	III	III	IV	III	III	III	III
Tetrahymena Pyriformis Toxicity	HT	HT	HT	HT	HT	HT	HT	HT	HT	HT	HT	HT
Biodegradation	RB	RB	RB	RB	RB	R	RB	RB	RB	RB	RB	NRB
Honey Bee Toxicity	HT	HT	HT	HT	HT	HT	HT	HT	HT	HT	HT	HT
Carcinogens	NC	C	C	NC	C	C	NC	NC	NC	C	C	NC

+: positive, -: negative, NS: Non-substrate, S: Substrate, NI: Non-Inhibitor, I: Inhibitor, WI: Weak Inhibition, NT: Non-Toxic, NC: Non- Carcinogen, C: Carcinogen, HT: High Toxic, RB: Readily Biodegradable, NRB: Not Readily Biodegradable

CONCLUSION

Using the GOLD software, the molecular docking analysis of the twelve compounds from the methanolic extract of A. indica leaves with the LXRα indicated their potential for receptor interaction. To the best of our knowledge, this is the first report on A. indica leaves' potential anti-hyperglycemic effects. The best drug (nimbolide) for the management and treatment of hyperglycemia has been found from this investigation; however, additional bench testing on potential candidates is still required to corroborate the findings. Thus, additional testing of these compounds in a wet lab environment is one of the future objectives in order to confirm their possible inhibitory ability. The database created in this study may play an important role in future studies of this plant compared to other biological targets, which in turn enables exploration of other therapeutic targets.

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