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Screening of *Phyto- molecules* against *Brugia malayi* asparaginyl tRNA synthetase - An *in silico* approach towards anti filarial leads

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ABSTRACT

Lymphatic Filariasis is one of the most neglected tropical diseases caused by Brugia malayi. The existing classical drugs act mostly on the larval stages of the parasite. The target Asparaginyl tRNA synthetase is an excellent molecular target due to its pivotal role in protein synthesis. Literature based evidence provided clue to explore the phyto-molecules as potential anti-filarial leads, which led to the scope for this computational study. The computational parameters such as docking score, drug likeliness prediction, intermolecular hydrogen bond interaction and the identical amino acids prove that plant derived molecules could serve as better anti filarial agents than the synthetic compounds. The phyto-molecules, Kaempferol and Luteolin have provided promising results. The outcomes prove that they can be explored further in invitro and invivo studies to validate their claim as potential anti filarial agents.

Keyword: *Brugia malayi, anti-filarial leads, Asparanginyl tRNA synthetase, Kaempferol and Luteolin*

INTRODUCTION

Lymphatic Filariasis (LF) is one of the most neglected tropical diseases in many countries. It causes a major public health problem such as physical disability, disfiguring and chronic morbidity[1]. It is ranked among the World Health Organization's (WHO) top 10-neglected tropical diseases [2]. According to a WHO report, India, Indonesia and Bangladesh alone contribute to about 70% of the infection worldwide. Apart from these countries, six other countries, such as Maldives, Myanmar, Nepal, Sri Lanka, Thailand and Timor-Leste are the other endemic countries, having more number of LF cases. LF is caused primarily by two related parasites, *Brugia malayi* and *Wuchereria bancrofti* represents a worldwide health crisis with over 20% of the global population at risk for infection. *B. malayi*, the most common causative organism for the filarial infection is transmitted by the mosquitoes (vector) like Mansonia, Anopheles, Culex, and Aedes. Development and replication of *B. malayi* occurs in two discrete phases: in the mosquito vector and in the human. Both stages are essential to the life cycle of the parasite. In humans, the adult worms can survive in the lymphatic system for 5–15 years. The male and female adult worms mate and the females produce an average of 10,000-sheathed eggs (microfilaria) daily. The microfilariae enter the blood stream

and exhibit the classic nocturnal periodicity and super periodicity[3]. LF results from mosquitoes transferring the nematode *B. malayi*, to host lymph nodes, leading to swelling of affected limbs.

For the past 20 years, three classical drugs named Diethylcarbamazine, Ivermectin and Albendazole are in practice to cure this disease. They are effective against the larval stages of the parasite but are unable to kill the adult filarial worms [4]. It is noticeable that currently these drugs are being used in the Global Programme to Eliminate Lymphatic Filariasis (GPELF) program, which has targeted to eliminate this disease by 2020[5].However, emergence of drug resistance to the currently available treatment is a potential threat to the LF elimination program. Hence, there is an urgent need to identify improved anti filarial drugs to prevent and treat this disease.

Aminoacyl-Trna synthetases (AARSs) were the first filarial targets for anti-parasite drug discovery embraced by WHO and are generally regarded as excellent therapeutic targets since they play a key role in protein synthesis. Among the AARSs, in particular, asparaginyl tRNA synthetase (AsnRS) in *B. malayi* is considered as the best, which catalyzes the specific attachment of amino acids to their cognate tRNAs in protein bio-synthesis [6]. AsnRS is expressed in both male and females during all the stages of *B. malayi* life cycle – adult, microfilariae, and larval stages. Particularly in females, AsnRS levels are significantly higher than those of other AARS[7].

In *B. malayi* and *W. bancrofti*, the AsnRSs are identical at the amino acid level and exist as multi-copy genes encoding an immuno-dominant antigen that produces a strong antibody response in the serum of humans with *LF*. Hence, AsnRS is now considered as a valid molecular target for anti-filarial drug discovery. It has already been reported by a few groups [6,7] the identification of inhibitors for the above said target. At present, the crystal structure of AsnRS in complex with the ligand 5'-O-[N-(L-asparaginyl) sulfamoyl]adenosine (NSS) is available in the Protein Data Bank. NSS is a synthetic non-hydrolysable analogue of the native intermediate compound AsnAMP. In screening analogues of NSS containing all 20 proteinogenic amino acids, the analogues were found to have potent immunosuppressive activity and act as potent inhibitors of AARSs [8, 9].

Plant-derived natural products play a significant role by being a lead molecule in the development of drug candidates. Herbal extracts represent the primary form of health care for a major proportion of the world population and are an important source of single-molecule drug leads. A prominent example is the anti-malarial activity of *Artimisiaannua* discovered by Professor Tu, of China Academy of Chinese Traditional Medicine, recipient of the 2015 Nobel Prize for Physiology and Medicine[10].

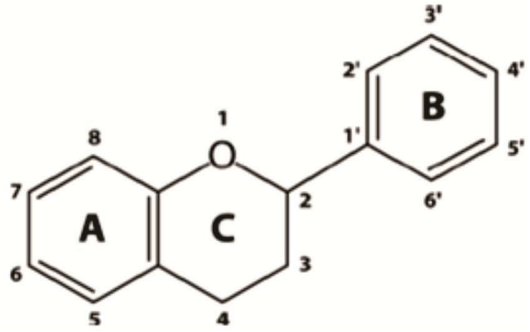


Figure 1. Basic chemical structure of flavonoids

Among the Plant-derived natural products, flavonoids are endowed with a wide range of biological benefits to human health that include not only anti-inflammatory, antioxidant, antibacterial, antifungal and antiviral activities but also anti filarial activity. Flavonoids are biologically active polyphenolic compounds providing health benefits, commonly found in fruits, vegetables, tea, and wine. They are benzo-γ-pyrone derivatives with phenolic and pyrane rings (Figure 1)[11]. Based on the substitutions, they are classified as flavonols (quercetin, kaempferol), flavones (apigenin, luteolin), flavanones (hesperidin, naringenin), flavan-3-ols, (catechin, theaflavin, and gallic esters of catechin and theaflavins), anthocyanidins (pelargonidin, cyaniding) and isoflavones (genistein, daidzein). Mainly they differ based on the distribution of hydroxyl groups (-OH) in the B ring. Quercetin is a catechol with 2 hydroxyl groups on neighboring carbon atoms of their B rings; and myricetin, a pyrogallol, has 3-OH groups, whereas both apigenin and kaempferol have only one isolated -OH group in the B ring.

Numerous studies have successfully shown various types of flavonoids such as Baicalein, Quercetin, Luteolin, Kaempferol, Plumbagin and Rutinto that are very active against various pathogens. The quest for identification of the leads for various drug targets led us to investigate the above flavonoids as anti filarial leads for our present study.

Among the Insilico computational methods applied in Drug Discovery, Molecular Docking accelerates the drug design process. It is used in the biopharmaceutical industry to discover and develop new lead compounds. It enables to visualize the possibilities of binding of potential small molecules as ligands/inhibitors. It analyses different docked conformations using the scores / energies based on their binding affinity as parameters to evaluate the ideal ligand. Therefore, we have designed the present study using computational approaches to discover the potential of these flavonoids, targeting the inhibitory effect against asparaginyl tRNAsynthetase.

MATERIALS AND METHODS

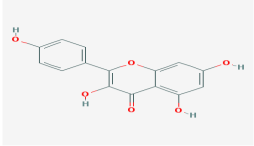
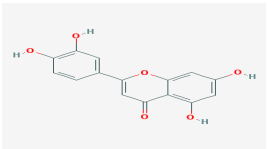
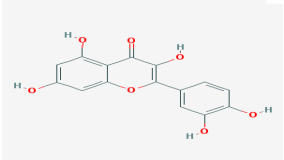
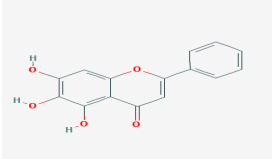
Protein Preparation

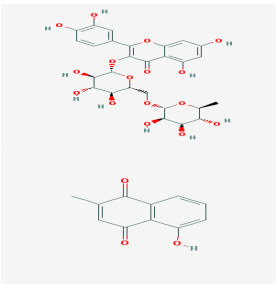
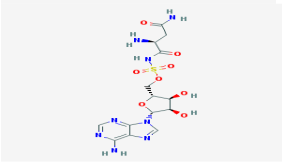
The X-ray crystallographic coordinates of AsnRS in its complex form with the ligand NSS, at a resolution of 1.9A^0 was retrieved from the Protein Data Bank. Water molecules, ligands and other heteroatoms were removed from the protein molecule along with the chain B. Addition of hydrogen atoms to the protein was performed using CHARMm force field. Energy minimization was performed by using conjugate gradient method with an RMS gradient of 0.01kcal/(A^0 mol) on Accelyrs Discovery studio client (version 2.5) (2009) San Diego.

Ligands Preparation

Six phytochemical ligands, namely Kaempferol, Luteolin, Baicalein, Plumbagin, Rutin and Quercetin, the plant-derived flavonoids were chosen for the docking against the target AsnRS. The phytochemical molecules were retrieved from the pubchem database and the chemical structures were generated using SMILES notation (Simplified Molecular Input Line Entry Specification) with Discovery Studio 2.5 version. The structural details namely, the chemical name, PubChem ID, Molecular formula and the 2D structure of the selected phytochemicals are given in Table 1.

Table 1 Structural details of the selected phytochemicals

S.No	Chemical name	PubChem ID	Molecular Formula	2D Structure
1	Kaempferol	5280863	$C_{15}H_{10}O_6$	
2	Luteolin	5280445	$C_{15}H_{10}O_6$	
3	Quercetin	5280343	$C_{15}H_{10}O_7$	
4	Baicalein	5281605	$C_{15}H_{10}O_5$	
5	Rutin	5280805	$C_{27}H_{30}O_{16}$	

6	Plumbagin	10205	$C_{11}H_8O_3$	
7	5'-O-[N-(L-Asparaginyl)Sulfamoyl]Adenosine (NSS)	6852127	$C_{14}H_{20}N_8O_8S$	

Drug likeliness prediction

Drug like properties of the ligands were predicted by using the Discovery Studio 2.5 version. Lipinski's rule helps in distinguishing drug-like and nondrug-like properties and predicts high probability of success or failure due to drug likeliness of the molecules. The Lipsinki's filter helps in early preclinical assessment and thereby avoiding costly late stage preclinical and clinical failures. Lipinski's rules state that ideal drug molecules possess 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, Molecular weight not more than 500 and LogP not more than 5 [12].

Molecular Docking

The grid-based molecular docking method is used here using the program Cdocker (Accelrys) that employs CHARMm force field. The target is held rigid while the ligands are allowed to be flexible during the refinement. Since the ligand was already docked, the binding site info is already known. Hence, it is possible, however, to specify the ligand placement in the active site using a binding site sphere. For this purpose, the NSS ligand present in the active site of the target protein was used to generate the sphere around the active site. Then the prepared ligands are docked to the active site using default parameters. The results of the docking enabled the ranking of the docked conformation of the ligands according to their Cdocker energy values. The top Cdocker energy ligands were selected as hits for the target protein.

Analyses of the ligand binding sites

The docking poses were ranked according to their docking energies. The scoring function in Cdocker was used to predict the binding affinity of one ligand to the target molecule. In addition to the structural information, each record includes the Cdocker energy reported as negative value, where the higher value indicates a more favorable binding. This enables the energy to be used like a score. This score includes internal ligand strain energy and receptor-ligand interaction energy, and is used to sort the poses of each input ligand. The molecular visualizations of the docked complexes were analyzed using the Discovery Studio 2.5 version.

RESULTS

In this study, *B. malayi* AsnRS was considered as the target protein towards the lead identification. It is a catalytically active fragment lacking the N-terminal extension, containing residues from 112 to 548. It has been complexed with the ligand NSS and the structure of the complex solved by X-ray crystallography to 1.9 A resolution has been considered for the docking study. The active site that has been occupied by the ligand NSS was used for the docking of the phytochemical ligands considered in this study. Six phytochemical ligands that were derived from flavonoids were considered as the ligands for this computational study. The drug likeliness of the selected ligands along with that of NSS has been presented in Table 2.

Table 2 The predicted Drug likeliness of the selected ligands

S.No	Name	Molecular Weight	Acceptors	Donors	ALogP
1	Kaempferol	286.23	6	4	1.9
2	Luteolin	286.23	6	4	1.4
3	Quercetin	302.23	7	5	1.5
4	Baicalein	270.23	5	3	1.7
5	Plumbagin	188.17	3	1	2.3
6	Rutin	610.51	16	10	-1.3
7	NSS	460.42	13	6	-4.8

The table provides the details of the molecular weight, number of donor and acceptor atoms and AlogP values. The data would reveal the likelihood of the ligands that could form a potential lead for further analysis. Table 3 lists the results of the docking analysis by providing the Cdocker energy for all the selected ligands with AsnRS as the target.

Table 3 Docking energies of Phyto-ligands against AsnRS of *B. malayi*

S.No	ligands	Cdocker Energy(Kcal/mol)
1	Kaempferol	-57.57
2	Luteolin	-54.43
3	Quercetin	-52.92
4	Baicalein	-44.05
5	Plumbagin	-19.77
6	Rutin	-3.11
7	NSS	-58.55

Higher the negative energy corresponds to the stable binding with the target. The Cdocker energy for NSS was also given and it is proved a better complex with AsnRS. The comparison of the energies of the selected ligands with that of NSS would give an indication about the strength of the ligand binding with the target.

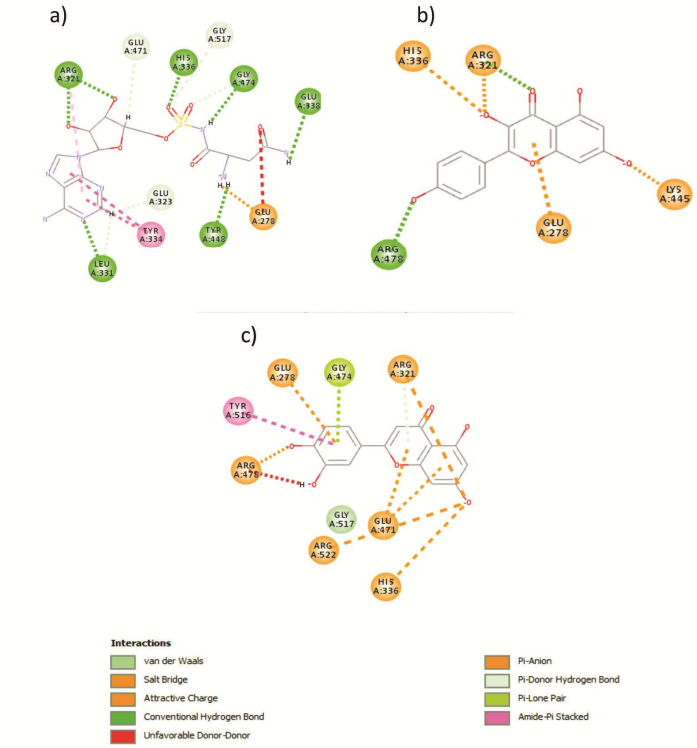


Figure 2 The 2D representations of the intermolecular interactions of the target AsnRS residues with the ligands, (a) NSS (b)Kaempferol (c)Luteolin

From the docked poses of the ligands with AsnRS, one gets a clear picture about the binding of the ligand with the target. The stability of the docking is decided based on the number of hydrogen bonds formed. The closer the

hydrogen bond distance corresponds to the greater strength of the interaction. A review by Szatylowicz classified the energy borders setting for strong, moderate and weak H-bonds, with 1.2–1.5 Å considered strong, >1.5–2.2 Å moderate and >2.2 Å weak[13]. The intermolecular conventional hydrogen bonds distances for the Kaempferol and Luteolin interactions with AsnRS are given in Table 4. Further, the intermolecular interactions between these ligands with AsnRS have been shown as a 2D representation in Figure 2 (a) for NSS, (b) for Kaempferol and (c) for Luteolin.

Table 4 Inter-molecular conventional H bond distances between Kaempferol and Luteolin with *B. malayi* AsnRS

Compound	Interacting target residue atom / Ligand atom	Distance (Å)
Kaempferol	LYS445:HZ2 - Kaempferol:O18	1.65
	ARG321:HH11 - Kaempferol:O10	1.81
	ARG321:HH22 - Kaempferol:O10	2.65
	ARG321:HH22 - Kaempferol:O20	1.68
	ARG478:HH22 - Kaempferol:O21	1.88
	HIS336:HE1 - Kaempferol:O20	3.08
Luteolin	ARG478:HH12 - Luteolin:O21	1.80
	ARG478:HH22 - Luteolin:O21	1.75
	ARG321:HH22 – Luteolin:O21	3.09

From the analyses of these data, a Venn diagram has been plotted and as shown in Figure 3. In this figure, the interactions of the atoms of the above said three ligands against AsnRS residues are presented independently. The common residues interacting with all the three ligands form the pocket for the pharmacophore. This will provide the uniqueness for the ligands to interact with AsnRS. These common interacting residue sites were brought out nicely by the display of the Venn diagram representation as shown in Figure 3.

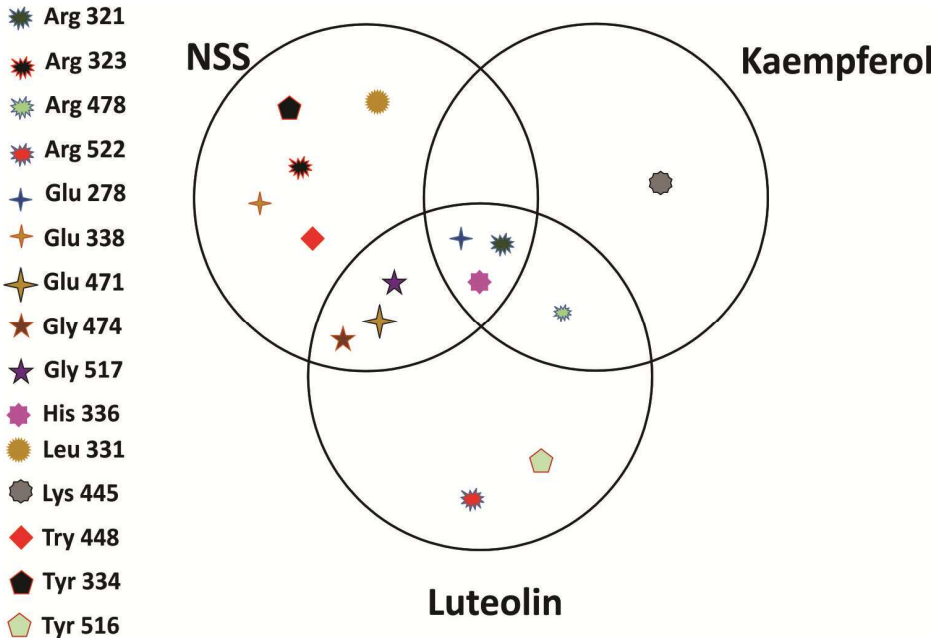


Figure. 3 Venn Diagram representation of AsnRS residue interactions with the ligands Kaempferol, Luteolin and NSS

DISCUSSION

The results of this computational study were analyzed through five different routes, namely, structural visualization and comparison with NSS, the drug like property analysis, analysis of Cdocker energy and the hydrogen bonding interactions and finally analysis of the ligand interactions with AsnRS.

Simple visualization of the ligand structures given in Table 1 indicates clearly that there are two clusters among the six phytochemicals considered in this study. The first four ligands from the table form cluster one and have

structural similarity among themselves having benzo-γ-pyrone derivatives with phenolic and pyrane rings. The fifth and sixth ligands form the second cluster and consist merely the benzopyran rings and the bulky multiple ring structure. The variation in the molecular weights for the first four ligands is by mere oxygen and one could expect a consistency of the derivatives that could bind with AsnRS. When comparing these ligands with the original ligand for which the crystal structure is available, viz., NSS, they all have a similarity with NSS with the benzo γ pyrone rings corresponding to the adenosine moiety rings, respectively. With the observation of this similarity, the first four-ligand structures are expected to bind with the target AsnRS more tightly when compared with the other group of benzopyran or bulkier multiple ring structures.

The analysis of the Drug Likeliness of the selected phytochemicals indicate that all the ligands except Rutin the Lipinski's rules and found to be potential candidates to be the leads[12]. Rutin's molecular weight being >500 and having 10 hydrogen acceptor atoms, it has deviated the rules. Surprisingly, the ligand NSS doesn't satisfy the Lipinski's rules of acceptor and donor atoms. Though NSS fits nicely into the catalytic site of AsnRS, it could not be a proper candidate towards the making of a drug. Thus, the first five ligands in the table are suitable to be the leads that have the potential to form the drugs

The analyses of the Cdocker energies of the ligands obtained by interacting with AsnRS clearly support the observation of the above two clusters made out of the structural analysis (Table 1). The four ligands (Kaempferol; Lutelin; Quercetin and Baicalein,) in the first group show Cdocker energies in the range of -44 to -57 kcal/mol in contrast to the second group ligands Plumbagin and Rutin having values of -19 and -3 kcal/mol respectively. Rutin shows a poor value of Cdocker energy because of its bulky multi ring structures and Plumbagin shows a low value as it doesn't have the complete benzo-γ-pyrone fragment. Comparing these with the Cdocker energy value of -58.55 kcal/mol for NSS, the first group of four ligands is expected to behave like NSS in binding with AsnRS. In fact, their binding strength would be in the following order Kaempferol; Lutelin; Quercetin and Baicalein, with the values of Cdocker energies -57.57; -54.43; -52.92 and -44.05 kcal/mol, respectively. Overall, the docking study suggests that ligand 1 and 2 interacted with the target AsnRS in a fashion similar to the X-ray studies of the ligand NSS. Since Kaempferol and Lutelin are showing a higher negative value of Cdocker energy comparable to NSS and are having the same molecular formula, they would be the likely candidates to be selected for further studies.

In support of the previous analyses and results, Kaempferol interacted with AsnRS by making five H-bonds with a distance range of 1.65 to 2.65 Å; among them, H-bond interactions with Glu278, Arg321 and His336 were also present between NSS and AsnRS. Similarly, Luteolin interacted with AsnRS by making six H-bonds; among them, H-bond interactions with Glu278, Arg321, His336, Glu471, Gly474 & Gly517 were also present between NSS and AsnRS. All intermolecular hydrogen bonds between Kaempferol and AsnRS in this study fell under the moderate bond group with one exception, interaction with the His336 residue, which was categorized as a weak bond. Also Luteolin interact with six amino acid residues similar to NSS. Kaempferol showed interaction with four residues of AsnRS, His336, Lys445, Arg321 and Arg478, with distance ranges from 1.65 to 1.88.

We were interested to find out the common motifs / residues in AsnRS that interact with all the ligands through the inference of the Venn diagram. NSS is found to interact with 11 residues of AsnRS; Luteolin and Kaempferol have 9 and 6 interacting residues respectively. Among these, NSS has six residues in common with Luteolin and three residues with Kaempferol. Luteolin and Kaempferol have four residues common among themselves. Only three residues (Glu278, Arg 321 and His336) form the common interacting partners among the three ligands. Thus, this analysis strongly supports the earlier observation that Kaempferol and Luteolin are the best possible candidates that can be taken up further in search of a drug candidate.

CONCLUSION

Thus, natural products are regaining popularity for drug discovery because they overcome various restrictions of synthetic libraries, including limited chemical diversity, novelty and safety norms. Furthermore, natural products have proved to be very successful leads for drug development in the past, with 34% of drugs approved by the FDA from 1981 to 2010 being either based on or derived from natural products. Based on the structural visualization, drug-likeliness prediction, docking energy, intermolecular hydrogen bond interactions and the common amino acid interaction overlaps, one can substantiate the claim that plant derived molecules could serve as better anti filarial agents. The phyto-molecules, Kaempferol and Luteolin have provided promising results. The outcomes prove that they can be explored further in invitro and in vivo studies to validate their claim as potential anti filarial agents.

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