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Screening of *Acorus calamus Phytocompounds against* Zika Virus (NS5B) using Molecular Docking Studies

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ABSTRACT

In 1947, the Zika virus, a mosquito-borne flavivirus, was identified in Uganda. This virus was later placed in the monkey and spread worldwide to the human population. But still, particular medicine and treatment are not available. Common antiviral synthetic drugs, such as sofosbuvir, boceprevir, etc., produce more side effects. To overcome this problem, we move on to alternative medications. In this study, the medicinal plant *Acorus calamus* had antiviral activity. It belongs to the family of Acoraceae. In ancient times *Acorus calamus* was widely used in traditional therapeutic systems. The rapidly developing field of Molecular Docking study approach predicts the plant *Acorus calamus* phytoconstituents against the Zika virus. In this study, we determine the novel potential active principle to inhibit the Zika virus's extension using molecular modelling using the Schrodinger Maestro 12.7 version. Qikprop tool also performs ADME screening. We have taken 60 phytochemicals from the *Acorus calamus* plant. The top-hit phytoconstituent of Galangin shows a high docking score compared to other phytoconstituents. The drug-likeness property of the Galangin obeyed in all parameters. The docking score of Galangin (-7.391kcal/mol) is higher than the reference drug sofosbuvir (-5.5 kcal/mol). The results reveal that Galangin could benefit as the lead drug candidate for inhibitors for the Zika virus.

Keywords*:* *Acours calamus,* Zika virus, Molecular Docking, Maestro and NS5BPolymerase.

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INTRODUCTION

The Zika virus (ZIKV) was discovered in the forest of Uganda. Scientists observed yellow fever in the species of Rhesus macaque in 1947. It is one type of monkey in the Uganda forest. The fever had developed in the monkey, and researchers isolated serum from the monkey. They were the first to characterize Zika virus [1]. Zika virus is a single-stranded RNA virus and has a place in the flavivirus genus and Flaviviridae family. Fever and congenital ZIKV syndrome were the two primary forms of ZIKV infection. In 80% of cases reported, Zika fever is an asymptomatic infection [2]. It is a mosquito-borne viral disease, and they are daytime biters. The disease-transmitted agents were female Aedes Mosquitoes *Aedes aegypti* and *Aedes albopictus*[3]. ZIKV is not only transmitted by mosquitoes, and they are also shared via mother to fetus during pregnancy, sexual transmission and blood transfusion. ZIKV is also transmitted by dengue, chikungunya and urban yellow fever. The primary symptoms are fever, rashes, headache, joint pain, red eyes, and muscle pain. Therefore, the infection of ZIKV death rate is rare[4,5].

The *Acorus calamus* is one of the most common plants used in the Indian traditional medicine system. It is a tall perennial wetland monocot plant. It belongs to the Acoraceae family[6]. They are also known as Vacha, sweet calomel, sweet flag, and sweet sedge[7]. The plants were grown in India, Sri Lanka, Europe, East Asia and North America[8]. The rhizomes of plants were indefinite, branched, smooth, pinkish or pale green. The leaves have scars on brown, white, and spongy. Leaf size was between 0.7cm and 1.7cm wide, averaging 1cm. The flowers are cylindrical, greenish-brown, 3 to 8 cm long and contain many rounded spikes. The fruits were small and berry-like, with few seeds[6]. The rich quantities of Essential oils, phenylpropanoids, sesquiterpenes, monoterpenes, xanthone glycosides, lignans and steroids were the chemical constituents of the *Acorus calamus*[9]. Pharmacologically, *Acours calamus* was reported to possess antimicrobial, anti-inflammatory, neuroprotective, antioxidant, anti-asthmatic, colic pain, diarrhoea, diabetes, tumours, insecticidal, enhancer, antitoxic, anti-cholinergic, anti-cancer, anti-hyperlipidemic and antispasmodic[10]. The in-silico study is a method which analysis the conformation and orientation of molecules into the binding site of a macromolecular target. The In-silico modelling approach has considerably minimised time and requirement of resources for biological testing and chemical. Molecular Docking is a pivotal component of the computer-aided drug design toolbox. It is a part of the "structure-based drug design" method. Molecular docking techniques were developed during the later '80s and earlier '90s. A few software were developed during the last ten years, amongst which are well-known examples such as AutoDock, AutoDock vina, DockThor, GOLD, FleXX and Molegro Virtual. Molecular Docking includes all

the theoretical or computational methods used for modelling or mimicking the molecule's behaviour[11]. This technique predicts the best match between two molecules when they are bound to each other to generate a stable complex[12].

MATERIALS AND METHOD

In this study, we use the Schrodinger Maestro software 12.7 version. New York LLC, USA. Academic version.

Protein Preparation:

This study Crystal Structure Zika Virus (NS5B) PDB ID (6LD5) [13] and Resolution 1.94 Å was downloaded from the protein data bank and placed in Maestro Suite. We used the protein preparation wizard for protein preparation. Here we used Maestro software 12.7. From that, preprocessing is applied to prepare protein. The following processes are preprocessing minimization and optimization. The force field of OPLS-3e was fixed. RMSD value reached 0.3 [14]. Finally, the grid was generated at the centroid of the centre in the workspace. This part is suitable for docking all ligands. The grid box ranges are X = 13; Y = 12; Z = 15; applied.

Ligand Preparation:

The 2D structures (SDF) of 60 phytoconstituents were selected from the plant *Acorus calamus*. The Ligprep tool was used in Maestro software for ligand preparation. The force filed OPLS-3e was used. The ligand was arranged in the proper bond orders by using tools. The final output minimized ligands were created. These ligands were utilized for further molecular docking analysis [15].

Docking analysis:

A molecular docking study of selected 60 phytocompounds of *Acorus calamus* medicinal plant for high though the put virtual screening was performed against the Zika NS5 polymerase domain (PDB ID: 6LD5). ZIKV with flexible Docking was conducted on Maestro between ligands and the target protein's active site. It is performed with an "extra precision" mode for the docking process. Among ligands, the best configuration with on highest docking score of virtual screening was sorted by extra precision flexible docking [16].

ADME Analysis:

The prediction of "drug-likeness" properties for selecting phytoconstituents using the Qikprop tool in the Maestro suite. Based on Lipinski's rule of five and "Rule of three" (Jorgenson's rule), selected phytoconstituents and filtered the unfit phytoconstituents [17].

RESULTS AND DISCUSSION:

Phytochemical library construction:

Maestro's modern molecular docking software did the docking studies of the ligands to active target site of protein to determine compounds' binding energy. In this study, we have collected 60 phytochemicals from the plant *Acorus calamus* and were examined to recognize the prospects of phytochemicals that can act as a drug against viral disease-causing agents. That all phytochemicals are mentioned in Table 1.

Table 1: Phytoconstituents name, PubChem Id, Molecular formula of *A.calamus* [9 and 18]

S.no	Phytoconstituent Name	PubChem ID	Molecular Formula
1	Alpha-pinene	6654	$C_{10}H_{16}$
2	Camphene	6616	$C_{10}H_{16}$
3	Myrcene	31253	$C_{10}H_{16}$
4	Alpha-limonene	440917	$C_{10}H_{16}$
5	Limonene	22311	$C_{10}H_{16}$
6	1,8-cineol	2758	$C_{22}H_{42}0$
7	Linalool	6549	$C_{10}H_{18}O_2$
8	Rosoride	27866	$C_{10}H_{18}O$
9	Citronellal	7794	$C_{10}H_{18}O$
10	alpha-terpineol	443162	$C_{10}H_{18}O$
11	(iso)-borneol	89306	$C_{10}H_{18}O$
12	beta-citronella	14535352	$C_{10}H_{20}O$
13	(2z)-2-decenal	5354834	$C_{10}H_{18}O$
14	Noral	643779	$C_{10}H_{16}O$
15	Nerol	643820	$C_{10}H_{18}O$
16	Geraniol	637566	$C_{10}H_{18}O$
17	linalool formate	61040	$C_{12}H_{20}O_2$
18	bornyl acetate	6448	$C_{12}H_{20}O_2$
19	2,4,undecadienal	5367531	$C_{11}H_{18}O$
20	2-(acetyl methyl)-3-carene	576614	$C_{13}H_{20}O$
21	Farnesan	19773	$C_{15}H_{32}$
22	alpha-duprezianene	101985700	$C_{15}H_{24}$
23	gamma-cadinene	12306058	$C_{15}H_{22}$
24	alpha-farnesene	5281516	$C_{15}H_{24}$
25	Acordiene	90351	$C_{15}H_{24}$
26	germacrene-d	91723653	$C_{15}H_{24}$
27	(e)-methylisoeugenol	637776	$C_{11}H_{14}O_2$
28	Tridecanol	8207	$C_{13}H_{26}O$
29	Cuparene	86895	$C_{15}H_{22}$
30	Pentadecane	12391	$C_{15}H_{32}$
31	Ledol	92812	$C_{15}H_{26}O$
32	caryophyllene oxide	1742210	$C_{15}H_{24}O$
33	13-tertadecenal	522841	$C_{14}H_{26}O$
34	beta-asarone	5281758	$C_{12}H_{16}O_3$
35	7-hexadecyne	549042	$C_{16}H_{30}$
36	alpha-asarone	636822	$C_{12}H_{16}O_3$
37	Heptadecane	12398	$C_{17}H_{36}$
38	cis-lanceol	6536796	$C_{15}H_{24}O$
39	Acorone	5316254	$C_{15}H_{24}O_2$

40	palmit aldehyde	984	$C_{16}H_{32}O$
41	pentadecanoic acid	167165	$C_{15}H_{30}O_2$
42	farnesyl acetone	171945	$C_{18}H_{30}O$
43	palmitic acid	985	$C_{16}H_{32}O_2$
44	Eicosane	8222	$C_{20}H_{42}$
45	2-methyleicosane	56936258	$C_{21}H_{44}$
46	Tetracosane	12592	$C_{24}H_{50}$
47	Paracymene	7463	$C_{10}H_{14}$
48	alpha-terpinen	7462	$C_{10}H_{16}$
49	beta-phellandrene	443161	$C_{10}H_{16}$
50	gamma-terpinene	7461	C10H16
51	Thujene	79017	$C_{10}H_{18}$
52	beta-sitosterol	222284	$C_{29}H_{50}O$
53	Galangin	5281616	$C_{15}H_{10}O_5$
54	Methyleugenol	7127	$C_{11}H_{14}O_2$
55	Camphor	2537	$C_{10}H_{16}O$
56	Acorenone	12480741	$C_{15}H_{24}O$
57	Shyobunone	5321293	$C_{15}H_{24}O$
58	Cryptoacorone	101861552	$C_{15}H_{24}O_2$
59	Isoshyobunone	5318673	$C_{15}H_{24}O$
60	Sesquilalavandulol	5352145	$C_{15}H_{26}O$

ADME Study Reports *Acorus calamus* Phytochemicals:

The captured selected phytochemicals were then subjected to ADME testing using Qikprop software. The forecasted ADME property of phytochemicals based on their structure, functional groups and molecular properties such as molecular weight, Central Nervous System, log HERG, QPlogBB, (#metab) several likely metabolic reactions and (% Human) Predicted human oral absorption. Few Compounds transgressed drug-likeness tests were removed as those compounds have poor ability to cross the Biological Membrane. At the end of this study, only fourteen phytochemicals passed within the limit. These selected phytocompounds are listed in Table 2.

Table 2: **Results of ADME Analysis of *A.calamus*Phytoconstituents**

Phytoconstituents name	MW	CNS	Log HERG	QP LOG BB	# metab	% HOA	Ro5	Ro3	QPlogPo/w
Alpha-pinene	136.236	2	-4.995	1.015	2	100	1	0	5.278
Methyleugenol	178.23	0	-2.662	-0.063	1	100	0	0	1.281
Myrcene	136.236	2	-3.007	0.891	1	100	0	0	4.232
Alpha-asarone	208.257	-1	-2.354	-0.444	1	92.4	1	0	1.183
Geraniol	154.252	1	-4.086	0.128	1	100	0	0	1.607
Methylisoeugenol	178.23	0	-2.383	-0.108	1	100	0	0	1.027
Neral	152.236	1	-3.026	0.138	0	100	0	0	1.639
Nerol	154.252	1	-4.19	0.122	1	100	0	0	1.572
Farnesylacetone	262.434	1	-4.766	0.22	1	100	0	1	4.289
Galangin	270.241	-2	-4.443	-1.007	3	76.8	0	0	6.524
Beta-Asarone	208.257	-1	-2.927	-0.416	1	93.36	1	0	1.341
(E)-Sesquilavandulol	222.37	1	-4.649	0.309	1	100	0	0	3.173
(Z)-2-Decenal	154.252	1	-3.738	0.096	0	100	0	0	2.133
2,4-Undecadienal	166.263	1	-3.933	0.083	0	100	0	0	2.273
limit	≤ 500	-2 to 2	< -5	-3-1.2	1-8	100	Max 4	Max3	-2 to 6

MW = Molecular Weight, **CNS**= central nervous system, **Log HERG=**Human ether –a-go-go- related gene, **QPLogBB** =Blood Brain Barrier, parameters of the compound,**# metab** = Number of metabolic reactions,**% human** = predicted human oral absorption.**Ro5**= Rule of Five, **Ro3**=Rule of Three. **QPlogPo/w**= Solubility

Molecular docking result of *Acorus calamus* phytochemicals:

In ZIKV, the C-terminal domain of the non-structural protein NS5 an essential for viral genome synthesis. It encodes with NS5-RdRP. The ZIKV NS5 protein architecture comprises three fields: fingers, thumb, and palm parallel right-handed, the latter of which contains the highly conserved aspartates that represent the active site in most of the reported flaviviral RdRps, as well as hepatitis C virus (HCV). In silico approach between selected ligands and Protein Data Bank ID: 6LD5 of the Zika virus. The good drug-likeness properties contain the ligand's docking score, Glide energy, and Glide $_{evdw}$ are shown in Table 3.

Table 3: The Docking results of *A. calamus* selected phytoconstituents

S.NO	Phytoconstituents Name	Docking score kcal/mol	Glide energy kcal/mol	Glide $_{evdw}$
1.	Galangin	-7.391	-33.166	-29.262
2.	Geraniol	-4.934	-23.337	-19.849
3.	Methylisoeugenol	-4.632	-25.83	-26.021
4.	Alpha-asarone	-4.39	-28.866	-27.964
5.	Nerol	-4.383	-23.793	-18.29
6.	Beta-Asarone	-4.319	-24.579	-24.125
7.	Methyleugenol	-4.255	-24.774	-23.923
8.	Neral	-3.761	-19.839	-18.091
9.	Myrcene	-2.899	-15.802	-15.356
10.	(E)-sesquilavandulol	-2.813	-24.842	-19.285
11.	2,4-Undecadienal	-2.066	-21.99	-19.565
12.	Farnesyl acetone	-1.928	-23.395	-23.279
13.	(Z)-2-Decenal	-1.466	-19.934	-18.413
14.	Alpha-pinene	-1.27	-13.346	-14.189
Reference drug	Sofosbuvir	-5.5	-54.668	-47.488

From the docking results, the docked ligand Galangin has a low crucial binding energy with ZIKV Polymerase. The docking score is -7.391kcal/mol, and The Galangin glide energy is -33.166kcal/mol. The significance of van der Waals was shown at -29.262kcal/mol. All docked ligands values listed and generated an H-bond bound with amino acid residue SER798 via water bridge. The two molecules of pi-pi stacking interact with ARG 731, illustrated in Figure 1. Sofosbuvir interaction with four amino acid residues involved TYR 609, ASN 612, and 2ASP 665, shown in Figure 2.

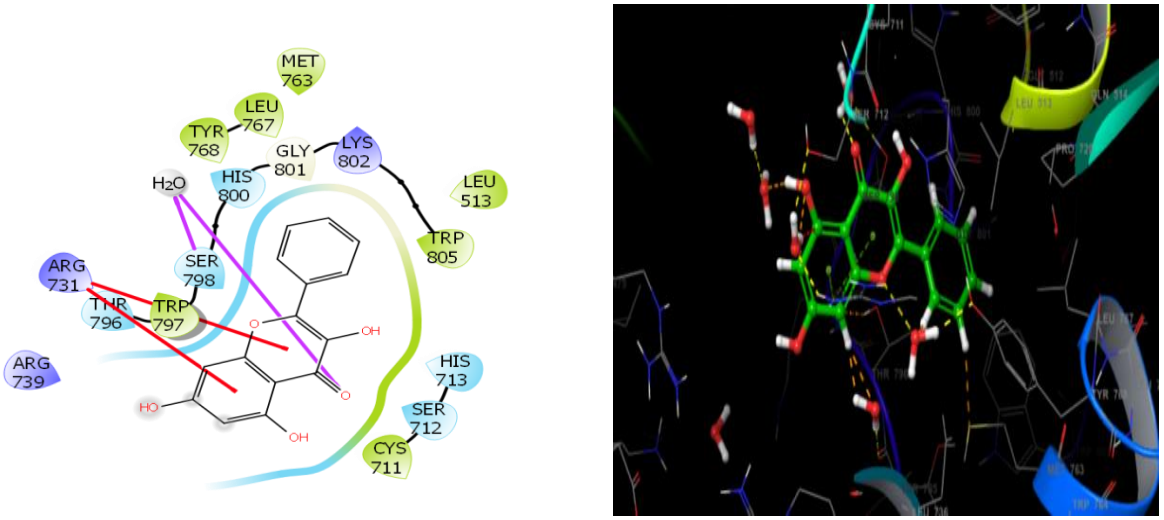

Figure 1: 2D and 3D structure of docking interaction of Galangin with Zika virus (NS5) Polymerase.

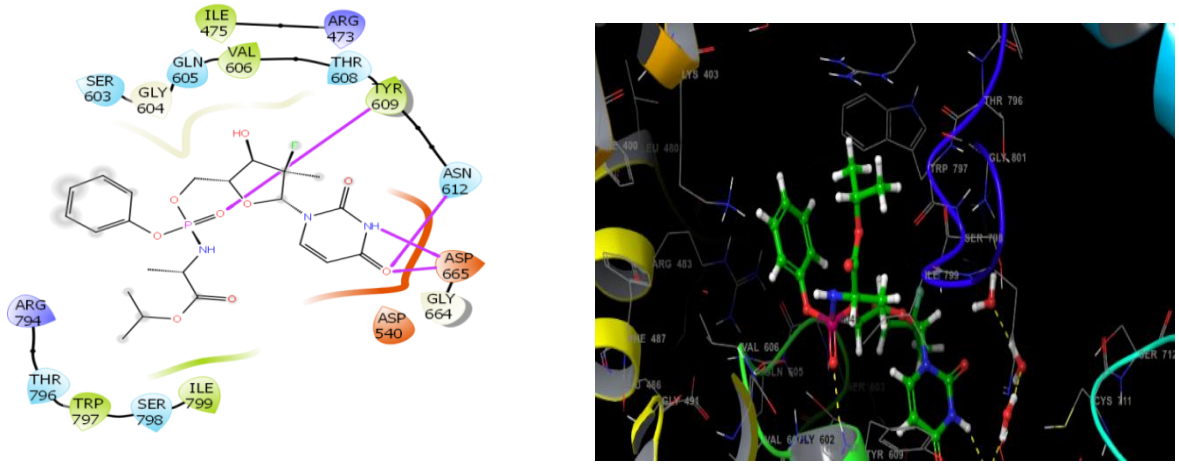

Figure 2: 2D and 3D structure of docking interaction of sofosbuvir with Zika virus (NS5) polymerase.

Compared to other Phytocompound, Galangin had low binding energy. This top hit phytocompound was compared with the standard drug sofosbuvir docking score is -5.5 kcal/mol. The docking score of Galangin contains low binding energy of -7.391 kcal/mol. Moreover, Galangin includes a molecular weight of 270.29. It can easily be transported compared to heavy molecular weight molecules. The CNS activity is inactive (-2). So it does not cause any side effects in CNS. The log HERG is also (-4.44) less than -5no cardiac toxicity. The Log BB passed within the limit. The Galangin human oral absorption was 76.8%. From the above discussion, we suggested Galangin had more effectiveness against ZIKV infection.

CONCLUSION:

The viral RNA-dependent RNA polymerase (NS5) catalytic domain, the NS2B-NS3 trypsin–serine protease and the NS3 helicase is responsible for the Zika virus. In our traditional system,

the plant *Acorus calamus* is used to treat viral diseases. This activity proved that the plant phytoconstituents act against the Zika virus. From the results, our active principle of Galangin can perform at the polymerase domain. So, we conclude that Galangin could be a promising drug candidate for suitable attachment inhibitors. Hence it can be utilized as a lead compound to control the Zika virus. Therefore, it is predicted to be fit for human consumption without side effects.

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