

Preliminary *in silico* studies of phytochemical compounds from *Vernonia amygdalina* as potential inhibitor of Chikungunya virus

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INTRODUCTION

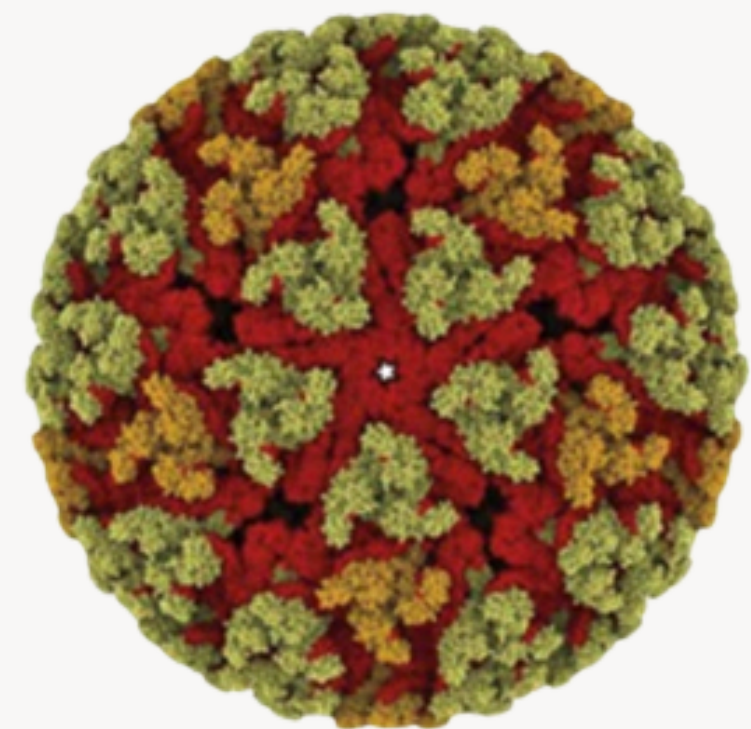


Figure 1 CHIKV virus particle

- CHIKV- mosquito-borne alphavirus causing fever, rash, and severe joint pain; severe cases occur in neonates, elderly, and those with comorbidities (Gao et al., 2019; Chan et al., 2016).
- Transmitted by *Aedes aegypti* and *Aedes albopictus* (Kovacikova & Van Hemert, 2020).
- The treatment of CHIKV infection focuses on relieving symptoms, as there is no specific cure. Majority of fatalities occur in neonates, elderly individuals, and adults with pre-existing medical conditions (Chan et al., 2016).
- A study by Chan et al., (2016) found that the ethyl acetate extract of *Vernonia amygdalina* (VA) could potentially exert a direct virucidal effect on CHIKV. This bioactivity may be attributed to the phytochemical diversity of VA, as a collection of previous studies has identified nearly 37 compounds isolated from different parts of the plant—such as its leaves, flowers, stems, and more—using techniques like nuclear magnetic resonance (NMR) and gas chromatography-mass spectrometry (GC-MS) analysis (Degu et al., 2024)

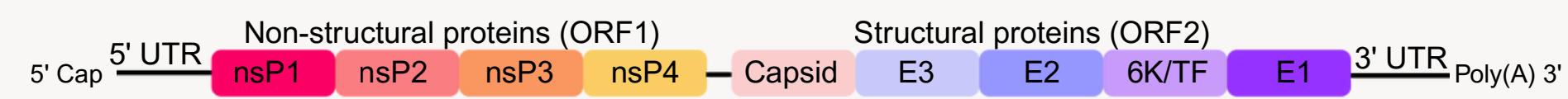


Figure 2 CHIK Virus genome

OBJECTIVE

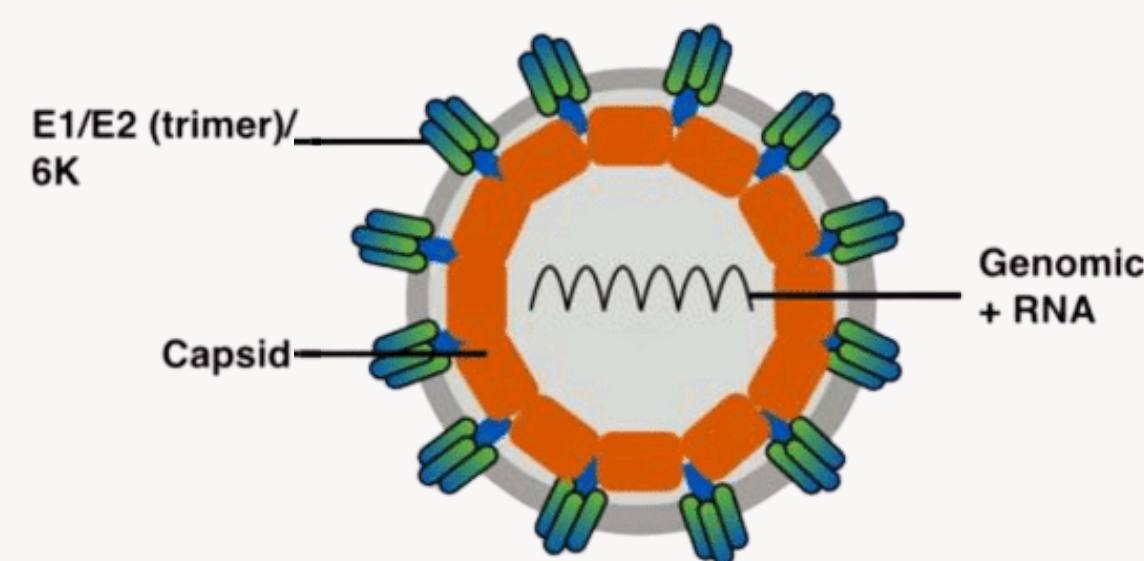


Figure 3 CHIKV structure

To evaluate the potential inhibitory effects of phytochemical compounds from VA against the non-structural protein 3 (nsP3) of the CHIKV through preliminary *in silico* studies.

SPECIFIC OBJECTIVE



METHODOLOGY

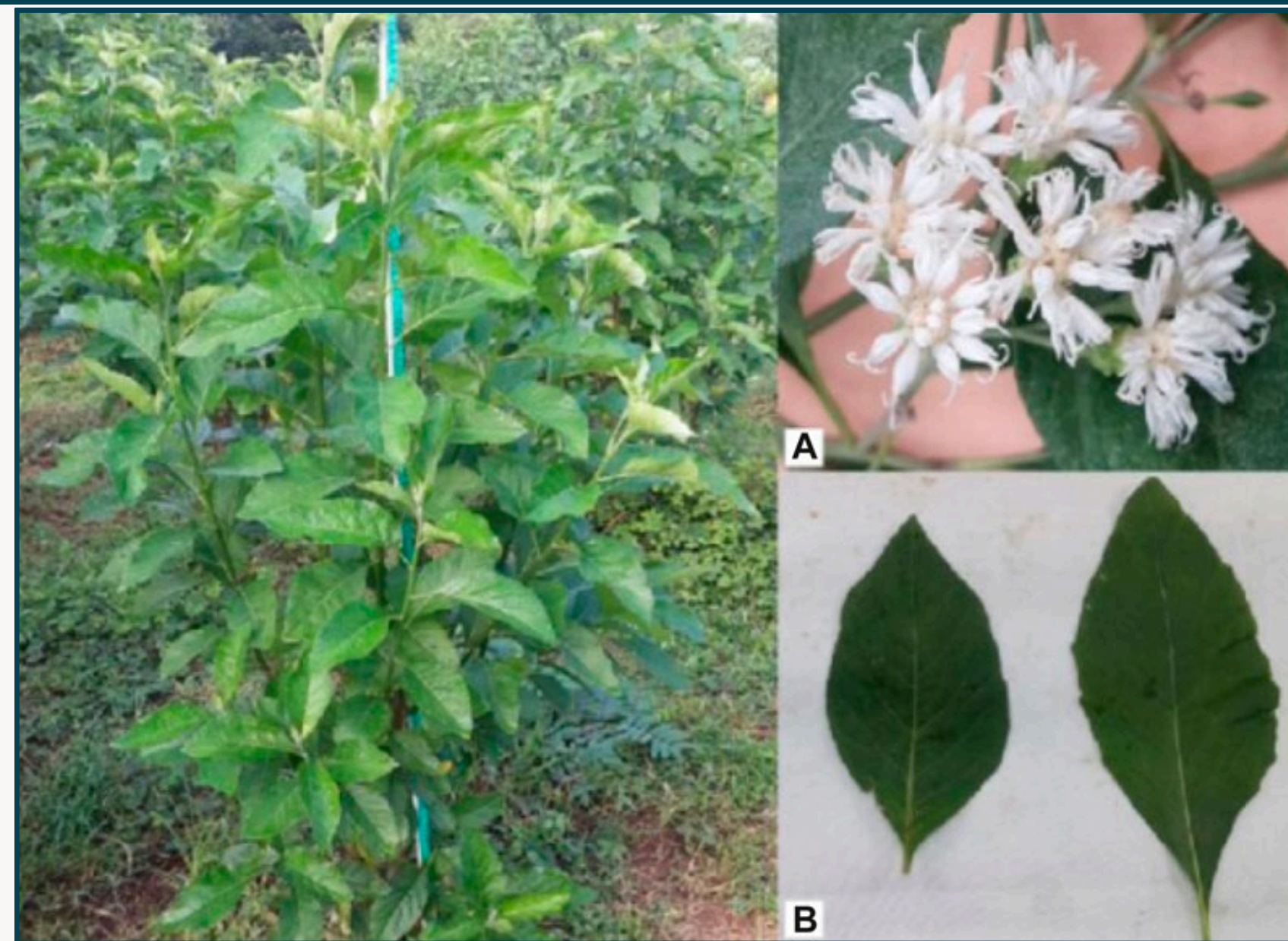


Figure 4 *Vernonia amygdalina* (VA) Tjhia et al., (2018)

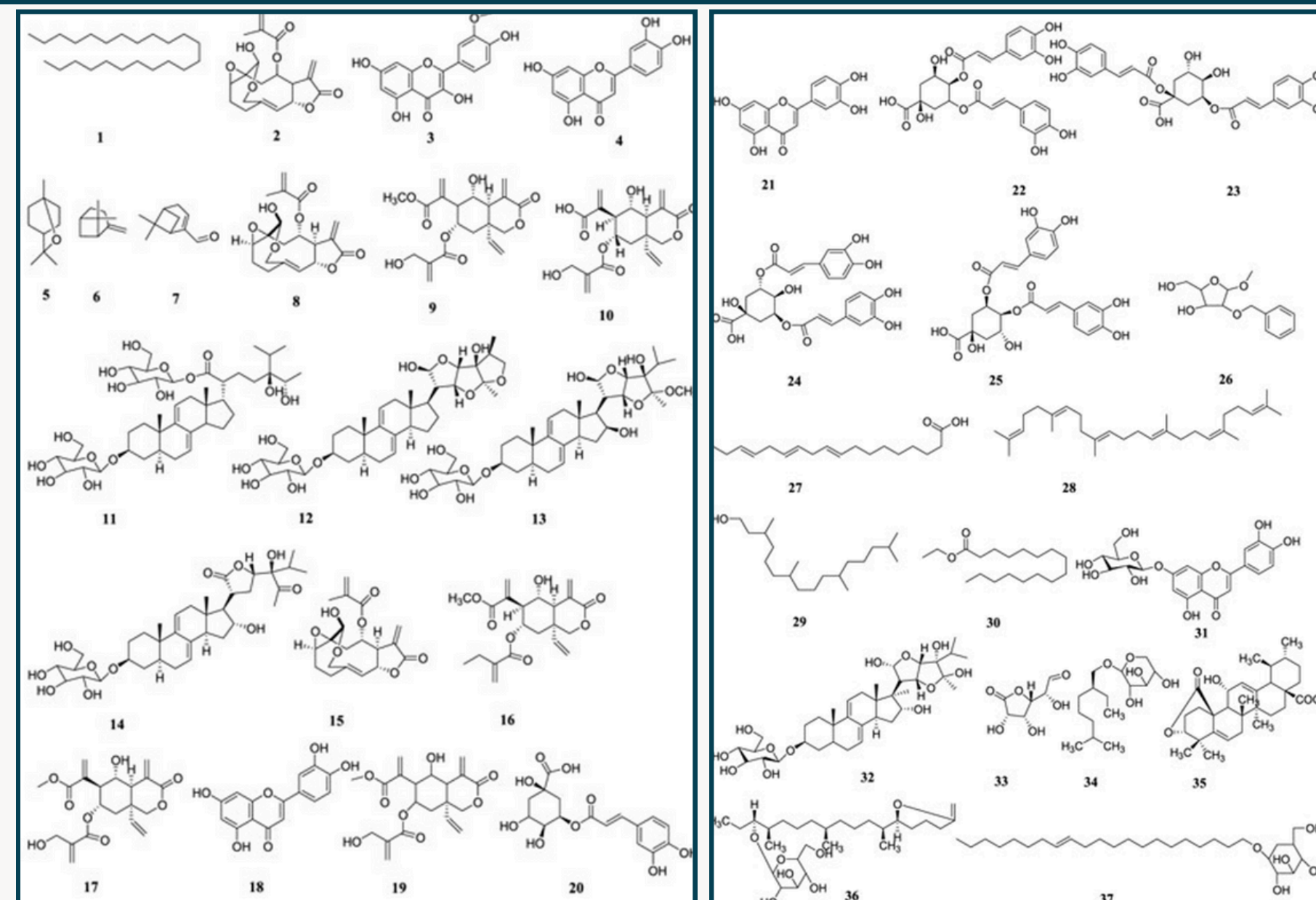


Figure 5 Chemical structures of phytochemicals from VA (Degu et al., 2024)

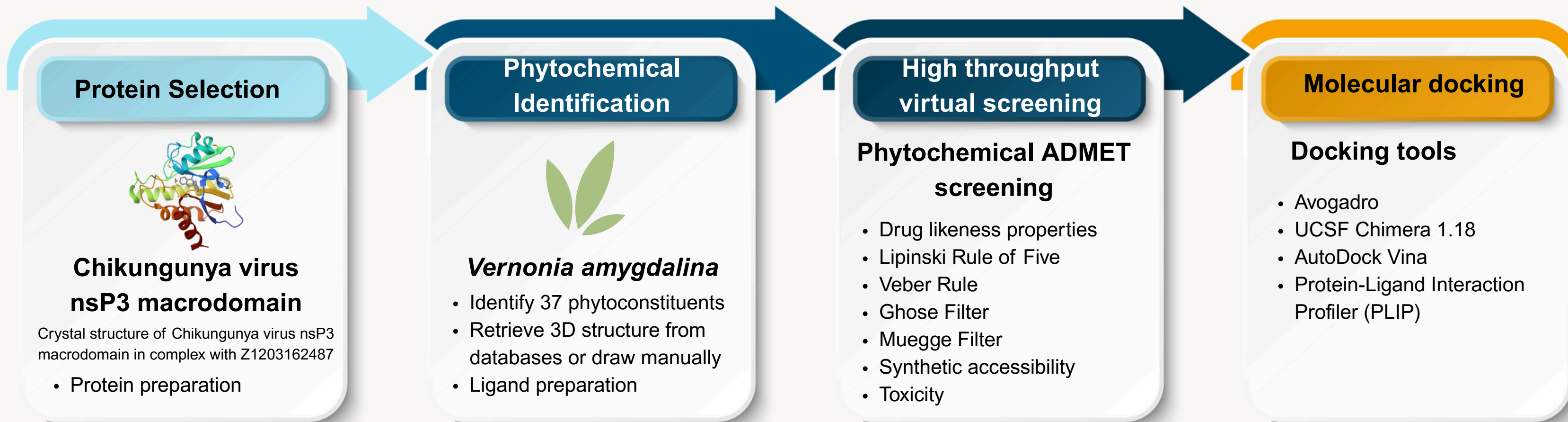


Table 1: Grid box size

Parameter	X	Y	Z
Coordinate	19.4007	7.1188	9.1839
Size	15.6569	28.6902	22.8341

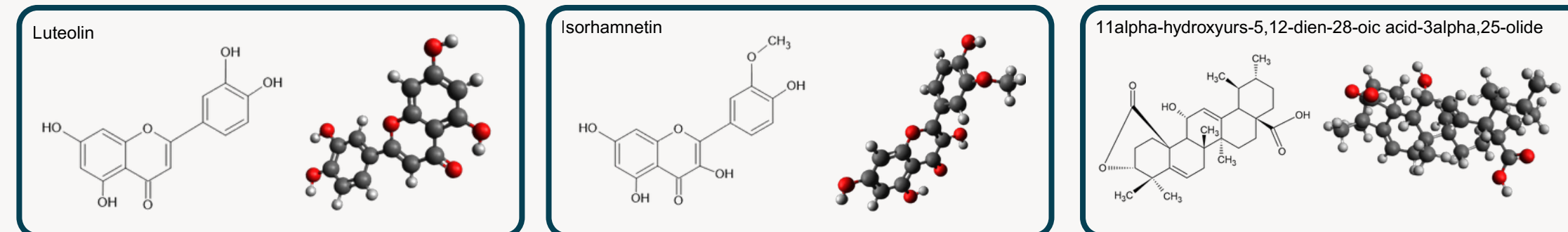


Figure 6 Chemical and 3D molecular structures of selected VA phytochemicals (National Center for Biotechnology Information, 2025)

Phytochemical Identification

- 37 phytochemical compounds from all parts of *Vernonia amygdalina* (VA) (eg: flowers, stems, leaves, and roots) were identified from previous studies, and their 3D structures and SMILES strings were retrieved from databases (eg: PubChem).
- The ChemSketch tool was used to manually draw the structures that were not available in the database. And OpenBabel GUI was utilised to generate its SMILES strings.

Protein Selection

- Crystal structure of Chikungunya virus nsP3 macromodomain in complex with Z1203162487 (CHKIV) (PDB ID: 7H9G) obtained from RCSB PDB.
- UCSF Chimera 1.18 was utilised to remove non-essential water molecules and extra subunits, add hydrogens, optimise geometry, energy minimisation, assign Gasteiger charges.
- Define grid box before removing Z1203162487.

High throughput virtual screening

- All the SMILES strings of the phytochemical compounds were inserted into SwissADME and Prottox 3.0 for ADMET screening.
- 12 ligands fulfilled the druglikeness criteria, Lipinski Rule of Five, Veber rule, Ghose Filter, Muegge Filter and subsequently underwent optimisation using Avogadro.

Molecular docking

- Performed using AutoDock Vina integrated with Chimera.
- Grid box coordinates as shown in Table 1.
- Docking simulation generated 9 poses for each ligand; lowest binding energy pose selected.

Post-Docking Analysis

- Inhibition Constant (Ki):
- Calculated from docking scores: $Ki = e^{AG/RT}$, prioritising Ki < 1 μ M.

Protein-Ligand Interaction Profiler (PLIP)

- Analysed hydrogen bonds, hydrophobic interactions, salt bridges, key residues, and interaction distances.

RESULT AND DISCUSSION

Table 2: Molecular properties and predicted toxicity of selected phytochemical compounds from VA

Phytochemical compounds	Formula	MV (Da)	H-bond acceptors ≤ 10	H-bond donors ≤ 5	CLogP ≤ 5	RB Rotatable bond ≤ 10	MR 40–130	XLOGP3 -2 to 5	TPSA $\leq 140 \text{ \AA}^2$	SA 1=easy 10=difficult	Toxicity	Docking Scores (kcal/mol)	Inhibition constant (Ki) (μ M))
11alpha-hydroxyurs-5,12-dien-28-oic acid-3alpha,25-olide	$C_{32}H_{42}O_5$	482.65	5	2	3.64	Yes	135.61	4.96	83.83	7.40	Respi, Immuno, Nutrition, PXR	-8.5	0.58
Isorhamnetin	$C_{23}H_{14}O_7$	316.26	7	4	1.65	Yes	82.50	1.87	120.36	3.26	Cardio, AhR, CYP1A2, MMP	-8.4	0.69
Luteolin	$C_{21}H_{20}O_5$	286.24	6	4	1.73	Yes	76.01	2.53	111.13	3.02	Respi, ER, AhR, TTR, CYP1A2, CYP2C19	-8.4	0.69
Luteolin hexoside	$C_{29}H_{30}O_8$	286.24	6	4	1.73	Yes	76.01	2.53	111.13	3.02	AhR, ER, ER-LBD, MMP, TTR, CYP1A2	-7.4	3.74
Vernodalol (different orientation)	$C_{20}H_{24}O_8$	392.40	8	2	1.58	Yes	98.05	1.17	119.36	4.88	Immuno, PXR, Nutrition	-7.0	7.34
Vernodalinol	$C_{19}H_{22}O_8$	378.37	8	3	1	Yes	93.73	0.85	130.36	4.77	Immuno, Nutrition, PXP	-6.9	8.69
Vernodalol (OH)	$C_{20}H_{24}O_8$	392.37	8	2	1.37	Yes	98.05	1.17	119.36	4.88	Immuno, Nutrition, PXP	-6.8	10.29
Vernodalol (CH3)	$C_{21}H_{26}O_7$	390.43	7	1	2.46	Yes	101.69	2.86	99.13	4.93	Immuno, BBB, PXR	-6.7	12.18
Epivernodalol	$C_{20}H_{24}O_8$	392.4	8	2	1.37	Yes	98.05	1.17	119.36	4.88	Immuno, PXR, Nutrition	-6.6	14.43
Ethyl-2-O-benzyl-d-arabinofuranoside	$C_{13}H_{18}O_5$	254.28	5	2	0.71	Yes	63.71	0.45	68.15	3.96	Nephro, BBB, TTR	-6.1	33.57
10-Geranylanyl-O-Beta-D-xyloside	$C_{19}H_{30}O_5$	290.40	5	3	1.66	Yes	77.76	1.94	79.15	5.16	Nephro, Cardio, NADHox	-6.0	39.75
1-Heneicosenol-O-beta-D-glucopyranoside	$C_{32}H_{62}O_5$	470.73	5	4	5.78	No	139.85	8.07	90.15	6.26	Immuno	-5.7	65.97

*MV: Molecular Weight *CLOG P: Consensus LOG P: Partition Coefficient *RB: Rotatable Bond XLOGP3: Lipophilicity *MR: Molecular Refractivity *TPSA: Total Polar Surface Area *SA: Synthetic Accessibility *BBB: Blood-brain barrier *Immuno: Immunotoxicity *ER: Estrogen Receptor Alpha *Cardio: Cardiotoxicity *AhR: Aryl hydrogen Receptors *CYP1A2: Cytochrome P450 family 1 subfamily A member 2 *MMP: Mitochondrial Membrane Potential *Respi: Respiratory toxicity *TTR: Transthyretin *CYP2C19: Cytochrome P450 family 2 subfamily C member 19 *Nutrition: Nutritional toxicity *PXP: Pregnane X receptors *ER-LBD: Estrogen Receptor Ligand Binding Domain *Nephro: Nephrotoxicity *NADHox: NADH-quinone oxidoreductase

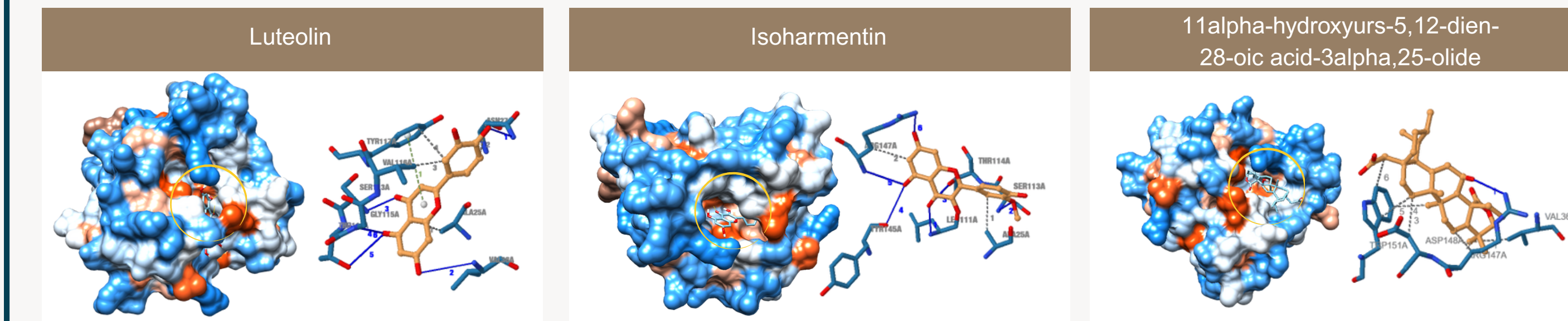


Figure 7 Docking pose and binding sites of the three selected candidates

Table 3: Selected three candidates with lowest docking scores (kcal/mol) and inhibition constant, Ki (μ M) with their respective CHIKV Protein Receptor Residue Interaction

Phytochemicals selected	Luteolin	Isoharmentin	11alpha-hydroxyurs-5,12-dien-28-oic acid-3alpha,25-olide
Docking scores (kcal/mol)	-8.4	-8.4	-8.5
Inhibition constant, Ki (μ M)	0.69	0.69	0.58
Hydrogen bond	6	6	1
Hydrophobic bond	4	2	6
Pi stacking	1	-	-

CONCLUSION AND RECOMMENDATION

- Identified **luteolin**, **isorhamnetin**, and **11alpha-hydroxyurs-5,12-dien-28-oic acid-3alpha,25-olide** as potential CHIKV inhibitors.
- **Luteolin** is the lead candidate.
- Findings support computational approaches as a **cost-effective, early-stage method for plant-based antiviral drug discovery**.
- Recommended to employ **advanced computational techniques**, such as **molecular dynamics simulations**, in conjunction with **in vitro and in vivo studies to validate the in silico findings**.

ACKNOWLEDMENT

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SCAN FOR REFERENCES

