

Metabolites profiling and molecular docking identification of putative leads from endophytic *Phyllosticta capitalensis* as modulators of key druggable structural targets of rotavirus A

Taofeeq Garuba, Rayhaan Govender, Habibah Abdulsalam Isah & Saheed Sabiu

To cite this article: Taofeeq Garuba, Rayhaan Govender, Habibah Abdulsalam Isah & Saheed Sabiu (2023): Metabolites profiling and molecular docking identification of putative leads from endophytic *Phyllosticta capitalensis* as modulators of key druggable structural targets of rotavirus A, Transactions of the Royal Society of South Africa, DOI: [10.1080/0035919X.2022.2158387](https://doi.org/10.1080/0035919X.2022.2158387)

To link to this article: <https://doi.org/10.1080/0035919X.2022.2158387>



Published online: 04 Jan 2023.



Submit your article to this journal [↗](#)



View related articles [↗](#)



View Crossmark data [↗](#)

Metabolites profiling and molecular docking identification of putative leads from endophytic *Phyllosticta capitalensis* as modulators of key druggable structural targets of rotavirus A

Taofeeq Garuba¹, Rayhaan Govender², Habibah Abdulsalam Isah¹ & Saheed Sabiu^{2*}

¹Plant Biology Department, University of Ilorin, Ilorin, Nigeria, ²Department of Biotechnology and Food Science, Faculty of Applied Science, Durban University of Technology, Durban, South Africa

*Author for correspondence Saheed Sabiu. Email: sabius@dut.ac.za

Rotavirus-induced gastroenteritis is a global health concern and the leading cause of death in low-income countries and children under the age of five. While rotavirus A (RVA) vaccines are available, there accessibility and efficacy remain challenging, especially in the third world countries. This has necessitated the need to develop therapeutics to manage RVA infection. This study investigated gas chromatography-mass spectrometry-derived *Phyllosticta capitalensis* metabolites as potential therapeutics against the key structural proteins [VP8* portion of the VP4 (spike protein), the VP7 (capsid protein), and the VP1 (RNA-dependent RNA polymerase)] of RVA using molecular docking and ADME approaches. The results revealed that four [lup-20(29)-en-one, A'-neogammacer-22(29)-ene, stigmasta-4,7,22-trien-3- α -ol, and fucosterol] of the 67 metabolites had favourable affinity for the three structural proteins and belonged to classes of metabolite of antiviral importance. Further analysis and pharmacokinetic profiling showed that the pentacyclic triterpenoids, lup-20(29)-en-one and A'-neogammacer-22(29)-ene, had the potential to be developed as antivirals against RVA infection and effort is underway in this direction.

Keywords: Anti-rotavirus metabolites; Molecular docking; Pharmacokinetics; Rotavirus; Structural proteins

INTRODUCTION

Rotavirus A (RVA) infections are a global public health concern and a leading cause of severe, dehydrating gastroenteritis in children < 5 years, accounting for approximately 128 500 deaths annually (Troeger *et al.*, 2018). In 2015 alone, an estimated 190,000 children aged < 5 years died from RVA diarrhoea, with majority of these occurring in low-income countries of Africa and Asia (GBD, 2018). While the number of RVA deaths in paediatric patients is declining globally due to vaccine introduction and use, RVA-associated gastroenteritis remains a significant health challenge in sub-Saharan Africa, where there is lack or limited access to vaccination (Troeger *et al.*, 2018). Even with access to the existing RVA vaccine(s), the efficacy has remained in the range of 50 to less than 90% in severe diarrhoea with moderate efficacy in lower socioeconomic countries (Troeger *et al.*, 2018). Another concern consistent with the available RVA vaccines is the slightly elevated risk of a rare, serious condition- intussusception, which may provoke potentially fatal bowel obstruction (Nyaga *et al.* 2020). Unfortunately, except for the application of oral rehydration therapy (ORT) for the management of acute diarrheal cases, there is lack of an FDA approved drug for treatment/management RVA- induced diarrhoea to date. Hence, the development of novel drug candidates that can stand the test of time in offering therapeutics

that will be globally acceptable, affordable, and easily accessible, is crucial. Drug development from endophytes has been demonstrated against several debilitating diseases including viral infections (Li *et al.*, 2008; Kaul *et al.*, 2012; Linnakoski *et al.*, 2018; Peters, 2020) and studies have lent credence to exploration of secondary metabolites of plants and endophytes as leads against druggable targets of diseases using computational approaches (Rajamanikyan *et al.*, 2007; Jamiu *et al.*, 2021; Shode *et al.*, 2021; Li *et al.*, 2022).

Of the druggable targets in RVA replication and pathogenesis, the structural proteins such as the VP7 (Glycoprotein [G-type]), VP4 (Protease Sensitive [P-type]) and VP1 (Viral RNA-dependent RNA Polymerase [RdRp] [R-type]) are crucial (Estes and Kapikian, 2007). While the VP7 is highly immunogenic, induces the formation of neutralising antibodies, and essential in viral attachment to the host cell (Nyaga *et al.*, 2020; Rasebotsa *et al.*, 2020), the VP4, also immunogenic, plays a major role in attachment and cellular penetration of the host cell (Jayaram *et al.*, 2004). In the presence of trypsin, VP4 splits into VP5* and VP8*, which increases the viral infectivity and penetration of the virus into the host cell (Nyaga *et al.*, 2020). Therefore, these RVA structural proteins could be said to represent attractive targets for the development of RVA therapeutics against the viral genome as they are implicated in the attachment, replication, and pathogenesis of the virus.

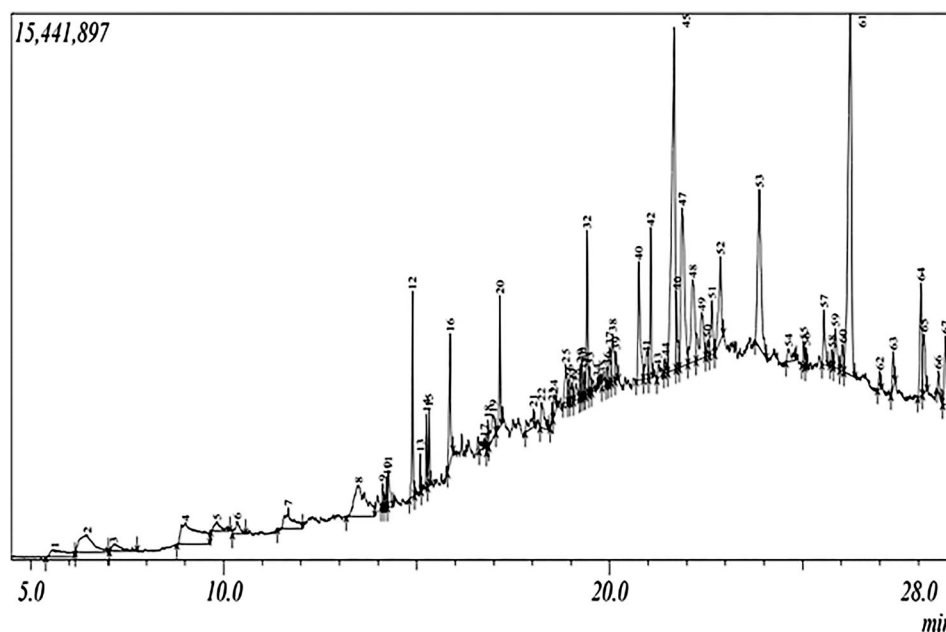


Figure 1. GC-MS chromatogram of methanol and ethyl acetate extract of endophytic *Phyllosticta capitalensis*.

As an effort to develop anti-RVA drugs, the current study adopted a computational structure–activity relationship approach exploring 67 secondary metabolites from *Phyllosticta capitalensis*—an endophytic fungus, with proven therapeutic benefits against several diseases (Zonno *et al.*, 2008; Ai *et al.*, 2014; Chukeatirote *et al.*, 2015), focusing on the key druggable RVA structural proteins.

MATERIALS AND METHODS

Collection and authentication *Phyllosticta capitalensis*

Phyllosticta capitalensis was collected from the stock in Plant Pathology Laboratory of the Department of Plant Biology,

University of Ilorin, Ilorin, Nigeria. The identification of the endophytic fungus had been previously authenticated using morphological and molecular methods (Lateef *et al.*, 2019) and kept under the voucher specimen number UIL/MYCOL-OGY2018/PM004.

Extraction and profiling of secondary metabolites from *Phyllosticta capitalensis*

Fungal inoculums obtained from pure culture of *P. capitalensis* were inoculated into 250 ml Erlenmeyer flask containing 100 ml potato dextrose broth and incubated for 18 d at $29 \pm 1^\circ\text{C}$ at 120 rpm/min with intermittent shaking.

Table 1. Docking scores of the top metabolites and reference standards against the binding pockets of the VP8*, epitopes A, C, and D of the VP7, and the VP1.

		Docking score (kcal/mol)				
Rank	Compound	VP8*		VP7		VP1
			A	C	D	
1	lup-20(29)-en-3-one	−7.1	−5.1	−7.9	−7.2	−10.5
2	A'-neogammacer-22(29)-ene	−6.0	−5.1	−8.0	−6.7	−10.5
3	stigmasta-4,7,22-trien-3-α-ol	−6.2	−5.1	−7.3	−6.2	−9.3
4	Fucosterol	−6.0	−5.6	−7.3	−6.2	−9.1
5	Stigmasterol	—	−5.4	−7.5	−6.3	−9.0
6	stigmasta-5,22-dien-3-ol, acetate, (3-β)-	—	−5.0	—	−6.2	−9.1
7	β-amyrin	−6.8	−5.0	−8.2	—	—
8	4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b	−6.3	-	−8.0	−6.5	—
9	campesterol	—	—	—	—	−8.9
10	γ-tocopherol	—	−5.6	—	—	—
11	9,19-cyclolanostan-3-ol, 24-methylene-(3-β)-	—	−5.4	—	—	—
12	cyclohexane, [6-cyclopentyl-3-(3-cyclopentylpropyl)hexyl]	—	−5.2	—	—	—
13	benzeneacetic acid, 3-pentadecyl ester	—	−5.1	—	—	—
14	bis (cyclohex-3-enylmethyl) amine	—	−5.1	—	—	—
control	sofosbuvir	—	—	—	—	−7.7
control	remdesivir	—	—	—	—	−7.5
control	tizoxanide	−5.4	−4.4	−5.9	−4.6	-

—: Not applicable

The fungal culture was filtered. Equal volume of methanol was added and homogenised. The secondary metabolites were extracted with ethyl acetate (1:1 ratio). The organic phase was evaporated and stored at 4°C for further use (Garuba *et al.*, 2018b).

Subsequently, the metabolite profiling of the extract obtained was achieved using Gas Chromatography- Mass Spectrometry (GCMS) as described by Garuba *et al.* (2018a). Briefly, the homogenised extract of *P. capitalensis* was treated with methanol until it was fully dissolved and the GCMS analysis was carried out using Agilent Technologies 7890A Gas Chromatograph equipped with a mass detector Agilent Technologies 5975C inert MSD with Tripple-Axis Detector, 30 m × 0.25 mm ID × 1 µm of capillary column. The instrument was set to an initial temperature of 110°C and maintained at this temperature for 2 min. At the end of this period the oven temperature rose to 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow as one ml/min. The ionisation voltage was 70 eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45–450 m/z. Using computer searches on a National Institute Standard and Technology (NIST v2.1 MS) data library and comparing the spectrum obtained through GC-MS, compounds present in the sample were identified. The names, retention time, molecular formula of the components of the test materials were ascertained.

In silico evaluation

Ligand and protein acquisition and preparation

The 3D structures of the 67 ligands (metabolites from GC-MS analysis) were obtained from PubChem [<https://www.pubchem.ncbi.nlm.nih.gov>] (accessed on 23 July 2022)]. For ligands without 3D representations their 2D structures were converted to the optimised 3D versions using Avogadro software v1.95 (Hanwell *et al.*, 2012). For the structural proteins, the crystal structures of the VP8* (ID: 4DS0) portion of the VP4, VP1 (ID: 3FMG) and VP7 (ID: 2R7Q) were obtained

from the RSCB Protein Data Bank [<https://www.rcsb.org/pdb/>] (accessed on 23 July 2022)] (Lu *et al.*, 2008; Aoki *et al.*, 2009; Hu *et al.*, 2012) and prepared through the removal of nonstandard molecules and chains using UCSF Chimera v1.16 (Butt *et al.*, 2020). Thereafter, the proteins, ligands, and reference standards (tizoxanide for the VP8* and VP7 epitopes, and sofosbuvir and remdesivir for the VP1) were optimised using PyRx v0.8 (Dallakyan and Olson, 2015).

Grid preparation and molecular docking

The amino acid residues of the binding pocket of VP8* were elucidated from the A-type histo-blood group antigen - VP8* co-crystal using Discovery Studio 2021, whilst those for the VP7 epitopes and VP1 active site were acquired from literature (Lu *et al.*, 2008; Aoki *et al.*, 2009; Ghosh *et al.*, 2012; Hu *et al.*, 2012; Sabiu *et al.*, 2021) and presented in Supplementary Table S1. Thereafter, the ligands and reference standards were docked with the respective protein target at their binding pockets using the Autodock vina plugin in PyRx v0.8 (Dallakyan and Olson, 2015). The individual ligand poses for each metabolite and protein structure were evaluated based on estimated free binding energy ($\Delta G \rightarrow$ kcal/mol) within the binding pocket. Thereafter, a comparative analysis was done to determine which ligands interacted strongly and efficiently with the VP8*, VP7 and VP1 and the intermolecular interactions between the top-ranking ligands across the structural protein binding pockets were visualised using Discovery Studio 2021 to determine the nature and strength of the interactions (Sabiu *et al.*, 2021).

Autodock vina validation

The molecular docking validation was done to ensure the confidence of the docking scores and intermolecular interactions obtained. Briefly, the top-ranked metabolites and reference standards were re-docked across the binding pockets of each structural protein as earlier reported (Dallakyan and Olson, 2015) before superimposition on UCSF Chimera v1.16 (Butt *et al.*, 2020) and the results obtained

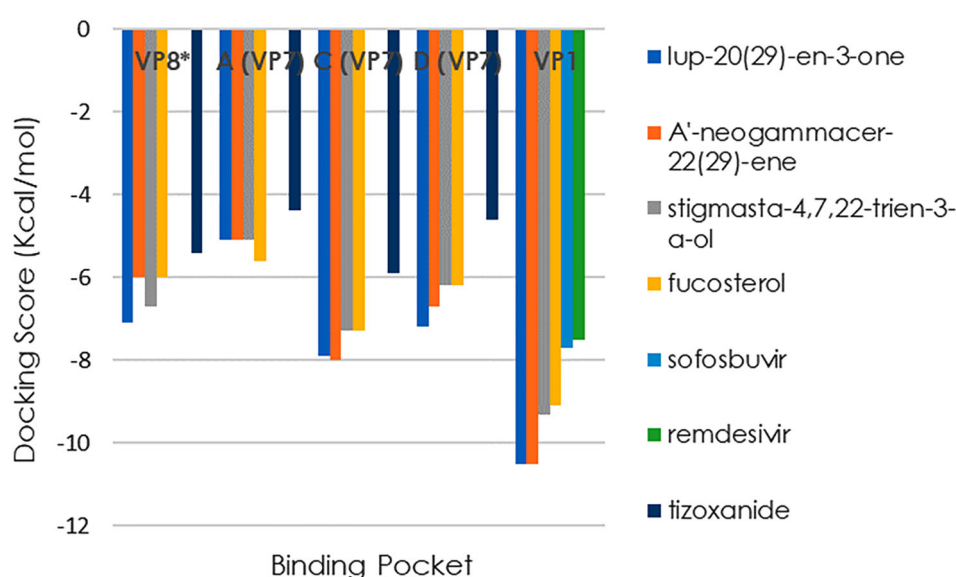


Figure 2. Docking scores of the four putative leads and reference standards against the binding pockets of the VP8*, VP1 and epitopes A, C, and D of the VP7.

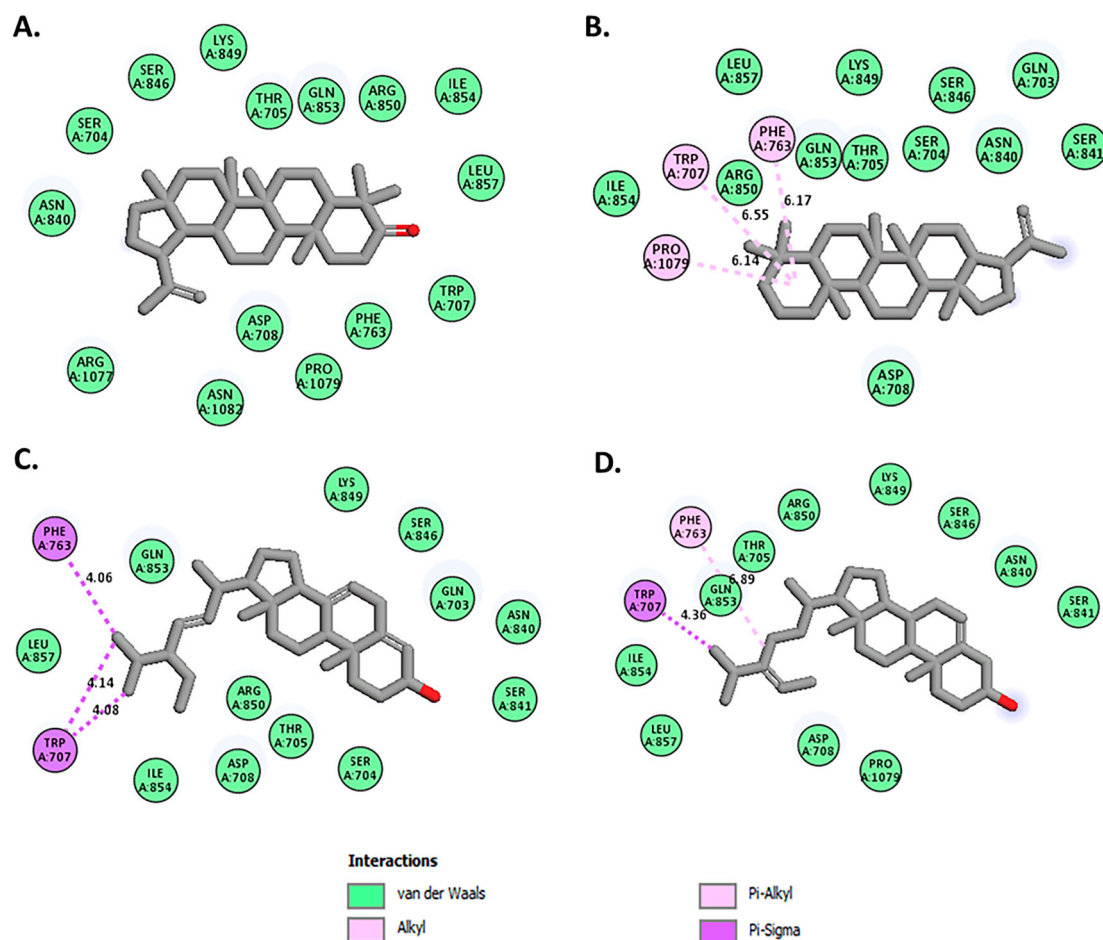


Figure 4. 2D interaction plots of (A) lup-20(29)-en-one, (B) A'-neogammacer-22(29)-ene, (C) stigmasta-4,7,22-trien-3 α -ol, and (D) fucosterol interacting with the active site amino acid residues of the VP1.

were evaluated on the accepted range of 0–2 Å deviation (Diller and Merz, 2001).

Pharmacokinetic evaluation

The SwissADME web tool [<http://swissadme.ch/index.php>] (accessed on 03 August 2022) was utilised to predict the absorption, distribution, metabolism, and excretion (ADME) properties of the putative leads and reference standards, while the ProTox-II webserver [https://tox-new.charite.de/protox_II/] (accessed on 03 August 2022) was used to determine their toxicity profiles (Aribisala *et al.*, 2021).

RESULTS AND Discussion

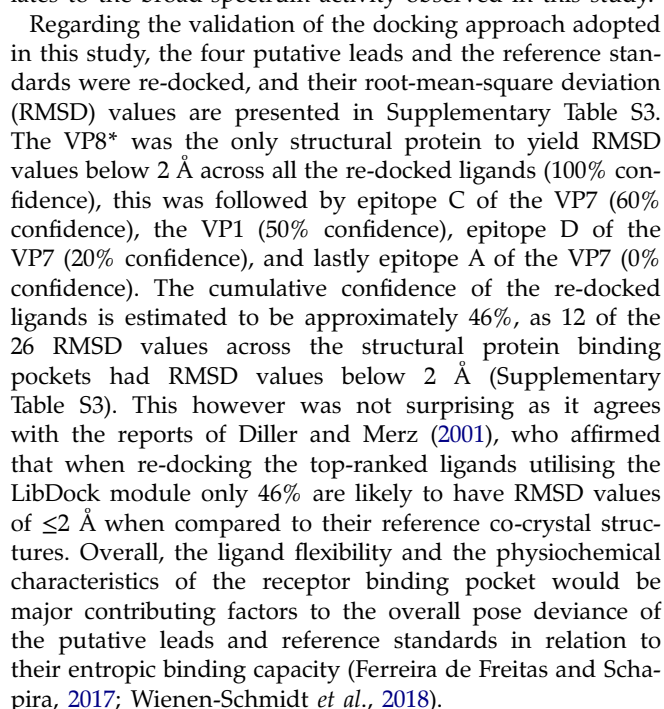
GS-MS analysis

The GC-MS chromatogram of *Phyllosticta capitalensis* extract, showed the presence of 67 secondary metabolites with phytol (9.57%) being the most abundant, followed by β -sitosterol (9.08%) (Figure 1, Supplementary Table S2).

Molecular docking analyses

Molecular docking provides insight into the interplay between a ligand and a receptor molecule. This is measured by assessing the possible poses a ligand can take within a designated binding pocket and ranking them from the highest to lowest negative value (Aribisala and Sabiu, 2022). The pose

ranking is done to determine the ligand conformation with the best affinity (lowest free binding energy) to the receptor in relation to the physical forces that mediate the ligand–receptor interactions (Kitchen *et al.*, 2004). The docking scores of all the metabolites identified in the GC-MS analysis are presented in Supplementary Table S2. Thereafter, the metabolites were ranked based on the top five docking scores within a binding pocket, and their occurrence within the top five docking scores across the binding pockets (Table 1). The docking scores per binding pocket ranged from –7.1 to –6.0 kcal/mol, –5.6 to –5.0 kcal/mol, –8.2 to –7.3 kcal/mol, –7.2 to –6.2 kcal/mol, and –10.5 to –8.9 kcal/mol against VP8*, epitopes A, C, and D of the VP7, and VP1, respectively (Table 1). The docking scores of the reference standards used in this study are also presented in Table 1 with sofosbuvir and remdesivir scoring –7.7 and –7.5 kcal/mol for VP1, respectively, and tizoxanide scoring –5.4, –4.4, –5.9, and –4.6 kcal/mol for VP8* and epitopes A, C, and D of the VP7, respectively. It was interesting to note that the docking scores of the ranked metabolites were far greater than that of the reference standards. The nucleotide analogues, sofosbuvir and remdesivir, were chosen for the VP1 standard as they can disrupt the RdRp of RNA viruses (De Clercq, 2004), while the other reference standard, tizoxanide was selected as it is the bioactive form of nitazoxanide which has been proven to have the potential to manage RVA infection (Rossignol *et al.*, 2006).



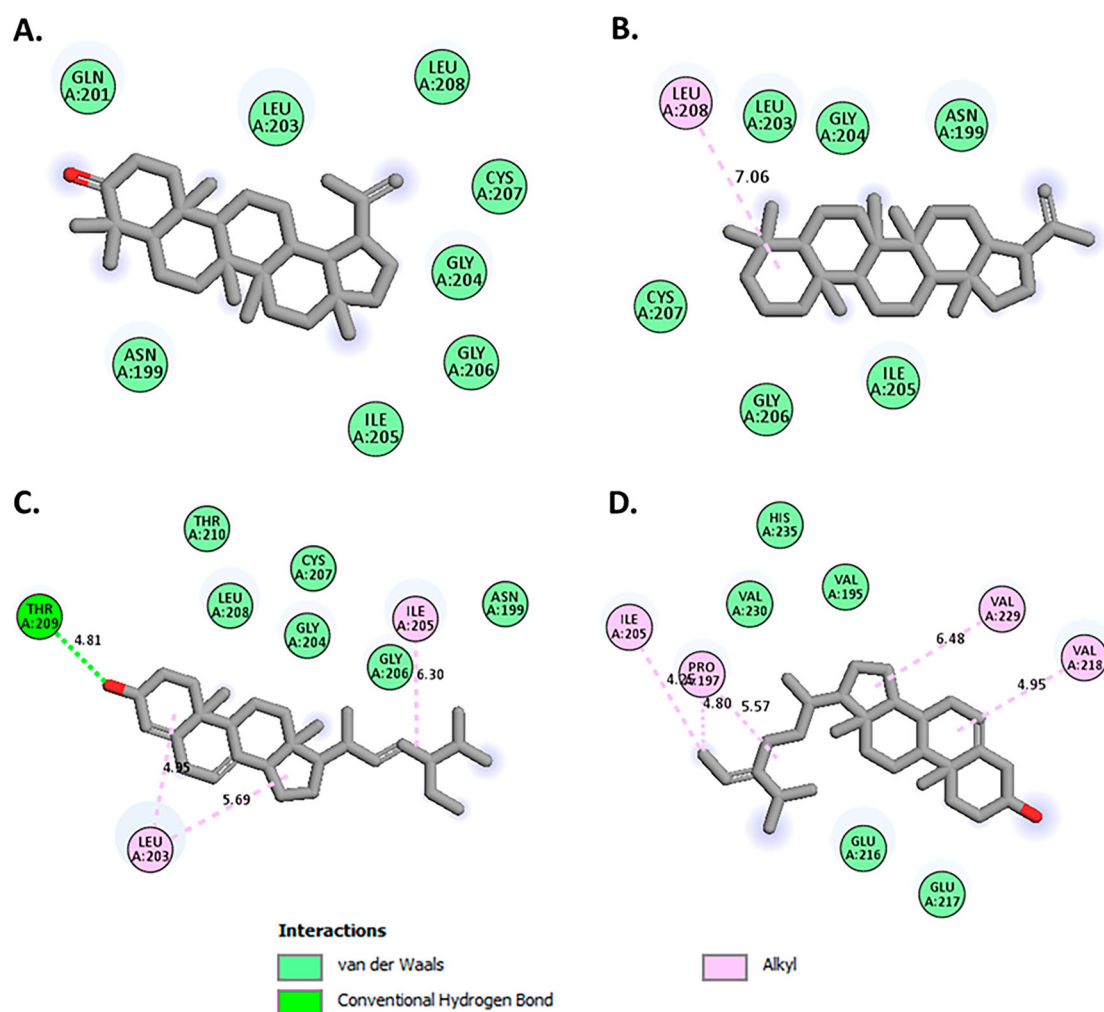


Figure 5. 2D interaction plots of (A) lup-20(29)-en-one, (B) A'-neogammacer-22(29)-ene, (C) stigmasta-4,7,22-trien-3 α -ol, and (D) fucosterol interacting with the VP7 amino acid residues of epitope A.

The intermolecular interactions that mediate the binding affinity and overall inhibitory potential of the putative leads are presented in Figures 3–7. Different degrees of bond interactions between the ligands and the amino acid residues such as van der Waals, alkyl, π -alkyl, π -sigma, hydrogen bonding, and carbon–hydrogen bonding were observed with most of them being van der Waals and hydrophobic. It has been demonstrated that weak intermolecular interactions (i.e., hydrophobic) play a pivotal role in the energetic stabilisation of a ligand on a protein interface. Similarly, hydrophobic ligands, which is the case for the putative leads, bind to their target at a higher efficiency than that of their hydrophilic counterparts (Ferreira de Freitas and Schapira, 2017). The docking score of lup-20(29)-en-one for the VP8* was -7.2 kcal/mol which is comparatively higher than that of the other putative leads (Figure 2). The ligand–receptor complex was stabilised by eight van der Waals interactions (Leu101, Ile144, Leu146, Ser187, Tyr188, Tyr189, Leu190 and Thr191) and one π -alkyl interaction (Tyr155) as shown in Figure 3(A). The overall number and positioning of these interactions could have contributed to why lup-20(29)-en-one yielded such a high docking score relative to the other putative leads (Figure 3). A similar trend was observed in the interactions between the VP1 and lup-20(29)-en-one where the

15 van der Waals interactions (Ser704, Trp707, Asp708, Phe763, Asn840, Ser846, Lys849, Arg850, Gln853, Ile854, Leu857, Thr705, Arg1077, Pro1079 and Asn1082) appear to encircle the ligand thus facilitating its relatively high docking score (Figure 4(A)). A'-neogammacer-22(29)-ene which had the same docking score as lup-20(29)-en-one (-10.5 kcal/mol) for the VP1 appeared to have been stabilised by the alkyl and π -alkyl forces (Trp707, Phe763 and Pro1079) exerted on its hexane ring fixing it within the binding pocket (Figure 2, Figure 4(B)). Similarly, most of the interactions exerted on the putative leads for the VP7 epitopes are typically van der Waals and hydrophobic, thus this could have contributed to affinity, strength, and stability of the resulting complexes (Figures 5–7). The results above coincide with that of previous *in silico* studies detailing the intermolecular interactions between pentacyclic triterpenoids and sterols against their respective receptors. A study by Chrobak *et al.* (2021) demonstrated that their top five 3,28-O, O'-diacetylbetulin derivatives were stabilised largely by van der Waals and hydrophobic interactions against the Akt1 kinase. Another study by Raju *et al.* (2021) revealed that 7-dehydrosiosgenin had the highest binding score (-10 kcal/mol) across three protein targets and among the top-ranked phytosterols when bound to the epidermal growth factor

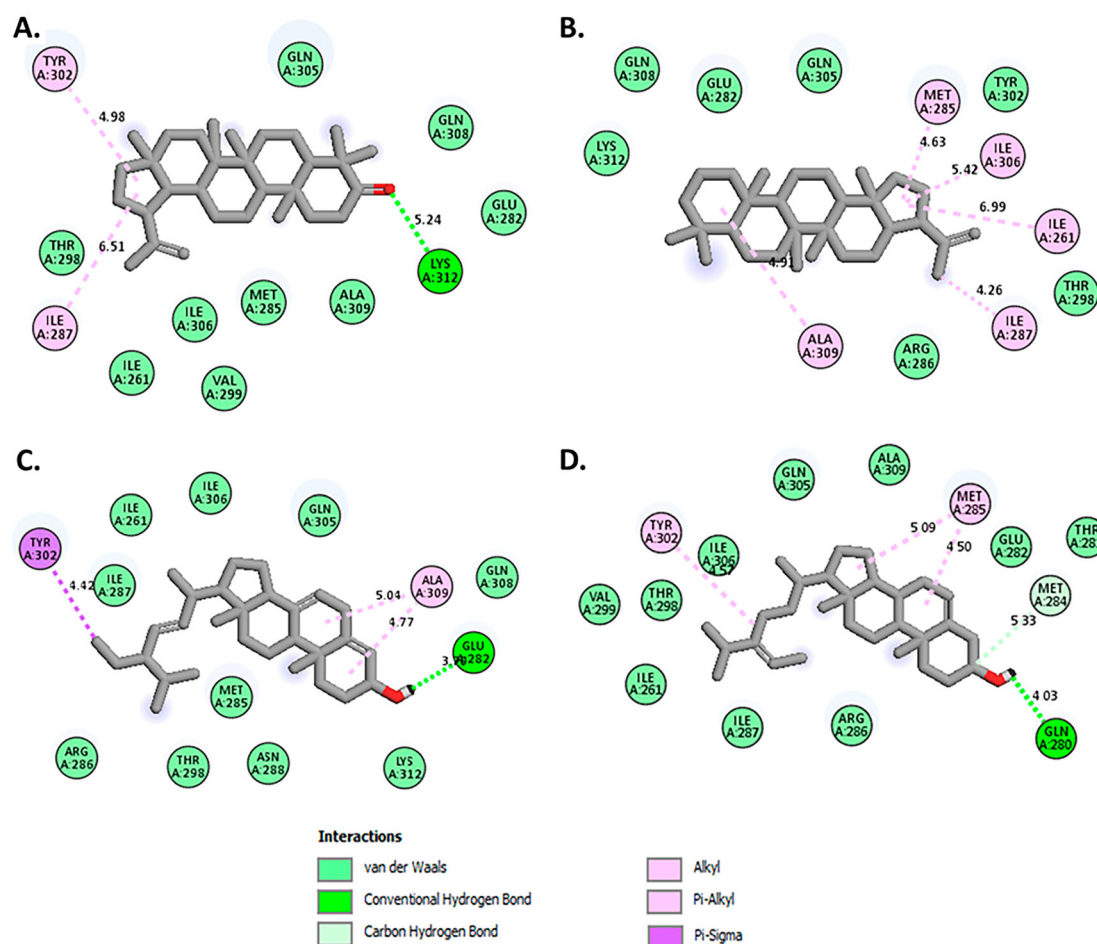


Figure 6. 2D interaction plots of (A) lup-20(29)-en-one, (B) A'-neogammacer-22(29)-ene, (C) stigmasta-4,7,22-trien-3 α -ol, and (D) fucosterol interacting with the VP7 amino acid residues of epitope C.

receptor; furthermore the complex was solely stabilised by ten van de Waals interactions. Considering the foregoing, it could be suggested that lup-20(29)-en-one and A'-neogammacer-22(29)-ene have the potential to be developed as probable therapeutics against RVA, with lup-20(29)-en-one capable of modulating the VP8*, epitope D of the VP7, and the VP1 and A'-neogammacer-22(29)-ene exhibiting modulatory effect towards epitope C of the VP7 and the VP1.

Pharmacokinetic properties

The classical drug discovery pipeline is often bottlenecked by compounds that perform outstandingly in *in vitro* studies but fail to meet the required attributes needed for a drug to be suitable for administration. Therefore, the use of *in silico* pharmacokinetic screening can drastically hasten the process and minimise factors such as cost and time by screening the compound's likeness against a library of compounds with known attributes (Ekins *et al.*, 2007). In this study, the four putative leads (lup-20(29)-en-one, A'-neogammacer-22(29)-ene, stigmasta-4,7,22-trien-3 α -ol, and fucosterol) were subjected to Lipinski's rule of five (Lipinski *et al.*, 2001), to estimate their physiochemical properties as potential oral drugs relating to absorption and permeation within the body. The results revealed that their molecular weights (<500 g/mol), topological surface areas (<140 Å²), number of hydrogen bond donors (<5) and acceptors

(<10), as well as the number of rotatable bonds (<10) were all within the acceptable limit (Table 2). However, there was a single violation across the putative leads as their lipophilicity (mlogP) was above the limit of 5 (Table 2). The absorption and permeation characteristics of the reference standards (sofosbuvir, remdesivir, and tizoxanide) on the other hand revealed that sofosbuvir and remdesivir violated four of the five rules as their molecular weights, topological surface areas, number of hydrogen bond acceptors, and number of rotatable bonds exceeded the acceptable limit (Table 2). However, the inverse was observed with tizoxanide as the compound obeyed all the rules (Table 2). These results coincide with the predicted bioavailability scores of the putative leads and reference standards. The scores for the putative leads and tizoxanide were 0.55, and this could be an indication that these compounds have a moderate level of distribution within the body (Table 2). The reason why tizoxanide had a moderate level of bioavailability despite obeying Lipinski's rule of five could be due to its topological surface area being extremely close to 140 Å² (Table 2). The other two reference standards, sofosbuvir and remdesivir, had an extremely low bioavailability of 0.17, and was highly expected due to their multiple Lipinski violations (Table 2). Potential drug candidates that exhibit low to moderate levels of bioavailability are often administered at higher doses or augmented by drug

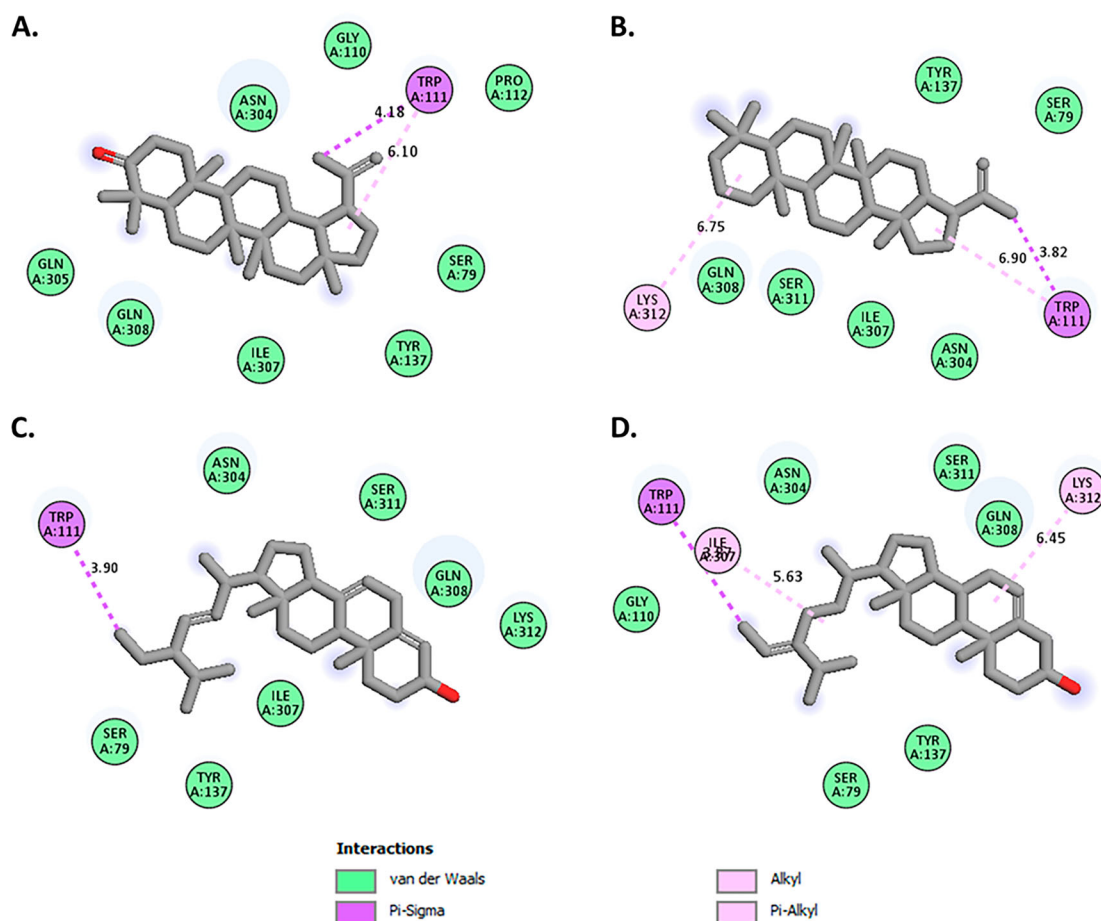


Figure 7. 2D interaction plots of (A) lup-20(29)-en-one, (B) A'-neogammacer-22(29)-ene, (C) stigmasta-4,7,22-trien-3 α -ol, and (D) fucosterol interacting with the VP7 amino acid residues of epitope D.

delivery systems to increase the likelihood of them reaching their target location (Alqahtani *et al.*, 2021).

The molecular weight and hydrogen bond characteristics of a compound tend to determine its passive cell membrane penetration and blood-brain barrier (BBB) permeation, respectively, while its lipophilicity largely determines its overall toxicity as there is an increased likelihood of the compound binding to unwanted cellular components (Meanwell, 2011; Aribisala *et al.*, 2021). A study by Hughes *et al.* (2008) demonstrated that lipophilic compounds tend to have a higher degree of promiscuity and are more likely to cause toxic side effects. The toxicity profiles of the putative leads and reference standards are described in Table 3. The pentacyclic triterpenoids, lup-20(29)-en-one and A'-neogammacer-22(29)-ene, had a favourable toxicity class score of 5 and a high predicted LD₅₀ of 5000 mg/kg (Table 3). They both also exhibited no predicted hepatotoxicity, immunotoxicity, cytotoxicity, mutagenicity, and carcinogenicity hence making them potentially safe for administration (Table 3). The sterols on the other hand were predicted to be immunotoxic, with stigmasta-4,7,22-trien-3 α -ol having a toxicity class score of 5 and a predicted LD₅₀ of 2100 mg/kg, and fucosterol having a toxicity class score of 4 and a predicted LD₅₀ of 890 mg/kg (Table 3). The immunotoxic potential of the sterols could be a result of their immunomodulating capacity which appears to be highly prevalent in

this metabolite class (Salehi *et al.*, 2021). A study by Desai *et al.* (2009) demonstrated that when peripheral blood mononuclear cells from individuals with multiple sclerosis were cultured with 4 and 16 μ M of β -sitosterol, there was a 27% and 30% decrease in the T-helper cell 1-stimulating cytokine interleukin 12, respectively. While this may prove beneficial for individuals with autoimmune and inflammatory diseases, healthy individuals could be at risk of a dampened immune system if dosages of the drug are too high. The nucleotide analogues, sofosbuvir and remdesivir, had variable toxicity profiles with sofosbuvir having a toxicity class score of 6, a predicted LD₅₀ of 12000 mg/kg, and the potential to be hepatotoxic, while remdesivir had a toxicity class score of 4, and a predicted LD₅₀ of 1000 mg/kg (Table 3). The other reference standard, tizoxanide, had a toxicity class score of 4, a predicted LD₅₀ of 1000 mg/kg, and proved to have the potential to be mutagenic and carcinogenic (Table 3).

Metabolism and potential interactions a drug may have with cytochrome P450 (CYP) enzymes is an important parameter used to determine a compound's clearance and toxicity (McDonnell and Dang, 2013). The potential inhibitory effect of the putative leads and reference standards are described in Table 2. Among the compounds, stigmasta-4,7,22-trien-3 α -ol, sofosbuvir, and remdesivir displayed potential inhibitory activity against the CYP enzymes as

Table 2. ADME and CYP-toxicity profiles of the investigated putative leads and reference standards.

Ligands							
Parameters	lup-20(29)-en-one	A'-neogammacer-22(29)-ene	stigmasta-4,7,22-trien-3- α -ol	fucosterol	sofosbuvir	remdesivir	tizoxanide
Molecular weight (g/mol)	424.70	410.72	410.67	412.69	529.45	602.58	265.25
Topological surface area ($\text{\AA}^2$)	17.07	0.00	20.23	20.23	167.99	213.36	136.28
Number of hydrogen bond donors	0	0	1	1	3	4	2
Number of hydrogen bond acceptors	1	0	1	1	11	12	5
Number of rotatable bonds	1	1	5	5	11	14	4
Lipophilicity (mlogP)	6.82	8.91	6.53	6.62	0.82	0.18	-0.21
Bioavailability score	0.55	0.55	0.55	0.55	0.17	0.17	0.55
CYP1A2 inhibition	No	No	No	No	No	No	No
CYP2C19 inhibition	No	No	No	No	No	No	No
CYP2C9 inhibition	No	No	Yes	No	No	No	No
CYP2D6 inhibition	No	No	No	No	No	No	No
CYP3A4 inhibition	No	No	No	No	Yes	Yes	No
Synthetic accessibility	5.38	5.59	6.19	6.15	6.12	6.43	2.63

Table 3. Toxicity profiles of the putative leads, and reference standards.

Ligands							
Parameters	lup-20(29)-en-one	A'-neogammacer-22(29)-ene	stigmasta-4,7,22-trien-3- α -ol	fucosterol	sofosbuvir	remdesivir	tizoxanide
Toxicity class	5	5	5	4	6	4	4
Predicted LD ₅₀ (mg/kg)	5000	5000	2100	890	12000	1000	1000
Hepatotoxicity	No	No	No	No	Yes	No	No
Immunotoxicity	No	No	Yes	Yes	No	No	No
Cytotoxicity	No	No	No	No	No	No	No
Mutagenicity	No	No	No	No	No	No	Yes
Carcinogenicity	No	No	No	No	No	No	Yes

the metabolite displayed inhibition against CYP2C9 and the reference standards showed inhibition against CYP3A4 (Table 2). The inhibition of CYP enzymes could pose a grave issue if the administered drug is co-administered with other drugs resulting in drug-drug interactions and an increase in serum concentrations of the competing drug (Aribisala *et al.*, 2021). The synthetic accessibility of the investigated metabolites is moderate and are ranked as follows: lup-20(29)-en-one (5.38) > A'-neogammacer-22(29)-ene (5.59) > fucosterol (6.15) > stigmasta-4,7,22-trien-3- α -ol (6.19) (Table 2). The cumulative pharmacokinetic data between the putative leads and reference standards indicates that lup-20(29)-en-one and A'-neogammacer-22(29)-ene have the potential to be developed into therapeutic agents to treat RVA infection due to their favourable ADME and toxicity profiles.

CONCLUSION

In the present study, the metabolites from *P. capitalensis* were elucidated through GC-MS analysis and docked against the major RVA structural proteins. The four putative leads (lup-20(29)-en-one, A'-neogammacer-22(29)-ene, stigmasta-4,7,22-trien-3- α -ol, and fucosterol) of the 67 metabolites outclassed

the reference standards and exhibited potential broad-spectrum activity against RVA. Majority of the intermolecular interactions were mediated by van der Waals and hydrophobic interactions and the pentacyclic triterpenoids (lup-20(29)-en-one and A'-neogammacer-22(29)-ene) had the most promising binding affinities of the four putative leads. The ADME and toxicity profiles of the putative leads proved to be more favourable than those of the reference standards, however they exhibited a high degree of lipophilicity. The sterols (stigmasta-4,7,22-trien-3- α -ol and fucosterol) were predicted to be immunotoxic and it was also observed that stigmasta-4,7,22-trien-3- α -ol had the potential to be a CYP2C9 inhibitor. However, based on the cumulative data lup-20(29)-en-one and A'-neogammacer-22(29)-ene look to be extremely promising in the development of a broad-spectrum antiviral drug to combat RVA. This will need to be followed up with *in vitro* and *in vivo* studies to validate their efficacy as drugs and their pharmacokinetic properties.

ACKNOWLEDGEMENTS

The authors specially acknowledge the financial assistance of the Directorate of Research and Postgraduate Support, Durban University of Technology, South Africa.

References

- AI, W., WEI, X., LIN, X., SHENG, L., WANG, Z., TU, Z., YANG, X., ZHOU, X., LI, J. & LIU, Y. 2014. Guignardins A-F, spirodioxynaphthalenes from the endophytic fungus *Guignardia* sp. KcF8 as a new class of PTP1B and SIRT1 inhibitors. *Tetrahedron* **70**: 5806–5814.
- ALQAHTANI, M.S., KAZI, M., ALSENAIDY, M.A. & AHMAD, M.Z. 2021. Advances in Oral Drug Delivery. *Frontiers in Pharmacology* **12**: Article 618411.
- AOKI, S.T., SETTEMBRE, E.C., TRASK, S.D., GREENBERG, H.B., HARRISON, S.C. & DORMITZER, P.R. 2009. Structure of rotavirus outer-layer protein VP7 bound with a neutralizing Fab. *Science* **324**: 1444–1447.
- ARIBISALA, J.O., NKOSI, S., IDOWU, K., NURAIN, I.O., MAKOLOMAKWA, G.M., SHODE, F.O. & SABIU, S. 2021. Astaxanthin-mediated bacterial lethality: Evidence from oxidative stress contribution and molecular dynamics simulation. *Oxidative Medicine and Cellular Longevity* **2021**: 1–24.
- ARIBISALA, J.O. & SABIU, S. 2022. Cheminformatics identification of phenolics as modulators of penicillin-binding protein 2a of *Staphylococcus aureus*: A structure–activity-relationship-based study. *Pharmaceutics* **14**: 1818. <https://doi.org/10.3390/pharmaceutics14091818>.
- BUTT, S.S., BADSHAH, Y., SHABBIR, M. & RAFIQ, M. 2020. Molecular docking using Chimera and Autodock Vina software for nonbioinformaticians. *JMIR Bioinformatics and Biotechnology* **1**(1): e14232.
- CHROBAK, E., BEBENEK, E., MARCINIEC, K., KADELA-TOMANEK, M., SIUDAK, S., LATOCHA, M. & BORYCZKA, S. 2021. New 30-substituted derivatives of pentacyclic triterpenes: preparation, biological activity, and molecular docking study. *Journal of Molecular Structure* **1226**: 1–11.
- CHUKEATIROTE, E., WIKKEE, S. & HYDE, K.D. 2015. Diversity and antibacterial activity of *Phyllosticta* species. *Micologia Aplicada International* **27**: 1–9.
- DALLAKYAN, S. & OLSON, A.J. 2015. Small-molecule library screening by docking with PyRx. *Methods in Molecular Biology* **1263**: 243–250.
- DE CLERCQ, E. 2004. Antiviral drugs in current clinical use. *Journal of Clinical Virology* **30**: 115–133.
- DESAL, F., RAMANATHAN, M., FINK, C.S., WILDING, G.E., WEINSTOCK-GUTTMAN, B. & AWAD, A.B. 2009. Comparison of the immunomodulating effects of plant β -sitosterol to simvastatin in peripheral blood cells from multiple sclerosis patients. *International Immunopharmacology* **9**: 153–157.
- DILLER, D.J. & MERZ, K.M. 2001. High throughput docking for library design and library prioritization. *Proteins* **43**: 113–124.
- EKINS, S., MESTRES, J. & TESTA, B. 2007. In silico pharmacology for drug discovery: methods for virtual ligand screening and profiling. *British Journal of Pharmacology* **152**: 9–20.
- ESTES, M. & KAPIKIAN, A. 2007. Rotaviruses. In Knipe D., Griffin D., Lamb R., Martin M., Roizman B. & Straus S. (Eds.) *Fields Virology*. Philadelphia, PA, Wolters Kluwer Health; Lippincott, Williams and Wilkins. pp. 1917–1975.
- FERREIRA DE FREITAS, R. & SCHAPIRA, M. 2017. A systematic analysis of atomic protein-ligand interactions in the PDB. *Medicinal Chemistry Communications* **8**: 1970–1981.
- GARUBA, T., OLAYINKA, B.U., ABDULKAREEM, K.A., BELLO, M.O., HAMID, A.A. & OLALEYE, F.Y. 2018a. A study on endophytic fungi, proximate and chemical composition of a local variety of mango fruit (*Mangifera indica* L.). *Journal of the University of Ruhuna* **6**: 61–69.
- GARUBA, T., SABIU, S., LATEEF, A.A., ADEKANMBI, A.T. & ADEKANMBI, M.K. 2018b. In vitro cytotoxicity and antioxidant activities of *Pestalotiopsis microspora* culture filtrate. *Malaysian Journal of Applied Sciences* **3**: 24–33.
- GHOSH, A., CHATTOPADHYAY, S., CHAWLA-SARKAR, M., NANDY, P. & NANDY, A. 2012. In silico study of rotavirus VP7 surface accessible conserved regions for antiviral drug/vaccine design. *Plos ONE* **7**: 1–12.
- GLOBAL BURDEN OF DISEASES. 2018. Causes of death collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **392**: 1736–88.
- HANWELL, M.D., CURTIS, D.E., LONIE, D.C., VANDERMEERSCH, T., ZUREK, E. & HUTCHISON, G.R. 2012. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Journal of Cheminformatics* **4**: 1–17.
- HU, L., CRAWFORD, S.E., CZAKO, R., CORTES-PENFIELD, N.W., SMITH, D.F., LE PENDU, J., ESTES, M.K. & PRASAD, B.V. 2012. Cell attachment protein VP8* of a human rotavirus specifically interacts with A-type histo-blood group antigen. *Nature* **485**: 256–259.
- HUGHES, J.D., BLAGG, J., PRICE, D.A., BAILEY, S., DECRESCENZO, G.A., DEVRAJ, R.V., ELLSWORTH, E., FOBIAN, Y.M., GIBBS, M.E., GILLES, R.W., GREENE, N., HUANG, E., KRIEGAR-BURKE, T., LOESEL, J., WAGER, T., WHITELEY, L. & ZHANG, Y. 2008. Physiochemical drug properties associated with in vivo toxicological outcomes. *Bioorganic and Medicinal Chemistry Letters* **18**: 4872–4875.
- JAMIU, A.T., POHL, C.H., BELLO, S., ADEDOJA, T. & SABIU, S. 2021. A review on molecular docking analysis of phytocompounds against SARS-CoV-2 druggable targets. *All Life* **14** (1): 1100–1128.
- JAYARAM, H., ESTES, M.K. & PRASAD, B.V.V. 2004. Emerging themes in rotavirus cell entry, genome organization, transcription and replication. *Virus Research* **101**: 67–81.
- KAUL, S., GUPTA, S., AHMED, M. & DHAR, M.K. 2012. Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. *Phytochemistry Reviews* **11**: 487–505.
- KITCHEN, D.B., DECORNEZ, H., FURR, J.R. & BAJORATH, J. 2004. Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature Reviews Drug Discovery* **3**: 935–949.
- LATEEF, A.A., GARUBA T., SA'AD, G., OLESIN, M., EPERETUN, G.G. & TIAMIYU, B.B. 2019. Isolation and molecular identification of dominant fungal endophytes from green leaves of physic nut (*Jatropha curcas*) from Unilorin Plantation, Ilorin, Nigeria. *Sri Lankan Journal of Biology* **4**: 1–13. <https://sljb.sljol.info/articles/abstract/10.4038sljb.v4i1.33/>
- LI, E., TIAN, R., LIU, S., CHEN, X., GUO, L. & CHE, Y. 2008. Pestalotiols A-D, bioactive metabolites from the plant endophytic fungus *Pestalotiopsis theae*. *Journal of Natural Products* **1**: 664–8. doi:10.1021/np700744t. Epub 2008 Feb 28. PMID: 18303847.
- LI, Z., WEN, W., QIN, M., HE, Y., XU, D. & LI, L. 2022. Biosynthetic mechanisms of secondary metabolites promoted by the interaction between endophytes and plant hosts. *Frontiers in Microbiology* **13**: 1–15.
- LINNAKOSKI, R., RESHAMWALA, D., VETELI, P., CORTINA-ESCRIBANO, M., VANHANEN, H. & MARJOMÄKI, V. 2018. Antiviral agents from fungi: Diversity, mechanisms and potential applications. *Frontiers in Microbiology* **9**: 1–18. <https://doi.org/10.3389/fmicb.2018.02325>.
- LIPINSKI, C.A., LOMBARDO, F., DOMINY, B.W. & FEENEY, P.J. 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery* **46**: 3–26.
- LU, X., MCDONALD, S.M., TORTORICI, M.A., TAO, Y.J., VASQUEZ-DEL CARPIO, R., NIBERT, M.L., PATTON, J.T. & HARRISON, S.C. 2008. Mechanism for coordinated RNA packaging and genome replication by rotavirus polymerase VP1. *Structure* **16**: 1678–1688.
- MCDONNELL, A.M. & DANG, C.H. 2013. Basic review of the cytochrome P450 system. *Journal of Advanced Practitioner in Oncology* **4**: 263–268.
- MEANWELL, N.A. 2011. Improving drug candidates by design: A focus on physiochemical properties as a means of improving compound disposition and safety. *Chemical Research in Toxicology* **24**: 1420–1456.
- NYAGA, M.M., SABIU, S., NDZE, V.N., DENNIS, F.E. & JERE, K.C. 2020. Report of the 1st African enteric viruses genome initiative (AEVGI) data and bioinformatics workshop on whole-genome

- analysis of some African rotavirus strains held in Bloemfontein, South Africa. *Vaccine* **38**: 5402–5407.
- PETERS, L.P., PRADO, L.S., SILVA, F.I.N., SOUZA, F.S.C. & CARVALHO, C.M. 2020. Selection of endophytes as antagonists of *Colletotrichum gloeosporioides* in açai palm. *Biological Control* **150**: Article 104350.
- RAJAMANIKYAM, M., VADLAPUDI, V., AMANCHY, R. & UPADHYAYULA, S.M. 2007. Endophytic fungi as novel resources of natural therapeutics. *Brazilian. Achieves. of Biology and Technology* **60**.
- RAJU, L., LIPIN, R. & ESWARAN, R. 2021. Identification, ADMET evaluation and molecular docking of phytosterols from Banaba (*Lagerstroemia speciosa* (L.) Pers) seed extract against breast cancer. *In Silico Pharmacology* **9**: 1–9.
- RASEBOTSA, S.P., MWANGI, P.N., MOGOTSI, M.T., SABIU S. & MAGAGULA, N.B. 2020. Whole genome analyses of G1P[8] rotavirus strains from pre- and post- vaccination periods in Rwanda. *Scientific Report* **10**: 1–22.
- REZANKA, T., SIRISTOVA, L. & SIGLER, K. 2009. Sterols and triterpenoids with antiviral activity. *Anti-Infective Agents in Medicinal Chemistry* **8**: 193–210.
- ROSSIGNOL, J.F., ABU-ZEKRY, M., HUSSEIN, A. & SANTORO, M.G. 2006. Effect of nitazoxanide for treatment of severe rotavirus diarrhoea: randomized double-blind placebo-controlled trial. *Lancet* **368**: 124–129.
- SALEHI, B., QUISPE, C., SHARIFI-RAD, J., CRUZ-MARTINS, N., NIGAM, M., MISHRA, A.P., KONOVALOV, D.A., OROBINSKAYA, V., ABU-REIDAH, I.M., ZAM, W., SHAROPOV, F., VENNERI, T., CAPASSO, R., KUKULA-KOCH, W., WAWRUSZAK, A. & KOCH, W. 2021. Phytosterols: from preclinical evidence to potential clinical applications. *Frontiers in Pharmacology* **11**: 1–18.
- SABIU, S., BALOGUN, F. O. & AMOO, S. O. 2021. Phenolics Profiling of *Carpobrotus edulis* (L.) N.E.BR. and Insights into Molecular Dynamics of Their Significance in Type 2 Diabetes Therapy and Its Retinopathy Complication. *Molecules*. **26**: 4867. <https://doi.org/10.3390/molecules26164867>
- SHADY, N.H., YOUSSEF, K.A., SAYED, A.M., BELBAHRI, L., OSZAKO, T., HASSAN, H.M. & ABDELMOHSEN, U.R. 2021. Sterols and triterpenes: antiviral potential supported by in-silico analysis. *Plants* **10**: 1–34
- SHODE, F.O., IDOWU, A.S.K., UHOMOIBHI, O.J. & SABIU, S. 2021. Repurposing drugs and identification of inhibitors of integral proteins (Spike protein and Main protease) of SARS-CoV-2. *Journal of Biomolecular Structure and Dynamics* **40**: 6587–6602. doi:10.1080/07391102.2021.1886993.
- TROEGER, C., KHALIL, I.A. & RAO, P.C. 2018. Rotavirus vaccination and the global burden of rotavirus diarrhea among children younger than 5 years. *JAMA Pediatrics* **172**: 958–65.
- WIENEN-SCHMIDT, B., JONKER, H.R.A., WULSDORE, T., GERBER, H., SAXENA, K., KUDLINZKI, D., SREERAMULU, S., PARIGI, G., LUCHINAT, C., HEINE, A., SCHWALBE, H. & KLEBE, G. 2018. Paradoxically, most flexible ligands bind most entropy-favored: intriguing impact of ligand flexibility and solvation on drug-kinase binding. *Journal of Medicinal Chemistry* **61**: 5922–5933.
- ZHOU, W.B., TAO, J.Y., XU, H.M., CHEN, K.L., ZENG, G.Z., JI, C.J., ZHANG, Y.M. & TAN, N.H. 2010. Three new antiviral triterpenes from *Aster tataricus* Z. *Nature* **65**: 1393–1396.
- ZONNO, M.C., VURRO, M., LUCRETTI, S., ANDOLFI, A., PERRONE, C. & EVIDENTE, A. 2008. Phyllostictine A, a potential natural herbicide produced by *Phyllosticta cirsii*: in vitro production and toxicity. *Plant Science* **175**: 818–8.