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Computational Discovery of Potential Drug Candidates from Phytochemicals of Withania somnifera and Saraca asoca Targeting Ebola and Marburg Viruses Infection

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Abstract

Ebola and Marburg viruses are extremely lethal and cause hemorrhagic fever, with deaths from cases exceeding 90%. Currently, just a few licensed drugs for these diseases. This necessitates extensive research on natural compounds having putative therapeutic effects, particularly phytochemicals derived from medicinal plants. In this study, the molecular properties of six *Withania somnifera* and six *Saraca asoca* phytochemicals were analyzed. The aim was to assess their potential to bind and possibly inhibit the VP24 protein (vital for the viral replication) of both Ebola and Marburg viruses using computational approaches. The drug-likeness properties of the phytochemicals were determined using a Mol soft server, Further Prediction of pharmacokinetics was studied using a ProTox web server and pKCSN server, Molecular docking studies were carried out using PyRx software. The Insilico analysis revealed Withaferin A, Withanolide D, Withanolide F phytochemicals of *Withania somnifera* and Catechin, Epicatechin, and Isolariciresinol of *Saraca asoca* to have high binding affinity with both VP24 Proteins of Ebola and Marburg viruses, this suggests their potential to bind with the VP24 protein, disrupt its normal function, and consequently impede viral replication. These findings strongly imply that these phytoconstituents could possess antiviral properties. However, to validate and establish the antiviral potential of these phytochemicals, further investigations are imperative. Methods such as molecular dynamics simulations, in-vivo and in-vitro studies should be conducted. Upon successful validation, these phytochemicals could emerge as viable candidates for the development of drugs aimed at treating Ebola and Marburg diseases.

Introduction

Ebola virus and Marburg virus are highly pathogenic viruses, that are categorized as category A pathogens by the CDC (Centers for Disease Control and Prevention) [1]. Ebola virus disease, also referred to as Ebola hemorrhagic fever, is a severe and fatal illness caused by the Ebola virus. The outbreaks of this disease were initiated with a single probable zoonotic transmission, leading to subsequent human-to-human transmission through direct contact or exposure to infected body fluids. This virulent disease predominantly triggers outbreaks in Africa [2, 3]. Furthermore, this illness is devastating, with a fifty percent average mortality rate [4]. headache, Fever, vomiting, lack of appetite, weakness, hiccups, diarrhea and conjunctivitis are the most typical Ebola symptoms. In more extreme situations, patients could experience unexplainable hemorrhage, renal and liver malfunction, hypovolemic shock and discomfort [5]. From 2014 to 2016, West Africa experienced the most severe Ebola outbreak, with approximately 28,000 infections and 11,000 fatalities [6]. Although various medicines were suggested to treat this pathogenic condition [7, 8], less progress was reported, a few progress was achieved which include FDA approved Ervebo ebola virus vaccine, no licensed Ebola antiviral medications in the market, and almost all of the treatments used to treat Ebola patients were designed to alleviate symptoms rather than specifically target the virus [9].

Marburg virus disease is a deadly infection caused by the Marburg virus. This virus is highly pathogenic and causes infectious diseases resulting in hemorrhagic fever [10]. Reports of Marburg virus disease outbreaks originate from African nations such as the Congo, Democratic Republic of Angola, Uganda, Kenya, Guinea and South Africa. it was also reported in the Netherlands, the United States, Russia and Yugoslavia. The most recent epidemic was recorded in Ghana in the year 2022 [11, 12]. The greatest Marburg virus disease epidemic to date was observed in Western Africa between the years 2004 and 2005 [13]. There were approximately 252 cases documented, with 227 deaths [9]. The condition causes hemorrhagic fever as well as organ dysfunctions such as renal system tissues, liver failure, and infection associated with the brain and it also triggers coagulation disorders [9]. Despite significant studies, no approved vaccines or effective treatments to manage the Marburg virus are currently available.

There is a necessity to assess and evaluate the effectiveness of phytochemicals, plant metabolites and new chemical compounds for their antiviral properties that could potentially be used against Ebola and Marburg viruses. This approach has proven effective against other significant emerging viral diseases such as COVID-19 caused by SARS-CoV-2 [14]. due to their intriguing bioactivities, natural products are regarded as the most promising sources of lead molecules. Furthermore, many pharmaceuticals that are marketed are either obtained directly from plant-based sources or are compounds obtained from natural sources [15].

Withania somnifera is a plant that is generally known as Ashwagandha. It is an important medicinal plant found across the Indian subcontinent that has been widely used in Ayurvedic and traditional systems for over 3,000 years [16]. *Withania somnifera* has been reported to have anti-inflammatory, anti-arthritic, analgesic, hepatoprotective, anti-epileptic, anti-cancer, anti-Alzheimer, cardioprotective, antiparkinson, neuroprotective, anti-fungal, anti-microbial, anti-diabetic, properties [17]. The majority of *Withania somnifera*'s biological activities have been associated withaferin-A and withanolide-A. withanolide-D, withanone, withanolide-E and withanolide-F [18, 19]

Saraca asoca has been considered one of the most significant herbal remedies, its phytoconstituents have been reported to possess antiviral, anti-diabetic, antidepressant, antibacterial, anti-inflammatory, and anti-cancer effects [20, 221]. Some of its important phytoconstituents include Catechin [22], Epicatechin [23], Leucocyanidin [24], Procyanidin [25], Isolariciresinol [26], Lyoniside [27].

Insilico study which involves the application of bioinformatics tools and computational approaches is generally used by researchers to discover and study the efficacy of new medications. Target lead identification represents one of the most significant aspects of current early drug development. Target discovery seeks to identify and validate appropriate targets for drug development for therapeutic application, whilst lead discovery seeks to identify novel compounds that act on such targets [28]. Insilico analysis also includes the discovery and optimization of novel molecules with affinity to a target and the evaluation of their molecular, toxicity and physiochemical properties [29].

This research utilized computational methods and bioinformatics tools to assess the antiviral potential of specific phytoconstituents found in *Withania somnifera* and *Saraca asoca*. The focus was on inhibiting the VP24 protein in both Ebola and Marburg viruses. VP24 is the smallest protein in these viruses, plays a crucial role in replication, transcription of the virus and formation of viral nucleocapsids when co-expressed with NP and VP35 [30]. Hence, targeting the VP24 protein in both viruses offers a promising strategy for restraining their replication and potentially treating the diseases they cause.

Materials and methods

Target Protein and preparation

Crystal structure of Marburg virus VP24 protein (PDB ID: 4or8) with **Resolution**: 2.65 Å and Ebola virus VP24 structure (PDB ID: 4D9O) with **Resolution**: 2.0 Å [31] were selected as the target protein were obtained from PDB database (https://www.rcsb.org) [32]. These proteins molecules were prepared by removing water molecules, and addition of polar hydrogen ions for molecular docking using Biovia Discovery software (Biovia 2015) [33]

Determination of target proteins binding sites

The active pockets of the selected target proteins were identified using the Computed Atlas of Surface Topography of proteins (CASTp) server [34].

Ligands compounds

The 3D structures of phytochemicals from *Withania somnifera* and *Saraca asoca* were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The phytoconstituents of *Withania somnifera* included in this study include: Withaferin-A (CID 265237), Withanone (CID 21679027), Withanolide-A (CID 11294368), Withanolide-D (CID 161671), Withanolide-E (CID 301751), and Withanolide-F (CID 44562999). Selected *Saraca asoca* phytochemicals were sourced from the same database and comprise: Catechin (CID 9064), Epicatechin (CID 72276), Procyanidin B-2 (CID 122738), Leucocyanidin (CID 71629), Lyoniside (CID 14521039), and Isolariciresinol (CID 160521) [35]

Molecular properties of phytochemicals

The drug-likeness properties of the selected phytoconstituents (ligands) of *Withania somnifera* and *Saraca asoca* were evaluated using Mol soft server (http://molsoft.com/mprop) the InChI of the ligands were obtained from the PubChem

database, uploaded and calculated by the Mol soft server. The results were subsequently analyzed based on Lipinski's rule of five (Molecular weight ≤ 500 Dalton, Hydrogen bonds donor ≤ 5, Hydrogen bonds acceptor ≤ 10 and Log P ≤ 5) [36].

Prediction of toxicity risk and oral toxicity (LD50)

The ProTox web server was used for predicting the phytochemicals oral toxicity (LD50). The ProTox server predicts the toxicity level of the compounds by correlating the two-dimensional chemical orientation of the compounds with the toxicity level of known authorized compounds and by identifying over-represented fragments in the toxic compound [37]

ADME prediction

Phytochemicals with deemed acceptable LD50 values underwent a comprehensive analysis to predict their pharmacokinetic properties, encompassing absorption, distribution, metabolism, excretion, and toxicity. Utilizing the pKCSN web server [38] SMILES of evaluated phytochemicals were obtained from the PubChem database and utilized to predict pharmacokinetics parameters using the webservers. A range of pharmacokinetic parameters, such as intestinal absorption (human), volume of distribution at steady state (Vss), metabolism, excretion, maximum tolerated dose, hepatotoxicity, and AMES toxicity, were systematically predicted. This extensive evaluation aimed to provide insights into the potential bioavailability, metabolic fate, and safety profiles of the examined phytochemicals, contributing valuable information for their prospective therapeutic applications.

Molecular Docking

The PyRx software (https://pyrx.sourceforge.io), which incorporates Autodock Vina, AutoDock, and Open Babel tools, was utilized for the preparation and molecular docking of selected target proteins with ligands.[39] The 3D structures of the Marburg virus VP24 protein (PDB ID: 4or8) and Ebola virus VP24 protein (PDB ID: 4D9O) were uploaded to PyRx, and charges were subsequently assigned. The structures were then minimized using the steepest descent algorithm and converted to the pdbqt format. Simultaneously, all ligands underwent a similar optimization process, involving minimization through the steepest descent algorithm and conversion to the pdbqt format, ensuring compatibility for docking using AutoDock Vina. For the docking simulations with the Marburg virus VP24 protein (PDB ID: 4or8) and ligand, the center points of the grid box were set to X: 14.0490, Y: 8.4397, and Z: 30.7368, with dimensions (Angstrom) of X: 29.9025, Y: 43.8297, and Z: 31.7038. Similarly, for the docking with the Ebola virus VP24 protein (PDB ID: 4D9O) and ligand, the center points of the grid box were set to X: 40.1741, Y: 14.2775, and Z: -11.2692, with dimensions (Angstrom) of X: 39.7891, Y: 25.0709, and Z: 29.9815.

BIOVIA Discovery Studio was employed to visualize the bonding interactions between the docked protein–ligand complexes and analyze the docking pose. The best conformation was selected based on the lowest docking score (in kcal/mol) [33].

Results and Discussion

Drug-likeness analysis of the phytochemicals

Roughly 60% of potential drug-like compounds face rejection in the clinical phase due to unfavorable molecular properties. Traditionally, drug likeliness and toxicity assessments were conducted at the conclusion of the drug discovery process. However, pre-assessing the molecular features of drug-like substances earlier in the drug development process can accelerate drug discovery. With the aid of computational algorithms and experimental data, drug likeliness can now be predicted, offering a means to streamline the drug development process and reduce both costs and time [40]

Lipinski’s Rule of Five was employed to assess the drug-likeness of the 12 selected phytochemicals. This comparative method enables the exclusion of certain phytochemicals based on their physicochemical properties. Phytochemicals that violated one or more parameters were excluded, leaving the remaining compounds as ligands for the docking study. Among the selected phytochemicals, leucocyanidin violated one rule (having several hydrogen Bond donors of more than 5), and Procyanidin B-2 and Lyoniside violated three of Lipinski’s rules (both having several hydrogen bond donors of more than 5, number of hydrogen bond acceptors more than 10 and molecular weight values greater than 5000 Da). Consequently, they were not

considered for further analysis. Conversely, Withaferin-A, Withanolide-A, Withanolide-D, Withanolide-E, Withanolide-F, Withanone, Catechin, Epicatechin, and Isolariciresinol did not violate any of Lipinski's rules (Table 1) [41]. Hence, they were regarded for further analysis, as they exhibit the recommended drug-likeness properties.

Table 1
Illustrating the molecular properties of selected phytochemicals of *Withania somnifera* and *Saraca asoc*a

Phytochemicals Ligands	MW (Molecular weight g/mol)	MLogP (Calculated Lipophillicity)	nHBA (Number of Hydrogen Bond Acceptor)	nHBD (Number of Hydrogen Bond donors)	Lipinski's Rule Violations
Withaferin-A	470.27	3.35	6	2	0
Withanolide-A	470.27	3.94	6	2	0
Withanolide-D	470.27	3.66	6	2	0
Withanolide-E	486.26	2.79	7	3	0
Withanolide-F	470.27	3.51	6	3	0
Withanone	470.27	3.63	6	2	0
Catechin	290.08	0.53	6	5	0
Epicatechin	290.08	0.53	6	5	0
Procyanidin B-2	578.14	1.44	12	10	3
leucocyanidin	306.07	0.25	7	6	1
Lyoniside	552.22	0.09	12	6	3
Isolariciresinol	360.16	1.74	6	4	0

Oral toxicity (LD50) analysis of the phytochemicals

The PROTOX tool was employed to predict the LD50 values of the selected phytochemicals that adhere to Lipinski's rule. Among these, both Catechin and Epicatechin exhibited the highest predicted LD50 values of 10,000 mg/kg, indicating their safety for administration. Isolariciresinol followed with an LD50 value of 5,000 mg/kg, is also considered safe. Notably, Withanolide-F displayed an LD50 value of 400 mg/kg, while Withaferin-A and Withanolide-D showed predicted LD50 values of 300 mg/kg, suggesting their safety for administration at a lower concentration. However, Withanolide-A, Withanolide-E, and Withanone exhibited lower predicted LD50 values of 34 mg/kg, 7 mg/kg, and 7 mg/kg, respectively (Table 2). These values indicate potential toxicity at low concentrations, leading to their exclusion from further analysis to ensure safety and reliability in subsequent studies.

Table 2
Presenting oral toxicity of Selected *Withania somnifera* and *Saraca asoca* phytochemicals

Selected Plants phytochemicals	Prediction Accuracy (%)	lethal dose 50% LD50 (mg/kg)	Toxicity Class (1–6)	Average Similarity (%)
Withaferin-A	69.26	300	3	78.45
Withanolide-A	69.26	34	2	79.06
Withanolide-D	69.26	300	3	76.07
Withanolide-E	70.97	7	2	81.05
Withanolide-F	69.26	400	4	70.80
Withanone	70.97	7	2	80.51
Catechin	100	10000	6	100
Epicatechin	100	10000	6	100
Isolariciresinol	68.07	5000	5	69.37

ADME analysis of the phytochemicals

Natural compounds represent a valuable reservoir for drug development, owing to their vast diversity and intricate structures. This significance is further underscored by the limited number of plant species investigated for pharmacological purposes [42]. ADMET analysis is used to study the pharmacokinetics of these phytochemicals thereby evaluating their risk or negative effect on the human body [43].

In order to select the best, druggable and lead-like phytochemicals for the progression of this study, the selected phytochemicals were subjected to ADME analysis using the pKCSN server.

Subsequent toxicity studies unveiled that all the chosen phytochemicals were predicted to be devoid of hepatotoxic effects and mutagenic potential, as indicated by the negative results in the Ames test which is a key assay designed to identify compounds with mutagenic properties [44]. The determination of the Maximum Tolerated Dose (MRTD) values, representing the highest dose of a phytochemical that can be administered to a patient without inducing undesirable side effects or toxicity (with a cutoff value of 0.477 for categorizing MRTD), was pivotal. All the examined phytochemicals exhibited MRTD values below 0.477, signaling their safety for administration at high doses without causing side effects or toxicity. Specifically, Withaferin A, Withanolide D, and Withanolide F showcased MRTD values of -0.695, -0.867, and − 0.701 log mg/kg/day, respectively, indicating a higher tolerance for elevated doses. In contrast, Catechin, Epicatechin, and Isolariciresinol displayed MRTD values of 0.438, 0.438, and 0.134, respectively, suggesting a comparatively lower tolerance for higher doses (Table 3).

Table 3
Presenting pharmacokinetic properties of Selected *Withania somnifera* and *Saraca asoca* phytochemicals

Selected Plants phytochemicals	Intestinal absorption (human)	volume of distribution at steady state (human) (log L/kg)	Metabolism	Excretion	Max. tolerated dose (human) (log mg/kg/day)	Hepatotoxicity	AMES toxicity
Withaferin-A	85.345	-0.131	CYP3A4 (cytochrome P450 3A4) substrate	Renal OCT2 substrates.	-0.695	NO	NO
Withanolide-D	99.2	-0.048	CYP3A4 (cytochrome P450 3A4) substrate	Total clearance 0.347 (log ml/min/kg)	-0.867	NO	NO
Withanolide-F	95.535	0.067	CYP3A4 (cytochrome P450 3A4) substrate	Total clearance 0.486 (log ml/min/kg)	-0.701	NO	NO
Catechin	68.829	1.027	NOT METABOLIZED BY CYP2C19 and CYP3A4	Total clearance 0.183 (log ml/min/kg)	0.438	NO	NO
Epicatechin	68.829	1.027	NOT METABOLIZED BY CYP2C19 and CYP3A4	Total clearance 0.183 (log ml/min/kg)	0.438	NO	NO
Isolariciresinol	73.973	-0.145	CYP3A4 (cytochrome P450 3A4) substrate	Total clearance 0.177 (log ml/min/kg)	0.134	NO	NO

The excretion pharmacokinetic parameter of the phytochemicals was assessed, focusing on the Total Clearance—a metric that signifies the overall rate at which a substance is eliminated from the body, encompassing both renal and non-renal processes. Notably, the total clearance values for Withanolide-D, Withanolide-F, Catechin, Epicatechin, and Isolariciresinol were determined as 0.347, 0.486, 0.183, 0.183, and 0.177 log ml/min/kg, respectively, indicating a comparatively higher rate of elimination from the body. In contrast, Withaferin-A was predicted to undergo renal excretion, supported by its classification as a Renal Organic Cation Transporter 2 (OCT2) substrate. OCT2 is a crucial protein involved in the renal excretion process of compounds categorized as its substrates [45].

The metabolic profile of the phytochemicals was assessed, revealing distinctive patterns. Withaferin-A, Withanolide-D, Withanolide-F, and Isolariciresinol were anticipated to undergo metabolism mediated by cytochrome P450 3A4 (CYP3A4), an enzyme within the cytochrome P450 family known for its involvement in the metabolism of diverse endogenous compounds and drugs [46]. Compounds metabolized by CYP3A4 are classified as CYP3A4 substrates. Conversely, Catechin and Epicatechin were identified as non-substrates for both CYP2C19 and CYP3A4 enzymes. However, it is noteworthy that these phytochemicals could potentially be metabolized by other enzymes, necessitating further investigations to elucidate their complete metabolic pathways.

The distribution profiles of these phytochemicals within the human body were examined, with a focus on the prediction of the volume of distribution at steady state (VDss). The findings, where VDss values below − 0.15 log L/kg are considered high and values above 0.45 log L/kg are deemed low, indicated distinctive distribution patterns. Withaferin-A, Withanolide-D,

Isolariciresinol, and Withanolide-F demonstrated VDss values of -0.131, -0.048, -0.145, and 0.067 log L/kg, respectively. These values suggest a propensity for low distribution in tissues and organs, with a higher concentration expected in the bloodstream. Catechin and Epicatechin exhibited predicted VDss values of 1.027 log L/kg each, indicative of high distribution in tissues and organs. These varying distribution profiles underscore the diverse pharmacokinetic behaviors of the studied phytochemicals within the biological system.

The intestine stands out as the principal site for drug absorption from orally administered solutions [47]. In our study, the predicted intestinal absorption of these phytochemicals demonstrated uniformly high rates, ranging between 68% and 99.2%. Notably, Withanolide-D exhibited the highest predicted absorption rate, reaching 95%, followed closely by Withanolide-F and Withaferin-A with absorption rates of 95.5% and 85.3% respectively.

Molecular docking analysis

Molecular docking is an in-silico approach designed to predict and calculate the chemical interactions between macromolecules (proteins) and small compounds (ligands). It has proven to be an exceptionally successful method for screening a diverse range of compounds and discovering novel medicines targeting specific proteins. Of particular interest is protein-ligand docking, widely employed in the pharmaceutical industry [48].

In our investigation, the selected proteins (VP24) play a crucial role in the survival of Ebola and Marburg viruses [30]. *Withania somnifera* and *Saraca asoca* are recognized for possessing medicinal properties, including anti-viral effects [21, 49]. To explore their potential as antiviral agents, we focused on six key phytochemicals from each plant, conducting analyses for drug-likeness, oral toxicity, and their binding affinity with the designated target proteins.

The selection criteria ensured that the six chosen phytochemicals adhered to Lipinski's rules and exhibited predicted non-toxicity along with optimal absorption, distribution, metabolism, and excretion profiles. These compounds were then designated as ligands for molecular docking studies with the target proteins. The objective of the docking analysis was to identify ligands capable of binding to the target protein's binding site and to discern their preferred and most advantageous binding poses. This systematic approach aimed to assess the potential therapeutic efficacy of the selected phytochemicals against the targeted proteins crucial for the survival of Ebola and Marburg viruses. The vina docking result generated eight conformations of each ligand that were ranked based on their energies and the conformation with the highest binding affinity was selected for further study.

Based on molecular docking analysis, any active compound with a minimum binding energy of -6.0 kcal/mol is considered to have the potential to induce pharmacological effects [50, 51]. The molecular docking results for Withaferin-A, Withanolide D, Withanolide F, Catechin, Epicatechin, and Isolariciresinol (ligands) revealed low binding energies of -10.0, -10.1, -9.1, -7.4, -7.2, and − 7.2 kcal/mol respectively with the Ebola virus VP24 protein (Fig. 1 and Fig. 2) indicating high binding affinity with the target protein and predicting a potential pharmacological effect (Table 4). Notably, Withanolide D exhibited the lowest binding energy, indicating the highest binding affinity toward the Ebola virus VP24 protein, followed by Withaferin-A and Withanolide F.

The strength of the protein-ligand interaction is intricately shaped by the array of bonds participating in complex formation. Specifically, the formation of hydrogen bonds between the ligand and target protein serves as a pivotal determinant of interaction strength. It follows that a higher count of hydrogen bonds corresponds to a stronger interaction [52], Emphasizing the crucial role of these molecular bridges in strengthening the bond between the ligand and its target protein, our investigation revealed robust hydrogen bonding interactions for all ligands with specific amino acids of the Ebola virus VP24 protein. These interactions involved key residues, such as ARG B:154, ARG A:154, GLU B:46, GLY A:44, GLY B:44, and PRO B:229, all situated at the active site of the protein.

Notably, Withaferin A formed hydrogen bonds with ARG B:154 and GLU B:46, Epicatechin formed bonds with ARG B:154 and GLY B:44, and Isolariciresinol established bonds with PRO B:229 and GLY A:44. Ligands Withanolide-D and Withanolide-F formed hydrogen bonds with ARG B:154 and ARG A:154, respectively, while Catechin formed a bond with GLU B:46. Importantly, ligands Withaferin A, Epicatechin, and Isolariciresinol established two hydrogen bonds, signifying a more potent

interaction with the Ebola VP24 protein, compared to the ligands forming a single hydrogen bond. This detailed analysis highlights the specific amino acid residues involved and the varying strengths of interactions across different ligands. Furthermore, various additional bonds, such as Van der Waals, Pi-Anion, Pi-Cation, Alkyl, Pi-Alkyl, and unfavorable Donor-Donor bonds (refer to Table 4) were observed in some complexes, these bonds contribute significantly to the overall stability of the ligand-Ebola VP24 protein complexes. These diverse bonding interactions collectively enhance the binding affinity and structural integrity of the protein-ligand complexes, thereby substantiating the robustness of the predicted interactions.

As observed in our molecular docking analysis, Withaferin-A, Withanolide D, and Withanolide F exhibited notably lower binding energies with the Ebola VP24 protein compared to certain Indonesian natural compound ligands, as reported by Tambunan and Nasution [53]. Specifically, our results indicated − 10.0 kcal/mol, -10.1 kcal/mol, and − 9.1 kcal/mol binding energies for Withaferin-A, Withanolide D, and Withanolide F, respectively, in contrast to the binding energies of some Indonesian natural compounds such as Cycloartocarpin (-7.4847 kcal/mol), Letestuianin B (-6.4799 kcal/mol), Lissoclin A (-6.4729 kcal/mol), Varamine A (-6.4660 kcal/mol), and Lissoclibadin 4 (-6.3958 kcal/mol) in the study conducted by Tambunan and Nasution [53]. This suggests that Withaferin-A, Withanolide D, and Withanolide F could be anticipated to have a higher affinity for the Ebola VP24 protein, potentially leading to a more pronounced inhibitory effect.

The results of drug-likeness and molecular properties of the selected phytochemicals (Withaferin-A, Withanolide D, Withanolide F, Catechin, Epicatechin, and Isolariciresinol) coupled with high binding affinity and the strong interactions formed with the Ebola virus VP2 protein underscore the potential of these ligands in binding with the protein. This suggests a possible hindrance to the normal biological function of the virus protein, making them promising lead molecules for further investigation in antiviral activity through MD simulation, in vitro, and in vivo studies. These studies are crucial for the development of therapeutic drugs targeting diseases caused by the Ebola virus.

There are very few active clinical studies for the development of Marburg virus therapies/vaccines. Some of the prospective studies include modified vaccinia Ankara, chimpanzee adenovirus type 3, Marburg DNA plasmid vaccine, galidesivir and antisense phosphorodiamidate morpholino oligomers. Despite this, there are no licensed vaccines or treatments for the Marburg virus due to viral genetic diversity [54].

The interaction and binding energy of ligands with the Marburg VP24 protein, as presented in Table 5, revealed that Withaferin-A, Withanolide D, Withanolide F, and Catechin exhibited binding energies of -6.2, -6.7, -6.3, and − 6.5 kcal/mol respectively (Table 5), indicating a high binding affinity with the Marburg VP24 protein. Notably, Catechin formed three hydrogen bonds with GLY B:36, THR A:52, and GLU A:226, in addition to Alkyl bonds with PRO B:50, ALA A:225, B:227, and MET A:195, as well as Pi-anion bonds with specific amino acids of the Marburg VP24 protein.

Considering the collective impact of binding energy and the formation of these bonds, resulting in a robust interaction strength, the prediction is for the formation of a stable Catechin-Marburg VP24 protein complex. Thus, Catechin stands out as a potential lead molecule. Although Withanolide-D exhibited the lowest binding energy (highest binding affinity), it formed only an Alkyl bond with PRO B:67. Withanolide-F formed H-bonds with SER B:34 and THR A:55, while Withaferin A formed an H-bond with SER B:34.

However, Epicatechin and Isolariciresinol exhibited binding energies of -5.4 kcal/mol and − 5.2 kcal/mol with the Marburg VP24 protein, respectively, which are relatively higher than those of other ligands, suggesting lower binding affinity with the target protein. Nevertheless, Epicatechin formed three hydrogen bonds with THR A:39, GLU B:68, and PHE B:64, along with two Alkyl bonds with VAL B:66 and VAL A:35, and a Van der Waals bond with PRO A:33. On the other hand, Isolariciresinol formed H-bonds with GLN B:71 and GLU B:68, Pi-Anion bonds with LYS A:237 and GLU B:68, and Alkyl bonds with PRO A:240 and HIS A:48, along with van der Waals bonds with PRO A:240 and GLU A:231. These suggest that despite the lower binding affinity, both Epicatechin and Isolariciresinol have the potential to form strong and stable complexes with the Marburg VP24 protein. The 3D interaction of the ligands with the Murburg virus is illustrated in Figs. 3 &4.

The molecular properties of the ligands exhibit drug-likeness characteristics, and the docking results suggest that these phytochemicals could be considered as lead molecules with a binding affinity for the Marburg VP24 protein. This implies the potential for inhibitory effects, disrupting the normal functioning of the VP24 protein, and subsequently hindering virus replication. To validate and enhance our understanding of the antiviral potential, further investigations such as MD simulation, in vitro, and in vivo studies are imperative. These additional studies will provide comprehensive insights into the efficacy and safety of these phytochemicals, facilitating the development of potential drugs for the treatment of diseases caused by the Marburg virus.

Conclusion

The predictions from in-silico studies offer promising avenues for novel target identification, potentially leading to compounds with predicted biological activity and enhancing preclinical safety testing in the pharmaceutical industry. Notably, the phytoconstituents Withaferin A, Withanolide D, and Withanolide F from *Withania somnifera*, as well as Catechin, Epicatechin, and Isolariciresinol from *Saraca asoca*, exhibited encouraging results in our in-silico analyses. Our comprehensive assessments, including drug-likeness, oral toxicity tests, and ADME predictions, demonstrated that these phytochemicals align well with drug-like properties. Furthermore, through docking analyses, we observed a high binding affinity between these phytoconstituents and the VP24 proteins of both Ebola and Marburg viruses. This suggests their potential to bind with the VP24 protein, disrupt its normal function, and consequently impede viral replication. These findings strongly imply that these phytoconstituents could possess antiviral properties. However, to validate and establish the antiviral potential of these phytochemicals, further investigations are imperative. Methods such as molecular dynamics (MD) simulations, in vivo, and in vitro studies should be conducted. Upon successful validation, these phytochemicals could emerge as viable candidates for the development of drugs aimed at treating Ebola and Marburg diseases.

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Author contribution

The study conceptualization and design is carried out by Munir Ibrahim. Data curation, methodology and analysis were performed by, Asmita Detroja, Avani Bhimani, Tirth Chetankumar Bhatt. Supervision of the research has been done by Gaurav Sanghvi and Ashok Kumar Bishoyi. The first draft was written by Munir Ibrahim and revised by the other authors. All Authors approved the final manuscript.

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References

1. Centers for Disease Control and Prevention & National Institutes of Health. (2020) Biosafety in Microbiological and Biomedical Laboratories (6th ed.)
2. World Health Organization (2023) Ebola virus disease.WHO. https://www.who.int/news-room/fact-sheets/detail/ebola-virus-disease
3. Centers for Disease Control and Prevention. (n.d.) 2014–2016 Ebola Outbreak in West Africa. https://www.cdc.gov/vhf/ebola/about.html

4. Park C (2022) Lessons learned from the World Health Organization's late initial response to the 2014-2016 Ebola outbreak in West Africa J Public Health Afr 13:(1)
5. Centers for Disease Control and Prevention (2016) Ebola (Ebola Virus Disease) http://www.cdc.gov/vhf/ebola/symptoms
6. World Health Organization. (2016) Ebola Data and Statistics. http://apps.who.int/gho/data/view.ebolasitrep.ebola-summary-latest?lang=en
7. Madrid PB, Chopra S, Manger ID, Gilfillan L, Keepers TR, Shurtleff A C, ... & Tanga MJ (2013) A systematic screen of FDA-approved drugs for inhibitors of biological threat agents PloS one 8(4):e60579
8. Oestereich L, Lüdtke A, Wurr S, Rieger T, Muñoz-Fontela C, & Günther S (2014) Successful treatment of advanced Ebola virus infection with T-705 (favipiravir) in a small animal model Antiviral res 105:17-21
9. Marzi A, Feldmann H (2014) Ebola virus vaccines: an overview of current approache Expert rev vaccines 13(4):521-531
10. Mehedi M, Groseth A, Feldmann, H & Ebihara H (2011) Clinical aspects of Marburg hemorrhagic fever Future virol 6(9):1091-1106
11. Koundouno FR, Kafetzopoulou LE, Faye M, Renevey A, Soropogui B, Ifono K, ... & Magassouba NF (2022) Detection of Marburg virus disease in Guinea N Engl J Med 386(26):2528-2530
12. World Health Organization. (2022) Marburg virus- Ghana, 22 July, 2022. https://www.who.int/emergencies/disease-outbreak-news/item/2022- DON402
13. Languon S, Quaye O (2019) Filovirus disease outbreaks: a chronological overview Virol res and treat 10 1178122X19849927
14. Saied AA, Nascimento MSL, do Nascimento Rangel, AH, Skowron, K, Grudlewska-Buda K, Dhama K, ... & Metwally AA (2022) Transchromosomic bovines-derived broadly neutralizing antibodies as potent biotherapeutics to counter important emerging viral pathogens with a special focus on SARS-CoV-2, MERS-CoV, Ebola, Zika, HIV-1, and influenza A virus J Med Virol 94(10):4599-4610
15. Patrick GL (2015) An introduction to drug synthesis Oxford University Press USA
16. Behl T, Sharma A, Sharma L, Sehgal A, Zengin G, Brata, R, ... & Bungau S (2020) Exploring the multifaceted therapeutic potential of withaferin A and its derivatives Biomedicines 8(12):571
17. Parihar S (2022) A literature review on pharmacological activities of *Withania somnifera* j biosci 2(1):147-154 doi:10.55006/biolsciences.2022.2105
18. Saleem S, Muhammad G, Hussain MA, Altaf M, & Bukhari SNA (2020) *Withania somnifera* L.: Insights into the phytochemical profile, therapeutic potential, clinical trials, and future prospective Iran J Basic Med Sci 23(12):1501. doi:10. 22038/IJBMS.2020.44254.10378
19. Paul S, Chakraborty S, Anand U, Dey S, Nandy S, Ghorai M, ... & Dey A (2021) *Withania somnifera* (L.) Dunal (Ashwagandha): A comprehensive review on ethnopharmacology, pharmacotherapeutics, biomedicinal and toxicological aspects Biomed Pharmacother 143:112175 doi:10.1016/j.biopha.2021.112175
20. Pal SC, Maiti AP, Chatterjee BP, Nandy A (1985) Antibacterial activity of flowers & flower buds of Saraca indica Linn The Indian J Med Res 82:188-189
21. Pradhan P, Joseph L, George M, Kaushik N, & Chulet R (2010) Pharmacognostic, phytochemical and quantitative investigation of *Saraca asoca* leaves J Pharm Res 3(1):776-780
22. Borokar AA, Pansare TA (2017) Plant profile, phytochemistry and pharmacology of Ashoka (*Saraca asoca* (Roxb.), De. Wilde)-A comprehensive review Int J Ayurvedic Herb Med 7(2):2524-2541
23. Ambasta SP (1986) The useful plants of India
24. Bhalerao SA, Verma DR, Didwana VS, Teli NC (2014) *Saraca asoca* (Roxb.) de. Wild: an overview Annals of plant sci 3(7):770-775
25. Nadkarni KM, Nadkarni AK (1982) Indian materia medica. Bombay: Popular Prakashan 1:278-279

26. Pradhan P, Joseph L, Gupta V, Chulet R, Arya H, Verma R, Bajpai A (2009) *Saraca asoca* (Ashoka): a review J chem pharm res 1(1):62-71
27. Warrier PK, Nambiar VP, Ganapathy PM (2001) Some Important Medicinal Plants of the Western Ghats, India-A Profile. Canada: Medicinal and Aromatic Plants Program in Asia (MAPPA), International Development Research Centre (IDRC) South Asia Regional Office (SARO) 87-101
28. Terstappen GC, Reggiani A (2001) In silico research in drug discovery Trends pharmacol 22(1):23-26
29. Ekins S, Mestres J, Testa B (2007) In silico pharmacology for drug discovery: applications to targets and beyond Br j pharmacol 152(1):21-37
30. Bamberg S, Kolesnikova L, Möller P, Klenk HD, Becker S (2005) VP24 of Marburg virus influences formation of infectious particles virol j 79(21):13421-13433
31. Edwards MR, Johnson B, Mire CE, Xu W, Shabman RS, Speller LN, ... & Basler CF (2014) The Marburg virus VP24 protein interacts with Keap1 to activate the cytoprotective antioxidant response pathway Cell rep 6(6):1017-1025
32. Rose PW, Prlić A, Altunkaya A, Bi C, Bradley AR, Christie CH, ... & Burley SK (2016) The RCSB protein data bank: integrative view of protein, gene and 3D structural information. Nucleic acids res gkw1000
33. Biovia DS (2015) Materials Studio Modeling Environment Dassault Systèmes San Diego
34. Tian W., Chen C, Lei X, Zhao J, Liang J (2018) CASTp 3.0: Computed atlas of surface topography of proteins. Nucleic Acids Res 46(W1) W363–W367. https://doi.org/10.1093/nar/gky473
35. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, ... & Bolton EE (2023) PubChem 2023 update. Nucleic acids res 51(D1) D1373-D1380 https://doi.org/10.1093/nar/gkac956
36. Lipinski CA (2000) Drug-like properties and the causes of poor solubility and poor permeability. J pharmacol toxicol methods 44(1):235-249
37. Drwal MN, Banerjee P, Dunkel M, Wettig MR, & Preissner R (2014) ProTox: a web server for the in silico prediction of rodent oral toxicity. Nucleic acids res 42(W1) W53-W58
38. Pires DE, Blundell TL, Ascher DB (2015) pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. J Med Chem 58(9):4066-4072
39. Dallakyan S, Olson AJ (2015) Small-molecule library screening by docking with PyRx. Methods Mol Biol 1263:243–250. https://doi.org/10.1007/978-1-4939-2269-7_19 - DOI - PubMed
40. Ertl P, Rohde B, Selzer P (2000) Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. J med chem 43(20):3714-3717
41. Baell J, Congreve M, Leeson P, Abad-Zapatero C (2013) Ask the experts: past, present and future of the rule of five Future med chem 5(7):45-752
42. Saklani A, Kutty SK (2014) Herbal medicine as inducers of apoptosis in cancer treatment. Adv Pharm Bull 4(Suppl1):421–427. https://doi.org/10.5681/apb.2014.062
43. Cheng F, Li W, Liu G, Tang Y (2013) In silico ADMET prediction: recent advances, current challenges and future trends. Current topics in medicinal chemistry 13(11):1273-1289
44. Zeiger E (2019) The test that changed the world: The Ames test and the regulation of chemicals. Mutat Res Genet Toxicol Environ Mutagen 841:43-48
45. Fujita T, Urban TJ, Leabman MK, Fujita K, Giacomini KM (2006) Transport of drugs in the kidney by the human organic cation transporter, OCT2 and its genetic variants. J Pharm Sci 95(1):25-36
46. Sevrioukova IF, Poulos TL (2013) Understanding the mechanism of cytochrome P450 3A4: recent advances and remaining problems. Dalton Trans 42(9):3116-3126
47. Barthe L, Woodley J, Houin G (1999) Gastrointestinal absorption of drugs: methods and studies. Fundam Clin Pharmacol 13(2):154-168

48. Wang W, Donini O, Reyes CM, & Kollman PA (2001) Biomolecular simulations: recent developments in force fields, simulations of enzyme catalysis, protein-ligand, protein-protein, and protein-nucleic acid noncovalent interactions. Annu rev bioph biom 30(1):211-243
49. Khanal P, Chikhale R, Dey YN, Pasha I, Chand S, Gurav N, ... & Gurav S (2022) Withanolides from Withania somnifera as an immunity booster and their therapeutic options against COVID-19. J. Biomol 40(12):5295-5308.
50. Kumer A, Chakma U, Matin MM, Akash S, Chando A, Howlader D (2021) The computational screening of inhibitor for black fungus and white fungus by D-glucofuranose derivatives using in silico and SAR study Org Commun 14:(4)
51. Rahman MM, Islam MR, Akash S, Mim SA, Rahaman MS, Emran TB, ... & Wilairatana P (2022) In silico investigation and potential therapeutic approaches of natural products for COVID-19: Computer-aided drug design perspective Front Cell Infect Microbiol 12:929430 https://doi.org/10.3389/fcimb.2022.929430
52. Menendez CA, Accordino SR, Gerbino DC, Appignanesi GA (2016) Hydrogen bond dynamic propensity studies for protein binding and drug design. PloS One, 11(10) e0165767 https://doi.org/10.1371/journal.pone.0165767
53. Tambunan USF, Nasution MAF (2017) Identification of novel Ebola virus (EBOV) VP24 inhibitor from Indonesian natural products through in silico drug design approach. In AIP Conference Proceedings (Vol. 1862, No. 1). AIP Publishing
54. Sharma G, Sharma AR, Kim JC (2024) Recent Advancements in the Therapeutic Development for Marburg Virus: Updates on Clinical Trials. Curr Infect Dis Rep 1-11

Table 4 and 5

Table 4 and 5 are available in the Supplementary Files section.

Figures

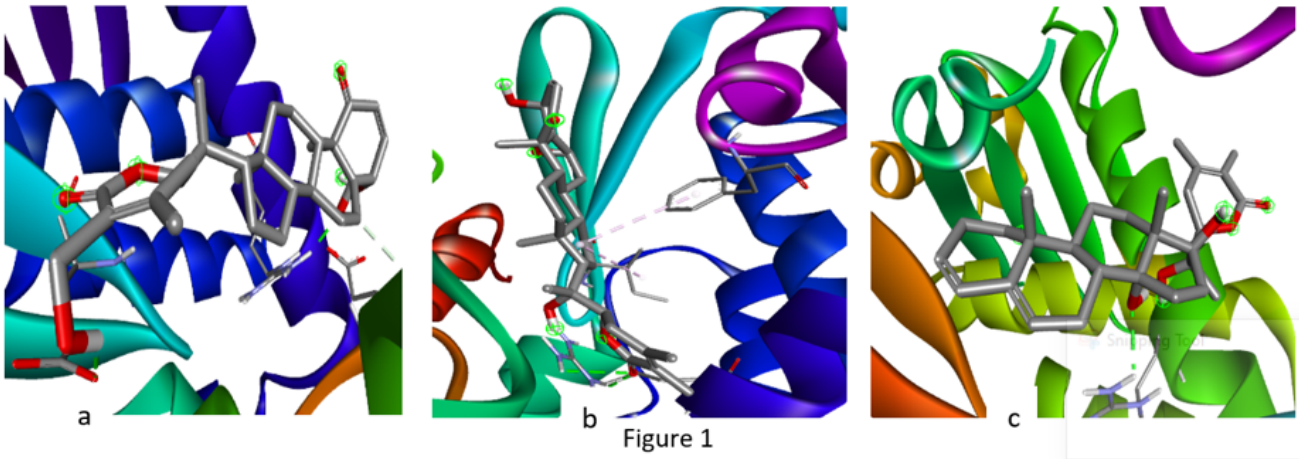

Figure 1

Illustrates the 3D structure interaction of Withaferin A (a), Withanolide D (b) and Withanolide F (c) with the Ebola virus VP24.

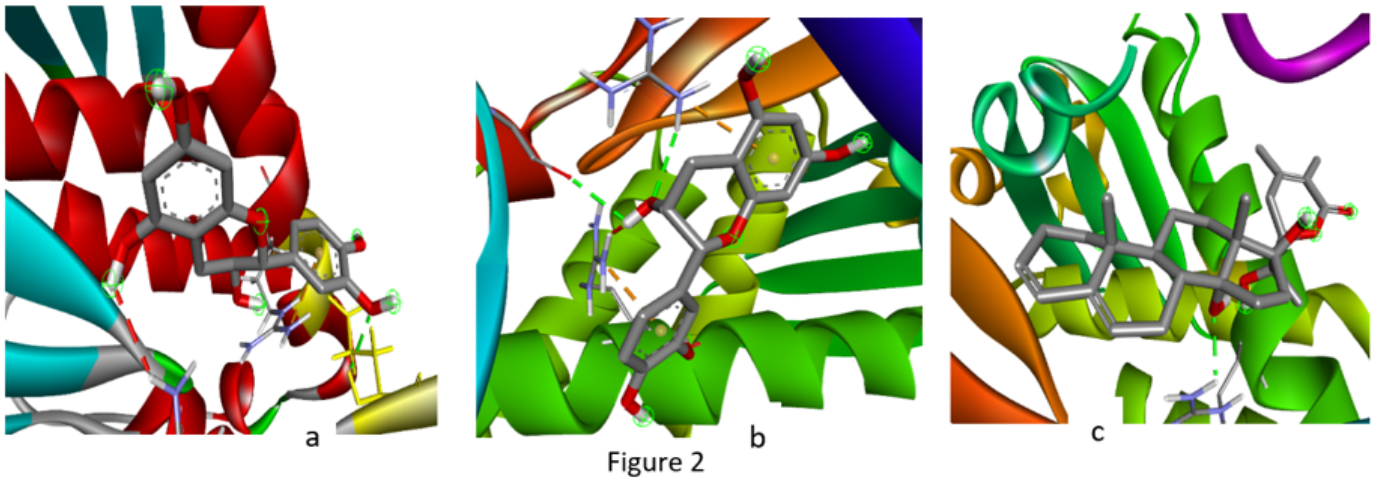

Figure 2

Figure 2

Illustrates the 3D structure showing the interactions of Catechin (a), Epicatechin (b) and Isolariciresinol (c) with Ebola virus VP24.

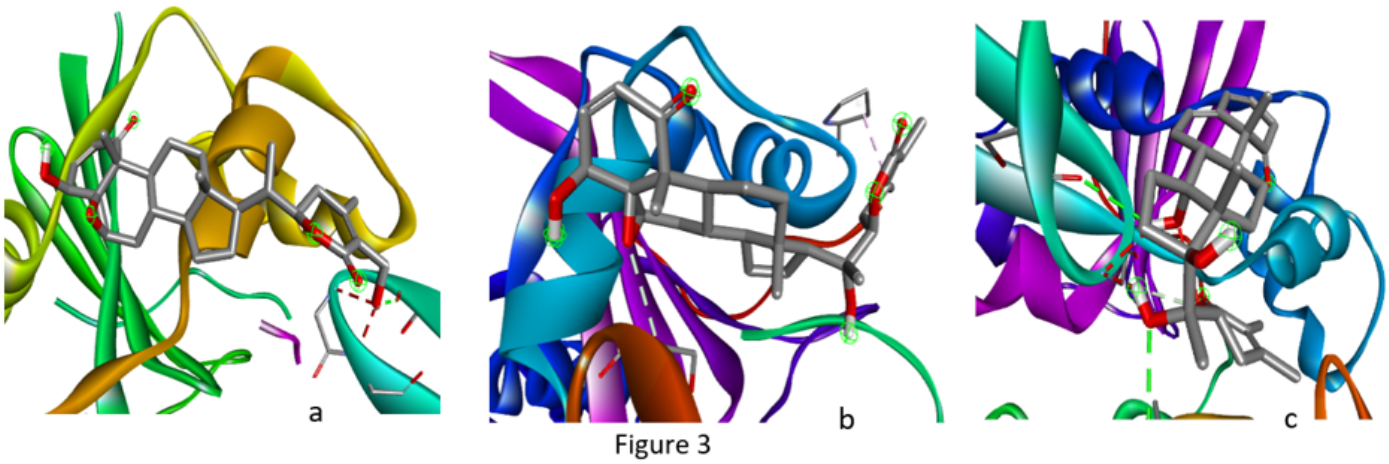

Figure 3

Figure 3

Illustrates the 3D structure interaction of Withaferin A (a), Withanolide D (b) and Withanolide F (c) with the Murburg virus VP24.

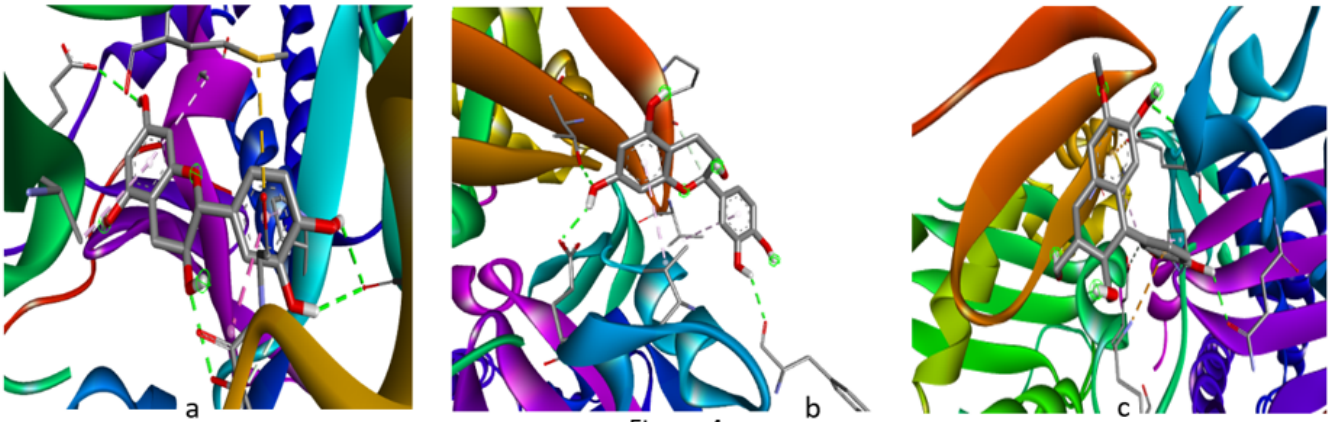

Figure 4

Illustrates the 3D structure showing the interactions of Catechin (a), Epicatechin (b) and Isolariciresinol (c) with Murburg virus VP24.

Supplementary Files

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