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HEALTH SCIENCES

Two phytocompounds from *Schinopsis brasiliensis* show promising antiviral activity with multiples targets in Influenza A virus

PEDRO HENRIQUE SETTE-DE-SOUZA, MOAN J.F. COSTA, FÁBIO A.C. ARAÚJO, EVERTON N. ALENCAR & LUCAS AMARAL-MACHADO

Abstract: Influenza A virus, the main flu agent, affects billions of people worldwide. Conventional treatments still present limitations related to drug-resistance and severe side effects. As a result, natural product-derived molecules have been increasingly investigated as prospect drug candidates. Therefore, the aim of this study was to investigate the possible anti-flu activity and to evaluate the toxicity and pharmacokinetic parameters, by *in silico* approaches, of the Schinopsis brasiliensis Engl. phytochemical compounds. Nine phytocompounds and six antiviral drugs (Amantadine, Umifenovir, Favipiravir, Nitazoxanide, Oseltamivir, Zanamivir) were selected for the analyses against four Influenza A proteins: neuraminidase, polymerase basic protein 2, hemagglutinin and M2 ion channel protein. The molecular docking, the predicted antiviral activity, the predicted toxicity and the pharmacokinetics investigations were conducted. The obtained results demonstrated that Syringaresinol and Cycloartenone display promising *in silico* antiviral activity (binding energy < 5.0 and ≥ 9.0 kcal/mol) and safety (low toxicity than commercial anti-flu drugs). Overall, this study corroborated the hypothesis that S. brasiliensis barks extract has a biological activity against Influenza A virus. Additionally, Syringaresinol and Cycloartenone have multiple targets in Influenza A virus and showed themselves as the most promising phytocompounds to be isolated and considered for the therapeutic arsenal against the flu.

Key words: Bioinformatics, *In silico* modeling, natural products, pharmacokinetics, protein binding.

INTRODUCTION

The flu, a common infectious disease caused by the influenza virus, affects annually billions of people worldwide (Huang et al. 2020). This infection has gained notoriety in the public health scenario due to antiviral resistance and its high mutation rate, which leads to the inefficacy of the pharmacological treatment and evasion of the human immune system. Based on this remark, the epidemics caused by the Influenza A virus results in high mortality, mainly in older people (Iuliano et al. 2018), which evidences the importance of studies to develop new strategies intended to improve the treatment methods (Liu et al. 2018, Kadam & Wilson 2017, Sheu et al. 2011).

Regarding this issue, it is possible to observe that the development of delivery systems/dosage forms against Influenza A has been targeting (i) neuraminidase inhibitors, (ii) M2 ion channel inhibitors, and/or (iii) hemagglutinin protein inhibitors (Kadam & Wilson 2017). However, this technological pharmaceutic approach directly impacts in the medicine cost and undesirable side effects, such as headache,

diarrhea, heart failure, photophobia, dyspnea, and hypertension, which impair patient access and compliance (Smith et al. 2002).

In order to overcome these drawbacks, traditional and ethnobotanical knowledge plays an essential role in the search for therapeutic alternatives intended for the treatment of Influenza A infections. Therefore, the use of natural products/compounds has been deeply studied since these products are described as renewable sources with noteworthy biological effects, reduced side effects, easy access, and low cost (Amaral-Machado et al. 2020).

In this context, many studies have investigated plants as the leading natural products for flu treatment. They contributed not only to the scientific field in the development of new drugs/medicines but also to the management of Influenza A infections in vulnerable populations (Gasparotto Junior et al. 2019, Picking et al. 2015, Yang et al. 2014, Tabuti et al. 2012, Pieroni & Gray 2008).

Concerning this subject, *Schinopsis brasiliensis* Engl., a tree of the Anacardiaceae family, endemic in Brazil semiarid regions (Caatinga Biome) and popularly known as "braúna" or "baraúna", has been widely studied in the ethnobotanical field due to its traditional use to treat the flu and other affections (Sette-de-Souza et al. 2020a, b). Although studies have demonstrated the popular use of its bark extract, its biological effects using *in vitro* or *in vivo* approaches against Influenza A infection have not been disclosed (Albuquerque et al. 2007, Albuquerque 2006).

Therefore, this study aimed to investigate the possible anti-flu activity and evaluate the toxicity and pharmacokinetic parameters of the phytochemical compounds from *S. brasiliensis* by *in silico* approaches. The overall rationale of this work is that the obtaining of data by computational analyses that could support this natural product use against Influenza A would contribute to the anti-flu therapeutic arsenal improvement.

MATERIALS AND METHODS

Compounds Identification and Ligands Obtaining

The *Schinopsis brasiliensis* bark phytocompounds, previously identified by Moreira (2009), were selected for *in silico* analyses. In addition, six antiviral drug models (Umifenovir, Favipiravir, Nitazoxanide, Amantadine, Zanamivir, Oseltamivir) were used as positive controls due to their noteworthy biological activity against the Influenza A virus (Shah et al. 2020) (Figure 1).

The structure of fifteen compounds was downloaded from the National Center for Biotechnology Information (NCBI) chemical structure library (PubChem, RRID:SCR_004284). The files were saved and imported in 3D SDF format and converted to Protein Data Bank format (PDB) by the Open Babel (RRID:SCR_014920). The following compounds were used: β-Sitosterol ($C_{29}H_{50}O$ – PubChem CID: 222284), Stigmast-4-en-3-one ($C_{29}H_{48}O$ – PubChem CID: 5484202), Cycloartenone ($C_{30}H_{48}O$ – PubChem CID: 12305360), Vanillin ($C_8H_8O_3$ – PubChem CID: 1183), 3,4,5-Trimethoxybenzyl alcohol ($C_{10}H_{14}O_4$ – PubChem CID: 77449), Methyl 2,4-dihydroxy-3,6-dimethylbenzoate ($C_{10}H_{12}O_4$ – PubChem CID: 78435), Methyl gallate ($C_8H_8O_5$ – PubChem CID: 7428), Gallic Acid ($C_6H_2(OH)_3COOH$ - Pubchem CID: 370), Syringaresinol ($C_{22}H_{26}O_8$ – PubChem CID: 100067), Umifenovir ($C_{22}H_{25}BrN_2O_3S$ – PubChem CID: 131411), Favipiravir ($C_5H_4FN_3O_2$ – PubChem CID: 492405), Nitazoxanide ($C_{12}H_9N_3O_5S$ – PubChem CID: 41684), Amantadine ($C_{10}H_{17}N$ – PubChem CID: 2130), Zanamivir ($C_{12}H_{20}N_4O_7$ – PubChem CID: 60855), and Oseltamivir ($C_{16}H_{28}N_2O_4$ – PubChem CID: 65028).

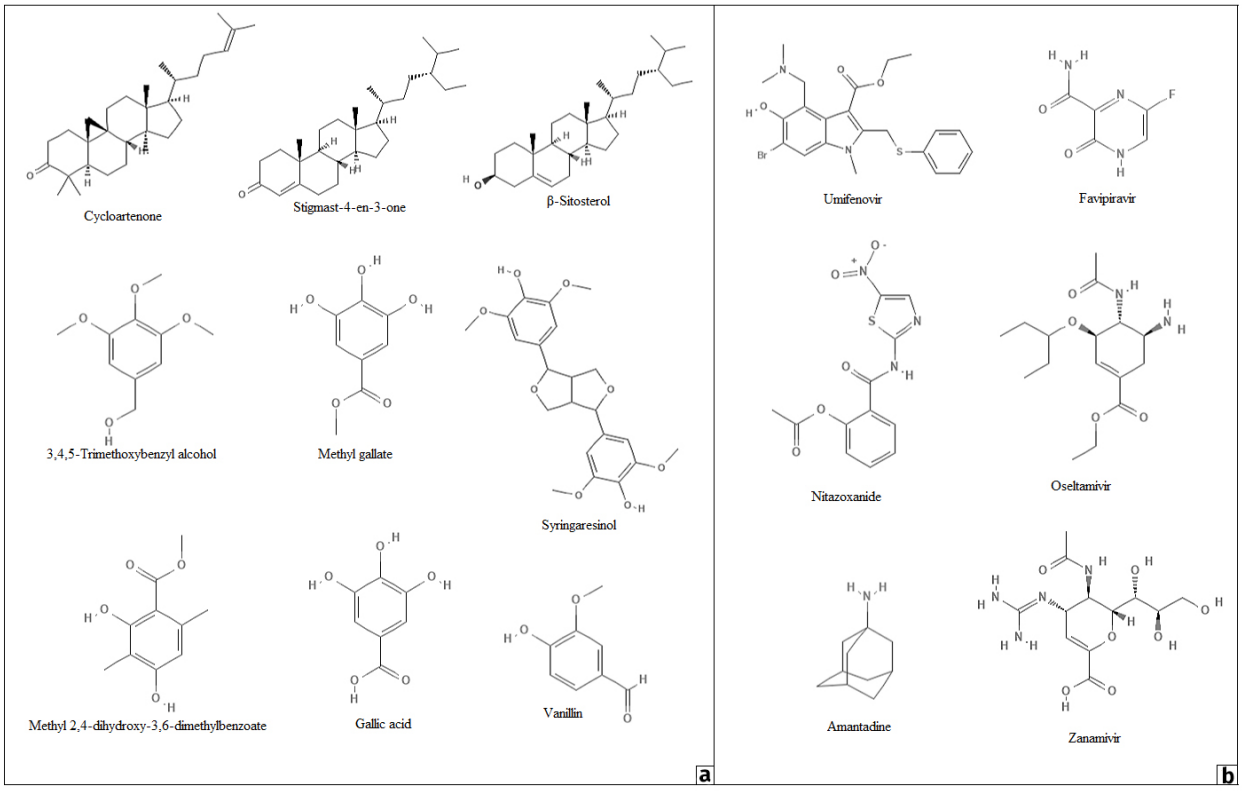

Figure 1. ***S. brasiliensis*** **phytocompounds (a) and antiviral drugs' chemical structures (b).**

Retrieval and Proteins Preparation

The crystal structures of Influenza A virus' proteins were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank – RCSB PDB (RRID:SCR_012820) in PDB file format. In this study, four influenza proteins were used: 1L7F (neuraminidase), 2VY6 (polymerase), 5T6N (hemagglutinin) and 6BKK (M2 ion channel).

AutoDock (RRID:SCR_012746) was used to delete repeated chains (we used only chain A), to add polar hydrogens atoms, and to add Gasteiger charge to all atoms in protein structure (Sette-de-Souza et al. 2021). Hence, the grid coordinates of the position X, Y, and Z to each protein were obtained (Table I).

In silico analyses

Molecular docking was carried out using AutoDock Vina (RRID:SCR_011958), and the best ligand/protein model was identified based on the binding energy (ΔG – kcal/mol) (Trott & Olson 2010). According to the protein, the docking parameters were set (table I), however, all analyses were carried out with "exhaustiveness = 8". To validate this procedure, we performed a redocking study to observe if the protein-ligand conformation would be the same. Then, we added hydrogens to the proteins by use of UCSF Chimera (RRID:SCR_004097), and then converted pdb file format to pdbqt by AutoDock Tools. So, we reperformed the analyses using AutoDock Vina.

The 2D interactions of the complex protein-ligand structure, including hydrogen bonds and the bond lengths, were analyzed by Ligplot+ (RRID:SCR_018249) for the high-affinity bindings and the standard drug on the specific receptor (Abdel Bar et al. 2020).

Table I. Grid parameters of studied Influenza proteins.

Proteins	Center			Size		
	X	Y	Z	X	Y	Z
1L7F*	19	21	55	82	82	82
2VY6*	10	9	12	74	96	80
5T6N*	28	38	-44	58	126	62
6BKK*	73	14	47	116	40	40

*** Influenza proteins: 1L7F (neuraminidase), 2VY6 (polymerase), 5T6N (hemagglutinin) and 6BKK (M2 ion channel).**

Antiviral activity was predicted using the webserver Antiviral Compound Prediction (AVCpred) (RRID:SCR_018505) (Qureshi et al. 2017). The SFD files were uploaded individually, and the predicted viral inhibition analysis was performed.

In silico toxicity study was performed using ProTox-II (RRID:SCR_018506), wherein the organ toxicity, carcinogenicity, mutagenicity, cytotoxicity and toxicity class were evaluated (Shah et al. 2019). The "Toxicity class" is a definition according to the globally harmonized system of classification of labeling of chemicals – GHS (UN, 2011). The GHS has five categories according to acute toxicity through LD50. The classification process refers to the hazard arising from the intrinsic properties of substances or mixtures, whether natural or synthetic. The classification mentioned above may improve the toxicity analyses since it indicates the toxicity degree through a worldwide standard, facilitating comparison among compounds or mixtures.

The Absorption, Distribution, Metabolism, and Excretion (ADME) studies were performed using ADMETlab (Dong et al. 2018). Important ADME descriptors, including the Blood-Brain Barrier (BBB) penetration, Human Intestinal Absorption (HIA), and Human Colon Adenocarcinoma Cells (Caco-2) permeability, were analyzed. Additionally, other pharmacokinetic parameters were calculated using the Molinspiration online software tool (RRID:SCR_018525), and the percentage of absorption was calculated as described by Zhao et al. (2002).

RESULTS AND DISCUSSION

Antiviral prediction evaluation

Ethnopharmacological surveys report *Schinopsis brasiliensis* as a suitable natural product with antiviral activity and usefulness in the flu treatment (Albuquerque et al. 2007, Albuquerque 2006). However, there is a lack of scientific evidence supporting this biological activity. Based on this remark, an *in silico* approach was used to elucidate and demonstrate the possible anti-flu activity of *S. brasiliensis* bark compounds. The therapeutic arsenal against Influenza A infections is restricted and, until 2018, only two classes of antiviral drugs were developed and used worldwide to treat Influenza A: adamantanes – target the virus M2 ion channel protein - and neuraminidase inhibitors (Tilmanis et al. 2020).

In this study, all tested phytocompounds presented affinity with Influenza A virus proteins (Table II). Moreover, Amantadine (positive control) showed the most outstanding predicted percentage inhibition (78.02 %), interpreted as potential antiviral activity, compared to all tested compounds. On the other hand, it is

Table II. Binding energy (ΔG - kcal/mol) and predicted percentage inhibition of the *S. brasiliensis* phytocompounds and drug models (positive controls).

Compound	Binding energy - ΔG (kcal/mol)				% Inhibition
	1L7F*	2VY6*	5T6N*	6BKK*	
β-Sitosterol	-6.3	-5.2	-7.0	-4.7	42.70
Stigmast-4-en-3-one	-6.1	-5.4	-8.4	-5.3	41.65
Cycloartenone	-6.8	-6.6	-9.0	-5.7	42.79
Vanillin	-5.0	-4.7	-5.9	-3.7	26.49
3,4,5-Trimethoxybenzyl alcohol	-5.2	-4.6	-5.4	-3.3	6.81
Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	-5.8	-5.2	-6.4	-3.8	56.16
Methyl gallate	-5.5	-4.8	-5.9	-3.3	58.93
Gallic Acid	-5.8	-5.5	-6.0	-3.5	42.39
Syringaresinol	-7.3	-6.6	-7.5	-5.1	35.56
Umifenovir	-5.6	-5.5	-6.8	-4.7	45.72
Favipiravir	-5.6	-4.7	-5.8	-3.5	24.81
Nitazoxanide	-7.1	-5.9	-7.6	-4.5	35.95
Amantadine	-5.8	-4.8	-5.8	-3.5	78.02
Zanamivir	-6.9	-5.8	-6.6	-3.9	43.93
Oseltamivir	-6.5	-5.1	-5.8	-4.3	24.10

*** Influenza proteins: 1L7F (neuraminidase), 2VY6 (polymerase), 5T6N (hemagglutinin) and 6BKK (M2 ion channel).**

essential to note that the phytocompounds Cycloartenone, β-Sitosterol, Stigmast-4-en-3-one, and Syringaresinol showed not only predicted percentage inhibition (42.79 %, 42.70 %, 41.65 % and 35.56 %, respectively) as expressive as the antiviral drugs Nitazoxanide (35.95 %), Zanamivir (43.93 %), and Umifenovir (45.72 %), but also smaller binding energies than these drugs.

In light of these results, it is possible to infer that these compounds, mainly Syringaresinol and Cycloartenone, are promising bioactive molecules with potential antiviral activity. Moreover, it is important to point out that AVCpred is not a specific webserver to evaluate the antiviral activity against influenza viruses. This server groups provide several viruses' data such as influenza A, influenza B, H1N1, SARS-coronavirus and respiratory syncytial virus (Qureshi et al. 2017). However, the combined results (molecular docking and predicted percentage inhibition) suggest that Syringaresinol and Cycloartenone are possible anti-flu compounds with multiple targets.

Additionally, the binding energies to the selected Influenza A proteins were also evaluated (Table II). The neuraminidase plays a crucial role in the Influenza A virus replication cycle, and a new generation of inhibitors would be important to overcome the emergence of resistant strains to Zanamivir and Oseltamivir (Zhang et al. 2020). In this context, both the Cycloartenone

(ΔG= - 6.8 kcal/mol) and the Syringaresinol (ΔG= - 7.3 kcal/mol) become promising candidates to inhibit the neuraminidase of the Influenza A virus, since they displayed lower and/or similar binding energies than the marketable antiviral agents. This superior affinity may have resulted from the numerous hydrophobic interactions between Cycloartenone and neuraminidase.

Moreover, the Zanamivir and Oseltamivir antiviral agents and the tested compounds (Cycloartenone and Syringaresinol) act in the same protein cavity (Figure 2, Table III). They bind to the amino acid residues chain by hydrogen bonds or hydrophobic interactions (Arg118, Glu119, Asp151, Trp178, Ser179, Ile222, Arg224, Ala246, Glu276, Glu277, Asn294, Tyr406). This finding is also supported by Zhang et al. (2020) study, who demonstrated noteworthy binding of Oseltamivir with amino acid residues chains and, thus, contribute to the direction of further studies in the development of new neuraminidase inhibitors. Solely based on the binding energies, among all compounds, Syringaresinol could be potentially considered for further *in vitro* studies.

Indeed, there is limited research using this protein as a target for antiviral molecules, to this date. Furthermore, there are no polymerase

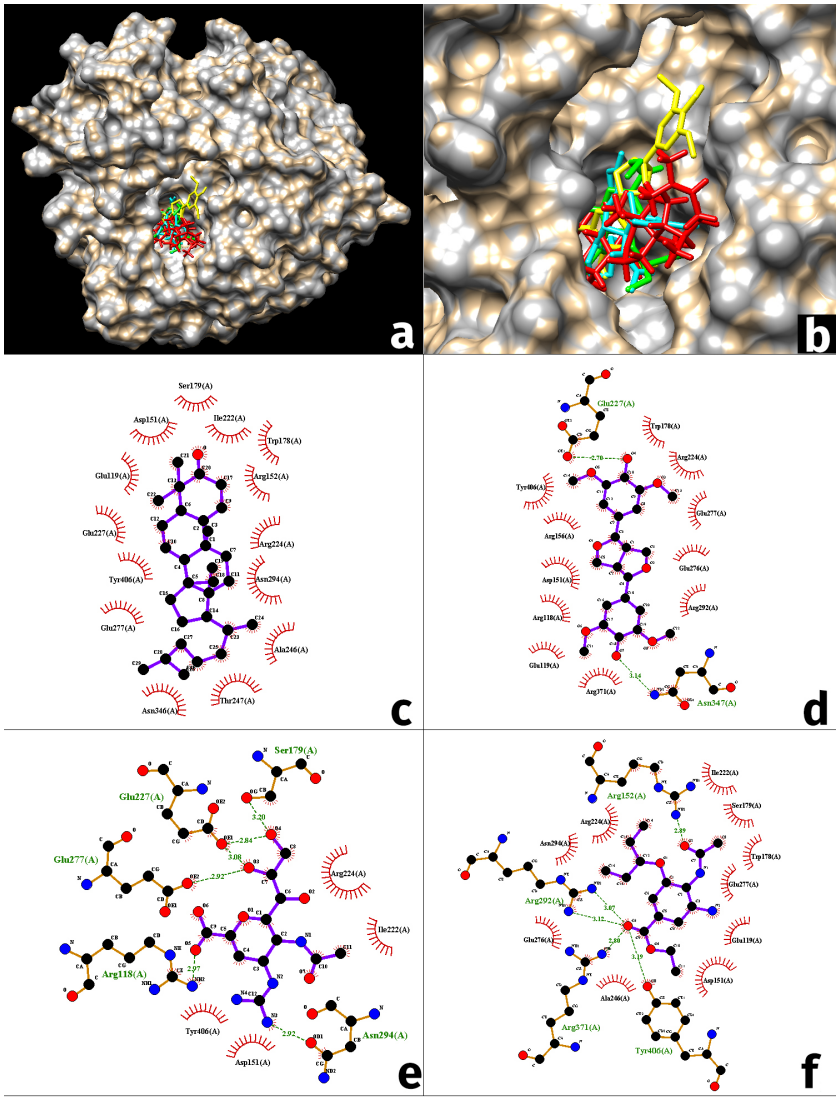

Figure 2. Interactions between the *S. brasiliensis* phytocompounds, positive controls and the neuraminidase (PDB 1L7F). Protein binding site and ligands (a, b): Cycloartenone (red), Syringaresinol (yellow), Zanamivir (cyan) and Oseltamivir (green), 2D representation of ligand and receptor interaction plots of docked molecules into binding site: Cycloartenone (c), Syringaresinol (d), Zanamivir (e) and Oseltamivir (f)

basic protein 2 (PB2) inhibitors approved for clinical use, and only Pimodivir is in Clinical Trials – Phase III (Takashita 2020, Hayden & Shindo 2019). Nonetheless, the obtained data reveal the expressive PB2 inhibitory potential of Cycloartenone (ΔG= - 6.6 kcal/mol) and Syringaresinol (ΔG= - 6.6 kcal/mol), since they can bind to several RNA subunits, as can be seen in Figure 3 and Table III. The better affinities presented by Cycloartenone and Syringaresinol than Nitazoxanide might be explained due to the greater hydrophobic interactions between the phytocompounds and the PB2. Cycloartenone interacts hydrophobically with eight residues (Ile539, Val545, Asn548, Thr549, Trp552, Glu576, Pro579, Ser582) and Syringaresinol with 12 (Asp611, Thr612, Gln614, Val649, Arg650, Gly651, Tyr658, Lys660, Lys663, Leu675, Glu677, Glu687), while the Nitazoxanide with only five (Pro568, Arg597, Pro626, Gln628, Ser741). These results suggest that these compounds may be further studied as potential candidates in developing of new medicines intended for the control of influenza A infections.

This study revealed that the β-Sitosterol, the Stigmast-4-en-3-one, the Cycloartenone, and the Syringaresinol exhibited remarkably and also more expressive binding energy with hemagglutinin than the Umifenovir (Table II). The Umifenovir is the standard drug that targets influenza's hemagglutinin since this drug effectively inhibits the viral envelop fusion with the endosome membrane, mainly at low pH media (Kadam & Wilson, 2017, Zeng et al. 2017). Moreover, the obtained data also indicates that the binding site has a hydrophobic profile. Accordingly, the strongest affinities were observed in hydrophobic interactions between the studied compounds and influenza's hemagglutinin (Table II, Figure 4). Finally, these results show themselves as preliminary and promising data that may support the development of drugs to prevent cell infection by the Influenza A virus.

The interactions between Influenza A proteins and the *Schinopsis brasiliensis* bark phytocompounds were also assessed using a protein-associated viral replication matrix 2 (M2) ion channel. The M2 is widely targeted by drugs, such as Amantadine and Rimantadine, during the treatment of Influenza A infections (Karthick & Ramanathan 2014, Pinto & Lamb 2006). The adamantyl-amine derivates inhibit M2 ion channel due to high affinity, ligand efficiency, and specificity (Thomaston et al. 2018, Karthick & Ramanathan 2014, Pinto & Lamb 2006). Based on this remark, the obtained results allow us to suggest that the tested phytocompounds show themselves as promising M2 ion channel inhibitors since their noteworthy and greater affinity (smaller binding energies) with M2 ion channel as Amantadine (Table II) was observed. Furthermore, the results also evidenced that *S. brasiliensis* bark compounds present the same binding site (His37, Leu38, Trp41) than Amantadine, implying a similar mechanism of action to this drug (Figure 5, Table III).

In silico toxicity assessment

Toxicity screening studies are one of the most important steps in discovering and developing of new active compounds/medicines for diseases treatment. However, experimental assays require high financial investments and time (Parasuraman 2011). Based on this, computational tools, such as *in silico* analyses, stand out as faster and more economically advantageous approaches than traditional *in vitro* and *in vivo* methods (Shah et al. 2019). In fact, Protox-II is a highly reliable web-served that displays high accuracy, sensitivity, and specificity for the investigated endpoints.

In silico toxicity study (Table IV) showed that all selected phytocompounds belonged to

Table III. Interactions between the *S. brasiliensis* phytocompounds, positive controls and the protein targets. AMA: Amantadine; UMI: Umifenovir; BTS: β-Sitosterol; CYC: Cycloartenone; NIT: Nitazoxanide; OSE: Oseltamivir; STI: Stigmast-4-en-3-one; SYN: Syringaresinol; ZAN: Zanamivir.

Protein	Ligand	Interactions
1L7F	CYC	Hydrophobic interaction: Glu119(A), Asp151(A), Arg152(A), Trp178(A), Ser179(A), Ile222(A), Arg224(A), Glu227(A), Ala246(A), Thr247(A), Glu277(A), Asn294(A), Asn346(A), Tyr406(A)
	SYR	Hydrogen bond: Glu227(A), Asn347(A) Hydrophobic interaction: Arg118(A), Glu119(A), Asp151(A), Arg156(A), Trp178(A), Arg224(A), Glu276(A), Glu277(A), Arg292(A), Arg371(A), Tyr406(A)
	NIT	Hydrogen bond: Arg292(A), Tyr406(A) Hydrophobic interaction: Arg118(A), Asp151(A), Asp152(A), Trp178(A), Ile222(A), Arg224(A), Glu227(A), Glu276(A), Glu277(A), Arg371(A)
	OSE	Hydrogen bond: Arg152(A), Arg292(A), Arg371(A), Tyr406(A) Hydrophobic interaction: Glu119(A), Asp151(A), Trp178(A), Ser179(A), Ile222(A), Arg224(A), Ala246(A), Glu276(A), Glu277(A), Asn294(A)
	ZAN	Hydrogen bond: Arg118(A), Ser179(A), Glu227(A), Glu277(A), Asn294(A) Hydrophobic interaction: Asp151(A), Ile222(A), Arg224(A), Tyr406(A)
2VY6	CYC	Hydrogen bond: Arg555(A) Hydrophobic interaction: Ile539(A), Val545(A), Asn548(A), Thr549(A), Trp552(A), Glu576(A), Pro579(A), Ser582(A)
	SYR	Hydrogen bond: Thr613(A) Hydrophobic interaction: Asp611(A), Thr612(A), Gln614(A), Val649(A), Arg650(A), Gly651(A), Tyr658(A), Lys660(A), Lys663(A), Leu675(A), Glu677(A), Glu687(A)
	NIT	Hydrogen bond: Tyr572(A), Thr598(A), Arg604(A) Hydrophobic interaction: Pro568(A), Arg597(A), Pro626(A), Gln628(A), Ser741(A)
5T6N	BST	Hydrophobic interaction: Ile58(A), Leu59(A), Asp60(A), Ile62(A), Asp63(A), His75(A), Val78(A), Phe79(A), Glu82(A)
	CYC	Hydrophobic interaction: Pro103(A), Gln211(A), Tyr233(A), Trp234(A), Ile236(A), Lys238(A), Asp241(A)
	STI	Hydrophobic interaction: Pro103(A), Asp104(A), Gln211(A), Tyr233(A), Trp234(A), Ile236(A)
	SYN	Hydrogen bond: Asp104(A), Thr206(A) Hydrophobic interaction: Arg208(A), Ser209(A), Gln211(A), Tyr233(A), Trp234(A), Ile236(A), Lys238(A), Asp241(A)
	NIT	Hydrogen bond: Ile236(A) Hydrophobic interaction: Pro103(A), Asp104(A), Ser209(A), Gln210(A), Gln211(A), Trp234(A), Thr235(A)
	UMI	Hydrogen bond: Ser209(A), Gln211(A), Ile236(A) Hydrophobic interaction: Pro103(A), Asp104(A), Tyr233(A), Trp234(A), Thr235(A)
6BKK	BST	Hydrophobic interaction: Ile35(A), Leu38(A), Ile39(A), Ile42(A), Leu43(A)
	CYC	Hydrophobic interaction: Ile35(A), Leu38(A), Ile39(A), Ile42(A), Leu43(A)
	STI	Hydrophobic interaction: Ser31(A), Gly34(A), Ile35(A), Leu38(A), Trp41(A)
	SYN	Hydrophobic interaction: Gly34(A), Ile35(A), His37(A), Leu38(A), Trp41(A)
	AMA	Hydrophobic interaction: His37(A), Leu38(A), Trp41(A)

toxicity classes 4 and 5, with a LD_{50} greater than 800 mg/kg. On the other hand, most control antiviral drugs belonged to class 3 and 4, with a LD_{50} lower than 600 mg/kg, except for Zanamivir (class 5 – LD_{50} 5000 mg/kg) and Nitazoxanide (class 5 – LD_{50} 1350 mg/kg). These findings support the hypothesis of Amaral-Machado et al. (2020), who described the use of natural products as an alternative therapeutic source with low cost, easy access and low toxicity. Therefore, our findings highlight the potential of those compounds as an alternative to the treatment of Influenza A infections due to their remarkable biological activity, and reduced toxicity compared to marketed synthetic drugs.

Additionally, the obtained results of Gallic Acid (phytocompound), Favipiravir and Nitazoxanide (antiviral drugs) could indicate that these compounds are carcinogenic agents and the Nitazoxanide is a mutagenic compound (Table IV). Toxicological endpoints were obtained from Protox-II. Although these molecules are already used for human therapeutics, the potential for carcinogenesis and mutagenesis cannot be disregarded. Protox-II displays an accuracy above 80 % for these parameters, which

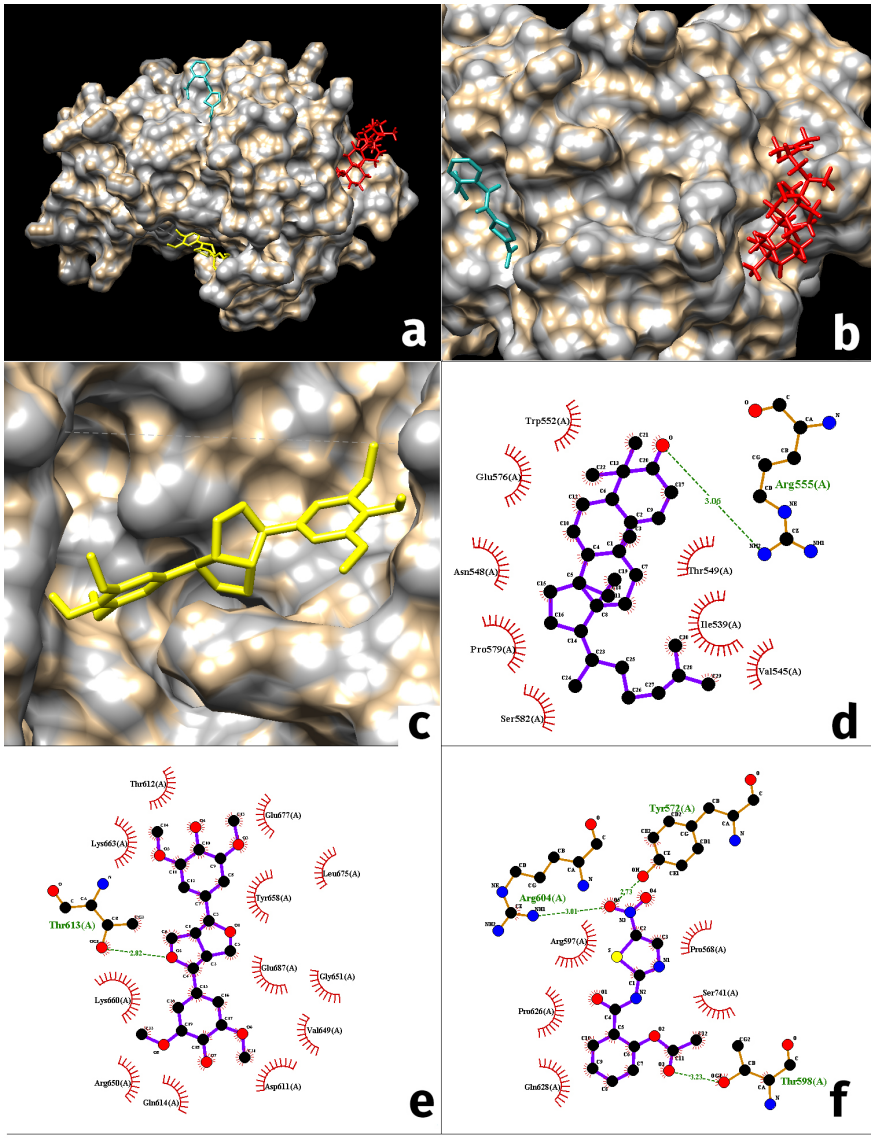

Figure 3. Interactions between the *S. brasiliensis* phytocompounds, positive controls and the polymerase basic protein 2 - PB2 (PDB 2VY6). Protein binding site and ligands (a, b, c): Cycloartenone (red), Syringaresinol (yellow) and Nitazoxanide (light sea, 2D representation of ligand and receptor interaction plots of docked molecules into binding site: Cycloartenone (d), Syringaresinol (e) and Nitazoxanide (f).

highlights the potential damage that could be caused by such drugs (Banerjee et al. 2018). The obtained results can be corroborated by *in vivo* studies performed for isolated molecules. Indeed, Murphy & Friedmann (1985) investigated the toxicity of Nitazoxanide in animal models and found an acute oral LD50 of 1.4 g/kg in mice, similar to the estimated data from Protox-II for this drug.

Finally, the high LD_{50}, in addition to the absence of hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity observed in the majority of tested phytocompounds, highlight their therapeutic potential as promising new bioactive. These results are especially relevant for the phytocompounds considering that FDA-approved drugs, such as Nitazoxanide, displayed toxicological limitations and are available to the market.

Pharmacokinetics parameters prediction evaluation

In silico pharmacokinetics analyses were performed to evaluate the absorption, distribution, metabolism and excretion (ADME)

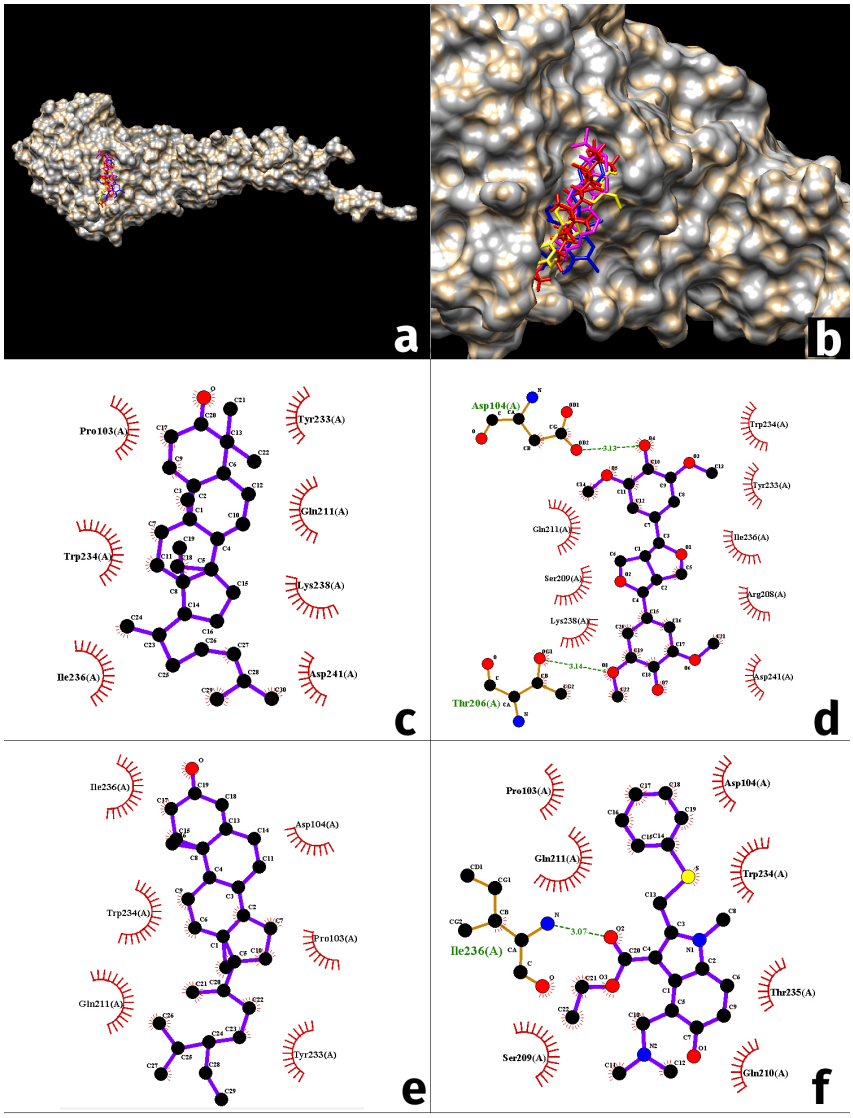

Figure 4. Interactions between the *S. brasiliensis* phytocompounds, positive controls and the hemagglutinin (PDB 5T6N). Protein binding site and ligands (a, b): Cycloartenone (red), Syringaresinol (yellow), Stigmast-4-en-3-one (magenta) and Umifenovir (blue), 2D representation of ligand and receptor interaction plots of docked molecules into binding site: Cycloartenone (c), Syringaresinol (d), Stigmast-4-en-3-one (e) and Umifenovir (f).

of the phytocompounds by ADMETlab and Molinsipiration information.

Pharmacokinetics (PK) assessment via ADME analyses is required in the screening of possible drug molecule candidates. These analyses help to determine whether drugs will bind to protein, their half-live, among other parameters. Such studies are relevant once several drug clinical trials fail due to PK issues rather than pharmacodynamics. However, current experimental methods for PK studies are money- and time-consuming. Most preliminary methods are based on *in-vivo* experiments, which require the extensive use of animals. When triaging several different molecules, especially from natural compounds, such methods could become a limitation for the advance of research. Hence, *in silico* models for ADME assessment provide reliable, fast and preliminary results for screening of possible drug candidates (Dong et al. 2018).

Therefore, the data revealed that only Gallic Acid and Zanamivir had a negative ADME evaluation (Table V), which means that these compounds, based on this *in silico* analysis, have poor absorption ability. These results are

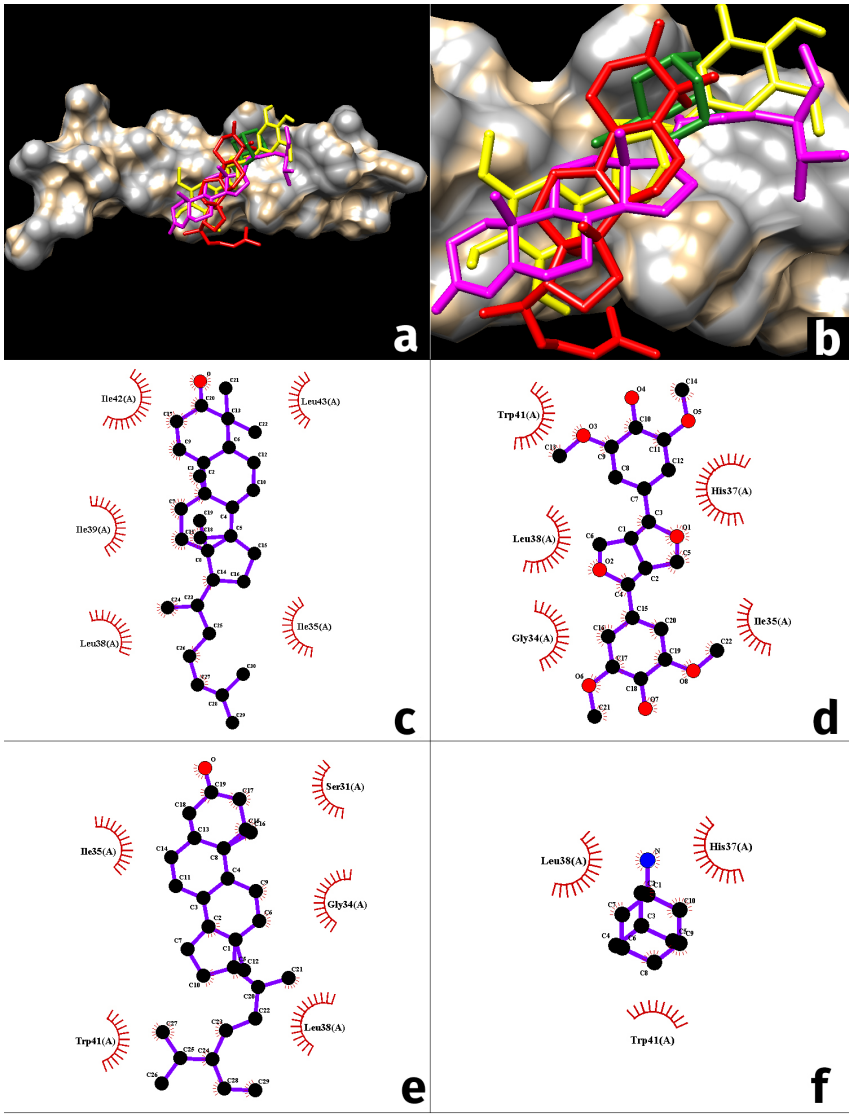

Figure 5. Interactions between the *S. brasiliensis* phytocompounds, positive controls and the M2 ion channel protein (PDB 6BKK). Protein binding site and ligands (A, B): Cycloartenone (red), Syringaresinol (yellow), Stigmast-4-en-3-one (magenta) and Amantadine (forest green), 2D representation of ligand and receptor interaction plots of docked molecules into binding site: Cycloartenone (c), Syringaresinol (d), Stigmast-4-en-3-one (e) and Amantadine (f).

related to the high polar surface area, which implies increased water solubility and low lipophilicity, which reduces the chances of absorption via passive diffusion (Arnott & Planey 2012). On the other hand, β-Sitosterol, Stigmast-4-en-3-one, Cycloartenone and Amantadine showed predicted percentage absorption (% ABS) values higher than 100% (Table V). These molecules display higher lipophilicity based on their chemical structure. The *in silico* results corroborate the biopharmaceutical and pharmacokinetics rationale that molecules with cyclic and mostly nonpolar groups, such as Cycloartenone, are easily absorbed once they are chemically favorable to go through the membranes via passive diffusion.

The obtained results allowed us to suggest that these compounds show the required biopharmaceutical characteristics that contribute to their tissue permeation and, as a result, availability to promote their biological effect. However, it is important to highlight that encouraging pharmacokinetic profiles, obtained by *in silico* analyses, are only observed when an association of all positive ADME descriptors and % ABS are obtained. ADME descriptors analyses by *in silico* studies should be regarded as predictors and triage tools for further *in vivo* study. This requirement is especially relevant once permeability itself is a complex process that is based on multiple mechanisms, such as passive diffusion (trans- and para-cellular),

Table IV. *In silico* toxicity studies of *S. brasiliensis* phytocompounds and positive controls. LD_{50}: Lethal Dose; HPT: Hepatotoxicity; CCN: Carcinogenicity; MTG: Mutagenicity; CYT: Cytotoxicity.

Compound	LD_{50} (mg/kg)	Class	HPT	CCN	MTG	CYT
β-Sitosterol	890	4	Inactive	Inactive	Inactive	Inactive
Stigmast-4-en-3-one	2450	5	Inactive	Inactive	Inactive	Inactive
Cycloartenone	2420	5	Inactive	Inactive	Inactive	Inactive
Vanillin	1000	4	Inactive	Inactive	Inactive	Inactive
3,4,5-Trimethoxybenzyl alcohol	1496	4	Inactive	Inactive	Inactive	Inactive
Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	1900	4	Inactive	Inactive	Inactive	Inactive
Methyl gallate	1700	4	Inactive	Inactive	Inactive	Inactive
Gallic Acid	2000	4	Inactive	Active	Inactive	Inactive
Syringaresinol	1500	4	Inactive	Inactive	Inactive	Inactive
Umifenovir	340	4	Inactive	Inactive	Inactive	Inactive
Favipiravir	555	4	Inactive	Active	Inactive	Inactive
Nitazoxanide	1350	4	Inactive	Active	Active	Inactive
Amantadine	157	3	Inactive	Inactive	Inactive	Inactive
Zanamivir	5000	5	Inactive	Inactive	Inactive	Inactive
Oseltamivir	260	3	Inactive	Inactive	Inactive	Inactive

active diffusion, active secretion and active uptake (Yang & Hinner 2015).

Moreover, Table V displays Caco-2 *in silico*-predicted permeability results. Caco-2 cells have been used as a gold standard model for *in vitro* drug permeability. This monolayer cell culture has successfully predicted the behavior obtained by the human intestine. Accordingly, *in silico* assessments have been used to reduce the *in vitro* tests for screenings of several molecules (Pham et al. 2011). Among the tested compounds, Gallic Acid and Zanamivir were found to display poor permeability, whereas the target molecules for this study, Syringaresinol and Cycloartenone, showed positive permeability, which can be correlated to a predicted adequate intestinal permeability (Pham et al. 2011). Furthermore, other ADME endpoints, such as CYPs inhibitor and substrate could be assessed *in silico* for analyses for investigations related to these compounds. However, for the purposes of our study, the overall permeation and absorption profiles were targeted.

Several drug dosages forms have been designed to deliver molecules with biopharmaceutical limitations. In recent years, the use of nanotechnology has been widely explored due to its advantages. The increased logP found in our studies suggests that β-Sitosterol, Stigmast-4-en-3-one and Cycloartenone can be considered highly lipophilic molecules (Yang & Hinner 2015). Therefore, nanosystems could be an alternative to allow the use of these molecules.

Table V. ADME properties and pharmacokinetic parameters of *S. brasiliensis* phytocompounds and positive controls. BBB: Blood-Brain Barrier; HIA: Human Intestinal Absorption; TPSA: Total Polar Surface Area; % ABS: Percentage of Absorption.

Compound	BBB	HIA	Caco-2	logP	TPSA	% ABS
β-Sitosterol	+	+	+	11.595	20.23	102.02
Stigmast-4-en-3-one	+	+	+	11.588	17.07	103.11
Cycloartenone	+	+	+	11.314	17.07	103.11
Vanillin	+	+	+	0.589	46.53	92.95
3,4,5-Trimethoxybenzyl alcohol	+	+	+	0.680	47.93	92.46
Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	+	+	+	1.510	66.76	85.97
Methyl gallate	+	+	+	1.285	86.99	78.99
Gallic Acid	-	+	-	0.964	97.98	75.20
Syringaresinol	+	+	+	1.796	95.86	75.93
Umifenovir	+	+	+	3.328	54.70	90.13
Favipiravir	+	+	+	-0.822	88.85	78.35
Nitazoxanide	+	+	+	0.485	114.12	69.63
Amantadine	+	+	+	2.312	26.02	100.02
Zanamivir	-	-	-	-3.012	200.72	39.75
Oseltamivir	+	+	+	1.052	90.66	77.72

Nanosystems are pharmaceutical formulations that display individual units in the nanoscale (< 1000 nm). They can be used to improve drugs' biopharmaceutical aspects. Lipophilic molecules can be incorporated into solid-solid, solid-liquid, and liquid-liquid dispersions. Among the most used systems to deliver lipophilic molecules, nanocapsules and emulsified systems are worth mentioning (Kashyap et al. 2019). Nanocapsules are nanoparticles surrounded by a polymeric shell with the ability the compartmentalize oil cores, wherein drugs and natural products could be dissolved (Xavier-Junior et al. 2018). On the other hand, emulsified systems, such as nanoemulsions and microemulsions, are dispersions of immiscible liquids, often oil-in-water, wherein lipophilic molecules could be solubilized in the oil compounds (Morais et al. 2016). Overall, this type of approach could improve the apparent solubility of molecules in pharmaceutical formulations (Kashyap et al. 2019).

CONCLUSIONS

This study carried out *in silico* analyses to evaluate the anti-flu activity and obtain data that support the traditional knowledge regarding the use of *S. brasiliensis* barks in the treatment of flu. Therefore, the obtained findings of this study corroborated the hypothesis that this extract has noteworthy biological activity against the Influenza A virus. The performed analyses allowed us to predict the antiviral activity of the *S. brasiliensis* phytocompounds by its binding affinity with influenza A virus' proteins. Additionally, the observed low toxicity and high absorption percentage suggest that these phytocompounds may be promising raw materials developing new anti-flu herbal-derived drugs. Furthermore, Syringaresinol and Cycloartenone displayed affinity to multiple targets in the Influenza A virus. Finally, it is essential to highlight that *in silico* analyses are important tools to perform the screening of compounds that may present themselves as effective products for treating diseases, reducing the cost and time of experiments. Nonetheless, these analyses do not exclude the need for further *in vitro* and *in vivo* studies to confirm and better understand the therapeutic activity and mechanisms of action. Therefore, the overall results demonstrated the possible anti-flu activity of *S. brasiliensis* phytocompounds, mainly Syringaresinol and Cycloartenone, and contributed to the direction of further studies regarding the development of anti-flu *S. brasiliensis*-based medicines.

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