In Silico Potential Antiviral Activity of Polyphenols From Plants Used in Traditional Medicine in La Libertad Región, Peru

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Article

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Abstract: Traditional medicine is one of the main bases for studying and discovering natural sources of phytometabolites with antiviral properties. This research aims to demonstrate that the medicinal plants used as a treatment of viral diseases in the La Libertad region have, in fact, antiviral activity. The study evaluated the ethnobotany of the 8 most widely used medicinal plants in the region (*Azadirachta indica* A. Juss. "paraíso", *Caesalpinia spinosa* (Molina) Kuntze "tara", *Citrus limon* (L.) Osbeck "limón", *Clinopodium pulchellum* (Kunth) Govaerts "panizara", *Cordia lutea* Lam. "overo", *Ocimum basilicum* L. "albahaca", *Schinus molle* L. "molle", and *Taraxacum campylodes* G.E. Haglund "diente de león"). The phytometabolites responsible for the antiviral activity were identified by LC-MS and evaluated in silico against the viral proteins NS2B/NS3 (DENV-2), NS5B (HCV), and ICP27 (HSV-1) using molecular docking in Autodock Vina and UCSF Chimera. The presence of five polyphenols (chlorogenic acid, gallic acid, caffeic acid, rosmarinic acid, and rutin) was found and, in the *in silico* test, the antiviral activity of chlorogenic acid stood out against DENV-2 and HCV, rutin against HCV and HSV-1, rosmarinic acid against DENV-2 and HCV. Therefore, it is verified that the medicinal plants studied have antiviral activity, which supports their use in traditional medicine.

Keywords: phytometabolites; ethnobotany; polyphenols; antiviral activity; molecular docking

1. Introduction

Synthetic drugs often cause adverse effects and are harmful when the treatment lasts for a prolonged period [1]. On the contrary, natural products regulate the damaging side effects and relieve the harm of various therapies. Moreover, natural products have been used as a source for drug design and development [2]. All these factors make them a possible choice when looking for an efficient treatment [2,3].

In times of health crisis, due to the ineffectiveness of therapies and the lack of medicines to combat a disease, traditional knowledge about medicinal plants and herbs plays an important role [4] During the recent COVID-19 pandemic, this fact was highlighted [5].

As the current pandemic underscored the importance of herbal therapies in most countries [6], ethnobotany took relevance. Ethnobotany is the interrelation of human beings with plants. Nevertheless, this interrelation has been lost multiple times [7,8]. As stated before, this field can add up new medication sources and provide other health prevention benefits. Therefore, there is a need to identify suitable and reliable natural compounds that could be used as drugs [9].

Medicinal plants with potential molecules for ethnobotanical use must be researched and selected with a disease treatment approach. Also, they must comply with traditional pharmacopeias

[10], ethnopharmacological evaluations, and therapeutic efficacy reports [11,12]. Likewise, the family of the plant species is not only an ethnomedical indicator but also a key to giving value to plant usage [13,14].

Some plant species are used as traditional medicines to treat diseases of viral origin [15–18]. Antiviral active principles described include alkaloids, terpenes, polysaccharides, flavonoids, phenolic acids, and steroids [19]. Thus, investigation of new species is necessary to find a source rich in molecules with antiviral activity [20,21]. However, choosing the most biologically active one remains a challenge.

From the COVID-19 pandemic and the global health crisis, not only multiple variants of the Coronavirus (SARS-CoV-2) have emerged, but also latent viruses have reappeared. Some of them, like Hepatitis C Virus (HCV) [22] and Herpes Simplex Virus (HSV) [23], cause the most widespread and harmful chronic infections. Others like the Dengue virus [24] are less harmful. Anyway, they constitute a concern for triggering and increasing the health problem.

On the other hand, the development of new antiviral drugs with efficacy in blocking host-specific functions had stopped. However, the advancement in detection assays, prediction tools, and virtual screening [25], brought the aim back [26]. Therefore, there is a need to reinvestigate medicinal plants and herbs using modern techniques and methods [27].

The perspectives of the study give a vision of the most frequently used plants in traditional medicine. Also, it describes the most relevant species indicated when conditions or symptoms correspond to a disease of viral origin.

The research work carried out aims to demonstrate that the medicinal plants used to treat viral diseases in the La Libertad region have, in fact, antiviral activity.

2. Results and Discussion

2.1. Taxonomic and Ethnobotany of Medicinal Plants

The results of the ethnobotanical study indicate the eight plants that are used and sold the most as natural treatments for viral diseases. Table 1 presents the collected information about their usage and preparation. Also, it shows the *Herbarium Truxillense* (HUT) code that demonstrates their taxonomical validation.

Table 1. Taxonomical and ethnobotanical identification of medicinal plants sold by herbalists in markets of the city of Trujillo, La Libertad region.

Plant species	Family	Vernacular name [1]	HUT code [2]	Type of viral affection	Part used [1]	Way of preparation [1]	Way of use [1]
Azadirachta indica A. Juss.	Meliaceae	Paraíso	60828	Mosquito-borne	Le	Inf	A liter, three times a day
Caesalpinia spinosa (Molina) Kuntze	Fabaceae	Tara	60820	Dermatological, respiratory	Po, WP	Inf, De, Wa, Ba	Gargle, two times a day Wa, Ba: a time a day
Citrus limon (L.) Osbeck	Rutaceae	Limón	60821	Hepatic, dermatological, respiratory, COVID-19	Le, Fr	Inf, Ba	Time water
Clinopodium pulchellum (Kunth) Govaerts	Lamiaceae	Panizara	60830	Mosquito-borne	WP	Inf, De	Time water
Cordia lutea Lam.	Boraginaceae	Overo	60823	Hepatic	Fl	Inf, De	A liter, three times a day
Ocimum basilicum L.	Lamiaceae	Albahaca	60826	Respiratory, COVID-19, Mosquito-borne	WP	Inf, Ba	A liter, three times a day Ba: A time a day
Schinus molle L.	Anacardaceae	Molle	60836	Dermatological, respiratory, mosquito-borne	Le, Se, WP	Inf, Ba, Ru	A liter, three times a day Ba: three times a week

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Taraxacum campylodes G. E. Haglund	Asteraceae	Diente de león	60838	Hepatic, respiratory, COVID-19	AP, WP	Inf, De	A liter as time water

[1] Information provided by market herbalists through interviews; [2] Identified in the *Herbarium Truxillense* (HUT). Part Used: AP, aerial parts; Fl, flowers; Fr, fruits; Le, leaves; Po, pod; Se, seeds; WP, whole plant. Preparation: Ba, Baths; De, decoction; Inf, infusion; Ru, Rub; Wa, washes.

Figure 1. Most used medicinal plants for viral deases in La Libertad region: *Azadirachta indica* (A), *Caesalpinia spinosa* (B), *Citrus limon* (C), *Clinopodium pulchellum* (D), *Cordia lutea* (E), *Ocimum basilicum* (F), *Schinus molle* (G), *Taraxacum campylodes* (H).

Herbalists from local markets played an essential role in the gathering of information. They provided traditional knowledge about plants that people buy, prepare, and use to treat viral diseases. Interestingly, the eight species are used as infusion or decoction.

The most common viral affections treated with these plants are hepatic, dermatological, and mosquito-borne. In line with our data, the bibliography describes these and related plant species with biological activity against a hepatic virus (hepatitis), a dermatological virus (herpes simplex), and a mosquito-borne virus (dengue), as summarized in Table 2.

Table 2. Review of the antiviral activity reported from plant species.

Virus	Plant species	Reference
Denge virus	*Azadirachta indica*	[28,29]
	Clinopodium gracile	[30]
	Ocimum basilicum	[31–33]

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	Schinus molle	[34–36]
Herpes simplex virus	*Caesalpinia ferrea*	[37]
	Caesalpinia pucherrima	[38]
	Citrus limon	[39–41]
	Schinus terebinthifolia	[42–44]
Hepatitis virus	*Caesalpinia crista*	[45]
	Caesalpinia sappan	[46]
	Citrus limon	[22]
	Cordia lutea	[47,48]
	Taraxacum officinale	[49]

Literature shows that *Azadirachta indica*, *Citrus limon*, *Cordia lutea*, *Ocinum basiculum*, and *Taraxum campylodes* have evidence of antiviral activity. On the other hand, the antiviral activity reported belongs to plants with the same genre but different species. That is the case of *Caesalpinia spinosa* with *C. pucherrima*, *C. sappan,* and *C. crista*; *Clinopodium pulchellum* with *C. gracile*; and *Schinus molle* with *S. terebinthifolia*.

Scientific studies validate traditional medicine knowledge by demonstrating its effectiveness. Therefore, the next step in the study was to identify the molecules and phytochemicals responsible for the antiviral effect attributed to these eight plants.

2.2. Antiviral Activity of Phytometabolites Polyphenolics of Medicinal Plants

To identify possible antiviral compounds, the LC-MS/MS method was used. The presence of only five molecules was reported, as stated in Table 3.

Table 3. Identification of potential antiviral compounds by LC-MS/MS.

No	Rt (Min)	Chemical formula	$[M-H]^-$ (*m/z*)	Fragment ions (*m/z*)	Identified compound	Plant species
1	1.87	$C_7H_6O_5$	169	125, 78, 69	Gallic acid	*Azadirachta indica* *Caesalpinia spinosa*
2	3.89	$C_{16}H_{18}O_9$	353	191, 179	Chlorogenic acid	*Clinopodium pulchellum* *Schinus molle* *Taraxacum compylodes*
3	5.97	$C_9H_8O_4$	179	135, 107	Caffeic acid	*Azadirachta indica* *Ocimun basilicum* *Taraxacum compylodes*
4	10.31	$C_{27}H_{30}O_{16}$	609	301, 169	Rutin	*Azadirachta indica* *Citrus limon* *Clinopodium pulchellum* *Cordia lutea* *Schinus molle*
5	13.91	$C_{18}H_{16}O_8$	359	197, 179, 161	Rosmarinic acid	*Clinopodium pulchellum* *Ocimun basilicum*

The identified phytometabolites are polyphenols. Specifically, one trihydroxy benzoic acid: gallic acid (1); three hydroxycinnamic acids: chlorogenic acid (2), caffeic acid (3), and rosmarinic acid (5); and one flavonol glycoside: rutin (4).

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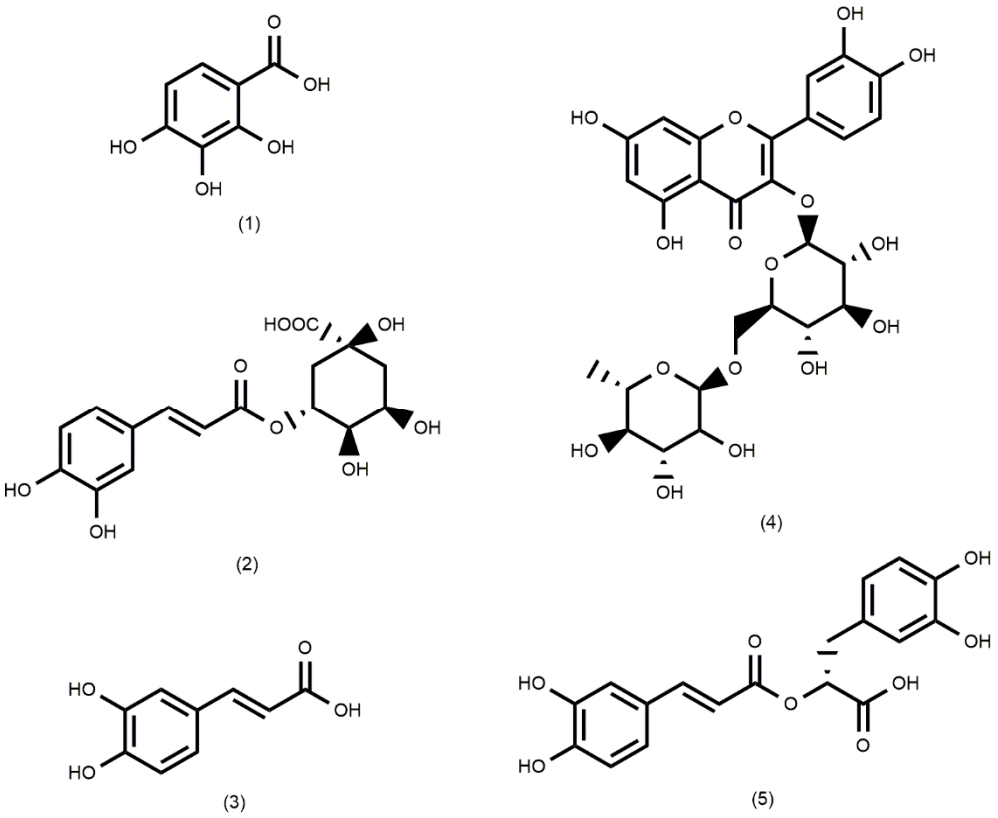

Figure 2. Chemical structures of polyphenols identified in medicinal plants from La Libertad region.

Polyphenols are recognized for their antiviral potential [50–52]. Their presence in plants and herbs is extensive [53]; and due to their diverse biological and pharmacological activity, are indicated as therapeutic sources with medicinal and pharmaceutical applications [54,55]. Several studies demonstrate that these phytochemicals have antiviral activity [4,56–58]. The evidence includes not only laboratory *in vitro* and *in vivo* studies but also clinical trials [57].

Due to what has been described, *Azadirachta indica*, *Caesalpinia spinosa*, *Citrus limon*, *Clinopodium pulchellum*, *Cordia lutea*, *Ocimun basilicum*, *Schinus molle*, and *Taraxacum compylodes* are considered possible natural antiviral agents because they contain polyphenol-type molecules with active characteristics against several types of viruses. These results confirm what is described in the literature, endorsing the effectiveness of their usage.

2.3. Molecular Docking Analysis

Five polyphenolic compounds found in the most consumed medicinal plants in traditional medicine in the La Libertad region were evaluated *in silico* using molecular docking in Autodock Vina. Through this program, it is possible to predict the binding affinities and molecular interactions between the compounds and the viral targets. Table 4 shows the results.

Table 4. Molecular docking values of the polyphenols with potential antiviral activity.

Pubchem ID	Ligand	Parameters	DENV-2 (PDB: 2FOM)	HCV (PDB: 4EO6)	HSV-1 (PDB: 4YXP)
1794427	Chlorogenic acid	Score	-8.6	-8.2	-6.8
		H-Bonds	4	3	0
		N. torsions	11	11	11
		Bindig residues	TRP83, LEU149	ASN142, GLU398, ASN411	-

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5280805	Rutin	Score	-7.1	-9.9	-7.8
		H-Bonds	2	2	3
		N. torsions	16	16	16
		Bindig residues	GLY153(2)	ASP444, GLY557	ARG345, ARG393
0370	Gallic acid	Score	-5.7	-5.9	-5.6
		H-Bonds	2	0	3
		N. torsions	5	5	5
		Bindig residues	ALA164, ASN152	-	ARG417, ARG435, ARG439
5281792	Rosmarinic acid	Score	-7.9	-7.9	-6.8
		H-Bonds	2	2	1
		N. torsions	12	12	12
		Bindig residues	ALA164, LEU149	ALA97, GLY449	GLU358
689043	Caffeic acid	Score	-6.2	-6.1	-5.7
		H-Bonds	1	0	1
		N. torsions	5	5	5
		Bindig residues	LEU149	-	GLU351

In the molecular docking carried out, the affinity between the identified polyphenols and the domain of the following viral proteins was evaluated: NS2B/NS3 (PDB: 2FOM) for DENV-2, NS5B (PDB: 4EO6) for HCV and ICP27 (PDB: 4YXP). for HSV-1.

In the case of DENV-2, several studies have NS2B/NS3 protease as the evaluated molecular target [59,60]. NS2B is a required cofactor of the NS3 serine protease [61]. Together with a cellular protease, this complex cleaves the viral precursor polyprotein into functional viral proteins [62]. Thus, the formation of this complex is essential for the virus replication process. The ligand-protein binding reported by the compounds and the NS2B/NS3 protease ranged from -8.6 kcal/mol to -5.7 kcal/mol. The higher the affinity to the macromolecular target, the lower the binding energy value [63]. Under this tenor, chlorogenic acid showed the highest binding affinity, while gallic acid had the lowest. The results obtained are consistent with those found for other compounds with antiviral potential for DENV-2 [64].

Hydrogen bonding and hydrophobic protein-ligand interactions are also determining factors for molecular docking values [65]. Chlorogenic acid achieved its stability with residues showing six hydrophobic interactions with LEU, 76, TRP83, LEU85, LEU149, VAL 154, ILE123, two anionic interactions with LYS 73, LYS74, three polar interactions with THR118, THR120, and ASN152. Likewise, rutin showed three hydrophobic interactions with VAL72, VAL154, and VAL155, five polar interactions with TRH118, ASN119, THR120, HIS51, and ASN152, two anionic interactions with LYS 73, and LYS74 and one ionic interaction with ASP75.

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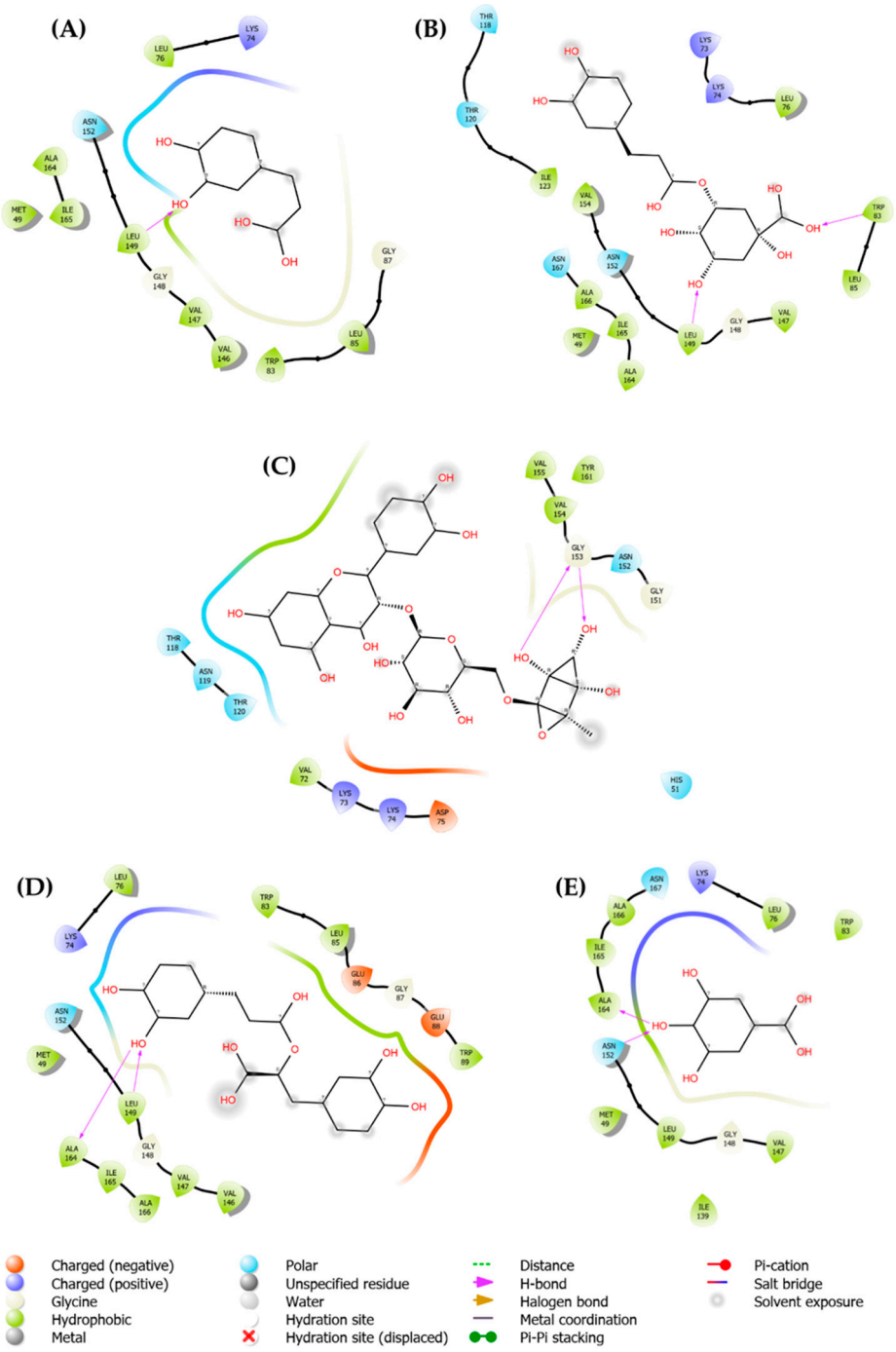

Figure 3. 2D docked complexes of the molecular interaction between NS2B/NS3 (PDB: 2FOM) domain and chlorogenic acid (A), rutin (B), gallic acid (C), rosmarinic acid (D), and caffeic acid (E).

Gallic acid also presented interactions with residues. Six hydrophobic interactions with VAL147, LEU149, ALA164, ILE165, ALA166, LEU76, two polar interactions with ASN152, ASN167 and one anionic interaction with LYS74. Similarly, rosmarinic acid achieved its stability with the interaction of the residues, nine hydrophobic interactions were shown with TRP89, LEU85, TRP83, LEU76, MET49, LEU149, ALA164, VAL147, and VAL146, two ionic interactions with GLU86, GLU88, one interaction polar and anionic with ASN152 and LYS74 respectively.

Likewise, caffeic acid reported hydrophobic interactions through six unions with the residues LEU85, TRP83, VAL146, VAL147, LEU149, and LEU76, an anionic interaction and a polar one with LYS74 and ASN152 respectively.

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In agreement with previous studies [66], the interactions found are close to residues LEU76, LEU149, TRP83, and ASN152 stabilizing the active site of DENV-2.

Notably, the chlorogenic acid-NS2B/NS3 interaction had the shortest distance (2.035 Å/2.183 Å RMSD) to a hydrogen bond between the ligand and the target protein (Figure 4). Another remarkable compound for this aspect is rosmarinic acid, whose distance was 2.116 Å/2.179 Å RMSD (Figure 5).

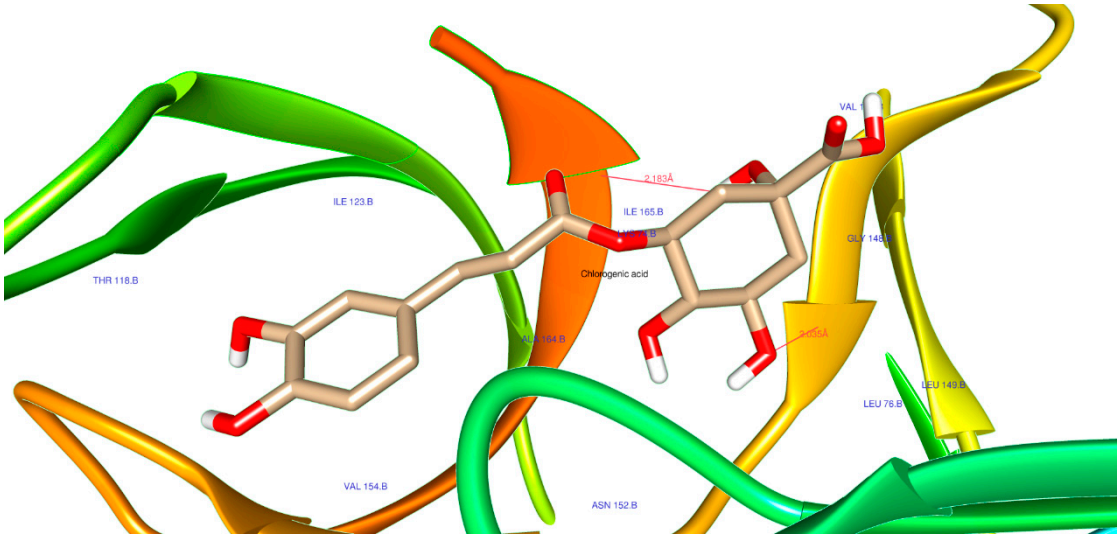

Figure 4. 3D Simulation by molecular coupling between the DENV-2 protease NS2B/NS3 (PDB: 2FOM) versus chlorogenic acid using Chimera software V. 1.16.

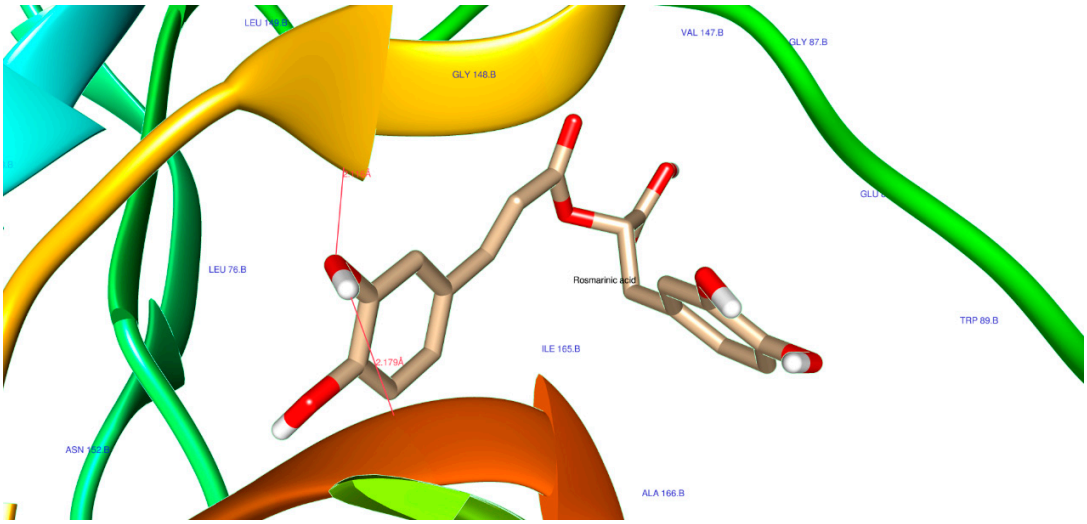

Figure 5. 3D Simulation by molecular coupling between the DENV-2 protease NS2B/NS3 (PDB: 2FOM) versus rosmarinic acid using Chimera software V. 1.16.

The non-structural protein NS5B is the core of HCV replication [67]. It is an RNA-dependent RNA polymerase (RdRp) that, when interacting with other proteins, promotes the formation of the viral RNA replication complex [68]. Since blocking the function of NS5B can inhibit HCV replication [69], this polymerase is a widely evaluated target. In this investigation, ligand-protein binding ranged from -9.9 kcal/mol to -5.9 kcal/mol. Rutin stood out for its high binding affinity, while gallic acid showed the lowest affinity. Previous studies on the anti-HCV activity of phenolic compounds and flavonoids [70–72] support our results. Furthermore, the binding energy value of rutin, chlorogenic acid, and rosmarinic are similar to potential antiviral compounds reported previously [73].

Some interactions with the residues stabilize polyphenols in the 2D simulation of the complexes formed (Figure 6).

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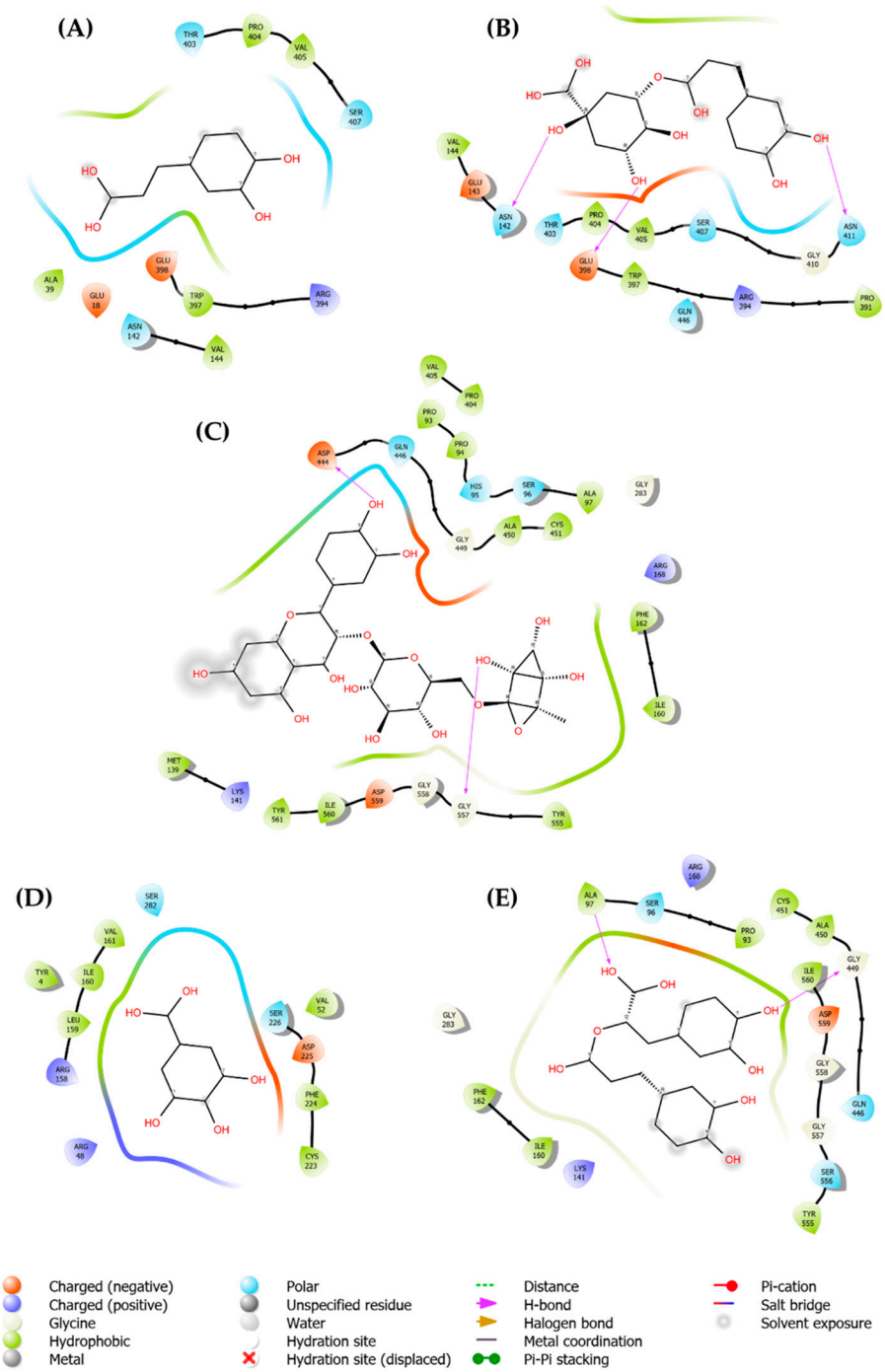

Figure 6. 2D docked complexes of the molecular interaction between NS5B (PDB: 4E06) domain and chlorogenic acid (A), rutin (B), gallic acid (C), rosmarinic acid (D), and caffeic acid (E).

Chlorogenic acid showed three hydrophobic interactions with VAL144, PRO404, and VAL405, four polar interactions with ASN142, THR403, SER407, and ASN411, and two ionic interactions with GLU143 and GLU398. In turn, rutin reported eight hydrophobic interactions with ALA450, CYS451, PHE162, ILE160, TYR555, ILE560, TYR561, MET139, two ionic interactions with ASP444 and ASP559, two anionic interactions with LYS 141 and ARG168, and one polar interaction with GLN446. Gallic acid presented hydrophobic interactions with residues VAL161, ILE160, LEU159, CYS223, and PHE224, two anionic interactions with ARG158 and ARG48, two polar interactions with SER282 and SER226, and one ionic interaction with ASP225. Likewise, rosmarinic acid showed six hydrophobic interactions with ALA97, PRO93, ILE560, ILE555, ILE160, and PHE162, two polar interactions with SER96 and SER556, and one ionic and anionic interaction with ASP559 and LYS141 respectively.

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Similarly, the interactions of the residues with caffeic acid showed four hydrophobic bonds with PRO404, VAL405, ALA39, and TRP397, three polar interactions with ASN142, SER407, and THR403, two ionic interactions with GLU18 and GLU398, and one anionic interaction with ARG394.

As mentioned, the interactions at the active site are close to the residues LEU419, THR229, and PRO133. This fact was previously reported [49], which reinforces the results found in the present study.

Regarding the hydrophilic bonds, the compounds rutin (2,098 Å RMSD) (Figure 7), chlorogenic acid (1,947 Å RMSD) (Figure 8), and rosmarinic acid (1,936 Å RMSD) (Figure 9) stood out with favorable distances.

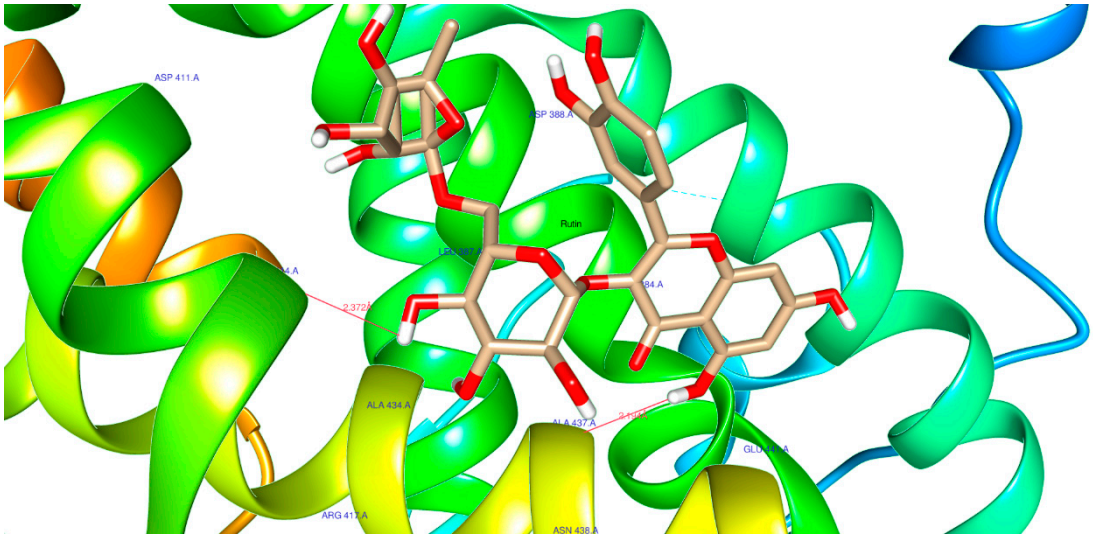

Figure 7. 3D Simulation by molecular coupling between the HCV protease NS5B (PDB: 4E06) versus rutin using Chimera software V. 1.16.

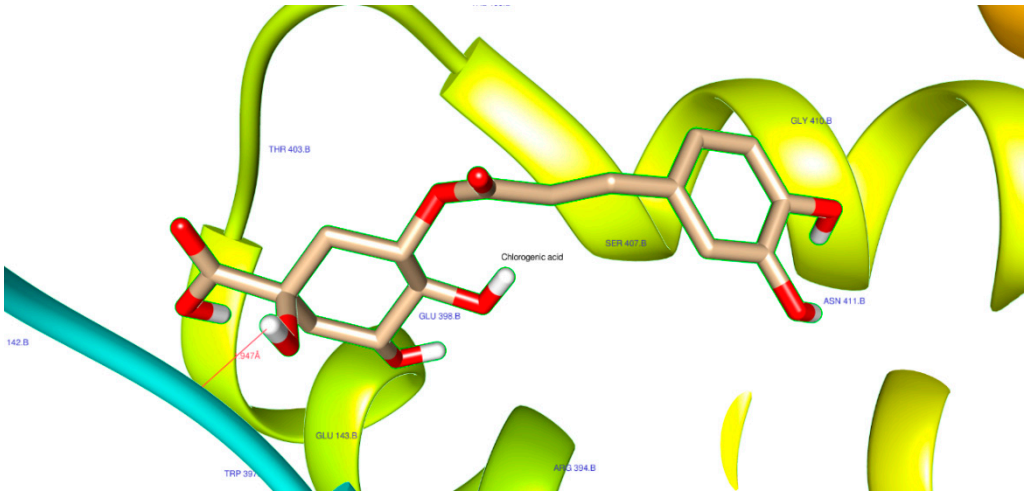

Figure 8. 3D Simulation by molecular coupling between the HCV protease NS5B (PDB: 4E06) versus chlorogenic acid using Chimera software V. 1.16.

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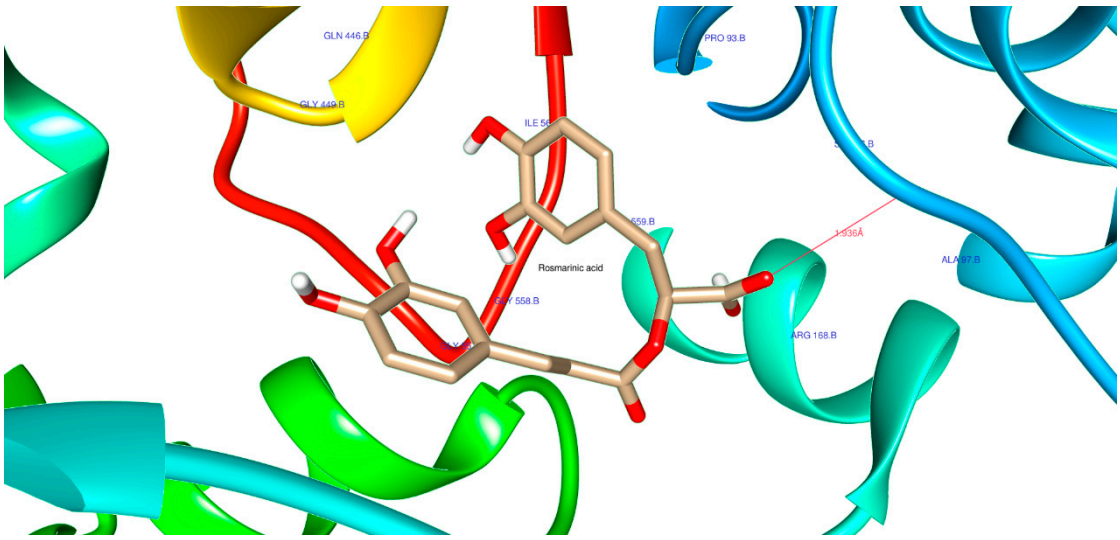

Figure 9. 3D Simulation by molecular coupling between the HCV protease NS5B (PDB: 4E06) versus rosmarinic acid using Chimera software V. 1.16.

Finally, the docking between the identified polyphenols and the ICP7 viral target of herpes simplex 1 (HSV-1) was performed. ICP7 regulates SRPK1 with the goal of changing host mRNA by modulating kinase activity, favoring viral transcripts [74]. In other words, ICP7 mediates host shut-off during HSV-1 infection. Also, this protein can interact with and hijack cellular proteins that function throughout the RNA maturation process [75,76].

When performing molecular docking, ligand-protein binding ranged from -7.8 kcal/mol to -5.6 kcal/mol. Rutin exhibited the highest binding affinity and gallic acid the lowest. These results prove that polyphenols block HSV-1 infection by inhibiting ICP27 synthesis [77–79].

In the 2D modeling (Figure 10) the stability of the polyphenols was evidenced by the interactions with nearby residues.

Chlorogenic acid showed nine hydrophobic interactions with ALA467, LEU464, TYR463, PHE462, PHE428, ILE425, ILE422, LEU419, ILE432 and one ionic interaction with ASP424.

Likewise, rutin reported four hydrophobic interactions with ILE495, PRO498, PHE349 and PRO396, four polar interactions with THR491, HIS494, GLN346, ASN389 and THR392 and two anionic interactions ARG345, ARG393.

Gallic acid presented one hydrophobic interaction with LEU436, five anionic interactions with ARG416, ARG417, ARG418, ARG435, ARG439, and two polar interactions with THR451 and ASN438.

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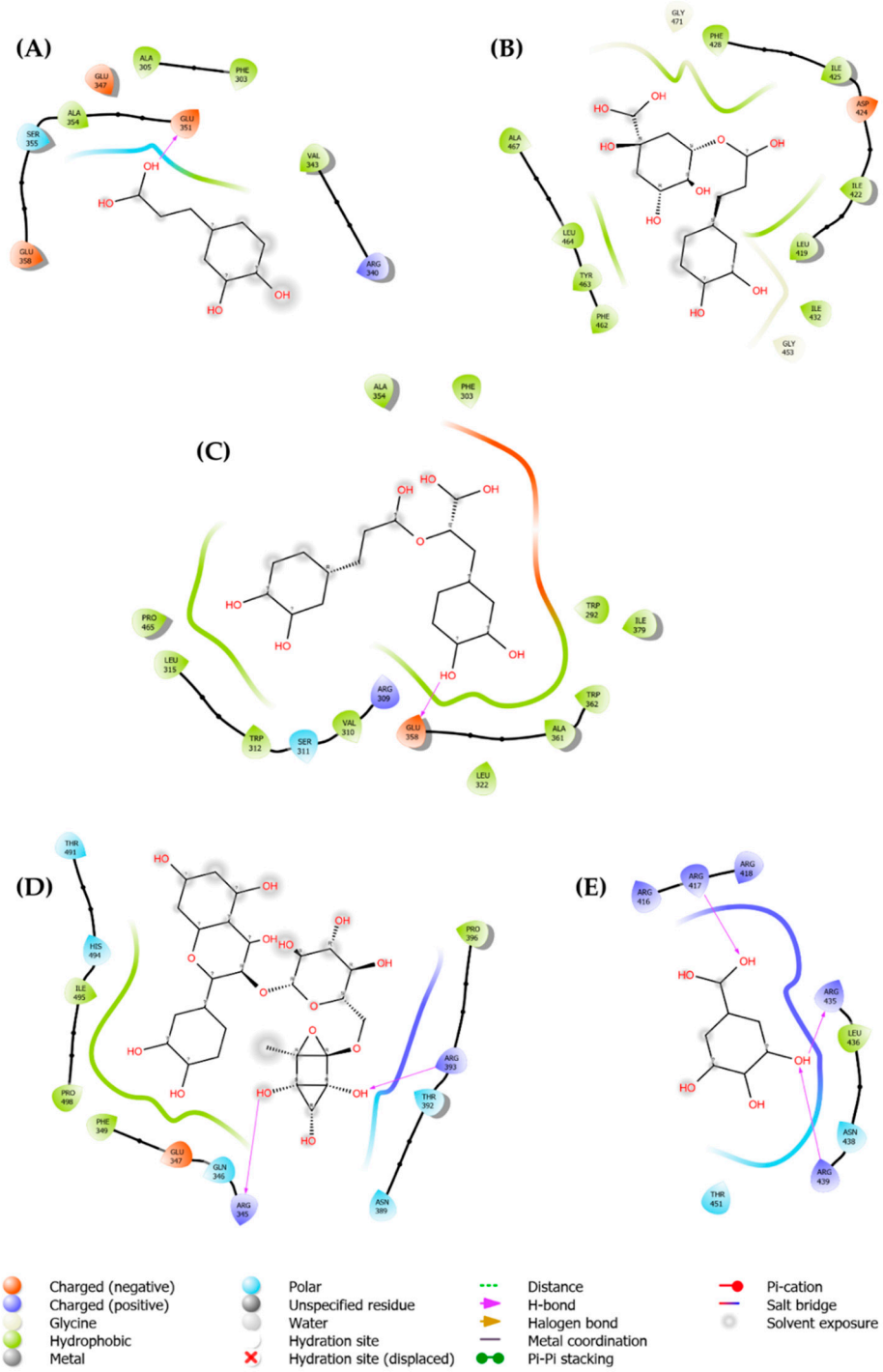

Figure 10. 2D docked complexes of the molecular interaction between ICP7 (PDB: 4YXP) domain and chlorogenic acid (A), rutin (B), gallic acid (C), rosmarinic acid (D), and caffeic acid (E).

In turn, rosmarinic acid showed nine hydrophobic interactions with PRO465, LEU315, TRP312, VAL310, ALA361, TRP362, TRP292, PHE303 and ALA354 and one ionic, anionic and polar interaction with GLU358, ARG309 and SER311 respectively.

Regarding caffeic acid, it highlighted two hydrophobic interactions with ALA354 and VAL343, three ionic interactions with GLU358, GLU347, and GLU351, and an anionic and polar interaction with ARG340 and SER355 respectively.

The interactions in the active site showed close binding to GLU358, TRP392, and ALA354 residues, a fact previously reported in other investigations [80,81]. Regarding the distance to a hydrophilic bond, a favorable distance between the viral protein and rutin (2.033 Å RMSD) stood out (Figure 11).

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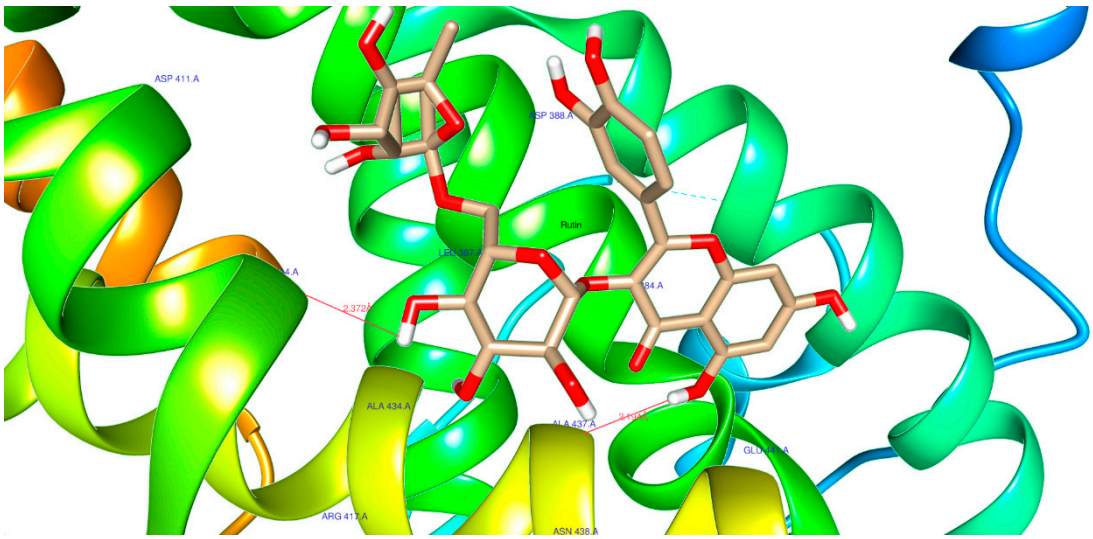

Figure 11. 3D Simulation by molecular coupling between the HSV-1 protease ICP7 (PDB: 4YXP) versus rutin using Chimera software V. 1.16.

All the ligand-protein interactions found in this research work are favorable since the binding energy values were negative [82]. Likewise, the distances found between the bonding atoms are relevant and of biological importance [83].

The compounds considered relevant and with potential for drug development are those that present good interaction and stability in the active site [84]. The results of this work indicate that chlorogenic acid (for DENV-2 and HCV), rutin (for HCV and HSV-1), and rosmarinic acid (for DENV-2 and HCV) meet said criteria. Therefore, its use as the main molecule to develop new pharmacological compounds aimed at inhibiting specific proteins caused by infections of viral origin should be further studied.

4. Materials and Methods

4.1. Plant Material

Medicinal plants were bought from herbalists of the wholesale markets "La Hermelinda", "Mayorista", and "La Unión". The eight plant species used were *Azadirachta indica* A. Juss. "paraíso", *Caesalpinia spinosa* (Molina) Kuntze "tara", *Citrus limon* (L.) Osbeck "limón", *Clinopodium pulchellum* (Kunth) Govaerts "panizara", *Cordia lutea* Lam. "overo", *Ocimum basilicum* L. "albahaca", *Schinus molle* L. "molle", and *Taraxacum campylodes* G.E. Haglund "diente de león". The aerial parts of medicinal plants were used and plant organs such as stems and secondary branches were excluded.

4.2. Selection, Collection, and Stabilization of Plant Drugs

For the selection of medicinal plants, the traditional medicine of the region La Libertad was considered. The medicinal plants were collected in packages of approximately 0,5 kg of plant material. Subsequently, a complete specimen of each medicinal plant was taken to the *Herbarium Truxillense* (HUT) for identification and taxonomic classification. The plant material was transported to the Pharmacognosy laboratory of the Faculty of Pharmacy and Biochemistry. They were cleaned with running water; dried at room temperature, and later in an oven at 40 °C; crushed and grinded in a mill (Corona); and finally, stored in airtight jars until later use [85].

4.3. Ethnobotanical Study

The ethnobotanical study was carried out between January and March 2021. The study area was the three most popular markets for the sale of medicinal plants (Hermelinda, Unión, Mayorista). These markets were visited to inform the study's objectives and hold conversations with herbalists, prior to informed consent from each participant. The representative population was twelve people who had knowledge about medicinal plants and sold them on the markets. For the age range, only adults were considered. The research followed the Code of Ethics of the International Society for Ethnobiology (International Society for Ethnobiology. Code of Ethics of the International Society for

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Ethnobiology (with 2008 additions). 2006; http://www.ethnobiology.net/what-we-do/core-programs/ise-ethics-program/code-of-ethics/code-in-english/) and the protocol was approved by the Ethics Committee of the National University of Trujillo (code N°: PR003P-2022/CEIFYB). A standard questionnaire was applied through structured semi-open interviews in which the plants used to treat some viral diseases were inquired about, taking into account the vernacular name, uses and traditional medicinal properties, forms, and uses of preparation, dose, and administration of the plant used [86,87].

4.4. Identification of Phytometabolites Polyphenolics from Medicinal Plants Referred to in the Literature and Their Antiviral Activity

An extensive literature search (database: Scopus, ScienceDirect, Google scholar) was performed to identify molecules from plant species and some other traditional medicinal plants that have been reported to contain potential antiviral activity. It was compared and a match was made with each plant species under study. From those studies, the effectiveness against the type of virus and active sites of interaction was also collected, and information from the virtual selection was used to identify phytometabolites that can serve as possible clues for the development of antiviral drugs [88–90].

4.5. Identification of Polyphenols by UPLC–PDA–ESI–MS/MS

The profiling of the chemical constituents was performed according to [91], using mass spectrometric analysis carried out on a Waters® ACQUITY Xevo® TQ-XS system composed of an ACQUITY® UPLC HSS T3 system and a Xevo® TQ-XS triple-quadrupole tandem mass spectrometer ESI (-ve mode) as the electrospray ionization (ESI) interface. The column used was a C18 100 mm × 2.1 mm column (p.s., 1.7 µm). The concentration was 1 mg/mL for dry extracts, the mobile phases used were 0.1% water in formic acid LC-MS Merck (Germany) (A) and 0.1% acetonitrile LC-MS Merck (Germany) (B) in formic acid in LC-MS Merck (Germany). The gradient was in phase A: 0-10.50 min from 90-78%, 10.5-19 min from 78-50%, 19-23 min from 50-10%, 23-25 min from 10-5%, 25 -26.50 min 5-90%, 26.50-30 min 90-90%, at 300 µL/min flow. The Survey Scan function was used with scanning in Scan Wave MS, scanning was from 50-1500 m/z. The mass detector parameters were 2.5 (kV) capillary voltage, 40 V cone voltage at a gas flow of 150 L/h, desolvation temperature of 480 °C, and collision energy of 20 eV.

4.6. In Silico Antiviral Activity

4.6.1. Ligand preparation: For molecular docking, six molecules previously identified by LC-MS/MS (CID_1794427: chlorogenic acid, CID_5280805: rutin, CID_370: gallic acid, CID_689043: caffeic acid, CID_5281792: rosmarinic acid) with a 3D-structure were used. SDF from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Using the Open Babel software, the 3D-SDF structures were converted to the Mol2 format [92]. The energy minimization of the ligands was done using the MMFF94 force field, using the Avogadro software [93].

4.6.2. Protein preparation: Three viral proteins DENV (PBD ID: 2FOM), HCV (PBD ID: 4EO6), and HSV (PBD ID: 4YXP) were selected from 3D structures of the Protein Data Bank RCSB (https: http://www.rcsb.org/search). Proteins were prepared using the UCSF Chimera V 1.16 program by removing ligands, ions, and water molecules, then parameterized by adding polar hydrogens, removing nonpolar hydrogens, and adding Kollman charges [94].

4.6.3. Molecular docking: Molecular docking between selected molecules and proteins was performed using Autodock Vina and UCSF Chimera [95], based on the Lamarckian genetic model. The highest negative scores were identified and the best poses with UCSF Chimera were saved. Grid box was created using the Grid box preparation module with desired dimensions, for PDB ID: 2FOM it was set to center_x = -11.02, center_y = -12.97, center_z = 9.95 and size_x = 25.21, size_y = 23.10, size_z = 22.62, for PDB ID: 4EO6 was set to center_x = -22.02, center_y = 50.32, center_z = 1.18 and size_x = 32.82, size_y = 51, 05, size_z = 34.88 and for PDB ID: 4YXP was set to center_x = -76.93, center_y = 7.79, center_z = 23.25 and size_x = 37.78, size_y = 38.08, size_z = 35,19. Ten AutoDock Vina runs were performed in each scenario for each ligand with a completeness value of eight. The CASTp server was used to find the binding site of viral proteins [96]. The ligand-protein interactions were visualized using the Maestro V 13.1 software [97].

5. Conclusions

Chlorogenic acid, rutin, gallic acid, caffeic acid, and rosmarinic acid were identified in the eight medicinal plants evaluated. In the *in silico* evaluation, the antiviral activity of chlorogenic acid stood out against DENV-2 and HCV, rutin against HCV and HSV-1, and rosmarinic acid against DENV-2 and HCV. Therefore, *Azadirachta indica*, *Caesalpinia spinosa*, *Citrus limon*, *Clinopodium pulchellum*, *Cordia lutea*, *Ocimum basilicum*, *Schinus molle*, and *Taraxacum campylodes* have antiviral activity.

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