

Identification of polyphenols by UPLC-MS/MS and their potential *in silico* antiviral activity from medicinal plants in Trujillo, Peru

[Identificación de polifenoles por UPLC-MS/MS y su potencial actividad antiviral *in silico* de plantas medicinales en Trujillo, Perú]

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Abstract

Context: Given the growing public health crisis caused by viral diseases, traditional medicine stands as one of the fundamental pillars for the study and discovery of phytometabolites with antiviral properties. It is necessary to investigate and evaluate these compounds, mostly phenolic, that could be used as drugs.

Aims: To identify the polyphenols by UPLC-MS/MS and the potential *in silico* antiviral activity of medicinal plants in Trujillo, Peru.

Methods: The ethnobotany of the eight most widely used medicinal plants in the city of Trujillo was described (*Azadirachta indica* A. Juss. "paradise", *Caesalpinia spinosa* (Molina) Kuntze "tara", *Citrus limon* (L.) Osbeck "lemon", *Clinopodium pulchellum* (Kunth) Govaerts "panizara", *Cordia lutea* Lam. "overo", *Ocimum basilicum* L. "basil", *Schinus molle* L. "molle", and *Taraxacum campyloides* G.E. Haglund "dandelion"). 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) by molecular docking using Chimera 1.16 software and molecular interaction by Maestro 13.1 software to identify the position and type of interaction.

Results: Five polyphenols (chlorogenic acid, gallic acid, caffeic acid, rosmarinic acid, and rutin) were 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.

Conclusions: It is demonstrated that the medicinal plants studied contain phytometabolites that make them possible antiviral candidates, which would support their use in the traditional medicine of the province of Trujillo, Peru.

Keywords: antiviral; ethnobotany; molecular docking; polyphenols; Tandem Mass Spectrometry.

Resumen

Contexto: Ante el creciente problema de salud pública originado por enfermedades virales, la medicina tradicional representa uno de los pilares fundamentales para el estudio y descubrimiento de fitometabolitos con propiedades antivirales. Se requiere investigar y evaluar estos compuestos, mayoritariamente fenólicos, que puedan ser utilizados como fármacos.

Objetivos: Identificar los polifenoles mediante el método UPLC-MS/MS y la potencial actividad antiviral *in silico* de las plantas medicinales en Trujillo, Perú.

Métodos: Se describió la etnobotánica de las ocho plantas medicinales más utilizadas en la ciudad de Trujillo (*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", y *Taraxacum campyloides* G.E. Haglund "diente de león"). Los fitometabolitos responsables de la actividad antiviral se identificaron mediante LC-MS y se evaluaron *in silico* frente a las proteínas virales NS2B/NS3 (DENV-2), NS5B (HCV) e ICP27 (HSV-1) mediante acoplamiento molecular con el software Chimera 1.16 e interacción molecular con el software Maestro 13.1 para identificar la posición y el tipo de interacción.

Resultados: Se encontraron cinco polifenoles (ácido clorogénico, ácido gálico, ácido cafeico, ácido rosmarínico y rutina) y, en el ensayo *in silico*, destacó la actividad antiviral del ácido clorogénico frente a DENV-2 y HCV, la rutina frente a HCV y HSV-1, el ácido rosmarínico frente a DENV-2 y HCV.

Conclusiones: Se demuestra que las plantas medicinales estudiadas contienen fitometabolitos que las convierten en posibles candidatos antivirales, lo que apoyaría su uso en la medicina tradicional de la provincia de Trujillo, Perú.

Palabras Clave: acoplamiento molecular; antiviral; etnobotánica; espectrometría de masas en tándem; polifenoles.

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INTRODUCTION

Ethnobotany studies the interrelation of human beings with plants developed over millennia (Lalama et al., 2016). 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 (Yasmin et al., 2020). This fact was highlighted during the recent COVID-19 pandemic (Mehmood et al., 2021), which underscored the importance of herbal therapies in many countries (Sharma et al., 2021). Natural products can help regulate the side effects of synthetic drugs, especially when the treatment lasts for a prolonged period (Lim et al., 2017). Moreover, natural products are a source of molecules for drug design and development (David et al., 2015). All these factors make them a possible choice when looking for an efficient treatment (Bernardini et al., 2018).

During the COVID-19 pandemic and the resulting global health crisis, not only have multiple variants of the coronavirus (SARS-CoV-2) emerged, but latent viruses have also reappeared. Some of them, including the hepatitis C Virus (HCV) (Yousaf et al., 2018) and herpes simplex virus (HSV) (Krenn et al., 2021), cause widespread chronic infections. Others, like the dengue virus, are potentially less harmful (Yan et al., 2020) but constitute a concern for triggering and increasing the public health problem.

A variety of plant species are used as traditional medicines for treating diseases of viral origin (Denaro et al., 2020; Huaccho-Rojas et al., 2020). Antiviral active principles derived from those plants include alkaloids, terpenes, polysaccharides, flavonoids, phenolic acids, and steroids (van de Sand et al., 2021). The family to which a plant species belongs is often an ethnomedical indicator (Kutal et al., 2021b; Oliveira et al., 2022). In this regard, medicinal plants with potential molecules for ethnobotanical use should be researched by applying a disease treatment approach. They also must comply with traditional pharmacopeias, ethnopharmacological evaluations, and therapeutic efficacy reports (Kutal et al., 2021a; Robles et al., 2020). The investigation of new species is necessary to find possible sources for molecules with antiviral activity (Mukherjee, 2019; Tida et al., 2020).

Over the last decades, however, the development of new antiviral drugs with efficacy in blocking host-specific functions has almost entirely stopped, despite great advancements in detection assays, prediction tools, and virtual screening (Martinez et al., 2015; Rolta et al., 2021). Therefore, there is a need for renewed research on medicinal plants and herbs using modern techniques and methods to identify suitable

and reliable natural compounds that could be used as drugs (Thanh et al., 2020).

Our research presented here aimed to identify polyphenols by UPLC-MS/MS and the potential *in silico* antiviral activity of medicinal plants sold in markets in Trujillo, Perú.

To achieve this goal, an ethnobotanical study was conducted to identify relevant medicinal plants. This study was complemented by an extensive bibliographic review. Subsequently, the polyphenols present in the selected medicinal plants were identified using the UPLC-MS technique. Finally, their antiviral potential was evaluated through *in silico* assays. Aqueous extracts were chosen for the analysis of the plants because it was documented that this was the most common form that the population of Trujillo used to treat viral diseases.

The outcomes of this research could contribute to the development of novel antiviral therapies from natural sources and validate the use of these medicinal plants as a possible treatment for diseases of viral origin.

MATERIAL AND METHODS

Chemicals

All chemicals and solvents were of MS-grade. Methanol, dichloromethane, acid formic, and acetonitrile were supplied by Sigma-Aldrich (St. Louis, MO, USA).

Plant material

Medicinal plants were bought from herbalists of the wholesale markets “La Hermelinda”, “Mayorista”, and “La Unión” located in the province of Trujillo, La Libertad region, Peru. *Azadirachta indica* A. Juss. “paraíso” (7°57'08" S, 78°31'00" W), *Caesalpinia spinosa* (Molina) Kuntze “tara” (8°06'46" S, 78°08'25" W), *Citrus limon* (L.) Osbeck “limón” (7°50'07" S, 78°01'59" W), *Clinopodium pulchellum* (Kunth) Govaerts “panizara” (7°50'07" S, 78°01'59" W), *Cordia lutea* Lam. “overo” (7°57'08" S, 78°31'00" W), *Ocimum basilicum* L. “albahaca” (7°57'08" S, 78°31'00" W), *Schinus molle* L. “molle” (7°50'07" S, 78°01'59" W), and *Taraxacum campylodes* G.E. Haglund “diente de león” (7°57'08" S, 78°31'00" W) were the plant species selected for further study.

Taxonomic identification

A complete specimen of each medicinal plant was deposited in the Herbarium Truxillense (HUT) and was identified by the Biologist-Botanist Eric Rodri-

guez. Each species was assigned a voucher number (Table 1). The plant material was transported to the Pharmacognosy Laboratory of the Faculty of Pharmacy and Biochemistry to pre-treat the samples.

Ethnobotanical study

The ethnobotanical study was carried out between January and March 2021. The study area covered the three most popular markets for the sale of medicinal plants (La Hermelinda, La Unión, and Mayorista). These markets were visited to inform on the study's objectives, obtain prior informed consent from each participant, and hold conversations with twelve herbalists who had knowledge about medicinal plants and sold them on the markets. For the age range, only adults were considered. The study protocol followed the Code of Ethics of the International Society for Ethnobiology (International Society for Ethnobiology. Code of Ethics of the International Society for 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 No. 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, considering the vernacular name, uses and traditional medicinal properties, forms, and uses of preparation, dose, and administration of the plant used (Bussmann et al., 2017).

Sample pretreatment

Samples were washed with water, aerated at room temperature, and then dried in an oven at 40°C for 72 hours. The dried samples were crushed and ground to obtain fine particles (Ruiz et al., 2018).

Extraction was carried out by subjecting 10 g of ground dry plant material to 100 mL of boiling ultrapure distilled water for 10 minutes. Then, the extracts were filtered, lyophilized, and stored at 6°C in amber bottles until further use. After that, the aqueous extracts were redissolved with MS-grade methanol, which allowed for the chemical identification of the polyphenolic compounds (Elshafie et al., 2023).

Identification of polyphenols by UPLC-PDA-ESI-MS/MS

The profiling of chemical constituents was performed according to (El-Zahar et al., 2022), 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

(negative 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 90-78%, 10.5-19 min 78-50%, 19-23 min 50-10%, 23-25 min 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, and scanning was from *m/z* 50-1500. 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 (Ortiz et al., 2023).

In silico antiviral activity

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. The reference drugs were CID_37542: ribavirin in the NS2B/NS3 protein for DENV-2, CID_135398513: acyclovir in the ICP27 protein of HSV-1 and CID_45375808: sofosbuvir in the NS5B protein of HCV. 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 (Hanwell et al., 2012). The energy minimization of the ligands was done using the MMFF94 force field and the Avogadro software (Pettersen et al., 2004).

Protein preparation

Three viral proteins DENV-2 (PBD ID: 2FOM), HCV (PBD ID: 4EO6), and HSV-1 (PBD ID: 4YXP) were selected from 3D structures of the Protein Data Bank RCSB (<https://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 (O'Boyle et al., 2011).

Molecular docking

Molecular docking between selected molecules and proteins was performed using Autodock Vina and UCSF Chimera (Tian et al., 2018), 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, for PDB ID: 4EO6 was set to center_x = -22.02, center_y = 50.32, center_z = 1.18 and for PDB ID: 4YXP was set to center_x = -76.93, center_y = 7.79, center_z = 23.25. Ten AutoDock Vina runs were conducted in each scenario for every ligand, employing a completeness value of eight. The CASTp server was employed to locate the binding sites of viral proteins. Maestro 13.1 software was used to analyze the position and type of interaction of the chemical bond (Bhachoo and Beuming, 2017).

Docking validation method

Validation was carried out to prove that the selected docking parameters can dock the viral receptor ligands. Validation was performed using natural ligand and redocking into the receptor's active site or protein target (Nur et al., 2023).

ADME properties and acute oral toxicity

ADME and drug-likeness prediction for polyphenols identified were developed by the SwissADME online server from the Swiss Institute of Bioinformatics (<https://www.sib.swiss>). In order to do this, the canonical SMILES chain was entered into the server, obtaining ADME parameters such as inhibitory cytochrome P450 (CYP450) family, blood-brain barrier permeability (BBB), and binding to P-glycoprotein (P-gp). Likewise, the metabolites were evaluated for acute oral toxicity using the GUSAR-Online server (Lagunin et al., 2011); inserting the canonical SMILE of molecules, followed by predictions of lethal dose 50 (LD₅₀) values for rats, and finally, classified in rodents based on the OECD Project (OECD, 2002).

Data analysis

The data from this study were analyzed descriptively, comparing the result with the standard. The UPLC-MS/MS spectra were verified using the spectra from the database in the NIST library version 02.L.

In silico data analysis was carried out in stage: (1) similarity analysis with polyphenol drugs using the Lipinski Rule of Five to identify compounds that could be used as drug candidates; (2) molecular docking to determine compounds with the lowest binding affinity; (3) after molecular docking, the compound with the lowest affinity was simulated to observe the molecular interaction between the compound and the three target proteins (compared to the control); (4) the prediction of absorption and distribution of the identified polyphenols was calculated using SwissADME;

and (5) the prediction of acute oral toxicity of the identified polyphenols was assessed using the GUSAR-Online server.

RESULTS

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.

Verification of medicinal plants with antiviral activity

The most common viral affections treated with these plants were hepatic and dermatological problems and mosquito-borne diseases. 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.

Identification of polyphenol antiviral compounds by UPLC-MS

The UPLC-PDA-ESI-MS/MS method was used to identify possible antiviral compounds. As mentioned before, the eight plants have infusion and decoction as traditional preparations. Following this criterion, the form of preparation selected for the used extracts was the infusion. In this way, the traditional use of the medicinal plants under study is followed. The presence of only five molecules was reported, as stated in Table 3 and Figs. S1-S16.

***In silico* molecular docking**

Five polyphenolic compounds found in the most consumed medicinal plants in traditional medicine in Trujillo 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.

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.

Table 1. Taxonomical and ethnobotanical identification of medicinal plants sold by herbalists in markets of Trujillo city, province of Trujillo, Peru.

Plant species	Family	Vernacular name ¹	HUT voucher number ²	Type of viral affection	Part used ¹	Way of preparation ¹	Way of use ¹
<i>Azadirachta indica</i> A. Juss.	Meliaceae	Paraíso	60828	Mosquito-borne	Le	Inf	A liter, three times a day
<i>Caesalpinia spinosa</i> (Molina) Kuntze	Fabaceae	Tara	60820	Dermatological, respiratory	Po, WP	Inf, De, Wa, Ba	Gargle, two times a day Wa, Ba: a time a day
<i>Citrus limon</i> (L.) Osbeck	Rutaceae	Limón	60821	Hepatic, dermatological, respiratory, COVID-19	Le, Fr	Inf, Ba	Time water
<i>Clinopodium pulchellum</i> (Kunth) Govaerts	Lamiaceae	Panizara	60830	Mosquito-borne	WP	Inf, De	Time water
<i>Cordia lutea</i> Lam.	Boraginaceae	Overo	60823	Hepatic	Fl	Inf, De	A liter, three times a day
<i>Ocimum basilicum</i> L.	Lamiaceae	Albahaca	60826	Respiratory, COVID-19, mosquito-borne	WP	Inf, Ba	A liter, three times a day Ba: A time a day
<i>Schinus molle</i> L.	Anacardiaceae	Molle	60836	Dermatological, respiratory, mosquito-borne	Le, Se, WP	Inf, Ba, Ru	A liter, three times a day Ba: three times a week
<i>Taraxacum campylodes</i> G. E. Haglund	Asteraceae	Diente de león	60838	Hepatic, respiratory, COVID-19	Le, Fl, WP	Inf, De	A liter as time water

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

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

Virus	Plant species	Reference
Dengue virus	<i>Azadirachta indica</i>	(Wandscheer et al., 2004; Dwivedi et al., 2021)
	<i>Clinopodium gracile</i>	(Chen et al., 2013)
	<i>Ocimum basilicum</i>	(Misra et al., 2020; Soonwera and Sittichok, 2020)
	<i>Schinus molle</i>	(Kanna and Krishnakumar, 2019; Tsegghai et al., 2019)
Herpes simplex virus	<i>Caesalpinia ferrea</i>	(Lopes et al., 2013)
	<i>Caesalpinia pucherrima</i>	(Chiang et al., 2003)
	<i>Citrus limon</i>	(Agrawal et al., 2021; Garber et al., 2021)
	<i>Schinus terebinthifolia</i>	(Nocchi et al., 2022; Oliveira et al., 2020)
Hepatitis virus	<i>Caesalpinia sappan</i>	(Holidah et al., 2022)
	<i>Caesalpinia crista</i>	(Gaurav and Govindasamy, 2021)
	<i>Citrus limon</i>	(Yousaf et al., 2018)
	<i>Cordia lutea</i>	(Hayashi et al., 1990; Ruiz-Reyes et al., 2021)
	<i>Taraxacum officinale</i> (<i>T. campylodes</i>)	(Rehman et al., 2016)

Table 3. Identification of phytometabolites with reported potential antiviral.

No.	Rt (min)	Chemical formula	[M-H] ⁻ (m/z)	Fragment ions (m/z)	Identified compound	Plant species
1	1.92-2.67	C ₇ H ₆ O ₅	169	125, 107	Gallic acid	<i>Azadirachta indica</i> <i>Caesalpinia spinosa</i>
2	3.20-4.80	C ₁₆ H ₁₈ O ₉	353	191, 179	Chlorogenic acid	<i>Clinopodium pulchellum</i> <i>Schinus molle</i> <i>Taraxacum campylodes</i>
3	5.92-6.02	C ₉ H ₈ O ₄	179	135, 134, 107	Caffeic acid	<i>Azadirachta indica</i> <i>Ocimum basilicum</i> <i>Taraxacum campylodes</i>
4	10.20-10.53	C ₂₇ H ₃₀ O ₁₆	609	301, 151	Rutin	<i>Azadirachta indica</i> <i>Citrus limon</i> <i>Clinopodium pulchellum</i> <i>Cordia lutea</i>
5	13.89-13.95	C ₁₈ H ₁₆ O ₈	359	197, 179, 161	Rosmarinic acid	<i>Clinopodium pulchellum</i> <i>Ocimum basilicum</i>

Chlorogenic acid achieved its stability with residues showing six hydrophobic interactions with LEU, 76, TRP83, LEU85, LEU149, VAL 154, and ILE123, two anionic interactions with LYS 73 and LYS74, three polar interactions with THR118, THR120, and ASN152. Likewise, 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 and GLU88, one interaction polar and anionic with ASN152 and LYS74 respectively for interactive with DENV-2 residues.

Rutin reported eight hydrophobic interactions with ALA450, CYS451, PHE162, ILE160, TYR555, ILE560, TYR561, and MET139, two ionic interactions with ASP444 and ASP559, two anionic interactions with LYS 141 and ARG168, and one polar interaction with GLN446. In turn, 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 for interaction with HCV residues.

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 with ARG345 and ARG393 for interaction with HSV-1 residues.

The molecular docking method was validated using UCSF Chimera, involving the removal and re-

docking at the active site. The validation parameter meets the criteria if the RMSD value is less than 2 Å. A lower RMSD value indicated a closer alignment of the ligand position from the redocking result to the crystallographic result (Kontoyianni et al., 2004). High values can lead to deviations and errors in interaction prediction (Maghfiroh et al., 2021). The RMSD values obtained for each ligand were less than 2 Å, confirming the validity of the molecular docking method (Shivanika et al., 2020).

The Lipinski's Rule of Five was employed to assess whether a molecule has the potential to be developed as a drug (drug-likeness). This rule predicts that a molecule is likely to be suitable for drug development if it has no more than two violations in the following criteria: no more than five hydrogen bond donors (HBD) and 10 hydrogen bond acceptors (HBA), a molecular weight (MWT) less than 500, and a calculated logarithm of the partition coefficient (CLogP) less than 5 (Lipinski, 2004; Naglah et al., 2020). The predicted outcome of the drug-likeness analysis is presented in Table 5.

Predictions of ADME properties showed that only two compounds could have viable gastrointestinal absorption, no compound with the possibility of permeable to BBB, only rutin indicated the possibility of a P-gp substrate, and one compound was related to possible inhibition of the isoform 3A4. Additionally, they report an *in silico* prediction classification of acute oral toxicity in rats between class IV and V (Table 6).

Table 4. Molecular docking values of the phytometabolites with potential antiviral activity and reference drugs.

Pubchem ID	Ligand	Parameters	DENV-2 (PDB: 2FOM)	HCV (PDB: 4EO6)	HSV-1 (PDB: 4YXP)
1794427	Chlorogenic acid	Free energy	-8.6	-8.2	-6.8
		H-bonds	4	3	0
		N. torsions	11	11	11
		Binding residues	TRP83, LEU149	ASN142, GLU398, ASN411	-
5280805	Rutin	Free energy	-7.1	-9.9	-7.8
		H-bonds	2	2	3
		N. torsions	16	16	16
		Binding residues	GLY153(2)	ASP444, GLY557	ARG345, ARG393
0370	Gallic acid	Free energy	-5.7	-5.9	-5.6
		H-bonds	2	0	3
		N. torsions	5	5	5
		Binding residues	ALA164, ASN152	-	ARG417, ARG435, ARG439
5281792	Rosmarinic acid	Free energy	-7.9	-7.9	-6.8
		H-bonds	2	2	1
		N. torsions	12	12	12
		Binding residues	ALA164, LEU149	ALA97, GLY449	GLU358
689043	Caffeic acid	Free energy	-6.2	-6.1	-5.7
		H-Bonds	1	0	1
		N. torsions	5	5	5
		Binding residues	LEU149	-	GLU351
135398513	Acyclovir	Free energy	-6.9	-	-
		H-bonds	2	-	-
		N. torsions	5	-	-
		Binding residues	GLY87, ALA164	-	-
37542	Ribavirin	Free energy	-	-	-6.1
		H-bonds	-	-	2
		N. torsions	-	-	6
		Binding residues	-	-	ARG417, ARG435
45375808	Sofosbuvir	Free energy	-	-8.0	-
		H-bonds	-	2	-
		N. torsions	-	12	-
		Binding residues	-	GLN446, ASP559	-

DISCUSSION

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 (Fig. 1). Interestingly, the eight species are used as infusion or decoction.

Literature shows that *Azadirachta indica*, *Citrus limon*, *Cordia lutea*, *Ocinum basiculum*, and *Taraxacum 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*.

Table 5. Lipinski’s rule of five for the phytometabolites with potential antiviral activity.

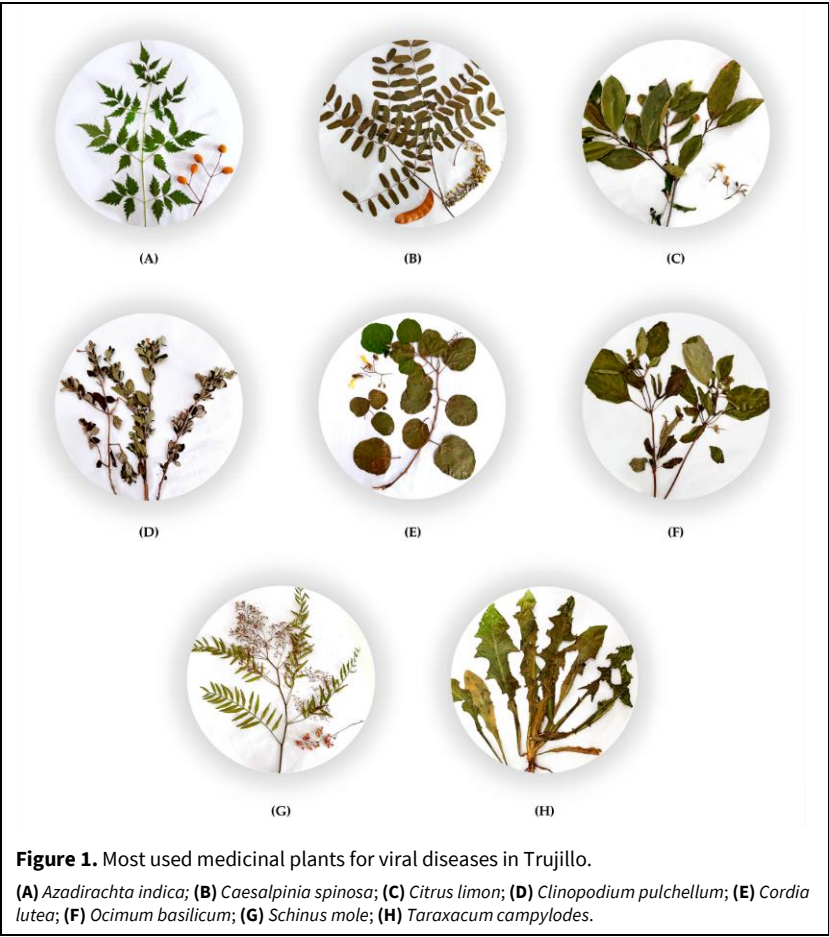
Ligands	Lipinski ’ s Rule of Five						Drug-Likeness
	MW	HBD	HBA	cLog P _{o/w}	MR	N° violations	
	≤500 Da	≤5	≤10	≤5	40-130	≤2	
Chlorogenic acid	Yes	No	Yes	Yes	Yes	1	Yes
Rutin	No	No	No	Yes	Yes	3	No
Gallic acid	Yes	Yes	Yes	Yes	Yes	0	Yes
Rosmarinic acid	Yes	Yes	Yes	Yes	Yes	0	Yes
Caffeic acid	Yes	Yes	Yes	Yes	Yes	0	Yes

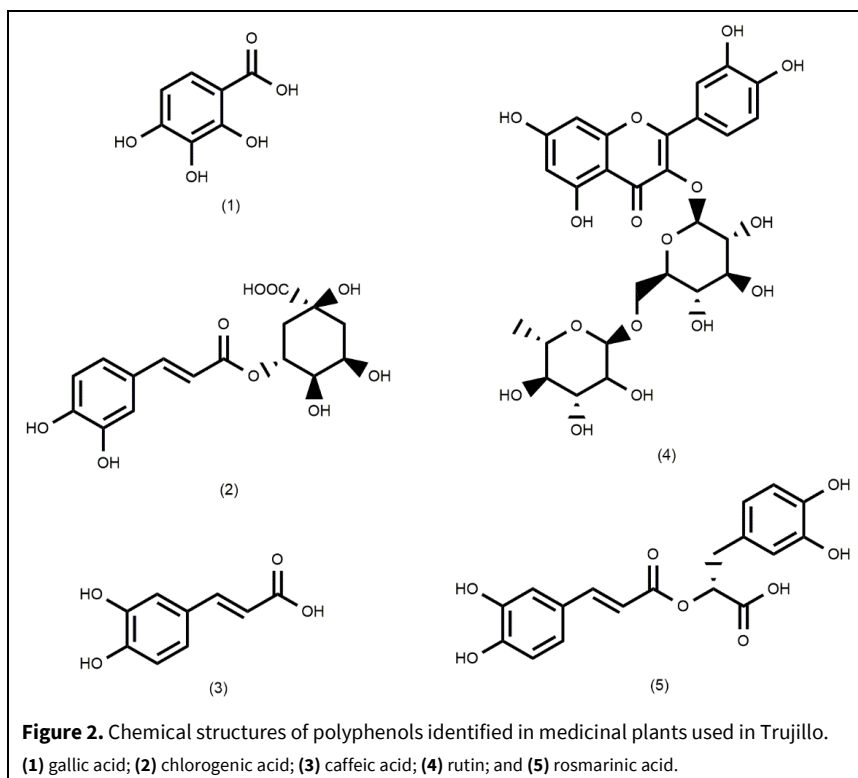
MW: molecular weight; HBD: hydrogen bond donors; HBA: hydrogen bond acceptors; MR: molar refractivity; CLogP: partition coefficient.

Table 6. ADME prediction, acute oral toxicity of the phytometabolites with potential antiviral activity.

Ligands	ADME					Toxicity		
	GI abs.	BBB	P-gp	Log Kp (cm/s)	CYP			Acute oral rats (mg/kg - OECD)*
					1A2	2D6	3A4	
Chlorogenic acid	Low	No	No	-8.76	No	No	No	3927(V)
Rutin	Low	No	Yes	-10.26	No	No	No	2953(V)
Gallic acid	High	No	No	-6.84	No	No	Yes	1606(IV)
Rosmarinic acid	Low	No	No	-6.82	No	No	No	2994(V)
Caffeic acid	High	No	No	-6.58	No	No	No	2386(V)

BBB: Blood–brain barrier; GI abs.: Gastrointestinal absorption; P-gp: P-glycoprotein. *I-II: Fatal if swallowed; III: Toxic if wallowed; IV: Harmful if swallowed; V: May be harmful if swallowed. NT: No toxic or NR.





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 (Fig. 2).

Polyphenols are recognized for their antiviral potential (Badshah et al., 2021; Renantha et al., 2022). Their presence in plants and herbs is extensive, and due to their diverse biological and pharmacological activity, they are indicated as therapeutic sources with medicinal and pharmaceutical applications (Kulkarni et al., 2019; Tungmunthum et al., 2018). Several studies demonstrate that these phytochemicals have antiviral activity (Riwu et al., 2022; Yasmin et al., 2020). The evidence includes laboratory *in vitro* and *in vivo* studies and clinical trials (Martin and Ernst, 2003).

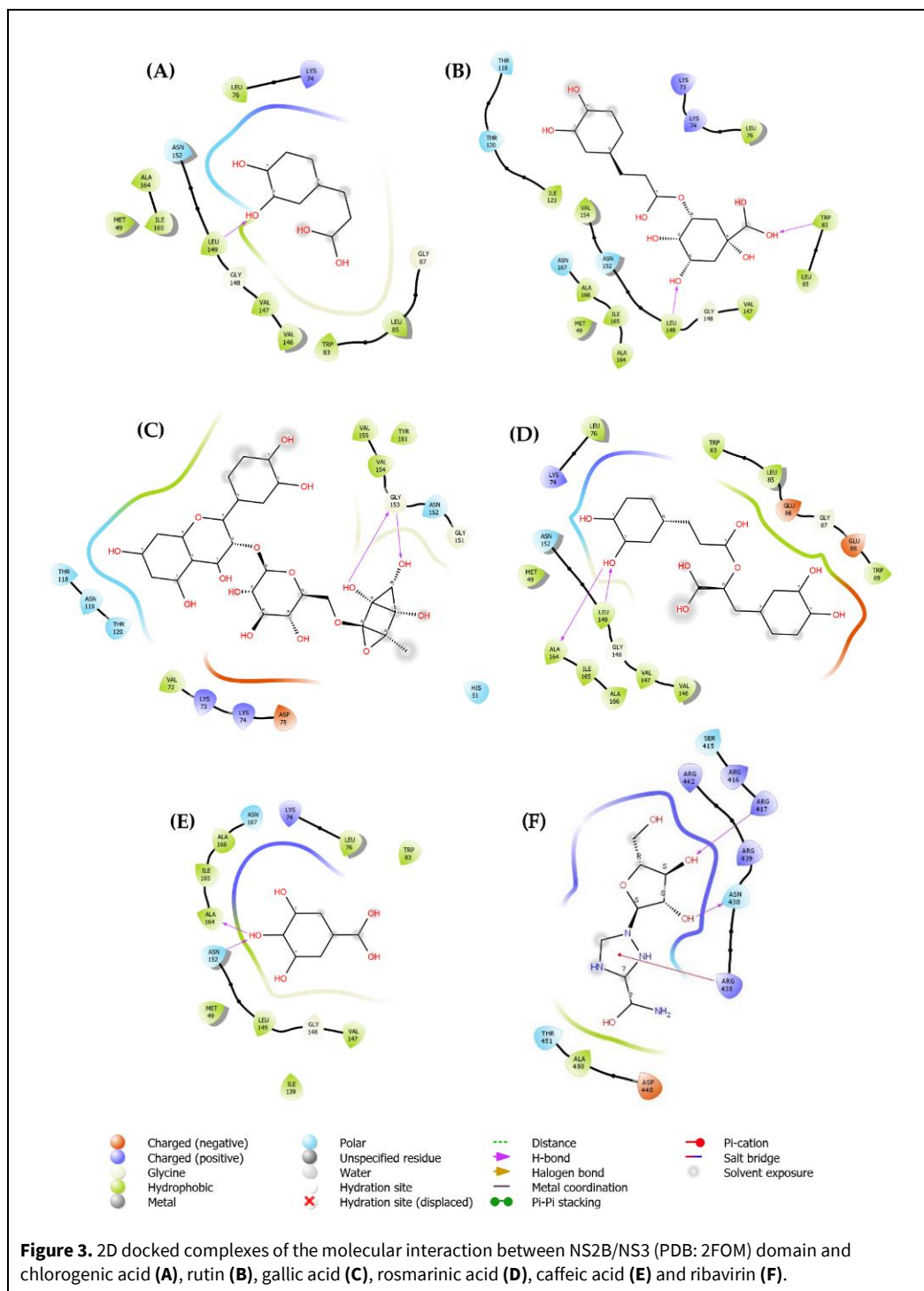
The identified phytometabolites are polyphenols. Specifically, one trihydroxy benzoic acid: gallic acid (1); three hydroxycinnamic acids: chlorogenic acid (2), caffeic acid (3), and rosmarinic acid (5); and one flavonol glycoside: rutin (4).

Due to what has been described, *Azadirachta indica*, *Caesalpinia spinosa*, *Citrus limon*, *Clinopodium pulchellum*, *Cordia lutea*, *Ocimum basilicum*, *Schinus molle*, and *Taraxacum campylodes* 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 de-

scribed in the literature, endorsing the effectiveness of their usage.

In the case of DENV-2, several studies have NS2B/NS3 protease as the evaluated molecular target (de Sousa et al., 2015; Lim et al., 2017). NS2B is a required cofactor of the NS3 serine protease (Henrich et al., 2010). Together with a cellular protease, this complex cleaves the viral precursor polyprotein into functional viral proteins (Wu et al., 2015). 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 (Herowati et al., 2017). Under this tenor, chlorogenic acid showed the highest binding affinity, while gallic acid had the lowest in comparison with ribavirin (-6.1 kcal/mol) as standard. The results obtained are consistent with those found for other compounds with antiviral potential for DENV-2 (Lima et al., 2021).

In some interactions with NS2B/NS3 (PDB: 2FOM), the residues stabilize polyphenols in the 2D simulation of the complexes formed (Fig. 3). Hydrogen bonding and hydrophobic protein-ligand interactions are also determining factors for molecular docking values (Trujillo-Correa et al., 2019). In agreement with previous studies (Hassandarvish et al., 2016), the interactions found are close to residues LEU76,



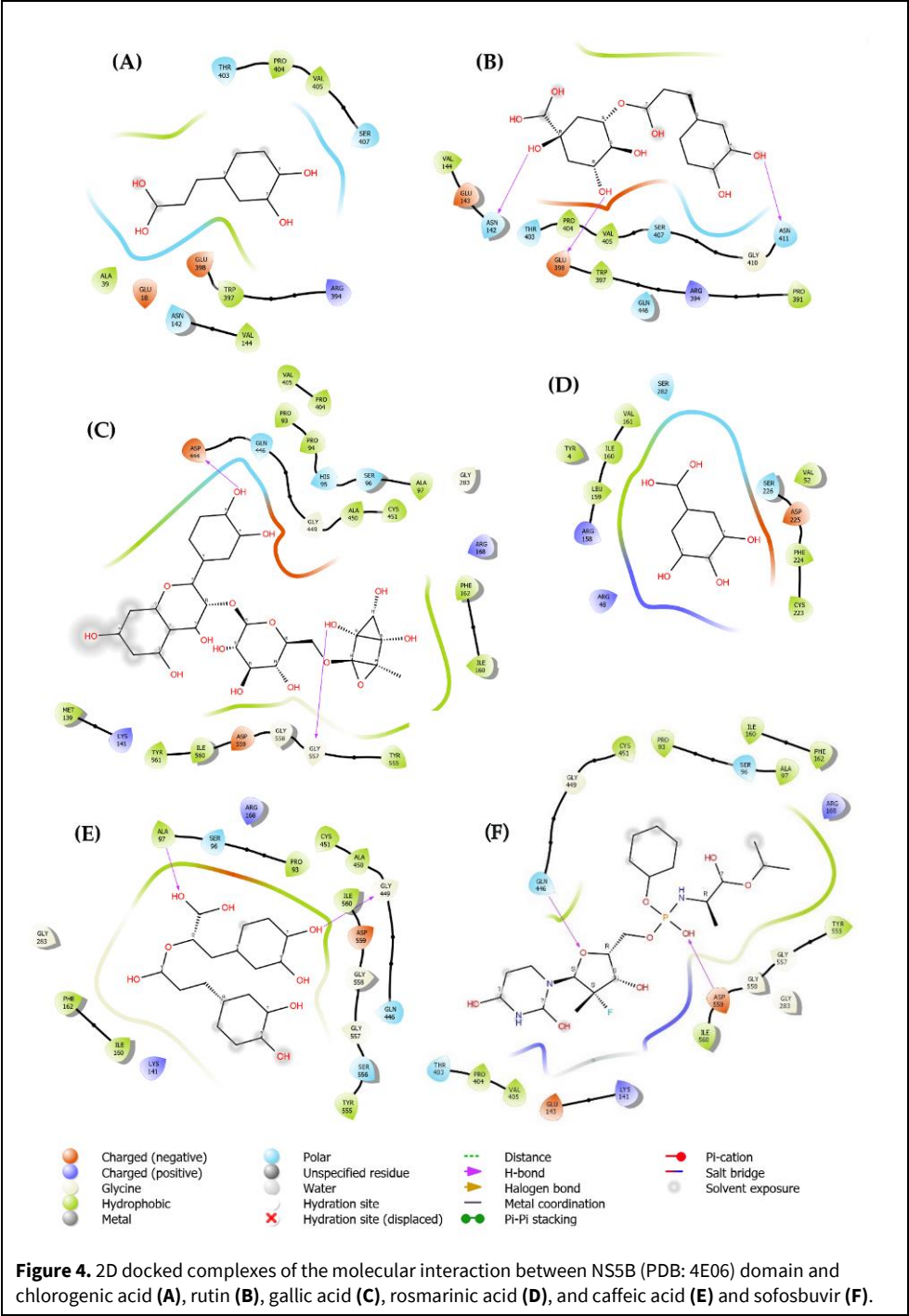
LEU149, TRP83, and ASN152, stabilizing the active site of DENV-2.

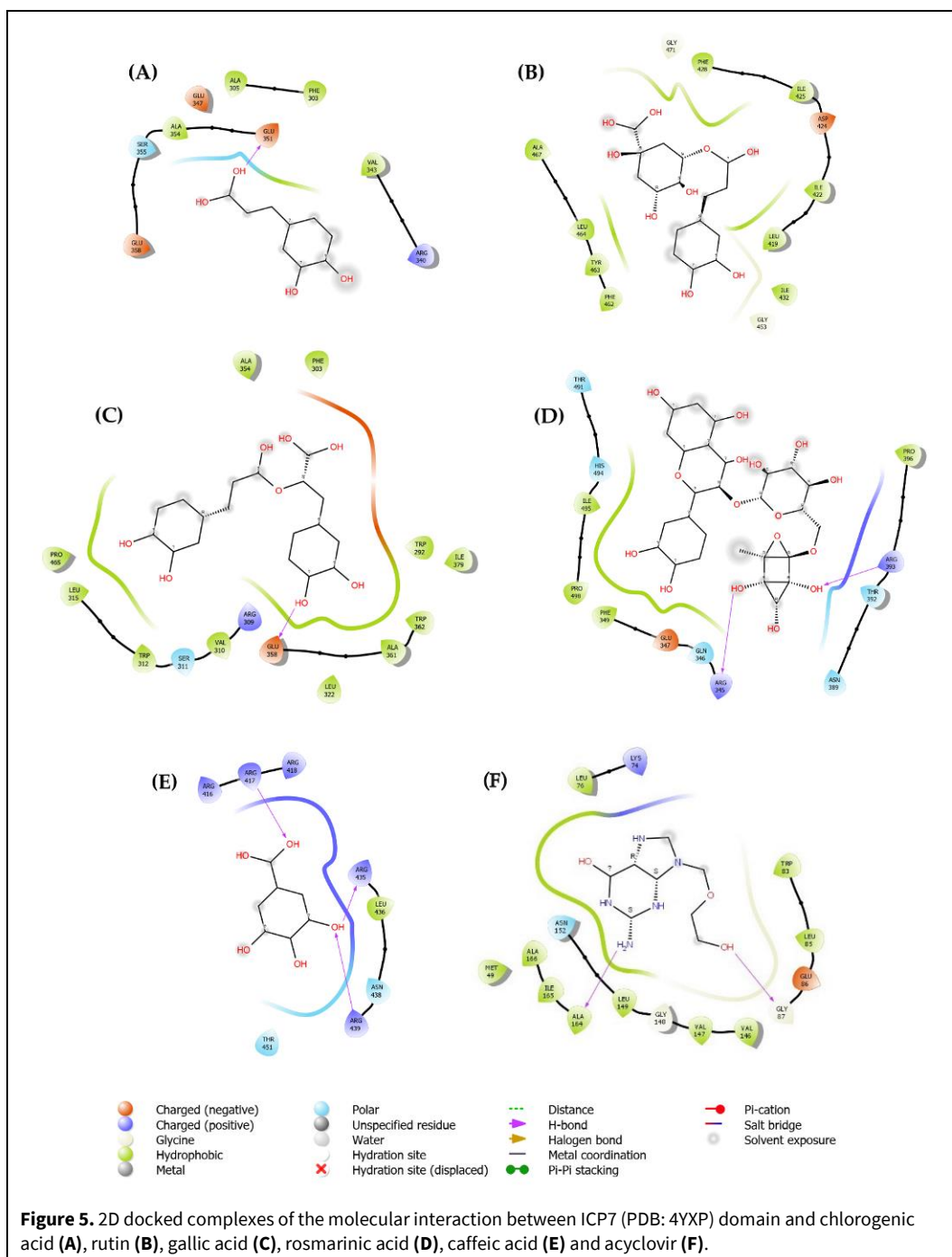
Notably, the chlorogenic acid-NS2B/NS3 interaction had the shortest distance (2.035 Å/2.183 Å RMSD) to a hydrogen bond between the ligand and the target protein. Another remarkable compound for this aspect is rosmarinic acid, whose distance was 2.116 Å/2.179 Å RMSD.

The non-structural protein NS5B is the core of HCV replication (Fadl and Salem, 2020). It is an RNA-dependent RNA polymerase (RdRp) that, when interacting with other proteins, promotes the formation of the viral RNA replication complex (Ahmed et al., 2021). Since blocking the function of NS5B can inhibit HCV replication (Zhou et al., 2022), 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 affini-

ty, while gallic acid showed the lowest affinity in comparison with the standard sofosbuvir (-8.0 kcal/mol). Previous studies on the anti-HCV activity of phenolic compounds and flavonoids (Arabi et al., 2022; Mustafa et al., 2020) support our results. Furthermore, rutin, chlorogenic acid, and rosmarinic acid binding energy values are similar to potential antiviral compounds reported previously (Lakshmi and Swapna, 2021).

Some interactions with NS5B (PDB: 4E06) the residues stabilize polyphenols in the 2D simulation of the complexes formed (Fig. 4). As mentioned, the interactions at the active site are close to the residues LEU419, THR229, and PRO133. This fact was previously reported (Rehman et al., 2016), which reinforces the results found in the present study. Regarding the hydrophilic bonds, the compounds rutin (2.098 Å RMSD), chlorogenic acid (1.947 Å RMSD), and rosmarinic acid (1.936 Å RMSD) stood out with favorable distances.





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 (Tunncliffe et al., 2019). 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 (Wang et al., 2020). 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 in comparison with the

standard acyclovir (-6.9 kcal/mol). These results prove that polyphenols block HSV-1 infection by inhibiting ICP27 synthesis (Trembl et al., 2020).

In the 2D modeling (Fig. 5), the stability of the polyphenols with ICP7 (PDB: 4YXP) was evidenced by the interactions with nearby residues. The interactions in the active site showed close binding to GLU358, TRP392, and ALA354 residues, a fact previously reported in other investigations (Tunncliffe et al., 2018; Yuan et al., 2018).

Regarding the distance to a hydrophilic bond, a favorable distance between the viral protein and rutin

(2.033 Å RMSD) stood out. All the ligand-protein interactions found in this research work are favorable since the binding energy values were negative (Amir-Hassan et al., 2017). Likewise, the distances found between the bonding atoms are relevant and of biological importance (Nittinger et al., 2017).

A good absorption capacity in the gastrointestinal tract relies on the drug's distribution within the body. A molecule exhibits good absorption if it is absorbed at least 90% into the human bloodstream (Borad et al., 2020). Such molecules may traverse the blood-brain barrier due to their lipophilicity and tendency to accumulate in the brain, potentially leading to neurogenesis and neurodegenerative diseases over time (Baldiissera et al., 2018). Furthermore, the activation of P-gp results in the efflux of the drug from cells, hindering the achievement of therapeutic effects (Ojo et al., 2021). On the other hand, the toxicity of the evaluated polyphenols indicated values ranging from 1606 to 3927 mg/kg, classified as low-risk toxicity according to the OECD (2002).

The compounds considered relevant and with potential for drug development are those that present good interaction and stability in the active site (Yadav et al., 2021). 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.

CONCLUSION

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, which reaffirms the biological studies that mention literature. Therefore, *Azadirachta indica*, *Caesalpinia spinosa*, *Citrus limon*, *Clinopodium pulchellum*, *Cordia lutea*, *Ocimum basilicum*, *Schinus molle*, and *Taraxacum campyloides* are possible phytomedicines with potential antiviral activity, it is suggested to determine the degree of biological effect.

CONFLICT OF INTEREST

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results

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Concepts or ideas	x	x			x	x
Design	x				x	x
Definition of intellectual content	x				x	x
Literature search	x	x	x	x	x	x
Experimental studies	x					x
Data acquisition	x	x	x	x		x
Data analysis	x	x	x	x	x	x
Statistical analysis	x	x				x
Manuscript preparation	x	x			x	x
Manuscript editing	x	x			x	x
Manuscript review	x	x	x	x	x	x

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Supplementary data

