

Polyphenolic-rich extracts from *Ilex paraguariensis* and *Larrea divaricata* leaves and their antioxidant and antiCOVID-19 potential

Extractos polifenólicos de las hojas de *Ilex paraguariensis* y *Larrea divaricata* y su potencial antioxidante y antiCOVID-19

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ABSTRACT

Yerba mate (*Ilex paraguariensis* A.St. Hil) and jarilla (*Larrea divaricata* Cav.) leaves are commonly used as tea infusions in some Latin American countries. This study was conducted to evaluate the antioxidant activity (FRAP, ABTS, and DPPH) and the inhibitory potential of yerba mate and jarilla extracts on the 3CL protease (Mpro) from coronavirus SARS-COV-2 by a molecular docking approach. The main bioactive compounds present in the plant extracts were identified by HPLC-MS. According to the results, the extracts of yerba mate and jarilla showed high antioxidant activity in DPPH (> 91 %), ABTS (> 90 %), and FRAP (> 47 mg TE/g) assays. Additionally, the phenolic compounds present in yerba mate, quercetin-3-O-rutinoside (rutin) (-9.60 kcal/mol) and 3,4-dicaffeoylquinic acid (- 8.20 kcal/mol) were more effective on Mpro than the antiviral drugs remdesivir and ribavirin. The compounds rutin and 3,4-dicaffeoylquinic acid have a high affinity and interaction with one of the catalytic residues Cys145 of Mpro. The glycosylation of phenolic compounds affects biological activities: positively anti-COVID-19 and negatively antioxidant. The results suggest that extracts of yerba mate and jarilla leaves could enhance the body's antioxidant defenses and can be used to improve health.

Keywords: COVID-19, phenolic compounds, *Ilex paraguariensis*, antioxidant activity, molecular docking.

RESUMEN

Las hojas de yerba mate (*Ilex paraguariensis* A.St. Hil) y jarilla (*Larrea divaricata* Cav.) se usan comúnmente como infusión de té en algunos países de América Latina. Este estudio se realizó para evaluar la actividad antioxidante (FRAP, ABTS y DPPH) y el potencial inhibitorio de los extractos de yerba mate y jarilla sobre la proteasa 3CL (Mpro) de coronavirus SARS-COV-2 por enfoque de acoplamiento molecular. Los principales compuestos bioactivos presentes en los extractos de plantas fueron identificados por HPLC-MS. De acuerdo con los resultados, los extractos polifenólicos de yerba mate y jarilla presentaron alta actividad antioxidante

en los ensayos DPPH (> 91 %), ABTS (> 90 %) y FRAP (> 47 mg TE/g). Además, los compuestos fenólicos presentes en la yerba mate, quercetina-3-O-rutinosido (rutina) (-9,60 kcal/mol) y ácido 3,4-dicafeoilquinico (- 8,20 kcal/mol) han demostrado ser más efectivos (en Mpro) que los medicamentos antivirales remdesivir y ribavirin. Los compuestos rutina y ácido 3,4-dicafeoilquinico tienen alta afinidad e interacción con uno de los residuos catalíticos Cys145 de Mpro. Estos resultados sugieren que las hojas de yerba mate y jarilla podrían potenciar las defensas antioxidantes del organismo y podrían beneficiar la salud.

Palabras clave: COVID-19, compuestos fenólicos, *Ilex paraguariensis*, actividad antioxidante, acoplamiento molecular.

INTRODUCTION

Natural products, especially medicinal plants and their phytochemicals, have been used for the treatment of various diseases since long time ago. They could be efficacious sources for prevention and treatment of diseases with new approaches, since approximately 60 % of the new medicines produced since 1981 come from plants (Newman and Cragg, 2020).

Medicinal plants have great potential in the prevention and treatment of infectious diseases. Despite the lack of strong evidence-based medicine, plants used in traditional medicine in many countries may have to contribute to the control of COVID-19 (Chen *et al.*, 2020). Medicinal plants are rich in bioactive compounds such as phenolic or polyphenolic compounds; these molecules are secondary metabolites of plants that have biological properties such as antioxidant (which helps to increase defenses) and antiviral activities (Torres-León *et al.*, 2017b) 2,3,4,6-Penta-O-Galloyl-β-D-Glucose (PGG). For example, bioactive compounds of traditional Mongolian medicine showed high anti-COVID-19 potential (Yu *et al.*, 2020) ETCM database and document mining methods were used to collect active compounds. Swiss TargetPrediction and SuperPred server were used to find targets of compounds with smiles number. Drugbank and Genecard database were used to collect antiviral drug targets. Then

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the above targets were compared and analyzed to screen out antiviral targets of Mongolia medicine. Metascape database platform was used to enrich and analyze the GO (Gene ontology). The antioxidant activity of phenolic compounds depends on the number of hydroxyl and methoxy groups present in the ring and its structural dispositions that generate stabilized free radicals (Olszowy, 2019).

Yerba mate (*Ilex paraguariensis* A.St. Hil) is a plant typical of subtropical and native regions of South America, with their leaves commonly used to produce tea infusions. Schinella *et al.* (2000), have suggested that the ingestion of yerba mate leaves is an effective and economic way to increase the antioxidant defenses. Pharmacological properties attributed to yerba mate have been related to its high content of polyphenolic compounds (Colpo *et al.*, 2016).

Jarilla (*Larrea divaricata* Cav.) is another medicinal plant of interest in Latin America. This plant is commonly known as creosote bush, and it is widely used in folk medicine, mainly in Argentina and Mexico (Agüero *et al.*, 2011).

These plants can be promising sources of phenolic compounds. However, the evaluation of antiviral activity against the COVID-19 protease (Mpro) has not been evaluated. Mpro is essential for virus replication and is in the life cycle of the coronavirus (Gimeno *et al.*, 2020) the scientific community has been under pressure to react and make progress in the development of an effective treatment against the virus responsible for the disease. Here, we implement an original virtual screening (VS. Kong *et al.* 2020a) recently introduced a docking server for docking molecules against potential targets Mpro of COVID-19. Molecular docking is an *in silico* technique for predicting the binding ability and binding mode of a protein and molecules (ligands) based on the geometry. There are two parameters to evaluate a better docking, a docking score and the type of interaction. Computational analysis has a primary role in the early drug discovery process (Kulkarni *et al.*, 2020). Therefore, the aim of this study was to evaluate the antioxidant activity *in vitro* and anti-covid potential of the bioactive compounds of yerba mate and jarilla on Mpro.

MATERIALS AND METHODS

Plant materials

Dried yerba mate (*I. paraguariensis* A.St. Hil) with leaf grinded and stems (1 kg package of Playadito con palo) was obtained from Cooperativa Agrícola de la Colonia Liebig Ltda. (Colonia Liebig's, Corrientes, Argentina). Dried jarilla (*L. divaricata* Cav.) leaf grinded (0.5 kg package) was purchased from Homeopathic Argentinian Pharmacy (60th avenue and 10th corner, La Plata, Buenos Aires, Argentina).

Polyphenolic extracts

Ultrasound-Assisted Extraction (UAE) (Branson, St. Louis, MO, USA) was performed according to Cárdenas-Hernández *et al.* (2020). Briefly, a 1 g sample of the plant was weighed and diluted in 20 mL of ethanol (90 %, v/v). The extraction was performed for 20 min at 35 °C (frequency: 60 Hz and power: 42 KHz). Samples were transferred to conical

tubes and taken to a centrifuge (SORVALL: Biofuge primo™ R) at 3500 x *g* at 20 °C (10 min). Subsequently, filtration of each sample was carried out (11 µm through filter paper) and the extracts were stored at -5 °C. The extraction yield was calculated by weight difference. Briefly, an extract of 5 mL was evaporated at room temperature (28 °C). The weight of the residue was multiplied by 4 to normalize the real volume of the experiment (20 mL).

Antioxidant activity

The antioxidant activity in the ethanol extracts was determined by: DPPH• (Molyneux, 2004), ABTS•+ (Re *et al.*, 1999), and FRAP (Benzie and Strain, 1996). Each sample was measured in triplicate. The results of DPPH• and ABTS•+ tests were expressed as a percentage reduction. FRAP assay was expressed as Trolox Equivalents (TE) with a calibration curve of Trolox (0.1–1.0 mg/mL).

Analytical RP-HPLC-ESI-MS

A Varian High-Performance Liquid Chromatography coupled to a mass spectrophotometer (Varian 500-MS IT Mass Spectrometer, USA; PDA detector Varian Pro Star 330, USA) was used to identify the main components in the extract according to Torres-León *et al.* (2017). Samples (5 µL) were injected into a Denali C18 column (150 mm x 2.1 mm, 3 µm, Grace, USA). The thermostat temperature was maintained at 30 °C. The eluents were as follows: formic acid (0.2%, v/v; A solvent) and acetonitrile (B solvent). The gradient was characterized by using initial, 3 % B with retention time of 0 – 5min, 9 % B linear with retention time of 5 – 15min, 16 % B linear with retention time of 15 – 45min, 50 % B linear.

Determination of physicochemical parameters of the main protease (Mpro)

Main Protease (Mpro; 6WQF_A) was taken from the NCBI database (<https://www.ncbi.nlm.nih.gov>). Analysis of the physical parameters of main protease (Mpro) is done by studying the number of amino acids and their composition, molecular weight, aliphatic index, theoretical pI, extinction coefficient, instability index, and grand average of hydrophobicity using ExPASy (<http://web.expasy.org/protparam>).

Molecular docking

A docking Server was used to predict the binding between the phytochemicals of yerba mate and jarilla extracts and the main COVID-19 protease (Mpro) (Torres-León *et al.*, 2020) (<https://ncov.schanglab.org.cn/index.php>), which is also named chymotrypsin-like protease (3CLpro) (Kong *et al.*, 2020b). The structures of bioactive compounds identified by the HPLC-MS analysis (used as ligands) were downloaded in SDF file format from: <https://pubchem.ncbi.nlm.nih.gov/>. The verified inhibitors were evaluated as positive controls (Remdesivir and Ribavirin). The main aim of this molecular interaction study was to identify the phytochemicals interacting with the crystal structure of protease (Mpro) *in silico*. All interaction visualization analysis studies were performed with the PyMol molecular visualization tool.

Statistical analysis

Results were expressed as mean values $\pm$ SD. The student's t-test was run to determine significant differences ($p < 0.05$). All statistical determinations were performed using Statistica 7.0 software (StatSoft, Tulsa OK, USA).

RESULTS AND DISCUSSION

Table 1 shows the extraction yield of yerba mate and jarilla leaves. The extraction yields (Ultrasound-Assisted Extraction, UAE) did not present a significant difference between the plants studied ($p > 0.05$). The results obtained for yerba mate are similar to a previous report (12 %) (Jacques *et al.*, 2007). These authors recommended UAE instead of maceration due to the considerable gain in time and enhanced extraction efficiency. The jarilla extraction yield was higher than that reported (2.9 %) by Aguirre-Joya *et al.* (2018).

Table 1. Extraction yields and antioxidant potential of *Ilex paraguariensis* A.St. Hil and *Larrea divaricata* Cav extracts.

Tabla 1. Rendimientos de extracción y potencial antioxidante de extractos de *Ilex paraguariensis* A.St. Hil y *Larrea divaricata* Cav.

Analysis	<i>Ilex paraguariensis</i> A.St. Hil	<i>Larrea divaricata</i> Cav.
Extraction yield (%)	12.0 $\pm$ 0.46	12.8 $\pm$ 0.57
DPPH (%)	91.5 $\pm$ 0.11	92.4 $\pm$ 0.56
ABTS* (%)	90.5 $\pm$ 1.10	93.0 $\pm$ 0.78*
FRAP (mg TE/g)	47.6 $\pm$ 4.00	108.0 $\pm$ 5.88*

Values are presented as means $\pm$ SD (n = 3). *Significantly different by t-test ($p < 0.05$).

Antioxidant activity

The results of the DPPH assay (Table 1) showed that both extracts have a high antioxidant activity (> 90 %); without presenting a significant difference between the plants. These results were similar (92 %) to those reported in *Larrea* genus (Aguirre-Joya *et al.*, 2018) and higher than those reported in yerba mate leaves from Brazil, Argentina, and Uruguay (< 80 %) (Colpo *et al.*, 2016). The results in the ABTS+ assay also showed high antioxidant activity in both samples (> 90 %). Extracts of jarilla (93 %) were significantly higher than those of yerba mate (90.5 %). Finally, the FRAP analysis showed that the extracts were able to reduce the ferric ion to ferrous ion. The jarilla extract showed a high antioxidant activity, significantly higher than yerba mate ($p > 0.05$). This trend was similar in the ABTS+ test; these assays are associated with its ability to scavenge free radicals. The results showed that yerba mate and jarilla leaves have high antioxidant activity. This biological activity is attributed to the presence of bioactive compounds such as polyphenols. The antioxidant activity of phenolic compounds depends on the number of hydroxyl and methoxy groups present in the ring and their dispositions in its structure. Generally, glycosylation of 3-hydroxyl groups reduces the antioxidant properties of compounds. For this reason, yerba mate has less antioxidant activity due to the presence of rutin (Olszowy, 2019). The

consumption of these plants can increase the antioxidant defenses in people (Schinella *et al.*, 2000).

Analytical RP-HPLC-ESI-MS

The identification of phenolic compounds of yerba mate and jarilla extracts was performed by comparing the retention time by HPLC analysis with MS. The molecular weight was compared with the literature and the database of the Food Research Department (DIA-UAdC). In this study, four phenolic compounds were separated and identified in each extract (Table 2). In general, yerba mate extract contained neochlorogenic acid, chlorogenic acid, rutin, and 3,4-dicaffeoylquinic acid. Chlorogenic acid is a compound that derives from caffeic acid and is present in higher concentrations in yerba mate extract. Thus corroborating the results presented in the literature (Agüero *et al.*, 2011; Zapata *et al.*, 2019).

The HPLC-MS analysis showed that jarilla leaves are a source of 3'-O-methyl-nordihydroguaiaretic acid, *p*-hydroxybenzoyl glucoside, meso (rel7S,8S,7'R,8'R)-3,4,3',4'-tetrahydroxy-7,7'-epoxylignan and nordihydroguaiaretic acid. These lignans and others have been previously reported in *Larrea* spp. extracts (Agüero *et al.*, 2011; Vargas-Arispuro *et al.*, 2005) nordihydroguaiaretic acid (NDGA). Variations in the phenolic profile may be due to factors such as the extraction method or growing conditions of the plants.

Determination of physicochemical parameters of the main protease (Mpro)

The main protease (Mpro) structure is composed of the I, II, and III domains (Kneller *et al.*, 2020); residues 8 – 10, 102 – 184, and 201 – 303, respectively. The number of amino acids is 306. In the present study, the theoretical isoelectric point (pI) was 5.92 – 5.95. The molecular weight of the protein is 33796.64 Da. The instability index is 27.65; a value < 40 indicates that the protein structure is stable. The average extinction coefficient is 33640. The aliphatic index is 82.12. The GRAVY value was - 0.019, this value indicates that the protein is hydrophilic and non-polar.

Molecular docking

The application of the COVID-19 docking server elucidated the interactions between the lead-likeness compounds and Mpro (Table 2).

All the identified compounds in the yerba mate and jarilla leaves extracts showed an inhibitory potential against Mpro. According to *in silico* results, all the compounds had a higher affinity against COVID-19 protease ($< - 7.20$ kcal/mol) than Ribavirin (- 6.40 kcal/mol). Additionally, the yerba mate compounds, rutin (-9.60 kcal/mol), and 3,4-dicaffeoylquinic acid (- 8.20 kcal/mol) had a high affinity that the broad spectrum anti-viral drug Remdesivir (- 8.10 kcal/mol). This molecule has anti-Covid19 activity (Singh *et al.*, 2020) and is currently used in COVID-19 management (Hung *et al.*, 2020). The docking energy is lower, and the binding is more stable, which indicates that yerba mate molecules probably

Table 2. Identification of the main *Ilex paraguariensis* A.St. Hil and *Larrea divaricata* Cav. compounds and their Mpro binding affinity.

Tabla 2. Identificación de los principales compuestos de *Ilex paraguariensis* A.St. Hil y *Larrea divaricata* Cav. y su afinidad vinculante contra Mpro.

Material	TR (min)	HPLC-MS		Reference	Mpro Docking Score	
		[M-H] ⁻ (m/z)	Name		PubChem	(kcal/mol)
Control	-	-	Remdesivir	-	121304016	- 8.10
Control	-	-	Ribavirin	-	37542	- 6.40
<i>Ilex paraguariensis</i> A.St. Hil	20.16	353	5-caffeoylquinic acid (neo-chlorogenic acid)	(Zapata <i>et al.</i> , 2019)	241164669	- 7.80
	25.86	353.1	3-caffeoylquinic acid (chlorogenic acid)	(Zapata <i>et al.</i> , 2019)	1794427	- 7.20
	34.88	609.1	Quercetin-3-O-rutinoside (Rutin)	(Perestrelo <i>et al.</i> , 2012)	5280805	- 9.60
	37.18	515.1	3,4-dicaffeoylquinic acid	(Zapata <i>et al.</i> , 2019)	5281780	- 8.20
<i>Larrea divaricata</i> Cav.	47.91	315	3'methyl-nordihydroguaiaretic acid	(Agüero <i>et al.</i> , 2011)	122821	- 7.60
	51.45	299	p-Hydroxybenzoyl glucoside	(Perestrelo <i>et al.</i> , 2012)	-	-
	52.59	329	meso-(rel 7S,8S,7'R,8'R)-3,4,3',4'-tetrahydroxy-7,7'-epoxylignan	(Agüero <i>et al.</i> , 2011)	-	-
	54.91	301.1	Nordihydroguaiaretic acid	(Agüero <i>et al.</i> , 2011)	4534	- 7.60

hinder the binding of substrate proteins more effectively. Das *et al.* (2020) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, reported that rutin could bind Mpro protein (PDB: 6Y84) with the highest affinity among 33 molecules screened (including natural products, antivirals, antifungals, and antiprotozoal agents). Furthermore, the docking energy of the rutin derivatives was determined for isoquercetin (monoglycosylated) and quercetin (deglycosylated), resulting in - 8.70 kcal/mol and - 7.80 kcal/mol, respectively. The docking energy decreases when the flavonoid loses glucose molecules.

Molecular docking predicted that polyphenols from yerba mate and jarilla have inhibitory potential against SARS-CoV-2 infection by interacting with the Mpro protein. The binding between Mpro and Remdesivir, Ribavirin, the bioactive compounds rutin and 3,4-dicaffeoylquinic acid (which presented the highest docking score) as a potential inhibitor of COVID-19 are shown in Figure 1. They have been represented in distinct colors for better visualization. Figure 1 corroborates the high-affinity potential listed in Table 1. There are two parameters to evaluate a better docking, a docking score and the type of interaction. A better docking score is one with more negative binding energy. The stability between ligands and the receptor is attributed to dispersion forces, hydrogen bonds, π - π interactions, and hydrophobic interactions (Mpiana *et al.*, 2020). Yu *et al.* (2020) ETCM database and document mining methods were used to collect active compounds. Swiss TargetPrediction and SuperPred server were used to find targets of compounds with smiles number. Drugbank and Genecard database were used to collect antiviral drug targets. Then the above targets were compared and analyzed to screen out antiviral targets of Mongolia medicine. Metascape database platform was used to enrich and analyze the GO (Gene ontology, demonstrated

that molecules present in traditional Mongolian medicine, such as chlorogenic acid, combine with SARS-CoV-2 protein in the form of hydrogen bonds.

The results confirmed that rutin and 3,4-dicaffeoylquinic acid interacted with His41 and Cys145, which are catalytic residues of Mpro by hydrogen bonding. Both rutin and 3,4-dicaffeoylquinic acid formed hydrogen bonds with Cys145 amino acid residues of Mpro. The rutin derivatives (quercetin and isoquercetin) form hydrogen bonds with the His41 amino acid residues of Mpro. In addition to these two key residues, several other Mpro active site amino acid residues were involved in hydrogen bonding (Glu166, Thr190, Gln189, Asn142, and Leu141) (Ghosh *et al.*, 2020; Kneller *et al.*, 2020). The distance between the ligands (≤ 3 Å) in relation to His41 and Cys145 within Mpro (active site) presents higher interaction. Given the affinity and interaction of the catalytic amino acids, they could inhibit/decrease the catalytic activity of the Mpro protease. These are important candidates for an alternative treatment of SARS-Cov-2, applying them in infusion or purified phenolic compounds. *In silico* experiments predict promising results.

CONCLUSION

The extracts of yerba mate and jarilla leaves presented a high antioxidant activity *in vitro*. The antioxidant activity is due to the hydroxyl and methoxy groups that form the structure of phenolic compounds and would be important to inhibit cell damage caused by free radicals. According to *in silico* results, the main compounds identified in these extracts showed an inhibitory potential against COVID 19 Mpro protease. However, the compounds of yerba mate, rutin, and 3,4-dicaffeoylquinic acid showed a high affinity and interaction with one of the catalytic residues (Cys145) and rutin

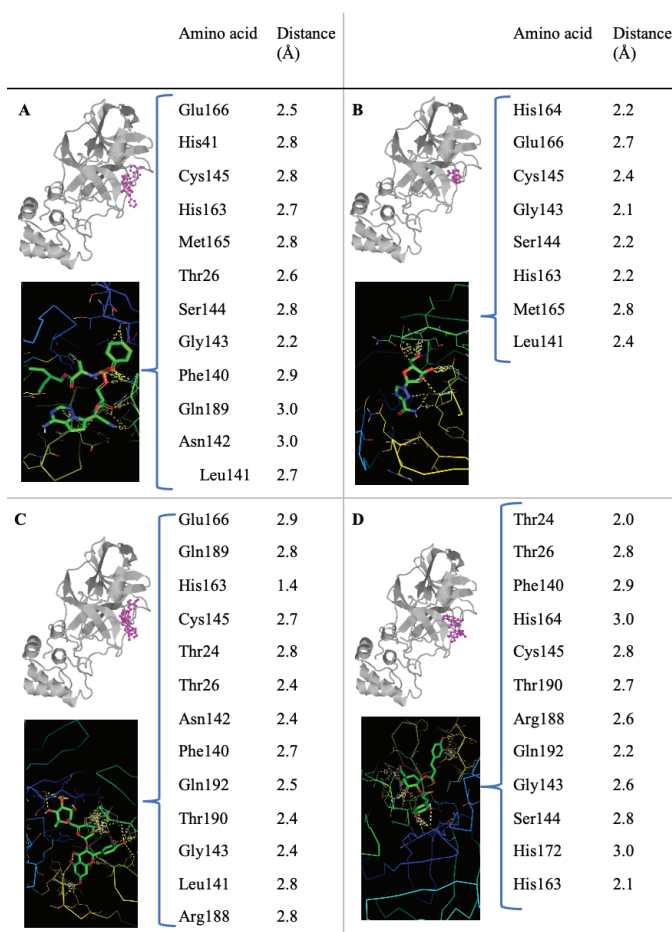


Fig. 1 2D representation of the interaction of Mpro COVID-19 with the anti-viral drug (A) Remdesivir, (B) Ribavirin, (C) Rutin, and (D) 3,4-dicaffeoylquinic acid.

Fig. 1 Representación 2D de la interacción de Mpro COVID-19 con el fármaco antiviral (A) Remdesivir, (B) Ribavirina, (C) Rutina y (D) ácido 3,4-dicaféoilquinico.

derivatives His41 when compared with the antiviral drugs Remdesivir and Ribavirin. The glycosylation of phenolic compounds affects biological activities: positively anti-COVID-19 and negatively antioxidant. These results suggest that yerba mate and jarilla leaves could be used as a potential source of bioactive compounds for potential use in the food and pharmaceutical industries.

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