



Chemical Profiling, Antioxidant, Anticholinesterase, and Antiprotozoal Potentials of *Artemisia copa* Phil. (Asteraceae)

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Artemisia copa Phil. (Asteraceae) (known as copa-copa) is a native species of Chile used as an infusion in traditional medicine by Atacameños people in the Altiplano, highlands of northern Chile. In this research, we have investigated for the first time the cholinesterase inhibition potential against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), and the chemical profiling of the infusions prepared from the aerial parts of *A. copa* by high resolution spectrometry. In addition, total phenolic, total flavonoid content, antioxidant (DPPH, FRAP, and ORAC) and antiprotozoal activity were tested. *Artemisia copa* showed good inhibitory activity against AChE and BChE ($3.92 \pm 0.08 \mu\text{g/ml}$ and $44.13 \pm 0.10 \mu\text{g/ml}$). The infusion displayed a total phenolics content of $155.6 \pm 2.9 \text{ mg}$ of gallic acid equivalents/g and total flavonoid content of $5.5 \pm 0.2 \text{ mg}$ quercetin equivalents/g. Additionally, trypanocidal activity against *Trypanosoma cruzi* was found (LD_{50} of $131.8 \mu\text{g/ml}$). Forty-seven metabolites were detected in the infusion of *A. copa* including several phenolic acids and flavonoids which were rapidly identified using ultrahigh performance liquid chromatography orbitrap mass spectrometry analysis (UHPLC-Orbitrap-MS) for chemical profiling. The major compounds identified in the infusions were studied by molecular docking against AChE and BChE. The UHPLC-MS fingerprints generated can be also used for the authentication of these endemic

Abbreviations: AAPH, 2,2'-azobis(2-amidinopropane) dihydrochloride; AChE, acetylcholinesterase; ANOVA, one-way analysis of variance; ATCI, acetyl-thiocholine iodide; BChE, butyrylcholinesterase; ChE, cholinesterase; DPPH, 2,2,1-diphenyl-1-picrylhydrazyl; DTNB, 5-dithio-bis(2-nitrobenzoic) acid; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; IC50, half maximal inhibitory concentration; MS, mass spectrometry; NO, nitric oxide; ORAC, oxygen radical absorbance capacity; OT, orbitrap technology; PDA, photodiode array detection; PTFE, polytetrafluoroethylene; QE, quercetin equivalent; ROS, reactive oxygen species; RP, reverse phase; TE, trolox equivalents; TEAC, trolox equivalent antioxidant capacity; TFC, total flavonoid content; TIC, total ion chromatogram; TPC, total phenolic content; TPTZ, 2,4,6-tri(2-pyridyl)-s-triazine; Trolox, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; UV, ultraviolet; UHPLC, ultra high performance chromatography; HESI II, electrospray heated ionization probe.

species. These findings reveal that *A. copa* infusions can be used as beverages with protective effects.

Keywords: *Artemisia copa*, Asteraceae, traditional medicine, HPLC-MS, anti-*Trypanosoma cruzi*, Anti-*Trypanosoma* activity, cholinesterase inhibition

INTRODUCTION

The Andean Altiplano is a South American high plateau located at about 3,700 m above sea level shared by Bolivia, Argentina, part of southern Peru, and Chile. These locations have ecological and environmental importance, and several ancient civilizations arose there, which uses the special fauna and flora as food and medicines for centuries. *Artemisia copa* Phil. (Asteraceae) also known as “copa-copa” is a Chilean altiplano plant characterized by having small green leaves (**Figure 1**) and used as an infusion in popular medicine (Giberti, 1983) in the Atacama Desert, North West of Argentina and Altiplano in Northern Chile. It grows between 3,500 and 4,250 m above sea level.

The *A. copa* infusion is used in traditional medicine for the treatment of colds, pneumonia, hypertension, pain, for gall bladder problems, as digestive, often combined with sodium bicarbonate. The infusion mixed with milk is used for stomach pain, cold, and colic. In the form of baths or incense, it serves to combat “mal de aire” and toothache. As a bath, it serves for bone pain. People attribute sedative properties to the species the ability to cause vivid dreams (Giberti, 1983; Montes and Wilkomirsky, 1985; De Nucci, 1988; Paniagua-Zambrana et al., 2020).

Different compounds have been identified in the extracts of aerial parts of *Artemisia copa*. In the dichloromethane and ethanolic extracts have been isolated the flavonoids spinacetin, jaceosidin, axillarin, penduletin, triclin, chrysoeriol (Moscatelli et al., 2006), chrysosplenetin (Morales et al., 2003), jaceidin (de la Fuente et al., 1986), 7-methyljaceidin, kaempferol-6-methylester-3-O-rhamnoglucoside, and luteolin (Catalán et al., 2007). The

guaianolides achillin and deacetylmaticarin have been isolated (de la Fuente et al., 1986). The coumarin scopoletin and phenolic acid *p*-coumaric acid have also been identified (Moscatelli et al., 2006). The essential oils of leaves, twigs, and flowers have been identified monoterpenoids as chrysanthenone, chrysanthenyl acetate, β -thujone, γ -terpinene, *cis*-2-menthenol, limonene and α -pinene, sesquiterpenoids as chamazulene, linalool and linalyl acetate, and homoterpene and other miscellaneous compounds as butyric acid (Catalán et al., 1982; Arze et al., 2004; Catalán et al., 2007; Larrazabal-Fuentes et al., 2019).

A. copa has been shown to possess significant biological activities such as antioxidant (Pérez-García et al., 2001; Catalán et al., 2007), analgesic and topical anti-inflammatory activities via inhibition of nitric oxide production, reduction of prostaglandin E2 levels and inhibition of cyclooxygenase-2 activity (Miño et al., 2004), inhibition of synovial phospholipase A2 activity (Moscatelli et al., 2006), spasmolytic activity (Gorzalczany et al., 2013a) and vasorelaxant and hypotensive effects through the inhibition of Ca^{2+} influx via membranous calcium channels and intracellular stores (Gorzalczany et al., 2013b).

Numerous results are reporting on the anti-trypanosomal activity the genus *Artemisia*, artemisinin, and its derivatives and other phytochemicals from *Artemisia* species (Sen et al., 2007; Aloui et al., 2016). However, more intensive research is required to explore the full potential of diverse *Artemisia* species and their chemical ingredients for the eradication of trypanosomal infections (Naß and Efferth, 2018).

Until now, no studies have been reported on the antiprotozoal and enzyme inhibitory properties against cholinesterase of *A.*



FIGURE 1 | *Artemisia copa* Phil (Asteraceae).

copa. In the same way, no metabolomic analyzes of the chemical compounds present in *A. copa* have been reported. Ultra-high-performance liquid chromatography (UHPLC) coupled with high-resolution mass spectrometry (HR-MS) is a modern and fast technique that allows the identification of accurate mass analysis. Moreover, is today a key tool for plant chemotaxonomy or metabolic profiling of plant samples (Simirgiotis et al., 2016b; Echiburu-Chau et al., 2017; Areche et al., 2020; Rodriguez et al., 2020).

Since herbal native infusions are receiving increasing attention for the number of physiological benefits they can bring to human health (Coelho et al., 2016) and as part of our continuing research of medicinal plants from Atacama Desert (Echiburu-Chau et al., 2017; Ardiles et al., 2018; Areche et al., 2020; Rodriguez et al., 2020) and native plants with potentiality as cholinesterase inhibitors (Barrientos et al., 2020a; Barrientos et al., 2020b), we investigated the infusions of the medicinal plant *A. copa* using screening tests to evaluate their antioxidant, antiprotozoal and enzyme inhibitory properties against cholinesterase and high-resolution UHPLC-MS/MS for the chemical profiling for the first time. Additionally, molecular docking of the major compounds was investigated.

MATERIALS AND METHODS

Chemicals and Regents

Solvents type UHPLC-MS, formic acid type LC-MS, and chloroform were from Merck (Santiago, Chile). Ultrapure water was obtained from a water purification system brand Millipore (Milli-Q Merck Millipore, Chile). Flavonol standards, (catechin, isoflavones, and flavonoids, all standards with a high purity: 95% by HPLC) were acquired from ChromaDex (Santa Ana, CA, United States), Sigma Aldrich (Saint Louis, Mo, United States), or Extrasynthèse (Genay, France). Folin-Ciocalteu reagent, NaOH, Na₂CO₃, AlCl₃, FeCl₃, HCl, NaNO₂, trichloroacetic acid, quercetin, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), sodium acetate, Gallic acid, TPTZ, nitroblue tetrazolium, DPPH (1,1-diphenyl-2-picrylhydrazyl radical), acetylcholinesterase (AChE), butyrylcholinesterase (BChE), 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH), were acquired from Sigma-Aldrich Chemical Company (Santiago, Chile).

Plant Material

Artemisia copa Phil. (Asteraceae) was collected in El Tatio, Antofagasta, Región de Antofagasta, Chile in November 2016. Voucher specimens for the herbarium are kept at the Laboratory of Natural Products of the Universidad de Antofagasta under the number: AC20161115.

Preparation of the Infusion of *A. copa*

The infusion was prepared using 3 g of dried milled aerial parts (stems and leaves) with 250 ml of boiling deionized water for 30 min. The infusion was filtered (Whatman 1), lyophilized (Labconco 2.5 L), and refrigerated until use at 4°C (the yield of the aqueous extraction was 8.6%).

Ultra-High-Performance Liquid Chromatography-Photodiode Array Detection-Orbitrap Technology-Mass Spectrometry Instrument

A UHPLC high-resolution MS Dionex Thermo Scientific Ultimate 3000 system connected with a Thermo Q-Exactive focus machine was used. For the analysis, 2 mg per ml of lyophilized material was dissolved in methanol-distilled water (1:1 v/v) and 10 µl of filtered solution (PTFE filter) was injected in the instrument, with the specifications set as already informed (Simirgiotis et al., 2016a).

LC Parameters and Mass Spectrometry Parameters

Liquid chromatography was run using an Acclaim UHPLC C18 column (150 × 4.6 mm ID, 2.5 µm, Thermo Fisher Scientific, Bremen, Germany) set at 25°C. The wavelength detection was 354, 254, 280, and 330 nm, and PDA was acquired from 200 to 800 nm for full characterization of peaks. Mobile phases employed were acetonitrile (B) and 1% formic aqueous solution (A) while the gradient program was: (time in minutes and % B), (0.00 min, 5% B); (5.00 min, 5% B); (10.00 min, 30% B); (15.00 min, 30% B); (20.00 min, 70% B); (25.00 min, 70% B); (35.00 min, 5% B) and 12 min for column equilibration before injections. The flow rate employed was 1.00 ml min⁻¹, and the injection volume was 10 µl. Standards dissolved in methanol and infusions were maintained at 10°C during storage in the auto-sampler. The HESI II and OT spectrometer parameters were set as informed previously (Simirgiotis et al., 2016c).

HPLC-Photodiode Array Detection Quantitation of Main Phenolic Compounds in *A. copa*

Some phenolic compounds were quantified using the UHPLC Dionex 3000 RS with photodiode array detector at flavonoid (255 and 265 nm) and phenolic acid (330 nm) wavelength as reported previously carried out according to ICH Guideline Q2 (R1) (Barrientos et al., 2020b). Some curves for representative compounds, each one covering six points from 0.01 to 0.5 mg/ml solutions, in triplicate, and injecting 10 ml. The linearity of the chromatographic method developed was confirmed by the coefficients of determination all around 0.999. For kaempferol based compounds the calibration curve was performed with kaempferol standard, ($R^2 = 0.9997$) and for quercetin-based compounds, the curve was performed with quercetin standard ($R^2 = 0.9989$), and other phenolic acid were quantified using a chlorogenic acid curve ($R^2 = 0.9994$). Using PDA, the lowest detection (LOD) and quantification (LOQ) limits, 0.0115 and 0.0322 mg/L respectively, were obtained for kaempferol, while the highest limits, 0.5543, and 1.476 mg/l were for chlorogenic acid. For the quantification the prepared infusion was injected (10 ml) in triplicate at 25°C.

Determination of Total Phenolic and Flavonoid Contents

The analyses of total phenolic compounds and flavonoid content were performed following a previous methodology of Folin-Ciocalteu (Morales and Paredes, 2014) and the aluminum chloride method (Simirgiotis et al., 2013) in microplate with some modifications. For total phenolic, a standard solution of gallic acid serial dilution (25–300 µg/ml in 10% EtOH) was used to prepare a calibration curve. Results were expressed as gallic acid equivalent (GAE)/g of dry plant. Flavonoid content was calculated using a quercetin standard calibration curve (2.5–250 µg/ml in ethanol) and the results were expressed as mg quercetin equivalents (QE)/g of dry plant. The absorbances for each methodology was determined using a microplate reader Synergy TM HT Multi-Mode.

Antioxidant Assays

1,1-Diphenyl-2-Picrylhydrazyl Radical Free Radical Scavenging Activity

The DPPH• (3.9 ml, 0.075 mM, in methanol) method reported by (Zeraik et al., 2016) was used to evaluate the antioxidant activity of *A. copa*. Briefly, 400 µl of a solution, (2 mg/ml for standards), plus 2 ml of DPPH solution were adjusted with the solvent methanol to an absorbance of 1.10 ± 0.02 at 517 nm. The mixture was homogenized using a vortex, allowed to react in the dark at room temperature for 20 min, after which time absorbance was measured at 517 nm. Gallic acid was used as a reference standard. The results were expressed as IC₅₀ (concentration of infusion or standard in µg/ml required to inhibit 50% of DPPH radical present in solution).

Ferric Reduction Ability-Antioxidant Power Test

For the Ferric Reducing Antioxidant Power (FRAP) test, the methodology determined by (Benzie and Strain, 1996) was performed with slight modifications (Morales and Paredes, 2014). The fresh working solution (FRAP reagent) included 300 mM acetate buffer (3.1 g C₂H₃NaO₂·3 H₂O and 16 ml CH₃COOH pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl₃·6H₂O solution were prepared. Trolox was used as standard solution. Briefly, to some 100 µl of the solution (or standards at 1 mg/ml), 3 ml of the FRAP solution was added and mixed using a vortex, allowed to react in the dark at room temperature for 5 min. The absorbance measurement of the colored Fe-TPTZ complex was performed at 593 nm. Absorbance values were replaced in the Trolox standard curve equation (µmol/l). The results were expressed as mg equivalents of Trolox/100 g of the dry plant.

Oxygen Radical Absorbance Capacity

The Oxygen radical absorbance capacity (ORAC) activity was determined according to the method previously reported by Huang with some modifications (Huang et al., 2002). Fluorescein (3',6'-dihydroxyspiro[2-benzofuran-3,9'-xanthene]-1-one) stock solution (4 µM) was made in 75 mM phosphate

buffer pH 7.4, stored at 4°C and used as the fluorescent probe (Zhang et al., 2009). AAPH (203.4 mg) was freshly prepared in 15 ml of 75 mM phosphate buffer and used to generate the peroxy radical. Trolox was used as internal standard. In all the experiments, 150 µl of fluorescein was added to each well. Blank wells received 25 µl of phosphate buffer, standards 25 µl of Trolox dilution, and samples 25 µl of the sample. Excitation was monitored at 485 nm and emission at 528 nm with a 20 nm bandpass on an HTX Multi-Mode Microplate Reader. The results were obtained by quadratic regression equation (Trolox/samples vs. fluorescence decay curves) and expressed as µM Trolox equivalents per 100 g of the dry plant.

Cholinesterase (ChE) Inhibition

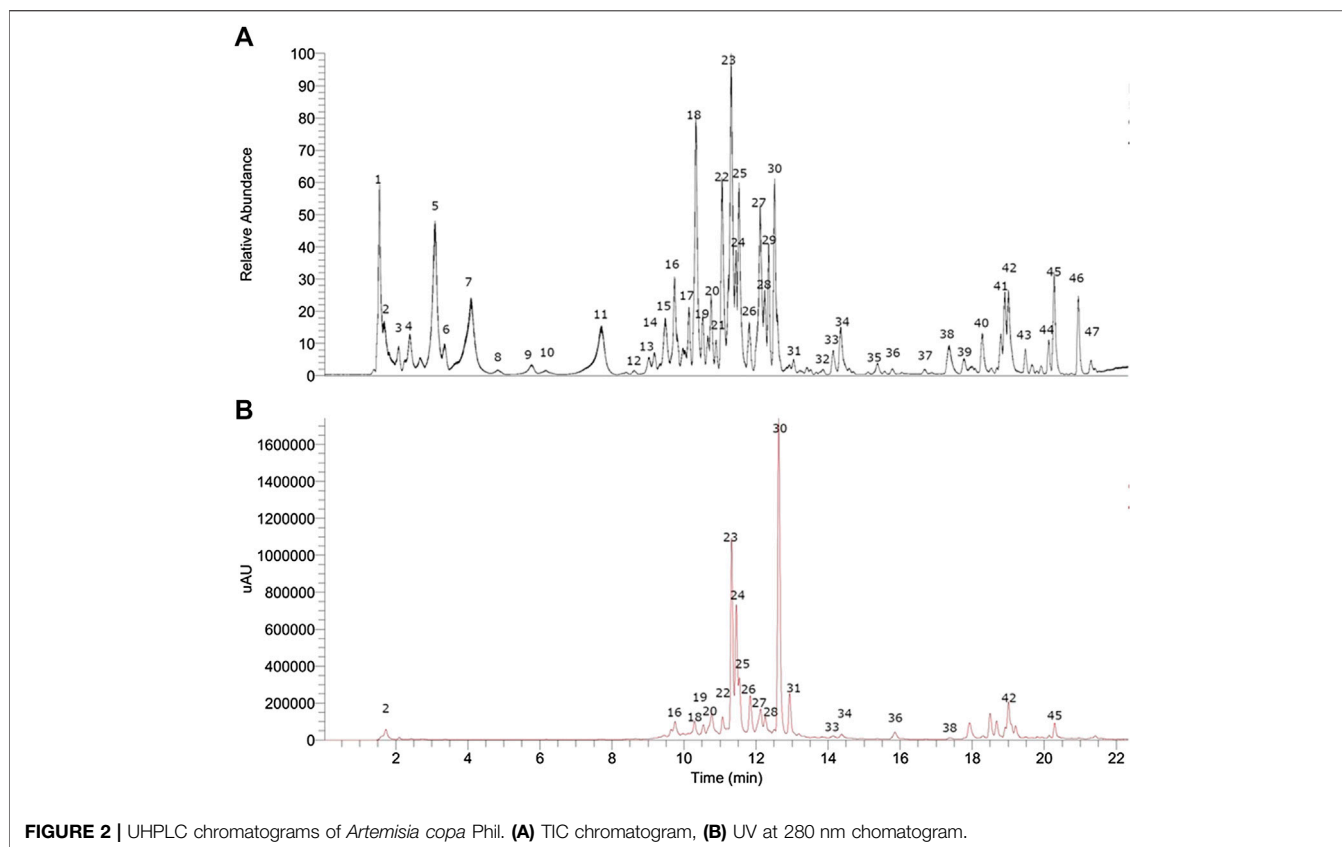
The infusions were evaluated by the Ellman's method, with some modifications (Ellman et al., 1961). Sample solution (50 µl, 2 mg/ml) was mixed with 120 µl DTNB (0.3 mM, 5,5-dithio-bis(2-nitrobenzoic acid) and AChE (0.026 U/ml, AChE from Electric eel), or BChE (0.026 U/ml, BChE from horse serum) solution (25 µl) in Tris-HCl buffer 50 mM (pH 8.0) in a 96-well microplate and incubated for 20 min at 37°C. The reaction was then initiated with the addition of 20 µl of ATCI (1.5 mM, acetylthiocholine iodide) or BTCl (1.5 mM, butyrylthiocholine chloride). A blank was prepared for all reaction reagents without enzymes solution. The absorbances were recorded at 405 nm for 30 min at 37°C and the results were expressed as IC₅₀ values (concentration range from 0.05 to 25 µg/ml).

Molecular Docking Studies

The geometries and partial charges of major flavonoids apigenin-7-O-glucoside, kaempferol-3-O-galactoside, kaempferol-3-O-acetyl-glucoside and kaempferol-7-rhamnoside contained in *A. copa* infusions, as well as the known cholinesterase inhibitor galantamine, was fully optimized using the DFT method with the standard basis set PBE0/ 6-311 + g* (Adamo and Barone, 1999). Molecular docking simulations over TcAChE and hBChE were performed using Autodock 4.2. All calculations were performed in Gaussian 09W software.

Parasites and Cell Cultures

T. cruzi (CL strain expressing beta-galactosidase) was used throughout this study (Andriani et al., 2011). Vero cells were infected with Tissue culture-derived trypomastigotes as previously described (San Francisco et al., 2017). Briefly, Vero cell monolayers were cultured in RPMI-1640 media supplemented with 10% fetal bovine sera. Culture flasks of 75 cm² (Nunc, Eas YFlasks, Thermo Scientific, Bremen, Germany) containing subconfluent cultures of Vero cells, were infected with Tissue culture-derived trypomastigotes (CL strain) in a parasite/cell ratio equal to 5:1. After 3 h of infection, cell cultures were washed with PBS to remove parasites that did not penetrate into the cell and cultures were re-incubated in RPMI-1640 medium at 37°C in a humidified 5% CO₂ atmosphere. Five days later, a pure population of *T. cruzi* trypomastigotes released in cell culture supernatants were harvested by centrifugation. *L. amazonensis* (IFLA/BR/67/PH8) was propagated as promastigotes at 26°C in M199 media supplemented with 5%



penicillin/streptomycin, 0.1% hemin (25 mg/ml in 0.1N NaOH), 10 mM adenine and 10% FBS, pH 7.5 as previously described (Sauter et al., 2019). Antiprotozoal activity against *T. cruzi* was evaluated as previously described (Gutiérrez et al., 2013). Briefly, serial dilutions of each infusion were performed in 96 wells microplates, in the range of 1000 to 0.48 $\mu\text{g/ml}$ in a final volume of 100 μl . Then, 100 μl of a suspension of trypomastigotes $1 \times 10^7 \text{ ml}^{-1}$ were added and microplates were incubated 48 h. Finally, a substrate, the β -D-galactopyranoside was added, and the mixture incubated 4 h and finally, the enzymatic activity was recorded at 570 nm in a Tecan Infinite M200 Pro (Tecan, Männedorf, Switzerland). Leishmanicidal activity was evaluated as described (Loyola et al., 2001). Briefly, serial dilutions of each infusion were performed as described above. Then, 100 μl of a suspension of 1×10^7 parasites ml^{-1} of *L. amazonensis* promastigotes were added and the microplates incubated 48 h. Finally, the number of live parasites were count in a Neubauer chamber.

Statistical Analysis

The results are expressed as mean values \pm standard error using GraphPad Prism 8 (GraphPad software Corporation, La Jolla, CA, United States). One-way analysis of variance (ANOVA; repeating three times each measurement of sample solutions) ($p < 0.05$) was used for comparison of means.

RESULTS AND DISCUSSION

Chemical Profiling

In this study, 47 compounds were detected and tentatively identified in the infusion of *A. copa* (Figure 2). Previously, from a Argentinean sample of this plant *p*-coumaric acid, luteolin, chrysoeriol, and vitexin were reported, to present vasodilation (Gorzalczany et al., 2013b) also, some flavonoids were isolated from the sample: spinacetin, tricrin, jaceosidin, axillarin, penduletin, and chrysoeriol which showed anti-inflammatory activity (Moscatelli et al., 2006), the compound: 3,5-dihydroxy 6,7,3',4'-tetramethoxyflavone was isolated previously and presented antimicrobial activity (Morales et al., 2003). Several sesquiterpene lactones such as chrysartemin A, were also reported previously from this species (de la Fuente et al., 1986). In this work several compounds are reported for the first time in this species and the determination of those metabolites in this Chilean sample were characterized based on UV absorption and high-resolution mass spectrometry fragmentation patterns for the first time (Table 1). The content of individual compounds (mg/Kg of dry plant) are depicted in Supplementary Table S1 (Supplementary Material) and the chromatograms are depicted in Supplementary Figure S1 (Supplementary Material). Main compound peak 30 was not quantified, due to lack of standard.

TABLE 1 | High-resolution UHPLC- MS Identification of metabolites from *Artemisia copa* infusion.

Peak number	UV max (nm)	Tentative identification	Formula [M-H] ⁻	Retention time (min)	Theoretical mass (m/z)	Measured mass (m/z)	Accuracy (ppm)	MS ⁿ ions (ppm)
1	272–345	Quinic acid	C ₇ H ₁₁ O ₆ ⁻	1.55	191.05501	191.05569	3.56	173.04527 (C ₇ H ₉ O ₅ ⁻) (M ⁻ -H ₂ O); 109.02870 (C ₇ H ₉ O ₅ ⁻)
2	371	Isoquinic acid	C ₇ H ₁₁ O ₆ ⁻	1.69	191.05501	191.05557	2.92	111.00788 (C ₅ H ₃ O ₃ ⁻)
3	351	Citric acid	C ₆ H ₇ O ₇ ⁻	2.07	191.01863	191.01929	3.44	111.00790 (C ₅ H ₃ O ₃ ⁻)
4	-	2-Hydroxyglutaric acid	C ₅ H ₇ O ₅ ⁻	2.39	147.02880	147.02917	2.55	133.01477 (C ₄ H ₅ O ₅ ⁻)
5	-	Lilioside A	C ₁₁ H ₁₉ O ₉ ⁻	3.08	295.10236	295.10349	3.81	133.04996 (C ₅ H ₉ O ₄ ⁻)
6	246	2,3-Butanediol apiosylglucoside	C ₁₅ H ₂₇ O ₁₁ ⁻	3.34	383.15479	383.15598	3.09	129.09160 (C ₇ H ₁₃ O ₂ ⁻)
7	231	Artemisinic acid	C ₁₅ H ₂₁ O ₂ ⁻	4.09	233.15361	233.15446	3.68	161.04453 (C ₆ H ₉ O ₅ ⁻)
8	299	2,4,6-Trihydroxyanisole	C ₇ H ₇ O ₄ ⁻	4.85	155.03389	155.03441	3.37	129.09145 (C ₇ H ₁₃ O ₂ ⁻)
9	228	Cinnamyl tiglate	C ₁₄ H ₁₅ O ₂ ⁻	5.77	215.10666	215.10745	3.70	197.09648 (C ₁₄ H ₁₃ O ⁻)
10	-	Pleioside	C ₁₅ H ₁₉ O ₉ ⁻	6.12	343.10346	343.10361	3.63	—
11	-	Methyl vanillate glucoside	C ₁₅ H ₁₉ O ₉ ⁻	7.71	343.10236	343.10352	3.37	—
12	217–281	Rehmanioside A	C ₁₉ H ₃₃ O ₈ ⁻	8.64	389.21809	389.21957	3.38	181.05011 (C ₉ H ₉ O ₄ ⁻)
13	221–284	Gaultherin	C ₁₉ H ₂₅ O ₁₂ ⁻	9.01	445.13405	445.13528	2.76	374.16104, 199.09721
14	222–285	4-Hydroxybenzoic acid	C ₇ H ₅ O ₃ ⁻	9.19	137.02442	137.02379	3.41	—
15	—	2-Isopropylmalic acid	C ₇ H ₁₁ O ₅ ⁻	9.48	175.06010	175.06079	3.94	—
16	218–325	Chlorogenic acid	C ₁₆ H ₁₇ O ₉ ⁻	9.74	353.08781	353.08789	3.34	191.05571 (C ₇ H ₁₁ O ₆ ⁻)
17	223–409	7-Demethylsuberosin	C ₁₄ H ₁₃ O ₃ ⁻	10.13	229.08592	229.08670	3.40	135.04437 (C ₈ H ₇ O ₂ ⁻)
18	270-329-427	Dihydro- <i>p</i> -coumaroylglucose	C ₁₅ H ₁₉ O ₈ ⁻	10.33	327.10744	327.10855	3.47	147.04445 (C ₉ H ₇ O ₂ ⁻)
19	259–431	Schaftoside	C ₂₆ H ₂₇ O ₁₄ ⁻	10.52	563.13953	563.14020	1.18	374.16104
20	269–346	Kaempferol-3-O-glucoside	C ₂₁ H ₁₉ O ₁₁ ⁻	10.77	447.09219	447.09329	2.47	175.03952
21	226–330	Feruloyl arabinobiose	C ₂₀ H ₂₅ O ₁₂ ⁻	10.88	457.13405	457.13535	2.82	215.10750 (C ₁₄ H ₁₅ O ₂ ⁻)
22	218–326	3-O-Feruloylquinic acid	C ₁₇ H ₁₉ O ₉ ⁻	11.06	367.10236	367.10364	3.48	173.04486 (C ₇ H ₉ O ₅ ⁻)
23	213–338	Apigenin 7-O-glucoside	C ₂₁ H ₁₉ O ₁₀ ⁻	11.30	431.09727	431.09845	2.74	197.09671 (C ₁₄ H ₁₃ O ⁻)
24	225–309-	Kaempferol 7-rhamnoside	C ₂₁ H ₁₉ O ₁₀ ⁻	11.44	431.09727	431.09851	2.87	163.03955 (C ₉ H ₇ O ₃ ⁻)
25	268–342	Kaempferol-3-O-galactoside	C ₂₁ H ₁₉ O ₁₁ ⁻	11.52	447.09219	447.09341	2.74	285.04037 (C ₁₅ H ₉ O ₆ ⁻ ; kaempferol)
26	296–439	Cynarine	C ₂₅ H ₂₃ O ₁₂ ⁻	11.81	515.11840	515.11932	1.78	135.04436 (C ₈ H ₇ O ₂ ⁻)
27	265–354	Kaempferol-3-O-acetyl-glucoside	C ₂₃ H ₂₁ O ₁₂ ⁻	12.12	489.10275	489.10376	2.05	285.04028 (C ₁₅ H ₉ O ₆ ⁻ ; Kaempferol)
28	336–462	Nepochlorogenic acid 1,3 di-O-caffeoyl quinic acid	C ₂₅ H ₂₃ O ₁₂ ⁻	12.23	515.11840	515.11945	2.03	191.05562 (C ₇ H ₁₁ O ₆ ⁻)
29	231-332-462	Hydroxyoctanoic acid-O-Glucoside	C ₁₄ H ₂₅ O ₈ ⁻	12.33	321.15439	321.15564	3.87	159.10207 (C ₈ H ₁₅ O ₃ ⁻ ; hydroxyoctanoic acid)
30	250–326	Chrysartemin A	C ₁₅ H ₁₇ O ₅ ⁻	12.51	277.10705	277.10809	3.75	135.08081 (C ₉ H ₁₁ O ⁻)
31	236	Unknown	C ₁₃ H ₁₃ O ₁₅ ⁻	13.05	409.02599	409.02380	-2.68	181.08667 (C ₁₀ H ₁₃ O ₃ ⁻)
32	228–427	trans-β-Damascenone	C ₁₃ H ₁₇ O ⁻	13.87	189.12739	189.12820	4.29	145.02859 (C ₉ H ₅ O ₂ ⁻)
33	232–309	Unknown	C ₁₃ H ₁₅ O ₄ ⁻	14.14	235.09649	235.09746	4.13	145.02879 (C ₉ H ₅ O ₂ ⁻)
34	265–354	Kaempferol	C ₁₅ H ₉ O ₆ ⁻	14.36	285.03936	285.04059	4.29	135.04439 (C ₈ H ₇ O ₂ ⁻)
35	239–340	3,7-Dimethylquercetagenin	C ₁₇ H ₁₃ O ₈ ⁻	15.37	345.06049	345.06183	3.89	129.09175 (C ₇ H ₁₃ O ₂ ⁻)
36	290–396	4-Propionylbenzoic acid	C ₁₀ H ₉ O ₃ ⁻	15.78	177.05462	177.05531	3.91	145.02884 (C ₉ H ₅ O ₂ ⁻)
37	239–382	Peuarenarine	C ₂₄ H ₂₅ O ₈ ⁻	16.68	441.15439	441.15570	2.96	145.02876 (C ₉ H ₅ O ₂ ⁻)
38	237–345	7, 3'-dimethoxyquercetin	C ₁₇ H ₁₃ O ₇ ⁻	17.36	329.06558	329.06683	3.81	299.01971 (C ₁₅ H ₇ O ₇ ⁻ ; quercetin)
39	233–340	3-O-Methylkaempferol	C ₁₆ H ₁₁ O ₆ ⁻	17.77	299.05501	299.05627	4.21	134.03671 (C ₈ H ₆ O ₂ ⁻)
40	255–367	Eupatolitin	C ₁₇ H ₁₃ O ₈ ⁻	18.28	345.06159	345.06189	4.04	183.08127 (C ₁₃ H ₁₁ O ⁻)
41	256–349	Irigenin	C ₁₈ H ₁₅ O ₈ ⁻	18.90	359.07724	359.07748	3.70	329.02969 (C ₁₆ H ₉ O ₈ ⁻)
42	262–425	Myristicin	C ₁₁ H ₁₁ O ₃ ⁻	19.01	191.07027	191.07101	3.89	145.02888 (C ₉ H ₅ O ₂ ⁻)
43	241–312	Calodendrolide	C ₁₅ H ₁₅ O ₄ ⁻	19.47	259.09758	259.09753	4.04	145.02895 (C ₉ H ₅ O ₂ ⁻)
44	269–338	Eupatorin	C ₁₈ H ₁₅ O ₇ ⁻	20.14	343.08123	343.08264	4.11	215.10728 (C ₁₄ H ₁₅ O ₂ ⁻)
45	254–354	3,6,7,8,3'-tetramethoxymyricetin	C ₁₉ H ₁₇ O ₈ ⁻	20.27	373.09289	373.09320	3.76	129.09129 (C ₇ H ₁₃ O ₂ ⁻)
46	248–392	Pentyl benzoate	C ₁₂ H ₁₅ O ₂ ⁻	20.95	191.10666	191.10741	3.94	427.21265(C ₂₅ H ₃₁ O ₆ ⁻)
47	291–396	5,7-Dimethoxy-2,2-dimethylchromene	C ₁₃ H ₁₅ O ₃ ⁻	21.30	219.10157	219.10248	4.14	145.02876 (C ₉ H ₅ O ₂ ⁻)

Sugars and Hydrocarbons

Peak 1 with a pseudo-molecular ion at m/z : 191.05569 was identified as quinic acid ($C_7H_{11}O_6^-$) and its isomer peak 2 as isoquinic acid at m/z 191.05557. Peak 3 was identified as citric acid ($C_6H_7O_7^-$). Peak 4 with a molecular ion at m/z : 47.02917 was identified as 2-hydroxyglutaric acid ($C_5H_7O_5^-$). peak 12 as rehmanioside A ($C_{19}H_{33}O_8^-$), a constituent of *Rehmannia glutinosa* (Gaertn.) DC. (Liu et al., 2020).

Phenolic Acids

Peak 5 with a $[M-H]^-$ ion at m/z : 295.10349 was identified as the coumaroyl glycerol lilioid A ($C_{11}H_{19}O_9^-$) (Kaneda, 1990), peak 6 was identified as 2,3-butanediol apiosylglucoside (383.15598, $C_{15}H_{27}O_{11}^-$). Peak 8 with a $[M-H]^-$ ion at m/z 155.03441 was identified as 2,4,6-trihydroxyanisole ($C_7H_7O_4^-$). Peak 9 was identified as cinnamyl tiglate ($C_{14}H_{15}O_2^-$), peak 10 with a molecular ion at m/z : 343.10361, was identified as pleoside ($C_{15}H_{19}O_9^-$) this compound was reported antiproliferative together with chlorogenic acid (Jafari et al., 2017), peak 11 as its isomer methyl vanillate glucoside, Peak 13 as gaultherin ($C_{19}H_{25}O_{12}^-$). Peak 14 with a $[M-H]^-$ ion at m/z : 137.02379 was identified as 4-hydroxybenzoic acid ($C_7H_5O_3^-$), peak 15 as 2-isopropylmalic acid ($C_7H_{11}O_5^-$). Peak 19 as schaftoside ($C_{26}H_{27}O_{14}^-$) peak 16 and 28 were identified as isomers of chlorogenic acid ($C_{16}H_{17}O_9^-$), peak 17 as 7-demethylsuberosin ($C_{14}H_{13}O_3^-$), and peak 18 as dihydro-*p*-coumaroylglucose ($C_{15}H_{19}O_8^-$), respectively. Peak 21 as feruloyl arabinobiose (457.13535), Peak 22 with a $[M-H]^-$ ion at m/z : 367.10364 was identified as 3-*O*-feruloylquinic acid ($C_{17}H_{19}O_9^-$), peak 26 as cynarine, peak 32 as trans- β -damascenone ($C_{13}H_{17}O^-$), and peak 36 as 4-propionylbenzoic acid (177.05531) and 37 as peuarenarine ($C_{24}H_{25}O_8^-$), peak 46 as pentyl benzoate ($C_{12}H_{15}O_2^-$) and finally, peak 47 as 5,7-dimethoxy-2,2-dimethylchromene ($C_{13}H_{15}O_3^-$).

Fatty Acids

Peak 29 was identified as hydroxyoctanoic acid-*O*-glucoside ($C_{14}H_{25}O_8^-$)

Flavonoids

Peak 20 with a $[M-H]^-$ ion at m/z : 447.09372 was identified as kaempferol-3-*O*-glucoside ($C_{21}H_{19}O_{11}^-$) while peak 23 with a pseudo-molecular ion at m/z 431.09845 was identified as apigenin 7-*O*-glucoside ($C_{21}H_{19}O_{10}^-$) (Taşkın et al., 2018) and peak 24 as kaempferol 7-rhamnoside ($C_{21}H_{19}O_{10}^-$). Peak 25 as kaempferol-3-*O*-galactoside ($C_{21}H_{19}O_{11}^-$) and peak 27 with a molecular ion at m/z : 489.10376 as kaempferol-3-*O*-acetylglucoside ($C_{23}H_{21}O_{12}^-$) a constituent of *Moringa oleifera* Lam. leaves (Mousa et al., 2019), finally, peaks 34, 35 and 38–42 and 44, 45 as: kaempferol ($C_{15}H_{19}O_6^-$), 3,7-dimethylquercetagenin ($C_{17}H_{13}O_8^-$), 7,3'-dimethoxyquercetin ($C_{17}H_{13}O_7^-$), 3-*O*-methylkaempferol ($C_{16}H_{11}O_6^-$), eupatolitin ($C_{17}H_{13}O_8^-$), irigenin ($C_{18}H_{15}O_8^-$), myristicin ($C_{11}H_{11}O_3^-$), eupatorin ($C_{18}H_{15}O_7^-$), and 3,6,7,8,3'-tetramethoxymyricetin ($C_{19}H_{17}O_8^-$), respectively.

TABLE 2 | Total phenolics, total flavonoids, and antioxidants activity of *Artemisia copa* infusion.

Assay	<i>Artemisia copa</i> infusion	Standard
Total phenolics ^a	155.6 ± 2.9	—
Total flavonoids ^b	5.5 ± 0.2	—
FRAP ^c	6.2 ± 0.2	—
ORAC ^d	1989 ± 5.2	—
DPPH ^e	89.72 ± 0.01	Gallic acid: 0.55 ± 0.01

All values were expressed as means ± SEM (n = 3).

FRAP, ferric reducing/antioxidant power; ORAC, Oxygen Radical Absorbance Capacity; DPPH, 2,2-diphenyl-1-picryl-hydrazyl-hydrate. All values in the column are significantly different (at $p < 0.05$).

^aexpressed in mg gallic acid equivalent per g of dry plant.

^bexpressed in mg quercetin per g of dry plant.

^cexpressed in mg trolox equivalent per 100 g of the dry plant.

^dexpressed in μ M Trolox equivalents per 100 g of the dry plant.

^eexpressed as IC_{50} in μ g of extract or standard per ml.

Sesquiterpene Lactones

Artemisinic acid peak 7 ($C_{15}H_{21}O_2^-$), main compound in famous Chinese medicinal plant *Artemisia annua* L. (Bora and Sharma, 2011), was found and identified with a molecular ion at m/z : 233.15446, and peak 30 as chrysartemin A ($C_{15}H_{17}O_5^-$) also constituent of the *Artemisia* genus.

Total Phenolic Content, Total Flavonoid Content, and Antioxidant Activity

Artemisia copa infusions were assessed *in vitro* for total phenolic content (TPC) and total flavonoid content (TFC) also, DPPH, FRAP, and ORAC assays were used to determine the antioxidant properties and are summarized in Table 2. These *in vitro* assays are simple and widely employed for the evaluation of antioxidant power and ROS quenching (Moon and Shibamoto, 2009). These results were compared to another very used Andean altiplano plant, *Aloysia deserticola* (Phil.) Lu-Irving and O'Leary (syn. *Acantholippia deserticola* Phil.) (Verbenaceae) previously reported by us (Larrazabal et al., 2018). Phenolic compounds are structurally diverse and comprise phenolic acids, flavonoids, tannins, coumarins, chalcones, iridoids, among others, most of them bearing phenolic moieties that give them antioxidant and beneficial health properties. In this study three main classes of compounds were tentatively identified: organic acids, feruloyl and coumaroyl derivatives, plus flavonoids. In addition, 47 compounds were detected in their corresponding infusion, that could be responsible for the bioactivities reported. The results observed for TPC and TFC of *A. copa* infusion were 155.6 ± 2.9 mg/g and the 5.5 ± 0.2 mg/g of dry plant, respectively. On the other hand, DPPH was performed to know the bleaching capacities of the infusion, and showed an IC_{50} of 89.72 ± 0.01 μ g/ml infusion. While the ABTS and DPPH values for *A. deserticola* infusions were 337 and 438 μ g/ml (Larrazabal et al., 2018). Also, the FRAP assay was conducted giving the lower antioxidant power obtained (6.2 ± 0.2 mg trolox equivalent per 100 g of the dry plant.) compared to the famous infusions of the arid plant *A. deserticola* (Larrazabal et al., 2018). This assay is based on the ability to reduce Fe^{+3} to Fe^{+2} by antioxidants in a sample. Regarding the ORAC assay, the infusion value was 1989 ±

TABLE 3 | Cholinesterase inhibitory activity of *Artemisia copa* infusion.

Assay	<i>Artemisia copa</i> infusion (IC ₅₀)	Galantamine (IC ₅₀)
AChE	3.92 ± 0.08	0.26 ± 0.03
BChE	44.13 ± 0.10	3.82 ± 0.02

All values were expressed as means ± SEM (n = 3). IC₅₀ as expressed in µg per ml. AChE, Acetylcholinesterase; BChE, Butyrylcholinesterase. Values in the same column marked with the same letter are not significantly different (at p < 0.05).

5.2 µM Trolox equivalents (TEAC) per 100 g of the dry plant. These values can classify these *A. copa* infusions as moderate antioxidants properties, like edible fruits such as cherries, strawberries, and plums (Rodríguez et al., 2014; Ramirez et al., 2015).

Cholinesterase Inhibitory Activity

In this study, *Artemisia copa* infusion was assessed for *in vitro* inhibitory activity against AChE and BChE using galantamine as the positive control (reference: galantamine 0.26 ± 0.03 µg/ml). The results are summarized in **Table 3** and are expressed as IC₅₀ values (µg/ml). The inhibition of key enzymes has been a very useful strategy to know the pharmacological potential of herbal medicines. Moreover, inhibition of enzymes linked to Alzheimer's disease (AD), such AChE and BChE (Pan et al., 2013). In the AChE assays, *A. copa* showed an IC₅₀ of 3.92 ± 0.08 µg/ml, while in the BChE assays the IC₅₀ results were 44.13 ± 0.10 µg/ml respectively. In previous reports the cholinesterase inhibitory activity of Algerian *Artemisia* spp: *A. absinthium* L., *A. fragrans* Willd. and *A. herba-alba* Asso were evaluated and showed similar results against AChE but not activity against BChE (Orhan et al., 2010), also Turkish *Artemisia*, *A. absinthium* was reported (Zengin et al., 2017). *A. copa* possessed the capacity to inhibit AChE and BChE enzymes.

Regarding the constituents identified in the infusion of *A. copa*, the literature has indicated that, for example, the increase of brain AChE activity was reported for citric acid, acting as a neuroprotective agent in animal models (Abdel-Salam et al., 2016). Regarding hydroxybenzoic acid, have evaluated the antioxidants and ChE inhibitors properties of hydroxybenzoic acid derivatives as dual-targets (Oliveira et al., 2018). In a previous report phenolic acids from malt, included hydroxybenzoic acid, showed efficient properties against AChE and BChE respectively (Szwajgier and Borowiec, 2012). Chlorogenic acid exert protective effects on learning and memory impairment. Furthermore, *ex vivo* experiments showed inhibition of AChE activity in hippocampus and frontal cortex. On the other hand, *in vitro* experiments inhibit AChE activity with a IC₅₀ = 98.17 µg/ml (Kwon et al., 2010). Also, the combination of chlorogenic acid with caffeic acid displayed an antagonist response against AChE and BChE. The neuroprotective properties have been associated to the ChE inhibition, as well to the prevention of oxidative damage (Obob et al., 2013). AChE activity have been reported for 7-demethylsuberosin isolated from genus *Angelica*, suggesting a potential agent for the AD (Kang et al., 2001; Kwon et al., 2017). In addition, Schaftoside isolated from the water layer of *Lycopodiella cernua* (L.) Pic.Serm. showed strong inhibitory activity against BChE (Hung et al., 2015).

The *in vitro* ChE inhibitory activities of different flavonoids derivatives (rutin, quercetin and kaempferol-3-O-galactoside) have been previously investigated. Docking experiments of quercetin showed a competitive inhibition mechanism against both enzymes (Khan et al., 2009). The principal active compound from *Cynara scolymus* L., cynarine, was purified from *Onopordum illyricum* L. and showed antioxidant, antiradical and inhibitory activities against AChE (Topal et al., 2016). On the other hand, the isoflavonoid irigenin was tested against AChE and BChE. The results showed a moderate activity against BChE (less to 40% of inhibition) (Ślusarczyk et al., 2019). Besides, inhibitory activity of AChE of myristicin have been investigated *in vitro* and *in vivo* experiments. The enzymatic results indicate that myristicin caused competitive and noncompetitive inhibition (Jaiswal et al., 2010). Finally, the liminoid calodrendolide showed neuroprotective effects against glutamate-induced neurotoxicity in rat cortical cells (Jeong et al., 2008).

This result suggests that infusion from *A. copa* could be explored as an alternative medicine for AD.

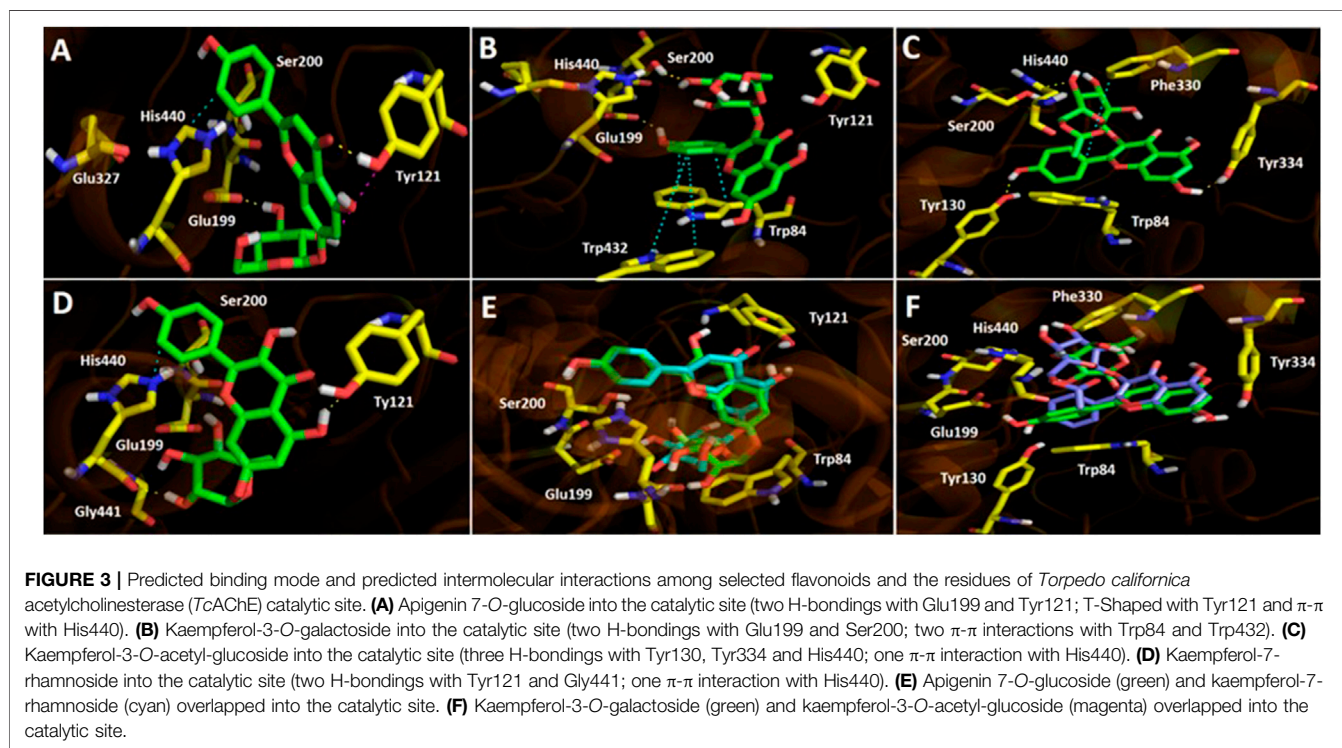
Molecular Docking Studies

To get insights on the intermolecular interactions, four flavonoids contained in the *Artemisia copa* infusion: apigenin-7-O-glucoside, kaempferol-3-O-galactoside, kaempferol-3-O-acetyl-glucoside and kaempferol-7-rhamnoside (which are in high amount and proportion in this species), as well as the known cholinesterase inhibitor galantamine (**Supplementary Figure S2**), were selected and subjected to molecular docking studies over AChE (*TcAChE*) and BChE (*hBChE*) (Full description, **Supplementary Material**). The relationship between flavonoids and their potential as cholinesterase inhibitors have been reported (Sevindik et al., 2015; Khan et al., 2018). **Table 4** shows the binding energies expressed in kcal/mol of every compound mentioned above.

In *TcAChE* the binding energy descriptors for flavonoids kaempferol-3-O-galactoside and kaempferol-3-O-acetyl-glucoside turned out to be better than for flavonoids apigenin-7-O-glucoside and kaempferol-7-rhamnoside. Apigenin-7-O-glucoside, as well as kaempferol-7-rhamnoside displayed deficient binding energies of 9.76 kcal/mol and 12.66 kcal/mol respectively, compared to -2.04 kcal/mol of kaempferol-3-O-galactoside and -1.27 kcal/mol of kaempferol-3-O-acetyl-glucoside, suggesting that the inhibitory activity over the enzyme lies predominantly in the first two flavonoids mentioned above. This could be explained due flavonoids kaempferol-3-O-galactoside and kaempferol-3-O-acetyl-glucoside are arranged in similar manners into the catalytic site of the enzyme. In the same way, apigenin-7-O-glucoside and kaempferol-7-rhamnoside are also closely overlapped between them. Therefore, both pairs of compounds carry out different interactions with the amino acids, although derivatives kaempferol-3-O-galactoside and apigenin-7-O-glucoside performed hydrogen bond interactions with the amino acid Glu199 (**Figure 3**). The differences in the abilities to inhibit the enzyme and the differences in the binding energies of the four flavonoids, must be due because of the greatest amount of

TABLE 4 | Binding energies obtained from docking experiments of selected flavonoids and Galantamine over cholinesterases *TcAChE* and *hBChE*.

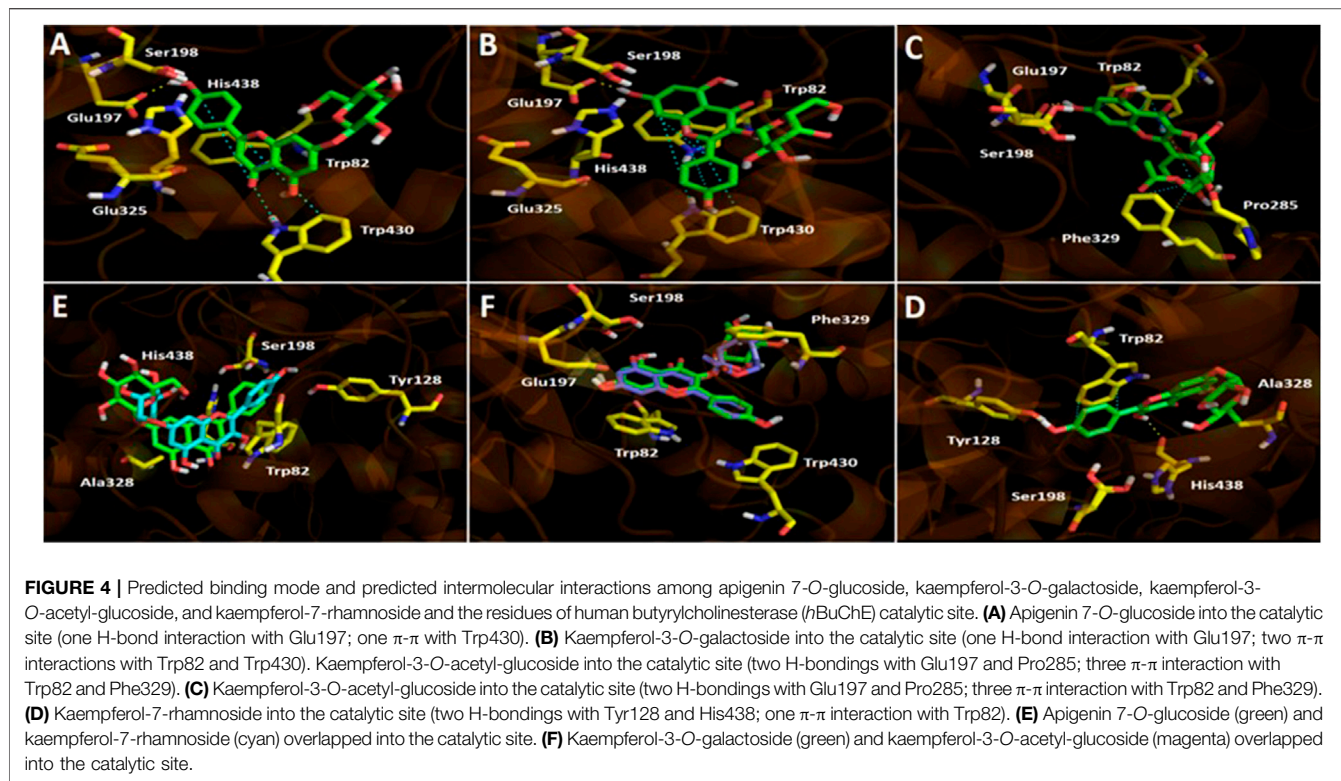
Compounds	Binding energy (kcal/mol) Acetylcholinesterase (<i>TcAChE</i>)	Binding energy (kcal/mol) Butyrylcholinesterase (<i>hBChE</i>)
Apigenin-7- <i>O</i> -glucoside	9.76	-5.93
Kaempferol-3- <i>O</i> -galactoside	-2.04	-8.92
Kaempferol-3- <i>O</i> -acetyl-glucoside	-1.27	-8.36
Kaempferol-7-rhamnoside	12.66	-3.38
Galantamine	-11.81	-9.50



hydrogen bond interactions performed by kaempferol-3-*O*-galactoside and kaempferol-3-*O*-acetyl-glucoside, as well as the π - π interactions (especially compared to T-shaped interaction in apigenin-7-*O*-glucoside and only one π - π interaction in kaempferol-7-rhamnoside) performed by the phenyl ring of the 4*H*-chromen-4-one in kaempferol-3-*O*-galactoside with the indole rings of Trp84 and Trp432 and with the same phenyl moiety in kaempferol-3-*O*-acetyl-glucoside and Phe330 of the *TcAChE*. The hydrogen bonding and the π - π interactions mentioned above must be stronger in kaempferol-3-*O*-galactoside and kaempferol-3-*O*-acetyl-glucoside than those for apigenin-7-*O*-glucoside and kaempferol-7-rhamnoside. For example, Trp bears electron-deficient aromatic rings, whereas the phenyl ring of kaempferol-3-*O*-galactoside possesses an electron-donating hydroxyl group (-OH) causing a stronger interaction than apigenin-7-*O*-glucoside.

Docking results on *hBChE* for the four flavonoids described above, behaved similarly than in *TcAChE*. The binding energies of kaempferol-3-*O*-galactoside and kaempferol-3-*O*-acetyl-

glucoside showed to be better (-8.92 and -8.36 kcal/mol respectively) compared to apigenin-7-*O*-glucoside and kaempferol-7-rhamnoside (-5.93 and -3.38 kcal/mol), suggesting once again that the inhibitory activity over *hBChE* lies predominantly in those derivatives which bear the sugar moieties at position 3- of the 4*H*-chromen-4-one core. The binding modes of compounds into the catalytic site of *hBChE* were different this time related to results seen in *TcAChE*. Kaempferol-3-*O*-galactoside and kaempferol-3-*O*-acetyl-glucoside are completely overlapped, as well as apigenin-7-*O*-glucoside and kaempferol-7-rhamnoside are arranged in the same manner with a slightly difference. This feature makes that the 4*H*-chromen-4-one moieties of the first two flavonoids to be in opposite positions related to the second ones (**Figure 4**). Binding energies for *hBChE* turned out to be better than for *TcAChE* in docking experiments, what is partially divergent with our experimental inhibition assays, where the *A. copa* infusion showed an $IC_{50} = 3.92 \pm 0.09 \mu\text{g/ml}$ for *TcAChE* and an $IC_{50} = 44.13 \pm 0.10 \mu\text{g/ml}$ for *hBChE*.



Antiprotozoal Activity Evaluation.

To assess the potential activity from *Artemisia copa* infusion, we have performed a screening test against *Trypanosoma cruzi* and *Leishmania amazonensis*. Protozoan parasites are a significant cause of infectious diseases with high morbidity and mortality. Besides, Chagas and Leishmaniasis diseases have a great impact on developing countries, and no new treatments have been developed in the last 40 years (Costa et al., 2018). *Artemisia copa* showed trypanocidal activity against *Trypanosoma cruzi* with a LD_{50} of 131.8 μ g/ml and against *Leishmania amazonensis* with a LD_{50} of 457 μ g/ml (Supplementary Table S2, Supplementary Figure S3, Supplementary Material). Artemisinic acid is a sesquiterpene isolated from *A. annua* and a very important precursor for the production of artemisinin (Kong et al., 2013). Moreover, the antiprotozoal properties of artemisinin derivatives have been reviewed in detail (Loo et al., 2017). Asteraceae family plants play an important contribution to the development of new trypanocidal and leishmanicidal agents (Moraes Neto et al., 2019). The *Artemisia* genus has been pointed out as interesting antiprotozoal agents (Emami et al., 2012). The essential oils from *Artemisia campestris* L. and *Artemisia herba-alba* showed IC_{50} of 44 and 68 μ g/ml against *Leishmania infantum* (Aloui et al., 2016). While, *Artemisia annua* showed IC_{50} of 22 μ g/ml against *Leishmania donovani* (Sen et al., 2007; van der Kooy and Sullivan, 2013). The results of *in vitro* studies indicate that the infusion from *Artemisia copa* displayed low trypanocidal activity against *Trypanosoma cruzi* and very low

activity against *Leishmania amazonensis*. The antiprotozoal properties of the flavonoid eupatorin isolated from the genus *Stevia* (Asteraceae) was investigated. Eupatorin displayed activity against *Tripanozoma cruzi* epimastigotes and trypomastigotes. Additionally, the activity against *Leishmania braziliensis* and the cytotoxicity on Vero cells were evaluated (Beer et al., 2016). The antiprotozoal properties of 7-demethylsuberosin isolated from the bark of *Citropsis articulata* (Willd. ex Spreng.) Swingle and M.Kellerm. (Rutaceae) was reported against *Plasmodium falciparum* (Lacroix et al., 2011). The antiprotozoal properties of the infusion of *A. copa* has not been previously reported and additional studies are necessary to explore the antiprotozoal activities.

CONCLUSION

The potential antioxidant, antiprotozoal, and cholinesterase inhibition activity plus the chemical profiling of *Artemisia copa* infusion were assessed. UHPLC-MS were used for the first time to detected 47 compounds in the infusion of the Altiplano plant, and to the best of our knowledge, is the first investigation on the cholinesterase inhibition activity. Our docking studies suggest that the inhibitory activity over the enzymes lies predominantly on kaempferol-3-O-galactoside and kaempferol-3-O-acetyl-glucoside. *A. copa* could be recommended as a supplementary beverage with cholinesterase inhibition potentials. The antiprotozoal evaluation showed no significant results against *T. cruzi* and *L. amazonensis*. However, should be

considered that activity was evaluated in extracts where the potential active product(s) against these protozoa would be highly diluted. This study may contribute to a better understanding of the chemistry and biological activity of this plant.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

MS, JG, MJL-F, JE, and C F-G wrote and revised the manuscript, JR-P, MJL-F, AG, and AP analyzed the data, AP, and JP-R performed the antioxidant assays, JG and KS performed the antiprotozoal assays.

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