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Research Article

Chemical Composition and Antioxidant Activity of *Aloe vera* from the Pica Oasis (Tarapacá, Chile) by UHPLC-Q/Orbitrap/MS/MS

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The chemical composition of *Aloe vera* growing in the small town of San Andrés de Pica, an oasis of warm waters and typical fruits, located in Tamarugal province in the Northern Chilean region of Tarapacá is reported. The chemical characterization was performed using liquid chromatography (UHPLC) coupled to PDA and high-resolution mass spectrometry (HESI-Q-Orbitrap®-MS) in four different plant parts of *Aloe* (peel, flowers, gel, and roots). Twenty-five phenolic compounds were identified, including cinnamic acids and other derivatives (e.g., caffeic and chlorogenic acids), chromones (e.g., aloesin and isoaloeresin D), anthracene compounds and derivatives (e.g., aloin A/B and emodin), and several C-flavonoids (e.g., orientin and isovitexin), among others. Total antioxidant activity of the ethanolic extracts of the peels, flowers, gel, and roots was measured as the capturing of the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals, while the iron-reducing antioxidant power (FRAP) was measured by spectroscopic methods. The peel had the highest antioxidant activity with values of 2.43 mM ET/g MF ($DPPH^{\bullet}$), 34.32 mM ET/g MF ($ABTS^{\bullet+}$), and 3.82 mM ET/g MF (FRAP). According to our results, the peel is the best part of the plant for the production of nutraceuticals or cosmetics products for its greatest number of bioactive compounds. This is a new and innovative finding since the only part used in traditional medicine is the gel of *Aloe*, and the peel is generally considered waste and discarded.

1. Introduction

In Tarapacá Region (I region, Northern Chile), a remote part of the Atacama Desert, there is a small town and oasis called San José de Pica. Pica has a lush greenery and thriving agriculture due to underground water sources surfacing in the middle of the desert [1]. This desert is one of the driest places on Earth resulting in extreme environmental conditions. These varying abiotic conditions, as seasonal fluctuations of chemical and physical water composition (e.g., nutrients, temperature, and salinity), are challenging for the biota and affect the species assemblages and ecosystem stability [2].

The different secondary metabolites produced by the plants are influenced by environmental conditions such as extreme light, water, soils, salts, chemicals, temperature, and geographical variations [3].

Environmental factors such as light intensity, temperature, water availability, type, and soil composition among others, have a substantial influence on the quality and productivity of medicinal plants. Plants of the same species occurring in different condition environments may differ significantly in their content of secondary metabolites [4].

Furthermore, the chemical composition of any plant depends upon the local geographical condition, type of soil, and its composition. For example, it has been reported that

the chemical composition and yield of the essential oil of *Mentha piperita* var., grown under different agroecological locations in Egypt, vary significantly according to the climate conditions. Plants growing in high temperatures showed high menthone/menthol contents and high antioxidant activity that could be attributed to their high number of phenolic compounds and flavonoids compared to other locations [5].

Aloe vera (*Aloe barbadensis* Miller) is a native species to South Africa, which has been widely distributed in the continent of Europe from where they have spread to almost the entire world [6]. This plant is also extensively distributed in South America [6, 7], where it is known for its therapeutic effects. *A. vera* has been studied for its clinical effectiveness against a great variety of affections and disorders of the skin [8], for example, wounds and burns [6, 9–11], for its effect as anti-inflammatory, antioxidant, healing, and antibacterial; these actions are biologically attributed to its chemical components [8]. The part of the plant that has been usually used for therapeutic purposes is the gel [6, 9]. Traditionally, *A. vera* gel has been used both externally for the treatment of wounds, minor burns, and irritations of the skin, and internally in different formats to treat constipation, cough, ulcers, diabetes, and headaches among others [12]. Regarding its chemical composition, *A. vera* gel consists mainly of water (>98%) and polysaccharides, including pectins, cellulose, hemicellulose, glucomannan, and acemannan, the latter being considered as the main functional component of *A. vera* gel, formed from a long chain of acetylated mannose [12]. *Aloe* latex, also known as *Aloe* juice, is a bitter yellow exudate of the pericyclic tubules in the outer skin of the leaf. The main active component of *Aloe* juice are hydroxyanthracenic derivatives, which represent between 15 and 40% of the total components, and among them are anthraquinone glycosides aloin A and B (also called barbaloin) along with *Aloe* emodin [12]. *A. vera* flowers have received little attention, although there are some studies that suggest the use of these flowers for phytotherapeutic purposes, due to the presence of several phenolic compounds such as caffeic acid, chlorogenic acid, and ferulic acid among others. The compound mannose-6 phosphate, which is a constituent of the sugar of *A. vera* gel, has been shown to have wound-healing properties as well. In addition, some glycoproteins present in the *A. vera* gel have antitumor and antiulcer effects and may increase the proliferation of normal human skin cells [12]. In the case of the root, some phenolic compounds, especially naphthoquinones and anthraquinones, have also been identified [13–15].

It is well known that the types and levels of the chemical components present in the plants can vary according to the geographical origin or variety; although there are some chemical characterization studies of *A. vera* from other countries, we were not able to find reports on the chemical composition of *A. vera* from the Chilean region of Tarapaca. In Chile, the studies carried out in *A. vera* have been scarce, focusing mainly on the farming conditions, the effect of high hydrostatic pressures (HHPs) on rheological properties [16], the effect of HHPs on functional properties and characteristics of the quality of *Aloe* gel [17], and the microbiological stabilization of *A. vera* gel using the treatment of HHP [18].

Moreover, other studies about the influence of temperature on drying kinetics, physicochemical properties, and antioxidant capacity of *Aloe* gel [19], plus the effect of temperature in the structural properties were also published [20]; however, studies covering the chemical composition of *A. vera* growing in extreme climatic conditions like the one growing in the region of Tarapacá have never been reported.

Several studies have shown that climatic conditions cause plants to develop metabolites that help in their survival; thus, species growing in extreme conditions, such as the Atacama Desert, can develop interesting metabolites to be studied. In this scenario, the use of state-of-the-art tools such as metabolomic mass fingerprinting can help to study the metabolomic processes in extreme systems like the one occurring among the "biodiversity of the Atacama Desert." For a complete chemical characterization of *A. vera* from Tarapaca Region, we used high-resolution hyphenated LC-MS (UHPLC-MS) techniques whose advantage is the rapid separation of compounds and the most accurate determination of the masses [21]. This technique is considered gold standard for the analysis of phenolic compounds, due to its versatility, precision, and relatively low cost [22]. The UHPLC machine can be coupled to several mass spectrometers, such as time-of-flight (TOF or Q-TOF), quadrupole-Orbitrap (Q-OT), or triple quadrupole (TQ) mass spectrometers. The Orbitrap is an ion trap mass analyzer that consists of a high-resolution hybrid mass spectrometer, which has recently been published as an innovative technology that offers high-resolution MS/MS fragments, for metabolomic analysis of a variety of metabolites, including toxins, pesticides, antibiotics, peptides, and several small organic molecules up to 2000 Daltons [21].

Based on this background, we have studied the chemical composition of *A. vera* from Tarapacá Region, given the geographic conditions and the possible influence on the secondary metabolites present in the species. The phenolic compounds of each part of *A. vera* were characterized using UHPLC-Q/Orbitrap/MS/MS, and the chemical composition was related with the antioxidant activity.

2. Materials and Methods

2.1. Chemicals and Plant Material. Folin-Ciocalteu phenol reagent (2 N), reagent-grade Na_2CO_3, HCl, $NaNO_2$, NaOH, $FeCl_3$, $AlCl_3$, quercetin, trichloroacetic acid, sodium acetate, HPLC-grade water, lichrosolv HPLC-grade acetonitrile, MeOH, reagent-grade MeOH, formic acid, CH_3COOH, CH_3COONa, potassium persulfate, and ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] were obtained from Merck (Darmstadt, Germany). Gallic acid, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), trolox, *tert*-butyl hydroperoxide, nitroblue tetrazolium, xanthine oxidase, and DPPH (1,1-diphenyl-2-picrylhydrazyl radical) were purchased from Sigma-Aldrich Chemical Co.

A. vera plant was collected in March 2016, from the sector La Concova in Pica, Tarapaca Region, Chile (latitude: −20.48612; longitude: −69.318967; 1379 metres above the sea

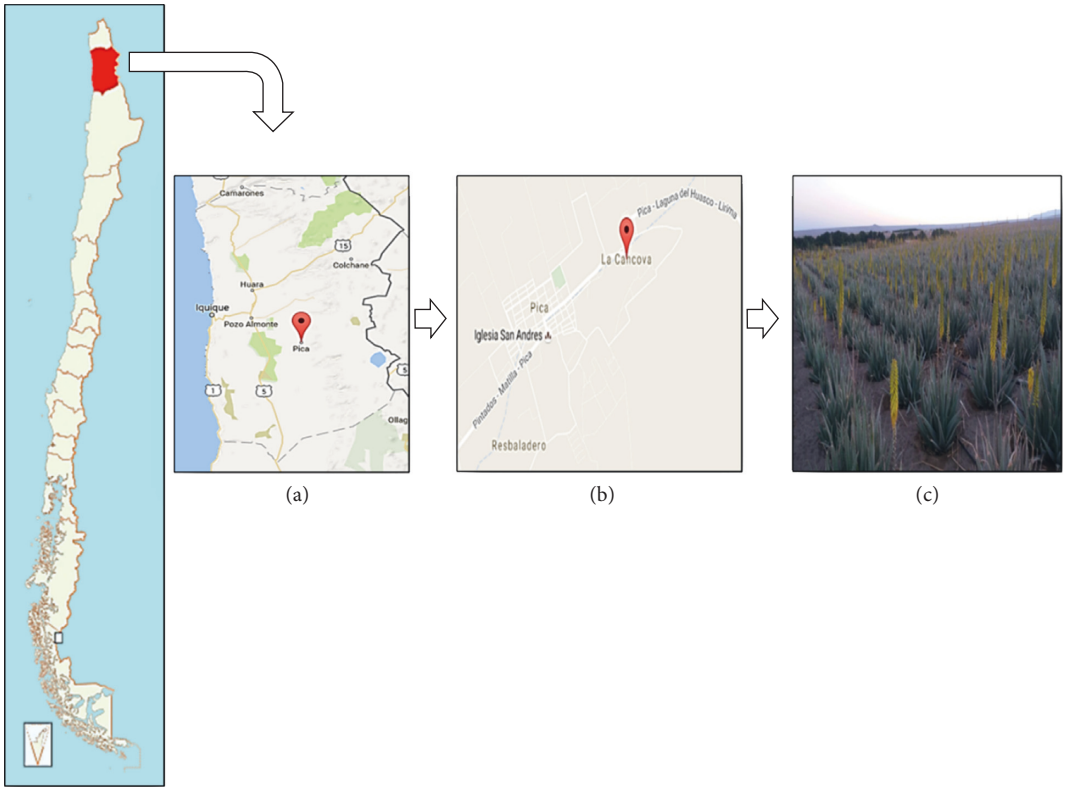


Figure 1: Map of Chile showing the location of Tarapaca Region: commune of Pica (a), La Concova sector (b), and the *A. vera* crops (c).

level) (Figure 1). The plant was identified by the botanist Alicia Marticorena, and voucher herbarium specimens are kept at the Natural Products Laboratory of the University of La Serena under reference number 14014.

2.2. Instrumentation. An ultrasonic bath Branson 3510, a vacuum filtration equipment Medi-Pump model N° 1136-THOMAS, a Rotavapor Laborota 4001, a −86°C Ultralow Temperature Freezer-Gene Xpress, a Labconco-Freezone 6-Plus Liophylizer, a Merck Spectroquant Pharo 300 spectrophotometer, a Agilent 1260 Infinity LC Quaternary high-performance liquid chromatography (HPLC) system, and a Thermo Scientific Dionex 3000 Ultrahigh-performance liquid chromatography (UHPLC, Thermo Fisher Scientific, Bremen, Germany) system with a modern PDA detector and quadrupole hybrid high-resolution mass spectrometer-Orbitrap Q Exactive® Focus were employed, using a quaternary Series RS pump and TCC-3000RS column compartments with a WPS-3000RS autosampler plus a rapid separations PDA detector controlled by Chromeleon 7.2 and Xcalibur 2.3. The chromatographic system was coupled to the MS with a heated electrospray ionization source II (HESI II). Nitrogen (purity >99.999%) obtained from a Genius NM32LA nitrogen generator (Peak Scientific, Billerica, MA, USA) was employed as both the collision and damping gas. Mass calibration for the Orbitrap was performed in both negative and positive modes, to ensure a working mass accuracy lower than or equal to 5 ppm. The calibration was done once a week. For calibration of the mass spectrometer, caffeine, N-butylamine, buspirone hydrochloride, sodium dodecyl sulfate and taurocholic acid (Sigma-Aldrich, St Louis, Missouri, USA) besides Ultramark 1621 (Alfa Aesar, Heysam, UK), were used as standards. The softwares Q Exactive 2.0 SP 2, XCalibur 2.3, and Trace Finder 3.2 (Thermo Fisher Scientific and Dionex Softron GmbH) were used to control the mass spectrometer and for data processing. For UHPLC-mass spectrometer control and data processing, Q Exactive 2.0 SP 2, XCalibur 2.3, and Trace Finder 3.2 software (Thermo Fisher Scientific and Dionex Softron GmbH Part of Thermo Fisher Scientific) were used, respectively [21].

2.3. LC Parameters. A portion of each extract (2.5 mg) obtained as explained above was dissolved in 1 mL of 1% formic acid in MeOH, filtered through a 0.45 μm micropore membrane (PTFE, Waters Milford, MA, USA) before use. Later, it was injected into the UHPLC-PDA and ESI-Orbitrap-MS equipment. The parameters of liquid chromatography were those previously published by our research group [21].

2.4. MS Parameters. The HESI parameters were optimized as reported previously [21]. Detection was based on calculated exact mass and on retention time of target compounds, presented in Table 1.

2.5. Sample Preparation. The *Aloe* leaves were carefully cut and washed, leaving the leaf upright to drain the exudate and

Table 1: Tentative identification of phenolic compounds in peel, flower, gel, and root of *A. vera* from Tarapaca Region detected by UHPLC-Q-OT-MS.

Peak no.	Tentative identification	Elemental composition $[M-H]^-$	Retention time (min)	Theoretical mass (m/z)	Measured mass (m/z)	Error (ppm)	Other ions (m/z)	Classification of the compound
1	Aloesin or aloeresin B	$C_{19}H_{21}O_9^-$	9.23	393.11911	393.11917	−0.15263	203.07106 ($C_{12}H_{11}O_3^-$) 245.08168 ($C_{14}H_{13}O_4^-$)	Chromone (C-glycosylated chromone)
2	Chlorogenic acid	$C_{16}H_{17}O_9^-$	9.54	353.08781	353.08752	0.82133	191.05534 ($C_7H_{11}O_6^-$)	Cinnamic acid
3	Caffeic acid	$C_9H_7O_4^-$	9.92	179.03498	179.03464	1.89907	135.04451 ($C_8H_7O_2^-$)	Cinnamic acid
4	*Aloe* emodin-diglucoside	$C_{27}H_{29}O_{15}^-$	9.98	593.15119	593.15063	0.94411	—	Anthracene compound (anthrone)
5	Isoquercitrin	$C_{21}H_{19}O_{12}^-$	9.98	463.08820	463.08752	1.46840	301.55185 (M-glucose)	Flavonoid (O-glycosylated flavonoid)
6	Kaempferol-3-*O*-hexosyl-*O*-pentoside	$C_{26}H_{27}O_{15}^-$	10.29	579.13554	579.13513	0.70795	—	Flavonoid (O-glycosylated flavonoid)
7	6-Methyl-1,3,8-trihydroxyanthraquinone (emodin)	$C_{15}H_9O_5^-$	10.40	269.04555	269.04538	0.63186	225.05438 ($C_{14}H_9O_3^-$) 241.73468	Anthracene compound (anthrone)
8	Luteolin-8-*C*-glucoside (orientin)	$C_{21}H_{19}O_{11}^-$	10.41	447.09329	447.09293	0.80520	327.05048 ($C_{17}H_{11}O_7^-$) 299.05582 ($C_{16}H_{11}O_6^-$)	Flavonoid (C-glycosylated flavonoid)
9	Feruloylquinic acid	$C_{17}H_{19}O_9^-$	10.46	367.10346	367.10309	1.00789	191.05547 ($C_7H_{11}O_6^-$)	Cinnamic acid
10	10-Hydroxyaloin A	$C_{21}H_{21}O_{10}^-$	10.90	433.11402	433.11392	0.23089	270.05280 ($C_{15}H_{10}O_5^-$)	Anthracene compound (anthrone)
11	Isovitexin	$C_{21}H_{19}O_{10}^-$	10.80	431.09837	431.09833	0.09279	311.05588 ($C_{17}H_{11}O_6^-$) 283.06058 ($C_{16}H_{11}O_5^-$)	Flavonoid (C-glycosylated flavonoid)
12	Chrysoeriol-7-*O*-glucuronide	$C_{22}H_{19}O_{12}^-$	11.11	475.08820	475.08856	−0.75775	—	Flavonoid (O-glycosylated flavonoid)
13	Caffeoyl ester of aloesin	$C_{29}H_{29}O_{12}^-$	11.74	569.16645	569.16626	0.33382	—	Chromone
14	Aloin A	$C_{21}H_{21}O_9^-$	12.18	417.11911	417.11908	0.07192	297.07687 ($C_{17}H_{13}O_5^-$) 268.07318 ($C_{16}H_{12}O_4^-$)	Anthracene compound (anthrone)
15	Isoaloeresin D	$C_{29}H_{31}O_{11}^-$	11.85	555.18719	555.18677	0.75650	—	Chromone
16	2′-*p*-Methoxycoumaroylaloeresin B	$C_{29}H_{29}O_{11}^-$	12.29	553.17154	553.17041	2.04277	—	Anthracene compound (anthrone)
17	4,5-Dimethyl ether of *Aloe* emodin	$C_{17}H_{13}O_5^-$	12.29	297.07685	297.07669	0.53858	—	Anthracene compound (anthrone)
18	Aloin B	$C_{21}H_{21}O_9^-$	12.36	417.11911	417.11893	0.43153	297.07669 ($C_{17}H_{13}O_5^-$) 268.07419 ($C_{16}H_{12}O_4^-$)	Anthracene compound (anthrone)
19	6′-Malonylnataloin	$C_{24}H_{23}O_{12}^-$	12.69	503.11950	503.11911	0.77516	459.12842 ($C_{23}H_{23}O_{10}^-$)	Anthracene compound (C-glycosylated anthrone)

Table 1: Continued.

Peak no.	Tentative identification	Elemental composition $[M-H]^-$	Retention time (min)	Theoretical mass (m/z)	Measured mass (m/z)	Error (ppm)	Other ions (m/z)	Classification of the compound
20	Naringenin-4′-methoxy-7-*O*-glucuronide	$C_{22}H_{21}O_{10}^-$	14.39	445.11402	445.11401	0.02247	—	Flavonoid
21	7-Methylether of 2′-feruloylaloesin	$C_{30}H_{31}O_{12}^-$	13.40	583.18210	583.18146	1.09743	—	Chromone
22	*Aloe* emodin-8-*O*-glucoside	$C_{21}H_{19}O_{10}^-$	14.52	431.09837	431.09793	1.02065	269.04495 ($C_{15}H_9O_5^-$)	Anthracene compound (anthrone)
23	5,3′-Dihydroxy-6,7,4′-trimethoxyflavone (eupatorin)	$C_{18}H_{15}O_7^-$	14.81	343.08233	343.08231	0.05830	—	Flavonoid
24	Trihydroxy octadecenoic acid	$C_{18}H_{33}O_5^-$	18.28	329.23335	329.23328	0.21262	—	Oxilipin
25	3,4-Di-*O*-caffeoylquinic acid	$C_{25}H_{23}O_{12}^-$	19.04	515.11950	515.11847	1.99954	—	Cinnamic acid

soaking it in distilled water overnight. Later, the peeled leaf and the gel inside was cut into pieces, soaked in water for half an hour, then passed through a strainer, and then liquefied. *A. vera* peel was also cut into small pieces and washed with distilled water. The roots were carefully cut into small pieces and washed with distilled water. Flowers were provided by the company "Mundo *Aloe vera*" from Pica, Tarapaca Region, and dried at room temperature (25–30°C).

2.5.1. Phenolic Compounds Extraction. Each part of *Aloe* obtained (peels, gel, roots, and flowers; previously weighed) were macerated with methanol for 48 hours (sample: methanol; 1 : 2 (w : w)) then sonicated for 30 minutes in a Branson 3510 ultrasonic apparatus. The extracts from each sample were combined, filtered, and evaporated in vacuo in the dark (40°C) in a rotavapor (Laborota 4001-efficient). The methanolic extracts were maintained at −86°C in an ultralow freezer for 24 hours and then freeze-dried in a Labconco-Freezone 6-Plus equipment. The extracts were suspended in 20 mL ultrapure water and loaded onto an XAD-7 (100 g) column. The column was rinsed with water (100 mL), and phenolic compounds were eluted with 100 mL of MeOH acidified with 0.1% HCl. The solutions were combined and evaporated to dryness under reduced pressure (40°C) to give dark-brown extracts from peels, gel, roots, and flowers. Samples were then analyzed by HPLC using the Agilent 1260 Infinity and by UHPLC using the Thermo machine coupled to the PDA detector Thermo Q Exactive Focus mass spectrometer.

2.6. Antioxidant Assays. The antioxidant activity of the peels, flowers, gel, and roots was determined by the following methods: DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] and FRAP (antioxidant potential of iron reduction), which are detailed below.

2.6.1. DPPH Assay. The $DPPH^\bullet$ radical decoloration activity of the *A. vera* extracts was determined using the DPPH solution methanol, following the modification method of Sogi et al. [23]. A portion of the DPPH stock solution (0.24 g/100 mL methanol) was diluted into 10 parts methanol at 80% (4 : 1 ratio of methanol and water, respectively) so that the working solution obtained an absorbance of 1.10 ± 0.02 at 515 nm. 3 mL of the working solution of DPPH was mixed with 0.6 mL of blank, standard, or sample, kept in the dark for 20 minutes, and the absorbance was recorded at 515 nm. Methanol at 80% (control) was used to calculate the radical decoloration activity of a standard curve, which was prepared with trolox solution (50–250 μM, R^2: 0.9905). Samples were analyzed in triplicate, and the results are expressed in units equivalent to trolox (ET), mM ET/g fresh weight (FW).

2.6.2. ABTS Assay. The $ABTS^{\bullet+}$ antioxidant activity of the extracts was carried out using the $ABTS^{\bullet+}$ radical cation discoloration test as described in Reference [23], with some modifications. The solution of 7 mM ABTS and 2.45 mM potassium persulfate was mixed in a 1 : 1 ratio, and the solution was allowed to stand in the dark for 12–16 hours to produce the $ABTS^{\bullet+}$ cation radical solution. The stock solution was then diluted ten times, with an 80% methanol solution, to reach the absorbance of 0.700 ± 0.020 at 734 nm. 3 mL of the $ABTS^{\bullet+}$ stock solution was mixed with 30 μL of blank, standard, or sample, and after 6 min, the absorbance at 734 nm was measured using a spectrophotometer. As a blank, 80% methanol was used, and the quantification was performed using a standard calibration curve of trolox antioxidant (0.30–1.5 mM, R^2: 0.9886). The samples were analyzed in triplicate, and the results were expressed in mM ET/grams of fresh mass (FM).

2.6.3. Ferric Reducing Antioxidant Power (FRAP) Assay. The determination of ferric reducing antioxidant power or ferric reducing ability (FRAP assay) of the extracts was performed as described by Sogi et al. [23], with some modifications. The stock solutions prepared were 10 mM TPTZ (2,4,6-tri (2-pyridyl)-s-triazine) solution in 40 mM HCl, 300 mM acetate buffer (pH 3.6), and 20 mM $FeCl_3{\cdot}6H_2O$ solution. The solution using in this assay was prepared with mixed buffer acetate, TPTZ solution, and

ferric chloride solution at a proportion of 10 : 1 : 1 (v : v : v), respectively. Plant extracts, standards, or methanolic trolox solutions (300 μL) were incubated at 37°C with 3 mL of the FRAP solution (prepared by mixing 25 mL acetate buffer, 5 mL TPTZ solution, and 10 mL $FeCl_3 \cdot 6H_2O$ solution) for 30 min in the dark. Absorbance of the blue ferrous tripyridyltriazine complex formed was then read at 595 nm. Quantification was performed using a standard calibration curve of antioxidant trolox (from 50 to 250 μM, R^2: 0.995). Samples were analyzed in triplicate and results are expressed in mM ET/g fresh weight (FW).

2.7. Analysis of Data. The statistical analysis was carried out using the SPSS program version 20. The determination was repeated at least three times for each sample solution. Analysis of variance was performed using ANOVA. Significant differences between means were determined by Tukey's comparison test (p values < 0.005 were regarded as significant).

3. Results and Discussion

3.1. Yield Percentage. The peels, flowers, gel, and roots were extracted three times ($n = 3$) with MeOH, and phenolics were retained on Amberlite XAD-7 to obtain the phenolic-enriched extract (PEE). The highest PEE was obtained from the peels (16.2%), while the extraction yields for flowers, gel, and roots were 12.6, 12.3, and 8.5%, respectively.

3.2. Antioxidant Activity Quantification. Table 2 shows antioxidant activity of the four methanolic extracts from several parts of *A. vera* using different methods for the antioxidant capacity. Three antioxidant assays were used to evaluate the antioxidant capacity of the samples, based on chemical aspects for the measurements of radical scavenging activity ($DPPH^\bullet$ and $ABTS^{\bullet+}$) assays and a method based on metals reduction (FRAP).

In the $DPPH^\bullet$ radical trapping capacity assay (Table 2), the extract of the peels showed the greatest antioxidant capacity (2.43 ± 0.14 mM ET/g FM), followed by the extract of the roots (1.43 ± 0.08 mM ET/g MF), then flowers (1.25 ± 0.03 mM ET/g FM), and finally with less antioxidant capacity, the gel extract (0.34 ± 0.01 mM ET/g FM), with a statistically significant difference between them ($p < 0.05$), except between the flower extract and the *Aloe* roots.

The $ABTS^{\bullet+}$ assay (Table 2, column (b)) showed that the peel has the highest antioxidant activity with average values of 34.32 ± 2.60 mM ET/g FM, followed by the roots (17.54 ± 0.77 mM ET/g FM), then the flowers with 16.55 ± 2.30 mM ET/g FM, and finally the *Aloe* gel with 2.06 ± 0.06 mM ET/g FM. There is a statistically significant difference between peel and gel extract ($p < 0.05$). This study allowed the measuring of the antioxidant capacity of polyphenols through the capture of free radicals. The antioxidant activity values obtained by the DPPH method were carried out using the trolox reagent as a standard. In the column (c), the same sample presented the highest activity in the FRAP assay, with values of 3.82 ± 0.23 mM ET/g FM for peel, followed by the root (2.67 ± 0.16), flowers (2.01 ± 0.10), and in the last place, the gel (0.38 ± 0.01 ET/g FM). There was a statistical correlation between the three antioxidant assays ($p < 0.05$).

Although the total phenolic content was not determined, when comparing the chromatographic profiles of the different parts of *Aloe*, under the same chromatographic conditions and sample concentration, we noticed that the greater variety, quantity, and abundance of the chemical compounds were in the peel, and these results correlate to the greater antioxidant activity.

3.3. Identification of Phenolic Compound to A. vera from Pica, Tarapaca Region

3.3.1. Fingerprinting from Phenolic Compounds. The phenolic profiles of PEE were assessed by UHPLC-PDA-QOT/MS (ultrahigh-performance liquid chromatography photo diode array quadrupole Orbitrap mass spectrometry); using the negative heated HESI mass detection mode, phenolic compounds were tentatively identified in the different extracts of *A. vera*. Comparative UHPLC-TIC (total ion current) chromatograms of *A. vera* parts are showed in Figure 2. The retention time (Rt), UV spectral maxima, MS fragmentation, and tentative identification of the compounds are summarized in Table 1.

The methanolic extracts of the peel, flower, gel, and root of *A. vera* retained in XAD-7 was analyzed by HPLC-PDA, to obtain the fingerprint chromatograms for each of the parts. Figure 2 indicates that the peel and the flowers had the greatest abundance of compounds, followed by the roots. The composition of the gel is scarce in phenolic compounds; thus, we could state differences in the composition of the organic compounds in each of the parts of the plant.

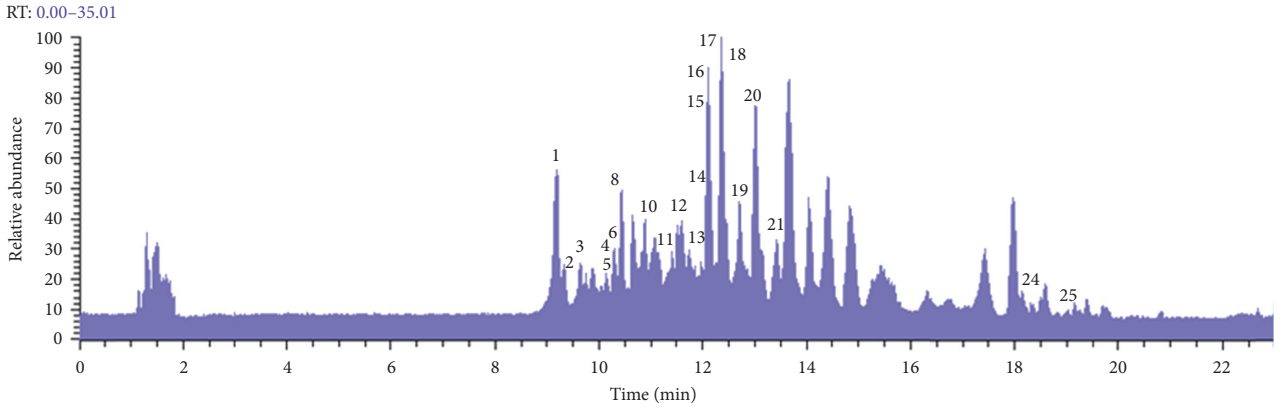
Twenty-five compounds (Table 1) were tentatively identified in different parts of *A. vera*. The highlighted compounds determined include various cinnamic acids and their derivatives, chromones, anthracene compounds, and flavonoids, some of which have been reported previously in *Aloe* species. The identification was performed based on its total mass compared to the theoretical mass (<5 ppm) and the characteristic fragments for each compound, finding differences and similarities between the samples analyzed. Peaks **1**–**6**, **8**, **10**–**21**, and **24**–**25** were detected in the peel, peaks **1**–**6**, **8**–**12**, **14**, **15**, **19**, and **22**–**24** in the flower, peaks **1**, **14**, **15**, **17**, **18**, **20**, **21**, **23**, and **24** in the gel, and peaks **1**–**4**, **6**, **7**, **10**–**15**, **23**, and **24** in the root of *A. vera*, shown in Figure 2. A detailed explanation of the characterization of these compounds, grouped based on their chemical characteristics, is given below.

3.3.2. Cinnamic Acids and Derivatives. Four cinnamic acids and their derivatives were tentatively identified in the negative mode. Peaks **2** and **9** were identified as chlorogenic acid (5-caffeoylquinic acid, $C_{16}H_{17}O_9^-$) and feruloylquinic acid ($C_{17}H_{19}O_9^-$), respectively. Peak **2** shows an $[M\text{-}H]^-$ ion at m/z of 353.08752 amu with retention times of 9.54 min, and peak **5** shows an ion $[M\text{-}H]^-$ at m/z around

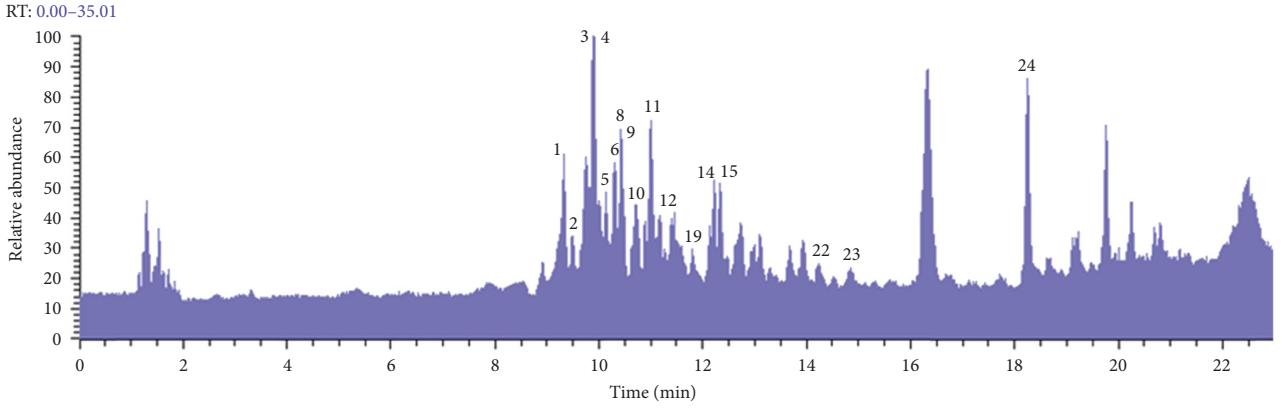
Table 2: Scavenging of the 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{+}$), ferric-reducing antioxidant power (FRAP), and extraction yield of four plant parts of *A. vera*.

Parts from *A. vera*	PEE (%)[a]	$DPPH^{\bullet}$[b]	$ABTS^{\bullet+}$[c]	FRAP[d]
Peel	16.2	2.43 ± 0.14	34.32 ± 2.60	3.82 ± 0.23
Flower	12.6	1.25 ± 0.03[f]	16.55 ± 2.30[e]	2.01 ± 0.10
Gel	12.3	0.34 ± 0.01	2.06 ± 0.06	0.38 ± 0.01
Root	8.5	1.43 ± 0.08[f]	17.54 ± 0.77[e]	2.67 ± 0.16

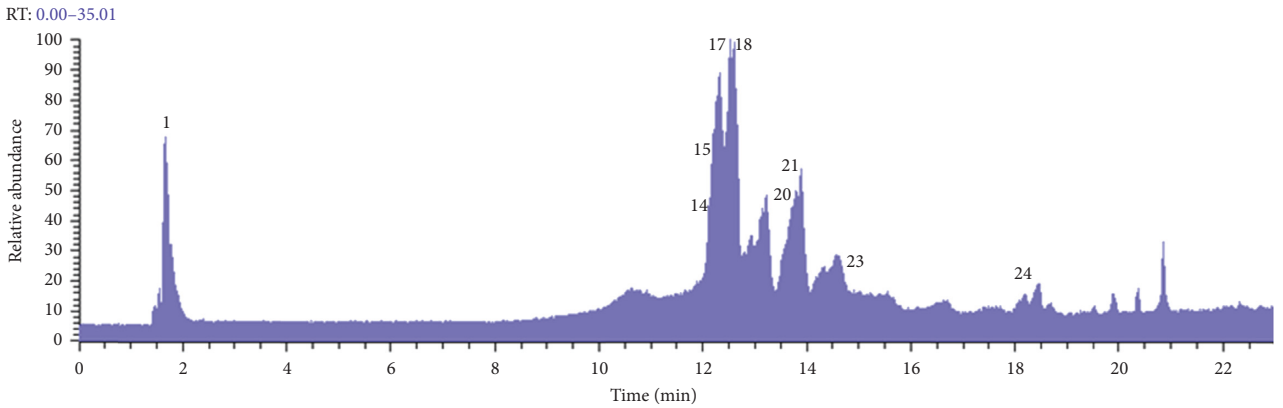
[a]Phenolic-enriched extract expressed in percentage; [b]antiradical DPPH activities are expressed as mM equivalent of trolox/g fresh weight, ; [c]ABTS expressed as mM equivalent of trolox/g fresh weight; [d]FRAP expressed as mM equivalent of trolox/g fresh weight. [e,f]Values are not significantly different (at $p < 0.05$). All measurements were repeated three times ($n = 3$).



(a)

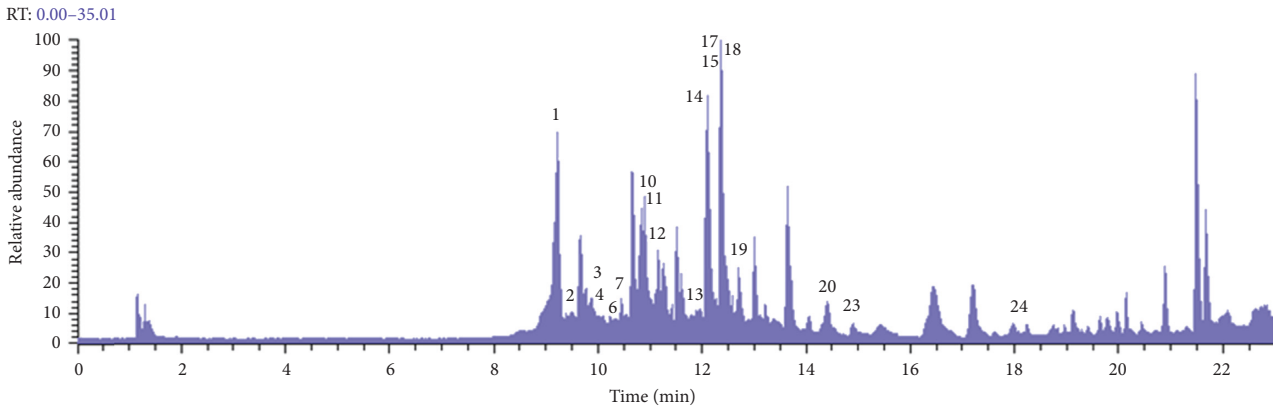


(b)



(c)

Figure 2: Continued.



(d)

Figure 2: UHPLC-chromatograms of (a) peel, (b) flower, (c) gel, and (d) root from Northern Chilean *A. vera*.

367.10309 amu (Rt of 10.46 min). These compounds were tentatively identified by their difference in mass of 0.82133 ppm and 1.00789 ppm with respect to the theoretical mass ion for each of the peaks. Both peaks showed a characteristic ion MS^n at *m/z* 191.05534 amu ($C_7H_{11}O_6^-$) [24, 25]. Peak **3** with a molecular anion at *m/z*: 179.03464 amu was identified as caffeic acid ($C_9H_7O_4^-$) by its difference in mass of 1.89907 ppm with respect to the theoretical mass ion and confirmed by producing an ion MS^n at *m/z* 135.04451 amu ($C_8H_7O_2^-$) due to the loss of a CO_2 molecule from the original ion [24]. Finally, peak **25** with an $[M\text{-}H]^-$ ion at *m/z*: 515.11847 amu was identified as 3,4-di-*O*-caffeoylquinic acid ($C_{25}H_{23}O_{12}^-$), compound identified by its difference in mass of 1.99954 ppm respect to the theoretical mass ion [26]. The presence of chlorogenic acid has been reported in *A. brevifolia* leaves [26] and caffeic acid was reported in leaves of *A. barbadensis* Miller and *A. arborescens* Miller [27]. In the case of 3,4-di-*O*-caffeoylquinic acid, its presence was reported in *A. saponaria* [26], and the feruloylquinic acid had not been reported in any *Aloe* species until this study.

3.3.3. Chromones. Four chromones were tentatively identified (peaks **1**, **13**, **15**, and **21**) using UHPLC-ESI-MS-MS analysis. Peak **1**, with an $[M\text{-}H]^-$ ion at *m/z*: 393.11917 (Rt 9.23 min) was described as aloesin or aloeresin B ($C_{19}H_{21}O_9^-$), recognized by their difference in mass of −0.15263 ppm with respect to the theoretical mass ion and by the identification of two typical fragments of MS^n; *m/z*: 203.07106 amu ($C_{12}H_{11}O_3^-$) and 245.08168 amu ($C_{14}H_{13}O_4^-$) [26]. The aloesin was reported in *A. grandidentata*, *A. perfoliata* [26], *A. ferox* Miller [28], and *A. barbadensis* Miller [29]. Peaks **13**, **15**, and **21** showed $[M\text{-}H]^-$ ions at *m/z* 569.16626 amu (Rt 11.74 min), 555.18677 amu (Rt 11.85 min), and 583.18146 amu (Rt 13.40 min) and were tentatively identified as a caffeoyl ester of aloesin [30], isoaloeresin D, and 7-methylether of 2′-feruloylaloesin [26], respectively. For these peaks, a great accuracy was observed demonstrated by their small differences in ppm (0.33382, 0.75650, and 1.09743, respectively) with respect to the theoretical mass ion [26]. Isoaloeresin D has been reported in *A. eru*, *A. grandidentata*, *A. perfoliata*, *A. brevifolia* [26], and *A. barbadensis* Miller [29]. The compound 7-methylether of 2′-feruloylaloesin was described in *A. eru*, *A. grandidentata*, and *A. saponaria* [26], and caffeoyl ester of aloesin was described in *A. broomii* [30].

3.3.4. Anthracene Compounds. Nine anthracene compounds corresponding to peaks **4**, **7**, **10**, **14**, **16**, **17**, **18**, **19**, and **22** were identified using UHPLC-ESI-MS-MS analysis. Peak **4** was identified as *Aloe* emodin-diglucoside ($C_{27}H_{29}O_{15}^-$), which showed an $[M\text{-}H]^-$ ion at *m/z* 593.15063 amu (Rt 9.98 min). This compound was tentatively identified by its difference in mass of 0.94411 ppm with respect to the theoretical mass ion. This compound was reported in *A. arborences*, *A. grandidentata*, and *A. ferox* [26]. The compound emodin ($C_{15}H_9O_5^-$) was assigned to peak **7** with an $[M\text{-}H]^-$ ion at *m/z* 269.04538 amu (Rt 10.40 min), identified by its difference in mass of 0.63186 ppm with respect to the theoretical mass ion and by the identification of the typical fragments MS^n, 225.05438 amu ($C_{14}H_9O_3^-$) and 241.73468 amu [31]. Peak **10** was identified as 10-hydroxyaloin A, ($C_{21}H_{21}O_{10}^-$, *m/z* 433.11392 amu). The major diagnostic daughter MS^n ion of this compound was at an *m/z* of 270.05280 amu ($C_{15}H_{10}O_5^-$) [29]. The 10-hydroxyaloin A was reported in *A. barbadensis* Miller, *A. grandidentata*, and *A. perfoliata* [26, 29].

Peak **14** was aloin A ($C_{21}H_{21}O_9^-$), which showed an $[M\text{-}H]^-$ ion at *m/z* 417.11908 amu (Rt 12.18 min). This peak was identified by its major diagnostic daughters, MS^n ions at *m/z* 297.07687 amu ($C_{17}H_{13}O_5^-$) and 268.07318 amu ($C_{16}H_{12}O_4^-$) [29]. Figure 3 shows TIC (total ion current, negative mode) and full high-resolution mass spectra showing the UHPLC chromatograms of $[M\text{-}H]^-$ ion and proposed structure of aloin A.

In the same manner, Peak **18** with an $[M\text{-}H]^-$ ion at *m/z* 417.11893 amu was identified as aloin B ($C_{21}H_{21}O_9^-$) isomer to aloin A. This peak was identified by the difference in mass of 0.43153 ppm with respect to the theoretical mass ion and by the identification of two typical ions MS^n; at

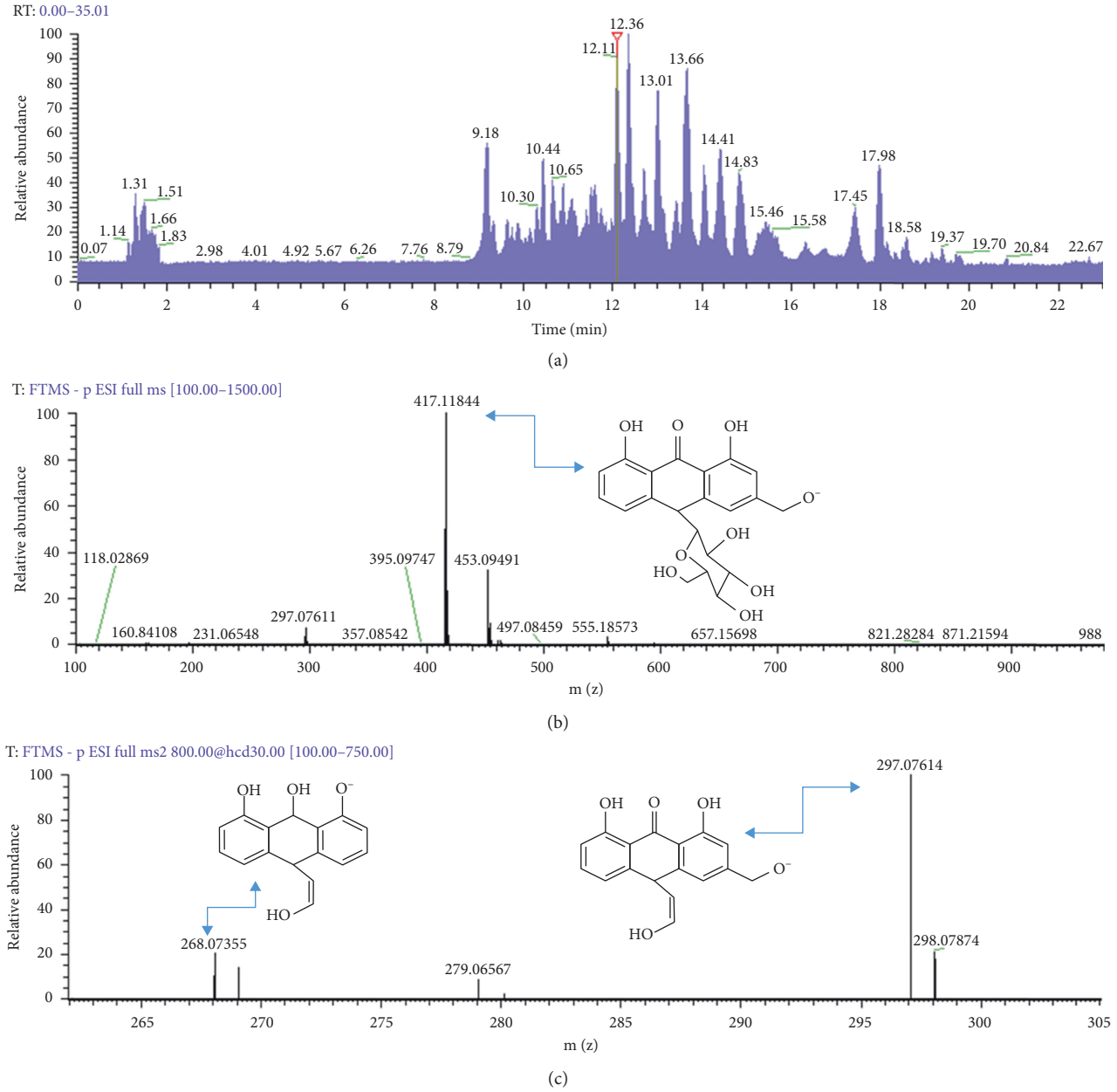


Figure 3: UHPLC chromatograms of *A. vera* peel: (a) TIC (total ion current, negative mode), (b) full high-resolution mass spectra showing the ion aloin A, and (c) fragmentation ions.

297.07669 amu ($C_{17}H_{13}O_5^-$) and 268.07419 amu ($C_{16}H_{12}O_4^-$) [24]; both isomers were confirmed by their characteristic UV max at retention time of 12.18 min for aloin A and 12.36 min for aloin B [32].

Aloin A has been reported in *A. barbadensis* Miller, *A. arborences*, and *A. grandidentata*, while aloin B was reported in *A. barbadensis* Miller and *A. grandidentata* [26]; both aloins (A and B) have also been reported in *A. ferox* Miller [28] and in *A. barbadensis* Miller [29]. Aloin is a mixture of aloin A (also called barbaloin) and aloin B (or isobarbaloin), corresponding to an anthraquinone glycoside to which attributed a characteristic of purgative effects, present in the *Aloe* leaf [26]. According to the International Aloe Science Council, the maximum concentration for human consumption of barbaloin present in derived products of *Aloe* is 10 mg/L [33].

Peaks **16** and **17** were 2′-*p*-methoxycoumaroylaloeresin B ($C_{29}H_{29}O_{11}^-$) and 4,5-dimethyl ether of *Aloe* emodin ($C_{17}H_{13}O_5^-$), which showed an $[M\text{-}H]^-$ ions at *m/z* 553.17041 amu and 297.07669 amu, respectively. These peaks were identified by their difference in mass of 2.04277 ppm and 0.53858 ppm with respect to the theoretical mass ion. The 2′-*p*-methoxy coumaroyl aloeresin B was reported in *A. eru*, *A. perfoliata*, and *A. saponaria* [26]. Mass spectra of peak **19** (Rt 1.69 min) showed $[M\text{-}H]^-$ ion at *m/z* 503.11911 amu, and it was identified as 6′-malonylnataloin (nataloin). This compound was identified by the difference in mass of 0.77516 ppm with respect to the theoretical mass

ion and detection of an MS^n ion at m/z 459.12842 amu ($C_{23}H_{23}O_{10}^-$) product of the loss of a CO_2 molecule [34]. This peak was detected in *A. barbadensis* Miller, *A. arborences*, *A. eru*, *A. grandidentata*, *A. brevifolia, A. ferox* [26], and *A. ellenbeckii* [34].

Aloe emodin-8-*O*-glucoside was detected as peak **22** (Rt 14.52 min) showing $[M-H]^-$ at m/z 431.09793 amu ($C_{21}H_{19}O_{10}^-$). This peak was identified by the difference in mass of 1.02065 ppm with respect to the theoretical mass ion and its daughters MS^n ion at m/z 269.04495 amu ($C_{15}H_9O_5^-$) [26]. Other studies reported the presence of this compound in *A. barbadensis* Miller, *A. arborences*, *A. eru*, *A. perfoliata*, *A. saponaria*, and *A. ferox*, both metabolites being considered as chemotaxonomic markers of the *Aloe* species in one study [26].

3.3.5. Flavonoids. Seven flavonoids were tentatively identified (peaks **5**, **6**, **8**, **11**, **12**, **20**, and **23**) using UHPLC-ESI-MS-MS analyses in the negative mode $[M-H]^-$. Peak **5**, with an $[M-H]^-$ ion at m/z 463.08752 amu, was tentatively classified as isoquercitrin ($C_{21}H_{19}O_{12}^-$) with Rt 9.98 min and their difference in mass of 1.46840 ppm with respect to the theoretical mass ion and its characteristic MS^n ion at m/z 301.55185 amu [24, 35]. This compound was reported in *A. arborences* and *A. eru* [26]. Peak **6** was recognized as kaempferol-3-*O*-hexosyl-*O*-pentoside ($C_{26}H_{27}O_{15}^-$) with an $[M-H]^-$ ion at m/z 579.13513 amu (Rt 10.29 min) with a difference in mass of 0.70795 ppm with respect to the theoretical mass ion. This compound was reported in *A. arborences* and *A. grandidentata* [26]. The retention time 10.41 min showed for peak **8,** identified it as luteolin-8-*C*-glucoside or Orientin ($C_{21}H_{19}O_{11}^-$) with an $[M-H]^-$ ion at m/z 447.09293. Peak 8 was characterized according to the small error of 0.80520 ppm with respect to the theoretical mass ion and its characteristic two ions at MS^n at m/z 327.04977 amu ($C_{17}H_{11}O_7^-$) and 299.05603 amu ($C_{16}H_{11}O_6^-$) [35]. This compound was reported in *A. barbadensis* Miller, *A. arborences*, *A. grandidentata*, *A. perfoliata*, and *A. ferox* [26]. The negative mode ESI-MS spectrum of peak **11** (Rt 10.80 min) showed a strong $[M-H]^-$ parent ion at m/z 431.09833 amu which yielded daughters ions at m/z 311.05588 amu ($C_{17}H_{11}O_6^-$) and 283.06058 amu ($C_{16}H_{11}O_5^-$) [35]. Peak **11** was determined as isovitexin, and this compound was only reported in *A. perfoliata* [26].

Peaks **12** (Rt 11.11 min), **20** (Rt 14.39 min), and **23** (14.81 min) showed $[M-H]^-$ at m/z 475.08856 amu, 445.11401 amu (Rt 14.39 min) and 343.08231 amu. Peaks **12**, **20**, and **23** were identified tentatively as chrysoeriol-7-*O*-glucuronide ($C_{22}H_{19}O_{12}^-$), naringenin-4′-methoxy-7-*O*-glucuronide ($C_{22}H_{21}O_{10}^-$), and 5,3′-dihydroxy-6,7,4′-trimethoxy-flavone (eupatorin) ($C_{18}H_{15}O_7^-$), respectively, due to its great accuracy demonstrated by their small differences in mass −0.75775, 0.02247, and 0.05830 with respect to the theoretical mass ion, respectively. Chrysoeriol-7-*O*-glucuronide was reported in *A. grandidentata* and 5,3′-dihydroxy-6,7,4′-trimethoxy-flavone in *A. arborences*, *A. eru*, *A. grandidentata*, and *A. brevifolia* [26], while naringenin-4′-methoxy-7-*O*-glucuronide had not been reported in any *Aloe* species until this study.

Table 3: Distribution of the phenolic compounds identified tentatively in methanolic extract of peel, flower, gel, and root from Chilean *A. vera*.

Peak no.	Tentative identification	Peel	Flower	Gel	Root
1	Aloesin and aloeresin B	X	X	X	X
2	Chlorogenic acid	X	X	—	X
3	Caffeic acid	X	X	—	X
4	*Aloe* emodin-diglucoside	X	X	—	X
5	Isoquercitrin	X	X	—	—
6	Kaempferol-3-*O*-hexosyl-*O*-pentoside	X	X	—	X
7	6-Methyl-1,3,8-trihydroxyanthraquinone (emodin)	—	—	—	X
8	Luteolin-8-*C*-glucoside (orientin)	X	X	—	—
9	Feruloylquinic acid	—	X	—	—
10	10-Hydroxyaloin A	X	X	—	X
11	Isovitexin	X	X	—	X
12	Chrysoeriol-7-*O*-glucuronide	X	X	—	X
13	Caffeoyl ester of aloesin	X	—	—	X
14	Aloin A	X	X	X	X
15	Isoaloeresin D	X	X	X	X
16	2′-*p*-Methoxycoumaroylaloeresin B	X	—	—	—
17	4,5-Dimethyl ether of *Aloe* emodin	X	—	X	X
18	Aloin B	X	—	X	X
19	6′-Malonylnataloin	X	X	—	X
20	Naringenin-4′-methoxy-7-*O*-glucuronide	X	—	X	X
21	7-Methylether of 2′-feruloylaloesin	X	—	X	—
22	*Aloe* emodin-8-*O*-glucoside	—	X	—	—
23	5,3′-Dihydroxy-6,7,4′-trimethoxyflavone (eupatorin)	—	X	X	X
24	Trihydroxy octadecenoic acid	X	X	X	X
25	3,4-Di-*O*-caffeoylquinic acid	X	—	—	—

X: presence of compounds; —: absence of compounds.

3.3.6. Oxylipins. An oxylipin corresponding to peak **24**, was identified as trihydroxy octadecenoic acid ($C_{18}H_{33}O_5^-$) with a m/z 329.23328 amu (Rt 18.28 min), determined by its small difference in ppm (0.21262) with the theoretical mass ion; this compound has been previously reported in *A. saponaria* [26].

The distribution of phenolic compounds identified in this study can be observed in Table 3, allowing a more graphical demonstration of the differences or similarities in the different plant parts. UHPLC-Q/Orbitrap/MS/MS analysis of the methanol extract of the peel, flower, gel, and root showed that the highest number of phenolic compounds is found in peel, flowers, and roots of *Aloe*. Peaks **1**, **14**, **15**, and **24** were detected in the peel, flowers, gel, and roots of the methanolic extract. Among the twenty-five compounds detected, only nine compounds were detected in the gel of *Aloe*.

4. Conclusions

Twenty-five compounds were tentatively identified for the first time in the native *A. vera* from Pica, Tarapacá Region, in Chile using UHPLC-Orbitrap-ESI-MS. Four were cinnamic acids and derivatives (peaks **2**, **3**, **9**, and **25**), four chromones (peaks **1**, **13**, **15**, and **21**), nine anthracene compounds and derivatives (peaks **4**, **7**, **10**, **14**, **16**, **17**, **18**, **19** and **22**), seven flavonoids (peaks **5**, **6**, **8**, **11**, **12**, **20**, and **23**) and an oxylipin (peak **24**).

The UHPLC fingerprints obtained indicate that the methodology developed in this study was appropriate for the analysis of *A. vera* from the Atacama Desert. This is the first study reporting a tentative identification of several phenolic compounds in this species. These findings could be used as quality control for the plant and for the chemical comparison with other *Aloe* species, as well as with cosmetics or dietary products made from the raw material.

The highest antioxidant activity was observed in the peel in the three assays used (measurement of $DPPH^{\bullet}$, $ABTS^{\bullet+}$, and FRAP resulting in 2.43 ± 0.14 mM ET/g MF, 34.32 ± 2.60 mM ET/g MF, and 3.82 ± 0.23 mM ET/g MF, respectively). The antioxidant capacity could be related to the presence of several phenolic compounds that were identified in the peel, being higher than in the other parts of *A. vera*. Based on these results, we could say that the waste material of the *Aloe* husk could be used more sustainably, which until now had not been used, given that the highest antioxidant activity was found in this part of the plant.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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