





# Potential use of medicinal plants from Argentinean highland as agent anti-photoaging

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## Abstract

**Background:** The overexposure to sun ultraviolet (UV) radiation produce photoaging by effect of free radicals on lipid, protein, and nucleic acid or by direct activation of responsible enzymes of cleavage of extracellular matrix components (EMC).

**Aims:** To develop new anti-photoaging agents, the anti-aging activity of four “jarillas” standardized extracts from Argentina (*Zuccagnia punctata* Cav., *Larrea divaricata* Cav., *Larrea cuneifolia* Cav., and *Larrea nitida* Cav.) were examined.

**Methods:** The effect on the activity and expression of enzymes related to photoaging (collagenase, hyaluronidase, elastase, tyrosinase) and the antioxidant capacity of four “jarillas” extracts were examined.

**Results:** The results suggest that “jarillas” extracts could protect EMC by inhibition of skin aging-related enzymes and suppression of the expression of metalloproteinases (MMP-1) in human skin fibroblasts from photoinduced damage. Modulation of MMP-1 expression induced by UV radiation in human skin cells could be associated, at least in part, with the ROS scavenging capacity of “jarilla” extracts. The extract of *Z punctata* was more active than *Larrea* species extracts in all the trials. The activity of *Z punctata* and *Larrea* extracts could be ascribed to chalcones and lignans, major constituents of “jarillas,” respectively.

**Conclusions:** The results obtained would indicate the potential of these adapted species to live in arid zones of Argentina, exposed to high ultraviolet radiation in production of cosmetic products and justify the promotion of their sustainable use and the development of crops.

## KEYWORDS

Argentinian “jarillas”, collagenase, elastase, hyaluronidase, photoaging

## 1 | INTRODUCTION

The skin is one barrier against the detrimental effects of the environment and toxic agents to protect the body.<sup>1</sup> The overexposure to sun ultraviolet radiation could damage skin and produce photoaging by increases of reactive oxygen species (ROS) that produce

alterations on macromolecules and produce enzyme activation that hydrolyze the EMC.<sup>2</sup> The EMC of epidermis and dermis, including type I collagen, elastin, proteoglycans, and fibronectin, are degraded by the action of enzymes such as matrix metalloproteinases (MMP) which are activated by the genetic induction caused by short UVB wavelengths.<sup>2</sup>

Nowadays, cosmetic or cosmeceutical products derived from plants are used as ingredients because they suppress or slow the aging processes of the skin and to contrast the extrinsic ones by ROS scavenging and modulating the activity of enzymes involved in the aging.<sup>2</sup>

In Argentina, in the biogeographic region of Monte, an arid ecosystem, with low temperature, low absolute humidity, and high solar radiation, grows the native plant species of the natural community of "jarillal" formed mainly by *Larrea* species and *Zuccagnia punctata* Cav. (Fabaceae). *Z punctata*, commonly named as "pus pus," "jarilla pispito," "jarilla macho," and "jarilla poposa" is a xerophytic and evergreen shrub. *Larrea divaricata* Cav. (Zygophyllaceae) (popular names: "jarilla," "jarilla hembra," "chamanilla," "jarilla del cerro," "yarilla"), *Larrea nitida* Cav. (popular names: "jarilla," "jarilla de la montaña," "crespa," "pisa o pispita," "jarilla fina"), and *Larrea cuneifolia* Cav. (popular name: "jarilla," "jarilla macho," "jarilla crespa," "jarilla norte-sur," "jarilla del campo") are the main species in the "jarillal." *L divaricata* exerts antibacterial, antioxidant, anticandida, and immunomodulatory effect.<sup>3-5</sup> It was also found that it slows induced apoptosis and cell division.<sup>6</sup> *L cuneifolia* extracts showed antioxidant, antifungal, and antibacterial activity.<sup>4,5</sup> Antifungal and antioxidant capacities were demonstrated in *L nitida*.<sup>5,7</sup> Lignans and flavonoids were identified in the three *Larrea* species.<sup>5</sup> In *L divaricata*, *L cuneifolia*, and *L nitida*, various lignans were identified, among them, nordihydroguaiaretic acid (NDGA).<sup>5</sup> This compound was assigned as chemical marker to obtain standardized extracts.<sup>5</sup>

*Z punctata* is an Argentine medicinal plant widely recognized by its functional properties (antibacterial, antifungal, anti-inflammatory, antigenotoxic, and antioxidant, between other activities).<sup>8-12</sup> Also, it was reported its activity as inhibitor of chemotherapeutic resistance factors such as efflux pump<sup>8</sup> and as agent that prevents cardiovascular diseases related to hypercholesterolemia and endothelial dysfunction.<sup>13</sup> The major bioactive components identified in *Z punctata* are 2',4'-dihydroxychalcone (DHC), 2',4'-dihydroxy-3'-methoxychalcone<sup>8-12</sup> that could be assigned as chemical markers.<sup>12</sup>

As the "jarillas" are plant species adapted to live in extreme conditions and have multiple properties that can be used industrially, in Argentina their cultivation in situ is being promoted. Currently, a product against the hair loss of one of the "jarilla" species is already commercialized in Argentina. Microcapsules containing "jarilla" extracts were realized and proved to be suitable for the treatment of vulvovaginal candidiasis.<sup>9</sup> Despite the knowledge of the chemistry of "jarilla" species and of the wide range of biological properties demonstrated, the anti-aging effects of the "jarillas" species are unknown.

The aim of this research work was to determine the anti-aging activity of standardized extracts of aerial parts of four "jarillas" species. The four hydroalcoholic extracts were previously chemically standardized.<sup>5,9,12</sup> Taking into account the EMA guidelines (2010), the hydroalcoholic extracts of aerial parts from four "jarillas" species could be used in herbal medicinal product as standardized extracts by the content of selected chemical markers.<sup>14</sup>



**FIGURE 1** Imagen of "jarillal" in Amaicha del Valle, Tucumán, Argentina. Photographs: by authors, in Amaicha del Valle, Tucumán, Argentina

## 2 | METHODOLOGY

### 2.1 | Plant material

The plant parts used were leaves and stems, according to the traditional use. *Z punctata* plants were collected in April 2017 at 2000 m above sea level (m.a.s.l.) in Amaicha del Valle, Tucumán, Argentina (26°35'27.99''S; 65°53'29.87''W) (Figure 1). *L cuneifolia* (26°35'27.99''S; 65°53'29.87''W) and *L divaricata* (26°62'64.09''S; 65°86'81.74''W) were collected in April 2017 at Amaicha del Valle, Tucumán, Argentina at 2000 m.a.s.l. The sample of *L nitida* was collected in Vinchina, La Rioja, Argentina at 3485 m.a.s.l. (28°34'03.74''S; 68°41'33.74''W). All the plants were identified by Dra. Soledad Cuello (INBIOFIV-CONICET-UNT). Voucher specimens (*L cuneifolia*: LIL 614 829; *L divaricata*: LIL 614 299; *L nitida*: LIL 615 845; *Z punctata*: LIL 605 935) are deposited at the Herbarium of Fundación Miguel Lillo (Tucumán, Argentina). The samples were dried in a forced air oven at 40°C.

### 2.2 | Preparation and chemical characterization of ethanolic extracts of "jarillas"

Ground air-dried "jarillas" aerial parts (10 g) were macerated in 60° ethanol (200 mL) for 1 h with ultrasound application in cycles of 10 minutes. The extracts were filtered, taken to dryness under reduced pressure at 37°C and lyophilized to afford the dry extracts.<sup>5,9</sup> Then, the extracts were placed in oxygen barrier bags and vacuum-packed until used (Multivac, D-8941, Germany) and named as ZP: *Z punctata*, LD: *L divaricata*, LC: *L cuneifolia*, and LN: *L nitida*.

These extracts were analyzed by HPLC (Waters Corporation, Milford, MA, USA) coupled to a diode array detector (Waters 2998 photodiode array detector) according to described conditions in previous reports.<sup>5,9,12</sup> A solution of 2 mg/mL of each extract was used. Data collection was carried out with Empower™ 2 software. The identification of compounds present in the extracts

was carried out by comparing retention times and spectral data (220–600 nm) of each peak with those of standards from Sigma-Aldrich (MO, USA) and Indofine Chemical Company, Inc. The main components or chemical markers 2',4'-dihydroxychalcone (DHC) and nordihydroguaiaretic acid (NDGA) were quantified by using plots relating areas and concentrations of commercial standards.

## 2.3 | Enzymatic assays with “jarillas” extracts

All the enzymatic assays were performed using until 250 µg dry weight (DW) of extract/mL and following the methodology described previously.<sup>15</sup> The percent inhibition of the enzymatic activity was calculated using Equation (1). The IC<sub>50</sub> values were determined and defined as the concentration of extract necessary to inhibit the 50% of enzymatic activity. All of them were expressed as µg DW of extract/mL.

### 2.3.1 | Anti-collagenase activity

The inhibitory capacity of “jarilla” extracts on collagenase was assayed by EnzCheck® Gelatinase-Collagenase kit (Thermo Fisher Scientific US). Different concentration of extracts or buffer as negative control was added. The fluorescence intensity was measured. Oleanolic acid (250 µM) was used as a reference inhibitor. The inhibition percent of collagenase enzyme by the extracts was calculated with the following equation:

$$\text{Inhibition (\%)} = (M_{\text{control}} - M_{\text{sample}}) / M_{\text{control}} \times 100 \quad (1)$$

Where M control and M sample are the slopes of the fluorescence graph vs time of incubation.

### 2.3.2 | Anti-elastase activity

Elastase inhibition was determined by measured using the EnzCheck® Elastase assay kit (Thermo Fisher Scientific US). Briefly, aliquots of 50 µL of different dilutions of “jarilla” extracts or buffer (negative control) were added to each well of a 96-well plate. Then, 50 µL of elastine substrate-fluorescein conjugate and 100 µL of elastase were added. The fluorescence was determined. Oleanolic acid was used as positive control.

### 2.3.3 | Anti-hyaluronidase activity

The effect of “jarillas” extracts on hyaluronidase enzyme was carried out according to Chompoo et al.<sup>15</sup> with preincubation extracts-enzyme during 10 minutes at 37°C. The positive control was epigallocatechin gallate (EGCG).

## 2.3.4 | Anti-tyrosinase activity

The assays of tyrosinase inhibition were carried out according to Bravo et al.<sup>16</sup> Different “jarillas” extract concentrations with and without tannins, buffer (negative control), kojic acid (positive control) were used. The percent inhibition of tyrosinase was calculated using Equation (1).

A size exclusion chromatography using Sephadex LH-20 was performed, in order to eliminate the tannins of the *Zuccagnia punctata* extract samples. Acetone: water (1:1; v/v) was used as mobile phase. Ethanol 80% was used with the purpose of recovery polyphenolic compounds. Then, the tannins free *Z punctata* extract was drying and named as ZP-WT. These extracts were assayed as tyrosinase inhibitors.

## 2.4 | Antioxidant capacity of dry extracts

### 2.4.1 | Oxygen radical absorbance capacity assay

Different dilutions of “jarilla” extracts or Trolox standard was mixed with 150 µL of fluorescein (1 µM) and incubated at 37°C during 30 minutes before the addition of 25 µL of AAPH solution (200 mM), peroxy radical generator. The fluorescence was measured every 2 minutes for 120 minutes using a Synergy HT Multi-Mode Microplate Reader.

## 2.5 | Assays in cell systems

### 2.5.1 | Cell culture

Dermal human fibroblasts (HDFs) isolated from adult human skin, as well as all the reagents for the culture and maintenance of the cells, were purchased in Life Technologies Inc, USA. HDFs were cultured in DMEM-F12 medium with 10% fetal bovine serum (FBS) and 100 U/mL penicillin–100 µg/mL streptomycin at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>, with change of medium every 48 h, up to 90% confluence.

Subsequently, the HDFs were transferred to 96-well plates (1 x 10<sup>4</sup> cells per well) for the respective analyzes.

### 2.5.2 | Cytotoxicity on human dermal fibroblasts by WST-8 assay

The cytotoxic effect of the extracts on HDFs was evaluated through of the reduction of 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium (WST-8, Dojindo Molecular Technologies Inc, USA) by cellular dehydrogenases. HDFs were treated with different concentrations of extracts in a range between 2.5 and 50 µg DW/mL, for 24 hours. After treatment, HDFs were washed with phosphate-buffered saline (PBS) and were incubated in

presence of reagent WST-8 at 37°C for 2 hours. The absorbance was measured at 450 nm by a multimodal Synergy microplate reader HT (BioTek Instruments, Inc, Winooski, USA).

### 2.5.3 | Production and quantification of metalloproteinase 1 (MMP-1) in human dermal fibroblasts

MMP-1 produced by HDF culture in presence or absence of “jarillas” extracts was determined by the ELISA technique (Human Total MMP-1 DuoSet Kit).

After this treatment, the cells were washed with PBS, then 100 µL of fresh medium without FBS was added, and the HDFs were subjected to a dose of UVB radiation of 100 mJ/cm<sup>2</sup> using a system of UV radiation Bio-Sun, from the Vilber Lourmat Company (France). Afterward, the dishes containing the cells were incubated at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> for 72 hours. The concentration of MMP-1 was determined following the instructions of maker. For reference, a solution of EGCG at 25 µM was used.

To determine the MMP-1 concentration after treatment with the sample, a response-dose model (log concentration vs. absorbance) was performed using a calibration curve with known concentrations of a recombinant human MMP-1 standard, in a range of 156 to 20 000 ng/mL.

### 2.5.4 | Effect of “jarillas” extracts on reactive oxygen species production in human dermal fibroblasts

HDFs (5x10<sup>4</sup>) were placed in 96-well black plates and treated for 2 hours at 37°C with different solutions of extract at a final concentration of 6.25, 12.5, and 25 µg DW/mL. Subsequently, 10 µL of 2',7'-dichlorofluorescein diacetate (DCFH-DA) was added and incubated again at 37°C during 45 minutes. HDFs were subjected to a dose of UVB radiation of 200 mJ/cm<sup>2</sup> using UV radiation system Bio-Sun and incubated again for 30 minutes at 37°C. Finally, the fluorescence (DCF) was measured (485 nm excitation and 520 nm emission wave) in a multimodal microplate reader Synergy HT. The levels of intracellular ROS are described as the increase in fluorescence with respect to nonirradiated cells.

## 3 | RESULTS AND DISCUSSION

Nowadays, the search for natural products that can reduce photoaging is booming in the field of dermatology. ZP, LC, LD, and LN, four “jarillas” species from region Monte in Argentina (Figure 2), are active as antibacterial, antifungal, antioxidant, and anti-inflammatory.<sup>4,5,8-12</sup> The hydroethanolic extracts obtained from them were previously chemically characterized and standardized.<sup>5,9,12</sup> In this

work, the potential use of these extracts in phytocosmetic or phytotherapy was analyzed.

### 3.1 | Identification and quantification of phenolic compounds by HPLC-DAD

The chemical composition of each plant extract was previously determined by HPLC-MS/MS, and chemical markers were identified, DHC in *Z punctata* extract and NDGA in *L cuneifolia*, *L nitida*, and *L divaricata* extracts.<sup>5,9,12</sup> In this work, the extracts were analyzed by HPLC-DAD to be compared with the previously standardized extracts.<sup>5,9,12</sup> In the extracts were identified and quantified the chemical markers, DHC (40 µg/mg DW) in *Z punctata* and NDGA in *Larrea* extracts (140 µg/mg DW in *L divaricata*; 268 µg/mg DW in *L nitida*; 158 µg/DW in *L cuneifolia*). The quantities of each of them were similar to previous determinations in standardized extracts.<sup>12</sup>

### 3.2 | Anti-aging activity of “jarillas” ethanolic extracts in cell-free systems

#### 3.2.1 | Effect on enzyme activity

In this frame, was evaluated the potential of Argentine native plant extracts to inhibit enzymatic activity of collagenase, elastase, hyaluronidase, and tyrosinase. The collagenase inhibition could represent a potential strategy for the prevention or treatment of photoaging induced by UV radiation.<sup>1,2</sup> The inhibition percentages of 100 and 250 µg/mL of ZP, LD, LC, and LN extracts are shown in Figure 3 and the IC<sub>50</sub> values of the collagenase activity in Table 1. ZP was the most active against the collagenase enzyme with IC<sub>50</sub> values of 2.50 µg/mL of extract. The IC<sub>50</sub> values of ZP without tannins were higher than ZP total extract on collagenase. These results are indicative that tannins would be one of the components responsible for the anti-collagenase activity of *Z punctata*.<sup>17</sup> Chalcones, major components of ZP extracts,<sup>8</sup> may also be responsible for the anti-collagenase effect since these generally have recognized anti-aging, antioxidant, and anti-inflammatory properties.<sup>18</sup> Due to reports on the effect of flavonoids with hydroxyl groups in rings A and B on collagenase activity, the activity of *Z punctata* extracts could be attributed to the interaction of hydroxyl groups present in tannins and chalcones with functional groups of amino acids present in the active site or the collagenase side chain.<sup>19</sup> Furthermore, the hydrophobic interaction that occurs between the benzene ring of these compounds and the collagenase enzyme could cause conformational changes that decrease enzyme activity.<sup>20</sup>

All *Larrea* species were active as inhibitors of collagenase but *L nitida* was more active than *L divaricata* and *L cuneifolia*. The presence of lignans and especially nordihydroguaiaretic acid (NDGA) in the extracts of the three *Larrea* species used in this work were previously demonstrated.<sup>5</sup> In fact, these compounds are major components of *L cuneifolia* extracts and could be responsible of collagenase



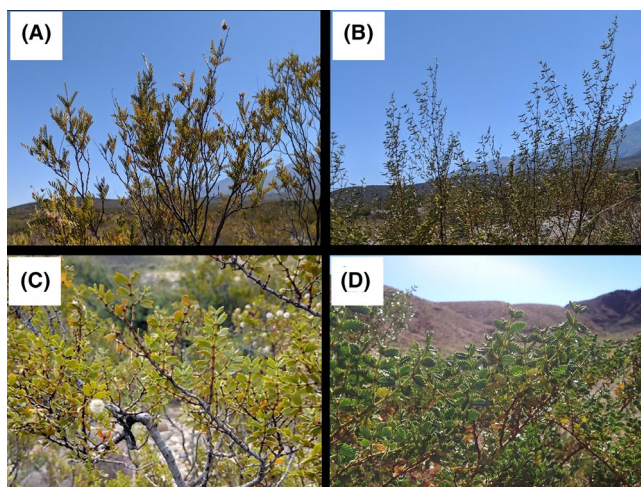
inhibitory effect. Recently was demonstrated by other authors that the NDGA can improve the stability of some types of collagen by improving resistance to hydrolysis caused by type I collagenase<sup>21</sup> and inhibiting the activity of MMP-1 in human fibroblast cultures.<sup>22</sup>

The elastin is a protein responsible of elasticity of the skin and lung and is hydrolyzed by elastase with a consequent loss of skin resistance and the appearance of wrinkles. Elastase activity has been reported to increase with age. The search elastase inhibitor could also be an important target to maintain the integrity of elastin during photoaging.<sup>10</sup> The IC<sub>50</sub> values of "jarillas" on elastase activity were not calculated due to that the inhibition percentages were low even at the maximal assayed concentration (250 µg/mL). Nevertheless, it is important to highlight that the elastase inhibition percentage by *Z punctata* was comparable with reported by Sacan et al<sup>23</sup> for the *Aloe vera* at same concentration of extract (100 µg/mL). The anti-elastase activity of *Z punctata* extract could be attributed to chalcone content.<sup>24</sup> However, the potency of extracts enriched in phenolic compounds was lower than oleanolic acid, a natural inhibitor of elastase with structural characteristics (carboxyl function at position 28, and a double bond) necessary to produce elastase inhibition.<sup>25</sup>

The skin requires certain components suitable to support dermal proliferation, migration, and regeneration. Hyaluronic acid (HA) is a natural molecule formed by glucose and considered important for skin rejuvenation, maintenance of moisture and viscosity and reduction of the permeability of extracellular fluid.<sup>26</sup> Hyaluronidase (Hyal) is a hydrolytic enzyme that acts degrading the HA present in the EMC of numerous tissues in response to various stimuli. Protect HA from the action of Hyal is a fundamental objective to maintain the structure of the connective tissues of the skin. All "jarilla" extracts were hyaluronidase inhibitors, with IC<sub>50</sub> values between 10.00 and 175.23 µg/mL (Table 1). The anti-hyaluronidase activity of *Zuccagnia* extracts was much higher than *Camelia sinensis* (74.85 µg/mL).<sup>27</sup> In inflammatory diseases of the lungs and skin, it has been found that Hyal is overexpressed. Low molecular mass fragments of hyaluronan

have also been shown to trigger inflammation by stimulating gene expression and the synthesis of pro-inflammatory mediators.<sup>28</sup> For this reason, extracts with enzyme inhibitory activity could also exhibit anti-inflammatory activity. In previous works was reported that *Z punctata* extracts could be inhibitors of pro-inflammatory enzymes such as cyclooxygenase-2, lipoxygenase, and phospholipase A2 and the authors attributed this inhibitory effect to chalcones, components of *Z punctata* extract.<sup>8</sup> However, in this paper has been shown that the anti-hyaluronidase activity of *Z punctata* extracts without tannins was lower than *Z punctata* extract with tannins (Table 1). This result indicates that the inhibitory activity of *Z punctata* extract could be attributed to both chalcones and tannins. As regards the behavior of *Larrea* extracts on hyaluronidase, its inhibitory activity could be attributed principally to NDGA with demonstrated anti-inflammatory activity on lipoxygenase activity.<sup>29</sup>

Tyrosinase is an enzyme involved in the melanin biosynthesis, responsible of skin hyperpigmentation. Therefore, it is important to search tyrosinase inhibitors as anti-hyperpigmentation agents. All "jarilla" extracts showed good activity as tyrosinase inhibitors (Figure 3), 97.08% of inhibition at 100 µg/mL with IC<sub>50</sub> values of 11.50 and 22.30 µg/mL for *Z punctata* and ZP-WT extracts, respectively (Table 1). No previous studies are reported on in vitro assays against skin enzymes in ZP extracts. However, the anti-tyrosinase activity of *Z punctata* could be attributed to chalcones and tannins present in the ZP extract. Khatib et al<sup>30</sup> reported that chalcones formed by a resorcinol moiety on one aromatic ring (hydroxyl groups in 2'- and 4'-positions) contribute to increase the inhibition power of chalcones through a mechanism of binding to the active site of the tyrosinase as would be the case of the DHC present in *Z punctata* extracts. Furthermore, it is documented through *molecular docking* methods that 4'-substituted chalcones at ring A exhibit greater anti-tyrosinase activity than those that are not substituted in this position, as well as, chalcones have greater inhibitory activity than other flavonoids such as flavonols.<sup>31,32</sup> *Larrea* extracts were also active against tyrosinase with IC<sub>50</sub> values between 39.33 and 76.55 µg/mL.

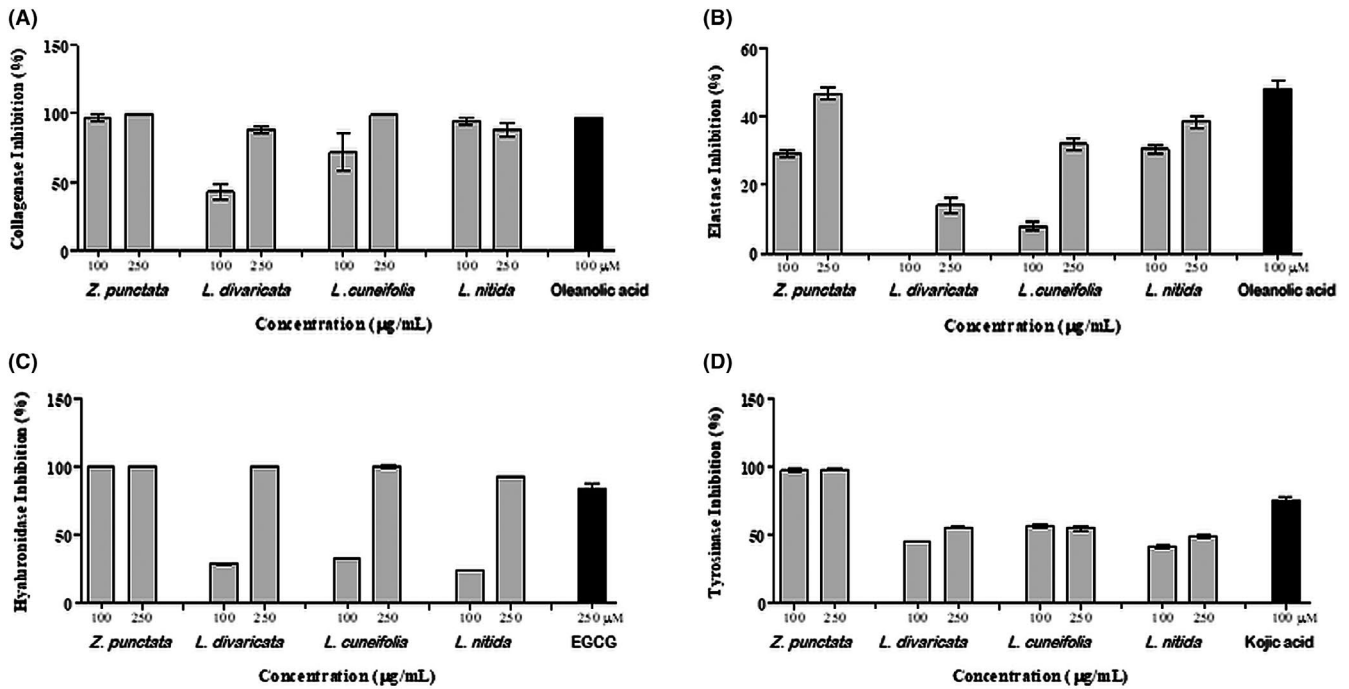


**FIGURE 2** "Jarillas" species: A, *Zuccagnia punctata*, B, *Larrea divaricata*, C, *Larrea cuneifolia*, and D, *Larrea nitida*. Photographs: by authors, in Amaicha del Valle, Tucumán, Argentina

### 3.2.2 | Antioxidant capacity

The increase in ROS production by sunlight UV radiation with the consequent macromolecules damage and skin photoaging has been reported.<sup>2</sup> Therefore, it is important to analyze whether the "jarilla" extracts, in addition to acting as enzyme inhibitors that lead to the degradation of EMC, are antioxidants or free radical scavengers. In previous papers has been reported the antioxidant activity of "jarillas" extracts.<sup>5,8-10</sup> The LD extract was more active as ABTS<sup>•+</sup> scavenger than LC and LN with SC<sub>50</sub> values (concentration of extract necessary for scavenged the 50% of ABTS radicals) of 2.68; 4.10; and 4.50 µg GAE/mL, respectively.<sup>5</sup>

Antioxidant capacity on ABTS<sup>•+</sup> to *Z punctata* extract was previously reported (SC<sub>50</sub> values of 3.8 µg GAE/mL).<sup>9</sup> In this report, the three maximum values in the ORAC assay were found in the three



**FIGURE 3** Enzymatic inhibition percentages of ethanolic extracts of native plants from northwestern Argentina. A, Collagenase, B, Elastase, C, Hyaluronidase, and D, Tyrosinase. Oleanolic acid was used reference inhibitor in collagenase and elastase assays. Epigallocatechin gallate (EGCG) was used as reference in hyaluronidase assay and kojic acid was used in tyrosinase assay

Sample	IC <sub>50</sub> (µg/mL)		
	Collagenase	Hyaluronidase	Tyrosinase
<i>Zuccagnia punctata</i>	2.50 ± 0.09 <sup>a</sup>	10.00 ± 0.52 <sup>a</sup>	11.57 ± 0.95 <sup>a</sup>
<i>Z punctata</i> WT	22.00 ± 2.56 <sup>b</sup>	15.03 ± 0.20 <sup>b</sup>	23.54 ± 1.20 <sup>b</sup>
<i>Larrea divaricata</i>	64.21 ± 0.90 <sup>d</sup>	104.25 ± 1.02 <sup>d</sup>	76.55 ± 5.20 <sup>d</sup>
<i>Larrea cuneifolia</i>	39.45 ± 1.45 <sup>c</sup>	122.00 ± 1.03 <sup>e</sup>	43.61 ± 3.45 <sup>c</sup>
<i>Larrea nitida</i>	31.90 ± 1.39 <sup>c</sup>	175.23 ± 0.63 <sup>f</sup>	39.33 ± 2.36 <sup>c</sup>
Control*	81.90 ± 6.65 <sup>e</sup>	30.23 ± 0.86 <sup>c</sup>	23.22 ± 0.96 <sup>b</sup>

Note: All data are shown as the mean ± SD of three independent experiments. Different letters in the same column show significant differences in IC<sub>50</sub> values between each extract according to Tukey's test ( $P \leq .05$ ). *Z punctata* WT: *Z punctata* extract–tannins free.

\*Control: Oleanolic acid for Collagenase and Hyaluronidase and kojic acid for Tyrosinase.

**TABLE 1** Effect of ethanolic extracts of “jarillas” on skin aging-related enzymes

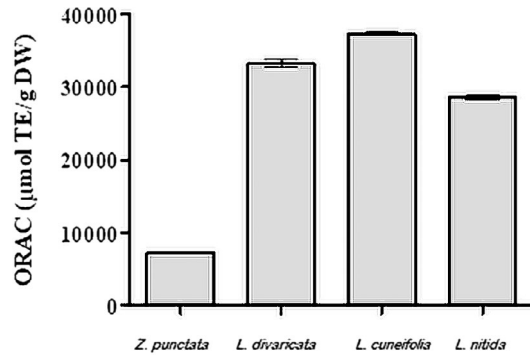
*Larrea* species, with higher values than 3300 µmol TE/g indicating the antioxidant efficacy of these extracts (Figure 4). *Z punctata* showed lowest ORAC values.

### 3.3 | Anti-aging activity of “jarillas” ethanolic extracts in cell systems

#### 3.3.1 | Effect on UV-B-induced MMP-1 production in HDFs

UV radiation induces the activity of MMP which causes damaging changes in the connective tissue of the skin.<sup>2</sup> Thus, bioactive “jarillas” extracts at concentrations of 6.25 and 12.5 µg/mL were

selected to measure the effect of MMP-1 production in UVB-irradiated HDFs. The UVB radiation increased about 4 times the MMP-1 production ( $P < .0001$ ), Figure 5. The pretreatment of HDF cultures with “jarillas” extracts reduced the MMP-1 expression after irradiation in a dose-dependent manner. Among extracts, *L divaricata* extract showed the greatest effect. At 12.5 µg/mL, this extract reduced the MMP-1 production by 82%. While the reference compound, EGCG, reduced it by 90% at 11.5 µg/mL (25 µM), other extracts reduced by 60% the MMP-1 production at 12.5 µg/mL. Based on these findings, the ZP extracts can inhibit UV-induced MMP-1 expression in fibroblasts, and this inhibitory effect could be due to the presence of chalcones.<sup>5</sup> The activity of *Larrea* extracts could be ascribed to NDGA and lignans reported by Moreno et al.<sup>9</sup>



**FIGURE 4** Antioxidant capacity of plant extracts expressed as µg of Trolox equivalent per gram of dry extracts

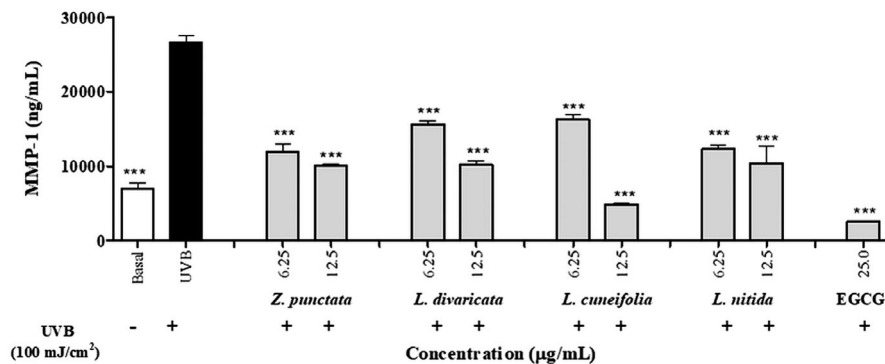
### 3.3.2 | Effect on UVB-induced ROS production by HDFs

The UV radiation drastically increased ROS production in human fibroblasts (more than 18-fold), Figure 6. All “jarilla” extracts

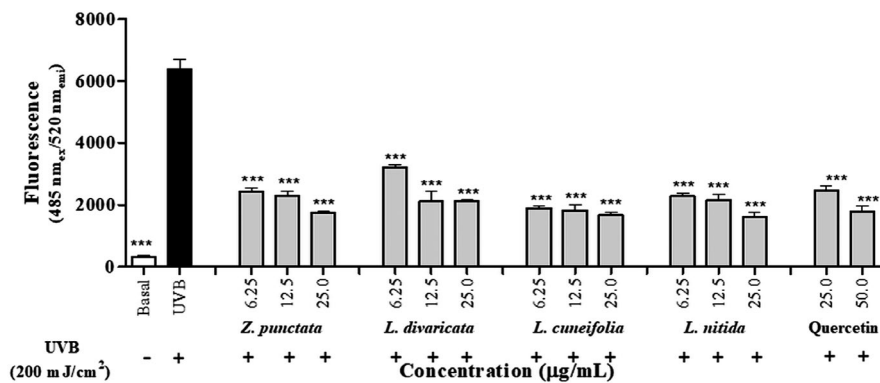
significantly reduced more than 50% of the UVB-induced ROS generation at concentrations between 6.25 and 25 µg/mL. At the highest concentration, the reduction was around 72%, except to LD which reduced 66% of ROS production at 25 µg/mL. The effects were similar to control quercetin which reduced 62 and 72% the ROS generation at 7.5 and 15 µg/mL, respectively (25 and 50 µM). The obtained results suggest the antioxidant capacity of “jarilla” extracts. Modulation of matrix metalloproteinase 1 expression induced by UV radiation in human skin cells could be associated, at least in part, with the ROS scavenging capacity of “jarilla” extracts.

## 4 | CONCLUSIONS

The results suggest the ability of some “jarilla” plant extracts to protect the main EMC through a route of inhibition of enzymes related to skin aging and its antioxidant capacity. In addition, the extracts cause the suppression of UV-induced MMP-1 expression in human skin fibroblasts. This result indicates the potential of these extracts



**FIGURE 5** Effect of plant extracts on MMP-1 in HDFa after UVB radiation.  $1 \times 10^4$  cells were treated with samples for 24 h and were then exposed to UVB (100 mJ/cm<sup>2</sup>) radiation. The MMP-1 production was determined by ELISA 72 h after irradiation. Epigallocatechin gallate (EGCG) 25 µM was used by reference. All data are shown as the mean ± SD of three independent experiments. \*\*\**P* < .0001, compared with the UVB-irradiated control



**FIGURE 6** Effect of plant extracts on intracellular ROS production in HDFa after UVB radiation.  $5 \times 10^4$  cells were co-treated with different concentrations of plant extracts for 2 hours. Then, treated and untreated cells were exposed to UVB (200 mJ/cm<sup>2</sup>) radiation. The ROS generation was measured by 2',7'-dichlorofluorescein diacetate assay. Quercetin 25 and 50 µM was used by reference. All data are shown as the mean ± SD of three independent experiments. \*\*\**P* < .0001, compared with the UVB-irradiated control

to prevent photoaging. *Z punctata* standardized extract is the most active in all tests and, therefore, is relevant for use in healthy cosmetic products. The benefic properties of the Argentinian plant extracts against the aging process could be attributed to a set of compounds previously described in them. However, this supports the need to examine extracts from these plant materials more extensively in *in vivo* models. The loss of biodiversity on the planet is the main incentive to deepen the study of our native species adapted to live in extreme conditions, high ultraviolet radiation and arid zones to promote the crop and sustainable use of them.

### CONFLICT OF INTEREST


The authors declare that there are no conflicts of interest.

### DATA AVAILABILITY STATEMENT

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

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