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Antioxidant Activity of A-Type Proanthocyanidins from *Geranium niveum* (Geraniaceae)

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Geranium niveum S. Watson (Geraniaceae) is a medicinal herb widely used by the Tarahumara Indians of Mexico. This species is rich in proanthocyanidins and other phenolics. Previous in vitro assays have demonstrated that proanthocyanidins exhibited antiinflammatory, antiviral, antibacterial, enzyme-inhibiting, antioxidant, and radical-scavenging properties. In view of its medicinal use and chemical composition, the aim of the present study was to determine the in vitro antioxidant activity of the extracts and two proanthocyanidins (geranins A and D) from the roots of *G. niveum* by using seven different assay systems, namely, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), hypochlorous acid (HOCl), and singlet oxygen ($^1O_2$). Two known antioxidants, resveratrol and ascorbic acid, were used as positive controls. The results showed that geranins A and D and the extracts were able to scavenge ABTS, DPPH, $O_2^{\bullet -}$, $OH^{\bullet}$, and HOCl. The scavenging ability of geranins A and D was similar to that of resveratrol and ascorbic acid in the following assays: ABTS, $O_2^{\bullet -}$, and HOCl. The scavenging capacity of ascorbic acid for DPPH was higher than that of both geranins and resveratrol. On the other hand, the $OH^{\bullet}$ scavenging action of both geranins and resveratrol was similar. The methanol−$CHCl_3$ (1:1) extract had a higher ability to scavenge ABTS, DPPH, and $O_2^{\bullet -}$ radicals than the chloroform extract. In turn, the latter was more potent than the methanol−$CHCl_3$ (1:1) extract as $OH^{\bullet}$ or HOCl scavenger agent. Neither geranins A and D nor the extracts were able to scavenge H_2O_2 and $^1O_2$. In conclusion, *G. niveum* roots have proanthocyanidins with powerful radical scavenging in vitro activity. This property may partially explain the wide use of this plant in the Tarahumara indigenous system of medicine for the treatment of gastrointestinal illnesses (other than spasms), pain, and fevers associated with oxidative stress.

KEYWORDS: ***Geranium niveum* S. Watson; Geraniaceae; proanthocyanidin; geranin A; geranin D; scavenger; antioxidants**

INTRODUCTION

In the past decade natural antioxidants have generated considerable attention in preventive medicine. Oxidation of cellular constituents by free radicals provokes several human diseases such as diabetes, arteriosclerosis, cardiovascular illnesses, cancer, several neurodegenerative disorders, and the aging process. Consequently, much attention has been directed toward the discovery of new natural antioxidants, including herbal products, aimed at quenching biologically harmful radicals. The study of the resources employed in complementary and alternative medicines around the world has being recognized as an important strategy for the discovery of such agents (*1*−*4*). Therefore, we initiated a systematic investigation of some Mexican medicinal plants for their potential antioxidant activity. Initially we selected *Geranium niveum* S. Watson (Geraniaceae) because of its high proanthocyanidin content and reputed medicinal properties. The proanthocyanidins are metabolites built by coupling of two or more flavanyl units. They are widely distributed in nature and often are the active compounds of the medicinal plants in which they occur (*5*). Reports of several in vitro assays have demonstrated that these metabolites exhibited antiinflammatory, antiviral, antibacterial, enzyme-inhibiting, antioxidant, and radical-scavenging properties. Their tendency

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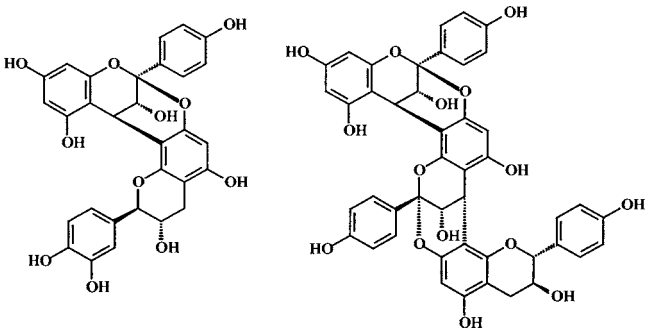

Geranin A Geranin D

Figure 1. Structures of geranins A and D.

to interfere with biological systems results, at least in part, from the characteristic ability to form complexes with biomolecules (*5*, *6*). The proanthocyanidins more investigated from a biological point of view belong to the B-type, where the constituent flavanyl moieties are linked via only one bond, in particular those related to procyanidins. These compounds as well as their monomeric units are well-known chemopreventive and antioxidant agents exhibiting noted radical scavenging properties for peroxyl, superoxide, and hydroxyl radicals (*3*, *5*−*9*). However, the A-type proanthocyanidins, which possess an unusual second ether linkage to C(2) of the terminal flavanyl unit, have not received considerable attention. To our knowledge the only report on the antioxidant potential of the A-type proanthocyanidins described the effect of epigallocatechin-(2β→ *O*→ 7, 4β→8)-epicatechin, isolated from the medicinal plant *Dioclea lasiophylla* (Leguminosae), on the oxidation of β-carotene in linolenic acid suspension; the antioxidant properties of the compound were higher than those of α-tocopherol, which was used as a positive control (*10*).

Geranium niveum is a silvery canescent-leaved herb which grows along the dry stream banks and grassy meadows of the pine-oak forests in the high mountains of western Chihuahua, Mexico. The Tarahumara Indians call this perennial herb "makiki" and employ the decoction of the roots as a remedy for pain, fevers, and gastrointestinal ailments. Previous investigation of this species led to isolation of four novel antiprotozoal A-type proanthocyanidins along with several known compounds. The new proanthocyanidins were characterized as epiafzelechin-(2β→ *O*→ 7, 4β→8,)-afzelechin (**1**), epicatechin-(2β→ *O*→ 7, 4β→8) afzelechin, epicatechin-(2β→ *O*→ 7, 4β→8) gallocatechin, and epiafzelechin-(2β→ *O*→ 7, 4β→8)-afzelechin-(2β→ *O*→ 7, 4β→8)-afzelechin (**2**) and given the trivial names of geranins A, B, C, and D, respectively (**Figure 1**). Geranins A−D exhibited moderate antiprotozoal properties against *Giardia lamblia* and *Entamoeba histolytica, G. lamblia* being the most sensitive protozoon (*11*−*13*).

On the basis of the above considerations the aim of the present study was to determine the antioxidant activity in vitro of the extracts and two proanthocyanidins (geranins A and D) from *G. niveum* using seven different assay systems, namely, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide anion, hydrogen peroxide, hydroxyl radical, hypochlorous acid, and singlet oxygen (*14*−*16*). Two well-known antioxidants, resveratrol and ascorbic acid, were used as positive controls in this study.

MATERIALS AND METHODS

Reagents. Resveratrol was purchased from Sigma-Aldrich (Mexico City, Mexico). Potassium persulfate, ammonium ferrous sulfate, trichloroacetic acid, HCl, NaOCl, H_2SO_4, and HPLC-grade solvents were purchased from JT Baker (Mexico City, Mexico). H_2O_2 was purchased from Mallinckrodt (Paris, KY). *N*,*N*-Dimethyl-*p*-nitrosoaniline was purchased from Aldrich (Milwaukee, WI). Xylenol orange, butylated hydroxytoluene (BHT), 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Tris-HCl, phenazine methosulfate (PMS), nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH), dimethyl sulfoxide (DMSO), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), histidine, sodium borohydride ($NaBH_4$), *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), ascorbic acid, ethylenediamine tetraacetic acid (EDTA), deferoxamin mesylate, tetramethoxypropane, 2-deoxy-D-ribose, and $FeCl_3$ were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were analytical grade and commercially available.

Plant Material, Isolation, and Identification of Proanthocyanidins. The extracts and geranins A and D from *G. niveum* were obtained and characterized as previously described (*12*, *13*). Purities (99%) of the geranins were determined by HPLC. The HPLC analyses were carried out with a Waters HPLC instrument equipped with Waters 996 UV photodiode array detector (900) set at 200−300 nm using a Spherisorb S5ODS2 column (10 × 250 mm) at a flow rate of 3.2 mL min^{-1}. The eluants were 5% formic acid and CH_3CN (7:3). Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium 2000 software program (Waters).

ABTS Radical Cation Decolorization Assay. The method of Re et al. (*17*) was followed. Briefly, a mixture of 7 mM ABTS and 2.45 mM potassium persulfate was prepared and allowed to stand at room temperature 12 h in the dark. The $ABTS^+$ solution was diluted to an absorbance of 0.70 at 734 nm in phosphate-buffered saline (PBS). A 15 μL amount of $ABTS^+$ solution was added to 985 μL of PBS without (0% scavenging tube) or with different concentrations of geranins A and D (from 1 to 20 μM) and extracts (from 1 to 20 μg/mL). The absorbance was read every 15 s for 1 min. The reference compounds (ascorbic acid and resveratrol) were used at the same concentration as the geranins and extracts.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Assay. DPPH radical scavenging activity was investigated according to the method of Yamaguchi et al. (*18*). Briefly, 0.4 mL of an ethanolic solution of 500 μM DPPH was mixed with 0.5 mL of 100 mM Tris-HCl buffer, pH 7.4, and 0.1 mL of Tris-HCl buffer without (% scavenging tube) or with different concentrations of geranins A and D (from 10 to 640 μM) or extracts (from 1 to 400 μg/mL). The tubes were incubated in the darkn at room temperature for 20 min, and the absorbance was recorded at 517 nm. The antioxidant capacity is given as percent (%) DPPH scavenging, calculated as [(optical density of control − optical density of geranin/extract)/(optical density of control) × 100].

Superoxide Anion Scavenging Activity in the NADH/PMS/NBT System. The nonenzymatic assay was performed according to Furuno et al. (*19*) with some modifications (*20*). A 10 μL amount of DMSO was added to 1.14 mL of 20 mM HEPES buffer, pH 7.2, containing 5 μM PMS, 50 mM NBT without (0% scavenging tube) or with different concentrations of geranins A and D (from 10 to 640 μM) or extracts (from 10 to 400 μg/mL). To the mixture 100 μL of 2.5 mM NADH solution was added to initiate the generation of superoxide anion. The absorbance was read at 560 nm every minute for 3 min and the △ optical density/min was obtained and compared with 0% scavenging tube.

Determination of H_2O_2 by the Ferrous Ion Oxidation−Xylenol Orange (FOX) Assay. A solution of 75 μM H_2O_2 was mixed (1:1 (v/v)) with water (0% scavenging tube) or different concentrations of pure compounds (from 10 to 640 μM) or extract (from 25 to 800 μg/mL) and incubated for 3.5 h at room temperature. Thereafter, the amount of H_2O_2 was measured by the method described by Long et al. (*20*). Briefly, nine volumes of 4.4 mM BHT in HPLC-grade methanol were mixed with one volume of 1 mM xylenol orange and 2.56 mM ammonium ferrous sulfate in 0.25 M H_2SO_4 to give the "working" FOX reagent. A 90 μL amount of the H_2O_2−samples solutions were pipetted in 1.5 mL Eppendorf tubes and mixed with 0.01 mL of HPLC-grade methanol immediately followed by the addition of 0.9 mL of FOX

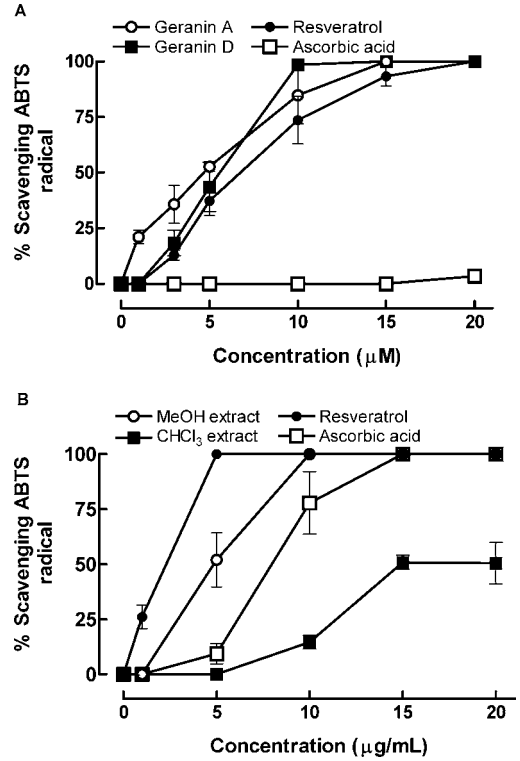

Figure 2. Reduction of the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation in the presence of geranins A and D (A), extracts from *G. niveum* (B), and resveratrol and ascorbic acid (A and B). Each point represents the mean of three determinations with the standard deviation.

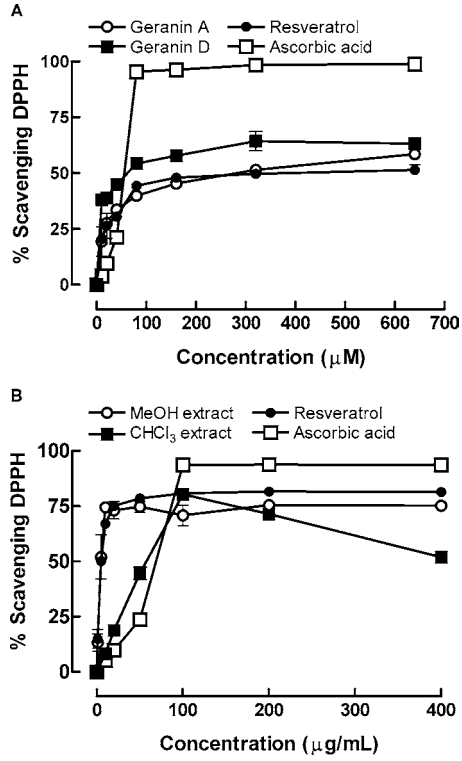

Figure 3. DPPH radical scavenging capacity of geranins A and D (A), extracts of *G. niveum* (B), and resveratrol and ascorbic acid (A and B). Values are expressed as percent inhibition of DPPH. Each point represents the mean of three determinations with the standard deviation.

Table 1. Scavenging Ability of Geranins A and D from *G. niveum*, Resveratrol, and Ascorbic Acid

	IC_{50} (μM)			
radical	geranin A	geranin D	resveratrol	ascorbic acid
ABTS	3.5 ± 1.5	4.8 ± 1.6	5.8 ± 1.4	57.0 ± 17.0
DPPH	258.1 ± 1.1	61.5 ± 1.4	323.9 ± 1.7	54.6 ± 1.9
$O_2^{\bullet -}$	20.9 ± 1.6	9.1 ± 2.0	>1000	>1000
$OH^{\bullet}$	0.2 ± 1.6	0.1 ± 1.7	0.4 ± 1.3	>1000 ND
HOCl	0.2 ± 2.5	0.1 ± 3.1	>1000	>1000

reagent, vortexed for 5 s, and then incubated at room temperature for 10 min. The tubes were centrifuged for 15 000*g* for 10 min, and absorbance at 560 nm was read against methanol blank. The H_2O_2 concentration was calculated from a H_2O_2 standard curve of the following concentrations: 2.5, 5, 10, 15, 25, 35, 50, and 75 μM.

Hydroxyl Radical Scavenging Assay. The ability of geranins A and D and geranium extracts to scavenge $OH^{\bullet}$ was conducted in the $Fe^{3+}-EDTA-H_2O_2-$deoxyribose system (*21*). A 900 μL amount of the reaction mixture 0.2 mM ascorbic acid, 0.2 mM $FeCl_3$, 0.208 mM EDTA, 1 mM H_2O_2, 0.56 mM 2-deoxy-D-ribose, and 20 mM phosphate buffer (pH 7.4) was mixed with 100 μL of distilled water (control tube) or 100 μL of different concentrations of geranins A and D (from 0.01 to 20 μM) or extracts (from 0.005 to 50 μg/mL). Hydroxyl radicals were generated by incubating the mixture at 37 °C for 60 min. The iron salt ($FeCl_3$) was mixed with EDTA before addition to the reaction mixture. The extent of 2-deoxy-D-ribose degradation by the formed $OH^{\bullet}$ was measured directly in the aqueous phase by the thiobarbituric acid test (*22*).

Hypochlorous Acid (HOCl) Scavenging Activity. Hypochlorous acid was prepared immediately before use by adjusting the pH of a 1% (v/v) solution of NaOCl to pH 6.2 with 0.6 M H_2SO_4. The concentration of HOCl was further determined spectrophotometrically at 235 nm using the molar extinction coefficient of 100 M^{-1} cm^{-1}. 5-Thio-2-nitrobenzoic acid (TNB) was prepared according to the procedure of Chin et al. (*23*) with some modifications. Briefly, 50 mM sodium borohydride ($NaBH_4$) was added to a 1 mM solution of DTNB in 50 mM potassium phosphate buffer, pH 6.6, containing 5 mM EDTA. The solution was incubated at 37 °C for 30 min. The concentration of TNB was determined by measuring the absorbance at 412 nm using a molar extinction coefficient of 13 600 M^{-1} cm^{-1}. The assay for scavenging HOCl was performed as follows: A blank tube containing 0.4 mL of 175 μM TNB, 0.1 mL of geranins (from 1 to 40 μM) or extracts (from 1 to 200 μg/mL), and 0.5 mL of 50 mM potassium phosphate, pH 6.6, and problem tubes containing 0.4 mL of 175 μM TNB, 0.1 mL of geranins (from 0.005 to 20 μM) or extracts (from 1 to 200 μg/mL), and 0.5 mL of 50 μM HOCl were vortexed, incubated at room temperature for 15 min, and read at 412 nm against 50 mM potassium phosphate, pH 6.6. The optical density of the blank tubes was subtracted from that of problem tube. This value was compared against the value obtained from tubes in which the sample was replaced by water, and the data are expressed as the percent inhibition of TNB oxidation to DTNB (*24*).

Singlet Oxygen Assay. The production of $^1O_2$ by sodium hypochlorite (NaOCl) and H_2O_2 was determined using a previously reported spectrophotometric method (*25*) with minor modifications in which *N*,*N*-dimethyl-*p*-nitrosoaniline was used as a selective acceptor of $^1O_2$. The bleaching of *N*,*N*-dimethyl-*p*-nitrosoaniline was monitored spectrophotometrically at 440 nm. The assay mixture contained 45 mM sodium phosphate buffer (pH 7.1), 10 mM histidine, 10 mM NaOCl, 10 mM H_2O_2, and 50 μM *N*,*N*-dimethyl-*p*-nitrosoaniline. The total reaction volume was 2.0 mL, and it was incubated at 30 °C for 40 min. The extent of $^1O_2$ production was determined by measuring the decrease in the absorbance of *N*,*N*-dimethyl-*p*-nitrosoaniline at 440 nm. The relative scavenging efficiency (% inhibition in production of $^1O_2$) was estimated from the difference in the absorbance of *N*,*N*-dimethyl-*p*-nitrosoaniline with and without addition of increasing amounts of geranins (from 10 to 640 μM) or extracts (from 25 to 800 μg/mL).

Statistical Analysis. All analyses were performed in triplicate. The data were recorded as mean ± SD and analyzed by ORIGIN (version 6.0 for Windows XP, Inc.).

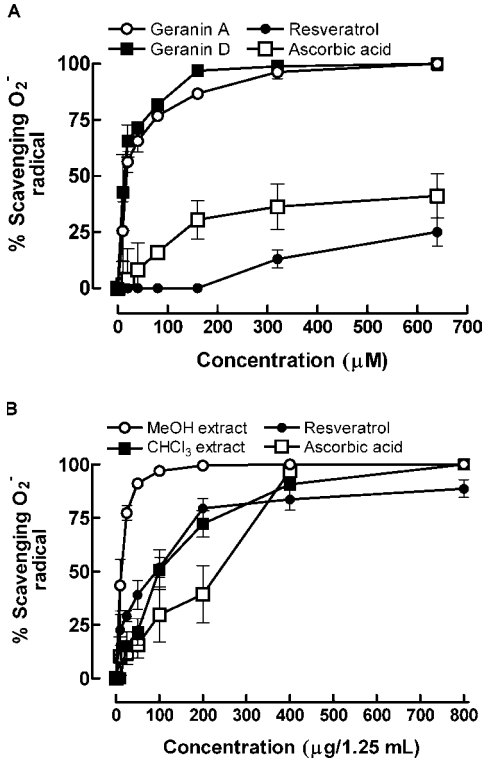

Figure 4. Effect of geranins A and D (A), extracts of *G. niveum* (B), and resveratrol and ascorbic acid (A and B) on reduction of nitroblue tetrazolium (NBT) by superoxide anions generated by PMS and NADH. Values are expressed as percent inhibition of NBT reduction. Each point represents the mean of three determinations with the standard deviation.

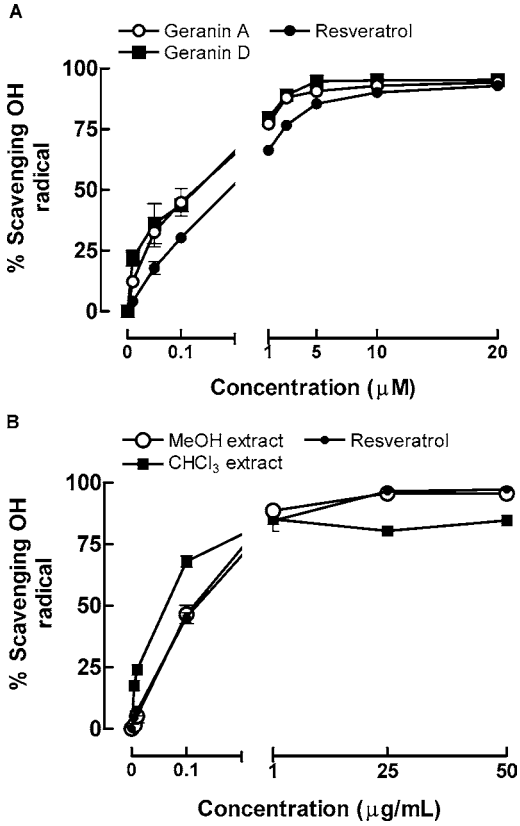

Figure 5. Effect of geranins A and D (A), extracts of G. niveum (B), and resveratrol (A and B) on 2-deoxy-D-ribose degradation stimulated with Fe^{3+}–EDTA + H_2O_2 + ascorbate. Values are expressed as percent inhibition of 2-deoxy-D-ribose degradation. Each point represents the mean of three determinations with the standard deviation.

RESULTS

To evaluate the radical scavenging activities of the extracts and compounds from *G. niveum* seven different assay systems were used, namely, ABTS, DPPH, $O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$, HOCl, and $^1O_2$. Two well-known antioxidant agents, resveratrol and ascorbic acid, were used as positive controls.

Antioxidant Activity on the ABTS Radical Cation Decolorization Assay. **Figure 2** shows the results of the ABTS assay for geranins A and D (**Figure 2A**) and extract of *G. niveum* (**Figure 2B**). Geranins A (IC_{50} = 3.5 μM) and D (IC_{50} = 4.8 μM) showed a similar ABTS radical scavenging activity. One hundred percent ABTS scavenging activity was reached using 15 μM geranins A and D and 20 μM resveratrol (IC_{50} = 5.8 μM). Ascorbic acid did not scavenge ABTS up to 20 μM. The scavenging activity was higher in the methanol−$CHCl_3$ (1:1) extract than in the chloroform extract (**Figure 2B**). The scavenging property of resveratrol was higher than that of the methanol−$CHCl_3$ (1:1) extract (IC_{50} = 17.8 μg), but the scavenging ability of ascorbic acid (IC_{50} = 7.7 μg) was higher than that of the chloroform extract, **Table 1**.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity. Figure 3 shows DPPH assay for geranins A (IC_{50} = 258.1 μM) and D (IC_{50} = 61.5 μM) (**Figure 3A**) and methanol−$CHCl_3$ (1:1) (IC_{50} = 7.3 μg) and chloroform extracts (IC_{50} = 92.0 μg) of *G. niveum* (**Figure 3B**). Ascorbic acid (IC_{50} = 54.6 μM), resveratrol (IC_{50} = 323.9 μM), and the tested samples reduced the stable radical DPPH to the yellow-colored diphenyl-picrylhydrazine.

Superoxide Assay. Figure 4 shows $O_2^{\bullet-}$ scavenging activity for geranins A and D (**Figure 4A**) and methanol−$CHCl_3$ (1:1) and chloroform extracts of *G. niveum* (**Figure 4B**). The scavenging activity of geranins A (IC_{50} = 20.9 μM) and D (IC_{50} = 9.1 μM) was much better than that of ascorbic acid (IC_{50} = 770.2 μM). The methanol−$CHCl_3$ (1:1) extract (IC_{50} = 6.5 μg) showed the strongest superoxide scavenging activity (**Figure 4B**).

Hydroxyl Radical Scavenging Assay. Figure 5 shows the $OH^{\bullet}$ scavenging activity for geranins A (IC_{50} = 0.2 μM) and D (IC_{50} = 0.1 μM) (**Figure 5A**) and both extracts of *G. niveum* (**Figure 5B**). Geranins A and D and resveratrol (IC_{50} = 0.5 μM) showed similar $OH^{\bullet}$ scavenging activity. The methanol−$CHCl_3$ (1:1) (IC_{50} = 0.2 μg) and chloroform (IC_{50} = 0.1 μg) extracts also showed good activity. Ascorbic acid was not used as a standard in this assay.

Hypochlorous Acid (HOCl) Scavenging Activity. Figure 6 shows HOCl scavenging activity for geranins A and D (**Figure 6A**) and extracts of *G. niveum* (**Figure 6B**). Geranins A (IC_{50} = 0.2 μM) and D (IC_{50} = 0.1 μM) showed strong and similar HOCl scavenging activity. Ascorbic acid and resveratrol were unable to scavenge HOCl up to 20 μM (**Figure 6A**). The extracts quenched HOCl but did not reach 50% scavenging activity.

Determination of H_2O_2 by the Ferrous Ion Oxidation−Xylenol Orange (FOX) and Singlet Oxygen Assay. Neither geranins A and D nor the extracts showed scavenging activity against hydrogen peroxide and singlet oxygen (data not shown).

DISCUSSION

The antioxidant activity of *G. niveum* extracts and compounds was initially tested by measuring their capacity to scavenge DPPH and ABTS radicals. Both radicals have been widely used to evaluate the antioxidant properties of natural products

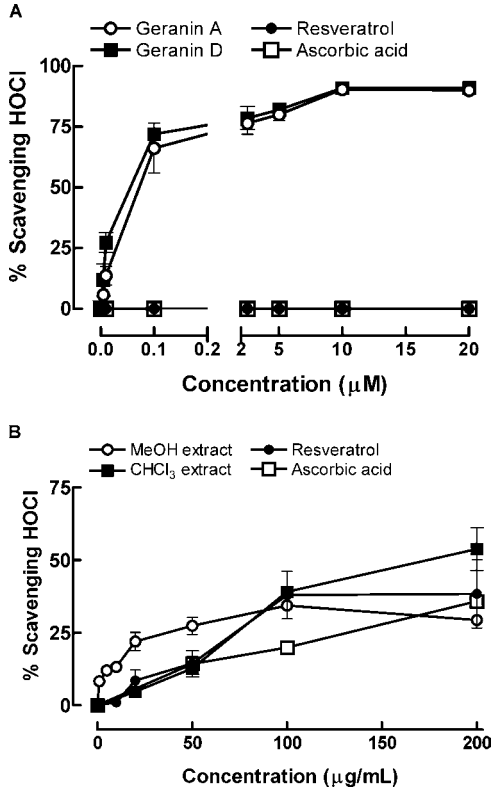

Figure 6. Effect of geranins A and D (A), extracts of *G. niveum* (B), and resveratrol and ascorbic acid (A and B) on TNB oxidation by HOCl. Values are expressed as percent inhibition of TNB oxidation. Each point represents the mean of three determinations with the standard deviation.

(*2*, *4*, *8*, *9*). In this work we found that all tested materials were able to quench these radicals in a concentration-dependent manner. In the DPPH assay the methanol−$CHCl_3$ (1:1) extract was found to be more effective than its main active components (geranins A and D), thus indicating a possible synergistic interaction of the constituents or the presence of more potent antioxidants not yet identified.

The potential chemopreventive properties of *G. niveum* extracts and pure compounds (geranins A and D) were further evaluated by testing their capacity to scavenge several reactive oxygen species (ROS); the ROS selected for testing have been implicated in the pathogenesis of several diseases and include $O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$, and HOCl (*2*, *3*, *9*). Geranins A and D had strong $OH^{\bullet}$, HOCl, and $O_2^{\bullet-}$ scavenging activity. The extracts of *G. niveum* were also good $OH^{\bullet}$ and $O_2^{\bullet-}$ scavengers. In all cases the effect was concentration dependent and higher than the positive standards. However, geranins A and D and the preparations were poor H_2O_2 scavengers. The ability of *G. niveum* preparations and compounds to scavenge $O_2^{\bullet-}$ radicals is important to prevent oxidative damage because in cellular oxidation reactions superoxide radicals produce other kinds of cell-damaging free radicals and oxidizing agents. On the other hand, since HOCl is produced in vivo by oxidation of chloride ion at the sites of inflammation, it is possible that geranins and the extracts of *G. niveum* could exert a protective effect in vivo during the inflammatory process. On the other hand, the extraordinary ability of geranins to quench $OH^{\bullet}$ radicals can reduce the oxidative stress provoked by these radicals such as DNA strand scission and lipid peroxidation through the Fenton reaction.

In conclusion, *G. niveum* roots are rich in phenolic compounds including proanthocyanidins A and D devoid of cytotoxic activity (*12*, *13*) with powerful radical scavenging in vitro activity. This property may partially explain the wide use of this plant in the Tarahumara indigenous system of medicine for the treatment of gastrointestinal illnesses (other than spasms), pain, and fevers associated with oxidative stress.

ABBREVIATIONS USED

ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl; $O_2^{\bullet-}$, superoxide anion; H_2O_2, hydrogen peroxide; $OH^{\bullet}$; hydroxyl radical; HOCl, hypochlorous acid; $^1O_2$, singlet oxygen; IC_{50}, concentration that gives 50% inhibition; HPLC, high-performance liquid chromatography; BHT, butylated hydroxytoluene; DTNB, 5,5′-dithio-bis-2-nitrobenzoic acid; PMS, Tris-HCl, phenazine methosulfate; NBT, nitroblue tetrazolium; NADH, nicotinamide adenine dinucleotide; DMSO, dimethyl sulfoxide; HEPES, 2,2′-histidine, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; EDTA, ethylenediamine tetraacetic acid.

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