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Evaluation of *in vitro* antioxidant properties of some traditional Sardinian medicinal plants: Investigation of the high antioxidant capacity of *Rubus ulmifolius*

Stefano Dall'Acqua [a,*], Rinaldo Cervellati [b], Maria Cecilia Loi [c], Gabbriella Innocenti [a]

[a] *Dipartimento di Scienze Farmaceutiche, Università di Padova, Via F. Marzolo 5, I-35131 Padova, Italy*
[b] *Dipartimento di Chimica 'G. Ciamician', Università di Bologna, Via Selmi 2, I-40126 Bologna, Italy*
[c] *Dipartimento di Scienze Botaniche, Università degli Studi di Cagliari, V.le S. Ignazio da Laconi 13, I-09123, Cagliari, Italy*

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Abstract

The antioxidant capacities of 11 botanical species used in the tradition of Sardinia as teas beverages or as decoction for medicinal purposes were evaluated using different *in vitro* methods (BR, TEAC, DPPH and FC). Among the various species, *Rubus ulmifolius*, resulted the more active with all the used methods. Phytochemical investigation on the extract yields in the isolation of several phenolic compounds namely caffeic acid, ferulic acid, quercetin-3-O-glucuronide, kaempferol-3-O-glucuronide, kaempferol-3-O-(6″-*p*-coumaroyl)-β-D-glucopyranoside, kaempferol-3-O-(6″-caffeoyl)-β-D-glucopyranoside, chlorogenic acid, 4-caffeoylquinic acid and 5-caffeoylquinic acid. The antioxidant activity of isolated compounds was also evaluated.

Keywords: Antioxidant; Plant extract; Phenolic derivatives

1. Introduction

A large number of naturally occurring compounds such as flavonoids, catechins, lignans and phenolic acids contained in edible plants and medicinal herbs have antioxidant properties. Dietary antioxidant intake has been associated for example with reduced risk of cardiovascular diseases, cancer, and neurodegenerative diseases (Nair, Li, & Kong, 2007). For these reasons, the natural antioxidants have recently become a major area of research. Different *in vitro* tests have been proposed for the evaluation of the antioxidant or radical scavenging power of natural compounds, but until now, few data are available about the comparison of various methods. Many herbal teas or decoction may act as a valuable source of dietary antioxidants but for several species, the information about the polyphenol content as well as the antioxidant activity is still lacking.

In this paper, we report the evaluation of the antioxidant properties of 11 plants used in the folk medicine of Sardinia (Italy) as teas or decoctions, for the treatment of several diseases (Table 1), moreover phytochemical investigation was performed on the most active extract. The selected plants are scarcely investigated regarding their antioxidant activity except for *Marrubium* spp. (Matkowsky & Piotrowska, 2006; VanderJagt, Ghattas, VanderJagt, Crossey, & Glew, 2002), *Teucrium* spp. (Kadifkova Panovska, Kulevanova, & Stefova, 2005; Ljubuncic et al., 2006) and *Urtica dioica* (Gulcin, Kufrevioglu, Oktay, & Buyukokuroglu, 2004). Few information are available about the polyphenol content of the investigated plant material. Phenylpropanoids esters were isolated from *Marrubium vulgare* leaves (Sahpaz, Garbacki, Tits, & Bailleul, 2002). Leaves of *Rubus* spp. are known for containing

* Corresponding author. Tel.: +39 0 49 8275332.
E-mail address: stefano.dallacqua@unipd.it (S. Dall'Acqua).

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Table 1
Considered botanical species and their traditional uses

Plant name	Family	Traditional uses	Extraction yield, %
Arundo donax L. leaves	Graminaceae	Vulnerary, otitis, reduction of the rise of the milk, emmenagogue	24.1
Calendula arvensis L. leaves	Compositae	Emmenagogue, diaphoretic, diuretic, sedative, anti inflammatory	29.6
Cerastium glomeratum L. whole plant	Cariophyllaceae	Diuretic, galactofuge, tonic	7.9
Eryngium campestre L. roots	Umbelliferae	Diuretic (renal calculus), break milk prodution, emmenagogue, urinary infection, cholagogue	2.0
Marrubium vulgare L. leaves	Labiatae	Maltese fever, malarial, fever, asthma, diaphoretic, dental abscesses, expectorant, emmenagogue, cirrhosis, hepatopathy, sedative, stomachic	13.8
Marrubium vulgare L. roots	Labiatae	Dysmenorrhea, antipyretic, stomach disorders	4.8
Mentha pulegium L. leaves	Labiatae	Helminthiasis, abdominal pains, antitussive, bronchodilator	8.3
Rubus ulmifolius Schott. leaves	Rosaceae	Diarrhoea, diuretic, intestinal astringent, menstrual pain, cycle regulator, menopause disorders, wounds, liver diseases, antitussive, antiseptic, aphtha, gingivitis, hypertension, toothache antidiabetic	7.5
Smilax aspera L. roots	Liliaceae	Cicatrizant, laxative, colagogue, diuretic, diaphoretic, asthma, cystitis, intestinal diseases and anti-inflammatory, cough, stomatitis, antispastic, febrifuge, emmenagogue antirheumatic, gout	8.0
Teucrium flavum L. flowers	Labiatae	Antipyretic, cicatrizant, antiseptic	14.2
Teucrium polium L. leaves	Labiatae	Myalgias, vulnerary, stomach pains, cold, menopause disorders, sedative for toothache, against insect bites	9.5
Urtica dioica L. leaves	Urticaceae	Antihemorrhoidal, carminative, digestive, antimyalgic, stomachic, hepatobiliary, hypoglycemic, antihyperuricemic, antirheumatic, liver diseases, cleansing agent, baldness, dandruff, neuralgias, antianemic, diuretic, hemostatic, local astringent (skin), galactogogue, revulsive, cough sedative	3.3
Vinca sardoa (Stearn) leaves	Apocynaceae	Antidiabetic, astringent, galactofuge, hypotensive, sedative (nausea), antitubercular, eupeptic, artritis (knee)	23.8

flavonoid glycosides and caffeic acid derivatives; the only paper on *Rubus ulmifolius* phytochemical composition described the isolation of anthrone derivatives with antimicrobial activity (Flamini, Catalano, Caponi, Panizzi, & Morelli, 2002).

2. Materials and methods

2.1. Chemicals, chromatographic and spectroscopic measurements

Malonic acid, manganese (II) sulphate monohydrate, $NaIO_3$, Na_2CO_3 anhydrous, resorcinol (1,3-benzenediol), (all reagent grade >99%) were purchased from Merck. Gallic acid (3,4,5-trihydroxy benzoic acid, Riedel-de Haën), 2,6-DHBA (2,6-dihydroxy benzoic acid, Aldrich), $K_2S_2O_8$, ABTS (2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid)), Folin–Ciocalteu reagent (FC), DPPH (2,2-diphenyl-1-picrylhydrazyl), $HClO_4$ and H_2O_2 were purchased from Fluka and Trolox (6-hydroxy-2,5,8-tetrametylchroman-2-carboxylic acid) from Aldrich. $HClO_4$ was analyzed by titration *vs.* a standard 0.1 M NaOH solution (from Merck). H_2O_2 was standardized daily by manganometric analysis. All stock solutions were prepared with double distilled (dd), deionized water.

For chromatographic separations Silica gel (Merck), Silica gel plates (Merck), Sephadex LH20 were used. Semipreparative HPLC was performed on a Gilson series 305 liquid chromatograph using a LiChrosphere 100 RP-18 column (particle size 10 μm, 250 × 10 mm ID, Merck).

Spectrophotometrical measurements were performed on a Perkin–Elmer Lambda-25 spectrophotometer operating with the UV-Winlab software. NMR spectra in CD_3OD or in $CDCl_3$ (Sigma) were obtained using a Bruker AMX-300, spectrometer, operating at 300.13 MHz for ^1H NMR and 75.03 MHz for ^{13}C NMR. 2D experiments, ^1H–^1H DQF-COSY, NOESY and inverse-detected ^1H–^{13}C HMQC and ^1H–^{13}C HMBC spectra were performed using UXNMR software. Exact masses were measured by an API-TOF spectrometer (Mariner Biosystem). Samples were diluted in a mixture of H_2O/AcCN 1/1 with 0.5% NH_3 and directly injected at a flow rate of 10 μL/min.

2.2. Plants source and extracts preparation

Plant materials were collected and identified by Dr. M.C. Loi of the Department of Botanical Sciences of the University of Cagliari (Italy) as indicated in Table 1. Plants were dried at room temperature. A voucher of each sample is deposited at the Department of Pharmaceutical Sciences of the University of Padova (Italy).

Dried powdered material (50 g) was extracted at room temperature for 5 min in an ultrasound bath, with metha-

nol (100 mL × 5 times). The solvent was removed under vacuum. Yields of extractions are reported in Table 1.

2.3. In vitro relative antioxidant activity

2.3.1. Antioxidant activity assay based on the Briggs–Rauscher (BR) reaction

The chemical *in vitro* method reported by Cervellati, Renzulli, Guerra, and Speroni (2002) is based on the inhibitory effects by free radical scavengers on the oscillations of the BR reaction. In brief, when antioxidant scavengers of free radicals are added to an active oscillating BR mixture there is an immediate quenching of the oscillations, an inhibition time (t_{inhib}) that linearly depends on the concentration of the antioxidant added, and a subsequent regeneration of the oscillations. Relative antioxidant activity (r.a.c.) with respect to a substance chosen as standard (resorcinol, Re) is determined on the basis of concentrations of sample and resorcinol that give the same t_{inhib}; r.a.c. is expressed as μg/mL resorcinol equivalents (Cervellati et al., 2002).

2.3.2. Antioxidant activity based on the TEAC assay

We used the protocol suggested by Re et al. (1999). Antioxidant activity is expressed as Trolox equivalent (mM).

2.3.3. Antioxidant activity based on the DPPH assay

The scavenging activity towards the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was measured as previously reported (Hatano, Kagawa, Yasuhara, & Okuda, 1988). A linear range of concentration *vs*. % decrease of absorbance was observed and was used for the determination of EC_{50} (μg/mL).

2.4. Determination of total phenolics (antioxidant reducing capacity quantification)

This test is based on the oxidation of phenolic groups by phosphomolybdic and phosphotungstic acids (FC reagent). After oxidation the absorbance of a green–blue complex can be measured at 765 nm. We used the procedure for 20 mL total volume of the reacting mixture (Singleton & Rossi, 1965). Total phenolic content is expressed as gallic acid equivalents (GAE) in mg/L.

2.5. Phytochemical investigation on R. ulmifolius

A methanol extract of *R. ulmifolius* (12 g) was suspended in methanol, applied to a Sephadex LH20 column (350 mL) and eluted with methanol. Fractions were pooled in seven groups on the basis of their chromatographic behaviour on TLC (using as eluents chloroform/methanol 90/10 or chloroform/methanol/water 10/5/1 or butanol/acetic acid/water 20/5/2). Fractions 5 (800 mg) and 6 (650 mg) were subjected to semipreparative HPLC (using as eluents mixtures acetonitrile/water 0.1% formic acid or methanol/water 0.1% formic acid). Different gradient conditions were used: from 90% water to 45% in 40 min; from 80% water to 55% in 45 min using methanol as organic phase; from 90% water to 60% in 15 min; from 60% water to 40% in 30 min using acetonitrile as organic phase. Solvents were removed under vacuum and residue aqueous layers were freeze dried yielding compounds **1**–**9** (Fig. 1). Compounds were characterized on the basis of MS spectra, both 1D and 2D NMR experiments (including HMQC, HMBC, COSY and NOESY) and by comparison with literature data or authentic samples. Isolated compounds were as follows: **1** kaempferol-3-O-glucuronide, 20.2 mg (Agrawal, 1989); **2** quercetin-3-O-glucuronide, 16.5 mg (Agrawal, 1989); **3** caffeic acid, 10.2 mg (spectral data compared with authentic sample from Sigma); **4** ferulic acid, 10.5 mg (spectral data compared with authentic sample from Sigma); **5** kaempferol-3-O-(6″-*p*-coumaroyl)-β-D-glucopyranoside, 3.3 mg (Calzada, Cedillo-Rivera, & Mata, 2001); **6** kaempferol-3-O-(6″-feruloyl)-β-D-glucopyranoside, 2.0 mg (Calzada et al., 2001); **7** 3-caffeoyl quinic acid, 22.5 mg (Chlorogenic acid) (spectral data compared with authentic sample from Sigma); **8** 4-caffeoyl quinic acid, 3.5 mg (Nakatani et al., 2000); **9** 5-caffeoyl quinic acid, 2.5 mg (Nakatani et al., 2000).

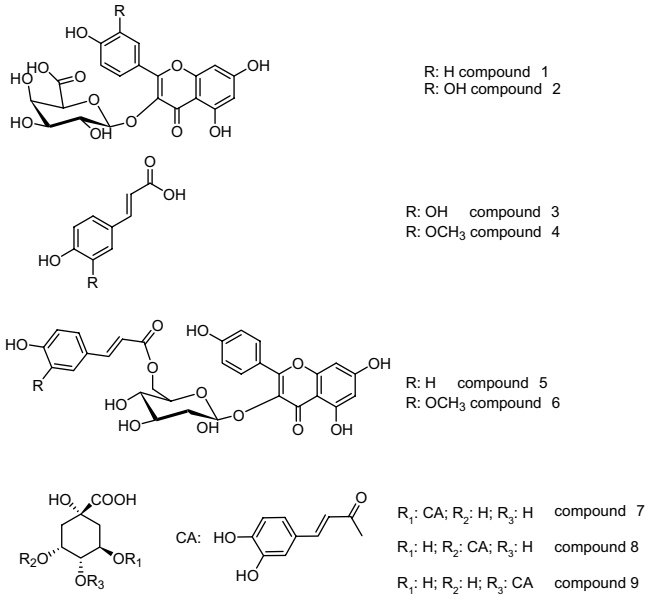

Fig. 1. Structures of compounds isolated from *Rubus ulmifolius*.

3. Results

3.1. In vitro relative antioxidant activity and total phenolic content

The antioxidant data for the examined plant extracts are reported in the first three column of Table 2. In the fourth column the values of the total phenolic content (total reducing power, GAE) are reported.

Table 2
Relative antioxidant capacity and total phenolic content (total reducing power) of the plant extracts

Botanical species	$(a.r.c.)_m \pm \sigma$, μg/mL Re	$(TEAC)_m \pm \sigma$, mM Trolox	(DPPH) $EC_{50} \pm \sigma$, μg/mL	$(GAE)_m \pm \sigma$, mg/L GA
Arundo donax leaves	0.020 ± 0.002	0.37 ± 0.05	159.4 ± 4.0	0.47 ± 0.02
Calendula arvensis	0.024 ± 0.002	0.46 ± 0.06	80.9 ± 1.5	0.47 ± 0.03
Cerastium glomeratum	0.021 ± 0.002	0.41 ± 0.03	203.2 ± 10.0	0.47 ± 0.04
Eryngium campestre	0.021 ± 0.002	0.59 ± 0.04	216.0 ± 4.0	0.44 ± 0.04
Marrubium vulgare leaves	0.018 ± 0.001	1.1 ± 0.2	25.4 ± 1.1	0.69 ± 0.09
Marrubium vulgare roots	0.018 ± 0.001	0.9 ± 0.2	69.0 ± 2.5	0.92 ± 0.03
Mentha pulegium	0.077 ± 0.002	1.4 ± 0.1	8.30 ± 0.50	1.3 ± 0.5
Rubus ulmifolius	0.09 ± 0.01	3.8 ± 0.3	5.10 ± 0.5	2.76 ± 0.08
Smilax aspera	0.021 ± 0.002	0.35 ± 0.02	84.2 ± 4.50	0.46 ± 0.01
Teucrium flavum	0.071 ± 0.007	1.0 ± 0.2	27.7 ± 0.5	1.72 ± 0.09
Teucrium polio	0.049 ± 0.003	0.9 ± 0.1	15.40 ± 0.90	0.9 ± 0.1
Urtica dioica	0.013 ± 0.001	0.46 ± 0.07	419.0 ± 10.0	0.35 ± 0.02
Vinca sardoa	0.033 ± 0.003	0.53 ± 0.07	44.5 ± 0.6	0.50 ± 0.01

The antioxidant activity at acidic pH (BR method, μg/mL Re eq.), was ranging from 0.09 ± 0.01 of *R. ulmifolius* to 0.013 ± 0.001 of *U. dioica*. For comparison purposes, the BR antioxidant activity of methanolic extracts of aerial and root parts of *Leontopodium alpinum* Cass. (Edelweiss) ranges from 0.040 ± 0.005 to 0.025 ± 0.006 (Speroni et al., 2006). The BR activity of a methanolic extract from *Wulfenia carinthiaca* Jacq. was determined as 0.15 ± 0.01, but this extract contains three very active phenylpropanoid glycosides (Cervellati et al., 2004). The activities at pH = 7.4 (TEAC method, mM Trolox eq.) show a great variability, ranging from 3.8 ± 0.3 for *R. ulmifolius* to 0.35 ± 0.02 of *Smilax aspera* extracts, respectively (Table 2).

With the DPPH method in methanolic solutions the results show also a great variability ranging from 8.3 ± 0.5 μg/mL for *R. ulmifolius* to 419 ± 10 μg/mL of *U. dioica*, respectively.

As far as the total phenolic content is concerned, here also data present a great variability, ranging from 2.76 ± 0.08 mg/L GAE for *R. ulmifolius* to 0.35 ± 0.02 mg/L GAE for *U. dioica*. Taking into account both the values of the antioxidant activity at acidic and physiological pH, and the values of the total phenolic content, the extract that probably contains the greater amount of polyphenols is that of *R. ulmifolius*, followed by *Teucrium flavum*, *M. vulgare* leaves and *Mentha pulegium*.

Table 3
Antioxidant activity of the isolated compounds

Compound	BR method ($(r.a.c)_m$, μg eq. Re)	TEAC method ($(TEAC)_m$, mM eq. Trolox)
1	0.103 ± 0.005	0.80 ± 0.04
2	0.143 ± 0.001	0.37 ± 0.01
3	1.68 ± 0.08	1.04 ± 0.06
4	2.0 ± 0.1	1.70 ± 0.05
5	6.0 ± 0.4	3.2 ± 0.1
7	3.2 ± 0.2	1.4 ± 0.1
8	5.0 ± 0.1	3.2 ± 0.1

Antioxidant activity of the isolated compounds was also measured (except for compounds **6** and **9** which have been isolated in too low quantity to perform the tests).

Among the tested compounds the chlorogenic acid derivatives (**7** and **8**) resulted the most active. The isolated flavonoids (**1**, **2** and **5**) resulted active too and in particular, the compound **5** showed remarkably high activity (Table 3). This effect could be explained because of the presence of the *p*-coumaric unit, which probably gave its contribution to the antioxidant activity of the compound **5**. The activity of isolated compounds is in agreement with previously published data on the phenolic derivatives rosmarinic acid, cynarin and phenylpropanoid glycosides (Cervellati et al., 2002, 2004).

4. Discussion

R. ulmifolius extract showed the higher antioxidant activity between the considered extracts with all the different *in vitro* methods used (BR, DPPH, TEAC) despite the different experimental conditions.

The BR method works at pH ≈ 2, similar to that of the human gastric juice. Kanner and Lapidot (2001) observed that some plant-derived antioxidants are able to prevent the lipid peroxidation, amplified in the acidic pH of gastric fluid. The conception of the stomach as a bioreactor, where ROS and food nutrients interact, underlines the importance of determining antioxidant activity of dietary sources at acidic pH. Moreover, since different testing methods give different ranking orders of antioxidant capacity due to different experimental conditions (radicals produced in the reacting mixture, pH of the mixture, solvent of the system, chosen standard, etc.), at least two different testing methods should be used in order to obtain a realistic estimate of the antioxidant activity of pure polyphenolic substances or mixtures (Prior, Wu, & Schaich, 2005). The Folin–Ciocalteu reagent method suffers from a number of interfering substances present in plants, such as ascorbic acid, sulphur-containing compounds, mono- and disaccharides, etc. (Huang, Ou, & Prior, 2005). Despite of its limitations for quantifying phenolic compounds in plant extracts, the

FC method is the recommended method for measurement of total reducing capacity (Prior et al., 2005).

Correlations between the different methods were obtained by Pearsons' correlation coefficient R in bivariate correlation. Good correlation were found between the GAE and BR and TEAC tests: $R = 0.8949$ ($p < 0.001$), $R = 0.9231$ ($p < 0.001$), respectively. Significant correlation was also observed between GAE and DPPH method: $R = 0.8804$ ($p < 0.005$). Those data are quite in agreement with the fact that the total reducing capacity is related to the antioxidant capacity of the extract. Satisfactory correlation was obtained between BR and DPPH: $R = 0.8721$ ($p < 0.005$), while poorer correlation was observed between BR and TEAC: $R = 0.7677$ ($p < 0.01$) suggesting, as mentioned above, that different experimental conditions yield different antioxidant ranking orders due to the variations of the polyphenols' behaviour at different pH and in different experimental conditions.

Our results on 11 species used in the traditional medicine of Sardinia showed that some of these species possess remarkable radical-scavenging activity. In particular, *R. ulmifolius* extract showed high activity compared to that of other extracts. Its strong antioxidant capacity could be related, at least in part, to the activity of caffeic acid, ferulic acid and caffeic quinic esters as well as quercetin-3-O-glucuronide, kaempferol-3-O-glucuronide found in this extract. Further studies are needed to investigate the *in vivo* pharmacological properties of this extract because with its high activity *R. ulmifolius* could be considered as a possible new antioxidant ingredient for the nutraceutical or functional-food market.

References

Agrawal, P. K. (1989). *Carbon-13 NMR of flavonoids*. Amsterdam: Elsevier.

Calzada, F., Cedillo-Rivera, R., & Mata, R. (2001). Antiprotozoal activity of the constituents of *Conyza filaginoides*. *Journal of Natural Products, 64*, 671–673.

Cervellati, R., Renzulli, C., Guerra, M. C., & Speroni, E. (2002). Evaluation of antioxidant activity of some natural polyphenolic compounds using the Briggs–Rauscher reaction method. *Journal of Agricultural and Food Chemistry, 50*, 7504–7509.

Cervellati, R., Speroni, E., Govoni, P., Guerra, M. C., Costa, S., Arnold, U. W., et al. (2004). *Wulfenia carinthiaca* Jacq. Antioxidant and pharmacological activities. *Zeitschrift für Naturforschung C, 59*, 255–262.

Flamini, G., Catalano, S., Caponi, C., Panizzi, L., & Morelli, I. (2002). Three anthrones from *Rubus ulmifolius*. *Phytochemistry, 59*, 873–876.

Gulcin, I., Kufrevioglu, O. I., Oktay, M., & Buyukokuroglu, M. E. (2004). Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). *Journal of Ethnopharmacology, 90*, 205–215.

Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: Their relative astringency and radical scavenging effects. *Chemical and Pharmaceutical Bulletin, 36*, 2090–2097.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*, 1841–1856.

Kadifkova Panovska, T., Kulevanova, S., & Stefova, M. (2005). *In vitro* antioxidant activity of some *Teucrium species* (Lamiaceae). *Acta Pharmaceutica, 55*, 207–214.

Kanner, J., & Lapidot, T. (2001). The stomach as a bioreactor: Dietary lipidic peroxidation in the gastric fluid and the effects of plant-derived antioxidants. *Free Radical Biology and Medicine, 21*, 1388–1395.

Ljubuncic, P., Dakwar, S., Portnaya, I., Cogan, U., Azaizeh, H., & Bomzon, A. (2006). Aqueous extracts of *Teucrium polium* possess remarkable antioxidant activity *in vitro*. *Evidence-Based Complementary and Alternative Medicine, 3*, 329–338.

Matkowsky, A., & Piotrowska, M. (2006). Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae. *Fitoterapia, 77*, 346–353.

Nair, S., Li, W., & Kong, A. T. (2007). Natural dietary anti-cancer chemopreventive compounds: Redox-mediated differential signaling mechanisms in cytoprotection of normal cells versus cytotoxicity in tumor cells. *Acta Pharmacologica Sinica, 28*, 459–472.

Nakatani, N., Kayano, S., Kikuzaki, H., Sumino, K., Katagiri, K., & Mitani, T. (2000). Identification, quantitative determination and antioxidative activities of chlorogenic acid isomers in Prune (*Prunus domestica* L.). *Journal of Agricultural and Food Chemistry, 48*, 5512–5516.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolic in foods and dietary. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. A. (1999). Antioxidant activity applying an improved $ABTS^{\cdot+}$ radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Sahpaz, S., Garbacki, N., Tits, M., & Bailleul, F. (2002). Isolation and pharmacological activity of phenylpropanoid esters from *Marrubium vulgare*. *Journal of Ethnopharmacology, 79*, 389–392.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Speroni, E., Schwaiger, S., Egger, P., Berger, A. T., Cervellati, R., Govoni, P., et al. (2006). *In vivo* efficacy of different extracts of edelweiss (*Leontopodium alpinum* Cass.) in animal models. *Journal of Ethnopharmacology, 105*, 421–426.

VanderJagt, T. J., Ghattas, R., VanderJagt, D. J., Crossey, M., & Glew, R. H. (2002). Comparison of the total antioxidant content of 30 widely used medicinal plants of New Mexico. *Life Sciences, 70*, 1035–1040.