Antioxidant Activity In Vitro of Selected Peruvian Medicinal Plants

O. Lock, P. Castillo, V. Doroteo and R. Rojas
Departamento de Química
Pontificia Universidad Católica del Perú
Aptdo. Postal 1761
Lima 100
Peru

Keywords: DPPH, free radicals, radical scavengers, traditional medicine

Abstract

Biomolecules can be oxidized by free radicals. This oxidative damage has an important etiological role in aging and the development of diseases like cancer, atherosclerosis, and other inflammatory disorders. Synthetic antioxidants, like butylated hydroxyanisole, are good free radical scavengers, however, the synthetic antioxidants can be carcinogenic. Therefore, there is an increasing interest in searching for antioxidants of natural origin. We report here the results of a screening for antioxidant activity of 53 ethanolic extracts from 40 Peruvian plants used in traditional medicine for the treatment of several infectious and inflammatory diseases. The antioxidant activity in vitro was measured by means of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. Rutin, a natural antioxidant, was used as a control. Twenty one (39.6%) of the plant extracts showed an EC_{50} lower than 50 μg/ml. The plants with the highest activity were *Gentianella nitida, Iryanthera lancifolia, Lepechinia meyenii, Oenothera multicaulis, Philodendron solimoesense* and *Tetracera volubilis*, which showed an EC_{50} of 13.70, 14.08, 16.65, 16.89, 8.80, and 5.29 μg/ml, respectively. The crude ethanolic extract of *Tetracera volubilis* has better antioxidant activity in vitro than the pure natural antioxidant rutin (EC_{50} = 7.16 μg/ml).

INTRODUCTION

The oxidative damage caused by free radicals is considered to be related to the development of diseases like atheroesclerosis, cancer, brain dysfunction, arthritis and other inflammatory disorders (Halliwell, 1991; Finkel and Holbrook, 2000). Several synthetic antioxidants, like butylated hydroxyanisole or butylated hydroxytoluene, are effective free radical scavengers, however, they are being restricted because they can be carcinogenic (Ito et al., 1983; Safer and Al-Nughamish, 1999). Thus, there is a growing interest in searching for antioxidants of natural origin, especially those present in medicinal plants.

The purpose of this study is to evaluate the in vital antioxidant activity of 40 plants used in Peruvian traditional medicine for the treatment of several infectious and inflammatory disorders. Plants that have been used extensively for the treatment of inflammatory or infectious diseases may contain radical scavengers compounds that could be used as natural antioxidants.

MATERIAL AND METHODS

Plant Material

Plants were collected between May and July 2001 from five different regions of Peru (Table 1). Plants were collected and identified by botanists Irma Fernández (IF), Joaquina Albán (JA), Alfredo Tupayachi (HV), Abundio Sagástegui (AS) and Genaro Yarupaitán (GY). The respective voucher specimens are deposited at the Department of Chemistry of the Pontificia Universidad Católica del Perú, in Lima.

Air-dried powdered plant material was extracted by percolation at room temperature with 95% ethanol. The solvent was then evaporated to dryness under reduced

pressure at a temperature lower than 40°C. Yield of extracts in terms of dry starting materials are listed in Table 1. For the antioxidant assay, the extracts were resuspended in ethanol.

Antioxidant Assay

The antioxidant activity in vital was measured by means of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. When DPPH is dissolved in ethanol at room temperature, DPPH produces a violet solution containing the stable DPPH radical. In the presence of an antioxidant compound, the DPPH radical is reduced, producing decoloration of the solution.

The DPPH free radical assay was carried out according to the method of Mensor et al. (2001), with some modifications. Briefly, one ml of a 0.3 mM DPPH ethanol solution was added to 2.5 ml of sample solutions (final concentrations of 10 and 50 μg/ml) and the mixtures were incubated at room temperature for 30 minutes. The absorbances were then measured at 517 nm using a spectrophotometer (Perkin Elmer, Lambda 2). The percentage antioxidant activity (%AA) was calculated according to the following formula:

$$\%AA = \left(\frac{Abs_{control} - (Abs_{sample} - Abs_{blank})}{Abs_{control}} \right) \times 100\%$$

Abs_{sample}= 1 ml 0.3 mM DPPH + 2.5 ml plant extract solution
Abs_{blank} = 1 ml ethanol + 2.5 ml plant extract solution
$Abs_{control}$ = 1 ml DPPH 0.3 mM in ethanol + 2.5 ml ethanol
Rutin (Sigma, St. Louis, MO) was used as a positive control.

Those extracts with the highest antioxidant activity (more than 25% AA at a plant extract concentration of 10 μg/ml) were diluted at 6 different concentrations between 1 and 30 μg/ml in order to assess their EC_{50}. The EC_{50} values were obtained by extrapolation from linear regression analysis and denote the concentration of plant extract required to scavenge 50% of the DPPH free radicals. The results shown in Table 1 are mean values of at least three independent determinations.

RESULTS AND DISCUSSION

Table 1 summarizes the antioxidant activities in vital of 53 ethanolic extracts from 40 Peruvian medicinal plants belonging to 21 different families. Twenty one (39.6%) of the plant extracts have an EC_{50} lower than 50 μg/ml. The plants with the highest antioxidant activities were *Gentianella nitida*, *Iryanthera lancifolia*, *Lepechinia meyenii*, *Oenothera multicaulis*, *Philodendron solimoesense* and *Tetracera volubilis*.

An important hypoglycemic activity was demonstrated for the *G. nitida* water extract in two rat models, which is attributed to the presence of several xanthones (Lacaille-Dubois et al., 1996; Callo et al., 2001). No antioxidant activities have been reported previously for this plant.

The *I. lancifolia* stems and bark ethanol extracts showed a strong DPPH radical scavenging activity. Silva et al. (1999) demonstrated that an extract from the pericarps of *I. lancifolia* inhibits the lipid peroxidation of rat brain homogenates. This activity is due to the presence of two flavonolignans and the compounds iryantherin K and L. It is not known yet if these compounds are also able to scavenge DPPH radicals.

L. meyenii is popularly used for the treatment of cough and bronchitis. Several terpenes have been isolated from this plant (Mango et al., 1990; Bruno et al., 1991), however, none of those compounds have been investigated for antioxidant actity.

No previous phytochemical or pharmacological investigations have been done on *O. multicaulis*, *P. solimoesense* and *T. volubilis*.

It is noteworthy that the crude extracts of the stems of *P. solimoesense* and the

inner bark of *T. volubilis* have EC_{50} values very similar to the pure compound rutin, a natural antioxidant present in several plants.

CONCLUSIONS

The screening for antioxidant activities in vitro of 53 ethanolic extracts from 40 Peruvian medicinal plants used for the treatment of infectious or inflammatory diseases have provided 21 extracts with strong DPPH free radical scavenging activity. The crude ethanolic extract of the inner bark of *T. volubilis* has even better antioxidant activity than the pure compound rutin, a natural antioxidant present in several plants.

An assay-guided fractionation of the most active plant extracts is currently in progress in order to isolate the respective active principles, which could be used as new natural antioxidants.

ACKNOWLEDGEMENTS

The authors wish to gratefully acknowledge the financial support of the Dirección Académica de Investigación of the Pontificia Universidad Católica del Perú; Grant DAI-010700000110. We would also like to thank the technical assistance of Milka Cajahuanca.

Literature Cited

Bruno, M., Savona, G., de la Torre, M.C., Rodríguez, B. and Marlier, M. 1991. Abietane diterpenoids from *Lepechinia meyenii* and *Lepechinia hasttata*. Phytochemistry 38:2339-2343.

Callo, N., Lock, O.R., Alvarez, C.M. and Jurupe, H. 2001. Xantonas y actividad hipoglicemiante de *Gentianella nitida* y *G. tristicha*. Bol. Soc. Quím. Peru 67:195-206.

Finkel, T. and Holbrook, N.J. 2000. Oxidants, oxidative stress and biology of ageing. Nature 408:239-247.

Halliwell, B. 1991. Reactive oxygen species in living systems: source, biochemistry and role in human disease. Am. J. Med. 91:14-22.

Ito, N., Fukushima, S., Hassegawa, A., Shibata, M. and Ogiso, T. 1983. Carcinogenicity of butylated hydroxyanisole in F 344 rats. J. Natl. Cancer. Inst. 70:343-347.

Lacaille-Dubois, M-A., Galle, K. and Wagner, H. 1996. Secoiridoids and xanthones from *Gentianella nitida*. Planta Med. 62:365-367.

Mango, R., Chávez, J. and Lock de Ugaz, O. 1990. Sesquiterpene guaiol from *Lepechinia meyenii*. Rev. Lat. Quím. 21:63-66.

Mensor, L.L., Menezes, F.S., Leitao, G.G., Reis, A.S., dos Santos, T.C., Coube, C.S. and Leitao, S.G. 2001. Screening of Brazilian plant extract for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 15:127-130.

Safer, A.M. and Al-Nughamish, A.J. 1999. Hepatotoxicity induced by the antioxidant food additive butylated hydroxytoluene (BHT) in rats: An electron microscopical study. Histol. Histopathol. 14:391-406.

Silva, D.H.S., Davino, S.C., de Moraes Barros, S.B. and Yoshida, M. 1999. Dihydrochalcones and flavonolignans from *Iryanthera lancifolia*. J. Nat. Prod. 62:1475-1478.

Tables

Table 1. Antioxidant activity in vitro of 53 ethanolic plant extracts.

Botanical species (Voucher #)	Family	Plant part[1]	Extract yield (%)[2]	%AA		EC_{50} (μg/ml)
				10 μg/ml	50 μg/ml	
Aloysia scorodonioides (IF1544)	Verbenaceae	AP	19.3	10.42	66.40	-
Ambrosia arborescens (IF1517)	Asteraceae	L,S	14.4	0.91	25.25	-
Aspidosperma rigidum (JA13629)	Apocynaceae	B	32.7	10.33	36.69	-
		L	8.0	2.62	3.60	-
		S	0.7	0.19	12.68	-
Cestrum auriculatum (JA13602)	Solanaceae	L	10.3	3.76	13.98	-
		S	5.9	1.14	7.72	-
Chuquiraga spinosa (IF1515)	Asteraceae	AP	25.5	6.36	28.70	-
Croton ruizianus (JA13626)	Euphorbiaceae	L	13.4	22.11	87.85	-
		S	8.2	10.46	47.82	-
Cynanchum corystephanum (IF1543)	Asclepiadaceae	AP	17.5	6.26	15.79	-
Desmodium molliculum (IF1529)	Fabaceae	AP	9.8	23.92	89.92	-
Euterpe precatoria (JA13630)	Arecaceae	R	2.3	6.69	35.90	-
Flaveria bidentis (IF1518)	Asteraceae	AP	44.8	15.27	57.71	-
Gentianella bicolor (AS14408)	Gentianaceae	WP	20.1	20.53	80.07	-
Gentianella nitida (GY2150)	Gentianaceae	WP	24.6	36.50	91.10	13.70
Gentianella weberbaueri (GY2165)	Gentianaceae	WP	24.4	25.49	87.54	24.15
Guatteria megalophylla (JA13660)	Annonaceae	L	8.7	0.30	5.93	-
		S	4.8	2.58	19.52	-
Guatteria modesta (JA13664)	Annonaceae	L,S	3.8	0.0	12.64	-
Hedychium coronarium (JA13665)	Zingiberaceae	WP	3.8	0.0	12.76	-
Iochroma umbellatum (JA13624)	Solanaceae	L	8.5	0.46	6.83	-
		S	6.4	2.20	21.70	-
Iryanthera lancifolia (JA13656)	Myristicaceae	L	10.3	15.41	67.02	-
		S	5.1	41.21	91.84	17.59
		B	12.9	41.49	90.90	14.08
Jungia paniculata (IF1523)	Asteraceae	AP	8.3	16.35	85.83	-
Justicia sericea (IF1541)	Acanthaceae	AP	22.9	1.44	14.84	-
Lavatera arborea. (IF1542)	Malvaceae	L	11.8	0.40	6.54	-
Lepechinia meyenii (IF1530)	Lamiaceae	AP	11.9	42.96	92.24	16.65
Mutisia cochabambensis (HV3020)	Asteraceae	AP	19.3	24.39	88.29	-
Oenothera multicaulis (IF1533)	Onagraceae	R	13.8	8.35	38.39	-
		AP	4.6	41.89	95.23	16.89
Ophryosporus peruvianus (JA13591)	Asteraceae	L,S	4.3	5.47	10.85	-
Philodendron solimoesense (JA13600)	Araceae	S	14.1	61.82	92.43	8.80
Sambucus mexicana (JA13594)	Caprifoliaceae	L	17.1	3.64	16.49	-
		S	3.2	5.07	14.47	-
		F	6.7	18.54	26.13	-
Sanguisorba officinalis (IF1526)	Rosaceae	AP	18.3	23.62	89.47	-
Satureja elliptica (IF1525)	Lamiaceae	AP	10.0	18.61	74.31	-
Senecio culcitioides (IF1531)	Asteraceae	AP	13.3	1.16	15.49	-
Senecio violaefolius (IF1532)	Asteraceae	AP	27.8	3.17	20.53	-
Tetracera volubilis (JA13528)	Dilleniaceae	S	13.1	69.14	95.47	6.92
		IB	17.9	83.96	94.73	5.29
Werneria caespitosa (IF1537)	Asteraceae	WP	3.1	9.09	42.10	-
Werneria digitata (GY1471)	Asteraceae	WP	13.8	8.77	38.25	-
Werneria nubigena (IF1535)	Asteraceae	WP	15.9	4.64	17.97	-
Werneria strigosissima (GY2158)	Asteraceae	WP	21.7	3.93	15.38	-
Wigandia urens (JA13592)	Hydrophyllaceae	L	8.5	9.63	44.25	-
		S	4.4	6.08	41.82	-
Xenophyllum ciliolatum (GY2157)	Asteraceae	WP	17.8	22.83	85.24	-
Xenophyllum dactylophyllum (GY2162)	Asteraceae	WP	19.4	12.76	64.50	-
Xenophyllum decorum (GY2156)	Asteraceae	WP	19.2	6.04	25.29	-
Rutin	-	-	-	-	-	7.16

[1] Plant part: AP: aerial parts, B: bark, F: flowers, IB: inner bark, L: leaves, R: roots, S: stems, WP: whole plant

[2] Dry residue of the ethanolic extract in terms of dry starting material