Antioxidant Activity of Selected Medicinal Plants

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Commonly used medicinal plant extracts with standardized content of polyphenols were investigated for their total antioxidant activity (TAA). Green tea, oligomeric procyanidins (from grape seed and pine bark), bilberry, and ginkgo exhibited TAA in the range of 5.12−2.57 mM Trolox, thereby indicating a valuable antioxidant capacity. Witch hazel, propolis EPID, artichoke, and hawthorn afforded lower TAA (1.54−0.44 mM Trolox), whereas echinacea, ginseng, passionflower, sweet clover, and eleuthero were rather uneffective (TAA < 0.32 mM Trolox). Excipients normally used to prepare the extracts did not interfere with the assay, and a good correlation between the content of polyphenols and the TAA was assessed. The measured TAA was higher than those calculated from the content and antioxidant potential of specific components, as exemplified for green tea and ginkgo extracts. This may be attributed to the presence in these extracts of other substances with antioxidant capacity. On the other hand, some components (such as ginkgolides in ginkgo extract) insensitive to the TAA assay played an important antioxidant role in vivo. These results suggest that TAA determination is of interest for a comparative evaluation of in vitro antioxidant potential, but it needs to be combined with in vivo data for adequate assessment of the antioxidant capacity of medicinal plant extracts.

Keywords: *Medicinal plants; polyphenols; total antioxidant activity; total radical-trapping antioxidant parameter*

INTRODUCTION

In recent years growing evidence has been accumulated indicating the involvement of reactive oxygen species (ROS) in the pathogenesis of a number of diseases (Halliwell et al., 1992). Among the cellular and extracellular components, lipids, proteins, enzymes, DNA, and RNA form the major targets for these reactive species, and the resulting damages are associated with degenerative ("oxidative") diseases (Ames et al., 1993). Most living organisms possess efficient enzymatic and nonenzymatic defense systems against excess production of ROS. However, different external factors (smoke, diet, alcohol, some drugs) and aging decrease the capability of such protecting systems, resulting in disturbances of the redox equilibrium that is established in healthy conditions. Therefore, antioxidants that scavenge ROS may be of great value in preventing the onset and/or the progression of oxidative diseases (Willet, 1994).

Interestingly, many medicinal plants contain large amounts of antioxidants other than vitamin C, vitamin E, and carotenoids. The antioxidative effect is mainly due to phenolic components, such as flavonoids (Pietta, 1998), phenolic acids, and phenolic diterpenes (Shahidi et al., 1992).

Over the past few years, a number of medicinal plants have been investigated for their quenching activity of specific ROS, such as the hydroxyl radical, the superoxide anion, singlet oxygen, and lipid peroxides (Masaki et al., 1995; Yen et al., 1995). The capacity to scavenge these ROS has been evaluated by using different techniques based on spectrophotometry (Nishikimi et al., 1972), electron paramagnetic resonance (Rosen et al., 1984), and chemiluminescence (Robinson et al., 1997).

On the contrary, the total antioxidant activity (TAA) of medicinal plants, that is, their capacity to scavenge all species of free radicals, has been not evaluated. TAA values may be measured either by the oxygen radical absorbance capacity (ORAC) (Wang et al., 1996) or by the Trolox equivalent antioxidant capacity (TEAC) (Miller et al., 1996) method. The latter, which is based on the ability of an antioxidant to scavenge the preformed radical cation ABTS+ 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$) relative to that of the standard antioxidant Trolox, has been applied to different phenolic compounds and some of their metabolites (Rice-Evans et al., 1997). Recently, this approach has been also proved to be valuable for the measurement of the antioxidant potential of selected Italian wines (Simonetti et al., 1997). Therefore, it was of interest to extend this assay for the evaluation of total antioxidant capacity of herbs, most of them containing polyphenols and regarded as valuable phytomedicines.

MATERIALS AND METHODS

Dry Extracts. Artichoke, bilberry, echinacea, eleuthero, ginseng, grape seed (Leucoselect), green tea (Greenselect), *Ginkgo biloba* (Ginkgoselect), hawthorn, passionflower, sweet clover, and witch hazel dry extracts were from Indena (Milano, Italy); propolis EPID and grape skin dry extracts were obtained from Specchiasol (Verona, Italy); pine bark extract (Oligopin) was from Dèrivès Rèsiniques et Terpèniques (Dax,

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Table 1. Examined Plant Dry Extracts

extracts	% of reference component(s)	method
Crataegus m. (hawtorn)	1.8% vitexin 2″-*O*-rhamnoside	HPLC (Pietta, 1998)
Camellia sinensis (green tea)	70% catechins	HPLC (Pietta et al., 1998a)
Cynara scolymus (artichoke)	14% caffeoyl derivates, as chlorogenic acid	CE (Pietta et al., 1998b)
Echinacea purpurea	4.5% echinacoside	CE (Pietta et al., 1998b)
Eleutheroccus s.	1.5% eleutheroside B	CE (Pietta et al., 1994)
Ginkgo biloba (ginkgo)	11−24% flavon glycosides	HPLC (Pietta, 1998)
Hamamelis virg. (witch hazel)	16.7% tannis, as hamamelitannin	spectrophotometric (Markham et al., 1998)
Melilotus off. (sweet clover)	17.9% coumarin	spectrophotometric (Markham et al., 1998)
Vaccinium myrtillus (bilberry)	25% anthocyanins	HPLC (Gao et al., 1994)
proanthocyanidins (oligopin)	22% total flavanols	spectrophotometric (Simonetti et al., 1997)
proanthocyanidins (grape seed OPC)	39.6% total flavanols	spectrophotometric (Simonetti et al., 1997)
anthocyanins (grape skins)	40% total phenols	spectrophotometric (Simonetti et al., 1997)
Passiflora incarnata (passionflower)	4% isovitexin	HPLC (Pietta, 1998)
Panax ginseng	15% ginsenosides	HPLC (Pietta et al., 1986)
Propolis EPID	3.1% flavonoids	HPLC (Markham et al., 1998)

France). Each extract was analyzed in our laboratory for the content of the antioxidant active compounds (all phenolics except for ginseng) according to the literature (Table 1).

Sample Preparation. Fifty milligrams of each extract was dissolved in 100 mL of 50% ethanol.

Measurement of TAA. This spectrophotometric technique measures the relative abilities of antioxidants to scavenge the $ABTS^{\bullet+}$ in comparison with the antioxidant potency of standard amounts of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Aldrich Chemical Co., Gillingham, Dorset, U.K.). The radical cation $ABTS^{\bullet+}$, produced by the ferrylmyoglobin radical generated from metmyoglobin and H_2O_2 in the presence of the peroxidase, is a blue/green chromogen with characteristic absorption at 734 nm. The determination of the TAA was carried out using the Randox kit (Randox Laboratories Ltd., Ardmore, Diamond Road, Crumlin Co., Antrim, U.K.). Twenty microliters of sample solution was added to 1 mL of chromogen solution previously incubated at 37 °C for 6 min. At the start of the reaction and after 3 min, the absorbance was measured and compared with that of 1.25 mM Trolox. The TAA of each extract was calculated according to the following equations:

$$A_2 - A_1 = A \text{ of blank or sample or 1.25 mM Trolox}$$

$$\text{TAA (mM Trolox)} = [(1.25 \text{ mM Trolox}) \times (A_{\text{blank}} - A_{\text{sample}})]/A_{\text{blank}} - A_{\text{Trolox}}$$

In these equations A_1 is the absorbance at the start of the reaction and A_2 is the absorbance at 3 min.

In Vivo Study. Twelve healthy young volunteers, 20−25 years old, with a habitual low flavonoid intake and not taking antioxidants, were divided in three groups. After an overnight fast, one group ingested ∼1.6 g of ginkgo extract (equivalent to 400 mg of ginkgo flavonoids and 100 mg of ginkgolides), the second group ingested 600 mg of green tea extract (equivalent to 400 mg of total catechins), and the third received a placebo (maltodextrin). Vein blood samples were collected in EDTA-Eppendorf before and 1, 2, 3, 4, 5, and 6 h after the intake. The blood was immediately centrifuged at 10000*g* for 1 min, and the plasma was separated and assayed for total radical-trapping antioxidant potential (TRAP).

TRAP Assay. The method employed to assess the in vivo antioxidant activity of green tea or *Ginkgo biloba* extracts is based on the protection afforded by plasma against the decay of a fluorescent target, R-phycoerytrhin (R-PE), during a controlled peroxidation reaction (Ghiselli et al., 1995).

The reaction mixture consists of 1.550 μL of phosphate buffer [75 mM, pH 7.0 (PBS)] added to 20 μL of R-PE (1.5×10^{-8} M in PBS, Molecular Probes, Eugene, OR) and 80 μL of plasma diluted 1:10 with PBS. The solution was maintained at 37 °C in 10 mm quartz fluorometer cells under agitation, and the oxidation reaction was started after 5 min by adding 100 μL of ABAP (0.2 M, Wako Chemicals USA, Inc.) preincubated for 3 min a 37 °C. The decay of R-PE fluorescence was monitored every 20 s on a Perkin-Elmer LS 50B luminescence spectrometer (495 nm excitation wavelength and 575 nm emission wavelength). After 25 min, 60 μL of Trolox was added and the lag phase was compared to that induced by plasma.

RESULTS AND DISCUSSION

All of the medicinal plants considered in this study have been widely studied and their chemistry is well-known (Pietta, 1998; Wagner et al., 1996). Standardized dry extracts of these plants (Table 1) with precise content of characteristic constituents are available, and this allows preparation of phytoceuticals with known potency and reproducible response.

Most extracts were previously checked for their fingerprinting and content of characteristic components by means of HPLC. Otherwise, the percentage of specific compound classes was determined according to spectrophotometric methods. Quantitative data and related methods are shown in Table 1.

The TAA of the extracts was obtained by preparing solutions in 50% ethanol (c = 0.5 mg/mL) and adding an aliquot (20 μL) of the sample solution to the colored reaction mixture containing the ABTS radical cation. The antioxidants present in the extract scavenged this radical, resulting in a decolorization of the mixture proportional to their concentration and antioxidant capacity. The percentage color remaining at 3 min yielded the TAA. The values shown in Figure 1 are the mean of five determinations and represent the concentration (millimolar) of Trolox having the same antioxidant capacity as 0.5 mg/mL solution of each extract.

The extracts of green tea, grape seeds, and pine bark (proanthocyanidins) and bilberry, ginkgo, and red grape skin (anthocyanins) were found to exhibit higher TAA than extracts from witch hazel, artichoke, passionflower, echinacea, hawthorn, sweet clover, and eleuthero. Extracts of the same plant with different content of phenolic compounds were assayed, and a good correlation between the concentration of the phenolics and the total antioxidant capacity was found, as exemplified for ginkgo. Two other extracts commonly used (ginseng without phenolic components, and propolis, which is not obtained from an herb, but from bee glue) were tested. Propolis EPID had the highest value, followed by ginseng (Figure 1).

Excipients normally used (i.e., lactose, maltodextrins, and maltose) to prepare dry extracts did not act as antioxidants in this assay, as resulted from preliminary tests done on each excipient. On the other hand, it was not surprising to find that the antioxidant capacity

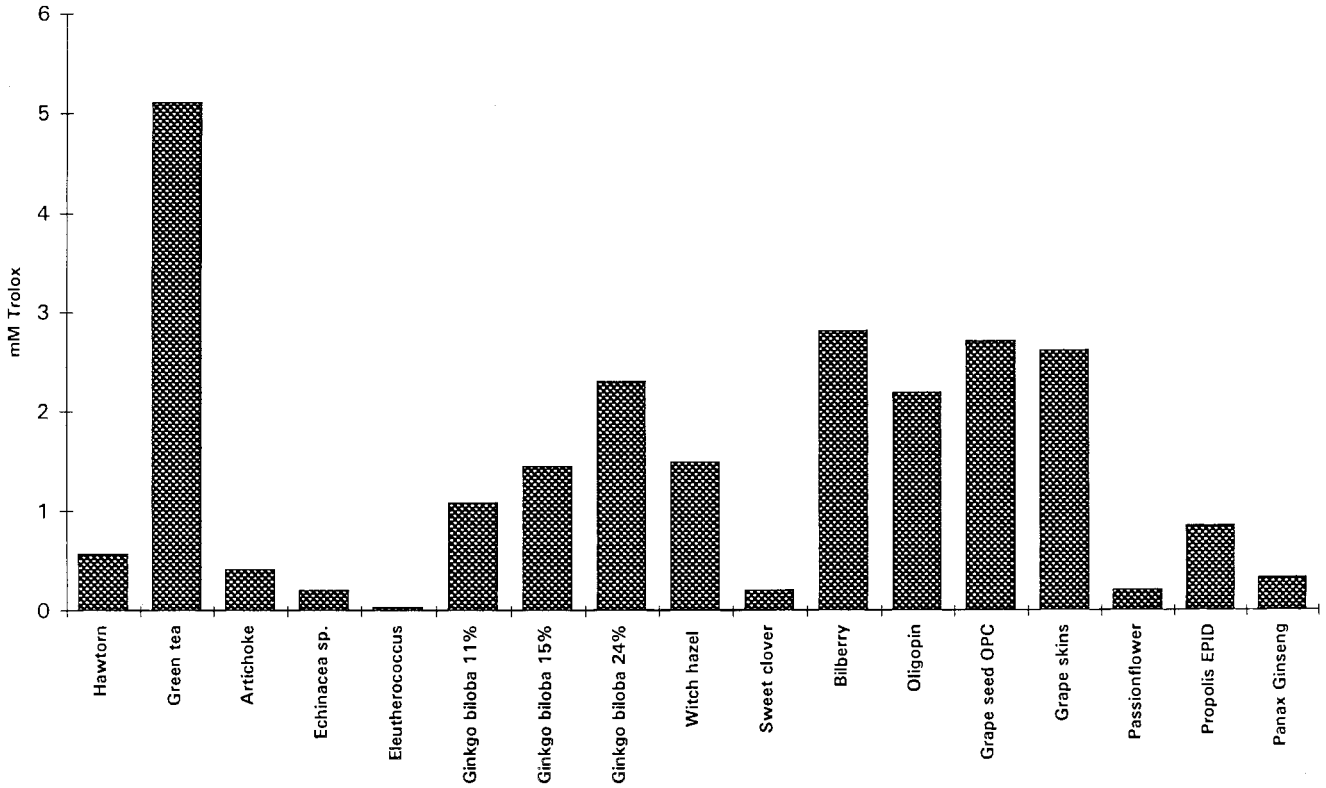

Figure 1. TAA of selected plant extracts ($c = 0.5$ mg/mL). Values are the mean of five determinations; relative standard deviations were in the range of 2−5%.

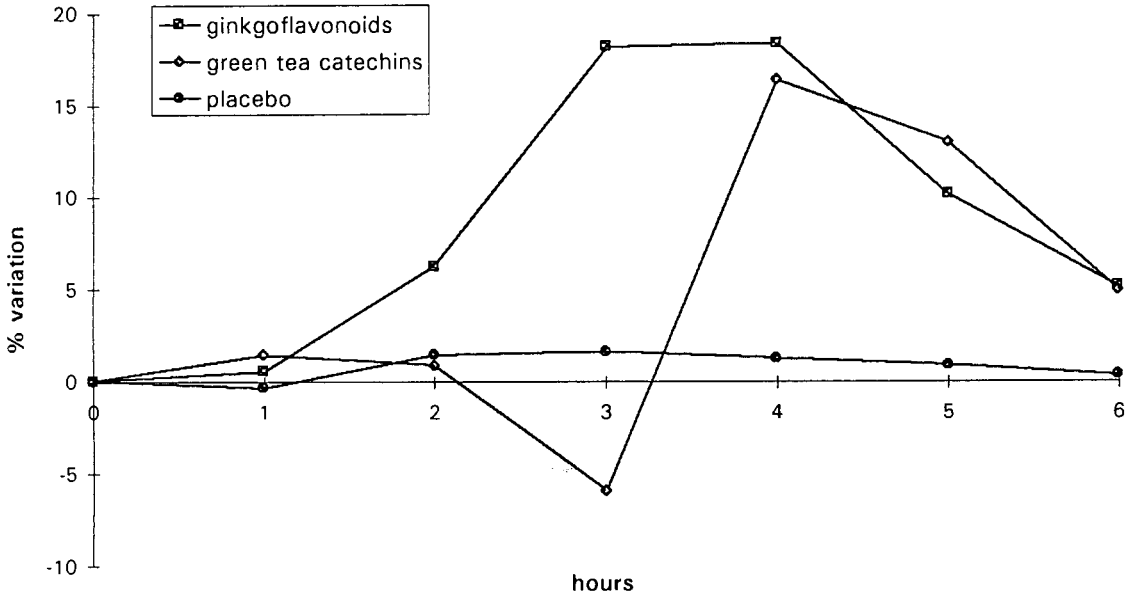

Figure 2. TRAP modification after intake of ginkgo extract (400 mg of ginkgo flavonoids plus 100 mg of ginkgolides), green tea extract (400 mg of total catechins), and maltodextrin (placebo). Values are the mean of three determinations for each subject; relative standard deviations were in the range of 6−12%.

calculated as the sum of contributions of single compounds with known antioxidant potential (Rice-Evans et al., 1997), such as catechins or flavonols detected in green tea or ginkgo, was lower (20−30%) than the measured antioxidant capacity of the related extract. The higher value of the extract can be ascribed to other substances present in the plant. For example, green tea extracts, in addition to the most abundant catechins, contain other related compounds (Graham, 1992). The chemical identity of these compounds has been already defined; however, their contents in green tea as well as their antioxidant capacity are unknown. Similarly, other phenolics (such as proanthocyanidins and biflavones) present in ginkgo extracts are structurally able to behave as radical scavengers. Unfortunately, the antioxidant capacity of these constituents has been not yet evaluated, making it impossible to quantify their contribution to the total antioxidant capacity of ginkgo extracts.

Analogous considerations can be made for other extracts, and this makes it even more problematic to extrapolate the in vitro antioxidant capacity to in vivo effects. One reason is that most polyphenols are poorly absorbed and largely degraded to metabolites (Pietta et

al., 1998) with antioxidant capacity only partly assessed (Merfort et al., 1996). In addition, other components of the extract with better bioavailability than polyphenols could play a major antioxidant role in vivo. Is this the case of ginkgolides? These terpenoids are present (6%) in ginkgo extracts (Pietta et al., 1992), and they are insensitive to the TAA assay. Nevertheless, they are largely bioavailable and may contribute to the in vivo radical scavenging activity of ginkgo (Pietri et al., 1997). To verify this possibility, two groups of volunteers were supplemented with the same polyphenol amounts from either ginkgo or green tea extracts, and a third group receved maltodextrin as a placebo. Despite its lower TAA value (2.4 mM Trolox), ginkgo extract produced a more rapid and lasting in vivo antioxidant potential than green tea extract (TAA, 5.2 mM Trolox), as was seen by following the plasma TRAP (Figure 2). This result may be ascribed to the presence of ginkgolides in ginkgo extract. No variation of plasma TRAP was detected in the placebo group.

Therefore, the TAA values presented in this work are of interest for a comparative in vitro evaluation of TAA of medicinal plant extracts. However, these values need to be combined with in vivo data to assess properly the antioxidant efficacy of medicinal plants.

ABBREVIATIONS USED

$ABTS^{\bullet+}$, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical cation; ABAP, 2,2′-azobis(2-amidinopropane) dihydrochloride; PBS, phosphate buffer, 75 mM, pH 7.0; TAA, total antioxidant activity; TRAP, total radical-trapping antioxidant parameter; ROS, reactive oxygen species; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; ORAC, oxygen radical absorbance capacity; HPLC, high-performance liquid chromatography; CE, capillary electrophoresis.

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