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Short communication

Trolox equivalent antioxidant capacity (TEAC) of *Ginkgo biloba* flavonol and *Camellia sinensis* catechin metabolites

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1. Introduction

Flavonoids are polyphenolic plant metabolites that have been found to be an important part of the human diet [1] and active principles of several medicinal plants [2].

Specific flavonoids, the most studied being flavonols and catechins, are preventive antioxidants because they scavenge reactive oxygen species (free radicals produced in the body as intermediates of the four-electron reduction of oxygen to water), terminate free radical chain reaction, and chelate transition metals involved in free radical generation [3].

According to their one-electron reduction potentials [4], these flavonoids quench superoxide anion, peroxyl, hydroxyl and alkoxyl species producing more stable (less energetic) radicals. However, whether this radical-scavenging capacity can be extended from in vitro to in vivo systems depends on flavonoid bioavailability.

Based on recent reports [5,6], flavonols and catechins are poorly absorbed from gut, but are extensively degraded by intestinal microflora. The C-ring may be broken along three different pathways yielding different phenolic acids (Fig. 1), which may undergo further dehydroxylation, methylation and β-oxidation. Unfortunately, little is known about the antioxidant potential of these acids. Only some metabolites from *Ginkgo biloba* have been studied following the inhibition of chemiluminescence, and the results indicated that metabolites with an *o*-dihydroxy group in the phenyl ring are as effective as the flavonol-glycoside precursor (rutin) in quenching radicals [7].

Recently, a new approach to establish the relative antioxidant capacity of a variety of polyphenols, including flavonols and catechin gallates , has been introduced [8]. According to this method, the capacity of hydrogen-donors antioxidant to scavenge the $ABTS^{\bullet+}$ radical cation compared with that of Trolox (TEAC) is assessed. We

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applied an improved version of this method [9] to establish the TEAC values of main metabolites from *G. biloba* flavonol-glycosides and green tea catechins, and the results are reported in this communication.

2. Materials and methods

Trolox™ (Hoffman-La Roche) (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Aldrich Chemical Co., The Old Brickyard, Gillingham, Dorset SP8) was used an antioxidant standard. 2.5 mM Trolox was prepared in 5 mM phosphate buffered saline, pH 7.4 (PBS), for use as a stock standard. Fresh working standards were prepared daily by mixing 2.5 mM Trolox with PBS (final concentrations 0–20 μM).

The metabolites from *G. biloba* flavonol-glycosides and green tea catechins were available in our laboratory or purchased from Fluka (Fluka Chemie AG CH-9470 Buchs) and Sigma (Sigma

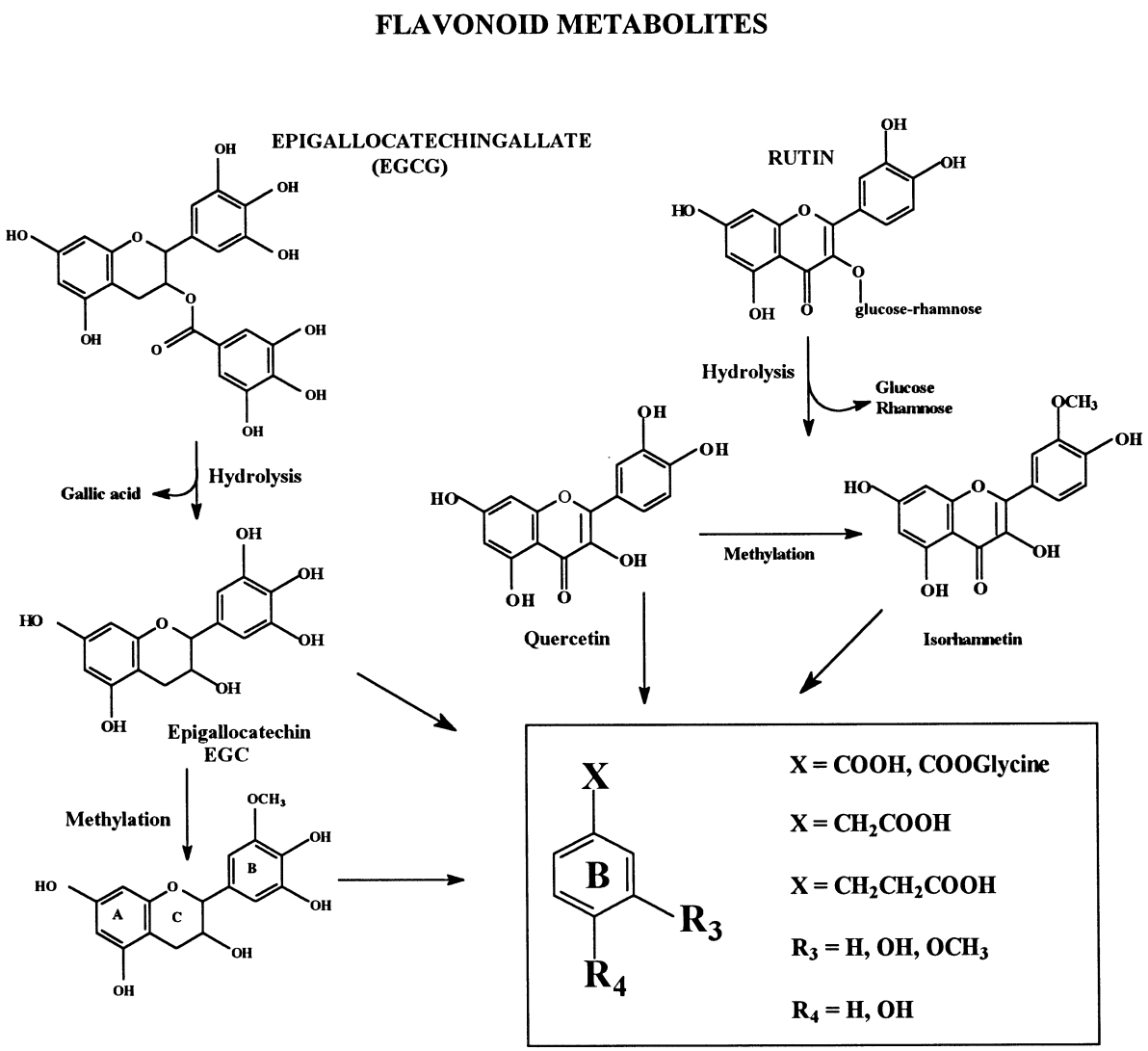

Fig. 1.

Table 1
TEAC values of selected flavonol and catechin metabolites[a]

Compound	Source	TEAC (mM)
4-Hydroxy benzoic acid (4-HBA)	Gb, GT	0.07 ± 0.01
4-Hydroxy hippuric acid (4-HHA)	Gb	0.20 ± 0.02
3,4-Dihydroxy benzoic acid (3,4-DHBA)	Gb, GT	1.01 ± 0.06
3-Methoxy-4-hydroxy benzoic acid (VA) (vanillic acid)	Gb, GT	1.19 ± 0.09
3-Methoxy-4-hydroxy hippuric acid (3-M-4-HHA)	Gb, GT	1.29 ± 0.09
3-Hydroxy phenyl acetic acid (3-HPAA)	Gb	0.97 ± 0.08
3,4-Dihydroxy phenyl acetic acid (3,4-DHPAA)	Gb	2.16 ± 0.15
3-Methoxy-4-hydroxy phenyl Acetic acid (HVA) (homovanillic acid)	Gb	1.63 ± 0.11
3-(3-Hydroxy phenyl) propionic acid (3-HPPA)	Gb	1.03 ± 0.08
3-(4-Hydroxy phenyl) propionic acid (4-HPPA)	Gb, GT	1.60 ± 0.10
4-Methyl catechol	GT	1.25 ± 0.08
Epigallocatechingallate		4.81 ± 0.21
Rutin		2.42 ± 0.18
Vitamin E		1.00 ± 0.08
Ascorbic acid		1.02 ± 0.07

[a] Gb, *G. biloba*; GT, Green tea (*C. sinensis*); mean ± S.D.

Chemical Co., St Louis MO 63178 USA). Stock solutions of the metabolites were prepared by dissolution in 50% ethanol (final concentration 10 μM). All investigated compounds were stable under the experimental conditions applied.

The Trolox equivalent antioxidant activity (TEAC) was evaluated applying the improved ABTS radical cation decolorization assay [8,9]. This spectrophotometric technique measures the relative ability of antioxidants to scavenge a long-lived specific radical cation chromophore in relation to that of Trolox, the water-soluble vitamin E analogue. Hence, the TEAC represents the concentration of Trolox with the same antioxidant activity as 1 mM solution of the examined metabolite. ABTS radical cation ($ABTS^{\bullet+}$) is produced by reacting ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) (7 mM) with potassium persulfate (2.45 mM). Trolox or metabolite solutions (10 μl) were added to 1.0 ml of diluted $ABTS^{\bullet+}$ solution (2.5 mM), and the absorbance readings at 734 nm were taken at 30°C exactly 6 min after initial mixing.

Absorbances were read with a Hewlett–Packard spectrophotometer (HP 8453) fitted with peltier temperature control, and a dose-response curve for Trolox over the range 0–20 μM (final concentrations) was obtained, using a logit/log 4 plot.

3. Results and discussion

The antioxidant properties of *G. biloba* against several reactive oxygen species, such as superoxide anion, hydroxyl and peroxyl radicals, have been observed in vitro. Furthermore, *G. biloba* standardized extracts have been reported to be beneficial on some diseases related to oxidative stress, including cardiac ischemia-reperfusion injury, diabetic retinopathy, cerebral ischemic damage and atherosclerosis [10]. The flavonoid fraction, mainly composed of flavonol-glycosides, is considered to be responsible in part for these protective effects.

Similarly, the powerful antioxidant capacity of epigallocatechingallate (EGCG) and related catechins [11] may explain the lower risk of atherosclerosis, coronary heart disease and diverse types of cancer associated with regular tea consumption [12].

Both ginkgoflavonol-glycosides and *Camellia sinensis* catechins undergo degradation in vivo, and main metabolites have been recently identified [6,12,13].

The TEAC assay provided an easy way to evaluate the antioxidant capacity of some of these metabolites, and the results are shown in Table 1. Among metabolites arising from EGb flavonol-glycosides, only 3,4-dihydroxyphenylacetic acid has a value (2.1 mM) near to that of a very common flavonol-glycoside, i.e. rutin. This result is in agreement with the radical-scavenging activity detected by chemiluminescence [7]. All the other metabolites yielded lower TEAC, and were comparable to the values of vitamin E and C (TEAC, 1 mM) or slightly higher.

Analogous values were obtained with green tea catechin metabolites, which were in the range 1–1.60 mM, except for the uneffective 4-hydroxybenzoic acid. In the case of catechin metabolites, none of these had a TEAC value comparable to that of their precursors (e.g. EGCG, TEAC 4.8 mM).

The results for these phenolic acids confirm that an *o*-hydroxy group is the main requirement for a strong radical scavenging capacity. However, the lenght of the alkyl chain, the position of the hydroxy group and methoxylation influence the antioxidant potential. Introduction of –OH, $-OCH_3$, and alkyl substituted groups into the phenyl ring makes the resulting phenols more oxidable. This is the case of 4-HPAA and VA versus 4-HBA, HVA versus VA, 3,4-DHPAA and 3,4-DHPPA versus 3,4-DHBA. On the contrary, the carboxyl group renders oxidation more difficult, as shown by the lower TEAC for 3,4-DHBA and VA as compared with 3,4-DHPAA, 3,4-DHPPA and HVA. Conjugation of 4-HBA and VA with glycine produces a slight increase of TEAC values, since the secondary amido group has lower electron-withdrawing capacity.

This behaviour satisfies the Hammett's correlation between antioxidant activity and electron-donating or withdrawing properties of the substituents. Better electron-donating groups (negative Brown's sigma$^+$ values) reduce the redox potential and increase the rate and efficay of phenolic antioxidants. Electron-withdrawing groups (positive Brown's sigma$^+$ values) increase the redox potential of phenols, disqualifying these particular phenols as antioxidants.

Concerning possible 'in vivo' effects of these metabolites, the amounts detected in the 0–48 h urine sample after intake of ginkgoflavonol-glycosides (1 g) or green tea catechins (0.4 g), were reported to be in the range of 0.6 mM [6,13]. Therefore, it is likely that these levels could account for a possible contribution to the body antioxidant potential.

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