

Role of Single-Electron Oxidation Potential and Lipophilicity in the Antiplasmodial *in vitro* Activity of Polyphenols: Comparison to Mammalian Cells

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Z. Naturforsch. **63c**, 445–450 (2008); received November 8/December 13, 2007

In spite of extensive studies, the structure-activity relationships in the action of polyphenols against the malaria parasite *Plasmodium falciparum* are poorly understood so far. As the mammalian cell cytotoxicity of polyphenols shows a negative dependence on the potential of the phenoxyl radical/phenol redox couple ($E_2^{\cdot}$), due to the involvement of prooxidant events, and a positive dependence on the octanol/water distribution coefficient at pH 7.0 (log D), we examined the role of these parameters in their antiplasmodial *in vitro* activity. We found that the concentrations of hydroxybenzenes causing 50% inhibition of the growth of *P. falciparum* strain FcB1 (IC₅₀) are described by the regression $\log \text{IC}_{50} (\mu\text{M}) = 0.36 + 1.81 E_2^{\cdot} (\text{V}) - 0.10 \log D$ [$n = 11$, $r^2 = 0.760$, $F(2.8) = 12.03$]. The IC₅₀ values of flavonoids ($n = 5$), comprising a separate less active series, did not depend on their $E_2^{\cdot}$ values, 0.33 V–0.75 V. These findings were similar to the mammalian cell cytotoxicity data. However, the mammalian cell cytotoxicity of hydroxybenzenes showed more pronounced dependence on their $E_2^{\cdot}$ values [$\Delta \log \text{CL}_{50} / \Delta E_2^{\cdot} = (6.9 - 5.1) \text{ V}^{-1}$, where CL₅₀ is the compound concentration for 50% cell survival] than on their antiplasmodial activity. Although it is unclear whether the prooxidant action is the main factor in the antiplasmodial action of polyphenols or not, our data showed that the ease of their oxidation (decrease in $E_2^{\cdot}$) may enhance their activity. On the other hand, the different sensitivity of the mammalian cell cytotoxicity and the antiplasmodial activity of the hydroxybenzenes to their $E_2^{\cdot}$ values implied that compounds with high oxidation potential may be used as relatively efficient antiplasmodial agents with low mammalian cell cytotoxicity.

Key words: *Plasmodium falciparum*, Flavonoids, Phenols

Introduction

Polyphenolic compounds (flavonoids, gallates, curcumin, other polyhydroxybenzenes) possess moderate activity against the malaria parasite *Plasmodium falciparum*, acting at micromolar or higher concentrations (Köhler *et al.*, 2002; Pradines *et al.*, 2002; Sannella *et al.*, 2007, and references therein). However, they are supposed to be

responsible for the antiplasmodial activity of plant and herbal extracts used in the ethnomedicine, and also may be important diet components for disease prevention. The action of polyphenols in *P. falciparum* is attributed to several possible mechanisms: (i) the chelation of Fe ions (Pradines *et al.*, 2002, and references therein); (ii) the inhibition of the enzymes of type-II fatty acid biosynthesis pathway (Tasdemir *et al.*, 2006, and references therein); (iii) the inhibition of dihydrofolate reductase (Sannella *et al.*, 2007, and references therein); and (iv) the formation of reactive oxygen species (ROS) during (auto)oxidation of polyphenols (Vennerstrom and Eaton, 1988; Cui *et al.*, 2007). However, in spite of extensive studies, the structure-activity relationships in the antiplasmodial action of polyphenols remain unclear.

Abbreviations: ROS, reactive oxygen species; $E_2^{\cdot}$, potential of phenoxyl radical/phenol redox couple at pH 7.0; log D , octanol/water distribution coefficient at pH 7.0; IC₅₀, the compound concentration causing 50% parasite growth inhibition; CL₅₀, the compound concentration for 50% survival of mammalian cells; DPPD, *N,N'*-di-phenyl-*p*-phenylene diamine; COMT, catechol-*o*-methyltransferase.

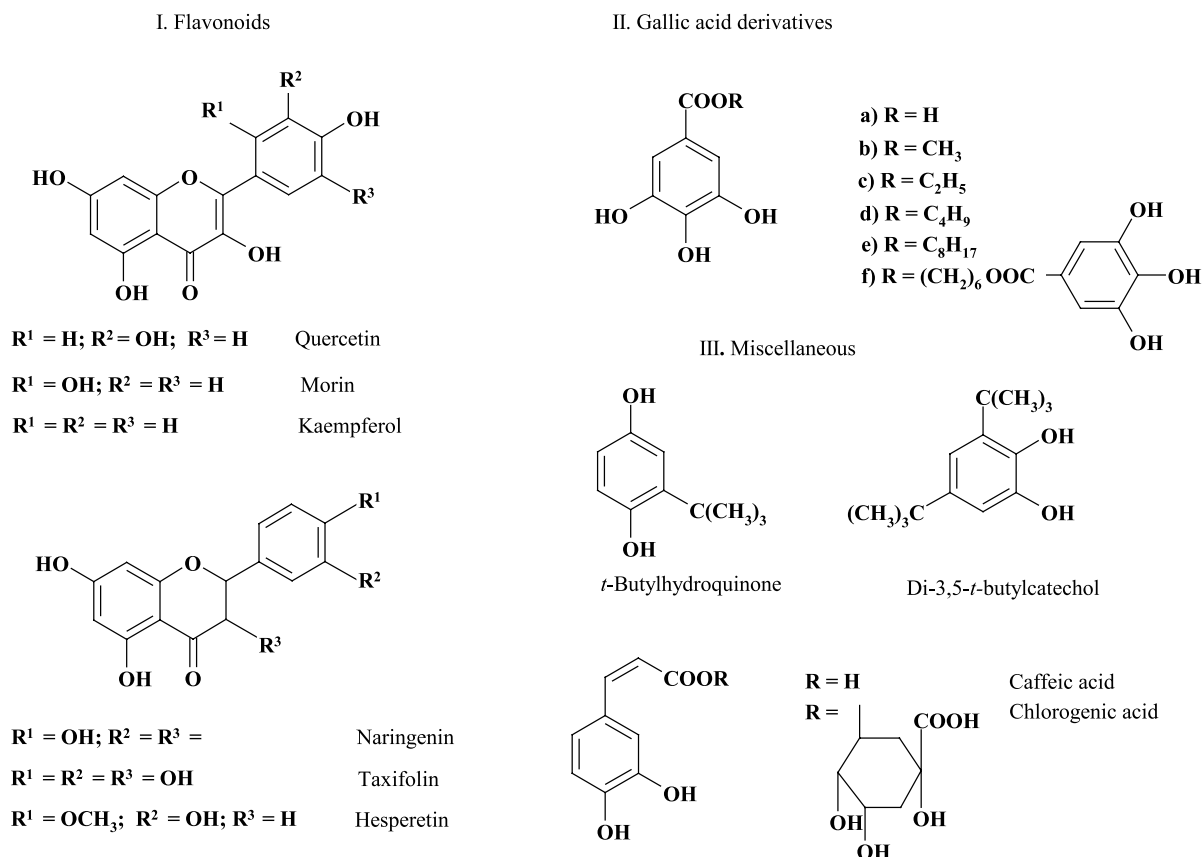


Fig. 1. Chemical structures of the polyphenolic antioxidants studied.

According to our data (Nemeikaitė-Čėnienė *et al.*, 2005a, and references therein), the cytotoxicity of polyhydroxybenzenes in mammalian cells may increase with a decrease in the potential of the phenoxyl radical/phenol redox couple ($E_2^{\cdot}$, available from pulse-radiolysis data). This shows that the cytotoxicity is mainly determined by the prooxidant action of polyphenols, *i.e.*, it increases with an ease of their (auto)oxidation, which is accompanied by the formation of ROS and the depletion of cellular $-SH$ groups by the quinone/quinomethide-type oxidation products (Metodiewa *et al.*, 1999; Boersma *et al.*, 2000; Galati *et al.*, 2001; Nemeikaitė-Čėnienė *et al.*, 2005b). The toxicity also increases with an increase in the lipophilicity of polyphenols, *i.e.*, in the octanol/water distribution coefficient at pH 7.0 ($\log D$).

These findings, taken together with the possible involvement of prooxidant events in the antiplasmodial action of polyphenols, prompted us to ex-

amine the possible relationship between $E_2^{\cdot}$ of polyphenols and their antiplasmodial activity.

In this work, we examined the antiplasmodial *in vitro* activity of a number of structurally diverse polyphenolic antioxidants and model phenols (Fig. 1) possessing a wide range of $E_2^{\cdot}$ and $\log D$ values. The results obtained were analyzed in the context of mammalian cell cytotoxicity data of polyphenols obtained in previous (Nemeikaitė-Čėnienė *et al.*, 2005a) and current studies.

Materials and Methods

The chloroquine-resistant *Plasmodium falciparum* strain FcB1 from Colombia was kindly provided by Dr. H. D. Heidrich (Max-Planck Institut für Biochemie, Martinsried bei München, Germany) and is deposited in the Protist Collection of Museum National d'Histoire Naturelle, Paris, France. *P. falciparum* FcB1 strain was maintained

Table I. The concentrations of polyphenols causing 50% growth inhibition of *Plasmodium falciparum* strain FcB1 (IC_{50}), their concentrations for 50% survival of mammalian cells (CL_{50}), their redox potentials of phenoxyl radical/phenol couples at pH 7.0 (E_7^2), and their octanol/water distribution coefficients at pH 7.0 ($\log D$).

No.	Compound	IC_{50} [μM] FcB1	CL_{50} [μM]			E_7^2 [V] ^a	$\log D$
			HL-60 ^b	FLK ^b	MH-22a		
1	Di-3,5- <i>t</i> -butylcatechol	4.5 $\pm$ 0.2	4.5 $\pm$ 0.5	14 $\pm$ 3.0	40 $\pm$ 6.0	0.39	4.26
2	<i>t</i> -Butylhydroquinone	6.1 $\pm$ 0.3	30 $\pm$ 5.0	56 $\pm$ 10	75 $\pm$ 12	0.46	2.30
3	4-Methylcatechol		16 $\pm$ 3.0	25 $\pm$ 6.0		0.46	1.34
4	Hydroquinone		18 $\pm$ 3.0	40 $\pm$ 6.0	38 $\pm$ 5.0	0.48	0.64
5	Catechol		23 $\pm$ 4.0	30 $\pm$ 8.0	75 $\pm$ 15	0.53	0.88
6	Caffeic acid	50 $\pm$ 2.0	300 $\pm$ 50	180 $\pm$ 20	560 $\pm$ 60	0.54	-0.08
7	Chlorogenic acid	39 $\pm$ 3.8	≥ 2000	1000 $\pm$ 80		0.54	-2.36
8	Gallic acid	31 $\pm$ 1.5	750 $\pm$ 150	750 $\pm$ 70	750 $\pm$ 80	0.56	-1.76
9	Methylgallate	6.3 $\pm$ 1.1	400 $\pm$ 100	112 $\pm$ 18		0.56	1.54
10	Ethylgallate	11 $\pm$ 1.7	160 $\pm$ 40	89 $\pm$ 11	300 $\pm$ 50	0.56	2.07
11	Butylgallate	18 $\pm$ 0.5	110 $\pm$ 30	45 $\pm$ 8.0		0.56	3.13
12	Bis(1,6-hexanediol)-gallate	9.6 $\pm$ 0.6			200 $\pm$ 40	0.56	4.44
13	Octylgallate		30 $\pm$ 10	56 $\pm$ 8.0	100 $\pm$ 20	0.56	5.26
14	<i>p</i> -Methoxyphenol	46 $\pm$ 3.0	1500 $\pm$ 400	≥ 2000	≥ 2000	0.73	1.30
15	<i>p</i> -Methylphenol	51 $\pm$ 5.0		$\gg 2000$		0.87	1.45
16	Quercetin	42 $\pm$ 1.0	120 $\pm$ 20	140 $\pm$ 18	160 $\pm$ 25	0.33	2.74
17	Taxifolin	51 $\pm$ 3.0	600 $\pm$ 150	780 $\pm$ 120	900 $\pm$ 100	0.50	1.22
18	Morin	56 $\pm$ 5.0	250 $\pm$ 40	112 $\pm$ 15	250 $\pm$ 35	0.60	1.97
19	Hesperetin		500 $\pm$ 100	750 $\pm$ 100		0.72	2.30
20	Kaempferol	50 $\pm$ 3.0	125 $\pm$ 20	185 $\pm$ 25	680 $\pm$ 80	0.75	2.69
21	Naringenin	54 $\pm$ 3.0	700 $\pm$ 100				2.59

^a From Wardman (1989) and Jovanovic *et al.* (1998).

^b From Nemeikaitė-Čėnienė *et al.* (2005a).

in continuous culture of human erythrocytes (Trager and Jensen, 1976; Grellier *et al.*, 2001). *In vitro* antiplasmodial activity was determined using a modification of the semiautomatic microdilution technique (Desjardins *et al.*, 1979). Stock solutions of test compounds in DMSO were serially diluted with culture medium and added to asynchronous parasite cultures (1% parasite-infected cells and 1% final hematocrit) for 24 h, at 37 °C, prior to the addition of 1.825 MBq of [³H]hypoxanthine (0.37–1.11 TBq/mmol) for 24 h. The growth inhibition for each compound concentration was determined according to the radioactivity incorporation into the treated culture as compared with that in the control culture. The experiments were repeated in triplicate. The culture of murine hepatoma MH-22a cells was grown and maintained at 37 °C in DMEM medium, supplemented with 10% fetal bovine serum (Shvemberger and Alexandrova, 2000). The viability of the cells after 24 h of growth on glass slides in the presence of polyphenols was examined by the Trypan blue exclusion test. The octanol/water distribution coefficients of the compounds at pH 7.0 ($\log D$) were calculated using an ACD/ChemSketch (version 4.02, Ad-

vanced Chemistry Development, Toronto, Ontario, Canada), while statistical and multiparameter regression analysis was performed using Statistica (version 4.3, Statsoft Inc., 1993).

Results and Discussion

The IC_{50} values of polyphenols (their concentration causing 50% *P. falciparum* growth inhibition) and their concentrations for 50% survival of mammalian cells (CL_{50}) are given in Table I. The cytotoxicity of polyphenols in human promyelocytic leukemia cells (line HL-60) and bovine leukemia virus-transformed lamb kidney fibroblasts (line FLK) has been examined previously (Nemeikaitė-Čėnienė *et al.*, 2005a), whereas murine hepatoma MH-22a cells were studied in the present work. For comparison, first the mammalian cell cytotoxicity was analyzed. The prooxidant character of polyphenol cytotoxicity in FLK and HL-60 cells was previously confirmed by the increase of lipid peroxidation, and the protective effects of the antioxidant *N,N'*-diphenyl-*p*-phenylene diamine (DPPD), catalase, and desferroxamine (Nemeikaitė-Čėnienė *et al.*, 2005a). In both cell lines, the

flavonoids were less toxic than polyhydroxybenzenes with similar E_7^2 and $\log D$ values, and comprised the separate series (Nemeikaitė-Čėnienė *et al.*, 2005a). The toxicity of polyhydroxybenzenes (compounds 1–11, 13, 14, Table I) was described by the following equations:

$$\begin{aligned} \log \text{CL}_{50} [\mu\text{M}] = & \\ & -(1.39 \pm 0.80) + (6.90 \pm 1.45) E_7^2 [\text{V}] \\ & -(0.20 \pm 0.05) \log D \\ [\text{HL-60}, r^2 = 0.817, F(2.10) = 22.33], \end{aligned} \quad (1)$$

$$\begin{aligned} \log \text{CL}_{50} [\mu\text{M}] = & \\ & -(0.67 \pm 0.64) + (5.46 \pm 1.17) E_7^2 [\text{V}] \\ & -(0.16 \pm 0.04) \log D \\ [\text{FLK}, r^2 = 0.810, F(2.10) = 21.33]. \end{aligned} \quad (2)$$

The same phenomena have been observed in MH-22a cells: (i) in the presence of 150 μM quercetin [cell viability, $(54.0 \pm 3.0)\%$], the addition of 3.0 μM DPPD, or 300 μM desferroxamine, or 100 U/ml catalase the cell viability increased up to $(84.0 \pm 5.0)\%$, $(73.4 \pm 5.5)\%$, or to $(87.0 \pm 4.4)\%$, respectively ($n = 3$, $p < 0.01$); (ii) the cytotoxicity of hydroxybenzenes (compounds 1, 2, 4–6, 8, 10, 12–14, Table I) was described by an analogous two-parameter regression:

$$\begin{aligned} \log \text{CL}_{50} [\mu\text{M}] = & \\ & -(0.33 \pm 0.68) + (5.10 \pm 1.21) E_7^2 [\text{V}] \\ & -(0.07 \pm 0.05) \log D \\ [r^2 = 0.783, F(2.7) = 11.85]; \end{aligned} \quad (3)$$

and (iii) the flavonoids comprised a separate series with lower cytotoxicity (Fig. 2). Thus, the observed dependences may be a general feature of the mammalian cell cytotoxicity of polyphenols.

The IC_{50} values of polyphenols against *P. falciparum* varied from 4.5 μM to 56 μM (Table I). The comparison of IC_{50} values of gallic acid and its esters indicates that the antiplasmodial activity of polyphenols in general increases with an increase in their $\log D$ value. We did not investigate the antiplasmodial activity of the most lipophilic compound octylgallate, because it causes erythrocyte lysis even at low concentrations, $\geq 10 \mu\text{M}$. The antiplasmodial activity of the flavonoids investigated almost did not depend on their E_7^2 values. This finding is further substantiated by the similar IC_{50} value of naringenin and other flavonoids (Table I). Although its E_7^2 value is not available, naringenin seems to be less readily undergoing oxidation than other flavonoids, because its voltammetric (two-electron) oxidation potential, 0.76 V, is much higher than that of kaempferol (0.39 V), morin

(0.34 V), and quercetin (0.29 V) (Jorgensen and Skibsted, 1998). For all the compounds examined, the correlation between $\log \text{IC}_{50}$ and $E_7^2 + \log D$ was almost absent ($r^2 = 0.384$, data not shown). On the other hand, the separate treatment of hydroxybenzenes (compounds 1, 2, 6–12, 14, 15, Table I) resulted in a regression, which was analogous to (1)–(3):

$$\begin{aligned} \log \text{IC}_{50} [\mu\text{M}] = & \\ & (0.36 \pm 0.35) + (1.81 \pm 0.57) E_7^2 [\text{V}] \\ & -(0.10 \pm 0.03) \log D \\ [r^2 = 0.760, F(2.8) = 12.03]. \end{aligned} \quad (4)$$

It was shown that the decrease in E_7^2 of hydroxybenzenes enhances their antiplasmodial activity. However, as desferroxamine and DPPD inhibited the *P. falciparum* FcB1 growth with IC_{50} values of $(16.0 \pm 1.0) \mu\text{M}$ and $(9.6 \pm 0.9) \mu\text{M}$, respectively, we were unable to test them in the protection experiments. Analogously to the data obtained in mammalian cells (Fig. 2), the antiplasmodial activ-

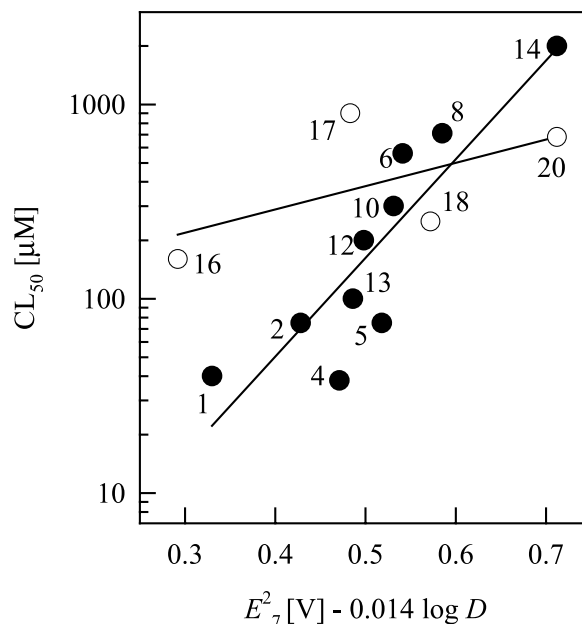


Fig. 2. Dependence of polyphenol cytotoxicity in MH-22a cells on their single-electron oxidation potential, E_7^2 , and octanol/water distribution coefficient, D , according to the multiparameter equation (3). The numbers of compounds are taken from Table I. The lower line represents a first-order regression describing the activity of hydroxybenzenes (solid circles); the activity of flavonoids (blank circles) is shown for comparison.

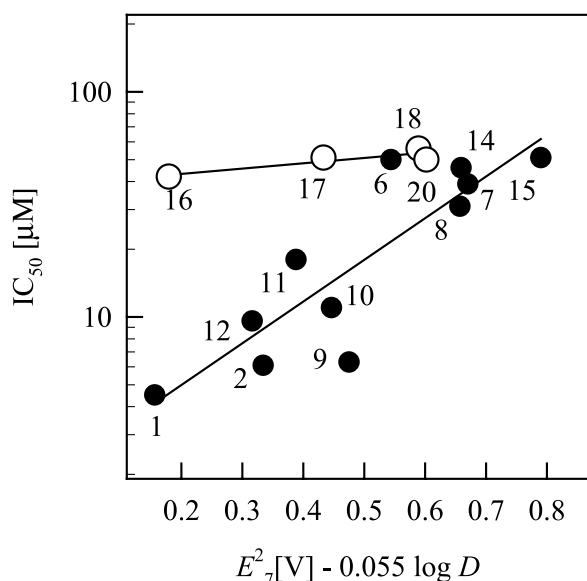


Fig. 3. Dependence of polyphenol antiplasmodial activity on their single-electron oxidation potential, E_7^2 , and octanol/water distribution coefficient, D , according to the multiparameter equation (4). The numbers of compounds are taken from Table I. The lower line represents a first-order regression describing the activity of hydroxybenzenes (solid circles); the activity of flavonoids (blank circles) is shown for comparison.

ity of flavonoids was lower than that of hydroxybenzenes (Fig. 3).

The data obtained indicate that there exist some similarities between the antiplasmodial activity and mammalian cell cytotoxicity of polyphenols: (i) In both cases, a decrease in the E_7^2 values of hydroxybenzenes, *i.e.*, increase in the ease of oxidation, increases their activity (Figs. 2, 3), that points to the possible prooxidant action mechanism. The prooxidant character of mammalian cell cytotoxicity of polyphenols has been demonstrated previously (Nemeikaitė-Čėnienė *et al.*, 2005a, b, and references therein), as well as in this work. The prooxidant character of antiplasmodial activity has been demonstrated for curcumin (Cui *et al.*, 2007), and has been proposed for quercetin (Vennerstrom and Eaton, 1988). Although the pathways of polyphenol oxidation in *P. falciparum* are unclear, these compounds may be oxidized by oxy-

hemoglobin in erythrocytes with the concomitant formation of ROS (Stolze and Nohl, 1999; Miyazaki *et al.*, 2004). (ii) Lower than expected mammalian cell cytotoxicity of flavonoids (Fig. 2) has been attributed to the action of catechol-*o*-methyltransferase (COMT, EC 2.1.1.6) (Nemeikaitė-Čėnienė *et al.*, 2005a, b), which methylates flavonoids with an *o*-dihydroxylated B-ring much faster than catechols (Lautala *et al.*, 2001), thus contributing to their detoxification. One should note that *o*-dihydroxybenzenes or flavonoids with an *o*-dihydroxylated B-ring may be formed from the starting compounds (Fig. 1) in cytochrome P-450-catalyzed hydroxylation/oxidative demethylation reactions (Nielsen *et al.*, 1998; Moridani *et al.*, 2003). In our opinion, lower than expected antiplasmodial activity of flavonoids (Fig. 3) may be explained analogously, because COMT is also present in erythrocytes (Masuda *et al.*, 2002), and cytochromes P-450 are also present in *P. falciparum* (Surolija *et al.*, 1993).

On the other hand, the activity of hydroxybenzenes in *P. falciparum* is less sensitive to the redox potential [$\Delta \log IC_{50} / \Delta E_7^2 = 1.81$, Eq. (4)] than in mammalian cells [$\Delta \log CL_{50} / \Delta E_7^2 = 6.9 - 5.1 \text{ V}^{-1}$, Eqs. (1)–(3)]. Interestingly, the latter coefficients are similar to the coefficients $\Delta \log (\text{rate constant}) / \Delta E_7^2 = -8.5$ to -7.4 V^{-1} in the single-electron oxidation of polyphenols by several cytochromes and Fe^{3+} (Rich and Bendall, 1980; Rich, 1982). This demonstrates the close parallelism between the oxidation rates of polyphenols and their mammalian cell toxicity. Evidently, the less expressed dependence of the antiplasmodial activity of polyphenols on their E_7^2 values shows that other mechanisms may be also partly responsible for their action. Nevertheless, this finding may be important in the design of new antiplasmodial agents, because it shows that polyphenols with a high E_7^2 value, 0.6 V–0.7 V, may possess substantial antiplasmodial activity (Fig. 3) and low mammalian cell cytotoxicity (Fig. 2).

Acknowledgements

This work was supported in part by Lithuanian Science and Studies Foundation and by Agency for International Science and Technology Development Programmes in Lithuania (COST B22).

- Boersma M. G., Vervoort J., Szymusiak H., Lemanska K., Tyrakowska B., Čenas N., Segura-Aguilar J., and Rietjens I. M. C. M. (2000), Regioselectivity and reversibility of the glutathione conjugation of quercetin quinone methide. *Chem. Res. Toxicol.* **13**, 185–191.
- Cui L., Miao J., and Cui L. (2007), Cytotoxic effect of curcumin on malaria parasite *Plasmodium falciparum*: inhibition of histone acetylation and generation of reactive oxygen species. *Antimicrob. Agents Chemother.* **51**, 488–494.
- Desjardins R. E., Canfield C. J., Haynes J. D., and Chulay J. D. (1979), Quantitative assessment of anti-malarial activity *in vitro* by a semiautomated microdilution technique. *Antimicrob. Agents Chemother.* **16**, 710–718.
- Galati G., Moridani M. Y., Chan T. S., and O'Brien P. J. (2001), Peroxidative metabolism of apigenin and naringenin versus luteolin and quercetin: glutathione oxidation and conjugation. *Free Rad. Biol. Med.* **30**, 370–382.
- Grellier P., Šarlauskas J., Anusevičius Ž., Marozienė A., Houee-Levin C., Schrevel J., and Čenas N. (2001), Antiplasmodial activity of nitroaromatic and quinoidal compounds: redox potential vs. inhibition of erythrocyte glutathione reductase. *Arch. Biochem. Biophys.* **393**, 199–206.
- Jorgensen L. V. and Skibsted L. H. (1998), Flavonoid deactivation of ferrylmyoglobin in relation to ease of oxidation as determined by cyclic voltammetry. *Free Rad. Res.* **28**, 335–351.
- Jovanovic S. V., Steenken S., Simic M. G., and Hara Y. (1998), Antioxidant properties of flavonoids: reduction potentials and electron transfer reactions of flavonoid radicals. In: *Flavonoids in Health and Disease* (Rice-Evans C. A. and Packer L., eds.). Marcel Dekker Inc., New York, Basel, Hong Kong, pp. 137–161.
- Köhler I., Jenett-Siems K., Siems K., Hernandez M. A., Ibarra R. A., Berendsohn W. G., Bienzle U., and Eich E. (2002), *In vitro* antiplasmodial investigation of medicinal plants from El Salvador. *Z. Naturforsch.* **57c**, 277–281.
- Lautala P., Ullmanen I., and Taskinen J. (2001), Molecular mechanisms controlling the rate and specificity of catechol-*o*-methylation by human soluble catechol-*o*-methyltransferase. *Mol. Pharmacol.* **59**, 393–402.
- Masuda M., Tsunoda M., Yusa M., Yamada S., and Imai K. (2002), Assay of catechol-*o*-methyltransferase activity in human erythrocytes using norepinephrine as a natural substrate. *Ann. Clin. Biochem.* **39**, 589–594.
- Metodiewa D., Jaiswal A. K., Čenas N., Sergedienė E., and Segura-Aguilar J. (1999) Quercetin may act as a cytotoxic prooxidant after its metabolic activation to semiquinone and quinone product. *Free Rad. Biol. Med.* **26**, 107–116.
- Miyazaki K., Arai S., Iwamoto T., Takasaki M., and Tomoda A. (2004), Metabolism of pyrogallol to purpurogallin by human erythrocytic hemoglobin. *Tohoku J. Exp. Med.* **203**, 319–330.
- Moridani M. Y., Cheon S. S., Khan S., and O'Brien P. J. (2003), Metabolic activation of 3-hydroxyanisole by isolated rat hepatocytes. *Chem.-Biol. Interact.* **142**, 317–333.
- Nemeikaitė-Čėnienė A., Imbrasaitė A., Sergedienė E., and Čenas N. (2005a), Quantitative structure-activity relationships in prooxidant cytotoxicity of polyphenols: role of potential of phenoxyl radical/phenol redox couple. *Arch. Biochem. Biophys.* **441**, 182–190.
- Nemeikaitė-Čėnienė A., Marozienė A., Pukalskas A., Venskutonis P. R., and Čenas N. (2005b), Redox properties of novel antioxidant 5,8-dihydroxycoumarin: implications for its prooxidant cytotoxicity. *Z. Naturforsch.* **60c**, 849–854.
- Nielsen S. E., Breinholt V., Justesen U., Cornett C., and Dragsted L. O. (1998), *In vitro* biotransformation of flavonoids by rat liver microsomes. *Xenobiotica* **28**, 389–401.
- Pradines B., Rolain J. M., Ramiandrasoa F., Fusai T., Mosnier J., Rogier C., Daries W., Baret E., Kunesch G., Le Bras J., and Parzy D. (2002), Iron chelators as antimalarial agents: *in vitro* activity of dicaticholate against *Plasmodium falciparum*. *J. Antimicrob. Chemother.* **50**, 177–187.
- Rich P. R. (1982), Electron and proton transfers in chemical and biochemical quinone systems. *Faraday Discuss. Chem. Soc.* **74**, 349–364.
- Rich P. R. and Bendall D. S. (1980), The kinetics and thermodynamics of the reduction of cytochrome c by substituted *p*-benzoquinols in solution. *Biochim. Biophys. Acta* **592**, 506–518.
- Sannella A. R., Messori L., Casini A., Vincieri F. F., Bilia A. R., Majori G., and Severini C. (2007), Antimalarial properties of green tea. *Biochem. Biophys. Res. Commun.* **353**, 177–181.
- Shvemberger I. N. and Alexandrova S. A. (2000), PCR-detected genome polymorphism in malignant cell growth. *Int. Rev. Cytol.* **199**, 117–159.
- Stolze K. and Nohl H. (1999), Free radical formation and erythrocyte membrane alterations during MetHb formation induced by the BHA metabolite, *tert*-butylhydroquinone. *Free Rad. Res.* **30**, 295–303.
- Surolia N., Karthikeyan G., and Padmanaban G. (1993), Involvement of cytochrome P-450 in conferring chloroquine resistance to the malarial parasite, *Plasmodium falciparum*. *Biochem. Biophys. Res. Commun.* **197**, 562–569.
- Tasdemir D., Lack G., Brun R., Ruedi P., Scapozza L., and Perozzo R. (2006), Inhibition of *Plasmodium falciparum* fatty acid biosynthesis: evaluation of FabG, FabZ, and FabI as drug targets for flavonoids. *J. Med. Chem.* **49**, 3345–3533.
- Trager W. and Jensen J. B. (1976), Human malaria parasites in continuous culture. *Science* **193**, 673–677.
- Vennerstrom J. L. and Eaton J. W. (1988), Oxidants, oxidant drugs, and malaria. *J. Med. Chem.* **31**, 1269–1277.
- Wardman P. (1989), Reduction potentials of one-electron couples involving free radicals in aqueous solutions. *J. Phys. Chem. Ref. Data* **18**, 1637–1755.