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Lipophilized epigallocatechin (EGC) and its derivatives: Inhibition of oxidation of β -carotene—linoleate oil-in-water emulsion and DNA strand scission

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Abstract

Tea epigallocatechin (EGC) was acylated with selected fatty acids, namely propionic acid [C3:0], caprylic acid [C8:0], lauric acid [C12:0], stearic acid [C18:0] and docosahexaenoic acid (DHA; C22:6 n-3). Antioxidant activity of EGC and its lipophilized derivatives were examined in various food (β -carotene—linoleate oil-in-water emulsion and bulk oil) and biological (supercoiled DNA and LDL) systems *in vitro* in order to evaluate the effect of increased lipophilicity on their antioxidant capacity. Lipophilized EGC derivatives were more effective in β -carotene—linoleate oil-in-water emulsion and bulk oil than their parent EGC molecule. Meanwhile, EGC and its derivatives showed more than 60% inhibition against DNA strand scission induced by hydroxyl or peroxy radical. Moreover, lipophilization of EGC had a negative effect on the inhibition of human LDL cholesterol peroxidation. Overall, this study revealed that EGC and its lipophilized derivatives could potentially be used as health promoting and disease preventing compounds.

Keywords: Acylation, Beta-carotene, DNA strand scission, Epigallocatechin (EGC), Fatty acids

1. Introduction

Catechins are polyphenolic flavonoids present in various plants. Epigallocatechin (EGC) has attracted much research interest due to its potential health benefits. EGC consists of a three-ring structure with six hydroxyl groups belonging to the flavan-3-ol/flavanol group of green tea catechins. Presence of hydroxyl groups renders greater hydrophilicity to the EGC molecule hence acting as a barrier towards its biological properties. Enhancement of lipophilicity could improve its efficacy which may potentially expand its nutraceutical applications.

Green tea, berries, grapes, apricots and red wine are some of the good sources of catechins. Catechins have been shown to exert various biological activities *in vitro* and *in vivo* such as anti-cancer, anti-

obesity, anti-glycation, anti-inflammatory, neuro-protective and cardioprotective effects. Anticancer activity of catechins has been attributed to its antioxidative property and its direct binding to proteins [1]. Green tea polyphenols have been shown to inhibit cancer cell proliferation, cancer related angiogenesis, metastasis as well as inducing apoptosis of cancer cells [1]. All these health benefits of catechins are greatly influenced by their bioavailability and bioaccessibility in the digestive tract [2]. Tarahovsky et al. [3] reported that passage through the apical membrane of the epithelial cell membrane is primarily determined by lipophilicity of catechins, which is decreased in the order of ECG > EGCG > EC > EGC. Ingestion of green tea by humans has been shown to produce ten different metabolites in plasma after 1.6–2.3 h, in the form of O-methylated, sulphated and glucuronide conjugates of (epi)catechins and (epi)gallocatechins, while 15 metabolites of (epi)catechins and (epi)

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gallo catechins were detected in urine sample after 24 h of ingestion [4,5]. Unmetabolized (–)-epigallocatechin-3-O-gallate and (–)-epicatechin-3-O-gallate were detected in plasma but not in the urinary sample, thus indicating that (epi)catechins are somewhat more bioavailable than most other flavonoids [4]. Roowi et al. [5] reported that 70% of the ingested catechins were present in the form of the parent compound (33%) and 23 metabolites (37%) in the ileal fluid, thus concluded that primary absorption occurred at the small intestine. In the large intestine, flavan-3-ols undergo cleavage by colonic microbiota, yielding epimers such as 1-(3',4')-dihydroxyphenyl)-3-(2'',4'',6''-trihydroxy)propan-2-ol, which is converted to (–)-5-(3',4'-dihydroxyphenyl)- γ -valerolactone and (–)-5-(3',4',5'-trihydroxyphenyl)- γ -valerolactone [6].

In this study, EGC was lipophilized with selected fatty acids, namely propionic acid [C3:0], caprylic acid [C8:0], lauric acid [C12:0], stearic acid [C18:0] and docosahexaenoic acid (DHA; C22:6 n-3). Antioxidative efficacy of EGC and its derivatives was examined in both food and biological model systems. Therefore, we hypothesized the increase in lipophilicity would improve the antioxidative capacity of EGC in food and biological systems.

2. Materials and methods

2.1. Materials

Epigallocatechin (EGC) sample was purchased from Chengdu Biopurify Phytochemicals Ltd (Chengdu, Sichuan, China). Acyl chlorides of fatty acids (propionyl [C3:0] chloride, capryl [C8:0] chloride, lauroyl [C12:0] chloride and stearoyl [C18:0] chloride) were purchased from Sigma–Aldrich Canada Ltd (Oakville, ON, Canada). DHA (docosahexaenoic acid) single cell oil (DHASCO) was obtained from DSM (Columbia, MD, USA). All other chemicals used were obtained from Sigma–Aldrich Canada Ltd or Fisher Scientific Ltd. (Ottawa, ON, Canada). The solvents used were of ACS grade, pesticide grade or HPLC grade and were used without any further purification.

2.2. Synthesis of EGC esters

Five acyl chlorides (propionyl [C3:0] chloride, capryl [C8:0] chloride, lauroyl chloride [C12:0], stearoyl chloride [C18:0] and DHA chloride [C22:6]) were used to esterify EGC. Detailed information about the esterification procedure was described elsewhere [7]. A high-performance liquid chromatography-electrospray ionization-time of flight-mass

spectrometry (HPLC-ESI-TOF-MS) and a proton NMR analysis were performed to identify the structure of esters. EGC-4'-O-caprylate (27%), EGC-3'-O-caprylate or EGC-5'-O-caprylate (12%) and EGC-3',5'-O-dicaprylate (16%) were the major resultant compounds from the acylation reaction of EGC with capryl [C8:0] chloride (Fig. 1) [7]. These compounds were purified using silica gel column chromatography with gradient elution of hexane/ethyl acetate/formic acid (90:10:2; 80:20:2; 70:30:2; 60:40:2 and 50:50:2; v/v/v) and analyzed for their antioxidant potential in various food and biological systems [7].

2.3. Antioxidant activity in oil-in-water emulsions

A β -carotene–linoleate oil-in-water emulsion model system was used to determine the antioxidant activity of EGC and its derivatives [8]. Ten milligrams of β -carotene were dissolved in chloroform (10 mL) followed by transfer of an aliquot (1.2 mL) of it into a flask containing linoleic acid (40 mg) and Tween 40 (400 mg). A blank devoid of β -carotene but containing 40 mg of linoleic acid and 400 mg of Tween 40 was prepared. Chloroform was removed under a stream of nitrogen; oxygenated distilled water (100 mL) was added to the flask and the mixture was stirred vigorously for 30 min. EGC and its derivatives (0.25 mg/mL; 0.5 mL), dissolved in ethanol, were mixed with the above emulsion (4.5 mL). A control without test compounds and a mixture of blank (without β -carotene) were prepared for each sample. The absorbance was read immediately after the addition of the emulsion at 470 nm using a spectrophotometer. The tubes were incubated in a shaking water bath at 50 °C and the

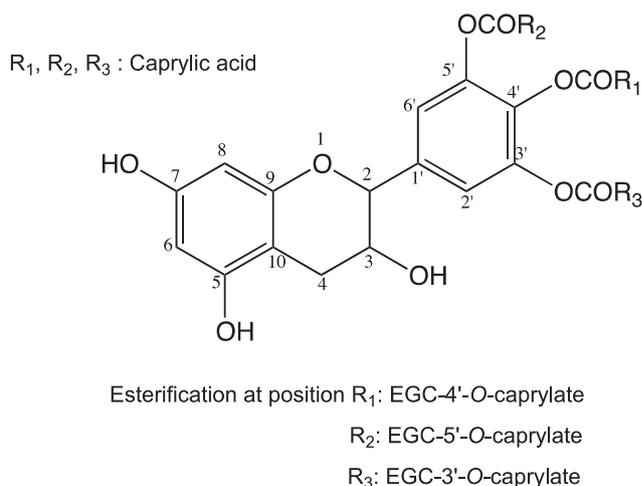


Fig. 1. Structures of EGC caprylate esters.

absorbance was read over a 75 min period at 15 min intervals. A kinetic curve was plotted against blank-corrected absorbance and time. Antioxidant activity of EGC esters in protecting β -carotene/linoleic acid oxidation was calculated using the following equation.

$$\text{Antioxidant activity (\%)} = [1 - (A_0 - A_t) / A_0^0 - A_t^0] \times 100$$

where A_0 and A_t are corrected absorbance values for test samples measured at zero time and after incubation, respectively; while A_0^0 and A_t^0 are corrected absorbance values for the control at time zero and at time t after incubation, respectively.

2.4. Antioxidant activity in bulk oil

A corn oil sample stripped of its minor components was used to measure the antioxidant activity of EGC and its derivatives in bulk oil [9]. The stripped corn oil (1 g) was weighed into a clear glass vial (10 mL) and capped loosely in order to induce oxidation of bulk oil. EGC and its derivatives (12.5 $\mu\text{g/mL}$, 100 μL) dissolved in ethanol were added followed by the removal of the solvent under a stream of nitrogen. The vials were wrapped in an aluminum foil in order to protect samples from light and stored in a forced air oven (Precision Scientific Co., Chicago, IL, USA) at 60 ± 0.5 °C. Sample vials were removed from the oven on day 0, 2, 4, 6, 8 and 10 for analyses and the formation of conjugated dienes [10] and p -anisidine values (pAV) [11] were monitored according to the AOAC [10,11] methods.

2.5. Inhibition of cupric ion-induced human low-density lipoprotein (LDL) peroxidation

The inhibitory effect of EGC esters on cupric ion-induced human low-density lipoprotein (LDL) peroxidation was determined according to the method described elsewhere [8]. Initially, LDL (5 mg/mL) was dialyzed against 100 volumes of freshly prepared phosphate buffer (10 mM, pH 7.4, 0.15M NaCl). A dialysis tube (MWCO of 12–14 kDa, Fischer, Carle and Kammerer Scientific, Nepean, ON, Canada) was used to dialyze LDL at 4 °C under a nitrogen blanket in the dark for 12 h. Diluted LDL cholesterol (0.04 mg LDL/mL) was mixed with the EGC extracts dissolved in ethanol (5 $\mu\text{g/mL}$, 10 μL) in an Eppendorf tube. The samples were pre-

incubated at 37 °C for 15 min and the reaction was initiated by adding a solution of cupric sulphate (0.1 mL, 100 μM). The samples were then incubated at 37 °C for 22 h. The formation of conjugated dienes (CD) was recorded at 234 nm using a diode array spectrophotometer (Agilent, Palo Alto, CA, USA). The appropriate blanks were run for each sample by replacing LDL cholesterol and CuSO_4 and with distilled water for background correction.

2.6. Inhibition of peroxy and hydroxyl radical-induced supercoiled DNA strand scission

EGC and its derivatives were examined for their inhibitory potential towards peroxy and hydroxyl radical-induced supercoiled DNA strand scission [9]. Supercoiled plasmid DNA (pBR 322; 50 $\mu\text{g/mL}$) was dissolved in a freshly prepared phosphate buffer (PBS, 10 mM, pH 7.4). EGC and its derivatives dissolved in ethanol (2.5 $\mu\text{g/mL}$, 2 μL), PBS (2 μL), pBR 322 (50 $\mu\text{g/mL}$, 2 μL) and 4 μL of 17.5 mM AAPH (2,2'-azobis-2-methylpropanimidamide dihydrochloride, prepared in PBS) were added in an Eppendorf tube (0.5 mL capacity) to generate peroxy radicals, while EGC samples (2.5 $\mu\text{g/mL}$, 2 μL), PBS (2 μL), pBR 322 (50 $\mu\text{g/mL}$, 2 μL), FeSO_4 (0.5 mM, 2 μL) and H_2O_2 (1 mM, 2 μL) were added to produce hydroxyl radicals. A blank with DNA alone and a control devoid of samples were also prepared. The mixture was incubated (37 °C, 1 h) in the dark followed by the addition of 1 μL of the loading dye containing bromophenol blue (0.25%), xylene cyanol (0.25%) and glycerol (50%). Ten microlitres of the above mixture were loaded onto agarose gel (0.7%) prepared in Tris-acetic acid-EDTA (TAE) buffer (40 mM Tris-acetate containing 1 mM EDTA, pH 8.5). Five microlitres of SYBR safe were added into the agarose gel solution (50 mL) as a gel stain. Electrophoresis was conducted at 80 V for 60 min using a model B1A horizontal mini gel electrophoresis system (Owl Separation Systems Inc., Portsmouth, NH, USA) and a model 300 V power supply (VWR International Inc., West Chester, PA, USA) in TAE buffer. The DNA bands were observed under transillumination of UV light using Alpha-Imager™ gel documentation system (Cell Biosciences, Santa Clara, CA, USA) and the intensity (area %) of bands was quantified with the Chemi-Imager 4400 software (Cell Biosciences, Santa Clara, CA, USA). The retention of supercoiled DNA strand (%) was

$$\text{DNA retention}(\%) = \frac{\text{Area of super coiled DNA with oxidative radical and extract}}{\text{Area of super coiled DNA in control}} \times 100$$

calculated using the following equation.

2.7. Statistical analysis

All experiments were replicated three times and mean values and standard deviations reported. One-way ANOVA was performed, and the mean separations were performed by Tukey's HSD test ($P < 0.05$) using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Antioxidant activity in oil-in-water emulsions

Antioxidant activity of EGC and its derivatives was examined in a β -carotene-linoleate oil-in-water emulsion model system (Fig. 2). All derivatives of EGC prevented the colour decomposition of β -carotene in β -carotene-linoleate oil-in-water emulsion model system compared to parent EGC molecule, which is evident from the measured absorbance at 470 nm (Fig. 2). This implies that an increase in lipophilicity enhances the antioxidant activity in an oil-in-water emulsion. The inhibition activity of EGC and its derivatives after 15 min of

incubation is shown in Table 1. EGC-C3:0, EGC-C8:0 and EGC-C12:0 showed nearly 79–85% inhibitory activity against β -carotene bleaching after 15 min of incubation. However, EGC itself showed only 12% inhibition in β -carotene–linoleate oil-in-water emulsion model system. This is in agreement with the results observed for EGCG and its derivatives [8]. Amarowicz and Shahidi [12] examined the antioxidant activity of individual green tea catechins in a β -carotene–linoleate, oil-in-water emulsion model system and found their antioxidant efficacy to be in the order of ECG > EGCG ~ EC > EGC. Moreover, Terao et al. [13] observed that EC and ECG had poor antioxidant activity in unilamellar liposomes compared to EGC and EGCG and concluded that the flavonoid-containing pyrogallol moiety in their B ring had low antioxidant activity compared to flavonoids containing other constituents.

In addition, it is noteworthy that the observed results followed the polar paradox theory, which states that the hydrophilic antioxidants are more effective in a non-polar media such as bulk oils, while lipophilic antioxidants work better in oil-in-water emulsions or liposomes [14]. Hence, increased lipophilicity of EGC derivatives showed better antioxidant activity in β -carotene–linoleate, oil-in-water emulsion model system regardless of the

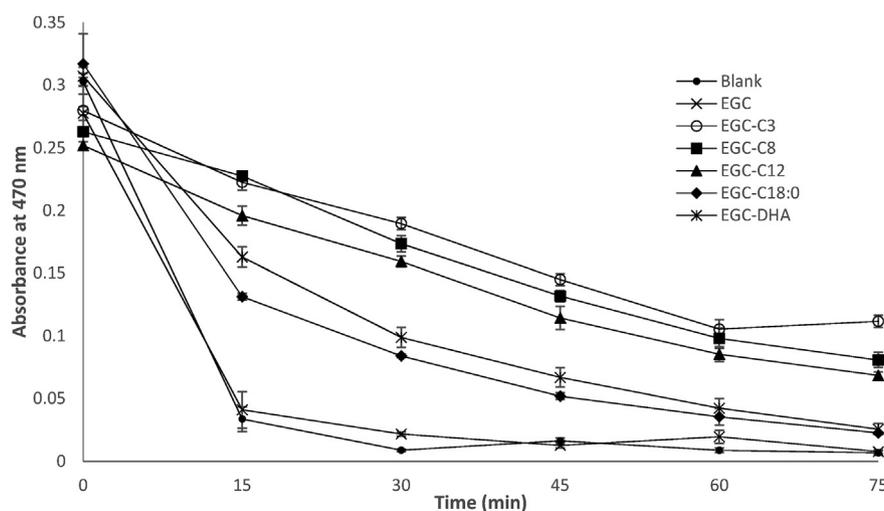


Fig. 2. Inhibition of beta-carotene bleaching by EGC and its derivatives.

Table 1. Inhibition of beta Carotene bleaching and human LDL cholesterol peroxidation by EGC and its derivatives¹.

Compound	Inhibition of β -carotene bleaching ² %	Inhibition of human LDL peroxidation ³ (%)
EGC	12.1 \pm 3.9 ^e	46.4 \pm 2.8 ^a
EGC-C3:0	85.5 \pm 2.0 ^a	39.2 \pm 2.5 ^a
EGC-C8:0	86.9 \pm 1.5 ^a	2.8 \pm 1.6 ^b
EGC-C12:0	79.2 \pm 2.2 ^b	5.6 \pm 2.6 ^b
EGC-C18:0	31.0 \pm 2.0 ^d	6.4 \pm 1.6 ^b
EGC-DHA	46.4 \pm 2.4 ^c	5.8 \pm 2.1 ^b

Values followed by the same superscript are not significantly different ($P > 0.05$) by Tukey's HSD test.

¹ All data represent the mean of triplicates.

² Inhibition (%) of beta Carotene bleaching measured after 15 min of incubation.

³ Inhibition (%) of human LDL peroxidation measured after 12 h of incubation.

absence of the chelation sites. The higher antioxidant effectiveness of lipophilic EGC derivatives could be attributed to their ability to form a protective membrane around the lipid droplet and scavenging free radicals at the interface whereas polar EGC molecule could predominantly get dissolved in the aqueous phase. Furthermore, EGC acylated with long chain unsaturated fatty acids could interfere with the alignment at the interface due to the bent structure, thus showing less antioxidant activity than EGC compounds acylated with short chain fatty acids. This is in agreement with the study reported by Laguerre et al. [15], where among the tested homologous series of chlorogenic acid esters (methyl, butyl, octyl, dodecyl, hexadecyl, octadecyl and eicosyl), dodecyl chlorogenate showed greater antioxidant activity in an emulsion system. This revealed that short- and medium-chain

lipophilic esters work better in emulsions than long chain esters. This effect is known as cut off effect or non-linear effect, which might be due to their partitioning, location and mobility in the multiphase system that is influenced by both polarity and molecular size [14].

3.2. Antioxidant activity in bulk oil

To examine the antioxidant effect of EGC and its derivatives, a non-polar medium such as bulk oil was used. A stripped corn oil devoid of its minor components such as monoacylglycerols (MAGs), diacylglycerols (DAGs), free fatty acids (FFAs), phospholipids, sterols, chlorophylls, carotenoids, tocopherols (tocopherols/tocotrienols), other phenolic compounds, and metal ions was used; its oxidative stability was evaluated in the presence of EGC and

Table 2. Antioxidant activities of EGC and its derivatives in bulk oil measured by the formation of conjugated dienes and *p*-anisidine value.

Conjugated dienes formation						
Samples	Storage period (days)					
	0	2	4	6	8	10
Control	0.08 \pm 0.00 ^a	0.43 \pm 0.01 ^a	0.93 \pm 0.00 ^a	2.11 \pm 0.02 ^a	5.63 \pm 0.01 ^a	5.36 \pm 0.05 ^e
EGC	0.02 \pm 0.01 ^b	0.21 \pm 0.00 ^d	0.75 \pm 0.01 ^d	1.03 \pm 0.01 ^f	2.94 \pm 0.03 ^d	7.09 \pm 0.30 ^{ab}
EGC-C3:0	0.07 \pm 0.01 ^a	0.24 \pm 0.01 ^{cd}	0.79 \pm 0.02 ^c	1.16 \pm 0.02 ^e	2.97 \pm 0.04 ^d	6.70 \pm 0.11 ^{bc}
EGC-C8:0	0.08 \pm 0.02 ^a	0.30 \pm 0.00 ^b	0.88 \pm 0.01 ^b	1.29 \pm 0.01 ^{cd}	3.73 \pm 0.03 ^b	6.97 \pm 0.12 ^b
EGC-C12:0	0.09 \pm 0.00 ^a	0.25 \pm 0.02 ^c	0.73 \pm 0.02 ^d	1.23 \pm 0.07 ^{de}	2.97 \pm 0.02 ^d	7.40 \pm 0.02 ^a
EGC-C18:0	0.07 \pm 0.01 ^a	0.31 \pm 0.01 ^b	0.81 \pm 0.01 ^c	1.43 \pm 0.05 ^b	3.62 \pm 0.06 ^c	6.54 \pm 0.18 ^{cd}
EGC-DHA	0.08 \pm 0.01 ^a	0.30 \pm 0.01 ^b	0.80 \pm 0.02 ^c	1.35 \pm 0.02 ^{bc}	3.65 \pm 0.01 ^{bc}	6.29 \pm 0.05 ^d
<i>p</i> -Anisidine values						
Samples	Storage period (days)					
	0	2	4	6	8	10
Control	11.7 \pm 1.4 ^b	14.2 \pm 1.7 ^e	26.7 \pm 1.5 ^a	43.0 \pm 1.8 ^a	167.4 \pm 6.2 ^a	614.9 \pm 3.7 ^a
EGC	22.8 \pm 2.0 ^a	19.9 \pm 0.4 ^{bc}	22.6 \pm 3.2 ^a	27.0 \pm 3.0 ^c	65.4 \pm 8.2 ^c	313.5 \pm 1.2 ^{ef}
EGC-C3:0	23.8 \pm 2.9 ^a	21.4 \pm 1.6 ^{abc}	22.4 \pm 1.3 ^a	30.2 \pm 1.3 ^{bc}	69.4 \pm 1.1 ^c	324.3 \pm 2.9 ^e
EGC-C8:0	20.9 \pm 2.4 ^a	25.3 \pm 2.5 ^a	24.4 \pm 3.9 ^a	31.8 \pm 2.2 ^{bc}	89.9 \pm 4.6 ^b	397.3 \pm 4.8 ^b
EGC-C12:0	22.3 \pm 3.2 ^a	23.8 \pm 1.3 ^{ab}	21.2 \pm 2.1 ^a	33.9 \pm 5.2 ^{abc}	70.1 \pm 1.4 ^c	302.7 \pm 3.0 ^f
EGC-C18:0	20.3 \pm 1.7 ^a	19.2 \pm 0.2 ^{cd}	24.3 \pm 4.4 ^a	35.8 \pm 4.7 ^{abc}	88.9 \pm 3.7 ^b	356.7 \pm 7.5 ^d
EGC-DHA	19.0 \pm 3.4 ^a	15.5 \pm 1.7 ^{de}	20.5 \pm 4.7 ^a	37.5 \pm 3.9 ^{ab}	91.4 \pm 5.3 ^b	380.2 \pm 6.8 ^c

its derivatives. The formation of conjugated dienes (CD) and *p*-anisidine values (*p*AV) (Table 2) were measured on day 0, 2, 4, 6, 8 and 10 of storage. Fatty acids (primarily linoleic acid) in corn oil get oxidized by the abstraction of a hydrogen atom from the bis-allylic carbon atom followed by delocalization and production of conjugated dienes as primary oxidation products, which can be measured spectrophotometrically at 234 nm. Unstable conjugated dienes further dissociate and generate aldehydes, ketones, alcohols, hydrocarbons and other molecules as secondary oxidation products that could be measured by *p*-anisidine value. The *p*-anisidine reagent reacts with aldehydes (2-alkenals and 2,4-alkadienals) and afford a yellow coloured chromophore that could be measured at 350 nm [16].

Table 2 shows the formation of conjugated dienes. The stripped con oil treated with EGC had a comparatively low number of conjugated dienes than its derivatives, which is contrary to the antioxidant activity observed in the β -carotene bleaching assay. A similar trend was observed for *p*-anisidine value (Table 2) of EGC and its derivatives. There is a 2 days lag phase in the generation of conjugated dienes, then the breakdown of primary oxidation products into secondary oxidation products which had a lag phase of 6 days as reflected in *p*-anisidine values. These results suggest that increased lipophilicity affects the antioxidant efficacy of EGC in bulk oil. This observation could be explained based on the polar paradox theory and interfacial phenomenon. Chaiyasit et al. [17] reported that bulk oils also contain small amounts of water, hence polar antioxidants tend to form colloidal structures (reverse micelles and lamellar structures). The hydrophilic EGC molecules tend to orient at the water–oil interface of the colloids and form a protective membrane, thus preventing oxidation of bulk oil better than lipophilic antioxidants. Moreover, the polar paradox theory states that polar antioxidants having a high hydrophilic-lipophilic balance (HLB) are more effective than nonpolar lipophilic antioxidants in bulk oils [8,17]. However, EGC derivatives also showed promising antioxidant activity the same as EGC in stripped corn oil, hence could be used as potential antioxidants in bulk oils. Furthermore, it is noteworthy that EGC esterified with unsaturated fatty acid (EGC-DHA) also inhibited the formation of conjugated dienes and secondary oxidation products to nearly the same level as other EGC derivatives despite its own oxidizable nature. This agrees with the results

observed for resveratrol and its esters with unsaturated fatty acids [9].

3.3. Inhibition of cupric ion-induced human low-density lipoprotein (LDL) peroxidation

Several studies have shown that green tea catechins are effective in protecting atherosclerosis which is caused primarily by the oxidation of LDL [18,19]. Berliner and Heinecke [20] suggested that the oxidation of LDL could be promoted by free metal ions, protein-bound metal ions, intracellular protein-bound iron, thiols, reactive oxygen intermediates, lipoxygenase, peroxyxynitrite as well as myeloperoxidase. The cardioprotective effect of green tea catechins proceeds via multiple mechanisms including the inhibition of LDL oxidation, vascular inflammation, thrombogenesis as well as improvement of lipid profile in plasma [21,22]. Moreover, lipid-soluble antioxidants have been shown to retard or inhibit atherosclerosis in hypercholesterolemic animals [23]. Antioxidants in general and polyphenols (catechins), in particular, have been shown to scavenge free radicals and other reactive oxygen species, chelate pro-oxidative metal ions as well as binding with apo-lipoprotein B, hence reduce LDL uptake by macrophages, inhibit LDL oxidation and decrease the susceptibility of LDL to aggregation [24,25].

In this study, EGC and its lipophilic derivatives were examined for inhibitory activity against cupric ion induced human LDL oxidation. Among tested compounds, EGC and EGC-C3:0 exhibited significantly ($p < 0.05$) higher inhibitory activity of 39–46% after 12 h of incubation (Table 1). Meanwhile, all other derivatives showed only 2–6% inhibition against LDL oxidation. These results demonstrated that increased lipophilicity decreased the inhibitory activity of EGC derivatives against LDL peroxidation. However, the observed results contradict those reported for EGCG and its derivatives, where improved lipophilicity increased the effectiveness of inhibition towards cupric ion-induced LDL peroxidation [8]. It is plausible that acylation of the hydroxyl group with a fatty acid could affect cupric ion chelating ability of EGC, consequently not being able to prevent LDL oxidation induced by cupric ion. However, EGC-C3:0 inhibited the LDL peroxidation to nearly the same extent as EGC, perhaps due to the fact that short-chain fatty acids might not affect the accessibility of cupric ion towards hydroxyl groups compared to the steric hindrance imposed by medium- and long-chain fatty acids. Moreover, improved lipophilicity of EGC-C3:0 could have enhanced the affinity towards the

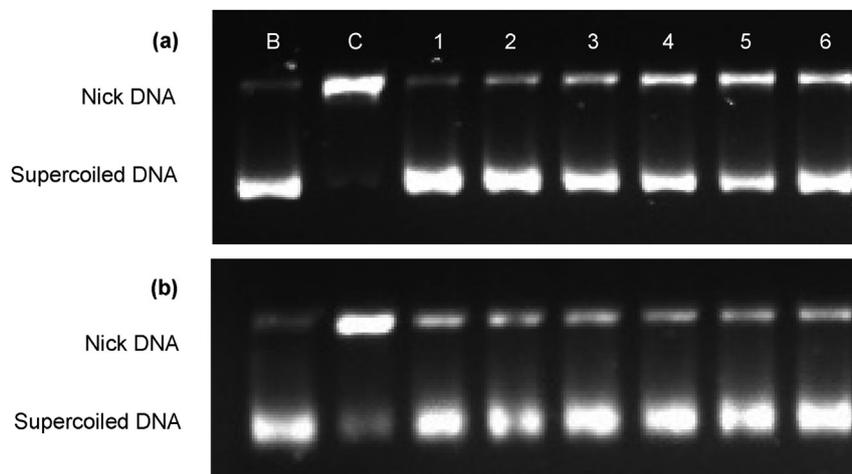


Fig. 3. Agarose gel electrophoresis of inhibition of peroxyl (a) and hydroxyl (b) radical induced supercoiled DNA strand scission by EGC and its derivatives; N-Nicked DNA, S-Supercoiled DNA, B-Blank, C-Control, 1-EGC, 2-EGC-C3:0, 3-EGC-C8:0, 4-EGC-C12:0, 5-EGC-C18:0 and 6-EGC-DHA.

surface of LDL (phospholipids), thus being more effective. Furthermore, the highly unsaturated fatty acid in EGC-DHA derivative could have oxidized itself and generated conjugated dienes, which might have counteracted the inhibitory effect of the molecule.

3.4. Inhibition of peroxyl and hydroxyl radical-induced supercoiled DNA strand scission

Several studies have shown that green tea catechins could exert anti-cancer properties via inhibiting cell viability, inhibiting DNA synthesis in cancer cells and inducing apoptosis in various cancer cell lines [26]. Kager et al. [27] observed a decrease in DNA damage in lymphocytes (blood), colonocytes (colon), and hepatocytes (liver) in rats fed a green tea polyphenol extract (500 mL of green tea/day for 5 days) compared to the control rats. The protective effect of green tea polyphenols towards DNA damage has been attributed to scavenging of free radicals, reducing glucose uptake in the gastrointestinal

tract that affects the mitochondrial function followed by a decrease in the production of reactive oxygen species and inducing antioxidant enzymes such as superoxide dismutase and glutathione-S-transferase by inducing transcription factors [27].

DNA could be damaged by superoxide or a combination of superoxide with other free radicals at both the phosphate backbone and the nucleotide bases that could lead to strand scission, sister chromatid exchange, DNA–DNA and DNA–protein cross-links [28,29]. Hydroxyl or peroxyl radicals cleave the single strands of supercoiled DNA (form I) into a nicked open circular form (form II) and a linear form (form III). Fig. 3 shows the image of agarose gel electrophoresis of inhibition of peroxyl (a) and hydroxyl (b) radical induced supercoiled DNA strand scission by EGC and its derivatives. In this study, the linear form (form III) was not observed, thus reflecting the absence of double strand breakage under the test conditions. As shown in Fig. 3, EGC and its derivatives retained more supercoiled DNA than the control sample (devoid of test compounds), which is reflected by the intensity of bands.

Inhibition of hydroxyl radical-induced DNA strand scission by EGC and its derivatives varied between 81 and 89% while peroxyl radical-induced DNA scission inhibition ranged from 66 to 99% (Table 3). The parent molecule, EGC showed significantly ($p < 0.05$) higher inhibition against DNA strand scission caused by peroxyl radicals. The results revealed that lipophilization has affected the ability to scavenge peroxyl radicals or the metal chelating capacity of EGC derivatives. EGC could

Table 3. Inhibition of DNA scission by EGC and its derivatives¹.

Samples	DNA scission inhibition %	
	Hydroxyl radical	Peroxyl radical
EGC	81.81 ± 1.90 ^c	99.16 ± 1.41 ^a
EGC-C3:0	82.44 ± 2.95 ^{bc}	88.96 ± 3.17 ^b
EGC-C8:0	86.69 ± 1.33 ^{abc}	77.64 ± 3.12 ^c
EGC-C12:0	87.88 ± 1.75 ^{ab}	72.23 ± 3.25 ^c
EGC-C18:0	88.60 ± 0.35 ^a	66.31 ± 1.98 ^d
EGC-DHA	88.80 ± 1.47 ^a	72.05 ± 3.39 ^c

Values followed by the same superscript are not significantly different ($P > 0.05$) by Tukey's HSD test.

¹ All data represent the mean of triplicates.

exert an antioxidant or pro-oxidant effect on hydroxyl radicals in the presence of iron or copper ions (Fenton reaction) [26]. Regardless of the generation of hydroxyl radicals, EGC and its derivatives inhibited hydroxyl radicals by more than 80% and showed promising antioxidant activity against DNA strand scission. Moreover, EGC could possibly reduce ferric ion to most reactive ferrous ion and enhance the generation of hydroxyl radicals thus compromising the overall DNA protective effect. This in agreement with the results observed for EGCG by Zhong and Shahidi [8]. However, an opposite trend was observed for peroxy radical-induced DNA strand scission, where the ester derivatives were less effective in protecting peroxy radical-induced DNA strand scission than the EGC molecule itself. Hence EGC and its derivatives showed more than 65% inhibition against DNA strand scission induced by hydroxyl or peroxy radical, thus they could be considered as novel antimutagenic compounds.

4. Conclusion

Lipophilized EGC derivatives showed better or comparable antioxidative potential compared to the EGC molecule itself in various food (β -carotene–linoleate oil-in-water emulsion and bulk oil) and biological (supercoiled DNA and LDL) systems *in vitro*. The antioxidative efficacy of EGC derivatives was mainly influenced by the lipophilicity, chain length of fatty acids, the reaction medium, mechanism of antioxidant action and the concentration of test compounds. This study demonstrated the potential utilization of lipophilized derivatives of EGC in various food and biological systems as a health promoting ingredient.

Conflict of interest

The authors declare no conflict of interest.

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