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Antioxidant and antimicrobial activity of individual catechin molecules: A comparative study between gallated and epimerized catechin molecules

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ABSTRACT

Protection of DNA samples against free radical damage was found to elevate with Gallocatechin gallate (GCG) followed by epigallo catechin (EGC). Epicatechin gallate and (+) catechin exhibited lower quantum of free radical scavenging activity. It is interesting to note that higher concentration of individual catechin molecules registered concurrent protection of DNA from free radical damage. Results showed that the epimerized form of gallocatechin gallate had high antagonistic effect against both the tested strains followed by epigallo catechin while, catechin gallate and (+) catechin recorded lower activity against microbes. Among the phenolic acids pyrogallic acid possessed high antimicrobial activity.

Keywords: Free radicals; DNA damage; phenolic components; antimicrobial activity.

INTRODUCTION

Plants contain a wide variety of free radical scavenging molecules, such as flavonoids, anthocyanins, carotenoids, dietary glutathiones, vitamins and endogenous metabolites which are natural products with antioxidant activities[1-4]. Various methods have been established to evaluate antioxidant activity of flavonoids such as active oxygen sepsis (superoxide anion, peroxyl radical and hydroxyl radical), 1,1-diphenyl-2-picrylhydraxyl (DPPH) radical and 2, 2 -azinobis (3-ethyl benzothiazoline)-6-sulfonate radical cation (ABTS). These methods are widely used to analyze the capacity of free radical scavenging activity of the phenolic components[5].

In laboratory animals, concentrated and purified polyphenol extracts of green tea had been shown to have anticarcinogenic activity against tumors of the duodenum [6], esophagus [7], lung [8] and skin [9]. Tanaka et al. (1997) suggested the reduction of chemically induced mammary gland carcinogenesis by green tea [10], but the results reported were not statistically significant. Furthermore, green tea was administered in the feed rather than the drinking fluid. In a series of three bioassays, a significant inhibitory effect of black tea on mammary tumorigenesis was found in rats fed at high fat diet [11]. The toxicity of tea extracts is low and they are potentially important for cancer chemotherapeutic agents.

Despite the apparently beneficial health effects of flavonoids, several studies indicated their improved vascular endothelial functions [12,13]. This may be due to their activity as pro-oxidants in generating free radicals that damage DNA or their inhibition of DNA associated enzymes such as topoisomerase. Unrepaired or misrepaired oxidative DNA damage can result in DNA strand breaks and mutations [14] that may lead to irreversible preneoplastic lesions. Furthermore, high intakes of these compounds may potentiate other deleterious effects due to their diverse pharmacological properties, which may alter drug and amino acid metabolism, modulate the activity of environmental genotoxicants and change the activity of other key metabolizing enzymes. Tea epicatechin molecules are responsible for such beneficial effects against various diseases but the compounds were converted frequently into its epimers. According to Chen et al. 2001[15] and Xu et al. 2003[16] the converted epimers amount was very high in some bottled products which are stated for health benefits. Thus, it is necessary to study the role of such epimers against free radical damages.

MATERIALS AND METHODS

DNA isolation from animal tissue: The DNA from animal cells, goat liver was taken and isolated according to Qi Wu et al. (1995) [17]. Goat liver was cut into small fragments, ground in Tris-EDTA (TE) buffer and centrifuged at 1200 rpm for 10 min. 0.2 g of tissue was lysed per 5ml of lysis buffer (400 mM NaCl. 1.00 mM Tris-HCl (pH 8.5), 5 mM EDTA, 0.2% SDS, 20 µg/ml RNase A and 500 µl/ml Proteinase K) overnight at 37°C in petri plates. After digestion, an equal volume of isopropanol was added to precipitate the DNA followed by centrifugation. The isolated DNA was washed twice with 70% ethanol. After washing, the DNA was air dried briefly (10 min), and dissolved in an appropriate amount of TE buffer and incubated at 37°C for 2 h or at 4°C overnight, DNA obtained with this technique consistently gives a 260/280 absorbance ratio of l.8-2.0 indicating good purity of the DNA.

DNA damage: To study the free radical damage, 4 µl (100 ng/µl) of DNA was mixed with 2 µl of TE buffer (Tris-HCl, 200 mM and EDTA, 5 mM) and 4 µl of 30% hydrogen peroxide. In this mixture, the individual catechin molecules along with epimers at 1.0 mM concentration were added and the volume was made up to 20 µl with sterile distilled water. One negative control was prepared in the same way without adding individual catechin molecules. All these samples were placed under UV light to generate free radicals for 30 min. One positive control was also prepared by taking 4 µl of DNA and volume was made up to 20 µl with sterile distilled water. No UV treatment was given to positive control.

Gel electrophoresis: All treated positive and negative control samples were mixed with 4 µl of 6 X loading dye and separated on 1% agarose gel in Tris-Acetate EDTA (TAE - Tris 89 mM, EDTA 89 mM. pH 8.3) buffer for 3 hours at 80 V. Staining of agarose gel was carried out with ethidium bromide for 1/2 hour. The gel was placed on UV-illuminator for photography and for calculating the free radical damages.

Calculating the scavenging activity: DNA damage was calculated based on gel photograph collected from three different gel images using Total Lab software, Version 1.0 (Amersham Biosciences) by comparing the band readings of treated samples with the untreated positive control.

Against human infecting microbes

Bacterial strains: The reference strains *Escherichia coli* and *Staphylococcus aureus* were purchased from (IMTEC, Chandigarh) and used to study the antimicrobial activity of the extracted individual molecules. Strains stored in sterile glycerol at -70^{o}C, were cultured on Luria Bertaini agar (LB) medium for activation and subcultured three times on the same medium to retain its virulence.

Susceptibility tests: The susceptibility tests were performed by the Mueller Hinton Agar well diffusion method [18]. The bacterial strains grown on nutrient agar at 37°C for 18 to 20 h were suspended in saline solution (0.85% NaCl, w/v). The suspension was spreaded in 90 mm diameter petri dishes containing LB medium, with a sterile non-toxic cotton swab on a wooden applicator. Wells (5 mm diameter) were punched in the agar and filled with 50 µl of individual phenolic (2 mM) extract. Plates were incubated at 37°C for 24 h. Antibacterial activities were evaluated by measuring inhibition zone diameters.

RESULTS AND DISCUSSION

Protection of animal DNA from free radical damage by catechin molecules: Electrophoretic movement of animal DNA after UV- photolysis of H_2O_2 in the presence of individual catechin molecules was presented in figure 1. Lane 1 indicated the control DNA (without free radical generation) and lane 2 represented the negative control (DNA without catechin molecules and treated with free radicals). The presence of individual catechin molecules at 1mM concentration have suppressed the degradation of DNA and prevented the DNA damage from free radicals (lane 3-9 in figure 1). The DNA images were analyzed with specific software Total lab *ver.* 1.0 to study the per cent of DNA degradation and also to find the per cent of DNA protection by individual catechin molecules (Table 1). The protection of DNA samples against free radical damage was found to be high in gallo catechin gallate (94%), followed by catechin gallate. The lowest degree of protection was found in (+) catechin followed by epi catechin gallate and the protection level was around 70%. Comparatively the degree of protection was found to be high in the case of gallated catechin molecules than epicatechins.

Earlier reports suggested that the free radical scavenging activity is high in EGCG using DPPH [19] and the epimers of the phenolic components were having high free radical scavenging activity than its original form. According to Tiefeng Xu et al. (2004) [20], the epimerized forms of catechins are comparable in scavenging activity with its original epicatechin forms. Our result

indicated that the epimers of individual catechin molecules GCG, CG provided better protection to DNA against free radical damage than ECG, EC, EGCG and EGC. Earlier studies of Jin Ze Xu et al. (2004) used spectrophotometric method of analyzing the antioxidant properties of individual catechin molecules [21]. But we had used animal DNA to measure the protection capacity of individual catechin molecules against free radicals.

Our results confirmed that epimers of individual catechin molecules are better for protecting the DNA from the free radicals. Higher concentration of individual fractions had high free radical scavenging activity.

Table 1. Protection (%) of animal DNA from free radical damage by catechin molecules at 1mM concentration

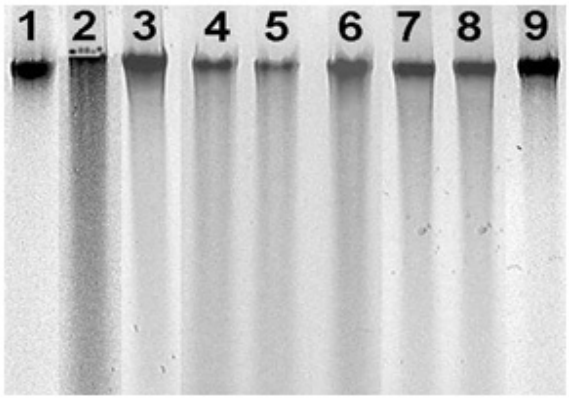
Components	**Protection of DNA %**	
DNA alone (positive control)	100.00	
DNA + H_2O_2 (negative control)	40.84	
DNA + H_2O_2 + EGC	89.75	
DNA + H_2O_2 + EGCG	72.45	
DNA + H_2O_2 + ECG	71.81	
DNA + H_2O_2 + (+) Cat	70.58	
DNA + H_2O_2 + CG	90.63	
DNA + H_2O_2 + GC	86.57	
DNA + H_2O_2 + GCG	94.03	
	CD @ 5%	
Between treatments (T)	0.73	
Between concentrations (C)		0.64
Interaction (T X C)		1.12

Table 2. Antagonistic effect of individual plant secondary products

	Compound purity (%)	***E.coli***	***Staph.aureous***
GA	71.68	+	+
PGA	68.33	++	++
EGCG	83.22	+++	+++
GCG		+++	+++
ECG	67.58	++	++
CG		++	++
EGC	82.50	++	+
GC		++	++
EC	78.75	++	++
(+)C	91.48	++	++

+	*low activity*
++	*medium activity*
+++	*high activity*

Fig. 1. Protection of animal DNA from free radical damage by catechin molecules

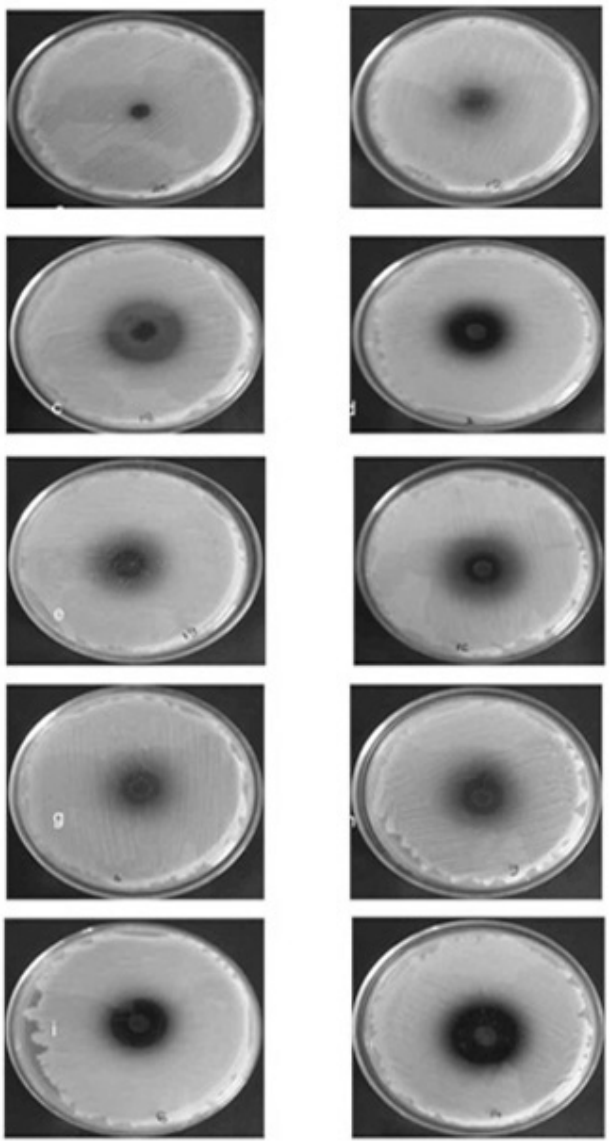


Lane 1: Positive control, Lane 2: Negative control, Lane 3: EGC, Lane 4: EGCG, Lane 5: ECG, Lane 6: (+) catechin, Lane 7: CG, Lane 8: GC, Lane 9: GCG

Antagonistic effect of individual phenolic components extracted from cell culture: The column eluted and identified samples were used against human pathogens like *E. coli* and *Staphylococcus aureus*. Result indicated that the epimerised form GCG had the highest antagonistic effect against both the organisms (*E. coli* (fig. 2) and *Staphylococcus aureus* (fig. 3) followed by epigallo catechins (figs. 2 and 3). The lowest activity was observed in the case of catechin gallate followed by (+) catechin. When comparing the results of phenolic acids, pyro gallic acid had higher anti microbial activity than gallic acid (Table 2).

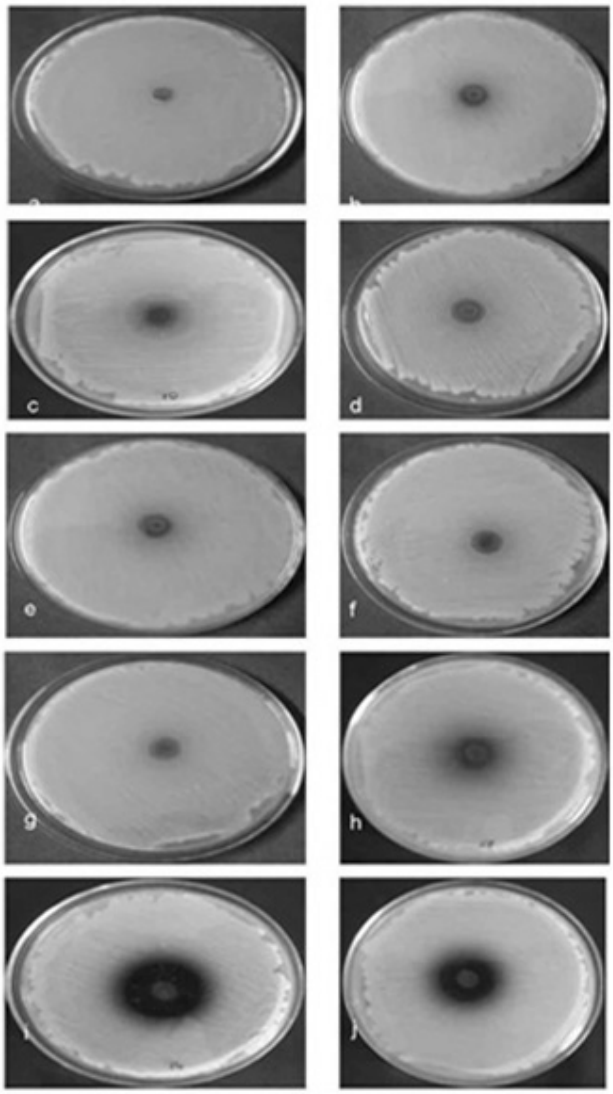
Phenolic components are found to have high antagonistic effects against microflora causing diarrhea, dental diseases and food poisoning [22,23, 24]. According to Puupponen et al. (2005) [25], the phenolic components had inhibited the growth of microflora in the intestine. *Escherichia coli* and *Staphylococcus aureus* are the two common microbes causing food poisoning and several dental problems to humans. The extracted plant secondary products were tested against these two strains to find the components having the highest antagonistic effect. Sakanaka (1997) [26] also reported that all the components possess antagonistic effect against the microbes. Strongest inhibitory activity was noted in the GCG, EGCG and ECG.

Figure 2. Antagonestic effects of individual plant secondary products against *E.coli*



a. GA, b. pyro GA, c. EC, d. (+)cat, e. ECG, f. CG, g. EGC, h. GC, i. EGCG, j. GCG

Figure 3. Antagonestic effects of individual plant secondary product against *Staphilococcus aurious*



a. GA, b. pyro GA, c. EC, d. (+)cat, e. ECG, f. CG, g. EGC, h. GC, i. EGCG, j. GCG

Based on the result, a strong correlation was obtained between tea individual catechin molecules against free radical scavenging activity and antimicrobial activity. It was observed that the epimers showed higher DNA protection against free radicals as well as antimicrobial activity.

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