# Effect of Tea Polyphenols on Glucan Synthesis by Glucosyltransferase from *Streptococcus mutans*[1)]

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**In the course of our studies on the development of anti-plaque agents for prevention of dental caries, we investigated effects of some of tea preparations and their individual components on the glucan synthesis catalyzed by glucosyltransferase (GTF) from *Streptococcus mutans*. Extracts of green tea and black tea, and polyphenol mixtures showed appreciable inhibition in the synthesis of insoluble glucan. Among the components isolated from tea infusions, theaflavin and its mono- and digallates had potent inhibitory activities at concentrations of 1—10 mM against GTF. (+)-Catechin, (−)-epicatechin and their enantiomers had moderate inhibitory activities at these concentrations, while galloyl esters of (−)-epicatechin, (−)-epigallocatechin and (−)-gallocatechin had increased inhibitory activities.**

**Keywords** anti-plaque agent; dental caries; (−)-epicatechin gallate; (−)-epigallocatechin gallate; glucosyltransferase; inhibitor; tea polyphenol; *Streptococcus mutans*; theaflavin; theaflavin gallate

Dental caries is one of the most ubiquitous infectious diseases in developed countries. *Streptococcus mutans* is the primary bacterium causing dental caries in experimental animals and humans.[1,2)] The bacterium produces water-soluble and water-insoluble glucans from sucrose by cell-bound or extracellular glucosyltransferase (GTF; EC, 2,4,1,4).[1,2)] The sticky insoluble glucan facilitates the accumulation of the microorganisms on smooth tooth surfaces (dental plaque) and the subsequent development of dental caries.[2,3)]

In attempts to find naturally occurring anti-plaque agents, we have screened various crude drugs used in Chinese and Ayurvedic medicines[4-6)] for antibacterial action against *S. mutans* and for inhibitory action in the adherence of *S. mutans* cells to smooth surfaces as well as anti-GTF action. Some of neolignans.[4,7-10)] coumarins[11)] and fatty acids[12)] were found to have potent antibacterial action against *S. mutans*, while gallotannins,[13)] procyanidins[14)] and some triterpenes[15)] were found to have appreciable anti-GTF action.

In the present paper, we describe additional inhibitory substances from tea infusions.

## Materials and Methods

**Chemicals** Bacto brain heart infusion (BHI) broth was a product of Difco Laboratories, Detroit, U.S.A. Uniformly labeled $^{14}$C-sucrose was purchased from New England Nuclear, Boston, U.S.A. ACS II (Amersham International Co.) was used as scintillation fluid. (+)-Catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate and (−)-epigallocatechin gallate were isolated from green tea. Theaflavin, theaflavin monogallate A, theaflavin monogallate B and theaflavin digallate were isolated from black tea. (−)-Catechin, (+)-epicatechin, (−)-gallocatechin and (−)-gallocatechin gallate were prepared by epimerization of catechins in hot aqueous solution.[16)]

**Polyphenon 30** Green tea leaves (30 g; processed in Shizuoka) were extracted with boiling water and the extract was spray-dried to give a green tea powder (instant green tea; yield, 10 g). The powder was dissolved in MeOH–water, and the solution was passed through an octadecyl silica (ODS) column for decolorization, followed by freeze-drying to give a powder (called polyphenon 30; yield, 6 g) which contains 35% catachins. The composition of polyphenon 30 was as follows: (−)-epigallocatechin (13.0%), (−)-epicatechin (3.8%), (−)-epigallocatechin gallate (15.0%), (−)-epicatechin gallate (3.5%) and others (64.7%).

**Polyphenon 100** Green tea leaves (3 kg) were extracted with boiling water. The extract was spray-dried to give a powder (1 kg; instant green tea), which was re-dissolved in hot water (1000 ml). An equal volume of $CHCl_3$ was added and the mixture was shaken. The aqueous layer was extracted with three volume of EtOAc. The EtOAc layer was evaporated and freeze-dried to give a residue (290 g; called polyphenon 100) which contains 91% catechins. Polyphenon 100 consisted of a mixture of (+)-gallocatechin (1.4%), (−)-epigallocatechin (17.6%), (−)-epicatechin (5.8%), (−)-epigallocatechin gallate (53.9%), (−)-epicatechin gallate (12.5%) and others (8.8%).

**Polyphenon B** Black tea leaves (30 g; processed in India) were extracted with boiling water and the extract was freeze-dried to give a powder (yield, 10 g; instant black tea). The powder was dissolved in MeOH–water, decolorized by passing through an ODS column and freeze-dried to give a powder (7 g; polyphenon B). The composition of polyphenon B was as follows: (−)-epicatechin (3.3%), (−)-epigallocatechin gallate (4.6%), (−)-epicatechin gallate (0.9%), theaflavin (0.3%), theaflavin monogallate A (0.6%), theaflavin monogallate B (1.0%), theaflavin digallate (0.5%) and others (88.8%).

**Polyphenon–Protein Complex** A solution of hydrolyzable soybean protein (10 g in 50 ml $H_2O$) was added to a solution of polyphenon 100 (5 g in 50 ml $H_2O$). The mixture was shaken and cooled overnight. Precipitates were washed and dried to give polyphenon–protein complex.

**Instant Green Tea Infusions** The water extract obtained during the preparation of polyphenon 30 was dissolved in dimethyl sulfoxide (DMSO) and used for experiments.

**Instant Black Tea Infusions** The water extract obtained during the preparation of polyphenon B was dissolved in DMSO and used for experiments.

**Crude Theaflavins** Instant black tea powder (10 g) was redissolved in hot water (500 ml) and the solution was extracted with an equal volume of $CHCl_3$. The aqueous layer was extracted with an equal volume of methyl isobutyl ketone. The organic phase was washed with an equal volume of 2.5% $NaHCO_3$, and evaporated *in vacuo* to give a residue. The residue was suspended in water and freeze-dried to give a mixture of theaflavins (300 mg). The mixture consisted of theaflavin (5%), theaflavin monogallate A (18%), theaflavin monogallate B (18%), theaflavin digallate (20%), (−)-epigallocatechin gallate (12%), (−)-epicatechin gallate (10%), other catechins (8%) and the rest.

**Microorganism** *S. mutans* OMZ 176 (serotype d; alias *S. sobrinus*) was provided by Professor S. Kotani of Osaka University Dental School.

**Crude GTF Preparation** Crude GTF from *S. mutans* OMZ 176 was prepared by a modified procedure of Mukasa and Slade.[17)]

**GTF-Inhibitory Activity** A mixture (20 $\mu$l) consisting of crude GTF (6.8 $\mu$g protein), 0.1 mM ($^{14}$C)sucrose (74 kBq), 50 mM phosphate buffer, pH 6.8, and a test sample dissolved in DMSO (final concentration of DMSO was approximately 10%) was incubated for 60 min at 37 °C. An aliquot (5 $\mu$l) of the reaction mixture (A, total glucans including soluble and insoluble glucans) was applied to filter paper (Toyo No. 51A). The rest of the reaction mixture was centrifuged at 5500 × *g* for 3 min, and an aliquot (5 $\mu$l) of the supernatant (B, soluble glucan) was also applied to the paper. The paper was developed with BuOH–pyridine–$H_2O$ (6:4:3) to the top and cut into strips. The radioactivity of glucans which remained at the original point of the paper was measured in a liquid scintillation counter. The amount of insoluble glucan (*C*, insoluble) was calculated by the difference in radioactivity of the above two ($A-B$). The glucans (total, *A*; soluble, *B*; insoluble, $A-B$) produced in the presence of a test sample were expressed in terms of % incorporation against the respec-

tive controls (total glucan = 4296 ± 113 dpm; soluble glucan = 158 ± 7 dpm; insoluble glucan = 4138 ± 116 dpm, in the absence of the sample). Percent inhibition against GTF was therefore indicated by (100 − % incorporation).

## Results

**Effect of Tea Preparations on Glucan Synthesis** Various tea preparations as listed in Table I were tested for inhibitory action in the glucan synthesis catalyzed by GTF from *S. mutans* OMZ 176 (serotype d; alias *S. sobrinus*). During the reaction, polyphenons B and 100 (10 mg/ml), polyphenon–protein complex (1 and 5 mg/ml) and crude theaflavins were precipitated, and instant black and green tea infusions (10 mg/ml) were slightly turbid. However, all

TABLE I. Effects of Tea Preparations on Total-, Soluble- and Insoluble-Glucan Formations Catalyzed by GTF

| Sample | Concentration (mg/ml) | % incorporation of ($^{14}$C)glucose[a] | | |
|---|---|---|---|---|
| | | Total glucan | Soluble glucan | Insoluble glucan |
| Instant green tea infusion[b] | 1.0 | 61.8 ± 5.1[c] | 109.0 ± 13.7 | 60.1 ± 5.5 |
| | 10.0 | 22.5 ± 1.7 | 90.4 ± 10.7 | 20.2 ± 1.5 |
| Instant black tea infusion[b] | 1.0 | 70.4 ± 5.1 | 101.0 ± 10.6 | 69.3 ± 5.0 |
| | 10.0 | 14.6 ± 0.6 | 160.7 ± 2.9 | 9.5 ± 0.7 |
| Polyphenon 30 | 1.0 | 77.9 ± 3.0 | 102.5 ± 8.6 | 77.0 ± 3.3 |
| | 10.0 | 15.2 ± 0.6 | 76.7 ± 1.2 | 13.3 ± 0.5 |
| Polyphenon 100 | 1.0 | 72.4 ± 3.1 | 82.7 ± 11.6 | 72.0 ± 3.7 |
| | 10.0[d] | 11.6 ± 0.3 | 133.3 ± 14.3 | 6.7 ± 1.1 |
| Polyphenon B | 1.0 | 79.8 ± 1.2 | 104.0 ± 4.9 | 79.0 ± 1.1 |
| | 10.0[d] | 14.8 ± 0.9 | 194.4 ± 15.2 | 9.2 ± 1.6 |
| Crude theaflavins | 1.0[d] | 25.6 ± 0.9 | 115.4 ± 17.1 | 23.2 ± 1.1 |
| | 10.0[d] | 1.3 ± 0.1 | 41.6 ± 3.1 | 0.2 ± 0.1 |
| Polyphenon–protein complex | 1.0[d] | 108.0 ± 9.8 | 224.0 ± 36.1 | 101.6 ± 11.3 |
| | 5.0[d] | 88.1 ± 13.2 | 258.7 ± 36.1 | 81.3 ± 14.5 |

*a*) Incorporation ratios into total, soluble and insoluble glucans relative to the respective controls are expressed as follows:

$$\% \text{ incorporation} = \left( \frac{\text{test } (^{14}\text{C-incorporation})}{\text{control } (^{14}\text{C-incorporation})} \right) \times 100.$$

*b*) Instant black and green tea preparations were not dissolved completely. The supernatant was used in this experiment but light precipitations occurred during the reaction. *c*) Mean ± S.E. ($n = 4$). *d*) Precipitation occurred during incubation.

TABLE II. Effects of Tea Polyphenols on Total-, Soluble- and Insoluble-Glucan Formations Catalyzed by GTF

| Sample | Configuration | Concentration (mM) | % incorporation of ($^{14}$C)glucose[a] | | |
|---|---|---|---|---|---|
| | | | Total glucan | Soluble glucan | Insoluble glucan |
| (+)-Catechin (**1**) | 2*R*,3*S* | 1.0 | 77.7 ± 5.7[b] | 78.2 ± 2.7 | 77.7 ± 5.9 |
| | | 10.0 | 39.1 ± 2.9 | 69.3 ± 1.8 | 38.3 ± 3.0 |
| (−)-Catechin (**2**) | 2*S*,3*R* | 1.0 | 84.9 ± 2.5 | 83.4 ± 12.0 | 85.1 ± 2.6 |
| | | 10.0 | 59.0 ± 2.2 | 104.0 ± 4.5 | 54.8 ± 2.3 |
| (+)-Epicatechin (**3**) | 2*S*,3*S* | 1.0 | 87.9 ± 8.0 | 89.7 ± 4.2 | 87.9 ± 8.4 |
| | | 10.0 | 57.7 ± 1.2 | 51.0 ± 3.5 | 58.1 ± 1.2 |
| (−)-Epicatechin (**4**) | 2*R*,3*R* | 1.0 | 98.7 ± 1.8 | 88.0 ± 2.5 | 94.5 ± 3.5 |
| | | 10.0 | 58.6 ± 4.9 | 92.7 ± 4.5 | 57.7 ± 5.0 |
| (+)-Gallocatechin (**5**) | 2*R*,3*S* | 1.0 | 83.6 ± 3.7 | 49.3 ± 2.3 | 85.5 ± 4.1 |
| | | 10.0 | 48.7 ± 1.0 | 48.2 ± 1.7 | 48.8 ± 1.1 |
| (−)-Gallocatechin (**6**) | 2*S*,3*R* | 1.0 | 80.4 ± 1.2 | 107.9 ± 8.9 | 79.6 ± 1.2 |
| | | 10.0 | 72.1 ± 1.1 | 91.4 ± 4.1 | 70.5 ± 0.9 |
| (−)-Epigallocatechin (**7**) | 2*R*,3*R* | 1.0 | 87.6 ± 3.4 | 110.8 ± 29.7 | 86.9 ± 4.4 |
| | | 10.0 | 77.6 ± 3.7 | 100.4 ± 5.9 | 75.3 ± 4.5 |
| (−)-Epicatechin gallate (**8**) | 2*R*,3*R* | 1.0 | 64.9 ± 6.1 | 75.0 ± 1.3 | 64.5 ± 6.2 |
| | | 10.0 | 23.4 ± 3.0 | 89.2 ± 1.3 | 17.0 ± 3.2 |
| (−)-Gallocatechin gallate (**9**) | 2*S*,3*R* | 1.0 | 53.9 ± 2.5 | 83.2 ± 1.8 | 52.8 ± 2.6 |
| | | 10.0 | 8.6 ± 0.1 | 111.7 ± 7.8 | 4.6 ± 0.1 |
| (−)-Epigallocatechin gallate (**10**) | 2*R*,3*R* | 1.0 | 59.9 ± 3.3 | 119.2 ± 14.6 | 58.4 ± 3.3 |
| | | 10.0 | 30.4 ± 1.9 | 89.1 ± 4.9 | 25.0 ± 1.9 |
| Free theaflavin (**11**) | | 1.0 | 46.2 ± 0.5 | 166.4 ± 44.0 | 43.2 ± 1.4 |
| | | 10.0[c] | 3.1 ± 0.1 | 55.2 ± 10.5 | 1.7 ± 0.2 |
| Theaflavin monogallate A (**12**) | | 1.0 | 38.2 ± 1.5 | 128.4 ± 9.5 | 35.5 ± 1.6 |
| | | 10.0[c] | 4.0 ± 0.6 | 49.1 ± 1.2 | 2.7 ± 0.6 |
| Theaflavin monogallate B (**13**) | | 1.0[c] | 55.9 ± 6.2 | 166.9 ± 20.8 | 52.9 ± 7.1 |
| | | 10.0[c] | 3.8 ± 0.4 | 59.8 ± 1.1 | 2.2 ± 0.4 |
| Theaflavin digallate (**14**) | | 1.0 | 46.4 ± 2.5 | 121.6 ± 3.8 | 44.1 ± 2.6 |
| | | 10.0 | 3.6 ± 0.3 | 48.8 ± 2.0 | 1.8 ± 0.3 |

*a*) % incorporation is defined as shown in the legend to Table I. *b*) Mean ± S.E. ($n = 4$). *c*) Precipitation occurred during incubation; in the presence of hexagalloyl glucose as a positive control, % incorporations into the total-, soluble- and insoluble-glucans were 8.8 ± 0.4, 127.1 ± 4.5 and 4.8 ± 0.3, respectively, under the same conditions.

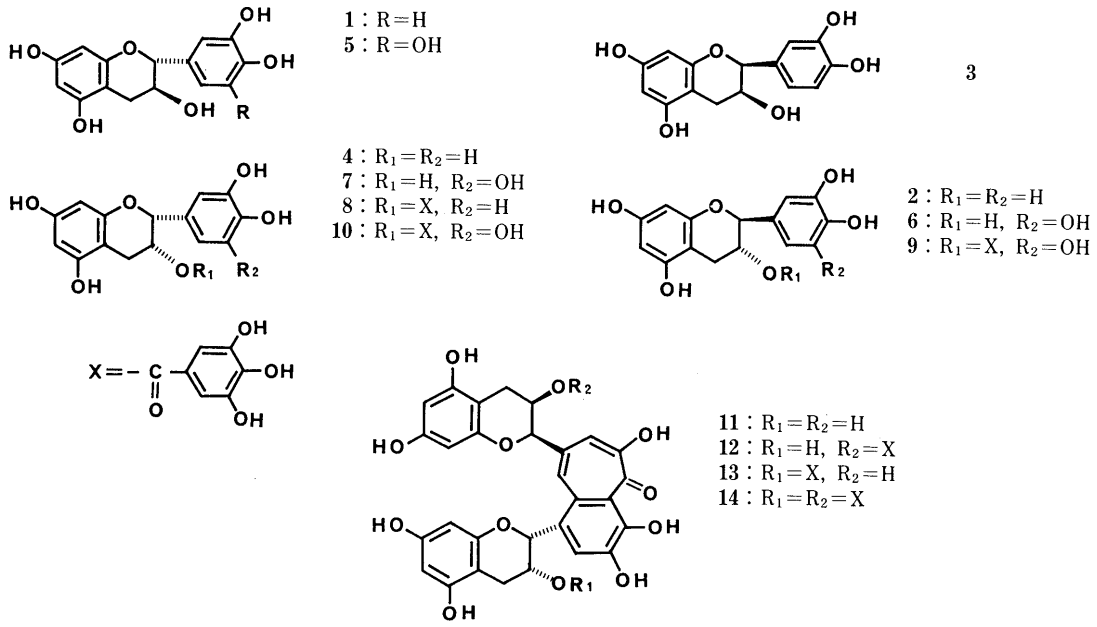


Chart 1. Structures of Tea Polyphenols and Related Compounds

preparations except polyphenon–protein complex inhibited total- and insoluble-glucan formations strongly at 10 mg/ml, but moderately at 1 mg/ml except for the case of crude teaflavins which had potent inhibition even at the low concentration. The tea infusions and polyphenons (30, 100 and B) were not inhibitory on soluble-glucan formation at 1 mg/ml, while the black tea infusion, polyphenon 100, polyphenon B and polyphenon–protein complex significantly stimulated the soluble-glucan formation compared to a control.

**Effect of Tea Polyphenols on Glucan Synthesis** Table II shows the effects of components isolated from green tea or black tea infusions and related compounds on total-, soluble- and insoluble-glucan formations. (+)-Catechin (**1**), (−)-epicatechin (**4**) and their optical isomers (**2** and **3**) showed moderate inhibition (40—60%) on total- and insoluble-glucan formations at 10 mM but weak inhibition (1—20%) at 1 mM. There were no appreciable differences in inhibitory potency among the respective stereoisomers (**1**—**4** and **5**—**7**). However, introduction of a galloyl group at 3-OH (**8**—**10**) resulted in the significant increases of inhibitory activities on both total- and insoluble glucan formations (35—50% inhibition at 1 mM and 70—95% inhibition at 10 mM). In this case, no significant difference in inhibitory potency was observed among the stereoisomers. Free theaflavin (**11**) and the mono- and digallates (**12**—**14**) had potent GTF-inhibition (45—65% inhibition at 1 mM and 95—100% inhibition at 10 mM). In comparison with the inhibitory activities among free theaflavin (**11**) and its gallates (**12**—**14**), no appreciable stimulatory effect was observed by introduction of galloyl group(s).

As in the case of crude polyphenol preparations, soluble-glucan formation was not significantly affected by (+)-catechin (**1**), (−)-epicatechin (**4**) or related compounds, while it was significantly stimulated by free theaflavin (**11**) and its mono- and digallates (**12**—**14**) at a low concentration (1 mM).

Figure 1 shows the relationship between percentages of total- and insoluble-glucan formations in the presence of

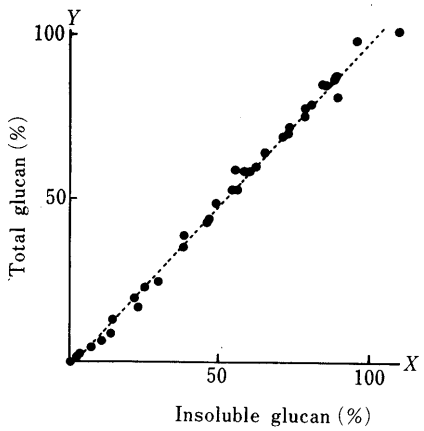


Fig. 1. Scatter Diagram of Total- and Insoluble-Glucans Formed by GTF in the Presence of Various Polyphenols

different tea components, relative to the respective controls (without samples). The percentages of total and insoluble glucans show a linear relation. The regression curve is indicated by an equation: $Y=-2.916+1.024X$ with a correlation coefficient ($r=0.997$, $p<0.01$), where $X$ and $Y$ represent the respective percentages of insoluble and total glucans. On the other hand, there is no significant correlation between percentages of total and soluble glucans. These findings clearly indicate that reduction of total-glucan formation relative to a control by individual tea phenols is mostly due to reduction of insoluble-glucan formation ($r^2=0.994$).

## Discussion

Tea is one of the most widely consumed beverages in the world. Tea consumption is said to decrease the incidence of caries in humans and the effect has been attributed to endogenous fluorine and tannins (polyphenols).[18] In fact, significantly high levels of fluoride (*ca.* 100—200 ppm, dry wt) were detected in a variety of tea products as reported in our previous paper.[19] Kashket *et al.*[18] reported that tea infusion inhibited GTF activity in *S. mutans* 6715 culture

fluids and its effect was significantly reduced by addition of gelatin, due to the precipitation of tea tannins.

A number of polyphenols from green and black tea infusions and from the leaves of *Camellia sinensis* (Theaceae) have been isolated and characterized,[20-26] and this encouraged the search for the major GTF-inhibitory substances from tea infusions.

The present experiments revealed that some tea preparations consisting of a mixture of tea polypenols and their components had inhibitory activity against GTF. However, polyphenon–protein complex which was prepared by mixing tea polyphenols and hydrolyzable vegetable protein had no significant inhibition on total- and insloluble-glucan synthesis, emphasizing the importance of the presence of any excess polyphenols capable of complexing with protein. This accords with the observation by Kashket *et al.*[18] Among the components, gallates of (−)-epicatechin, (−)-gallocatechin and (−)-epigallocatechin (**8**—**10**) and theaflavins (**11**—**14**) had potent inhibitory activities. Theaflavins, pigments of black tea infusions, possess a unique benzotropolon moiety but few studies on their biological activities have been reported so far.[27] This is additional evidence that theaflavins have enzyme-inhibitory actions. In comparing of the inhibitory activities of flavan-3-ols (**4**—**7**) and their 3-*O*-gallates (**8**—**10**), introduction of a galloyl (3,4,5-trihydroxybenzoyl) group was shown to potentiate inhibitory activity to a considerable extent. However, the effect of this galloyl group(s) was not significant in the theaflavin derivatives (**11**—**14**). On the other hand, (+)-gallocatechin (**5**), (−)-gallocatechin (**6**) and (−)-epigallocatechin (**7**) which possess a similar 3,4,5-trihydroxyphenyl moiety in ring B of the flavonoid carbon skeleton, showed almost equal or lower activity than (+)-catechin (**1**), (−)-catechin (**2**), (+)-epicatechin (**3**) and (−)-epicatechin (**4**). Furthermore, gallic acid and its methyl, ethyl and propyl esters were less inhibitory, but octyl gallate had potent inhibitory activity.[28] These findings reveal that the relationship between a galloyl group and its inhibitory potency against GTF is quite complex. Effect of the number of galloyl group in galloyl glucoses (gallotannins) on GTF-inhibitory activity was reported earlier; in this case, the inhibition was enhanced as the galloyl: glucose ratio in the galloyl glucoses increased.[13] It is of interest that most of the tea polyphenols inhibited the water-insoluble glucan formation, but polyphenon–protein complex, as well as polyphenon B and theaflavins (**11**—**14**) at a low concentration (1 mM), strongly stimulated the water-soluble glucan formation. However, we did not study the inhibitory and stimulatory actions of these compounds in detail, because our enzyme preparation consisted of a complex mixture of GTFs, which catalyze the formation of water-soluble, water-insoluble and both glucans.[29,30]

A mixture of tea polyphenols and the major component, (−)-epigallocatechin gallate (**10**), have been demonstrated to have a variety of biological activities such as antioxidant activity,[31] inhibitory activity against angiotensin converting enzyme,[27] a hypocholesterolemic action in cholesterol-fed rats,[32,33] and suppression of the growth of inoculated tumor cells in animals.[34] Our present result provides an additional example of the biological activity of tea polyphenols.

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