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Potential Chemopreventive Properties of Extract from Baked Sweet Potato (*Ipomoea batatas* Lam. Cv. Koganesengan)

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The extract from baked sweet potato (*Ipomoea batatas* Lam. cv. Koganesengan) showed potential cancer-preventing effects. The extract was partially fractionated to four fractions (I, II-a II-b, and III) by Sephadex G-25 gel chromatography. The cytotoxicity against human myelocytic leukemia HL-60 cells, the suppression of TPA-induced transformation in mouse skin JB6 C141 cells, the apoptosis inducing activity in HL-60 cells, and the scavenging capacity against DPPH radical were tested on the four fractions. Fractions II-a and III showed markedly strong radical scavenging effects on the DPPH radical, coinciding with the high content of total phenolic compounds in the fractions. Both of these fractions suppressed strongly the proliferation of HL-60 cells with apoptosis induction in a dose-dependent manner. Moreover, the two fractions markedly blocked TPA-induced cell transformation in the JB6 cell line. Taken together, these data suggest that the water extract from baked sweet potato had potential chemopreventive properties.

KEYWORDS: Baked sweet potato; cytotoxicity; apoptosis; antioxidant; antitumor

INTRODUCTION

Cancer is one of the most harmful diseases, causing high mortality worldwide. The identification and use of effective cancer chemopreventive agents have become important issues in public health and research (*1*−*4*). Many findings have shown that dietary vegetables and fruits and medicinal herbs contain many cancer chemopreventive compounds (*1*, *2*, *5*−*9*). The antimutagenetic activity of many vegetables, fruits, and plants was partly reduced by heating as reported previously (*10*−*12*). The sweet potato (*Ipomoea batatas*) is one of the most important foods in the world. Recently Yoshimoto et al. (*13*) found that the water extract from purple sweet potato (cv. Ayamurasaki) inhibited strongly the mutagenicity of *Salmonella typhimurium* TA 98, but those from cultivars Koganesengan, Joy White, Kyushu-114, and Ayamurasaki-mutant, which were anthocyanin-deficient, inhibited weakly the mutagenicity, suggesting that the antimutagenic activity of the extract of sweet potato should be due to anthocyanin pigments, which were abundant in the purple sweet potato, Ayamurasaki (*14*). More recent studies on the anthocyanins showed the potential of cancer chemoprevention by these compounds (*2*, *15*−*17*).

The free oxygen radicals are most responsible for the development of many diseases including tumor promotion and carcinogenesis, arthritis, atherosclerosis, disorder with advancing age, Alzheimer's and Parkinson's diseases, gastrointestinal dysfunctions, and AIDS (*18*−*20*). The antioxidants protect against free radical damage by scavenging reactive oxygen species (ROS), ending radical chain reactions, or chelating transition metals, which catalyze ROS formation from peroxides (*20*−*23*). The multistage skin carcinogenesis model in mice JB6 cells involves three well-defined stages: initiation, promotion, and progression. This model has been used to evaluate carcinogenesis and to investigate chemical and molecular events accruing in each stage (*15*, *23*, *24*). The JB6 cell model was sensitive to tumor promoters such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) or tumor necrosis factor-α (TNF-α) (*25*). The transformation of mouse JB6 cell by TPA is considered to be through the generation of ROS, especially superoxide anion (*2*, *15*, *26*). The antioxidant compounds were supposed to block this promotion by blocking the reactive oxygen radicals or by eliminating them.

Apoptosis or programmed cell death plays an important role in many biological processes including carcinogenesis, tumorigenesis, and cancer (*27*−*29*). The removal of damaged precancerous cells via apoptosis or terminal differentiation of malignant cells provides an important and valuable strategy for management of cancer (*6*, *30*). The cells that have undergone apoptosis showed typically chromatin condensation and DNA fragmentation (*16*). Apoptosis and carcinogenesis should be opposed phenomena, but ROS have been widely reported to play a key role in both (*19*, *31*, *32*). An increase in the concentration of ROS by depletion of antioxidant enhances

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apoptosis and thereby inhibits tumor growth (*33*). Therefore, further efforts are also necessary to fully elucidate the importance of free radical scavengers in the therapy of many diseases (*32*).

In the present work, we found that the water extract of baked sweet potato cv. Koganesengan showed a strong antioxidant activity and a potential cytotoxicity to human leukemia HL-60 cells with apoptosis-inducing activity. Moreover, the extract suppressed significantly TPA-induced cell transformation in mouse skin JB6 cells. These observations suggested the potential of cancer chemoprevention by the extract.

MATERIALS AND METHODS

Materials and Cell Culture. Eagle's minimum essential medium (EMEM), L-glutamine, and RPMI-1640 medium were purchased from Nissui (Tokyo, Japan). TPA, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), *p*-iodonitrotetrazolium violet (INT), protein kinase K, RNase A, and Folin−Ciocalteu phenol reagent were from Sigma (St. Louis, MO). Fetal bovine serum (FBS) was from BioWhittaker Co. (Walkersville, MD). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were from Aldrich (Milwaukee, WI). Human leukemia cell line (HL-60) was obtained from Cancer Cell Repository, Tohoku University, Japan, and was cultured at 37 °C, 5% CO_2 in RPMI-1640 medium containing 10% FBS and 1% penicillin−streptomycin−glutamine (PSG). The JB6 P^+ mouse epidermal cells, C141, were a kind gift from Dr. N. H. Colburn (National Institutes of Health, Bethesda, MD) and were cultured at 37 °C, 5% CO_2 in EMEM containing 5% FBS, 2 mM L-glutamine, and 25 μg/mL gentamicin.

Preparation of Baked Potato Extract (BPE). After 20 min of boiling at 95 °C, the sweet potato (*Ipomoea batatas* Lam. cv. Koganesengan) was freeze-dried and baked on the frying pan at about 180 °C for 20 min. The soluble materials in the baked sweet potato were extracted five times with equal volumes (v/w) of water at 95 °C for 1 h and obtained by centrifugation and then lyophilized.

Fractionation of BPE by Sephadex G-25 Gel Filtration. BPE was resolved (0.3 g/mL) in 0.1 M ammonium formate and applied to a Sephadex G-25 column (4 × 60 cm) at 4 °C (**Figure 1A**). Fraction II was concentrated and reapplied to the column (**Figure 1B**). The absorbance was measured at 280 and 500 nm with an Ultrospec 2000 UV−visible spectrophotometer (Biochrom Ltd., Camridge, U.K.).

DPPH Radical Scavenging Assay. The antioxidant capacity of the BPE fractions after gel filtration was assessed according to the DPPH method (*22*, *34*, *35*) with some modifications. One hundred micromolar DPPH was prepared in 80% aqueous MeOH. Ten microliters of the BPE fractions and the antioxidant standards were placed into each well of 96-well microtiter plates. Controls were treated with water. Ninety microliters of DPPH was added to each well and mixed vigorously. The mixture was allowed to react for 30 min in the dark, and then the absorbance was measured at 490 nm with a microplate reader (Bio-Rad model 550). The activity was converted to the Trolox equivalent antioxidant capacity (TEAC) from the standard curve of Trolox (**Figure 2A**).

Determination of Total Phenolic Contents. The concentration of the total phenolic substances was measured according to the methods described previously (*22*, *36*, *37*) with some modification as follows. Ten microliters of each the BPE fractions was mixed with 200 μL of 2% Na_2CO_3 in 96-well microtiter plates. After 3 min, 10 μL of 50% diluted Folin−Ciocalteu reagent was added to each well, and the mixture was allowed to stand for 1 h; then the absorbance was measured at 595 nm with a microplate reader (Bio-Rad model 550). The gallic acid was used as standard, and the total phenolic content was expressed as a gallic acid equivalent (GAE) in a milligrams per gram of BPE fractions.

Cell Viability Assay. Human leukemia HL-60 cells are a valid model and widely used to determine antileukemic or general antitumoral compounds (*6*, *16*). The MTT colorimetric assay, which is based on the reduction of MTT by the mitochondria dehydrogenase of intact cells to a purple formazan product (5), was used to assess the antiproliferation action of the baked sweet potato extract fractions in HL-60 cells. The cells were suspended at a density of 2 × 10^4 cells/mL in RPMI-1640 medium containing 10% FBS and 1% PSG, and then 100 μL of each was plated into each well of 96-well microtiter plates. After 24 h of incubation, the cells were treated with various concentrations of each fraction for 48 h. Controls were treated with water alone. MTT solution was then added to each well (0.5 mg/mL) and incubated for another 4 h. The resulting MTT−formazan product was dissolved by the addition of 100 μL of 0.04 N HCl−2-propanol and determined by measuring the absorbance at 595 nm with a microplate reader (Bio-Rad model 550). The results are expressed as the optical density ratio of the treatment to control. Student's *t* test was used to determine the difference between the treated samples and the control.

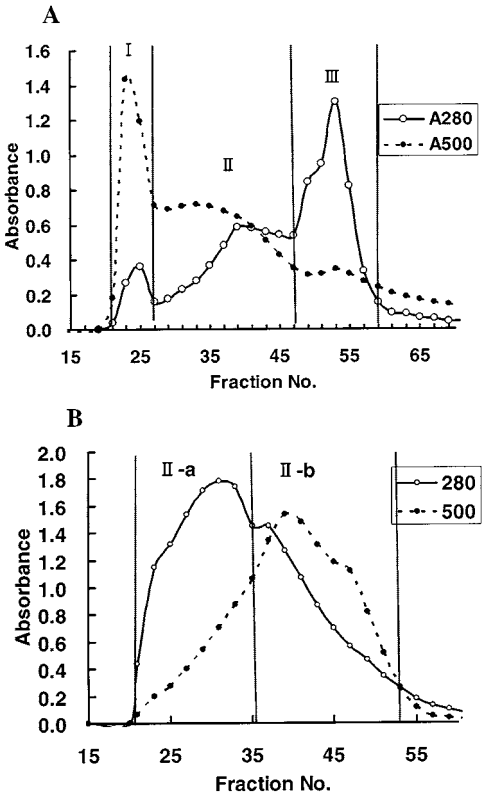

Figure 1. (**A**) Sephadex G-25 gel filtration. The BPE was dissolved in 0.1 M ammonium formate and was applied to a Sephadex G-25 column equilibrated with the same solution. (**B**) Sephadex G-25 re-gel filtration. Fraction II obtained from the first gel filtration was applied to a Sephadex G-25 column under the same conditions: column size, 4 × 65 cm; flow rate, 180 mL/h; fraction volume, 15 mL.

Analysis of DNA Fragmentation. The DNA fragmentation assay was carried out as described (*16*). HL-60 cells (2 × 10^6) were treated with various concentrations of the extract fractions for 6 h. The cells were harvested by centrifugation and washed in ice-cold PBS. The pellets were resuspended in lysis buffer (50 mM Tris-HCl, pH 8.0, 10 mM EDTA, and 0.5% SDS) plus 0.1 mg/mL RNase A. After 30 min of incubation at 50 °C, proteinase K (7 mg/mL) was added and incubated for another 1 h. DNA was separated on 2% agarose gel electrophoresis and digitally imaged after staining with ethidium bromide.

Anchorage-Independent Transformation Assay. The anchorage-independent transformation assay was carried out as described previously (*15*). Briefly, the effects of the antioxidants on TPA-induced cell transformation were investigated in the parental JB6 C141 cells. Cells (1 × 10^4) were suspended in 2 mL of 0.38% agar EMEM over 3 mL of 0.5% LMP agar EMEM containing 20 ng/mL TPA with or without different concentrations of the antioxidants. The cultures were maintained in an incubator at 37 °C and 5% CO_2/95% air for 14 days, and the anchorage-independent colonies were scored by a computerized

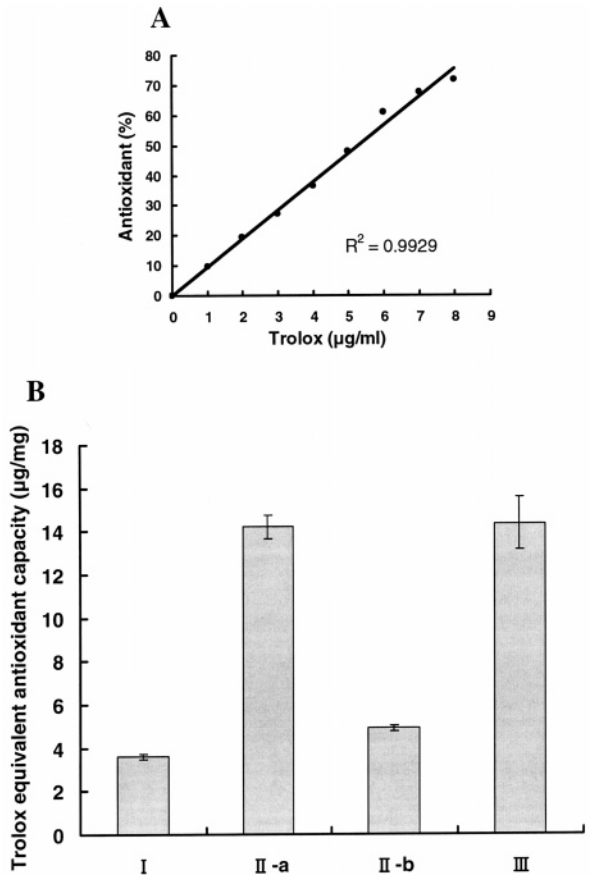

Figure 2. DPPH radical scavenging activity by BPE fractions: (**A**) standard curve of Trolox antioxidant; (**B**) activities represented with TEAC (μg/mg). Various concentrations of Trolox or the BPE fractions were added to DPPH solution, and the mixture was allowed to react for 30 min at room temperature in the dark. The reduction in absorbance at 490 nm was determined with a microtiter plate reader. The values represent the means ± SD. Vertical bars indicate the standard deviation.

image analyzer after staining with INT. The efficiency of the inhibition of TPA-induced cell transformation is presented as a percentage of the transformation frequency when the cells were treated with TPA alone.

Statistical Analysis. Difference between the treated samples and the control was analyzed by *t* test. A probability of $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Fractionation of BPE. Three fractions (I, II, and III) were obtained after the BPE was subjected to Sephadex G-25 gel filtration in ammonium formate (**Figure 1A**). Fraction II was re-gel filtered under the same conditions, and fractions II-a and II-b were obtained as shown in **Figure 1B**.

Antioxidant Activities and Total Phenolic Contents in BPE Fractions. Fractions II-a and III showed very strong antioxidant activities as shown in **Figure 2B**.

High contents of total phenolic compounds were found in fraction III (145.4 mg [GAE]/g) and in fraction II-a (109.5 mg [GAE]/g), respectively, as shown in **Figure 3**. Lower amounts of phenolic contents were obtained in fraction I (38.5 mg [GAE]/g) and in fraction II-b (49.5 mg [GAE]/g), respectively. These findings suggested that the level of antioxidant activity and the amount of phenolics were closely related to each other.

The Maillard reaction product mixtures could act as radical scavengers and as metal chelators (*38*). The roasted food and browned confectionery formed some Maillard products, which had antioxidant and cytotoxicity effects (*39*). In the present experiment, sweet potato was baked before extraction, so some Maillard products should be produced. The total phenolics amounts as well as the total antioxidant activity in the baked potato extract were markedly increased from those in the unbaked potato extract (data not shown). These findings showed that some polyphenolic compounds or some compounds sensitive to the Folin−Ciocalteu reagent, which showed antioxidant activities, should be produced during the baking process of the sweet potato.

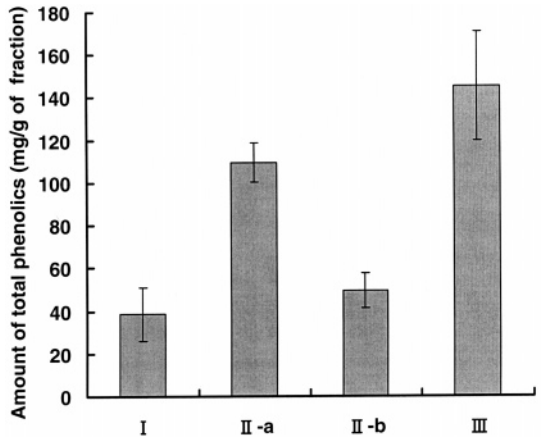

Figure 3. Total phenolic contents in BPE fractions. The total phenolics were determined by Folin–Ciocalteu method. Amounts are represented with GAE (mg/g). The values represent the means ± SD. Vertical bars indicate the standard deviation.

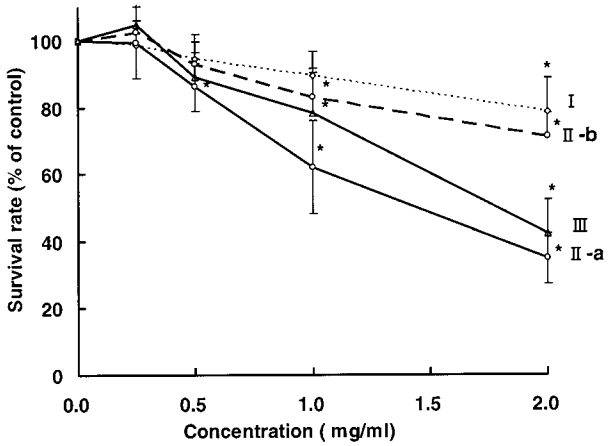

Figure 4. Effect of BPE fractions on the proliferation of HL-60 cells. The cells (2×10^4 cells/mL) were cultured in RPMI-1640 medium as described under Materials and Methods for 24 h and then exposed to the indicated concentrations of the BPE fractions for 48 h. The cell density was assessed colorimetrically after staining with MTT and expressed as absorbance at 595 nm. Each value represents the mean ± SD of four data. Vertical bars indicate the standard deviation. *, $p < 0.05$ versus control.

Cytotoxicity and Apoptosis-Inducing Activity in HL-60 Cells. The cytotoxicity of the BPE fractions against human promyelocytic leukemia cells (HL-60) was investigated by using an MTT assay. Fractions II-a and III showed significant inhibition of the cell growth of HL-60 as shown in **Figure 4**. The antiproliferative effect was in a dose-dependent manner, and the maximum inhibition was observed at the concentration of 2 mg/mL, which caused 65% inhibition with fraction II-a and 57% inhibition with fraction III, respectively. Fractions I and II-b had slight effects on cell viability.

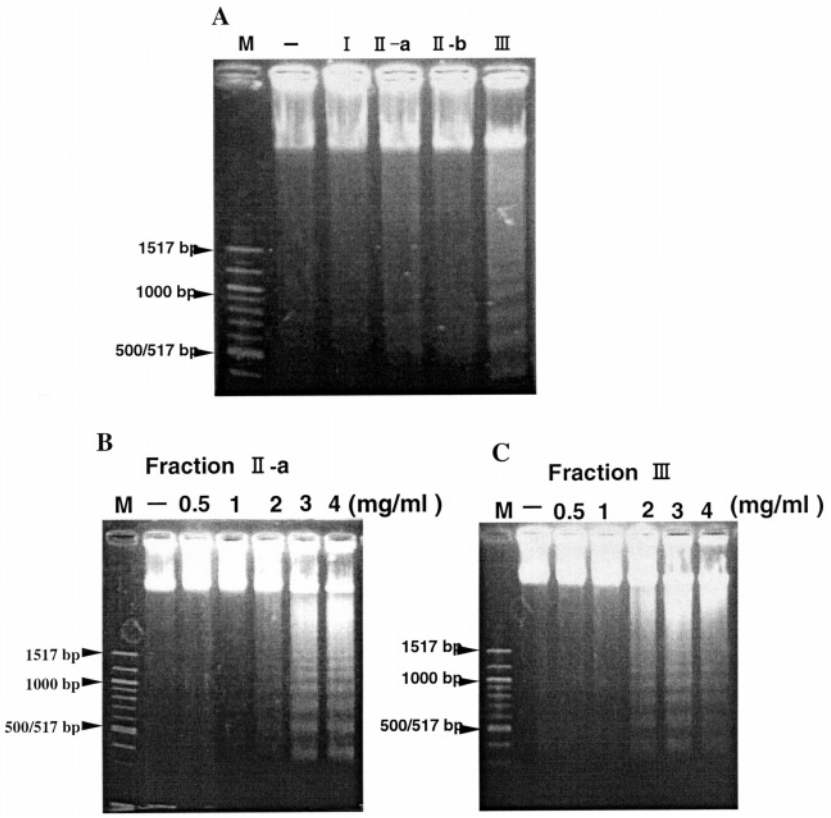

Figure 5. Apoptosis induction in HL-60 cells by BPE fractions. The cells (2×10^4) were grown in 3 mL of RPMI-1640 medium supplemented with 10% FBS and 1% PSG for 24 h and then were exposed to 2 mg/mL of each fraction (**A**) or to various concentrations of II-a (**B**) and III (**C**) for 6 h. Harvested DNA was separated on a 2% agarose gel and digitally imaged after staining with ethidium bromide. (–) control (no addition); M, DNA marker of 100 bp.

To investigate whether the BPE fractions decreased cell survival by the induction of apoptosis, the apoptotic characterization in HL-60 cells treated with the BPE fractions was monitored. After HL-60 cells were treated with 2 mg/mL of each fraction for 6 h, the DNA was obtained as described under Materials and Methods. Analysis of DNA integrity by agarose gel electrophoresis showed the typical DNA fragmentation pattern in the cells treated with fractions II-a and III as shown in **Figure 5A**. DNA fragmentation was observed at 6 h in a dose-dependent manner with 0.5−4 mg/mL of fractions II-a (**Figure 5B**) and III (**Figure 5C**). The minimum concentration that induced apoptosis was 2 mg/mL; efficacious induction of apoptosis was observed at 3 mg/mL of each fraction II-a and III. The results indicated that the BPE fractions suppressed the cell survival through the induction of apoptosis.

Apoptosis or programmed cell death plays an important role in many biological processes including carcinogenesis, tumorigenesis, and cancer and the removal of damaged precancerous cells (*27*). The induction of apoptosis or terminal differentiation of malignant cells provides an important valuable strategy for the management of cancer (2). Our results suggested that the extract of baked sweet potato involved some components that may able to prevent cancer.

BPE Fractions Suppressed TPA-Induced JB6 Cell Transformation. TPA is a tumor promoter that has been recently widely used in in vitro assays for determining antitumor compounds (*15*, *24*, *40*). The cells (1×10^4) were exposed to 20 ng/mL TPA with or without various concentrations (0.25−1.5 mg/mL) of the BPE fractions in soft agar for 14 days. TPA-induced transformation was significantly inhibited by fraction II-a and subsequently inhibited by fraction III as shown in **Figure 6**. The antipromotion activity was in a dose-dependent manner. Some investigators reported that the elimination of ROS produced by inflammatory cells could account for inhibition of TPA promotion by antioxidants (*15*, *41*). Our result showed that, at the concentration 1.5 mg/mL, the cell transformation was blocked almost absolutely by 98% with fraction II-a and subsequently was suppressed by 63% with fraction III, suggesting that both fractions II-a and III involved markedly strong antitransforming activity.

The mouse epidermal JB6 cell system of clonal genetic variants that are promotion sensitive (P^+) or promotion resistant (P^-) has enabled the study of genetic susceptibility to transformation promotion at the molecular level (*40*, *41*). This model has been used to evaluate carcinogenesis and to investigate chemical and molecular events occurring in each stage (*2*, *4*, *26*, *40*). TPA has been demonstrated to cause an induction of activator protein 1 (AP-1) activity in the skin (*41*), and AP-1 transactivation was required for TPA-induced cell transformation in JB6 cells (*15*, *41*, *42*). AP-1 might be used as a molecular target for the prevention of carcinogenesis (*41*). According to the hypothesis, the findings that fractions II-a and III blocked strongly TPA-induced cell transformation in JB6 cells show that the same components in fractions II-a and III should have a possible interference with the cancer prevention. More work should be done to characterize some active compounds in the extract and to study the cancer-preventing mechanisms in the molecular level.

In summary, our results involving the suppression of tumor promoter TPA-induced cell transformation and the anitiproliferation of human myelocytic leukemia (HL-60) cells) with the DNA strand breaks in these cells indicated the high potential of cancer prevention by the extract from baked sweet potato cv. Koganesengan. Moreover, the strong radical scavenging

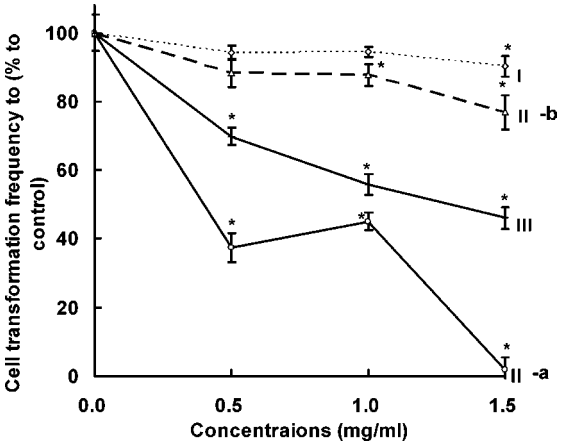

Figure 6. BPE fractions suppressed TPA-induced cell transformation in JB6 P^+ Cl41 cells. The cells (1×10^4) were suspended in 2 mL of 0.5% LMP agar medium over 3 mL of 0.3% LMP agar medium containing 20 ng/mL TPA with or without various concentrations (0.5–1.5 mg/mL) of the BPE fractions. The dishes were cultured at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air for 14 days. The colonies after staining with 0.8 mL of 0.25 mg/mL INT were scored by the counting under optical microscopy. The inhibition ratio of TPA-induced cell transformation was calculated against control cells (treated with TPA only). Each value represents the mean ± SD of three to four separate experiments. Vertical bars indicate the standard deviation. *, $p < 0.05$ versus control.

effects of this extract may account for more protection against other diseases such as arthritis, atherosclerosis, advancing age, and Alzheimer's and Parkinson's diseases. The investigation showed that the amount of fractionated compounds, as well as the contents of total phenolic compounds, gave a strong correlation with the activities. NMR spectrophotometery analysis indicated that the molecular mass of the active compounds ranges from 300 to 1000 Da (data not shown). However, detailed information about the structure of the most active compound is now under investigation. This will be reported in a future paper.

ABBREVIATIONS USED

BPE, baked potato extract; EDTA, ethylenediaminetetraacetic acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EGF, epidermal growth factor; FBS, fetal bovine serum; INT, *p*-iodonitrotetrazolium violet; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ROS, reactive oxygen species; TEAC, Trolox equivalent antioxidant capacity; TNF-α, tumor necrosis factor alpha; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; PSG, penicillin−streptomycin−glutamine.

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