# Cancer Preventive Effect of *Kumaizasa* Bamboo Leaf Extracts Administered Prior to Carcinogenesis or Cancer Inoculation

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**Abstract.** *Background: Kumaizasa bamboo found in Hokkaido is used for traditional medicine in Japan. The cancer preventive effect of vigorous (multistep) hot water extract of Kumaizasa was examined in relation to immunological conditioning and free radical scavenging activity. Materials and Methods: Cytokine induction in mice, free radical scavenging activity in vitro, and cancer preventive effect by oral administration of the vigorous extracts prior to tumor implantation or carcinogenesis by 7,12-dimethylbenz[a]anthracene (DMBA) were examined. Results: In tumor inoculated mouse models (S-180 sarcoma, Meth-A fibrosarcoma, B16-F10 melanoma), the vigorous extracts from Kumaizasa bamboo leaves suppressed tumor growth and prolonged survival significantly. In the chemical carcinogenesis model suppression of cancer incidence on day 100, tumor size and survival time were significantly improved with the vigorous extract, at/or above 0.03% in the diet, when given two weeks prior to the administration of the carcinogen. Conclusion: The vigorous extracts of bamboo leaf show immunopotentiating and radical scavenging effects and administration prior to carcinogen exposure or tumor inoculation significantly suppresses tumor incidence and tumor growth and prolongs survival.*

Cancer remains the first or second main cause of death in developed countries (1). Finding a cure for advanced cancer remains a long way off, and this situation has not improved over the past 50 years. Although cancer therapy is very important, it is also now recognized that cancer prevention is very important (2). In fact, alternative agents obtained from various plants to fight cancer have attracted great interest (3, 4).

Bamboo leaves are used in traditional medicine in Japan, and have shown various activities such as anti-oxygen radical activity, antioxidant activity (5-7) and antitumor activity (8, 9). We have recently reported an improved hot water extraction method for Kumaizasa (*Sasa senanesis*) leaves, which is a bush-type bamboo grown in Hokkaido, Japan, under vigorous steam conditions at high temperature and high pressure. The extracts obtained by this method (vigorous extract) contained a larger quantity of β-glucan (150-fold) and phenolics (2.5-fold) compared to the conventional hot-water extract (10). Furthermore, the vigorous extract also exhibited more potent antitumor activity against mouse sarcoma S-180 than the conventional hot-water extract when given orally at 0.1%w/w in the diet (10). Additionally, the production of Th1-type cytokines such as interleukin (IL)-2, IL-12, and interferon (IFN)-γ was significantly induced by the vigorous extract, which stimulated the cytotoxic activity of splenic natural killer (NK) cells and macrophage *in vitro* and *in vivo* (10). These immunological activations were probably mediated by β-glucans which is known to have various immunostimulating effects (11-16).

In the previous study, our primary interest was the therapeutic effect, rather than the suppressive and preventive effects, thus the vigorous extract was administered when the carcinomas had become established to a palpable size. The primary purpose of the present study was to clarify whether

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*Abbreviations:* IL, interleukin; SD, Sprague-Dawley; *s.c.*, subcutaneously; *i.p.*, intraperitoneal; FBS, fetal bovine serum; DMBA, 7,12-dimethylbenz[α]anthracene; DMEM, Dulbecco's modified Eagle's medium; DTPA, diethylenetriaminepentaacetic acid; $LOO^{\cdot}$, alkyl peroxy radical; NK, natural killer; NO, nitric oxide; T/C, treated group/control group; *t*-BuOOH, *tert*-butylhydroperoxide.

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*Key Words:* Bamboo leaf extract, anticarcinogenesis effect, breast cancer, pretreatment, cancer prevention, tumor growth suppression.

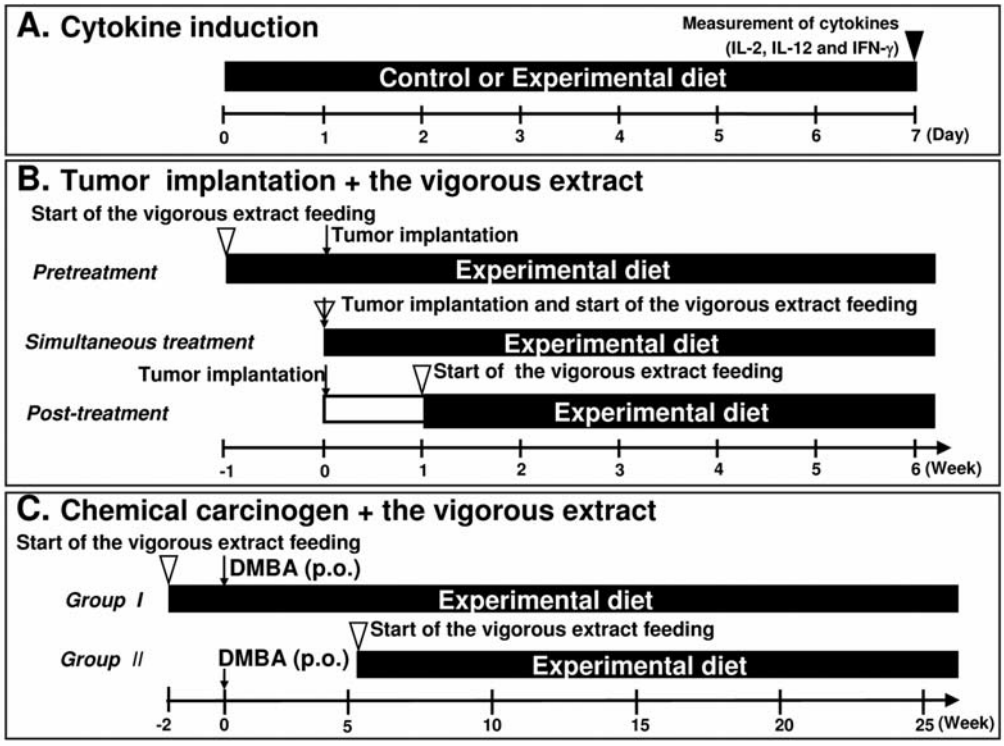


Figure 1. *Experimental protocols: (A) Dietary effect on cytokine induction. (B) Dietary schedule in tumor being mice. The mice in each tumor type were randomly divided into control, pretreatment, simultaneous treatment and post-treatment groups (10-11 mice per group). The vigorous extract (0.1%) was administered from 7 days before (pretreatment), simultaneously with, or (post-treatment) tumor implantation. Control animals were administered the basal diet only. (C) DMBA-induced rat breast cancer model.*

immunological conditioning administration (pretreatment, *i.e.*, before tumor implantation) of the vigorous extract has more potent tumor suppressive activity than post-treatment (administration of the vigorous extract after the tumor reached palpable size). Three mouse tumor models and a chemical carcinogen, 7,12-dimethylbenz[α]anthracene (DMBA)-induced rat tumor model were used.

## Materials and Methods

*Materials*. Luminol, diethylenetriaminepentaacetic acid (DTPA), DMBA and corn oil were purchased from Wako Pure Chemical Industries (Osaka, Japan). *tert*-Butylhydroperoxide (t-BuOOH) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Kumaizasa was provided by CosmoBios Research Institute (Akahira, Hokkaido, Japan). Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from Life Technologies (Carlsbad, CA, USA).

*Extraction method*. Briefly, Kumaizasa bamboo leaves were first extracted in water at 100˚C for 60 min; residues were then treated with water at 121˚C, 2 atm, for 30 min, followed by treatment with steam at 196˚C, 15 atm, for 5 min and finally extraction with water at 120˚C for 30 min (10). The extracts were lyophilized, and the obtained dark powder was stored *in vacuo* at –20˚C.

*Cells lines*. Meth-A cells (mouse fibrosarcoma) and S-180 cells (mouse sarcoma) were maintained as ascetic form by intraperitoneal (*i.p.*) passage in BALB/c mice and ddY mice respectively. These ascitic tumor cells were maintained by weekly passage. B16-F10 cells (mouse melanoma) were maintained *in vitro* at 37˚C in DMEM containing 10% FBS, 100 μg/mL streptomycin, and 100 U/mL penicillin in 5% $CO_2$/air.

*Animals*. Female Sprague-Dawley (SD) rats (age, 5 weeks), male ddY mice (age, 6 weeks), female BALB/c mice (age, 6 weeks), and male C57BL/6J mice (age, 6 weeks) were purchased from SLC Inc. (Shizuoka, Japan). The rats were housed three per cage for a group and the mice in a group of four or five per cage; they were maintained at 22±1˚C and 55±5% relative humidity, with an automatic 12 h light/dark cycle. All the experiments were carried out according to the Laboratory Protocol of Animal Handling, Sojo University.

*Diet*. The experimental diet was prepared by Funabashi Farm (F2, Funabashi, Chiba, Japan), and all the diets were vacuum sealed followed by γ-ray sterilization and then stored at 4˚C until use. The vigorous extract was mixed with the F2 diet at 0.03-0.5% (w/w).

*Cytokine induction*. To investigate the effect of both the conventional and vigorous bamboo extracts on the immune or host response of healthy animals before tumor implantation,

BALB/c mice were randomly divided into 4 groups: control (basal diet), or fed the basal diet supplemented with 0.1% conventional hot-water (100˚C, 30 min) extract, or 0.03-0.5% vigorous extract. After seven days (Figure 1A) the animals were sacrificed and the IL-2, IL-12, and IFN-γ levels were quantified by ELISA (10).

*Effect of the vigorous extract on solid tumors in mice*. In all the tumor models, $2\times10^6$ tumor cells were subcutaneously (*s.c.*) implanted dorsally. Our previous studies showed that *s.c.* inoculation of $2\times10^6$ tumor cells became a solid tumor mass with more than 90% probability (17-19). Because different mouse strains have different immune responses, the ddY strain was used for the S-180 tumors, the BALB/c strain for the Meth-A tumors, and the C57BL/6 strain for the B16-F10 tumors. These tumor implantation models may provide analogy to the tumor metastasis at ectopic sites. For the post-treatment group, the diet containing the vigorous extract was fed when the tumors reached a diameter of 5-6 mm.

*Effect of the vigorous extract against DMBA-induced breast cancer in rats*. The rats were randomly divided into group I, fed with the test diets containing 0.03, 0.1, or 0.5% of the vigorous extract for 2 weeks before exposure to DMBA (pretreatment), and group II fed with the test diet with 0.1% the vigorous extract 5 weeks after exposure to DMBA (post-treatment) (Figure 1C). For each experimental group, a control group of normal female SD rats were fed with the chemically defined basal diet (F2) only. DMBA was dissolved at 10 mg/mL in corn oil and administered to 7-wks old rats *via* gavage at 10 mg/body. The vigorous extract was given in the diet throughout the 20 week experimental period. The body weight, food intake, tumor volume, survival analysis (% T/C), number of rats with palpable tumors, tumor size, and number of tumors per rat were recorded daily or every third day for more than four months usually. In all the experiments, the tumor volume (V) was calculated by formula: $V=(L\times W^2)/2$, where L is the longitudinal cross-distance and W is the transverse width of the solid tumor in mm.

*Determination of lipid peroxy radical scavenging activity*. The scavenging activity of alkyl peroxy radical ($LOO^{\cdot}$) was determined using the method reported by Akaike *et al*. (20, 21). Briefly, the reaction mixture contained 1.7 mM DTPA, 16.7 μM luminol, 1 mM *t*-BuOOH and 1 mg/mL hemoglobin as the $LOO^{\cdot}$ generating system in 0.01M phosphate buffered 0.15M NaCl (PBS). The diet samples contained various concentrations of either the conventional hot-water extract, or the vigorous extract, or Sephadex G-50 gel column chromatography fractions obtained from the vigorous extract (10). The assay was started by adding hemoglobin solution to the reaction mixture, and chemiluminescence was measured using a Luminoskan RS (Thermo Fisher Scientific K.K., Yokohama, Japan). The suppression of radical generation (or chemiluminescence) was defined as $IPOX_{50}$ (50% inhibitory potential of peroxyl radicals), which indicates the concentration of the assay sample required to quench 50% of chemiluminescence.

*Statistical analyses*. All the data are expressed as means±SE. The student's *t*-test was used to determine the significance of differences between each experimental group; $p<0.05$ was considered significant.

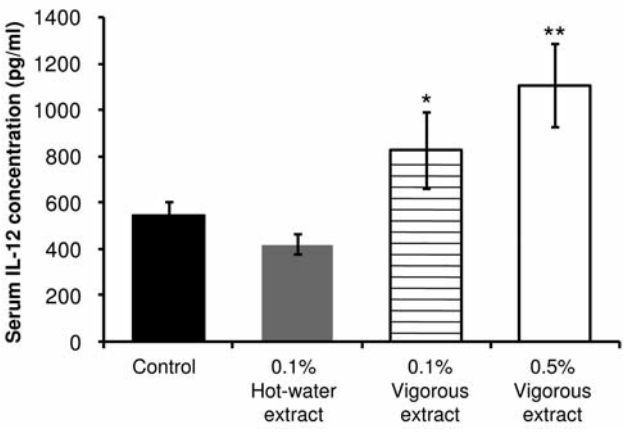


Figure 2. *Effect of bamboo leaf extracts obtained by two extraction methods on IL-12 production. Values are means±SEM (n=5). **:p<0.01, *:p<0.05 (the vigorous extract vs. control and vs. the hot-water extract).*

## Results

*Cytokine induction*. As shown in Figure 2, the vigorous extract significantly increased the serum IL-12 level compared to the normal (basal) diet and the conventional hot-water extract, in a dose-dependent manner. However, serum IL-2 and IFN-γ levels were not affected in all groups (data not shown).

*Effect of the vigorous extract in vivo against implanted tumors in mice. a) S-180 solid tumors in ddY mice:* Figure 3A demonstrates the significant suppression of S-180 tumor growth as judged by tumor volume (>50%) in both the pre- and post-treatment groups compared with the control group ($p<0.01$). The simultaneous treatment group showed significant suppression of tumor growth, but less than the pretreatment group ($p<0.05$). All the treatments improved the survival rate of the S-180 tumor-bearing mice in a dose-dependent manner (Figure 3B, D). Pretreatment with the vigorous extract resulted in the highest increase of survival time; the %T/C (treated group/control group) was 161.1% (Figure 3B). Furthermore, significant numbers of the mice showed complete regression of tumors with the vigorous extract: the numbers of tumor-free-mice to mice-with-tumors after 20 weeks (0.1% vigorous extract) were: 1/11 of the simultaneous treatment, 3/11 of the pretreatment and 2/11 of in the post-treatment groups, respectively. No significant difference in body weight was found between the vigorous extract treatment groups and the control group (data not shown).

*b) Meth-A solid tumors in BALB/c mice:* As shown in Figure 3C, pretreatment with the vigorous extract significantly suppressed tumor growth (to about 25%) compared to the control group ($p<0.01$). The group

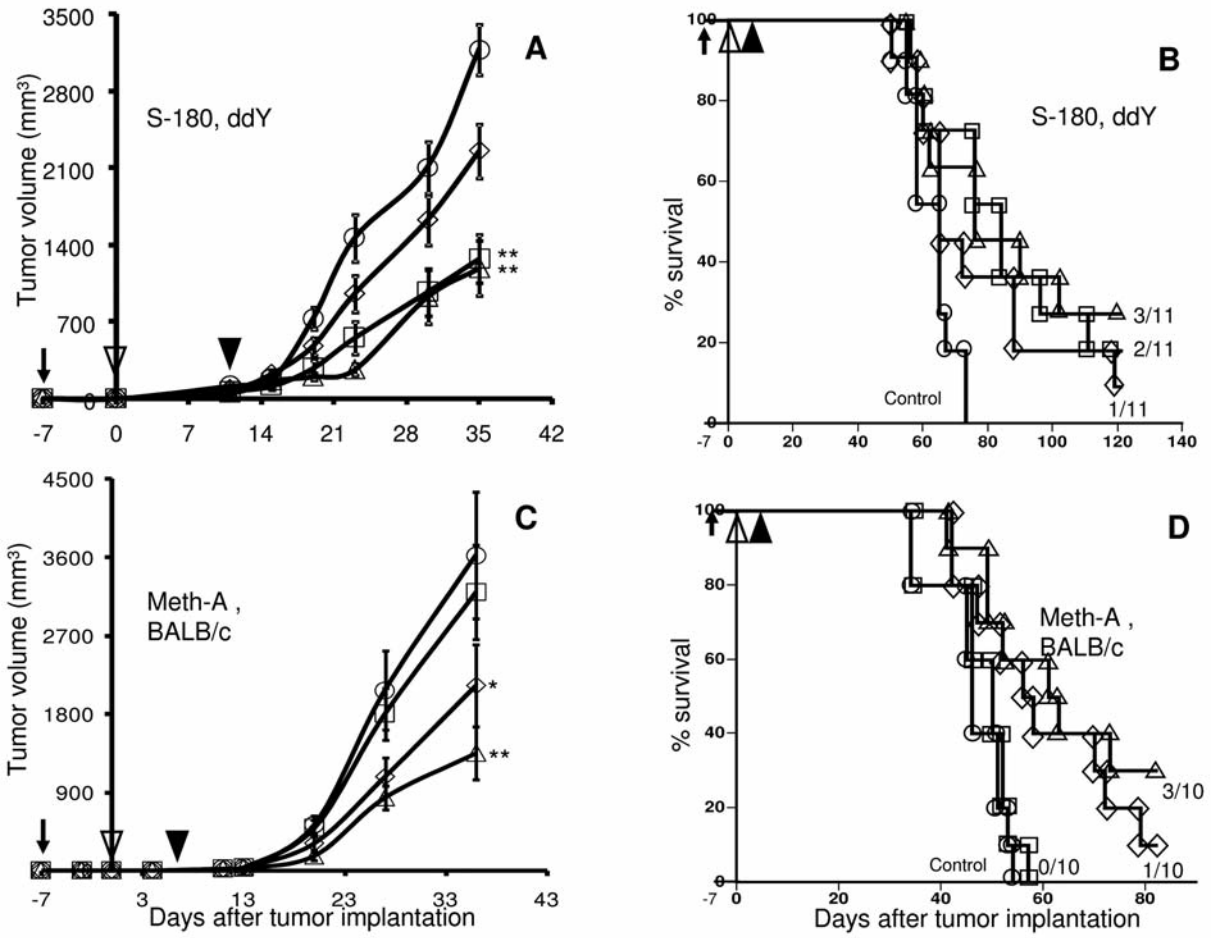


Figure 3. *Suppressive effect of the vigorous extract (0.1%) in different tumor models. S-180 and Meth-A cells (2×10⁶ cells) were implanted s.c. in ddY and BALB/c mice, respectively. (A) and (C), tumor growth after each treatment. (B) and (D), survival rates of tumor-bearing mice. ○, control (no extract); □, post-treatment; ◇, simultaneous treatment; △, pretreatment. Values are means±SEM (n=11, or 10). Black arrows, open arrowhead, and closed arroehead indicate the starting times of pretreatment, simultaneous treatment, and post-treatment with the vigorous extract, respectively. *p<0.05, **p<0.01 vs. controls. Numerals in (B) and (D) (e.g., 3/11) indicate the number of tumor-free mice surviving/total mice in the group.*

receiving simultaneous treatment also showed significant suppression of tumor growth, but to a lesser degree ($p<0.05$). Both the pretreatment and simultaneous treatment groups also showed improved 50% survival time (%T/C, 143.5% and 130.7%, respectively). The post-treatment group showed only marginal (non-significant) suppression of growth and improvement of 50% survival time (%T/C of 111%) (Figure 3D). Similar to the S-180 solid tumor model, administration before tumor implantation resulted in the best tumor growth suppression and survival benefit, as well as more tumor-free mice (3/10, Figure 3D) compared to the other treatments.

*c) B16-F10 solid tumors in C57BL/6 mice:* The vigorous extract at 0.1% did not show any significant effect on tumor growth and survival time in the B16-F10 implanted mice when compared to controls (data not shown).

*Effect of the vigorous extract against DMBA-induced breast cancer in rats.* The vigorous extract at 0.1% and 0.5% in the diet exhibited a significant anticarcinogenesis effect compared to the control. The incidence of breast cancer was significantly lower at about 50% and 60% on day 93, after 0.5% or 0.1% of the vigorous extract was fed, respectively (Figure 4A, both $p<0.01$). However, the vigorous extract at 0.03% showed no anticarcinogenic effect (Figure 4A). Moreover, the vigorous extract at 0.5% significantly suppressed tumor growth (by volume) on days 85-106 (Figure 4C, $p<0.01$). The dose-response effect in both the tumor-incidence and the cumulative tumor-number of the vigorous extract is shown significant in Figures 4A and B, and Figure 5 at concentrations higher than 0.03%.

The importance of the timing of administration of the vigorous extract (*i.e.* five weeks after or two weeks before the

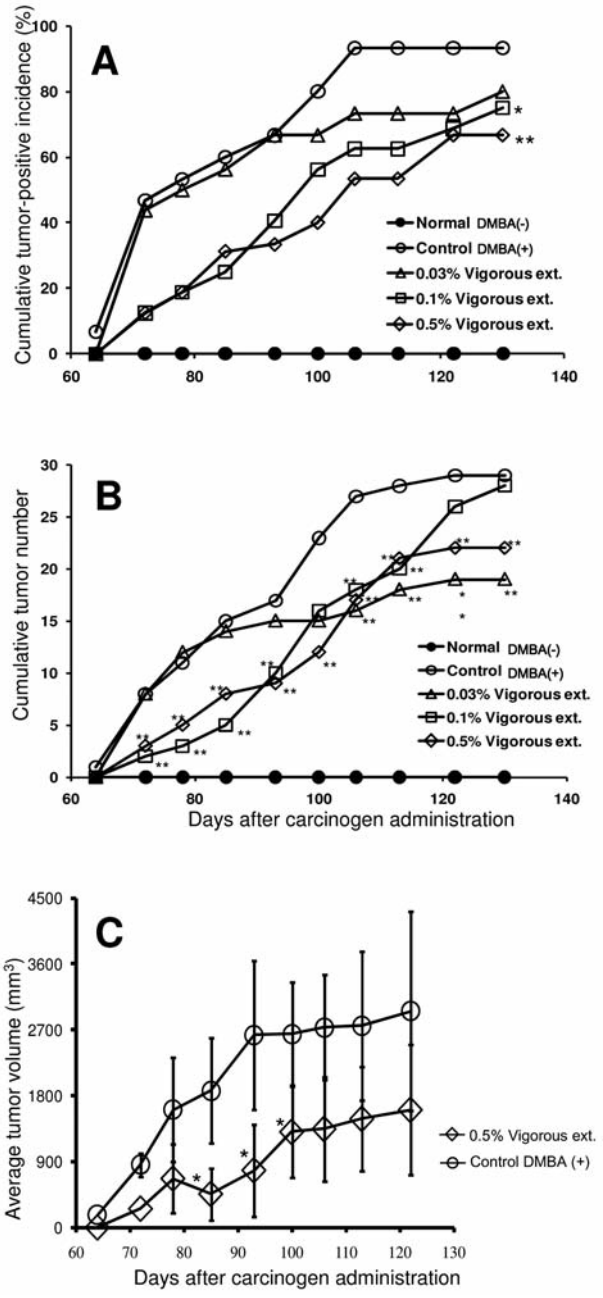


Figure 4. *Effect of the vigorous extract on DMBA-induced breast cancer in rats. (A) Incidence of detectable breast tumors (per group). (B) Total (cumulative) number of detectable breast tumors (per group). (C) Average tumor volume. Values are means±SEM. **p<0.01, *p<0.05 (the vigorous extract vs. control).*

exposure to carcinogen, DMBA) was further examined in the *in vivo* rat model where a more than 90% chance of developing DMBA-induced breast cancer was seen without the vigorous extract (control). As shown in Figure 6, the administration of the vigorous extract at 0.1% after DMBA treatment significantly suppressed the cumulative breast

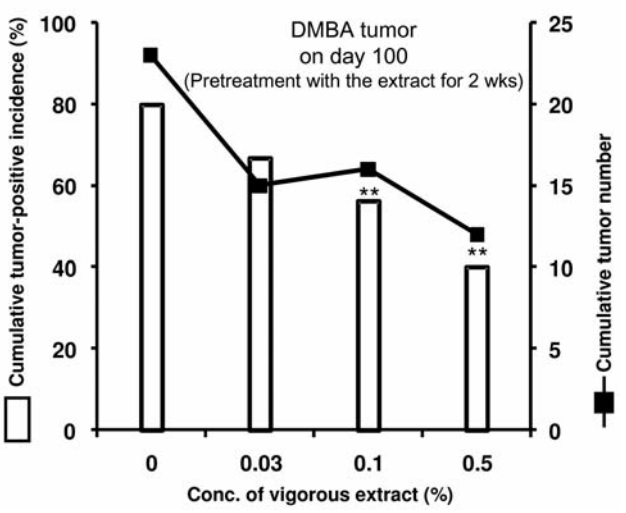


Figure 5. *Effect of the vigorous extract on day 100 in DMBA-induced rat breast cancer model. The vigorous extract was given 2 weeks before DMBA exposure.*

tumor numbers at same time-points, however, the anticarcinogenic effect was not as pronounced as pretreatment with the vigorous extract as seen in Figure 4C.

*LOO˙ -scavenging activity of the vigorous extract.* As shown in Table I, the vigorous extract exhibited scavenging activity of the LOO˙-radical to a significant extent, about two-fold more potent than that of the conventional hot-water extract. Three major fractions obtained by Sephadex G-50 gel column chromatography from the vigorous extract were also examined for LOO˙-scavenging activity. The F-I fraction showed two-fold more potent activity than the conventional hot-water extract. Furthermore, the lower molecular weight fractions F-II and F-III exhibited more potent LOO˙-scavenging activity than F-I which was considered to consist of primarily β-glucan (10).

## Discussion

In the present study, treatment with the vigorous extract significantly increased the serum IL-12 levels (Figure 2). This host-mediated immunological event considered as immunosurveillance, may effectively suppress newly formed cancer cells, tumor clones, or tumor cells newly metastasized to distant sites (10-13). This last possibility was examined by the use of ectopically implanted S-180 and Meth-A tumor cells.

Both the dose and the administration schedule of the vigorous extract were important in these models (Table II). The best effect was found by preconditioning of the hosts' immune system by administration of the vigorous extract before tumor transplantation, the pretreatment, followed by simultaneous treatment and then post-treatment. In both the S-180 and Meth-A solid tumor models, pretreatment

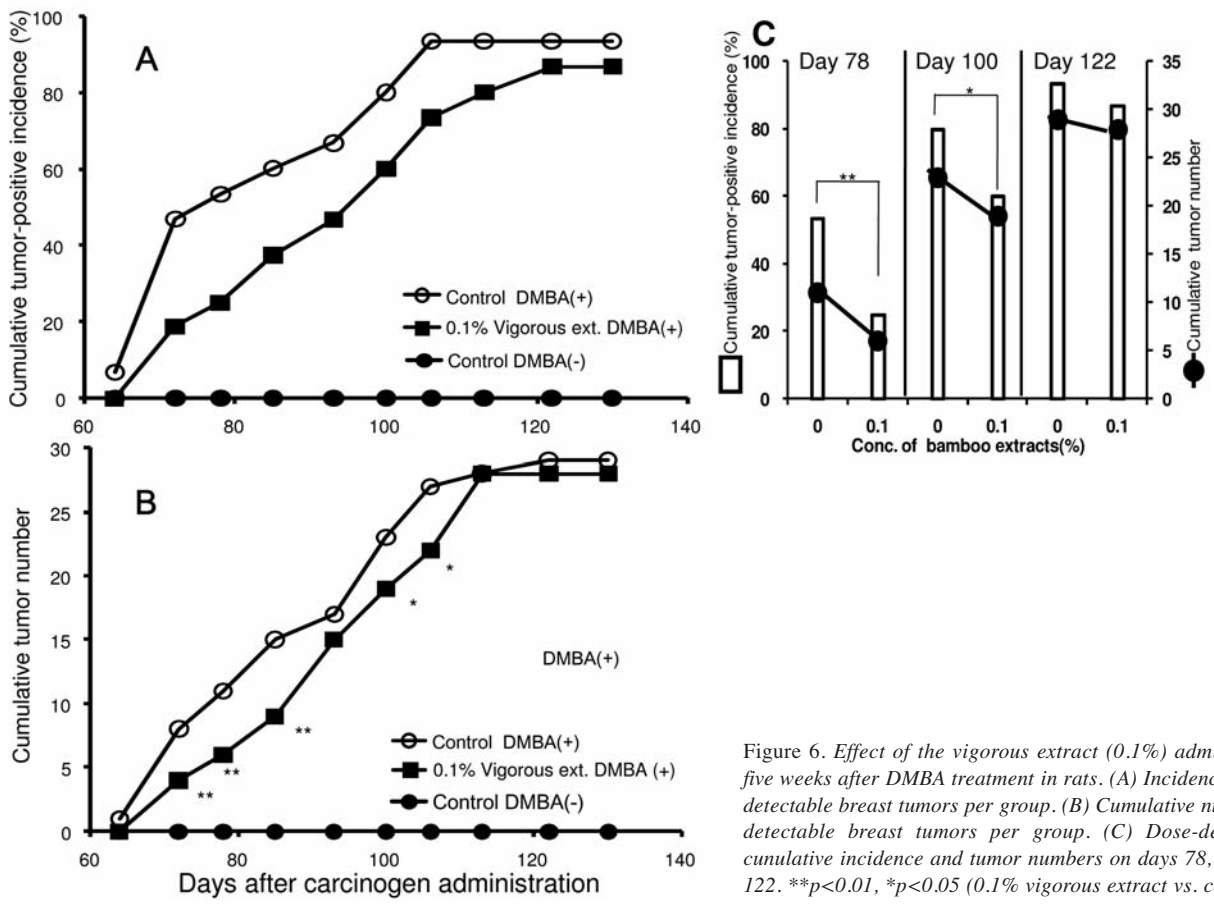


Figure 6. *Effect of the vigorous extract (0.1%) administered five weeks after DMBA treatment in rats. (A) Incidence (%) of detectable breast tumors per group. (B) Cumulative number of detectable breast tumors per group. (C) Dose-dependent cunulative incidence and tumor numbers on days 78, 100 and 122. **p<0.01, *p<0.05 (0.1% vigorous extract vs. control).*

significantly suppressed tumor growth by volume and pretreatment prolonged the survival rate of tumor-bearing mice (50% survival time, %T/C of 130% and 135%, respectively), whereas almost no activity was seen for post-treatment in the Meth-A model (Figure 3C and D). Pretreatment or earlier administration of the vigorous extract, when the tumor burden (or number of target cells) was small, gave more favorable results rather than post-treatment when the tumor burden became larger.

In contrast, all schedules of administration of the vigorous extract did not suppress growth of the B16-F10 tumor implanted in C57BL/6 mice to a significant level. In a separate study, the growth rate of the B16-F10 tumor was more rapid than that of Meth-A, S-180 or colon-38 adenocarcinoma (Seki and Maeda, unsubmitted data). This rapid growth rate suggested that the B16-F10 tumors became too big before the immune tumor suppressive effect could be sufficiently induced. Thus, the timing of administration of the vigorous extract, to allow immunological conditioning of the host seems crucial for better efficacy.

With others we have previously shown that chemical carcinogens induced free radicals in biological systems. In addition, we found that free radical or lipid peroxy radical scavengers (*e.g.* canolol, a phenolic compound from crude rape seed oil) suppressed DNA damage and mutation (22, 23). These free radicals include NO produced *via* iNOS (inducible form of

Table I. *LOO˙-scavenging activity in different bamboo extracts and Sepadex G-50 fractions obtained from the vigorous extract.*

| Extract or fraction | $IPOX_{50}$ (μg/mL)* |
|---|---|
| Conventional hot-water extraction | 6.47 |
| Vigorous extraction (unpurified) | 3.16 |
| Sephadex G-50 chromatography | |
| F-I | 6.80 |
| F-II | 3.93 |
| F-III | 2.23 |
| Sinapinic acid (control) | 0.314 |
| Syringic acid (control) | 1.12 |

*Suppression of generation of peroxy radicals (*t*-BuOO˙) expressed as $IPOX_{50}$, or inhibitory potential of 50% for peroxyl radicals, which indicates the concentration of the assay sample required to quench 50% of chemiluminescence.

Table II. *Effect of timing of administration of vigorous extract on tumor growth as judged on day 30 or 100, or on the incidence of carcinogenesis by DMBA.*

| | | Tumor volume (%) | | | DMBA carcinogenesis | |
|---|---|---|---|---|---|---|
| Timing and dose of bamboo extract [a] | | S-180 | Meth-A | DMBA (breast ca) | Tumor number [b] | Tumor incidence*(%) [c] |
| Evaluated day | | (day 30) | (day 34) | (day 100) | (day 100) | |
| Control | none | 100 | 100 | 100 | 23 | 80 |
| Simultaneous | 0.1% | 65.2* | 52.0* | – [d] | – | – |
| | 0.03% | – | | | 15** | 66.7[ns] |
| Pre-administration | 0.1% | 44.0** | 40.5** | 88.6[ns] | 16* | 56.3* |
| | 0.5% | – | – | 49.4* | 12** | 40.0** |
| Post-administration | 0.1% | 46.1** | 86.9[ns] | – | 19[ns] | 60.0* |

[a]Dose of the vigorous bamboo extract in the diet; [b]mean numbers of tumor observed per rat on day100 (n=6); [c]% of tumor development rats in a group (n=6) regardless of tumor numbers. 80% means 20% rats were tumor free; [d]–: not done; *:$p<0.05$, **:$p<0.01$, compared to the control, ns: non-significant.

NO synthase that occurs for instance in inflammatory tissue) and to an even greater degree, by peroxynitrite (ONOO–) when the superoxide anion radical is concomitantly produced by cytochrome P450 oxidase, cytochrome b5 reductase and iNOS (24-27). In addition, $LOO^{\cdot}$ is generated from lipid hydroperoxides predominantly *via* catalysis of heme or iron containing compounds (28-30). For example, we showed that the potent chemical carcinogens heterocyclic amines and 5-nitroguanosine, which is an inflammatory NO-derived endogenous product *in vivo*, could induce superoxide generation catalyzed by heme-containing enzymes (*e.g.*, iNOS and cytochrome b5 reductase etc.) (24, 25). Many phytochemicals have strong antioxidant activity and suppress either the initiation or promotion step of carcinogenesis (4). Therefore, the strong antioxidant activity (free radical scavenging) of the vigorous extract might be expected to suppress DNA damage, *i.e.* initiation of carcinogenesis, and inflammatory responses. The present vigorous extract contained a higher level of phenolics (10) and showed stronger antiradical activity compared to the conventional hot-water extract (Table I). More importantly, it suppressed in a dose-dependent manner, the growth of breast cancer induced by DMBA (Figures 4A, 4B and Table II). Usually chemically induced tumors are more difficult to control like human breast cancer than those of implanted experimental inbred tumors (S-180 and Meth-A). Thus, the vigorous extract may exhibit an anticarcinogenic effect *via* radical scavenging as well as immunopotentiation such as activation of NK cells and macrophage (8-16, 31). Namely, the vigorous extract significantly suppressed DMBA-induced breast tumor growth (Figure 4C) and the number of palpable tumor mass (Figure 4B) *via* activation of NK cells and macrophages as well.

The higher incidence of cancer in aged populations might be explained by reduced immune responses or immune surveillance, as well as accumulated errors in DNA. We believe that two factors, *i.e.*, the availability of free radical scavengers and immunosurveillance *in vivo*, are most crucial for cancer prevention. The former can protect DNA from damage; and the latter will eliminate transformed or carcinogenic cells *via* the immune system (NK cells, cytotoxic T cells, and macrophages). In this context, free radical scavengers such as flavonoids, phenolics from plants and microbial components or polysaccharides such as β-glucan are of great importance (16).

Other researchers have shown that bamboo extracts contain lignan (32) and lignin (33, 34), and lignan was found to exhibit antiestrogen activity and an inhibitory effect against breast cancer (35, 36). Lignin is also known to exhibit free radical-scavenging activity (34). Thus, if the vigorous bamboo leaf extract studied here contain lignan, the extract may also have antiestrogen activity in addition to the multiple effects described above.

In conclusion, dietary supplements of the vigorous extract suppress, in a dose-dependent manner, the carcinogenesis and growth of DMBA-induced breast cancer. Moreover, in S-180 and Meth-A implanted tumor models, treatment with the vigorous extract before implantation of tumors significantly suppresses solid tumor growth by more than 60% and significantly prolongs life span. However, it is ineffective against B16-F10 melanoma in C57BL/6J mice, which may be attributed to either the rapid growth rate of this tumor, or to a lesser response of this mouse strain to immunostimulation effect of the extract. These results strongly suggest the potential of this vigorous bamboo leaf extract as a tumor suppressive and cancer preventive food supplement.

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## References


1 "World Health Organization" fact sheet (2009). Available from: http://www.who.int/mediacentre/factsheets/fs297/en/index.html.

2 Danaei G, Vader Hoorn S, Lopez AD, Murray CJ and Ezzati M: Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors. Lancet *366*: 1784-1793, 2005.

3 Yian W, Zhongqiu Z, Joel RG *et al*: Chemoprevention of lung squamous cell carcinoma in mice by a mixture of chinese herbs. Cancer Prevention Res *2*: 634-640, 2009.

4 Surh YJ: Cancer chemoprevention with dietary phytochemicals. Nature Cancer Rev *3*: 768-780, 2003.

5 Park HS, Lim JH, Kim HJ, Choi HJ and Lee IS: Antioxidant flavones glycosides from the leaves of Sasa borealis. Arch Pharm Res *30*: 161-166, 2007.

6 Hu C, Zhang Y and Kitts DD: Evaluation of antioxidant and prooxidant activities of bamboo *Phyllostachys nigra* var. *Henonis* leaf extract *in vitro*. J Agric Food Chem *48*: 3170-3176, 2000.

7 Kweon MH, Hwang HJ and Sung HC: Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phyllostachys edulis*). J Agric Food Chem *49*: 4646-4655, 2001.

8 Suzuki S, Saito T, Uchiyama M and Akiya S: Studies on the anti-tumor activity of polysaccharides. Isolation of hemicelluloses from Yakushima-bamboo and their growth inhibitory activities against sarcoma-180 solid tumor. Gann *16*: 2032-2039, 1968.

9 Sakai S, Saito G, Sugayama J, Kamasuka T, Takano T and Takada S: Anticancer effect of polysaccharide fractioj prepared from bamboo grass. Gann *55*: 197-203, 1964.

10 Seki T, Kida K and Maeda H: Immunostimulation-mediated antitumor activity of bamboo (*Sasa senanensis*) leaf extracts obtained under "vigorous" condition. Evid Based Complement Alternat Med; Advance access published online on May 7, 2008, doi:10.1093/ecam/nen026

11 Hamuro J, Rollinghoff M and Wagner H: Induction of cytotoxic peritoneal exudate cells by T-cell immune adjuvants of the β(1→3) glucan-type lentinan and its analogues. Immunology *39*: 551-559, 1980.

12 Suzuki I, Hashimoto K, Oikawa S, Sato K, Osawa M and Yadomae T: Antitumor and immunomodulating activities of a β-glucan obtained from liquid-cultured *Grifola flondosa*. Chem Pharm Bull *37*: 410-413, 1989.

13 Kodama N, Komuta K, Sakai N and Nanba H: Effects of D-Fraction, a polysaccharide from Grifola frondosa on tumor growth involve activation of NK Cells. Biol Pharm Bull *25*: 1647-1650, 2002.

14 Takimoto H, Wakita D, Kawaguchi K and Kumazawa Y: Potentiation of cytotoxic activity in naive and tumor-bearing mice by oral administration of hot-water extracts from Agaricus brazei fruiting bodies. Biol Pharm Bull *27*: 404-406, 2004.

15 Suzuki I, Hashimoto K and Oikawa S: Antitumor and immunomodulating activities of a β-glucan obtained from liquid-cultured *Grifola flondosa*. Chem Pharam Bull *37*: 410-413, 1980.

16 Seki T, Morimura S, Ohaba H, Tang Y, Shigematsu T, Maeda H and Kida K: Immunostimulation-mediated antitumor activity by preconditioning with rice-shochu distillation residue against implanted tumor in mice. Nutr Cancer *60*: 776-783, 2008.

17 Fang J, Sawa T, Akaike T and Maeda H: Tumor-targeted delivery of polyethylene glycol-conjugated D-amino acid oxidase for antitumor therapy *via* enzymatic generation of hydrogen peroxide. Cancer Res *62*: 3138-3143, 2002.

18 Fang J, Sawa T, Akaike T *et al*: *In vivo* antitumor activity of pegylated zinc protoporphyrin: targeted inhibition of heme oxygenase in solid tumor. Cancer Res *63*: 3567-3574, 2003.

19 Greish K, Nagamitsu A, Fang J and Maeda H: Copoly(styrene-maleic acid)-pirarubicin micelles: high tumor-targeting efficiency with little toxicity. Bioconjugate Chem *16*: 230-236, 2005.

20 Kanazawa A, Sawa T, Akaike T, Morimura S, Kida K and Maeda H: Generation of lipid peroxyl radicals from edible oils and their biological activities: a need for consideration for anti-radical components and purification processing. Biofactors *13*: 187-193, 2000.

21 Akaike T, Ijiri S, Sato K, Katsuki T and Maeda H: Determination of peroxyl radical-scavenging activity in food by using bactericidal action of alkyl peroxyl radical. J Agric Food Chem *4*: 1864-1870, 1995.

22 Kuwahara H, Kanazawa A, Wakamatu D, Morimura S, Kida K, Akaike T and Maeda H: Antioxidative and antimutagenic activities of 4-vinyl-2,6-dimethoxyphenol (canolol) isolated from canola oil. J Agric Food Chem *52*: 4380-4387, 2004.

23 Cao X, Tsukamoto T, Seki T, Tanaka H, Morimura S, Cao L, Mizoshita T, Ban H, Toyoda T, Maeda H and Tatematsu M: 4-Vinyl-2,6-dimethoxyphenol (canolol) suppresses oxidative stress and gastric carcinogenesis in *Helicobacter pylori*-infected carcino-gen-treated Mongolian gerbils. Int J Cancer *122*: 1445-1454, 2008.

24 Maeda H, Sawa T, Yubisui T and Akaike T: Free radical generation from heterocyclic amines by cytochrome b5 reductase in the presence of NADH. Cancer Lett *143*: 117-121, 1999.

25 Sato K, Akaike T, Kojima Y, Ando M, Nagao M and Maeda H: Evidence of direct generation of oxygen free radicals from heterocyclic amines by NADPH/cytochrome P-450 reductase *in vitro*. Jpn J Cancer Res *83*: 1204-1209, 1992.

26 Nishino H, Tokuda H, Satomi Y *et al*: Cancer prevention by antioxidants. Biofactors *22*: 57-61, 2004.

27 Sawa T, Akaike T, Ichimori K *et al*: Superoxide generation mediated by 8-nitroguanosine, a highly redox-active nucleic acid derivative. Biochem Biophys Res Commun *311*: 300-306, 2003.

28 Sawa T, Akaike T, Kida K, Fukushima Y, Takagi K and Maeda H: Lipid peroxyl radicals from oxidized oils and heme-iron: implication of a high-fat diet in colon carcinogenesis. Cancer Epidemiol Biomarkers Prev *7*: 1007-1012, 1998.

29 Kanazawa A, Sawa T, Akaike T and Maeda H: Formation of abasic sites in DNA by t-butyl peroxyl radicals: implication for potent genotoxicity of lipid peroxyl radicals. Cancer Lett *156*: 51-55, 2000.

30 Akaike T, Sato K, Ijiri S *et al*: Bactericidal activity of alkyl peroxyl radicals generated by heme-iron-catalyzed decomposition of organic peroxides. Arch Biochem Biophys *294*: 55-63, 1992.

31 Fujii T, Maeda H, Suzuki F and Ishida N: Isolation and characterization of a new antitumor polysaccharide, KS-2, extracted from culture mycelia of Lentinus edodes. J. Antibiotics *31*: 1079-1090, 1978.

32 Suga A, Takaishi Y, Goto S, Munakata T, Yamauchi I and Kogure K: Two lignan dimers from bamboo stems (*Phyllostachys edulis*). Phytochemistry *64*: 991-996, 2003.

33 Nakamura W and Nozu Y: Studies on the biosynthesis of lignin. II. Purification and properties of peroxidases from bamboo shoot. J Biochem *62*: 308-314, 1967.

34 Lu FJ, Chu LH and Gau RJ: Free radical-scavenging properties of lignin. Nutr Cancer *30*: 31-38, 1998.

35 Messina MJ, Persky V, Setchell KD and Barnes S: Soy intake and cancer risk: a review of the *in vitro* and *in vivo* data. Nutr Cancer *21*: 113-131, 1994.

36 Touillaud MS, Thiébaut ACM, Fournier A, Niravong M, Boutron-Ruault MC and Clavel-Chapelon F: Dietary lignan intake and postmenopausal breast cancer risk by estrogen and progesterone receptor status. J Natl Cancer Inst *99*: 475-486, 2007.


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