Introduction: Functions of Plant extracts

Some plant extracts were found to have physiological functions, and recently have been used in functional foods and health foods. Green tea extract, Ginkgo biloba extract and grape seed extract are examples of functional plant extracts. Green tea extract is reported to prevent cancer, to lower blood pressure and cholesterol concentration, and to show antibacterial effects (1,2,3,4). Its active compound is polyphenol (catechin). It is used as an ingredient in food and animal feed. Ginkgo biloba extract improves angiopathy (5). It is sold as health food ingredient, and is also approved as a medicine in Europe. Grape seed extract, whose active component is a pigment (proanthocyanin) is sold as a functional food ingredient, and there are indications that it prevents arteriosclerosis and suppress the development of gastric ulcer and colon carcinoma (6). All of these extracts are also known to have antioxidative activities, and a relationship between antioxidative activities and other physiological functions have been noted by many researchers (7,8,9,10,11,12).

Although antioxidative activities and related functions, such as anticancer effects and regulation of blood pressure, have been topics of conversation for about 10 years, the effect of improving the immune system was noted only one or two years ago. Pale colored vegetables and fruit extracts have been proved to exhibit these activities. The relationship between antioxidative activities and immune reactions has not been clarified.

Sugar Cane

Okinawa is a sugar cane cultivating area in Japan and famous for the longevity of its residents. The average life span is about 85 years in women and 78 years in men. The people of Okinawa are healthy and continue to work as long as they live. Elderly people eat KOKUTOU, a kind of non-centrifugal sugar, with green tea at teatime. It is a unique diet habit that other Japanese dose not have.

Up until now, sugar cane has been reported to have various effective components (13,14,15,16). The components of KOKUTOU have antioxidative activity (17) and the ability to improve hyperlipemia. Molasses is reported to have a whitening effect on human skin (18) and anti-mutagenicity. It has been used in facial soap since the olden days. And octacosanol from cane wax can enhance the physical endurance (19). This substance is used in a health food.

Health-conscious Consumers and Desire for Natural Products

Recently, consumers have expressed regret and anger over the abuse of synthetic chemicals and
medicines, such as food additives, and the antibiotics or hormones used in the domestic animal feed. These feelings affect companies in the human food and animal feed industries, so their researchers tend to study natural materials with physiological functions. The plant extracts mentioned above are examples. It is also thought that sugar cane might have such effective components. Therefore, study of Sugar Cane Extracts was started (20,21).

**Physiological functions of Sugar Cane Extracts**

**Preparation**

There are four types of Sugar Cane Extracts: Sugar Cane Extracts 1, 2, 3, and 4. Sugar Cane Extracts 1 and 4 are prepared from cane juice. Extract 1 is prepared using synthetic adsorbent chromatography, and adsorbed substances are components of the extract. Extract 4 is obtained by chromatographic separation by ion exchange column. Extract 3 is prepared by hot water extraction from bagasse. Extract 2 consists of volatile substances that had adsorbed to synthetic adsorbent resin.

The number of the name is the order of the discovery of the extract’s effect. First we found the deodorant effects of Extracts 1 and 2 (20, 21). Most of Physiological functions of Extract 1, 3 and 4 were found by collaboration with Eisai Co., Ltd. [JAPAN], which produces medicines, food additives, and animal feed materials.

**Phylactic Effects**

First, phylactic effects as physiological functions were found. Phylactic effects in this case would mean promotion of resistance against viral and bacterial infections, and these effects may be exploited to reduce or in some cases eliminate the use of antibiotics. Therefore, feed companies are interested in these effects.

Ten mice (Slc:ICR, male, 5 weeks of age) were used in each experimental group. Minimum lethal dose of virus (*Pseudorabies* virus, originally a swine pathogenic virus) or bacteria (*Escherichia coli*, originally human) was inoculated subcutaneously into the mouse. Each of the Sugar Cane Extract was orally administrated once a day for three days in the viral experiment, and only once in the bacterial experiment. Dosage was 500 mg/kg per day. As for the control, distilled water was administered instead of Extracts. Survival rate was counted 7 days after the inoculation for the viral infection, and 4 days after inoculation for the bacterial infection.

In both the viral and bacterial experimental groups, all mice in control groups died. In all extract-administration groups, at least 7 of 10 mice survived (Figures 1. A and B). These results indicate that the
Vaccine Adjuvant Effect

Given at the same time as the vaccination, vaccine adjuvant stimulates the immune response and increases the effectiveness of the vaccine. Domestic animals, especially, are given many vaccines throughout their lives. To elevate the antibody titer level of all animals, vaccines are given many times, but repeated vaccinations stress domestic animals and affect their growth. Thus, all over the world, the direction has changed to reducing the number of vaccination by using adjuvant.

Ten mice (Slc:ICR, male, 5 weeks of age) were used in each group of experiment. Each Sugar Cane Extract was orally administered once a day for 6 days from the day of Pseudorabies virus vaccine inoculation. Extract dosage was 500 mg/kg per day. Pseudorabies virus attack occurred 2 weeks after vaccination, and the survival rate was counted on the 7th day of the virus attack.

All mice that were not vaccinated and did not receive the extract died. Only 20% of mice in the vaccinated group that did not receive an extract survived. But the survival rate were 80% in all extract-administered groups (Table 1). These results show that the extracts enhanced the effect of the vaccine significantly.

Protection Effects on Liver Injuries

The number of liver injuries such as hepatitis, fatty liver and cirrhosis has been increasing recently. Liver injuries are caused by various factors, such as food, alcohol, and chemicals, etc.

The protective effects of Sugar Cane Extract on liver injury models were estimated (22,23). Five mice (Slc:ICR, male, 5 to 6 weeks of age) were used in each experimental group. Carbon tetrachloride (CCl₄), CCl₄ with Phenobarbital, ANIT, and D-galactosamine were used to induce liver injuries. All the models are acute liver injury models, but the mechanisms by which liver injuries are induced are different. Extract 1 was administrated orally once a day for 5 consecutive days, and injury evocation was induced by oral administration of chemicals on the final day of Extract administration. Serum GOT and GPT activities were measured the day following injury evocation. When liver injury occurs, liver cells are damaged and release these enzymes into blood vessels.

The negative control column shows values for animals without administration of extract nor inducement of liver injury. In groups given chemical treatment without extract administration both GOT and GPT activities were higher than in those previously administered Extract 1.
The same experiments were also done by using Extracts 3 and 4. Results showed the same activities as for Extract 1 (Table 2).

### Table 2. Effects of Sugar Cane Extract 1 on Liver Injury Models

<table>
<thead>
<tr>
<th></th>
<th>Chemical treatments</th>
<th>CCl₄</th>
<th>CCl₄ with phenobarbital</th>
<th>ANIT</th>
<th>GaIN</th>
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<tr>
<td></td>
<td>Negative control</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Got Extract 1</td>
<td>4.3 ± 4.1</td>
<td>2,846 ± 802</td>
<td>4,460 ± 2,130</td>
<td>1,804 ± 616</td>
<td>5,061 ± 3,484</td>
</tr>
<tr>
<td>Got No extract</td>
<td>18.4 ± 4.1</td>
<td>1,083 ± 477</td>
<td>403 ± 219</td>
<td>136 ± 117</td>
<td>177 ± 50.7</td>
</tr>
<tr>
<td>Gpt Extract 1</td>
<td>18.4 ± 4.1</td>
<td>4,177 ± 1312</td>
<td>9,255 ± 2,272</td>
<td>903 ± 372</td>
<td>7,193 ± 4,064</td>
</tr>
<tr>
<td>Gpt No extract</td>
<td>18.4 ± 4.1</td>
<td>1,059 ± 679</td>
<td>1,382 ± 1,278</td>
<td>57.2 ± 55.7</td>
<td>195 ± 93.3</td>
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**Protection Effects on Involution of Lymphoid Organs Exposed to Cold Stress**

Two groups of ten mice each (Slc:ICR, male, 5 weeks of age) were exposed to cold stress in a low temperature room maintained at 5°C for 4, 7, 24 and 24hr on the 1st day, the 2nd day, the 3rd day and the 4th day, respectively, for 4 consecutive days. SCE was orally administered at a dose of 500mg/kg/day once daily after each exposure. In the negative control group (no exposure to stress) and the positive control group (exposure to stress), distilled water instead of SCE was orally administered at a dose of 0.5ml/mouse/day for 4 consecutive days. Increases in body weight and organ weights were individually measured 1 day after the last dose of SCE. In the mice exposed to the cold stress, increases in body weight were suppressed and spleen and thymus weights were decreased in the positive control group. However, the oral administration of SCE allowed for body weight increase. The spleen and thymus weights of the SCE-administered mice were also protected to the same degree as that of the negative control group. SCE is thought to maintain normal immune function and regulation in the mice under cold stress.

**Antioxidative Activities**

There are many kinds of free radicals and active oxygens in our body. Some of them, which are derived from NO that is released by some leucocytes, have the important function of attacking cancerous cells and cells infected with viruses. However, they simultaneously damage cells of various

### Table 3. Radicals and Non-radicals

<table>
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<th>Radical</th>
<th>Non-radical</th>
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<tr>
<td>Oxygen centered radical</td>
<td>¹O₂</td>
</tr>
<tr>
<td>HO · Hydroxyl radical</td>
<td>H₂O₂</td>
</tr>
<tr>
<td>HO₂ · Hydroperoxyl radical</td>
<td>HOCl</td>
</tr>
<tr>
<td>LO₂ · Peroxyl radical</td>
<td>O₃</td>
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<tr>
<td>O₂⁻ · Superoxide anion</td>
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organisms, and cause many kinds of diseases as well as aging. Free radicals and active oxygens (Table 3) are in our body all the time. At the same time, some enzymes like SOD (superoxide dismutase) scavenge them (24). If this balance goes wrong, diseases occur and aging progresses.

Currently, some plant extracts get attention because of their antioxidative activity and are used as dietary supplements, functional food, and medicines. The Sugar Cane Extracts were also evaluated for these activities (25).

**DPPH Radical Scavenging Activity**

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical is a stable free radical, and its scavenging activity can be representative of free radical scavenging activity (26). DPPH radical has maximum absorbance at 517 nm, but DPPH-H does not have this maximum absorbance. Therefore when DPPH exists together with antioxidants, absorbance is lower than without antioxidants.

We evaluated the DPPH radical scavenging activity of Sugar Cane Extracts 1, 3, and 4, catechin (reagent), apple extract, and cocoa powder. Figure 2 shows the antioxidant concentration that can scavenge 50% of DPPH radical, so the lower value indicates higher activity. Catechin reagent and apple extract, which are polyphenols and representative antioxidants, show a high level of activity. The graph shows that Extracts 1, 3 and 4 have DPPH activity; Extract 1 especially shows a high level of activity. Extracts 3 and 4 have the same level as cocoa powder, which is known to have abundant cacao polyphenol. These results indicate that Sugar Cane Extracts have relatively high DPPH scavenging activity.

**Superoxide Anion Scavenging Activity**

Superoxide anion is one of the active oxygens, and its scavenging activity is measured by using a method which measures superoxide dismutase activity. Antioxidants are not enzymes, but some show the same activity as the enzyme, SOD (superoxide dismutase), so in Table 4 indicating the scavenging activity converted into enzymatic activity (24).

Catechin reagent showed the highest activity, and apple extract and cocoa powder activity values were relatively high. Extracts 1 and 4 showed the same levels as apple extract.

<table>
<thead>
<tr>
<th>Table 4 . Superoxide Anion (O$_2^-$) Scavenging Activity</th>
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<tbody>
<tr>
<td>Catechin (Reagent)</td>
</tr>
<tr>
<td>Extract 1</td>
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<tr>
<td>Extract 3</td>
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<tr>
<td>Extract 4</td>
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<tr>
<td>Apple Extract</td>
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<tr>
<td>Cocoa Powder</td>
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<tr>
<td>Red Wine</td>
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which is sold as plant polyphenol. Extract 3 had a value of 6,700 U/g, which was not high. However, it can be said that it has scavenging activity.

The relationship between antioxidative activity and other physiological functions is not clear, as is mechanism of such effects. But it is known that plant extracts having such activities usually have other physiological functions, so recent attention to these activities is growing.

Other functions

Deodorizing effect

Extracts 1, 2, and 3 have a deodorizing effect (20). Figure 2 shows one of their effects. We used a steam humidifier in an old man’s room in a nursing home for elderly people. A 0.02% Extract 2 tap water solution was poured into a humidifier. Five people checked the strength of the offensive odor and discomfort index at the starting point, after 30 and 60 minutes of steaming. Figure 2 shows both the offensive odor and discomfort index strength were decreased remarkably. If the Extract has a strong specific smell (“a masking effect”), the offensive odor should be decreasing and the discomfort index should be increasing with the passage of time as the concentration of the Extract in the air is increasing. Extract 2 is a volatile component of sugar cane and is useful as a deodorant of room air, clothes, furniture, fabrics, livestock barns, etc., in addition to use in the food processing industry. Extracts 1 and 3 are useful as deodorizer of food products, such as fish and meat.

Taste Improvement effect

Both of the Extracts 1 and 2 have taste improvement effects (27). Figure 4 shows 10-ppm final concentration of the Extract 1 added to liquid yogurt improved factors in the index. Especially, quaff ability, off-taste, aftertaste, and stickiness were improved. Considering its concentration was extremely low, strength of this effect is amazing.
**Discussion**

Sugar Cane Extracts have various functions. These functions are very useful and make these extracts effective as ingredients in functional food, health food, and functional animal feed. The National Institute of Animal Health [JAPAN] has also investigated immune-related effects of Extract 4 in chickens. Growth promotion effect in commercially bred chickens (Deklb) and the immunopotentiation effect and anti-coccidial infection effect in inbred laboratory chickens have been studied (28). The immunopotentiation effects of Extract 4 can also be expected to be an animal feed material.

It is surprising and interesting that sugar cane components have various useful functions. Besides, they are safe natural products. Sugar cane is mass-cultivated in large areas of the world to make sugar, so it is easy to obtain raw materials from sugar cane for extracts compared with other extraction materials. This is advantageous industrially.

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*Shin Mitsui Sugar Co., Ltd., **Eisai Co., Ltd. and ***National Institute of Animal health.

**Reference**

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