Antioxidants and other functional extracts from sugarcane

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1.Functions of plant extracts

1.1. Introduction

Many plant extracts have been found to have physiological functions and are used in functional and health foods. Green tea, Ginkgo biloba and grape seed extracts are examples of functional plant products. Green tea extract is reported to prevent cancer, lower blood pressure and cholesterol concentration, and exhibit antibacterial effects (1,2,3,4). Its active compound is a polyphenol (catechin, etc.) and it is used as an ingredient in foods and animal feed. Ginkgo biloba extract improves angiopathy (5). It is sold as a 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 suppresses the development of gastric ulcer and colon carcinoma (6). A purified extract of bilberry is rich in anthocyanins and was found effective in human subjects for reducing the clinical symptoms of lowered capillary resistance and increased retinal sensitivity. Extracts of strawberry and spinach were found to enhance the age-related functions of brain in rats, while blueberry extracts reduced the lung damage in rats subjected to pure oxygen. All of these extracts are also known to have antioxidant activities, and a relationship between antioxidant activities and other physiological functions have been noted by many researchers (7,8,9,10,11,12,13).

Although antioxidant 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 more recently. Pale colored vegetables and fruit extracts have been proven to exhibit these activities. The relationship between antioxidant activities and immune reactions has not been clarified.

1.2 Sugarcane

Sugarcane is a tropical grass belonging to the same spices as sorghum. The objective of sugarcane harvest is to produce sugarcane stalks with the highest possible sucrose content, ranging from 10 to 15% of the weight of stalks. Most sucrose is stored in the inner portion of the stalks while majority of valuable sugarcane extracts, including antioxidants, concentrate in the outer component (rind fraction) of the stalks. Both sucrose and sugarcane extracts are recovered in the form of cane juice via "milling" of sugarcane stalks. The cane juice is further processed to produce white/refined sugar, sugarcane extracts and other products.

Okinawa is a sugarcane cultivating area in Japan, famous for the longevity of its residents. The average life span according to the 1995 data of the Health and Welfare Ministry of Japan is about 85.1 years in women and 77.2 years in men. The elderly people of Okinawa are healthy and continue to work as long as they live. The elderly people eat kokutou, a non-centrifuged sugar, with green tea at teatime. It is a unique diet habit that other Japanese do not follow.

Sugarcane has been reported to contain various effective components (14,15,16,17). The components of kokutou have antioxidant activity (18) and the ability to improve hyperlipemia. Octacosanol from cane wax can enhance physical endurance (19). This substance is used in health foods. Blackstrap molasses, a byproduct of processing of sugarcane has long been claimed to have therapeutic values albeit with little or no verifiable evidence. It is available in the health food industry and is also reported to have a whitening effect on human skin (20) and is known to possess anti-mutagenicity. In Japan, it has been used in facial soaps since ancient times.

Recently, increasing concerns among consumers over the use of synthetic chemicals and medicines, such as food additives, antibiotics or hormones used in the domestic animal feed led to studies of natural materials with physiological functions. The plant extracts mentioned above are some of the examples and sugarcane was thought to possess such effective components. Hence, study of sugarcane extracts was initiated (21,22,23,24).

1.3 Physiological functions of sugarcane extracts

1.3.1 Preparation

Four types of sugarcane extracts were produced. Extracts 1 and 4 were prepared from cane juice. Extract 1 was prepared using synthetic adsorbent chromatography whereby the adsorbed substances are concentrated in the extract. Extract 4 was obtained by chromatographic separation with ion exchange resin. Extract 3 was prepared by hot water extraction from bagasse, the fibrous residue of sugarcane. Extract 2 consisted of volatile substances that had been adsorbed on and stripped of a synthetic adsorbent resin. The number of the extract refers to the chronology of the discovery of its effect. First were found the deodorant effects of extracts 1 and 2 (21,22). Most of the physiological functions of extract 1, 3 and 4 were discovered in collaboration with Eisai Co., Ltd. [JAPAN], a producer of pharmaceuticals, food additives and animal feed materials (21, 22, 25,26).

1.3.2 Phylactic effects

Phylactic effects in this case refer to promotion of resistance to viral and bacterial infections. These effects can be exploited to reduce or in some cases eliminate use of antibiotics.

Ten mice (Slc:ICR, male, 5 weeks of age) were used in each experimental group (21,22). A minimum lethal dose of a virus (*Pseudorabies* virus, originally a swine pathogenic virus) or a bacterium (pathogenic *Escherichia coli*, a strain derived from human) was inoculated subcutaneously into the mice. Each sugar cane extract was orally administrated once a day for three days after the date of the viral challenge, and only once in the bacterial experiment (the day before the bacterial challenge). The dosage was 500 mg/kg per day. In the control groups, distilled water was administered instead of extracts. Survival rates were determined 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 groups that were administered the extract, at least 7 of 10 mice survived (Figures 1. A and B). These results indicate that the extracts have a marked phylactic effects because they did not substantively prevent pathogens from multiplying (*in vitro* test). At present, sugarcane extracts have been developed to be feed materials for chickens, swine, etc. to reduce or in some cases to eliminate use of antibiotics.

1.3.3 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 the animals and adversely affect their growth. Thus, all over the world, the direction has changed towards reducing the number of vaccination by using adjuvants.

Ten mice (Slc:ICR, male, 5 weeks of age) were used in each group of experiment (21). Each sugar cane extract was administered orally once a day for 6 days from the day of *Pseudorabies* virus vaccine inoculation. Extract dosage was 500 mg/kg per day. *Pseudorabies* virus challenge occurred 2 weeks after vaccination, and the survival rate was counted on the 7th day of the virus challenge.

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

1.3.4 Protective effects on liver injuries

The number of liver disorders such as hepatitis, fatty liver and cirrhosis has been increasing recently. Liver disorders are caused by various factors, including foods, alcohol, chemicals, pathogens, etc. The protective effects of sugar cane extract on liver injury models were estimated (25,26). Five mice (Slc:ICR, male, 5 to 6 weeks of age) were used in each experimental group. Carbon tetrachloride (CCl_4), CCl_4 with phenobarbital (orally administrated 4 days before evocation), ANIT (alfa-naphtyl-isothiocyanate), and D-galactosamine (GalN) were used to induce liver injuries. All models are acute liver injury models, but the mechanisms by which liver injuries are induced differ. Extract 1 was administrated orally once a day for 5 consecutive days, and injury evocation was induced by given administrations of chemicals on the final day of extract administration. Serum GOT (Glutamic oxaloacetic transaminase) and GPT (Glutamic pyruvic transaminase) activities (IU/l; JSCC method) were measured the day following induction of liver injury. When liver injury occurs, liver cells are damaged and release these enzymes into blood.

The negative control column shows values for animals not administered any extract and with no induced liver injury (Table 2). In the groups given chemical treatment without extract administration, both GOT and GPT activities were higher than in those that had previously been administered extract 1.

The same additional experiments were also conducted using extracts 3 and 4. The results showed the same activities as that of extract 1.

1.3.5 Protective 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 24 hrs on the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} days, respectively (22). Extract 4 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 extract 4 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 administration of the last dose of extract 4. In the mice exposed to 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 extract 4 resulted in a body weight increase. The spleen and thymus weights of the extract 4-administered mice were also protected to the same degree as those of the negative control group. Extract 4 is thought to maintain normal immune function and regulation in the mice under cold stress.

1.3.6 Antioxidant activity

There are many kinds of free radicals and active oxygen species in our bodies (Table 3). Some of them are derived from nitrous oxide (NO) that is released by leucocytes. They have the important function of attacking cancerous and virus-infected cells. However, they simultaneously damage cells of various organs and may cause many kinds of diseases and aging. At the same time, some enzymes such as superoxide dismutase (SOD) scavenge them (27) and protect the cells from the damage. If this balance is upset, diseases occur and aging progresses.

Currently, some plant extracts get attention because of their antioxidant activity and are used as dietary supplements, functional food, and medicines. Extracts from sugarcane were also evaluated for these activities (23, 28).

1.3.6.1 DPPH radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical is a stable free radical (29) that displays a maximum absorbance at 517 nm. As DPPH-H does not exhibit this maximum, the absorbance is lowered in the presence of DPPH-scavenging antioxidants.

The DPPH radical scavenging activity of sugarcane extracts 1, 3, and 4, catechin, apple extract, and cocoa powder was evaluated. Figure 2 shows the antioxidant concentration that can scavenge (reduce the concentration by) 50% of DPPH radical; lower values indicate higher antioxidant activity. Catechin reagent and apple extract, which are polyphenols and representative antioxidants, showed a high level of activity. Extract 1 especially showed a high level of activity (28). Extracts 3 and 4 had the same level of activity as cocoa powder, which is known to contain an abundant amount of cacao polyphenols. These results indicate that sugarcane extracts had a relatively high DPPH scavenging activity.

1.3.6.2 Superoxide anion scavenging activity

Superoxide anion is one of the active oxygen species, and the scavenging activity is measured by determination of superoxide dismutase (SOD) activity. Antioxidants are not enzymes, but some show the same activity as SOD. Table 4 shows the scavenging activity converted to enzymatic activity (28).

Catechin showed the highest activity, and apple extract and cocoa powder activities were relatively high. Extracts 1 and 4 showed the same levels of activity as apple extract, which is sold as a plant polyphenols. Extract 3 has a value of 6,700 U/g, which is not high although it does have scavenging activity.

The relationship between antioxidant activity and other physiological functions is not clear, and neither is the mechanism of such effects. It is known, though, that plant extracts having such activities usually have other physiological functions, so the attention to these activities is growing.

1.3.6.3 Oxygen radical absorbance capacity (ORAC)

ORAC (30,31,32), a quantitative method of measuring the antioxidant activity of plasma, foods, and natural extracts, among other has become a standard method and ORAC values, in µmole TE (Trolox - a soluble analogue of Vitamin E – equivalents) per 100 g are available in the literature (Table 5) for a number of common fruits, vegetables and other antioxidant rich food supplements. In addition, a more recent refinement has been the differentiation between “fast”, “slow” and total or “whole” antioxidant capacity, referred to in the following, respectively, as “95% ORAC”, “50% ORAC” and “whole ORAC”, respectively. (31).

Five common edible molasses products (Table 6) available in the American market were selected and characterized (Tables 6 and 7). Products A – D were sugarcane-based products, while E was probably a corn - based product with a minor amount of sugarcane liquor blended in.

Of the sugarcane products A – D, only B, based on its high color and sugar composition, corresponded to "blackstrap" molasses, the others were lower color products with higher levels of sugars and lower ash.

The antioxidant capacity of the five products correlated very well with their color (Figure 3) indicating that high antioxidant polyphenols formed a large part of the sugarcane colorants. With some variations, the "95%" and "50%" ORAC values were much lower than the "whole" ORAC. This suggests that a substantial part of the antioxidant capacity originated from components with very slow-acting functionality.

Blackstrap molasses is a final product of sugarcane processing, that has been subjected to a number of unit operations, and a possibility exists that some of the antioxidant activity has been lost in the process. Samples of Louisiana sugarcane juice and syrups, i.e. sugarcane juice clarified with two different procedures and concentrated under vacuum were analyzed (Table 9). These products have only been subjected to juice extraction, vacuum concentration and, in the case of syrups, to a pH adjustment and settling, and are products with about 80 % sucrose on dry solids and a color of about 15,000 ICUMSA units. The ORAC values found were substantially higher than those of the edible molasses and were identical for the concentrated juice and syrups, indicating that neither the lime nor soda ash clarification measurably reduced the antioxidant capacity.

As even prolonged heating of another sample of Louisiana syrup (Table 10) did not result in any reduction of its antioxidant capacity, the high antioxidant capacity of the syrup samples that does not conform to the pattern observed in Figure 3 for various edible molasses is yet unexplained. Geographical or varietal differences of sugarcane composition, ash components or process chemicals in the industrial process or other factors may be responsible.

Application of granulated activated carbon, bone char, ion exchange resins, crystallization and chromatographic method for separation of colorants, including polyphenols and flavonoids from sugarcane liquors are well established industrial processes. Therefore, some of these processes were explored to concentrate the antioxidant rich compounds contained in the sugarcane juices. An example of such an application is in Table 11, where the antioxidant capacity is given of one syrup and two kinds of extracts or concentrates. While the concentrate 1 exhibits only a minor improvement over the source syrup, the concentrate 2 is a very antioxidant-rich product. The very high proportion of the "fast" antioxidant capacity is remarkable, and augurs well for its therapeutic potential. Concentrate 1 and 2 are sugarcane extracts produced by deferent separation processes

The whole ORAC capacity of the concentrate 2 is comparable to such well known antioxidants as caffeic and gallic acids, and exceeds that of many existing commercial antioxidant supplements (34), and, by a factor of one hundred or more of such health food favorites (Table 5) as prunes. While its physiological functions still need to be established, it is believed that this natural extract could be produced, as a new natural or even organic product from sugarcane, at a sufficiently low cost and high volume to aid significantly the antioxidant intake of the population. A 250 mg capsule of this product would satisfy the daily recommended intake of 3,000 ORAC units (34) considered as minimum to sufficiently increase the serum antioxidant levels.

1.4 Other functions

1.4.1 Deodorizing effect

Extracts 1, 2, and 3 have a deodorizing effect (21). Figure 4 shows some of the effects of extract 2. A home steam humidifier was filled with a 0.02 % solution of extract 2 in tap water. Five people checked the intensity of the offensive odors and discomfort index at the starting point, and after 30 and 60 minutes of humidifier operation. Figure 4 shows both the offensive odor and discomfort index strength were decreased remarkably. If extract 2 has a strong specific smell ("a masking effect"), the offensive odor should decrease and the discomfort index should increase with the passage of time as the concentration of extract 2 in the air increase. Extract 2 is a mixture of volatile component of sugarcane and is useful as a deodorant for room air, clothes, furniture, fabrics, livestock barns, etc., in addition to its application in the food processing industry. Extracts 1 and 3 are useful as deodorizers of food products, such as fish and meat.

1.4.2 Taste and Texture improvement

Both extracts 1 and 2 have taste improvement effects (21,33). Figure 5 shows that 10-ppm final concentration of extract 1 added to liquid yogurt improved factors in the index. Organoleptic quality, such as off-taste, aftertaste, and stickiness in particular were improved. Considering its concentration was extremely low, the strength of this effect is conspicuous.

Discussion

Sugarcane extracts have various functions. These functions are very useful and make these extracts effective as ingredients in functional foods, health foods, and functional animal feed. The National Institute of Animal Health [JAPAN] has also investigated immunological effects of extract 4 in chickens. Growth promotion effect in commercially bred chickens (Dekalb) and an immunopotentiation effect and an anti-coccidial infection effect in inbred laboratory chickens were studied (24,35). These effects of extract 4 are also expected to be an animal feed material.

It is surprising and interesting that sugarcane components have various beneficial physiological functions. Furthermore, they are safe natural products. Sugarcane is mass-cultivated in large areas of the world for sugar production, so that raw materials from sugarcane for extracts are readily available for industrial exploitation and relatively inexpensive as compared with other extraction substrates.

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