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Application of the leaves of *Phellodendron amurense* on new type of foods (novel food)

Summary

Amur cork tree (*Phellodendron amurense* Rupr., "Kihada" in Japanese) has been one of the most important medicinal raw materials in Japan since ancient times, used in various mixed oriental herbal medicines (mainly gastrointestinal), e.g. "Sankogan".

We researched the leaves of *Phellodendron amurense* as a material for a new type of food (health-promoting food), in terms of ingredients and its property, suitability for food processing and acceptability for consumers. After cutting off, the leaves were so quickly steamed (for enzyme inactivation), freeze-dried, and turned to powder (up to 0.1 mm in diameter). Finally, they were packed and stored in the refrigerator. An analysis revealed that there is no caffeine in the leaves, and they are safer than coffee, black tea, green tea, and chocolate. However, they contain a high content of calcium, folic acid, dietary fiber, polyphenols, *B*-carotene, and lutein. Also, we found that a 50% ethanol extract of the leaves had an antibacterial effect against *Streptococcus mutans* (a cariogenic bacterium), comparable to that of green tea. We have been trying to develop food products using the leaves, such as cakes, cookies, and teas. The leaves did not change their

original green color even after baking. Any problem couldn't be observed before or after mixing, dissolving, baking, etc., necessary for manufacturing sweets. Preliminary sensory test revealed that tested sweets were all well accepted, and there was no complaint above the color, taste and flavor.

We are going to develop also other foods and drinks for pregnant women, children, and elderly people.

Keywords: Amur cork tree, *Phellodendron amurense* Rupr., Kihada, novel food, antibacterial activity, *Streptococcus mutans*, caffeine free

Introduction

Historical background

Amur cork tree (Phellodendron amurense Rupr., "Kihada" in Japanese) is a deciduous tree belonging to the Rutaceae family, that can reach a height of about 20 meters (Figure 1). In Japan, it is widely distributed from Hokkaido to Kyushu and blooms from May to July. The flowers are dioecious and produce cone-shaped corymbs at the tips of branches, with many small yellow-green 5-petaled flowers. The leaves are opposite, odd-pinnate compound leaves, about 20 cm to 45 cm long, with 5 to 13 leaflets, long oval, about 5 cm to 10 cm long, with wavy margins. Yellowfin fruit is a spherical drupe, about 1 cm in diameter, and ripens to a black color in autumn. The fruit has a citrus aroma. The powdered yellow bark obtained from a trunk of Kihada without the epidermis is the herbal medicine "Phellodendron bark" ("Obaku" in Japanese) (Figure 2). It has been one of the most important medicinal raw materials in Japan since ancient times used in various mixed oriental herbal medicines (mainly gastrointestinal), e.g., "Sankogan".¹ This, one of the famous seven hundred years old Kampo (kanpð²) traditional medicines, which is composed of: Swertia japonica Makino, Cinnamomum cassia Blume, Phellodendron amurense Rup., Glycyrrhiza *uralensis* Fischer and used habitually by many people all over Japan³

¹ I. Arabas, S. Asada, *Medicaments in Japanese Medicine-Boxes on the Turn of the* 19th century, [in:] Acta 34 Congressus Internationalis Historiae Pharmciae, Firenze 2001, pp. 121–122.

² H. Kawahara, M. Yano, *Japanese Contributions to the History of Chinese Science*, "Historia Scientiarum. International Journal of the History of Science Society of Japan" 1966, Vol. 6, No. 2(59), pp. 123–158; H. Nishi Yama, *Kampoyaku to Minkanyaku*, Osaka 1969; S. Hajime, *Nihonno Meiyaku*, Tokyo 1993; A. Tsumura, *Kampo*, Tokyo 1991; J.D. Keys, *Chinese Herbs, their Botany, Chemistry, and Pharmacodynamics*, Tokyo 1995.

³ I. Arabas, S. Asada, *Leki i metody lecznicze w japońskiej tradycji*, "Farmacja Polska" 1998, Vol. 54, pp. 461–468.

(Fig. 3). Kampo medicaments⁴ are now recognized as a medicine not only by the government, but also by scientific institutions. Kampo is not a folk medicament⁵ but despite traditional perception of health and illness, it has become a highly developed medical system that effectively contributes to the maintenance of the health of the Japanese.⁶

Although legally prohibited in the past, kampo has never been banished from common use. The fact is that in recent years, kampo has gained enormous recognition from the public and from the official structures.

Perhaps the basic reason why kampo has not only survived oppression, but has actually gained strength, is that throughout its long history in Japan, the basic premises and principles of kampo, as revealed by its diagnosis and treatment methods, have always been very close to the opinions held by most Japanese. To the Japanese public, kampo does not appear as a distinct or alien system of medicine, but rather as an integral part of their own health maintenance practices. Most Japanese roughly equate kampo with plant and animal medicine, without clearly differentiating between the plant and animal medicines of kampo and those of folk medicaments.

Although kampo has always been a significant component of Japanese health care, its popularity during the past ten years or so is quite remarkable. Not only has the public become more interested in and aware of its medical efficacy, but the Japanese government has taken the official position of recognizing and encouraging its practice. The factors responsible for this development are numerous and complex. Some of them constitute the response to the increasingly widespread worldwide dissatisfaction with official medical treatment, while others are sociocultural factors⁷ specific to Japan.

It offers an effective alternative to pharmacology, since the two systems are almost ideally complementary, the weakness of one is the strength of the other.

⁴ E. Ohnuki-Tierney, *Illnes and culture in contemporary Japan. An anthropological view*, New York 2005, pp. 91–121.

⁵ D. Penkala-Gawęcka, Antropologia medyczna a etnofarmakologia, "Lud" 1994, Vol. 77, pp. 13–21.

⁶ S. Arai, T. Osawa, H. Ohigashi, M. Yoshikawa, S. Kaminogawa, M. Watanabe, T. Ogawa, K. Okubo, S. Watanabe, H. Nishino, K. Shinohara, T. Esashi, T. Hiraharaa, *Mainstay of Functional Food Science in Japan—History, Present Status, and Future Outlook,* "Bioscience, Biotechnology, and Biochemistry" 2001, Vol. 65(1), pp. 1–13.

⁷ T. Nagayo, History of Japanese Medicine in the Edo Era. Its social and cultural backgrounds, Nagoya, Japan 1991.

Contemporary background

This can be, for instance, newly developed or innovative food, or the food produced using new technologies and production processes. Novel food also includes traditional food consumed in other countries/ cultures and not commonly consumed in a given country.

The concept of producing food containing, apart from basic nutrients, additional substances with a physiological effect on the body was created in Japan in the early 1980s. The inspiration to undertake such a concept of food production development was the intensive increase in research on the physiological properties of food ingredients and the demonstration of their health-promoting functions.⁸ Novel food⁹ is characterized by additional, special utility properties. The source of acquisition are raw materials, including by-products. For example, yeast, field bean seeds, lupines and leaves of some green plants are used in search for new protein sources (so-called unconventional proteins). In 1984, the first Japanese project on functional foods¹⁰ commenced under the sponsorship of its Ministry of Education, Science, and Culture (MESC). The food value criteria were defined by three categories: primary function identified as the function of ordinary nutrients in the body, secondary function referring to the function of tastants and/or odorants, and third, as directly or indirectly related to disease prevention.¹¹

At Kyoto Koka Women's University in Japan, a group of researchers attempted to prepare food products from *Amur cork tree* leaves, which therefore meet the conditions to be called a novel type of foods/ novel food.

Based on the European Union Regulation (No. 2015/2283), it means the food that was not used for human consumption to a significant extent before 15 May 1997. First of all, we are going to develop foods and drinks for pregnant women, children, and elderly people.

⁸ R. Olędzka, Nutraceutyki, żywność funkcjonalna – rola i bezpieczeństwo stosowania, "Bromatologia i Chemia Toksykologiczna" 2007, Vol. 40, No. 1, pp. 1–8.

⁹ Nowa żywność – nowe smaki i innowacyjne technologie produkcji żywności też mają swoje regulacje, pp. 1–4,

https://fairlegal.pl/publikacje-prawne/2022-rok/nowa-zywnosc-nowe-smaki-iinnowacyjne-technologie-produkcji-zywnosci-tez-maja-swoje-regulacje [Access: 12.02.2023].

¹⁰ T. Kubiński, Żywność funkcjonalna, "Życie Weterynaryjne" 2010, Vol. 85(11), pp. 932–935.

¹¹ I. Podolak, K. Grabowska, *Leki pochodzenia naturalnego – znaczenie i perspektywy rozwoju, interakcje*, pp. 1–5, https://docplayer.pl/39920995-Leki-pochodzenia-naturalnego-znaczenie-i-perspektywy-rozwoju-interakcje.html [Access: 1.02.2023].



Amur cork bark ("Kihada" in Japanese)



Leaves



Fruits in autumn

Fig. 1. Amur cork tree (*Phellodendron amurense* Rupr., "Kihada", in Japanese). Photos: provided by Sankogan company





Fig. 2. *Phellodendron* bark ("Obaku" in Japanese). Photos: provided by Sankogan company



Fig. 3. Medicinal raw materials in Japan, used in various mixed oriental herbal medicines (mainly gastrointestinal), e.g., "Sankogan". Photos: provided by Sankogan company

Materials and methods

1. Preparation of bark of Amur cork tree (Kihada) leaf powder

After harvested, the leaves were washed twice with tap water. Then the leaves were transferred to wooden vessel and steamed for 3 minutes. After cooled to room temperature, the leaves were freeze-



Fig. 4. Powder processing of Kihada leaf (leaves of Amur cork tree). Photos: taken by H. Suido

dried and divided into aluminum bags, and kept in refrigerator until use (Figure 4).

2. Components analyses of Kihada leaf powder.

General nutrition components, various minerals and other components of the powder were analysed at Japan Food Research Laboratories, Osaka, Japan.

3. Measurement of antibacterial activity against a cariogenic bacterium

The antibacterial activities of 50% ethanol extracts of the Kihada leaf powder, other herbal medicines and green tea were investigated through broth dilution method: minimal inhibitory concentration (MIC) assays against *Streptococcus mutans* ATCC 25175. Briefly, *S. mutans* ATCC 25175 was grown in Brain heart Infusion (BHI) broth at 37°C for 24 hr. On the other hand, each plant samples were dissolved in 50% ethanol at 8mg/mL and diluted to various concentra-

tions of the samples, prepared by making 2-fold serial dilutions of the extracts using BHI broth in test tubes. Then the *S. mutans* culture solution was added to each test tube containing various concentrations of plant samples at 1% concentration and incubated at 37°C for 48 hr. After incubation, the presence or absence of bacterial growth was judged visually. The minimum concentration at which no bacterial growth was observed was defined as the MIC [μ g/mL].

4. Preparation of sweets and drinks and their tasting evaluation

Various sweets and drinks were prepared using Kihada leaf or its powder. Then a tasting evaluation of the prototypes was conducted by volunteers.

Results

1. Characteristics of freeze-dried Kihada leaf powder

The powder had a bright green color, and even when heated, it did not turn brown like tea leaf, and retained its beautiful green color. Since the size of the powder was as small as about 100μ m, it was easy to mix and dissolve in water.

2. Components of Kihada leaf

Since Kihada is grown without pesticides, it does not contain pesticides. The amounts of general nutrition components of Kihada leaf powder in comparison with Black tea leaf, which is widely consumed all over the world, are shown in Table 1. Kihada leaf contained about 33 g of dietary fiber per 100 g dried powder. It is almost the same as black tea leaf.

Components	Kihada leaf	Black tea leaf (※1)
Energy (kcal)	318	234
Water (g)	3.5	6.2
Protein (g)	11.2	20.3
Lipid (g)	8.7	2.5
Carbohydrate (g)	65.2	51.7
Sugar (g)	32.3	
Dietary fiber(g)	32.9	38.1
Salt (NaCl) equivalents (g)	0.0335	0
Sodium (mg)	13.2	3
Ash (g)	11.4	5.4

Tab. 1. General nutrition components of Kihada leaf in comparison with Black tea leaf (per 100 g)

*1: As comparison; cited from "ted fromison; ivalents (g)omponents of Kihada leaf ith Edition)is The amounts of minerals in Kihada leaf and Black tea leaf are shown in Table 2. Calcium contents were very high in Kihada leaf.

Tab. 2. Mineral components of Kihada leaf in comparison with Black tea leaf (per 100 g)

Components	Kihada leaf	Black tea leaf (※1)
Ash (g)	11.4	5.4
Natrium (mg)	13.2	3
Karium (mg)	1900	2000
Calcium (mg)	2680	470
Magnesium (mg)	284	220
Phosphorus (mg)	901	320
Iron (mg)	4.33	17.0
Zinc (mg)	2.22	4.0
Copper (mg)	0.49	2.1
Manganese (mg)	1.93	21.0

%1: As comparison; cited from "Standard Tables of Food Composition in Japan 2020 (8th Edition)".

The amounts of other components are shown in Table 3. Kihada leaf contained $250 \,\mu g/100$ g of folic acid. The amount is almost the same as that of black tea leaf. Also, catechin amount of Kihada leaf was only 2.6 mg, but that of black tea leaf is 2900 mg. Thus, catechin content of Kihada leaf is very little. Kihada leaf contained high amounts of antioxidants such as β -carotene, lutein and chlorogenic acid. Surprisingly, Kihada leaf did not contain caffeine, compared with black tea leaf or coffee.

3. Antibacterial activities of the extracts of Kihada leaf, various herbal medicines and green tea against a cariogenic bacterium

We compared the antibacterial activity of Kihada leaf with those of Licorice, Cinnamon and Kihada fruit as herbal foods. Also, we used bark of Amur cork tree ("Obaku" in Japanese) and green tea leaf as positive controls, because they have been reported to have antibacterial activities among natural materials. Minimum inhibitory concentrations (MICs) of 50% ethanol extracts of three kinds of herbal medicine, Kihada leaf and fruit (edible parts) and green tea against *Streptococcus mutans* ATCC25175 are shown in Table 4. MICs of *Phellodendoron* bark and green tea leaf were $250 \sim 500 \,\mu$ g/mL and $500 \,\mu$ g/mL, respectively. On the other hand, MIC of Kihada leaf powder was 1000 μ g/mL. Thus, Kihada leaf shows antibacterial activity against

ieur (per 100 g)		
Components	Kihada leaf	Comparison
Folic acid	250 µg	Black tea leaf: $210 \mu g (\gg 1)$
Polyphenol	6.14 g	Black tea leaf: No data
Catechin	2.6 mg	Black tea leaf: 2900 mg(×2)
Chlorogenic acid	510 mg	Coffee: 3 mg
Total ascorbic acid	112 mg	0 mg
(Total vitamin C)		
Vitamin A	2.02 mg	Black tea leaf: $75 \mu g$
(retinol activity equivalent)		
α- carotene	0.7 mg	Black tea leaf: No data
β- carotene	23.9 mg	Spinach (fresh): 4.2 mg (×1)
Lutein	40 mg	Kale (fresh): 21.9 mg (※3)
Vitamin E (α-tocopherol)	185 mg	Black tea leaf: 9.8 mg (×1)
Chlorogenic acid	510 mg	Coffee: 3 mg (X1)
Caffeine	Not	Black tea leaf: 2.9 g (×1)
	detected	Coffee: 4.0 g (※1)

Tab. 3. Other components of Kihada leaf in comparison with Black tea leaf (per 100 g)

*1: cited from "Standard Tables of Food Composition in Japan 2020 (8th Edition)".

*2: cited from "Tea encyclopedia" [ITO EN website].

%3: cited from https://www.fancl.co.jp/aojiru/column/12.html.

Tab. 4. Minimal Inhibitory Concentrations (MICs) of 3 types of herbal medicines, Kihada leaf and fruit (edible parts) and green tea against a cariogenic bacterium

Sample	MIC (µg/mL)
Licorice	125~250
Cinnamon	500~1,000
Phellodendoron bark (No use for food)	250~500
Kihada leaf powder (Freeze dried)	1,000
Kihada fruit powder	500~1,000
Green tea leaf	500

※: All samples are dissolved in 50% Ethanol extract at 8 mg/mL and diluted to each concentrations with BHI broth.
※: Incubation: 37°C, 48~72 hours

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Roll cake and Castella with black beans







Castella Fig. 5. Various sweets using Kihada leaf powder. Photos: taken by H. Suido



Roasted Kihada leaf tea



A drink containing Kihada leaf powde

Fig. 6. Drinks using Kihada leaf. Photos: taken by H. Suido

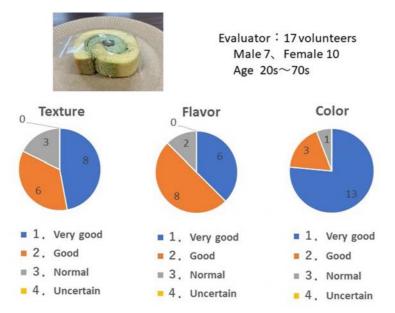


Fig. 7. Tasting evaluation of Roll cake with black beans using Kihada leaf powder. Photo: taken by H. Suido

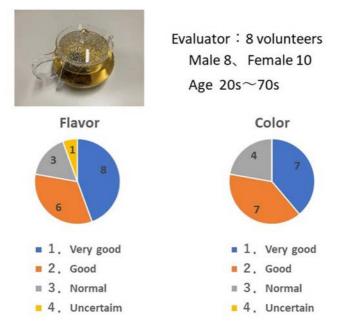


Fig. 8. Tasting evaluation of Roasted Kihada leaf tea. Photo: taken by H. Suido

a cariogenic bacterium, although the activity is slightly lower than *Phellodendoron* bark or green tea leaf.

4. Food prototypes using Kihada leaf and its powder, and their tasting evaluation

Various sweets such as Roll cake, Castella, Financie and Butter sand cookie were prepared using Kihada leaf powder (Figure 5). Also Roasted Kihada leaf tea and A drink containing Kihada leaf powder were prepared (Figure 6).

A tasting evaluation of the sweet prototypes using Kihada leaf powder was conducted by seventeen volunteers (male 7, female 10 and age 20s–70s). Figure 7 shows the result of "Roll cake with black beans". The texture, flavor and color were all well accepted (most were 'very good' or 'good'). Concerning other food samples, the results were almost the same as Roll cake.

A tasting evaluation of the drinks using Kihada leaf was conducted by 18 volunteers (male 8, female 10 and age 20s–70s). Figure 8 shows the results of "Roasted Kihada leaf tea". The flavor and color were all well accepted. Concerning another drink, the result was almost the same as Roasted Kihada leaf tea.

Summary of findings

As a result of component analysis of Kihada leaf, the followings were found.

- 1. No caffeine and no pesticide (suitable for pregnant woman and the elderly).
- 2. Very high content of calcium (good for the formation of bones and teeth).
- 3. High contents of antioxidants such as lutein, β -carotene and chlorogenic acid (good for eyes, contribute to protect skin and mucous membranes).
- 4. Very little content of catechins.
- 5. Slightly higher content of folic acid, compared with black tea (contributes to the normal development of the fetus).

Discussion

As a result of component analysis, no caffeine was found in Kihada leaf. Caffeine is naturally found in coffee beans, cocoa beans, and tea leaves, making coffee and tea the main sources of caffeine.¹² Caffeine has a calming effect on the nervous system, but ingesting too much caffeine can overstimulate the central nervous system, causing dizziness, rapid heart rate, agitation, anxiety, tremors, and insomnia. Further, it has been reported that long-term caffeine intake may increase the risk of high blood pressure for some people and that if a pregnant woman ingests high concentrations of caffeine, it may inhibit fetal growth (low birth weight).¹³ So Kihada leaf is thought to be safer than coffee, black tea and green tea in these points of view.

Also Kihada leaf contained high amounts of calcium and folic acid. Calcium is necessary for the formation of bones and teeth and is also a useful nutrient for preventing osteoporosis in the elderly. Folic acid is a vitamin that, along with vitamin B12, helps produce red blood cells. It is also an important vitamin for the development of the body, as it is involved in metabolism, promotes the biosynthesis of nucleic acids such as DNA and RNA, and proteins, and aids in cell production and regeneration. Folic acid greatly affects cell division and matura-

¹² Ministry of Agriculture, Forestry and Fisheries in Japan, *About excessive caffeine intake*, https://www.maff.go.jp/j/syouan/seisaku/risk_analysis/priority/hazard_ chem/caffeine.html [Access: 9.12.2022].

¹³ Australia New Zealand Food Authority, *The safety aspects of dietary caffeine*, "Report from the expert working group on The safety Aspects of Dietary Caffeine" 2000.

tion, so it can be said to be an important nutritional component, especially for fetuses.

Kihada leaf powder showed antibacterial activity (MIC: 1000 μ g/mL) against a cariogenic bacterium, but the activity was lower than *Phellodendoron* bark, "Obaku" in Japanese (MIC: 250~500 μ g/mL) and slightly lower than that of green tea powder (MIC: 500 μ g/mL). The main ingredient of *Phellodendoron* bark is berberine (a type of alkaloid), which has been reported to have antibacterial effects (against *Staphylococcus aureus*, Shigella, Cholera, Gonococcus, etc.).¹⁴ So, *Phellodendoron* bark has been applied as a stomachic and antidiarrheal. Japanese green tea has been known to have antibacterial activity.¹⁵ The antibacterial activity of green tea is thought to be due to catechins.¹⁶ But Kihada leaf has been reported to contain no berberine¹⁷ and did not contain catechins in this study. Therefore, it is presumed that the antibacterial activity of Kihada leaf is due to other components such as chlorogenic acid (a type of polyphenol), which was found in high amounts in the leaf.

Various sweets and beverages were made using Kihada leaf and its powder, and all the prototypes were highly evaluated by volunteers.

Thus, Kihada leaf seems to be a suitable food material for pregnant women and the elderly.

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¹⁵ M. Toda, Antibacterial and bactericidal activities of Japanese green tea, "Japanese journal of bacteriology" 1989, Vol. 44, No. 4, pp. 669–672.

¹⁶ S. Kusakabe, *Effect of Catechin Powdered Green tea on Oral bacterium*, "The Japanese Society of Conservative Dentistry" 2013, Vol. 56, No. 4, pp. 353–359.

¹⁷ S. Yamaguchi, *Berberine Contents in Phellodendron Leaves*, "Natural Medicines" 1998, Vol. 52, No. 5, pp. 452–454.

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