

Indonesian Biodiversities, from Microbes to Herbal Plants as Potential Functional Foods

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Abstract *L. plantarum* IS-10506 is one of very potential probiotic bacteria isolated from dadih, an Indonesia traditional fermented milk. The mechanisms and involvement of Toll-like Receptor (TLR) 2 and 4 in reducing allergic reaction by *Lactobacillus plantarum* IS-10506 probiotic *in vivo* through enhancement of the T_H1 and T_{reg} immune responses and forming a new T_H1-T_H2 equilibrium has been proved.

Noni (*Morinda citrifolia*), Buah Merah (*Pandanus conoideus*) and Extra Virgin Coconut Oil (EVCO, *Cocos nucifera*) are Indonesian indigenous herbal plants and have been focused on their usefulness as functional foods. Nutritional values and biological actions were studied. Scopoletin in Noni fruits plays a significant role in improving the blood rheology, and has remarkably strong quenching action of reactive oxygen species (ROS). Buah Merah is rich in carotenoids, especially β -cryptoxanthin, a novel chemopreventive agent against lung cancers of heavy smokers and may prevent and/or improve post-menopausal osteoporosis. EVCO does not contain trans-fatty acids and is rich in medium-chain fatty acids and saturated fatty acids.

Key word : dadih, probiotic, allergy, *L. plantarum*, Noni juice, blood fluidity, Buah Merah, β -cryptoxanthin, lung cancer, EVCO

Introduction

Dadih is Indonesian traditional fermented buffalo milk in West Sumatra prepared by pouring fresh raw buffalo milk into a bamboo tube capped with banana leaves and allowed to ferment spontaneously at room temperature (30°C) for two days (Akuzawa and Surono, 2002). Various indigenous lactic bacteria involved in the dadih fermentation, which may vary from time to time as well as from one to another place of dadih making. Some dadih lactic acid bacteria have antimutagenicity, hypocholesterolemic properties, antipathogenic properties and immunomodulatory properties (Surono and Hosono, 1996, Pato *et al.*, 2004 ; Zakaria *et al.*, 2005).

Morinda citrifolia (hereinafter Noni) is Indonesia-originated indigenous herbal plant of which fruits, leaves and roots have been utilized as traditional medicine, foods, natural dye and so

on for 6,000 years. We have already found that Noni fruit juice is rich in medium fatty acids, dietary fibers, vitamins and minerals, and disclosed some of biological and pharmacological actions including bactericidal action, inhibition of DNA damage, anti-mutagenicity, anti-oxidation or scavenging activity of reactive oxygen species, inhibition of type 1 diabetes mellitus etc (Surono *et al.*, 2000 ; Surono, 2003 ; Nishigaki and Waspdo, 2003).

Buah Merah (*Pandanus conoideus*) is exclusively distributed in Papua island and its neighboring areas, and was recently re-emerged and taken into account for its health benefits in Indonesia and some countries. Buah Merah has been utilized by Papuan inhabitants for 60,000 years as energy and vitamin sources rich in oil and carotenoids as well as vitamin E. Recently, Buah Merah was taken into account for health values in Indonesia and some countries (Ohtsuka, 1995 ; Laura, 2000).

Cocos nucifera, coconut is abundant in In-

Indonesia as a number one country in coconut oil production. Coconut oil has been refined with chemicals and high temperature, and used as cooking oil and cosmetic materials. Recently, Asian and Pacific Coconut Community (APCC) advocated to use virgin coconut oil that is not treated with chemicals and high heat and is emphasizing usefulness and benefit of virgin coconut oil for health.

Materials and Methods

Probiotic studies

Immune system, especially Th1, Th2, Treg for *in vivo* probiotics of *L. plantarum* IS-10506 from dadih and LGG of Valio were analysed biochemically. In the factorial design study, male Balb/c mice subjects were randomized into nine experimental groups and were sensitized with OVA. Probiotics (LGG, LIS and placebo) and TLR inhibitors (PD98059, LY294002 and placebo) are independent variable. The expression of innate immune response (TLR2, TLR4, NF- κ B p65, NF- κ B p105/p50); the activation of adaptive immune response (IFN- γ , IL-2, IL-4, IL-5, TGF- β and IL-10, sera OVA-specific IgG2a, IgA, IgE, ileum OVA-specific IgA and total IgE); and the allergic reaction (histamine and symptoms score) are dependent or effect variable.

The TLR2, TLR4, NF- κ B p65, NF- κ B p105/p50, IFN- γ , IL-2, IL-4, IL-5, TGF- β and IL-10-positive cells were measured on histological slices from the small intestine of mice in all groups. Levels of sera OVA-specific IgA, IgE, IgG2a and ileum OVA-specific IgA were determined with an indirect ELISA. Measurement of total IgE antibodies in sera was done with Sandwich ELISA. Histamine levels were determined with a competitive ELISA. The study protocol was approved by the Animal Care and Use Ethical Committee of the Veterinary Medicine Faculty of Airlangga University.

After final OVA sensitization, the LGG and LIS groups did not experience the severe and fatal allergic reaction, however, most of mice in control group did experience. There were significant

modulation of the innate and adaptive immune response in the LGG and LIS groups compared with the control group, except IL-4 and total IgE.

Traditional Herbal Plants

Nutritional analyses and bioactive compounds were analysed chemically. Other analyses were conducted chemically, biochemically or microbiologically. Noni juice was assessed for its antimutagenic activity and antioxidation, Buah Merah was determined for its fatty acid profiles, and the chemopreventive agents was also studied *in vitro* with lung cancer cells. While EVCO was studied clinically for its effect on serum cholesterol. A clinical study in Japan, with Japanese and Indonesian volunteers with help of Indonesian Embassy in Japan was conducted. Five males and 10 females were enrolled in this study. They took 5 or 10 ml of EVCO, 3 times a day, for 12 consecutive weeks. They were allowed to take foods freely and recorded their body weight and other parameters on obesity. The blood examinations were conducted 3 times for their total cholesterol, HDL- and LDL-cholesterols and other parameters for adverse events.

Results and Discussion

Effect of probiotics in reducing allergy *in vivo*

In control group, the low level of *innate* immune response (TLR2, TLR4, NF- κ B p65, NF- κ B p105/p60) was in conformity with Th2-skewed ($T_{H1} < T_{reg} < T_{H2}$) and IgE-mediated mast cell degranulation which produce histamine and clinical symptoms.

LGG probiotics enhanced the *innate* immune response (TLR2, TLR 4, NF- κ B p65, NF- κ B p105/p60), formed the new equilibrium of Th2-Th1 cytokine profile ($T_{H1} = T_{reg} = T_{H2}$), and reduced the mast cell degranulation (decreasing the level of histamine and symptoms score).

LIS probiotics enhanced the *innate* immune response (TLR2, TLR4, NF- κ B p65, NF- κ B p105/p60), formed the Th1-skewed ($T_{H1} > T_{reg} > T_{H2}$), and reduced the mast cell degranulation (decreasing the level of histamine and symptoms

score).

The involvement of TLR 2 and 4 in the effect of the probiotic LGG and LIS on decreasing of allergic reaction was proved by the interaction effect between those probiotics and inhibitor TLR in reducing allergic reaction.

Effect of Noni on blood flow time

Compared with before-dose blood time, blood flow time at 1 hr after Noni juice ingestion caused 3.2 to 6.7 seconds shorter, average 4.7 seconds, in blood flow time of all the persons in a single study. In a repeated study, blood flow time of all the persons was shortened, in average of 1.6 seconds. Blood flow times between the single and repeated studies were remarkably shorten by 3.9 to 7.1 seconds. The average shorter time was 5.1 seconds in about 3 months.

Effect of Noni on ADP-stimulation

ADP, adenosine diphosphate is stored in the platelets, and released by various stimulations. ADP can stimulate platelets resulting in aggregation, blood clotting or interrupting blood circulation.

Blood from the single and repeated dose studies was treated with ADP at 0.1 μ M concentration and blood flow time was measured.

In the single and repeated studies, the addition of ADP to blood caused blood flow times longer compared with those without ADP treatment. However, 1 hr after Noni juice intake, ADP-treated blood flow time was shortened. In the repeated study, blood flows of all the persons, except one man revealed inhibitory effects of Noni juice against flow time elongation of ADP-treated bloods.

Effects of Noni juice and scopoletin on ADP-stimulation

Blood was collected from M, 55 years person with heavy smoking, and pre-treated with PP grade Noni juice at 0.01% or scopoletin at 0.1 and 1 μ M. After addition of ADP, blood flow times for 20 and 100 μ L were measured with FC-FAN.

PP grade Noni juice pretreated to blood made

blood flow time shorter in comparison with ADP treatment only. Scopoletin too showed the same results in a dose-dependent manner.

Effect of scopoletin on PRP aggregation

In addition, action of scopoletin was examined by means of platelet-coagulometer. Platelet rich plasma (PRP) with 300,000/ μ L was prepared from M, 55 years person. Scopoletin ranged from 0.1-10 μ M was pretreated with PRP at 37 °C for 1 minute. Thereafter, 5 or 10 μ M of ADP was added and permeability indicating grades of coagulation was monitored for 5 minutes.

In the common experimental condition of 5 μ M ADP platelet aggregation, scopoletin inhibited ADP-stimulated platelet aggregation at range of from 0.1 to 5 μ M.

Indonesian Noni fruit juice inhibits damage of DNA and has anti-mutagenic action (Surono *et al.*, 2002; Nishigaki and Wasposito, 2003). These actions may be related to strong scavenging activity of reactive oxygen species (Nishigaki and Wasposito, 2003) which are believed to contribute to nuclear damages. Medium chain fatty acids cause bad odor, but their contents are comparable with mother's milk (Wakai, *et al.*, 2005) and may contribute to protect from bacterial and viral infections.

An additional feature of Noni fruits is to contain relatively higher level of scopoletin that is a coumarin derivative. We routinely determine the scopoletin contents in Noni juice from different origins and products. We found that scopoletin contents varied from areas of origin and from products (Ito *et al.*, 2005). So far, Indonesian Noni juice from PT Morindo International is highest at 61 g/mL. There are several reports that scopoletin is very active substance with many functions.

Nutritional Feature of Buah Merah

Table 1 shows typical nutritional and hygienic analyses of Buah Merah extract oil that was produced by the procedures developed at the CABI, Bogor and supplied from PT Rediss Papua.

The analyses were conducted at Japanese government-authorized laboratories in Japan.

About 94% in the extract is oil. The remaining around 5% is carbohydrates and no protein is detected. As carotenoids, α - and β -carotene, and β -cryptoxanthin are found. No lutein, zeaxanthin and lycopene are detected in the Buah Merah extract oil.

β -Cryptoxanthin is found in green-yellowish vegetables and fruits and now most notable carotenoid from the reason of possible chemopreventive agent for lung cancers. M&K Laboratories Inc., Japan is the first to discover its presence in Buah Merah.

Even using HPLC, it is very difficult to separate α - and β -cryptoxanthin each other from foods. This value of β -cryptoxanthin contains partly α -cryptoxanthin. Therefore, we are now developing a new method to separate and measure actual β - and α -cryptoxanthins contents from Buah Merah in Japan.

One of features of Buah Merah is rich in Vitamin E. Considering that almost all of food supplements of Vitamin E use synthetic chemical, but not natural origin, Buah Merah is a good source of natural Vitamin E.

There are no microbiological problems and no heavy metals are detected in Buah Merah oil.

Japanese Quarantine Office requires to be absent of artificial red dyes and anti-oxidative agents. Buah Merah extract oil from Indonesia meets Japanese regulation.

Lung Cancers and Buah Merah

Buah Merah extract oil was applied to human cancer cell line, A549, *in vitro* and demonstrated that 500 mg of Buah Merah oil/mL prohibited the growth of A549 cells. The concentration of beta-cryptoxanthin in Buah Merah oil was equivalent to 0.015 μ g/mL. From this study, it is suggested that very small quantity of β -cryptoxanthin may cause the growth inhibition of lung cancer cells.

We have found out that red fruits, Buah Merah (*Pandanus conideus*) naturally grown in Papua areas is a good source of carotenoids including α - and β -carotene, and β -cryptoxanthin. Of which all can be converted to essential Vitamin A in the body. Beta-cryptoxanthin level in Buah Merah is relatively high, more than 1 mg per 100 g extract oil. Buah Merah oil prohibits the growth of lung cancer cell, A549.

Conclusions

LGG and LIS probiotics are involving TLR2 and 4 in reducing allergic reaction through enhancement of the T_{H1} and T_{reg} immune responses and forming a new T_{H1} - T_{H2} equilibrium. LGG and LIS probiotics are suggested to be applied in human subject for preventing fatal and chronic allergic reaction.

Buah Merah must be a novel chemopreventive food for lung cancers. Main ingredient in Buah

Table 1. Nutritional profile of Buah Merah extract oil

Item	Value per 100 g	Item	Value per 100 g
Moisture	0.7 g	α -Carotene	130 μ g
Energy	868 kcal	β -Carotene	1,980 μ g
Protein	0	β -Cryptoxanthin	1,460 μ g
Lipid	94.2 g	Lutein	Not detected
Carbohydrate	5.1 g	Zeaxanthin	Not detected
Ash	0	Lycopene	Not detected
Sodium	3 mg	Vitamin E (α -Tocopherol)	21.2 mg

From the results of microbiology, heavy metals and additives, Buah Merah extract oil can be imported to use for food in Japan.

Merah is likely to be β -cryptoxanthin, while the mode of action remains further examined.

Body Mass Index (BMI), blood total cholesterol, and LDL/HDL ratio were not affected in males. 8 out of 10 females were improved in total cholesterol and LDL/HDL ratio, one had no change, and one had slightly unwanted changes.

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