**Pediococcus pentosaceus** as probiotic with cholesterol-lowering ability

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**ABSTRACT**

**Aims:** Hypercholesterolemia which is an elevated blood cholesterol level that considered as a major risk factor for cardiovascular disease, which is the leading cause of death in many countries. Therefore, lowering the cholesterol level is important to prevent the disease. Lactic acid bacteria (LAB) group are often used as probiotics for their health-promotion which include cholesterol-lowering effect. The purpose of this study was to evaluate the potency of *Pediococcus pentosaceus* as probiotic that could reduce cholesterol.

**Methodology and results:** All *P. pentosaceus* strains were able to survive in acid conditions and in the presence of 0.3% bile salts. These strains had antimicrobial activity against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Salmonella typhimurium* ATCC 14028. The LAB were also sensitive to chloramphenicol and showed autoaggregation and coaggregation ability. *Pediococcus pentosaceus* E5, E7, and E8 were able to remove cholesterol with the highest activity showed by *P. pentosaceus* E7 (49.00 ± 2.83%). Dead cells and resting cells of *P. pentosaceus* E5, E7, and E8 (6-22%) also able to reduce the cholesterol but not as effectively as growing cells. Cholesterol lowering is often associated with bile salt hydrolase (BSH) enzyme activity, however none of the isolates were found BSH positive in this study.

**Conclusion, significance and impact of study:** The present study suggests that *P. pentosaceus* E7 has beneficial probiotic properties which can be exploited for probiotic product with cholesterol-lowering effect.

**Keywords:** *Pediococcus pentosaceus*, probiotics, cholesterol-lowering

**INTRODUCTION**

Cholesterol is an important structural component of animal cell membrane. However, elevated cholesterol levels is a major risk factor for coronary heart disease (Anila et al., 2016). Cardiovascular disease is the leading cause of death for million people worldwide. The risk of developing cardiovascular disease is reduced by 2-3% when cholesterol level is reduced by 1% (Alba et al., 2018). Although drugs such as statins, effectively decrease cholesterol level but its side effects are commonly reported (Miremadi et al., 2014). Myalgia is the most common side effect from statin use with rates 1-10% of patients (Ramkumar et al., 2016).

It has been proposed that consumption of probiotics products able to lower the serum cholesterol level. Probiotics are defined as viable microorganisms that exerts various beneficial effects to the host when ingested in an appropriate concentration. Therefore, the interest in using probiotics to reduce cholesterol level has increased from the last few decades (Anila et al., 2016). Probiotic bacteria mainly belonging to lactic acid bacteria (LAB) included *Lactobacillus* and *Pediococcus*. LAB are often used as probiotics for their health-promotion such as lowering-cholesterol, a series of *in vitro* tests must be applied as the selection criteria to identify potential probiotics.

The results from previous studies showed *Pediococcus pentosaceus* VJ56 from Idly batter was able...
to reduce 63% cholesterol (Vidhyasagar and Jeevaratnam, 2013); Lactobacillus plantarum EM from kimchi was able to reduce 80.69% cholesterol (Choi and Chang., 2015); P. pentosaceus from fermented finger millet was able to reduce 34% cholesterol (Damodharan et al., 2015) and P. pentosaceus from breast milk was able to reduce the cholesterol for 15.76% (Nuraida et al., 2011). Several possible mechanisms for cholesterol removal by probiotics included cholesterol assimilation, adhesion of cholesterol to the cell membrane, conversion of cholesterol to coprostanol, and enzymatic deconjugation of bile salts (Tok and Aslim, 2010).

Hamida et al. (2015) had selected three strains of P. pentosaceus from spontaneous fermented corn as a probiotic candidate for chicken. However, this isolate has not been evaluated for its specific functional benefit and probiotic properties for human. Therefore, this study aimed to evaluate the potential of P. pentosaceus which isolated from spontaneous fermented corn as probiotics with cholesterol-lowering ability.

MATERIALS AND METHODS

Sources of microbes

Lactic acid bacteria (LAB) of Pediococcus pentosaceus E5, P. pentosaceus E7, P. pentosaceus E8 were isolated previously from spontaneous fermented corn and examined previously by Hamida et al. (2015). Pathogenic bacteria (Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Salmonella typhimurium ATCC 14028) were obtained from Animal Biotechnology and Biomedical Laboratory, RCBIO, IPB University, Indonesia. While, Lactobacillus rhamnosus R23 which used as reference strain for cholesterol lowering was obtained from SEAFAST Center Laboratory, IPB University, Indonesia. All the LAB were grown in the Mann Rogosa Sharpe (MRS) broth media (Merck), while pathogenic bacteria were grown in the nutrient broth (NB) media.

Acid tolerance test

A low pH tolerance test was carried out based on Manini et al. (2016). MRSB was adjusted to pH 2 using 37% hydrochloric acid (HCl) and inoculated with 1% (10^−10^a CFU/mL) LAB culture for 2 h and incubated at 37 °C. The total amount of LAB was determined by the plate count method on MRS agar and incubated for 48 h at 37 °C. LAB survival rate was determined by the following equation:

\[
\text{Survival} \% = \frac{\log \text{CFU of viable cells survived}}{\log \text{CFU of initial viable cells inoculated}} \times 100
\]

Bile salt tolerance test

Bile salt tolerance was carried out based on Tokatli et al. (2015). MRS broth containing 0.3% (w/v) bile salt (HIMEDIA) was inoculated with 1% (10^-7^-10^a CFU/mL) LAB culture and incubated for 6 h at 37 °C. Then, the total amount of LAB was determined by the plate count method on MRS agar after incubated for 48 h at 37 °C.

Antimicrobial activity assays

Antimicrobial activity was evaluated based on Shukla and Goyal (2014) and Wang et al. (2016). Briefly, the tested pathogenic bacteria (E. coli ATCC 25922, S. aureus ATCC 25923 and S. typhimurium ATCC 14028) were grown in NB medium and LAB was grown in MRS broth at 37 °C for 24 h. Then, the LAB culture was removed by centrifugation at 6000 × g, 4 °C for 10 min. The Petri dishes containing nutrient agar were prepared, previously inoculated with 200 μL the tested pathogenic bacteria. After the agar plates had been surface dried, sterilized paper disks were placed aseptically on the agar surface, then 20 μL CFS (cell-free supernatant) of LAB were applied to each disk. In another set of experiment, CFS was neutralized to pH 6.5 by 2 N sodium hydroxide (NaOH), then 20 μL of CFS were applied to each disk. The plates were incubated at 37 °C for 24 h and in the zone of inhibition (mm) was measured and data interpretation referred to Zommitti et al. (2018).

Autoaggregation and coaggregation ability test

Autoaggregation and coaggregation ability tests were performed based on Seddik et al. (2017) and Ladha and Jeevaratnam (2018). LAB strains were grown for 24 h at 37 °C in MRS broth. After centrifugation (8000 × g, 10 min), the cells pellet were washed twice with 0.01 M, pH 7.2 phosphate buffer saline (PBS) and resuspended in PBS. Then, cell suspensions were mixed by vortexing and autoaggregation was determined after incubation at 37 °C for 2 h and 4 h, respectively. Subsequently, an aliquot of these suspensions was carefully removed from the upper layer of the suspension and its absorbance was read at 600 nm using spectrophotometer. The percentage of autoaggregation was calculated using the formula:

\[
\text{Autoaggregation} \% = 1 - \left( \frac{A_t}{A_0} \right) \times 100
\]

where \(A_t\) represents absorbance value at time \(t = 2\) h or 4 h and \(A_0\) represents absorbance value at \(t = 0\) h.

Culture preparation for coaggregation test was carried out as previously described, 2 mL of LAB culture and 2 mL of pathogenic culture were mixed, then incubated at 37 °C for 2 h and 4 h. Subsequently, an aliquot of these suspensions was carefully removed, and its absorbance was read at 600 nm using spectrophotometer. The percentage of coaggregation was calculated using the formula:

\[
\text{Coaggregation} \% = \left( \frac{Ax + Ay}{2} - A(x + y) \right) \left( \frac{Ax + Ay}{2} \right) \times 100
\]
where $Ax$ represents absorbance value of LAB culture; $Ay$ represents absorbance value of pathogenic culture; $A (x + y)$ represents absorbance value of the mixture suspension.

Antibiotic susceptibility

The antibiotic susceptibility of P. pentosaceus E5, E7, and E8 were examined according to Lee et al. (2016) and Ilavenil et al. (2016). The strains were tested for their susceptibilities against streptomycin (25 μg), kanamycin (30 μg), chloramphenicol (30 μg) and ampicillin (25 μg). The Petri dishes containing MRS agar were prepared, previously inoculated with 200 μL ($10^7$-10$^8$ CFU/mL) LAB culture. After the agar plates had been surface dried, sterilized paper disks were placed aseptically on the agar surface, then 20 μL of antibiotics solution which had been filter sterilized with 0.22 μm membrane filter were applied to each disk. After 24 h of incubation at 37 °C, the diameters of inhibition zone (mm) were measured and data interpretation referred to Shukla and Goyal (2014).

Cholesterol removal by growing cells

The ability of LAB to reduce cholesterol was analyzed according to Shehata et al. (2016). In short, 1% ($10^7$-10$^8$ CFU/mL) of LAB cells grown overnight were inoculated into MRS broth supplemented with 0.3% (w/v) bile salt and 100 μg/mL of water-soluble cholesterol (PEG6000, Sigma-Aldrich) which had been filter sterilized with 0.45 μm membrane filter and incubated for 24 h at 37 °C. Following incubation, the remaining cholesterol concentration of broth was determined. The cells were harvested by centrifugation (7000 × g, 15 min) and 1 mL of CFS was added to 1 mL of 33% (w/v) potassium hydroxide (KOH) and 2 mL of absolute ethanol. The mixture was shaken well for 1 min and then heated for 10 min at 60 °C water bath. After cooling, 3 mL of hexane and 2 mL of distillate water were added then mixed. The mixture was incubated at room temperature (28 °C) for 10 min for phase separation, after which 1 mL of a separated hexane layer was transferred into clean tube which was then evaporated using a nitrogen stream. Subsequently, 2 mL of o-phthalaldehyde (Sigma-Aldrich) was added, mixed, and stand at room temperature for 10 min. Following the addition of 2 mL of concentrated sulfuric acid and incubation for 10 min, the absorbance at 550 nm was read using a spectrophotometer. The cholesterol removal percentage was obtained by comparing the absorbance value with the control (cholesterol standard) as formula below (Ooi and Liong, 2010).

\[
\text{Cholesterol removal (\%)} = \left( \frac{\text{Absorbance value of remaining cholesterol in cultures}}{\text{Absorbance value of cholesterol in cultures}} \right) \times 100
\]

Cholesterol removal by dead and resting cells

Cholesterol removal by dead and resting cells was determined according to Choi and Chang (2015). LAB was grown for 24 h in MRS broth, after centrifugation (7000 × g, 15 min) cell pellet was washed using sterile-distilled water. For cholesterol removal by resting cells, the cell pellet was suspended in 0.05 M PBS (pH 7.2) containing 0.3% (w/v) bile salt and 100 μg/mL of water-soluble cholesterol. For dead cell assay, the cell pellet was suspended in saline and heat-killed at 121 °C for 15 min. The dead cells were harvested, after which the pellet was suspended in MRS containing 0.3% (w/v) bile salt and 100 μg/mL of water-soluble cholesterol. All strains were incubated at 37 °C for 24 h, then remaining cholesterol concentration of broth was determined as previously described.

Qualitative determination of bile salts hydrolyase (BSH) activity

BSH activity was determined based on Choi and Chang (2015). Active LAB culture (10 μL) was spotted on MRS agar containing 0.5% (w/v) taurodeoxycholic acid sodium salt (TDCA) (Sigma, Aldrich) and 0.037% (w/v) calcium chloride (CaCl2). The agar plates were incubated at 37 °C for 48 h. The precipitation zone surrounding colonies indicated the presence bile salt hydrolyase activity of bacteria.

RESULTS AND DISCUSSION

Acid tolerance test

It is well known that probiotic bacteria should be capable of surviving passage through the gastrointestinal tract based on acid tolerance to human gastric juice in the small intestine (Choi and Chang, 2015). In this study, P. pentosaceus E5, P. pentosaceus E7, and P. pentosaceus E8 were shown to have more than 85% of survival rate in MRS broth with pH 2 for 2 h (Table 1). The results are in accordance with P. pentosaceus LJ1R1, P. pentosaceus LJ1R5, and P. pentosaceus LJ1R9 which have a survival rate of more than 75% (Ladha and Jeevaratnam, 2018), but in contrast to Osmanagaoglu et al. (2010), who reported P. pentosaceus OZF was not resistant to acidic condition. The decrease of the cells number can be associated with the impact of the production of H+ ions by acid in the cell wall and metabolism of the isolates, however, acid tolerance was strain dependent (Ayyash et al., 2018).

Table 1: Survival rate (%) of LAB in MRS broth pH 2 after incubated for 2 h at 37 °C.

| LAB         | Survival rate (%) |
|-------------|-------------------|
| P. pentosaceus E5 | 85.21 ± 0.82     |
| P. pentosaceus E7 | 88.68 ± 1.58     |
| P. pentosaceus E8 | 90.41 ± 1.18     |

*Means of triplicate ± standard deviation is shown.
Bile salt tolerance test

Tolerance to bile salt was a prerequisite for microbial colonization and metabolic activity in small intestine of the host (Shehata et al., 2016). *Pediococcus pentosaceus* E5, E7 and E8 strains exhibited good bile salt tolerance with increased in cells number for about 1 log CFU/mL from initial cell number of 7 log CFU/mL to 8 log CFU/mL (Table 2). Ladha and Jeevaratnam (2015) reported that *P. pentosaceus* LJR5 and *P. acidilactici* R01, R02 had more than 100% survival after 2 h incubated in MRS broth with bile salt, in contrast to *P. ethanolidurans* (0%) (Tokatli et al., 2015). Bile salt can act as antimicrobial agent via disintegration of bacterial membranes. Resistance to bile salt can involves several mechanisms including bile salt efflux, hydrolysis of bile salt, and changes in membrane and cell wall components (Ruiz et al., 2013).

Table 2: Increased in cell number (with initial cell number 7 log CFU/mL) of LAB in MRS broth with 0.3% bile salt after incubated for 6 h at 37 °C.

| LAB         | Increased in cell number (Log CFU/mL)* |
|-------------|----------------------------------------|
| *P. pentosaceus* E5 | 1.05 ± 0.03                            |
| *P. pentosaceus* E7 | 1.31 ± 0.03                            |
| *P. pentosaceus* E8 | 1.04 ± 0.05                            |

*Means of triplicate ± standard deviation is shown.

Table 3: Antimicrobial activity of LAB against pathogen on MRS agar after incubated for 24 h at 37 °C.

| LAB         | Without neutralization CFS (pH ± 4) | Neutralized CFS (pH 6.5) |
|-------------|------------------------------------|-------------------------|
|             | S. aureus | S. typhimurium | E. coli | S. aureus | S. typhimurium | E. coli |
| *P. pentosaceus* E5 | ++         | +            | ++       | +          | +            | +       |
| *P. pentosaceus* E7 | ++         | ++           | ++       | +          | +            | +       |
| *P. pentosaceus* E8 | +++        | +            | +        | +          | +            | +       |

Interpretation of inhibition zone diameter based on Zommiti et al. (2018): (-): no inhibition zone; (+): <3 mm; (;++): 3-6 mm; (+++): > 6 mm.

Antimicrobial activity assays

Table 3 showed that CFS without neutralization was able to inhibit all pathogenic bacteria with inhibition zone ranging from 2.06 ± 0.44 to 5.00 ± 0.35 mm. Damodharan et al. (2015) reported that *P. pentosaceus* KID7 was able to inhibit Gram positive and Gram negative pathogens with the highest activity against *S. aureus* while Noohi et al. (2016) found that *P. pentosaceus* P3 and P6 had the highest inhibitory activity against *S. typhimurium*. Neutralized CFS of *P. pentosaceus* E7 was unable to inhibit *E. coli* and *S. aureus* (Table 3), and this result was contradicting with *L. bulgaricus* (Georgieva et al., 2015). Overall, CFS without neutralization and neutralized CFS had different antimicrobial activity. CFS was neutralized in order to eliminate putative effect of produced organic acid. The observed inhibition for some strains after elimination of the putative effects of lactic acid raised the question for possible production of other inhibitor substances, such as hydrogen peroxide, bacteriocin and bacteriocin-like substances (Georgieva et al., 2015). Hamida et al. (2015) showed that neutralized CFS of *P. pentosaceus* E5, E7, and E8 lose their inhibitory activity against *Enterococcus casseliflavus* after added with proteinase-K, it seems that the antimicrobial compounds are protein such as bacteriocin.

Autoaggregation and coaggregation ability

The highest autoaggregation ability (54.85 ± 1.03 %) was found in *P. pentosaceus* E7 after 4 h and *P. pentosaceus* E8 (26.97 ± 0.15 %) after 2 h of incubation in PBS (Table 4). The autoaggregation ability of *P. pentosaceus* E7 was better than *P. pentosaceus* A24 (40.4%) reported by Lee et al. (2014), but lower than *P. pentosaceus* LJR1 (81%) (Ladha and Jeevaratnam, 2018) after 4 h of incubation. According to Wang et al. (2010), strong autoaggregation ability must be higher than 40% while weak autoaggregation is defined for 10% or less. In contrast, Rahman et al. (2008) claimed that strong autoaggregation ability is about 70%. Thus, it is difficult to categorize the standard autoaggregation value for *Pediococcus sp.* (Zommiti et al., 2018).

On the other hand, the strongest coaggregation ability at 2 h of incubation was found in *P. pentosaceus* E7 with *S. typhimurium*, which was 29.73% (Figure 1). The coaggregation ability of *P. pentosaceus* E7 was better than *P. pentosaceus* OZF with *S. typhimurium* (6.26%) after incubation of 5 h (Osmanagaoolu et al., 2010). *P. pentosaceus* E5 with *E. coli* showed the strongest

Table 4: Autoaggregation ability of LAB in PBS after incubated for 2 and 4 h at 37 °C.

| LAB         | Autoaggregation (%)* |
|-------------|----------------------|
|             | 2 h                  | 4 h                  |
| *P. pentosaceus* E5 | 15.46 ± 3.31 | 26.58 ± 2.48 |
| *P. pentosaceus* E7 | 13.03 ± 3.98 | 54.85 ± 1.03 |
| *P. pentosaceus* E8 | 26.97 ± 0.15 | 40.88 ± 2.04 |

*Means of triplicate ± standard deviation is shown.
coaggregation ability (71.01%) after incubated for 4 h as similar as reported by Osmanagaoglu et al. (2010). The coaggregation ability is important in inhibiting the growth of pathogens (Ladha and Jeevaratnam, 2018). It has been reported protein, glycoprotein, teichoic acid, and lipoteichoic acid of bacteria cell wall play important role in co- and auto-aggregation of pathogens and LAB (Tuo et al., 2013).

Figure 1: Coaggregation ability of LAB with pathogens in PBS after incubated for 2 and 4 h at 37 °C. SA: S. aureus; ST: S. typhimurium; EC: E. coli; E5: P. pentosaceus E5; E7: P. pentosaceus E7; E8: P. pentosaceus E8.

Antibiotics susceptibility test

All P. pentosaceus were sensitive to chloramphenicol, but resistant to ampicillin, streptomycin, and kanamycin (Table 5). The result of this study was in accordance with P. pentosaceus VJ35 (Vidhyasagar and Jeevaratnam, 2013) and P. pentosaceus KID7 (Damodharan et al., 2015) with slight variations. Pediococcus sp. intrinsically is resistant to various groups of antibiotics, including β-lactams, cephalosporins, aminoglycosides, glycopeptides, streptomycin, kanamycin, tetracycline, and sulfatrimethoprim (Zommiti et al., 2018). When resistance is intrinsic which acquired as the results of chromosomal mutation, probiotic bacteria do not constitute a safety concern because the antibiotic resistance is only passed onto the next generation via the organism’s genetic material (Pereira et al., 2018). Antibiotic resistance of probiotics is considered a safety issue when the risk of gene transfers is present (Gueimonde et al., 2013). The transmissible resistance genes are attributed to plasmid containing resistance genes, which may be transferred to intestinal pathogens, and may give rise to negative consequences when humans receive antibiotic therapy. In contrast to intrinsic resistance, it might be considered advantageous, because probiotics with the property of intrinsic resistance could maintain the natural poise of intestinal microbiota (Bacha et al., 2010). Therefore, LAB with intrinsic resistance to antibiotics is generally can be used as a probiotic microorganism (EFSA, 2012).

Table 5: Susceptibility of LAB to antibiotics on MRS agar after incubated for 24 h at 37 °C.

| LAB          | Chloramphenicol | Ampicillin | Streptomycin | Kanamycin |
|--------------|-----------------|------------|--------------|-----------|
| P. pentosaceus E5  | S               | R          | R            | R         |
| P. pentosaceus E7  | S               | R          | R            | R         |
| P. pentosaceus E8  | S               | R          | R            | R         |

*Interpretation of inhibition zone diameter was based on Shukla and Goyal (2014): Resistant (R): 0-2 mm; Moderate (M): 3-6 mm; Sensitive(S): 7-13 mm.

Cholesterol removal ability by growing, resting, and dead cells

All the tested strains exhibited higher ability to reduce cholesterol than L. rhamnosus R23, the reference strains (Figure 2). Pediococcus pentosaceus E7 had the highest cholesterol removal ability in growing cells (49%), dead cells (15%), and resting cells (22.2%). It shows that the cholesterol removal ability of P. pentosaceus E7 growing cells was lower than P. pentosaceus VJ56 (63%) (Vidhyasagar and Jeevaratnam, 2013) and P. pentosaceus LAB6 (58%) (Syakila et al., 2018) but better
than *P. pentosaceus* KACC 12311 (28%) (Damodharan et al., 2015). It has been reported that cholesterol removal by probiotics were strain dependent (Syakila et al., 2018). Growing cells were able to reduce cholesterol from media via incorporation and conversion of cholesterol to coprostanol (Lye et al., 2010).

Cholesterol removal by dead cells and resting cells has been reported previously (Vidhyasagar and Jeevaratnam, 2013; Iranmanesh et al., 2014). The ability of dead cells and resting cells to reduce cholesterol was due to binding of cholesterol to the cell membrane (Anila et al., 2016). Iranmanesh et al. (2014) reported that the decrease in cholesterol by dead cells increased with increasing number of cells. Cholesterol binding to LAB varies among strains and species and hypothesized that these differences in binding abilities can be attributed to chemical and structural properties of cell wall peptidoglycans containing amino acids capable of binding to cholesterol (Choi and Chang, 2015). Syakila et al. (2018) showed that cholesterol strongly adhered to cell membranes of *P. pentosaceus* LAB12 as observed by fluorescently tagged cholesterol under a confocal microscope.

**Qualitative determination of BSH activity**

In this study, none of the strains were found to be BSH positive and the results was similar to 12 isolates from cheese (Sedlackova et al., 2015) and 5 isolates from food fermentation (Anila et al., 2016). LAB from the gastrointestinal tract are more likely to be BSH positive, as compared to those without exposures to bile salts (Sedlackova et al., 2015). Miremadi et al. (2014) found that 14 isolates of *Lactobacilli* and *Bifidobacteria* from human possessed BSH activity, while *Pediococcus* from fermented vegetables were only 10% to be BSH positive (Abriouel et al., 2012). Other findings suggest that BSH activity is influenced by specific substrates, bile salt formed from glycine is more easily hydrolyzed than bile salt from taurine (Hu et al., 2018). A total of 243 isolates tested by Ru et al. (2018) showed higher BSH activity on the GDCA (glycodeoxycholic acid) substrate (68%) compared to TDCA (taurodeoxycholic acid) (23%). Tokatli et al. (2015) stated that the cholesterol-lowering effect of the strains tested is not always related to the ability of deconjugated bile salt such as *P. pentosaceus* VJ31 and VJ35, those which are BSH negative, but able to reduce cholesterol by more than 67% (Vidhyasagar and Jeevaratnam, 2013). Other mechanisms for cholesterol lowering was conversion of cholesterol to coprostanol (Lye et al., 2010).

**CONCLUSION**

In this study, three strains of *P. pentosaceus* have a good probiotic properties (acid tolerance, bile salt tolerance, antimicrobial activity, autoaggregation and coaggregation ability, and antibiotics susceptibility) and cholesterol-lowering ability. Although they are BSH negative, *P. pentosaceus* E7 showed the highest cholesterol-lowering ability. This strains could be potentially used in the development of probiotic product with their functional properties in cholesterol-lowering effect. Based on the finding from this study, further in vitro studies are needed to determine the mechanism involved in the reduction of cholesterol and in vivo study is necessary to prove the hypercholesterolemic effect.

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