

## Antimicrobial activity (*in vitro*) of polysaccharide gel from durian fruit-hulls

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### Abstract

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*In vitro* activity study of polysaccharide gel (PG) extracted from fruit-hulls of durian (*Durio zibethinus* L.) was performed to evaluate the activities against microorganisms. Inhibitory activity of PG against two bacterial strains, *Staphylococcus aureus* and *Escherichia coli*, and two yeast strains, *Candida albicans* and *Saccharomyces cerevisiae*, were determined by microbiological assay techniques using a simple agar diffusion and broth dilution method. PG at the concentration 0.32% in distilled water showed inhibition zone on TSA medium against *S. aureus*, and MIC of PG in TSB medium against *S. aureus* was 0.64 mg/ml. However, the lowest concentration of PG in distilled water at 1.25% and 2.50% produced inhibitory activity on MNG agar medium against *S. aureus* and *E. coli*, respectively, and an inhibition zone with sharp and clear delineated zone margins was obtained. Inhibitory activity of PG at the lowest concentration of 1% in peptone broth medium against *E. coli* and *S. aureus* was demonstrated: the colony count at 24 hours declined to zero and to 15%, respectively. However, both strains of test bacteria in NSS were inhibited in the presence of 0.1% PG: the colony count at 24 hours declined to zero. PG showed no inhibitory activity against two strains of test yeast in this study.

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**Key words :** *Durio zibethinus*, durian-rind extracts, durian polysaccharides, anti-microbial, bactericide

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broth) contained neopeptone 10.0 g per liter. NSS contained 9.0 g sodium chloride per liter.

### Polysaccharide gel

The polysaccharide isolated from fruit-hulls of durian was partially purified and its properties were previously described (Pongsamart and Panmaung, 1998). Solution of polysaccharide was freshly prepared from PG powder to make a series of two-fold dilutions of various concentrations of polysaccharide gel in distilled water before being added to the agar and broth media.

### Microorganisms

Two bacterial strains, *Staphylococcus aureus* ATCC 6538P and *Escherichia coli* ATCC 25922 were used. The bacterial colonies from MNG agar were suspended in sterile 0.9% NaCl, diluted in saline and the turbidity was adjusted equivalent to a 0.5 McFarland Standard before use.

Two yeast strains, *Saccharomyces cerevisiae* ATCC 9763 and *Candida albicans* ATCC 10230, were grown in Sabouraud dextrose agar. The colonies from this agar were suspended in normal saline and the turbidity was adjusted by the same method as described for the preparation of bacterial suspension.

### *In vitro* antimicrobial activities of polysaccharide gel

**Agar diffusion test** (Brock *et al.*, 1994; Lorian, 1991)

Agar diffusion testing was performed as follows: serial two-fold dilutions of various concentrations of polysaccharide gel (PG), 10, 5, 2.5, 1.25, 0.625 and 0.32%, in distilled water were freshly prepared.

Media containing mineral, nitrogen and glucose (MNG), and the enriched media TSA with 1% bacterial suspension was seeded over the solidified base layer of the same medium in petri dishes. Sterile stainless cups were placed over the surface of seeded media. The various concentrations of PG were filled into the cups (300 µl/cup) (the procedures were performed in triplicate for each dilution) and the plates were allowed for pre-

diffusion by leaving in room temperature for 1 hour. The plates were incubated at 37°C for 24 hours. The diameter of inhibition zones were measured after incubation. The distilled water and sterile normal saline filled in the cups were used as control.

The yeast suspensions (*S. cerevisiae* and *C. albicans*) were prepared by the same procedure as that for bacterial suspensions by using Sabouraud dextrose agar as the test culture media.

**Broth dilution test** (USP XXIII; Brock *et al.*, 1994)

Macro-dilution testing was carried out using media containing 4.5 ml of MN or peptone broth with various dilutions of PG (1, 0.5, 0.1%) and 0.5 ml of microorganism suspension for bacterial and yeast growth, respectively. Media without PG were used as performed as a culture control. The inoculated media with PG were incubated at 37°C for 7 days. MIC of PG testing was performed using TSB media with various dilutions of PG (2.5, 1.25 and 0.625%), the inoculated media were incubated at 37°C for 24 hours. The cultures were counted by drop plate method every day including the original concentration of microorganism suspension at day 0. NSS and MN media containing various dilutions of PG were performed by the same procedure to determine bacterial survival pattern in comparison to MN and MN with 0.1% glucose media.

### Results and Discussion

The *in vitro* activity of polysaccharide gel (PG) extracted from durian fruit-hulls was determined against 2 strains of bacteria and yeast (Table 1). Of the 2 bacterial strains, *S. aureus* represented a gram-positive bacteria that can cause skin infection and pus; and *E. coli* represented a gram-negative bacteria which can be found in gastrointestinal tract normal flora. Growth of tested bacteria were inhibited by PG. However, the two strains of yeasts were resistant to PG at concentration as high as 10%. The bacterial and yeast strains used in this study were found susceptible to amoxicillin and nystatin according to the pre-

**Table 1.** Activity of polysaccharide gel (PG) on growth of bacteria and yeasts in agar diffusion method. a, medium MNG agar; b, Sabouraud agar; NSS, normal saline solution; nz, no inhibition zone.

%PG	Diameter of inhibition zone, mm. (mean±SD)			
	<i>S. aureus</i> <sup>a</sup>	<i>E. coli</i> <sup>a</sup>	<i>C. albicans</i> <sup>b</sup>	<i>S. cerevisiae</i> <sup>b</sup>
10	16.53±1.07	19.00±1.31	nz	nz
5	14.13±0.92	16.17±0.47	nz	nz
2.5	10.50±0.50	12.97±0.55	nz	nz
1.25	9.97±0.15	nz	nz	nz
0.625	nz	nz	nz	nz
NSS	nz	nz	nz	nz

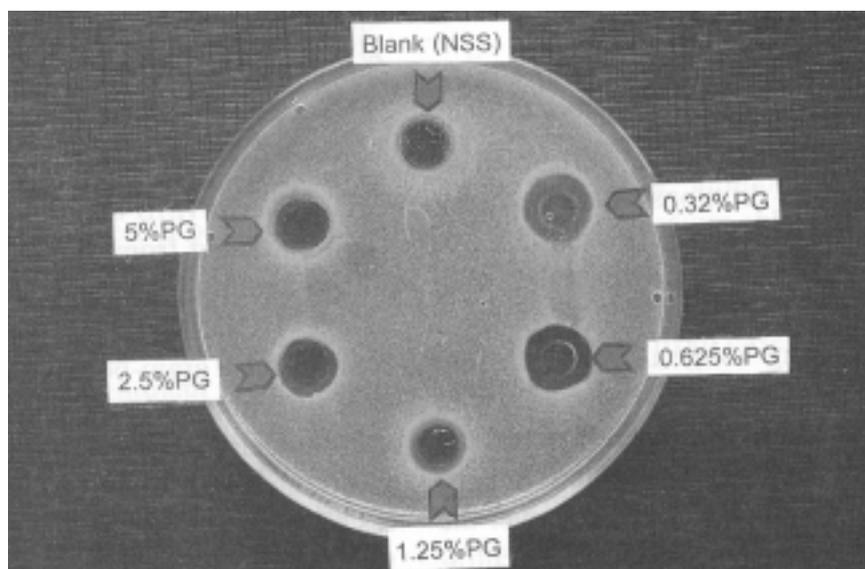
nz = no inhibition zone

vious study of Nantavutikul (1999).

#### Activity of PG by agar diffusion method

The inhibition zone was observed on media MNG agar with PG at the concentration 1.25% and 2.50% against *S. aureus* and *E. coli*, respectively. An increment of inhibition zone diameter was found with respect to increasing concentrations of PG as indicated in Table 1. The maximum

concentration of PG at 10% was performed according to the saturation solubility of PG. However, inhibition zone of 10.63±1.92, 11.08±1.95, 10.79±1.49, 11.65±1.43 and 11.88±2.20 mm in diameter was also observed on enriched TSA media with PG at the concentration of 0.32%, 0.625%, 1.25%, 2.5% and 5%, respectively, against *S. aureus* as shown in Figure 1. *E. coli* was not inhibited with PG on TSA media. The results sug-



**Figure 1.** Microbiological assay plate for *S. aureus* on medium TSA. Cups contain PG 0.32, 0.625, 1.25, 2.5 and 5%, and control cup filled with NSS.

gest that *S. aureus* was susceptible and being inhibited in the presence of PG from fruit-hulls of durian. Different effect of PG inhibition against *E. coli* was obtained depending on the media used. Two strains of tested yeasts were not inhibited by PG according to this assay, as shown in Table 1; no inhibition zone was obtained.

#### Broth dilution susceptibility test

PG at the concentration of 2.5% in TSB medium produced bactericidal activity against *S. aureus*, the colony count was declined to zero at 20 hours (Figure 2), The MBC value was 2.5 mg/ml. The MIC value of PG against *S. aureus* was found to be 0.64 mg/ml. The results suggest that *S. aureus* was susceptible to inhibition by polysaccharide gel extracted from durian fruit-hulls.

#### Survival test of microorganisms by broth dilution method

PG at the concentration of 1% produced inhibitory activity in MN broth against *E. coli* and *S. aureus*; the colony counts at 37°C, 24 hour incubation were declined to zero (Figure 3(A)) and to 15% count (Figure 4 (A)), respectively. Figure 3(A) and 4(A) show the growth pattern of both bacterial strains in MN broth containing 0.5% PG and 0.1% PG were similar to control growth in MN broth, and the same pattern of growth was

also observed (Figures 3(B) and 4(B)) in MN broth containing 0.1% glucose. Figures 3(B) and 4(B) illustrate that in the presence of PG at low concentrations of 0.1% in NSS killed *E. coli* and *S. aureus* within 1 day. Whereas, both strains of bacteria inoculated in NSS without PG survived until day 7 as shown in Figures 3(B) and 4(B). PG is composed of sugars including arabinose, rhamnose, glucose (Pongsamart and Panmaung, 1998) which might bind and interfere on the bacterial cell surface (Neu, et al., 1992) or Na<sup>+</sup> in NSS. Therefore, the normal bacterial function was inhibited in NSS.

Yeast strains, *C. albicans* and *S. cerevisiae*, were found resistant up to 1% PG in peptone broth, the colony counts were not lower than those in NSS without PG control, as illustrated in Figures 5 and 6, respectively.

#### Conclusion

The results demonstrate that bacterium *S. aureus* was susceptible and being inhibited by PG extracted from fruit-hull of durian. Inhibition zone of *S. aureus* on TSA medium was found at a concentration as low as 0.32% PG, and MIC of PG was 0.64 mg/ml against *S. aureus*. However, inhibition result in *E. coli* by PG according to agar diffusion test was found to depend on the medium used, the result of inhibition zone was obtained on

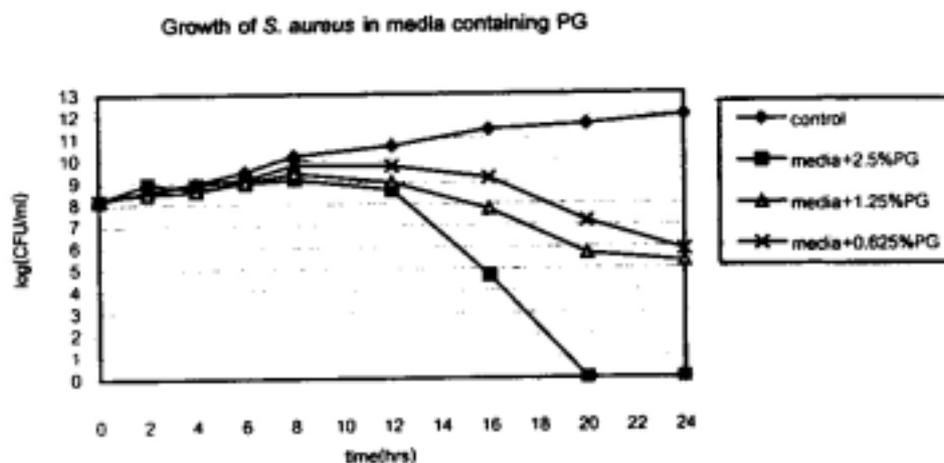


Figure 2. Time-kill analysis of polysaccharide gel (PG) in TSB medium for *S. aureus* by broth dilution method.

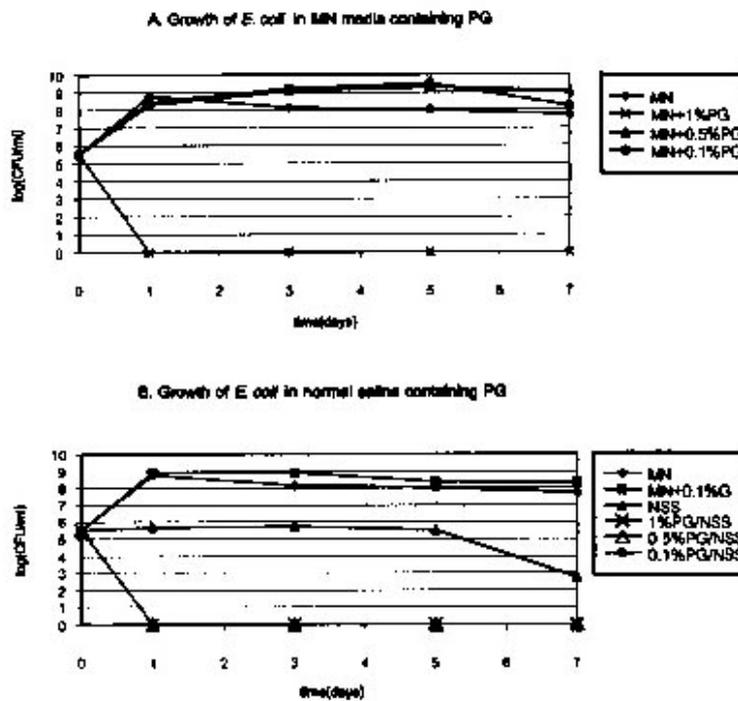


Figure 3. Activity of polysaccharide gel (PG) on growth of *E. coli* in broth dilution method.

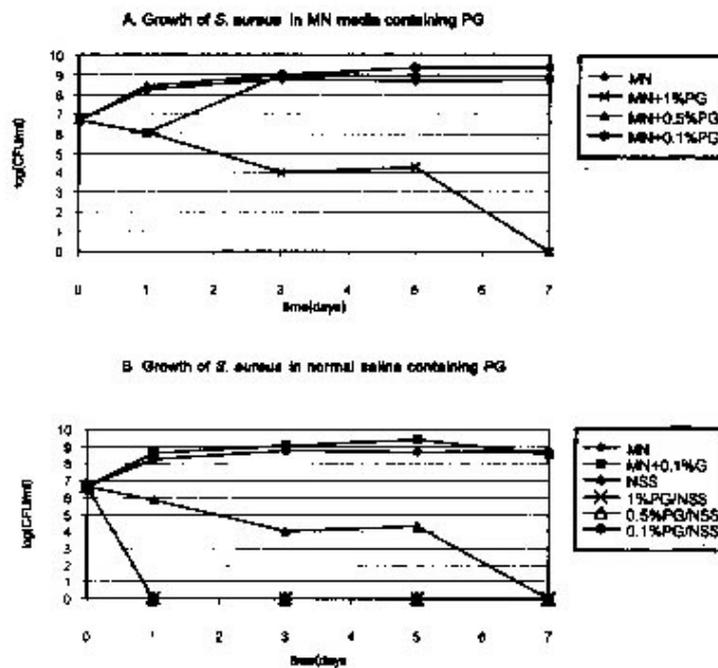


Figure 4. Activity of polysaccharide gel (PG) on growth of *S. aureus* in broth dilution method.

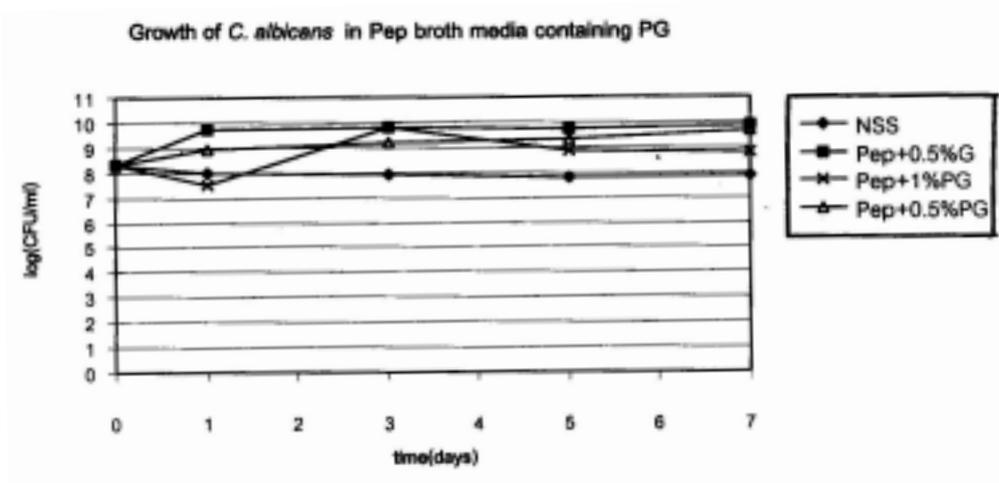


Figure 5. Activity of polysaccharide gel (PG) on growth of *C. albicans* in broth dilution method.

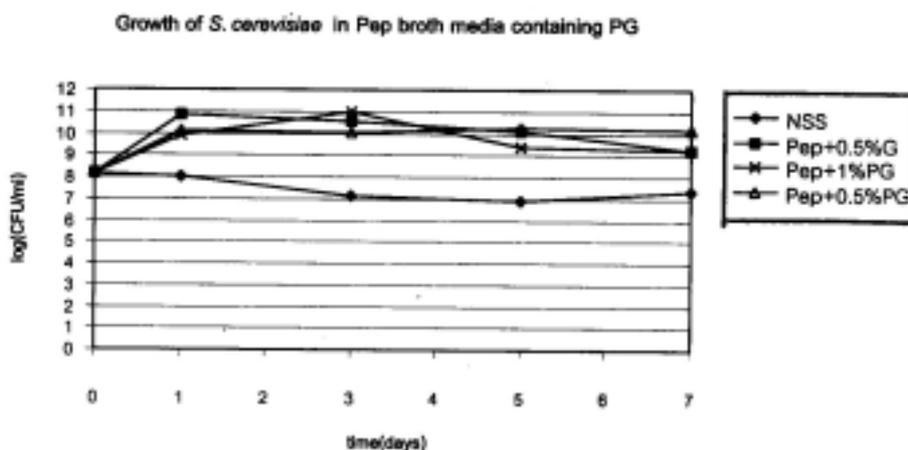


Figure 6. Activity of polysaccharide gel (PG) on growth of *S. cerevisiae* in broth dilution method.

MNG agar media whereas no inhibition zone was observed on enriched TSA media. Bacterial cells survival in NSS in the presence of PG was reduced to zero within 24 hours. Polysaccharide gel from durian fruit-hulls did not appear to produce inhibitory activity against *C. albicans* and *S. cerevisiae* in this study.

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