ORIGINAL ARTICLE

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

Vimolmas Lipipun[1], Nantawan Nantawanit[2] and Sunanta Pongsamart[3]

Abstract

Lipipun, V., Nantawanit, N. and Pongsamart, S.
Antimicrobial activity (*in vitro*) of polysaccharide gel from durian fruit-hulls
Songklanakarin J. Sci. Technol. 2002, 24(1) : 31-38

***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.**

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

[1]Ph.D. (Micro. and Immuno.), Assoc. Prof., Department of Microbiology, [2]B.Sc. (Biochem.), [3]Ph.D. (Med. Chem.), Assoc. Prof., Department of Biochemistry, Faculty of Pharmaceutical Sciences Chulalongkorn University, Bangkok 10330 Thailand.
Corresponding e-mail : Sunanta.Po.@chula.ac.th
Received, 30 May 2001 Accepted, 21 August 2001

บทคัดย่อ

วิมลมาศ ลิปิพันธ์[1] นันทวัน นันทวนิช[2] และ สุนันท์ พงษ์สามารถ[2]
การยับยั้งเชื้อจุลินทรีย์ในหลอดทดลองของสารโพลีแซคคาไรด์เจลจากเปลือกของผลทุเรียน
ว. สงขลานครินทร์ วทท. 2545 24(1) : 31-38

การศึกษาในหลอดทดลองของปฏิกิริยาของสารโพลีแซคคาไรด์เจลสกัดจากเปลือกของผลทุเรียน (*Durio zibethinus* L.) ทำการทดลองเพื่อประเมินผลการยับยั้งเชื้อจุลินทรีย์ของสารโพลีแซคคาไรด์เจล ทดสอบการยับยั้งการเจริญของจุลินทรีย์ด้วยสารโพลีแซคคาไรด์เจลต่อแบคทีเรียสองสายพันธุ์ ได้แก่ *Staphylococcus aureus* และ *Escherichia coli* และต่อเชื้อยีสต์สองสายพันธุ์ ได้แก่ *Candida albicans* และ *Saccharomyces cerevisiae* โดยเทคนิคจุลชีววิเคราะห์ใช้วิธี Simple Agar Diffusion Method และวิธี Broth Dilution Method โพลีแซคคาไรด์เจลที่ความเข้มข้น 0.32% ในน้ำกลั่น แสดงให้เห็น inhibition zone บนอาหารวุ้น TSA ต้านการเจริญของเชื้อ *S. aureus* และ MIC ของสารโพลีแซคคาไรด์เจลในอาหาร TSB ต่อเชื้อ *S. aureus* เท่ากับ 0.64 มก./มล. อย่างไรก็ดีความเข้มข้นต่ำสุดของสารละลายโพลีแซคคาไรด์เจลในน้ำกลั่นที่ 1.25% และ 2.50% ให้ผลยับยั้งการเจริญบนอาหารวุ้น MNG ต่อเชื้อ *S. aureus* และ *E. coli* ตามลำดับ ได้ผลของ inhibition zone ที่มีขอบเขตที่คมและชัดเจน ได้ผลของการยับยั้งของสารละลายโพลีแซคคาไรด์เจลในความเข้มข้นต่ำสุดที่ 1% ในอาหาร peptone broth ต่อเชื้อ *E. coli* และ *S. aureus* โดยเห็นผลของ colony count ที่ 24 ชั่วโมงลดลงเหลือ 0 และ 15% ตามลำดับ อย่างไรก็ดีสายพันธุ์ทั้งสองชนิดของแบคทีเรียที่ทดสอบพบว่าถูกยับยั้งการเจริญในสารละลาย NSS ที่มี 0.1% ของสารละลายโพลีแซคคาไรด์เจล พบมี colony count ที่ 24 ชั่วโมงลดลงถึง 0 สารโพลีแซคคาไรด์เจลไม่มีผลยับยั้งการเจริญต่อเชื้อยีสต์สองสายพันธุ์ที่ทดสอบในการศึกษาครั้งนี้

[1]ภาควิชาจุลชีววิทยา [2]ภาควิชาชีวเคมี คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย พญาไท กรุงเทพมหานคร 10330

Polysaccharide gel (PG) isolated from fruit-hulls of durian (*Durio zibethinus* L.) have been found to be useful in preparation of food product and also as pharmaceutical excipients in jelly, tablet, suspension and emulsion (Pongsamart *et al*., 1989 a.; Pongsamart, 1989; Umprayn *et al*. 1990 abc). The composition of sugars and properties of PG were previously described (Pongsamart and Panmaung, 1998). Toxicity test of polysaccharide gel was determined, a high oral dose (2 g/kg) did not induce severe toxicity in male mice and rats (Pongsamart *et al*., 2001 a.). No toxic effects were observed in subacute treatment in male mice (Pongsamart *et al*., 1989 b.) and subchronic studies in male and female mice confirmed the consumptive safety of PG (Pongsamart *et al*., 2001 b.). The purpose of this study was to determine the inhibitory activity of polysaccharide gel against microorganisms. Extracellular susceptibility testing by the methods of agar diffusion and macrodilution were investigated (Collin *et al*., 1995).

Materials and Methods

Chemicals and media

Magnesium sulfate, potassium chloride, sodium chloride and ammonium dihydrogen phosphate of purified grade were obtained from Merck Co. MNG agar contained 20.0 g agar (Difco Laboratories); 1.0 g, ammonium dihydrogen phosphate; 1.0 g, potassium chloride; 1.0 g, magnesium sulfate; 10.0 g, glucose ;10.0 g, peptone per liter. Tryptic soy agar (TSA) from Merck contained 15.0 g, peptone from casein; 5.0 g, peptone from soymeal; 5.0 g, sodium chloride; 15.0 g, agar per liter. Tryptic soy broth (TSB) contained 17.0 g, peptone from casein; 3.0 g, peptone from soymeal; 2.5 g, glucose; 5.0 g, sodium chloride; 2.5 g, potassium hydrogen phosphate per liter. MN broth contained the same ingredients as MNG described except agar and glucose were not added. Sabouraud dextrose agar contained 10.0 g, neopeptone ; 40.0 g, glucose ; 15.0 g, agar per liter. Peptone broth (pep

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 37ºC 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)

Macrodilution 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 was performed as a culture control. The inoculated media with PG were incubated at 37ºC 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 37ºC 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)			
	S. aureus[a]	*E. coli*[a]	*C. albicans*[b]	*S. cerevisiae*[b]
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-

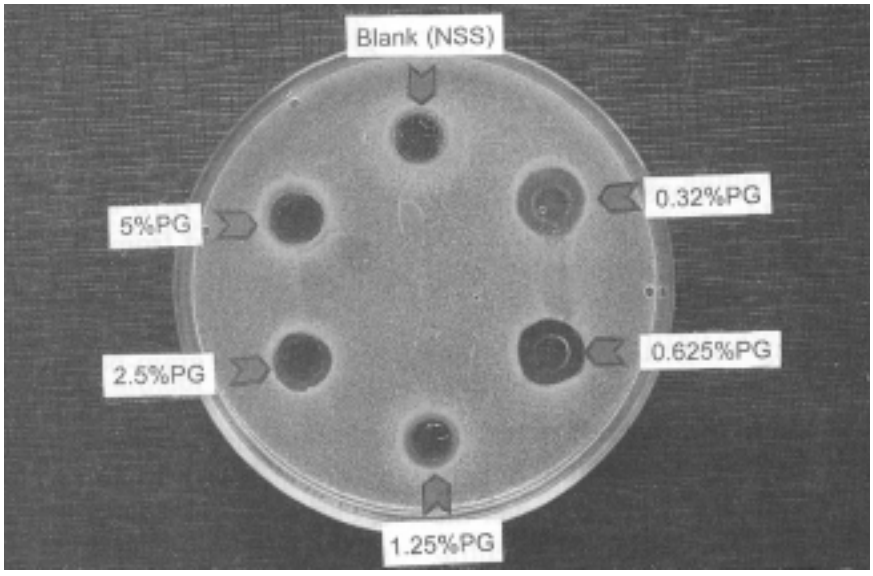

Figure 1. Microbiological assay plate for *S. aureus* on medium TSA. Cups contain PG 0.32, 0.625, 1.25, 2.5 and 5%, and control cup filled with NSS.

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 37ºC, 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^+ 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

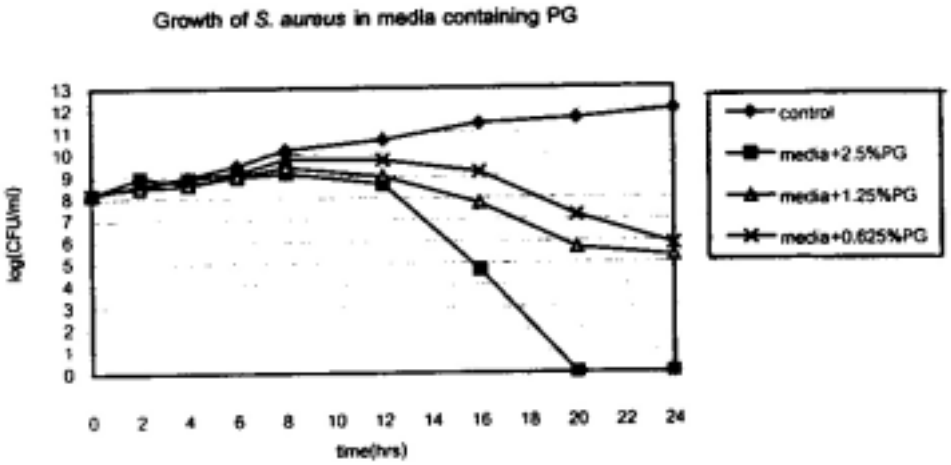

Figure 2. Time-kill analysis of polysaccharide gel (PG) in TSB medium for *S. aureus* by broth dilution method.

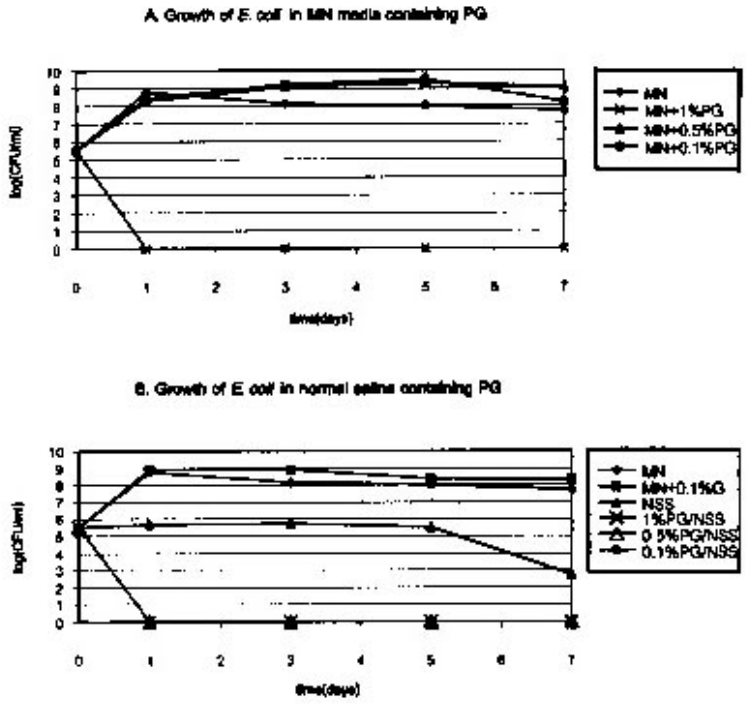

Figure 3. Activity of polysaccharide gel (PG) on growth of *E. coli* in broth dilution method.

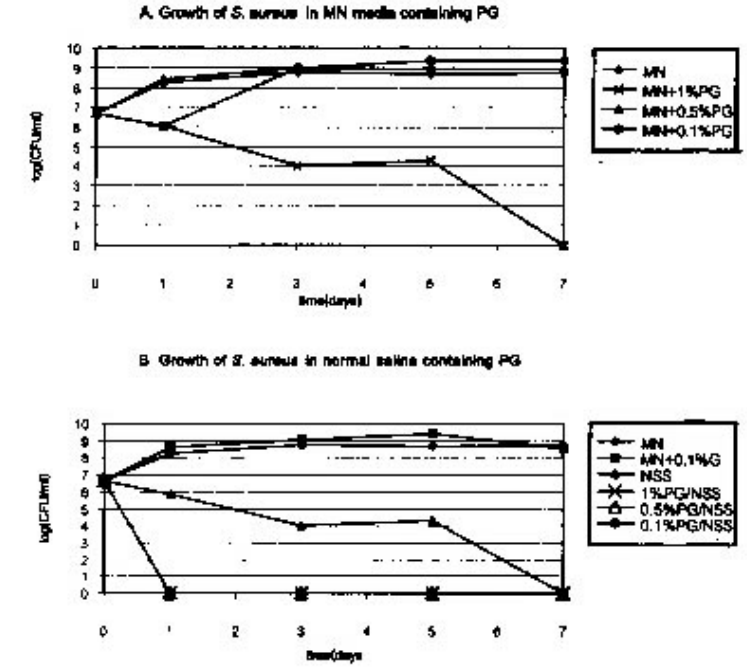

Figure 4. Activity of polysaccharide gel (PG) on growth of *S. aureus* in broth dilution method.

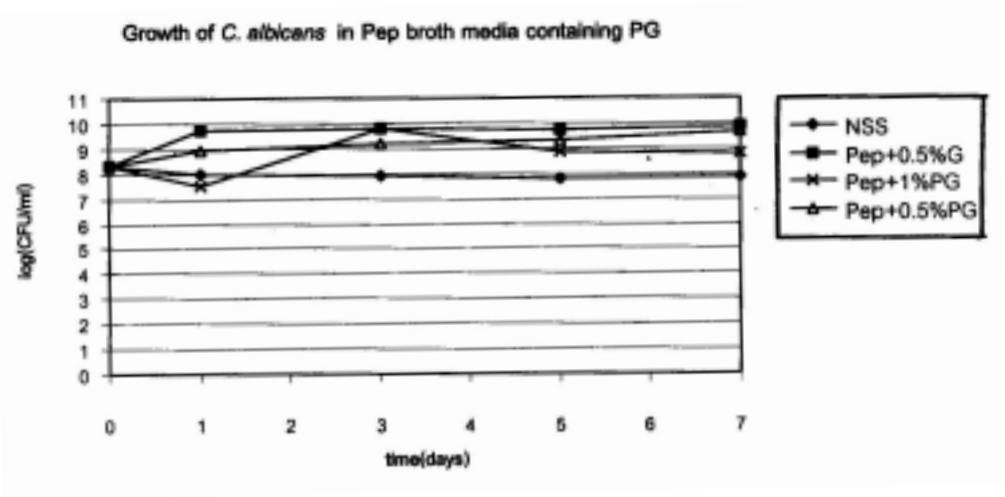

Figure 5. Activity of polysaccharide gel (PG) on growth of *C. albicans* in broth dilution method.

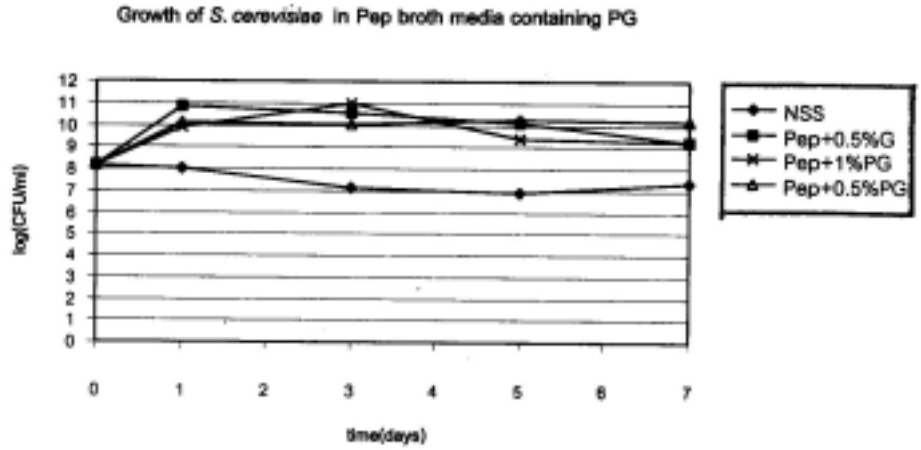

Figure 6. Activity of polysaccharide gel (PG) on growth of *S. cerevisia*e in broth dilution method.

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.

Acknowledgments

This work was supported by research budgeting of Faculty of Pharmaceutical Sciences, Chulalongkorn University. Thanks are extended to Miss Anuree Tung, Department of Microbiology, for her technical assistance.

References

Brock, T.D., Madigan, M.T., Martinko, J.M. and Parker, J. 1994. Biology of Microorganisms, 7th ed., Prentice Hall, Englewood Cloffs, N.J., pp. 118-124.

Collin, C.H., Lyne, P.M. and Grange, J.M. 1995. Collins and Lyne's Microbiological Method: Antimicrobial Susceptibility Test, 7th ed., Oxford: Butterworth-Heinemann, pp. 178-205.

Lorian, V. 1991. Antibiotics in Laboratory Medicine, Williams Wilkins, Baltimore, pp. 739-786.

Nantavutikul, S. and Farkprapai, J. 1999. Study of antimicrobial activity of medicinal plant extracts. Senior Project Report, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Neu, T.S, Dengler, T., Jann, B., and Poralla, K. 1992. Structural studies of an emulsion-stabilizing exopolysaccharides produced by an adhesive, hydrophobic *Rhodococcus* strain. J. Gen. Microbial. 138: 2531.

Pongsamart, S. 1989. The studies of carbohydrate extracts from durian rinds to use as suspending agent. Research Report, Department of Biochemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Pongsamart, S., Dhumma-Upakorn, R. and Panmaung, T. 1989 a. The studies of carbohydrate from durian rind for pharmaceutical and food preparations. Research Report, Rachadapisek-sompoach Research Funds, Chulalongkorn University.

Pongsamart, S., Jesadanont, S.N. and Markman, N. 1989 b. The studies on safety an toxicity of the consumption of pectin-like substance isolated from durian rinds Research Report, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Pongsamart, S. and Panmaung, T. 1998. Isolation of polysaccharides from fruit-hulls of durian (*Durio zibethinus* L.). Songklanakarin J. Sci. Technol. 20(3): 323-332.

Pongsamart, S., Sukrong, S. and Tawatsin, A. 2001 a. The dertermination of toxic effects at a high oral dose of polysaccharide gel extracts from fruit-hulls of durian (*Durio zibethinus* L.) in mice and rats. Songklanakarin J. Sci. Technol. 23(1): 53-62.

Pongsamart, S., Tawatsin, A. and Sukrong, S. 2001 b. Long-term consumption of polysaccharide gel from durian fruit-hulls in mice. (in press)

The United State Pharmacopoeia XXIII and The National Formulary XVII 1995, United State Pharmacopoeia convention Inc., Rockville, MD., pp. 1681-1686.

Umprayn, K., Kaimonkong, R. and Pongsamart, S. 1990 a. Evaluation of tablet disintegrating properties of durian rind extracts. NUS-JSPS Seminar, Chiba, Japan Oct 23-26,1990.

Umprayn, K., Chanpaparp, K. and Pongsamart, S. 1990 b. The studies of durian rind extracts as an aqueous binder I: Evaluation of granule properties. Th. J. Pharm. Sci. 15(2): 95-115.

Umprayn, K., Chanpaparp, K. and Pongsamart, S. 1990 c. The studies of durian rind extracts as an aqueous binder II : Evaluation of tablets properties. Th. J. Pharm. Sci. 15(3): 173-186.