# CHAPTER III

# RESULTS

## 1. Determination of antimicrobial activity of polysaccharide gel

### 1.1 Agar diffusion susceptibility test

The result of the initial screening of polysaccharide extract for antibacterial activity of *Staphylococcus* spp. 42 isolates , *Streptococcus* spp. 34 isolates, *E. coli* 13 isolates, *Klebsiella* spp. 5 isolates and *Pseudomonas* spp. 15 isolates are summarized in Table 3, 4, 5, 6 and 7, respectively. The inhibition zone was observed on agar media at PG concentration 3.12-50 mg/ml (w/v). The result showed inhibitory activity. PG appeared to be quite promising for their capacity to inhibit the growth of isolated bacteria from cow mastitis as well as for their broad spectrum activity against standard bacteria. In addition, the strains that exhibited resistance to gentamicin (MBC $\geqslant$8 µg/ml, Lorian 1991) were sensitive to PG. According to viscosity of PG, the highest allowable concentration of 50 mg/ml PG was used in this study.

All tested bacteria were susceptible to PG, and the result showed that 50 mg/ml PG produced 24.70-10.50 mm, 18.00-8.50 mm, 14.80-12.30 mm, 13.60-11.90 mm and 17.50-10.30 mm in diameter inhibition zone against *Staphylococcus* spp., *Streptococcus* spp., *E. coli, Klebsiella* spp., and *Pseudomonas* spp., respectively. Inhibition zone of sharp and clear margin was obtained. An increment of inhibition zone diameter was found with respect to increasing concentrations of PG. Whereas the control (NSS) showed no inhibition zone.

**Table 3: Inhibitory activity of polysaccharide gel (PG; Monthong) on growth of *Staphylococci* by agar diffusion method, no zone inhibition was observed in NSS (control), NZ = no zone, SP = *Staphylococcus spp.* isolated from cow mastitis**

| *Staphylococci* | Diameter of inhibition zone of PG, mm mean ± SD | | | | |
|---|---|---|---|---|---|
| | 50mg/ml | 25mg/ml | 12.5mg/ml | 6.25mg/ml | 3.12mg/ml |
| *S haemolyticus* (SP1) | 8.50±0.00 | NZ | NZ | NZ | NZ |
| *S haemolyticus* (SP24) | 8.50±0.00 | NZ | NZ | NZ | NZ |
| *S. aureus* (SP2) | 12.10±0.05 | 10.40±0.00 | 8.50±0.00 | NZ | NZ |
| *S. aureus* (SP3) | 11.10±0.12 | 9.90±0.02 | 8.50±0.00 | NZ | NZ |
| *S. aureus* (SP4) | 11.00±0.01 | 10.10±0.02 | 8.50±0.00 | NZ | NZ |
| *S. aureus* (SP7) | 13.10±0.10 | 9.00±0.00 | 8.50±0.00 | NZ | NZ |
| *S. aureus* (SP11) | 8.50±0.00 | NZ | NZ | NZ | NZ |
| *S. aureus* (SP13) | 11.80±0.06 | NZ | NZ | NZ | NZ |
| *S. aureus* (SP15) | 12.30±0.08 | 10.00±0.00 | 8.50±0.00 | NZ | NZ |
| *S. aureus* (SP23) | 8.50±0.00 | NZ | NZ | NZ | NZ |
| *S. simulan* (SP5) | 11.20±0.00 | 10.50±0.00 | 8.50±0.00 | NZ | NZ |
| *S. simulan* (SP9) | 11.80±0.02 | 8.50±0.00 | NZ | NZ | NZ |
| *S. simulan* (SP21) | | | | | |
| *S. simulan* (SP25) | 15.20±0.05 | 12.60±0.05 | 10.80±0.07 | 8.50±0.00 | NZ |
| *S. chromogenes* (SP6) | 14.70±0.06 | 11.10±0.13 | 8.50±0.00 | NZ | NZ |
| *S. chromogenes* (SP8) | 11.70±0.19 | 10.10±0.01 | 8.50±0.00 | NZ | NZ |
| *S. chromogenes* (SP12) | 24.70±0.20 | 19.90±0.15 | 17.30±0.16 | 17.00±0.02 | 11.20±0.03 |
| *S. chromogenes* (SP16) | 13.20±0.18 | 10.00±0.00 | 8.50±0.00 | NZ | NZ |
| *S. chromogenes* (SP18) | 12.70±0.06 | 10.10±0.01 | 8.50±0.00 | NZ | NZ |
| *S. chromogenes* (SP26) | 10.60±0.11 | 8.50±0.00 | NZ | NZ | NZ |
| *S. chromogenes* (SP27) | 22.70±0.14 | 20.40±0.13 | 16.20±0.0.12 | 13.90±0.09 | 8.50±0.00 |
| *S. chromogenes* (SP28) | 11.10±0.05 | 8.50±0.00 | NZ | NZ | NZ |
| *S. chromogenes* (SP31) | 11.50±0.14 | 8.50±0.00 | NZ | NZ | NZ |
| *S. chromogenes* (SP32) | 12.30±0.00 | 8.50±0.00 | NZ | NZ | NZ |
| *S. chromogenes* (SP33) | 8.50±0.00 | NZ | NZ | NZ | NZ |
| *S. chromogenes* (SP34) | 11.90±0.00 | 8.50±0.00 | NZ | NZ | NZ |
| *S. chromogenes* (SP35) | 11.70±0.11 | 8.50±0.00 | NZ | NZ | NZ |
| *S. chromogenes* (SP36) | 15.30±0.04 | 11.60±0.11 | 8.50±0.00 | NZ | NZ |
| *S. chromogenes* (SP37) | 14.30±0.08 | 10.30±0.03 | 8.50±0.00 | NZ | NZ |
| *S. chromogenes* (SP38) | 13.90±0.13 | 10.10±0.01 | 8.50±0.00 | NZ | NZ |
| *S. hominis* (SP10) | 10.90±0.11 | 8.50±0.00 | NZ | NZ | NZ |
| *S.caprae* (SP20) | 11.40±0.03 | 10.00±0.00 | 8.50±0.00 | NZ | NZ |
| *S. hyicus* (SP22) | 11.80±0.06 | 9.90±0.04 | 8.50±0.00 | NZ | NZ |
| *S. hyicus* (SP41) | 8.50±0.00 | NZ | NZ | NZ | NZ |
| *S. saprophyticus* (SP29) | 12.70±0.06 | 10.10±0.05 | 8.50±0.00 | NZ | NZ |
| *S. saprophyticus* (SP39) | 8.50±0.00 | NZ | NZ | NZ | NZ |
| *S. xylosus* (SP30) | 8.50±0.00 | NZ | NZ | NZ | NZ |

**Table 4: Inhibitory activity of polysaccharide gel (PG; Monthong) on growth of *Streptococci* by agar diffusion method, no zone inhibition was observed in NSS (control), NZ = no zone, SP = *Streptococcus spp.* isolated from cow mastitis**

| *Streptococci* isolates | Diameter of inhibition zone of PG, mm mean ± SD | | | | |
|---|---|---|---|---|---|
| | 50 mg/ml | 25 mg/ml | 12.5mg/ml | 6.25mg/ml | 3.12 mg/ml |
| *S. uberis* (SR 3) | 16.50±0.29 | 13.10±0.00 | 9.60±0.20 | 8.50±0.00 | NZ |
| *S. uberis* (SR 4) | 14.90±0.19 | 11.70±0.18 | 9.80±0.17 | 8.50±0.00 | NZ |
| *S. uberis* (SR 6) | 14.90±0.05 | 12.40±0.01 | 11.30±0.07 | 8.50±0.00 | NZ |
| *S. uberis* (SR 7) | 16.40±0.08 | 11.90±0.30 | 8.50±0.00 | 8.50±0.00 | NZ |
| *S. uberis* (SR 8) | 14.70±0.12 | 12.90±0.10 | 8.50±0.00 | NZ | NZ |
| *S. uberis* (SR 9 ) | 15.40±0.25 | 11.80±0.29 | 9.20±0.11 | 8.50±0.00 | NZ |
| *S. uberis* (SR 10) | 17.90±0.11 | 14.80±0.12 | 8.50±0.00 | NZ | NZ |
| *S. uberis* (SR 22) | 14.80±0.03 | 12.40±0.18 | 9.00±0.09 | 8.50±0.00 | NZ |
| *S. uberis* (SR 30) | 15.70±0.01 | 12.80±0.09 | 8.50±0.00 | NZ | NZ |
| *S. uberis* (SR 36) | 14.80±0.18 | 10.60±0.18 | 8.50±0.00 | NZ | NZ |
| *S. uberis* (SR 38) | 14.60±0.15 | 10.00±0.15 | 8.50±0.00 | NZ | NZ |
| *S. uberis* (SR 39) | 8.50±0.00 | 8.50±0.00 | NZ | NZ | NZ |
| *S. uberis* (SR 41) | 14.40±0.09 | 10.20±0.05 | 8.50±0.00 | 8.50±0.00 | NZ |
| *S. uberis* (SR 42) | 8.50±0.00 | NZ | NZ | NZ | NZ |
| *S. uberis* (SR 43) | 14.40±0.04 | 9.90±0.19 | 8.50±0.00 | NZ | NZ |
| *S. uberis* (SR 44) | 13.00±0.07 | 11.10±0.04 | 8.50±0.00 | NZ | NZ |
| *S. uberis* (SR 45) | 14.70±0.00 | 11.90±0.13 | 8.50±0.00 | NZ | NZ |
| *S. uberis* (SR 47) | 12.90±0.04 | 10.30±0.01 | 8.50±0.00 | NZ | NZ |
| *S. uberis* (SR 59) | 13.90±0.06 | 11.60±0.11 | 8.50±0.00 | NZ | NZ |
| *S. dys.dysagalactiae*(SR23) | 17.20±0.03 | 14.60±0.09 | 11.80±0.11 | 10.00±0.07 | 8.50±0.00 |
| *S. dys.dysagalactiae* (SR 24) | 14.50±0.01 | 10.00±0.13 | 8.50±0.00 | 8.50±0.00 | NZ |
| *S. bovisI* (SR 53) | 10.70±0.03 | 8.50±0.00 | 8.50±0.00 | NZ | NZ |
| *S. bovisII* (SR 26) | 16.50±0.18 | 12.70±0.05 | 9.00±0.09 | 8.50±0.00 | NZ |
| *S. bovisII* (SR 32) | 18.00±0.11 | 14.90±0.01 | 9.00±0.00 | 8.50±0.00 | NZ |
| *S. bovisII* (SR 37) | 13.40±0.15 | 10.10±0.19 | 8.50±0.00 | NZ | NZ |
| *S. dys.equisimilis* (SR 28) | 16.50±0.03 | 15.00±0.09 | 11.50±0.11 | 8.50±0.00 | NZ |
| *S. dys.equisimilis* (SR 29) | 13.10±0.19 | 8.50±0.00 | 8.50±0.00 | 8.50±0.00 | 8.50±0.00 |
| *S. dys.equisimilis* (SR 31) | 19.50±0.11 | 16.30±0.05 | 9.00±0.01 | 8.50±0.00 | NZ |
| *S. dys.equisimilis* (SR 35) | 14.00±0.03 | 11.00±0.07 | 8.50±0.00 | NZ | NZ |
| *S. acidominimus*(SR 21) | 9.90±0.03 | 8.50±0.00 | NZ | NZ | NZ |
| *S. acidominimus* (SR 25) | 8.50±0.00 | NZ | NZ | NZ | NZ |
| *S. acidominimus* (SR 33) | 9.20±0.06 | 8.50±0.00 | 8.50±0.00 | NZ | NZ |
| *S. agalactiae* (SR 1) | 14.50±0.09 | 11.20±0.00 | 9.50±0.00 | 8.50±0.00 | NZ |
| *S. porcinus* (SR 27) | 16.90±0.12 | 12.50±0.23 | 8.50±0.00 | 8.50±0.00 | NZ |

**Table 5: Inhibitory activity of polysaccharide gel (PG; Monthong) on growth of *Escherichia coli* by agar diffusion method, no zone inhibition was observed in NSS (control), NZ = no zone, CM = Clinical Mastitis,**

| *Escherichia coli* isolates | Diameter of inhibition zone of PG, mm mean ± SD | | | | |
|---|---|---|---|---|---|
| | 50mg/ml | 25 mg/ml | 12.5mg/ml | 6.25mg/ml | 3.12mg/ml |
| *E. coli* (CM 3) | 13.80±0.08 | 12.00±0.06 | 11.00±0.00 | 8.50±0.00 | NZ |
| *E. coli* (CM 4) | 13.00±0.05 | 12.50±0.00 | 11.80±0.03 | 11.50±0.05 | 9.00±0.00 |
| *E. coli* (CM 24) | 13.10±0.06 | 10.30±0.04 | 10.30±0.00 | 8.50±0.00 | NZ |
| *E. coli* (CM 40) | 12.30±0.07 | 12.30±0.09 | 10.50±0.00 | 8.50±0.00 | NZ |
| *E. coli* (CM 49) | 14.80±0.10 | 11.80±0.01 | 11.50±0.02 | 8.50±0.00 | NZ |
| *E. coli* (CM 55) | 12.70±0.01 | 12.60±0.05 | 10.80±0.00 | 8.50±0.00 | NZ |
| *E. coli* (CM 70) | 14.60±0.09 | 14.00±0.03 | 12.20±0.08 | 12.80±0.22 | 12.00±0.07 |
| *E. coli* (CM 84) | 13.20±0.00 | 11.30±0.00 | 11.00±0.04 | 8.50±0.00 | NZ |
| *E. coli* (CM 85) | 13.60±0.00 | 11.00±0.00 | 8.50±0.00 | NZ | NZ |
| *E. coli* (CM 90) | 12.50±0.03 | 12.40±0.00 | 10.70±0.03 | 10.00±0.00 | 8.50±0.00 |
| *E. coli* (CM 186) | 13.20±0.00 | 12.70±0.08 | 12.60±0.06 | 9.50±0.00 | 9.00±0.16 |
| *E. coli* (CM 194) | 12.50±0.02 | 11.80±0.01 | 10.50±0.00 | 8.50±0.00 | NZ |

**Table 6: Inhibitory activity of polysaccharide gel (PG; Monthong) on growth of *Klebsiella pneumoniae* by agar diffusion method, sc = clinical mastitis, no zone inhibition was observed in NSS (control), NZ= no zone**

| *K. pneumoniae* isolates | Diameter of inhibition zone of PG, mm mean ± SD | | | | |
|---|---|---|---|---|---|
| | 50 mg/ml | 25 mg/ml | 12.5 mg/ml | 6.25mg/ml | 3.12 mg/ml |
| *K. pneumoniae* (SC 73) | 13.60±0.00 | 12.30±0.00 | 11.20±0.00 | 10.10±0.00 | 8.50±0.00 |
| *K. pneumoniae* (SC74) | 13.50±0.00 | 12.80±0.09 | 10.70±0.12 | 9.00±0.36 | 9.00±0.23 |
| *K. pneumoniae* (SC 78) | 12.20±0.01 | 11.30±0.06 | 11.00±0.01 | 8.50±0.00 | NZ |
| *K. pneumoniae* (SC 82) | 11.90±0.01 | 8.50±0.00 | NZ | NZ | NZ |
| *K. pneumoniae* (SC 93) | 8.50±0.00 | NZ | NZ | NZ | NZ |

**Table 7: Inhibitory activity of polysaccharide gel (PG; Monthong) on growth of *Pseudomonas* spp. by agar diffusion method, no zone inhibition was observed in NSS (control), NZ = no zone, sc = subclinical mastitis**

| *Pseudomonas spp.* isolates | Diameter of inhibition zone of PG, mm ( mean ± SD) | | | | |
|---|---|---|---|---|---|
| | 50mg/ml | 25 mg/ml | 12.5mg/ml | 6.25mg/ml | 3.12mg/ml |
| *Pseudomonas* sp. (SC 71) | 10.50±0.00 | 8.50±0.00 | NZ | NZ | NZ |
| *Pseudomonas* sp. (SC 74) | 10.60±0.07 | 8.50±0.00 | NZ | NZ | NZ |
| *Pseudomonas* sp. (SC 75) | 13.90±0.08 | 11.70±0.04 | 10.50±0.00 | NZ | NZ |
| *Pseudomonas* sp. (SC 76) | 11.70±0.19 | 8.50±0.00 | NZ | NZ | NZ |
| *Pseudomonas* sp. (SC 184) | 11.70±0.02 | 8.50±0.00 | NZ | NZ | NZ |
| *Pseudomonas* sp. (SC 190) | 12.60±0.05 | 10.90±0.05 | 8.50±0.00 | NZ | NZ |
| *Pseudomonas* sp. (SC 228) | 14.30±0.05 | 14.10±0.06 | 10.30±0.03 | 8.50±0.00 | NZ |
| *Pseudomonas* sp. (SC 273) | 11.60±0.05 | 8.50±0.00 | NZ | NZ | NZ |
| *Pseudomonas* sp. (SC 360) | 16.40±0.02 | 15.00±0.02 | 8.50±0.00 | NZ | NZ |
| *Pseudomonas* sp. (SC 694) | 15.40±0.02 | 12.30±0.08 | 8.50±0.00 | NZ | NZ |
| *Pseudomonas* sp. (SC 697) | 13.60±0.04 | 11.90±0.03 | 8.50±0.00 | NZ | NZ |
| *Pseudomonas* sp. (SC 1001) | 10.70±0.12 | 8.50±0.00 | NZ | NZ | NZ |
| *Pseudomonas* sp. (SC 1002) | 14.90±0.06 | 11.50±0.02 | 11.00±0.03 | 8.50±0.00 | NZ |
| *Pseudomonas* sp. (SC 1021) | 17.50±0.09 | 14.50±0.06 | 10.20±0.03 | 8.50±0.00 | NZ |
| *Pseudomonas* sp. (SC 1135) | 10.30±0.00 | 8.50±0.00 | NZ | NZ | NZ |

1.2 Broth microdilution test of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC)

1.2.1 Determination of MIC and MBC of PG Monthong and galacturonic acid

The antibacterial activity of PG against bacteria isolated from cow mastitis have been further characterized by determining the MICs (minimum inhibitory concentrations) and MBCs (minimum bactericidal concentrations) using the broth microdilution method. Values corresponding to MIC of PG of *Staphylococcus* spp., *Streptococcus* spp., *E.coli, Klebsiella* spp. and *Pseudomonas* spp. were giving in Table 8, 10, 12, 14 and 16 along with the activity of gentamicin for comparison. The results showed that the PG had a significant influence on the MIC values. PG showed *in vitro* antimicrobial activity against all strains tested. The average MBC for most of the bacteria was 12.5-6.25 mg/ml.

MBC values were mostly one doubling dilution higher than MIC values, although occasionally they were identical. PG showed the the same activity against gram positive and gram negative bacteria. The MIC for PG was found to be 6.25 mg/ml for *Staphylococcus* spp. and *Streptococcus* spp. while *E. coli, Klebsiella* spp. and *Pseudomonas* spp. required about 12.5 mg/ml for effective inhibition. The gram negative bacteria with the higest MIC and MBC of PG was *E. coli* (12.5 mg/ml and 25 mg/ml). This fact was in agreement with the agar diffusion method with the results mentioned above (Table 5).

In addition, the strains that exhibited resistance to gentamicin (MBC $\geqslant$8 µg/ml, Lorian 1991) were sensitive to PG. Comparison of the finding PG showed difference result for gentamicin sulfate. The concentration of gentamicin used was 0.03-64 µg/ml. Gentamicin inhibited the growth of *Staphylococcus* spp. and *Streptococcus* spp. while *E. coli, Klebsiella* spp. and *Pseudomonas* spp. and Table 8, 10, 12, 14 and 16 showed the MIC and MBC values obtained, expressed in terms of the gentamicin concentration. Garm-positive strains had lower MBC values (0.25 µg/ml) than the Gram-negative strains tested (4-0.5 µg/ml). *Staphylococcus* spp. had the lowest MIC overall

(0.125 μg/ml), while *Streptococcus* spp. showed the highest MIC (4 μg/ml) of the Gram positive bacteria tested. *E. coli and Klebsiella* spp. had the lowest MIC overall (0.25 μg/ml), while *Pseudomonas* spp. showed the highest MIC (2 μg/ml) of the Gram negative bacteria tested.

The MIC of galacturonic acid against *Staphylococcus* spp., *Streptococcus* spp., *E.coli, Klebsiella* spp. and *Pseudomonas* spp. were showed in Table 9, 11, 13, 15 and 17, respectively. Galacturonic acid was employed in the range of 0.39-50 mg/ml. The average range of MIC for most of bacteria was 1.56-12.5 mg/ml. The gram negative-strains had higher MIC values (3.12-12.5 mg/ml) than the gram positive strains tested (1.56- mg/ml). MBC values were mostly one doubling dilution higher than the MIC values, although occasionally they were identical. The bacteria with the lowest MIC and MBC of galacturonic acid was *Staphylococcus* spp. (1.56 and 3.12 mg/ml). A summary of the MIC and MBC values of PG against those tested bacteria is also demonstrated in Figure 6-10.

**Table 8: MICs and MBCs of polysaccharide gel (PG) and gentamicin sulfate against mastitis causing bacteria, *Staphlococcus* spp. by broth microdilution test**

| *Staphylococci* | PG Monthong (mg/ml) | | Gentamicin sulfate (μg/ml) | |
|---|---|---|---|---|
| | MBC | MIC | MBC | MIC |
| *S. aureus* (SP2) | 50 | 25 | 4 | 2 |
| *S. aureus* (SP3) | >50 | >50 | 0.25 | 0.125 |
| *S. aureus* (SP4) | 25 | 12.5 | 0.25 | 0.125 |
| *S. aureus* (SP7) | 25 | 12.5 | 0.25 | 0.125 |
| *S. aureus* (SP11) | 25 | 12.5 | 0.25 | 0.125 |
| *S. aureus* (SP13) | 25 | 12.5 | 1 | 0.5 |
| *S. aureus* (SP15) | 50 | 25 | 0.5 | 0.25 |
| *S. aureus* (SP23) | 6.25 | 3.12 | 0.25 | 0.125 |
| *S. caprae* (SP20) | 12.5 | 6.25 | 0.5 | 0.25 |
| *S. chromogenes* (SP6) | 12.5 | 6.25 | 0.25 | 0.125 |
| *S. chromogenes* (SP8) | 50 | 25 | 1 | 0.5 |
| *S. chromogenes* (SP12) | 25 | 12.5 | 0.25 | 0.125 |
| *S. chromogenes* (SP16) | 6.25 | 3.12 | 0.06 | 0.03 |
| *S. chromogenes* (SP18) | 12.5 | 6.25 | 0.5 | 0.25 |
| *S. chromogenes* (SP26) | 12.5 | 6.25 | 0.25 | 0.125 |
| *S. chromogenes* (SP27) | 12.5 | 6.25 | <0.03 | <0.03 |
| *S. chromogenes* (SP28) | 12.5 | 6.25 | 0.5 | 0.25 |
| *S. chromogenes* (SP31) | 12.5 | 6.25 | 0.125 | 0.06 |
| *S. chromogenes* (SP32) | 6.25 | 3.12 | 0.125 | 0.06 |
| *S. chromogenes* (SP33) | 12.5 | 6.25 | 0.25 | 0.125 |
| *S. chromogenes* (SP34) | 6.25 | 3.12 | 0.5 | 0.25 |
| *S. chromogenes* (SP35) | 6.25 | 3.12 | 0.5 | 0.25 |
| *S. chromogenes* (SP36) | 6.25 | 3.12 | 0.25 | 0.125 |
| *S. chromogenes* (SP37) | 12.5 | 6.25 | 0.25 | 0.125 |
| *S. chromogenes* (SP38) | 6.25 | 3.12 | 2 | 1 |
| *S. chromogenes* (SP40) | 6.25 | 3.12 | 0.06 | 0.03 |
| *S. chromogenes* (SP42) | 25 | 12.5 | <0.03 | <0.03 |
| *S. haemolyticus* (SP1) | 50 | 25 | 1 | 0.5 |
| *S. haemolyticus* (SP24) | 12.5 | 6.25 | 1 | 0.5 |
| *S. hominis* (SP10) | 12.5 | 6.25 | 0.06 | 0.03 |
| *S. hyicus* (SP22) | 12.5 | 6.25 | 0.25 | 0.125 |
| *S. hyicus* (SP41) | 12.5 | 6.25 | 0.25 | 0.125 |
| *S. saprophyticus* (SP29) | 12.5 | 6.25 | 0.06 | 0.03 |
| *S. saprophyticus* (SP39) | 12.5 | 6.25 | 0.5 | 0.25 |
| *S. simulan* (SP5) | 50 | 25 | 0.5 | 0.25 |
| *S. simulan* (SP9) | 12.5 | 6.25 | 0.25 | 0.125 |
| *S. simulan* (SP21) | 12.5 | 6.25 | <0.03 | <0.03 |
| *S. xylosus* (SP30) | 12.5 | 6.25 | 0.5 | 0.25 |

**Table 9: MICs and MBCs of galacturonic acid against mastitis causing bacteria, *Staphylococcus spp.*, by broth microdilution test**

| *Staphylococci* | Galacturonic acid(mg/ml) | |
|---|---|---|
| | MBC | MIC |
| *S. aureus* (SP2) | 1.56 | 0.78 |
| *S. aureus* (SP3) | 6.25 | 3.12 |
| *S. aureus* (SP4) | 3.12 | 1.06 |
| *S. aureus* (SP7) | 6.25 | 3.12 |
| *S. aureus* (SP11) | 3.12 | 1.56 |
| *S. aureus* (SP13) | 6.25 | 3.12 |
| *S. aureus* (SP15) | 3.12 | 1.56 |
| *S. aureus* (SP23) | 3.12 | 1.56 |
| *S. caprae* (SP20) | 3.12 | 1.56 |
| *S. chromogenes* (SP6) | 3.12 | 1.56 |
| *S. chromogenes* (SP8) | 3.12 | 1.56 |
| *S. chromogenes* (SP12) | 3.12 | 1.56 |
| *S. chromogenes* (SP16) | 3.12 | 1.56 |
| *S. chromogenes* (SP18) | 6.25 | 3.12 |
| *S. chromogenes* (SP26) | 6.25 | 3.12 |
| *S. chromogenes* (SP27) | 3.12 | 1.56 |
| *S. chromogenes* (SP28) | 6.25 | 3.12 |
| *S. chromogenes* (SP31) | 12.5 | 6.25 |
| *S. chromogenes* (SP32) | 6.25 | 3.12 |
| *S. chromogenes* (SP33) | 3.12 | 1.56 |
| *S. chromogenes* (SP34) | 12.5 | 6.25 |
| *S. chromogenes* (SP35) | 1.56 | 0.78 |
| *S. chromogenes* (SP36) | 6.25 | 3.12 |
| *S. chromogenes* (SP37) | 1.56 | 0.78 |
| *S. chromogenes* (SP38) | 6.25 | 3.12 |
| *S. chromogenes* (SP40) | 0.78 | 0.39 |
| *S. chromogenes* (SP42) | 3.12 | 1.56 |
| *S haemolyticus* (SP1) | 12.5 | 6.25 |
| *S haemolyticus* (SP24) | 3.12 | 1.56 |
| *S. hominis* (SP10) | 3.12 | 1.56 |
| *S. hyicus* (SP22) | 1.56 | 0.53 |
| *S. hyicus* (SP41) | 3.12 | 1.56 |
| *S. saprophyticus* (SP29) | 3.12 | 1.56 |
| *S. saprophyticus* (SP39) | 3.12 | 1.56 |
| *S. simulan* (SP5) | 3.12 | 1.56 |
| *S. simulan* (SP9) | 3.12 | 1.56 |
| *S. simulan* (SP21) | 0.78 | 0.39 |
| *S. simulan* (SP25) | 12.5 | 6.25 |
| *S. xylosus* (SP30) | 3.12 | 1.56 |

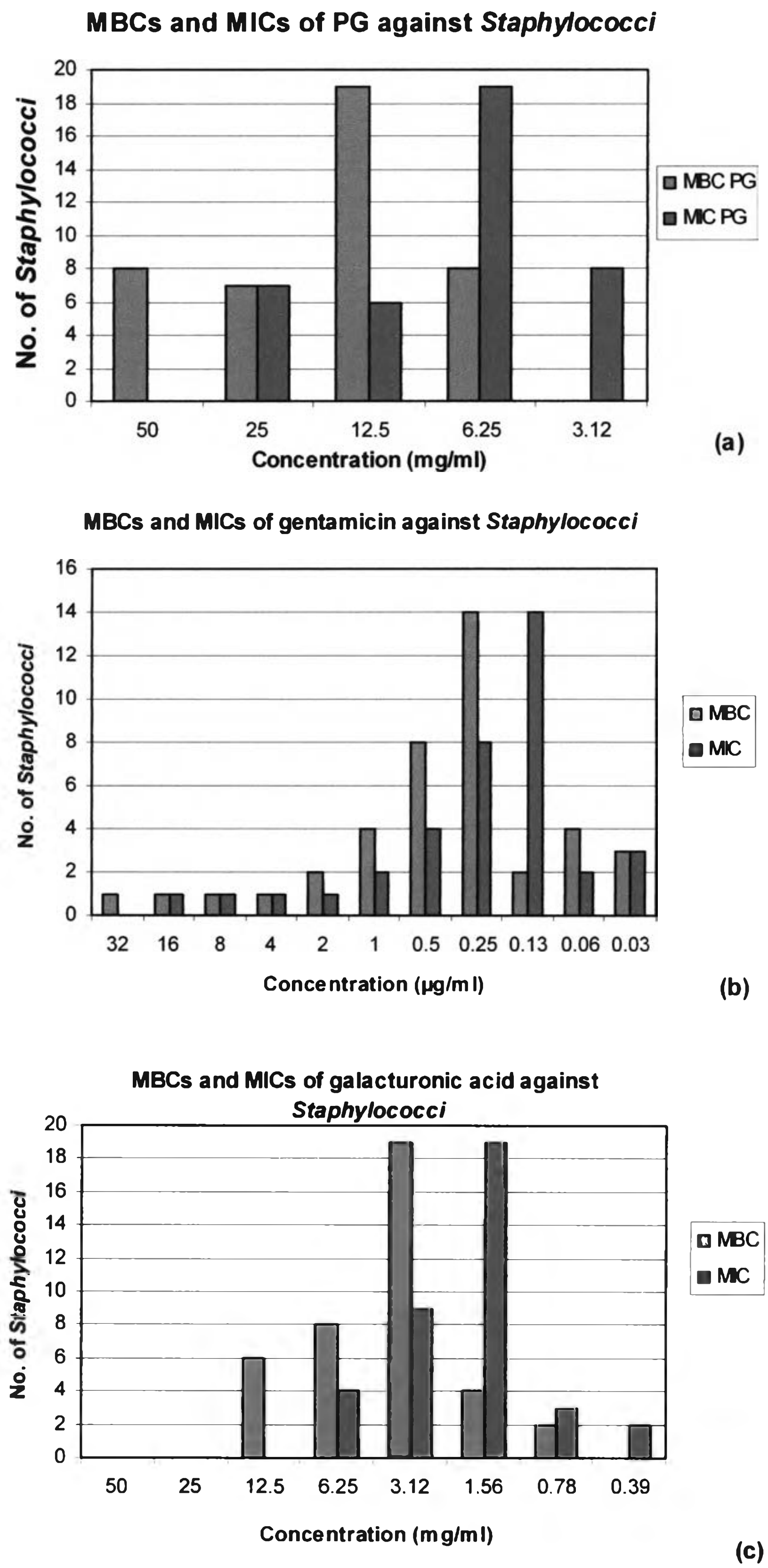



**Figure 6: A summary of MICs and MBCs of (a) polysaccharide gel (PG) Monthong cultivar (b) gentamicin and (c) galacturonic acid against tested mastitis causing bacteria, *Staphylococci***

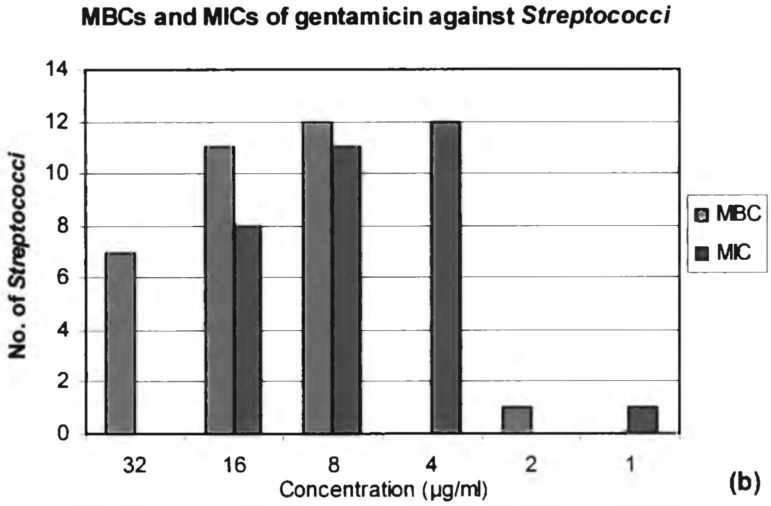
**Table 10: MICs and MBCs of polysaccharide gel (PG) and gentamicin sulfate against mastitis causing bacteria, *Streptococcus* spp., by broth microdilution test**

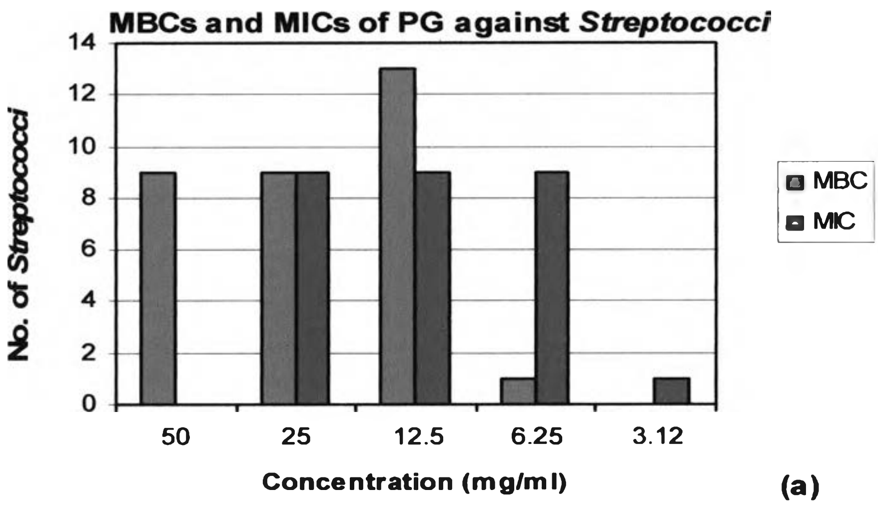
| | PG Monthong (mg/ml) | | Gentamicin sulfate(μg/ml) | |
|---|---|---|---|---|
| *Streptococci* isolates | MBC | MIC | MBC | MIC |
| *S. acidominimus*(SR 21) | 12.5 | 6.25 | 8 | 4 |
| *S. acidominimus* (SR 25)* | 50 | 25 | 2 | 1 |
| *S. acidominimus* (SR 33) | 25 | 12.5 | 32 | 16 |
| *S. agalactiae* (SR 1) | 50 | 25 | 32 | 16 |
| *S. bovisII* (SR 37) | 12.5 | 6.25 | 32 | 16 |
| *S. dys.dysagalactiae* (SR 5) | 50 | 25 | 16 | 8 |
| *S. dys.dysagalactiae* (SR23) | 25 | 12.5 | 8 | 4 |
| *S. dys.dysagalactiae* (SR 24) | 12.5 | 6.25 | 8 | 4 |
| *S. dys.equisimilis* (SR 28) | 50 | 25 | 16 | 8 |
| *S. dys.equisimilis* (SR 29) | 50 | 25 | 8 | 4 |
| *S. dys.equisimilis* (SR 31) | 25 | 12.5 | 8 | 4 |
| *S. porcinus*(SR 27) | 25 | 12.5 | 16 | 8 |
| *S. suis* (SR 60) | 50 | 25 | 16 | 8 |
| *S. uberis*(SR 3) | 25 | 12.5 | 32 | 16 |
| *S. uberis* (SR 4) | 12.5 | 6.25 | 16 | 8 |
| *S. uberis* (SR 6) | 25 | 12.5 | 16 | 8 |
| *S. uberis* (SR 7) | 12.5 | 6.25 | 8 | 4 |
| *S. uberis* (SR 8) | 12.5 | 6.25 | 16 | 8 |
| *S. uberis* (SR 9) | 6.25 | 3.12 | 8 | 4 |
| *S. uberis* (SR 10) | 12.5 | 6.25 | 8 | 4 |
| *S. uberis* (SR 22) | 12.5 | 6.25 | 8 | 4 |
| *S. uberis* (SR 30) | 50 | 25 | 32 | 16 |
| *S. uberis* (SR 34) | 12.5 | 6.25 | 32 | 16 |
| *S. uberis* (SR 36) | 25 | 12.5 | 8 | 4 |
| *S. uberis* (SR 38) | 25 | 12.5 | 16 | 8 |
| *S. uberis* (SR 39) | 25 | 12.5 | 16 | 8 |
| *S. uberis* (SR 40) | 12.5 | 6.25 | 32 | 16 |
| *S. uberis* (SR 41) | 12.5 | 6.25 | 8 | 4 |
| *S. uberis* (SR 42) | 50 | 25 | 32 | 16 |
| *S. uberis* (SR 44) | 12.5 | 6.25 | 16 | 8 |
| *S. uberis* (SR 45) | 12.5 | 6.25 | 16 | 8 |
| *S. uberis* (SR 46) | 12.5 | 6.25 | 16 | 8 |
| *S. uberis* (SR 47) | 12.5 | 6.25 | 8 | 4 |
| *S. uberis* (SR 59) | 50 | 25 | 8 | 4 |

* sensitive strains to gentamicin (MBC < 8 μg/ml)

**Table 11: MICs and MBCs of galacturonic acid against mastitis causing bacteria, *Streptococcus* spp., by broth microdilution test**

| *Streptococci* isolates | Galacturonic acid (mg/ml) | |
|---|---|---|
| | MBC | MIC |
| *S. acidominimus*(SR 21) | 6.25 | 3.12 |
| *S. acidominimus* (SR 25) | 6.25 | 3.12 |
| *S. acidominimus* (SR 33) | 6.25 | 0.3 |
| *S. agalactiae* (SR 1) | 3.12 | 0.15 |
| *S. bovisII* (SR 37) | 1.56 | 0.78 |
| *S. dys.dysagalactiae* (SR 5) | 6.25 | 3.12 |
| *S. dys.dysagalactiae* (SR23) | 3.12 | 0.15 |
| *S. dys.dysagalactiae* (SR 24) | 3.12 | 0.15 |
| *S. dys.equisimilis* (SR 28) | 6.25 | 3.12 |
| *S. dys.equisimilis* (SR 29) | 6.25 | 3.12 |
| *S. dys.equisimilis* (SR 31) | 3.12 | 0.15 |
| *S. porcinus*(SR 27) | 6.25 | 3.12 |
| *S. suis* (SR 60) | 6.25 | 3.12 |
| *S. uberis*(SR 3) | 6.25 | 3.12 |
| *S. uberis* (SR 4) | 1.56 | 0.78 |
| *S. uberis* (SR 6) | 6.25 | 3.12 |
| *S. uberis* (SR 7) | 1.56 | 0.78 |
| *S. uberis* (SR 8) | 1.56 | 0.78 |
| *S. uberis* (SR 9) | 1.56 | 0.78 |
| *S. uberis* (SR 10) | 1.56 | 0.78 |
| *S. uberis* (SR 22) | 6.25 | 0.3 |
| *S. uberis* (SR 30) | 6.25 | 3.12 |
| *S. uberis* (SR 34) | 6.25 | 3.12 |
| *S. uberis* (SR 36) | 1.56 | 0.78 |
| *S. uberis* (SR 38) | 3.12 | 0.15 |
| *S. uberis* (SR 39) | 6.25 | 3.12 |
| *S. uberis* (SR 40) | 1.25 | 6.25 |
| *S. uberis* (SR 41) | 1.56 | 0.78 |
| *S. uberis* (SR 42) | 25 | 12.5 |
| *S. uberis* (SR 44) | 6.25 | 3.12 |
| *S. uberis* (SR 45) | 1.56 | 0.78 |
| *S. uberis* (SR 46) | 6.25 | 3.12 |
| *S. uberis* (SR 47) | 1.56 | 0.78 |
| *S. uberis* (SR 59) | 3.12 | 0.15 |



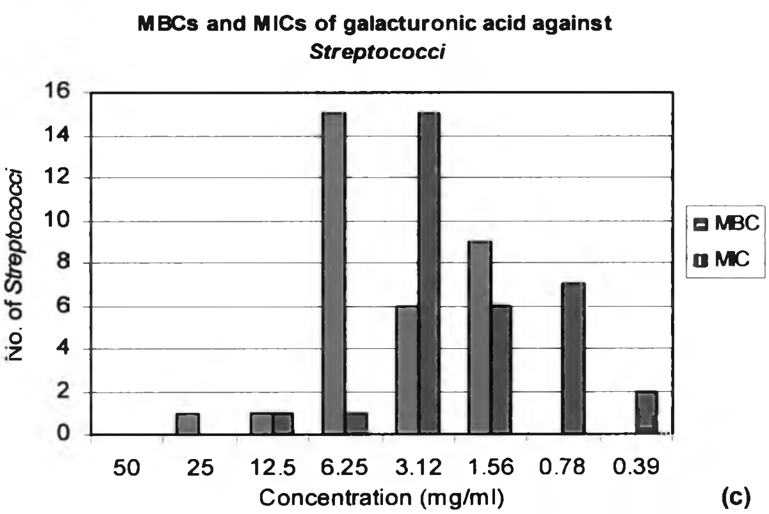




**Figure 7: A summary of MICs and MBCs of (a) polysaccharide gel (PG) Monthong cultivar (b) gentamicin and (c) galacturonic acid against mastitis causing bacteria, *Streptococci*, by broth microdilution test**

**Table 12: MICs and MBCs of polysaccharide gel (PG) and gentamicin against mastitis causing bacteria, *Escherichia coli*, CM = Clinical Mastitis, by broth microdilution test**

| *Escherichia coli* isolates | PG Monthong (mg/ml) | | Gentamicin sulfate (µg/ml) | |
|---|---|---|---|---|
| | MBC | MIC | MBC | MIC |
| *E. coli* (CM 3) | 25 | 12.5 | 2 | 1 |
| *E. coli* (CM 4) | 25 | 12.5 | 2 | 1 |
| *E. coli* (CM 24) | 25 | 12.5 | 1 | 0.5 |
| *E. coli* (CM 40) | 25 | 12.5 | 0.5 | 0.25 |
| *E. coli* (CM 49) | 12.5 | 6.25 | 0.5 | 0.25 |
| *E. coli* (CM 55) | 25 | 12.5 | 2 | 1 |
| *E. coli* (CM 70) | 25 | 12.5 | 0.5 | 0.25 |
| *E. coli* (CM 84) | 12.5 | 6.25 | 1 | 0.5 |
| *E. coli* (CM 85) | 50 | 25 | 1 | 0.5 |
| *E. coli* (CM 90) | 25 | 12.5 | 0.5 | 0.25 |
| *E. coli* (CM 186) | 25 | 12.5 | 1 | 0.5 |
| *E. coli* (CM 194) | 50 | 25 | 0.5 | 0.25 |

**Table 13: MICs and MBCs of galacturonic acid against mastitis causing bacteria, *E. coli*, by broth microdilution test, CM= Clinical Mastitis**

| *Escherichia coli* isolates | Galacturonic acid(mg/ml) | |
|---|---|---|
| | MBC | MIC |
| *E. coli* (CM3) | 12.5 | 6.25 |
| *E. coli* (CM4) | 25 | 12.5 |
| *E. coli* (CM24) | 25 | 12.5 |
| *E. coli* (CM40) | 50 | 25 |
| *E. coli* (CM49) | 25 | 12.5 |
| *E. coli* (CM55) | 25 | 12.5 |
| *E. coli* (CM70) | 25 | 12.5 |
| *E. coli* (CM84) | 50 | 25 |
| *E. coli* (CM85) | 50 | 25 |
| *E. coli* (CM90) | 25 | 12.5 |
| *E. coli* (CM186) | 25 | 12.5 |
| *E. coli* (CM194) | 12.5 | 6.25 |

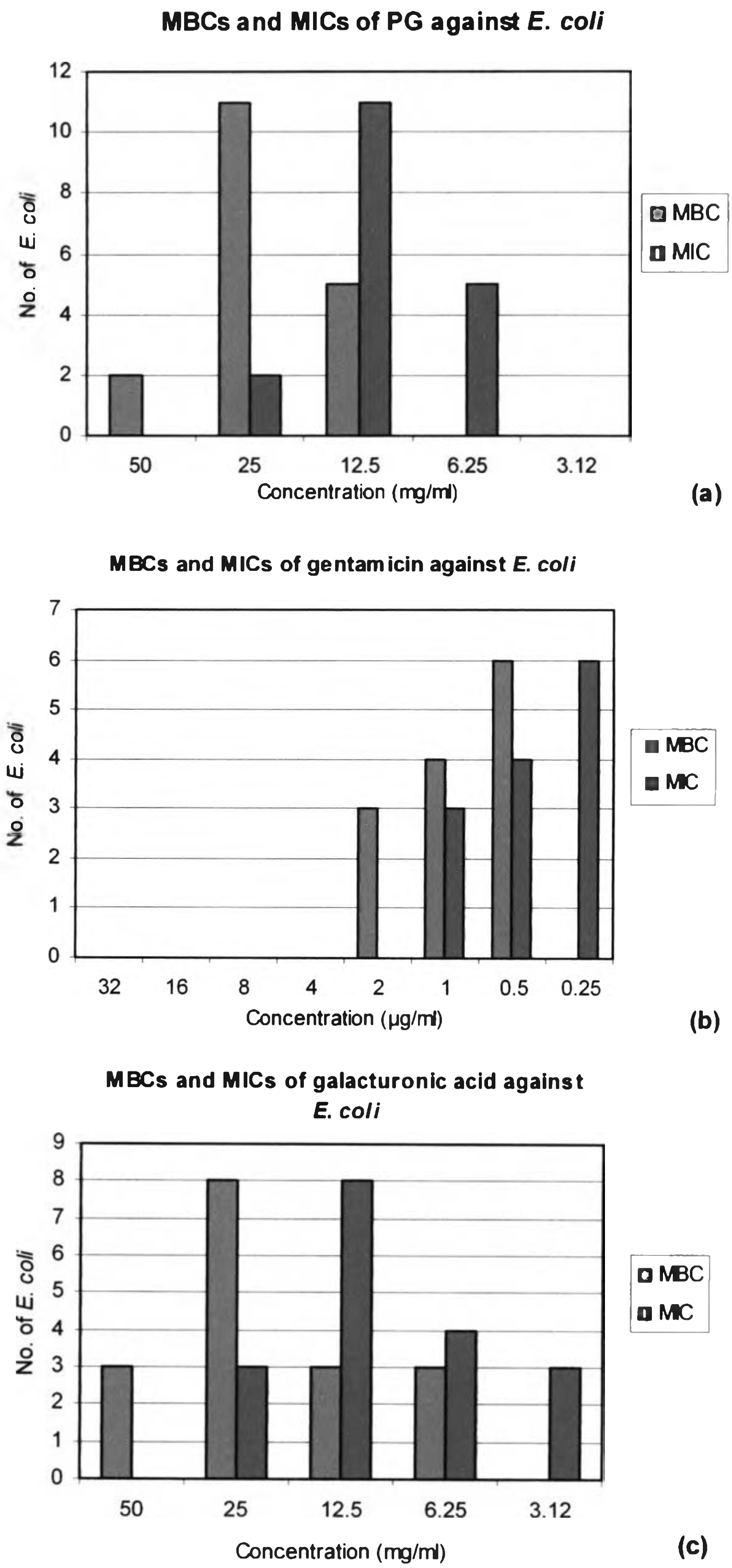



**Figure 8: A summary of MICs and MBCs of (a) polysaccharide gel (PG) Monthong cultivar (b) gentamicin and (c) galacturonic acid against mastitis causing bacteria, *Escherichia coli*, by broth microdilution test**

**Table 14: MICs and MBCs of polysaccharide gel (PG) and and gentamicin sulfate against mastitis causing bacteria, *K. pneumoniae*, by broth microdilution test, SC = clinical mastitis**

| | PG Monthong (mg/ml) | | Gentamicin sulfate (µg/ml) | |
|---|---|---|---|---|
| *K. pneumoniae* isolates | MBC | MIC | MBC | MIC |
| *K. pneumoniae* (SC 73)* | 25 | 12.5 | >64 | >64 |
| *K. pneumoniae* (SC 74) | 25 | 12.5 | 0.5 | 0.25 |
| *K. pneumoniae* (SC 78)* | 12.5 | 6.25 | 8 | 4 |
| *K. pneumoniae* (SC 82) | 25 | 12.5 | 0.5 | 0.25 |
| *K. pneumoniae* (SC 93) | 12.5 | 6.25 | 0.25 | 0.125 |

* resistant strains to gentamicin (MBC ≥8 µg/ml)

**Table 15: MICs and MBCs of galacturonic acid against mastitis causing bacteria, *K. pneumoniae* sc= clinical mastitis, by broth microdilution test**

| | Galacturonic acid (mg/ml) | |
|---|---|---|
| *K. pneumoniae* isolates | MBC | MIC |
| *K. pneumoniae* (SC 73) | 12.5 | 6.25 |
| *K. pneumoniae* (SC 74) | 6.25 | 3.12 |
| *K. pneumoniae* (SC 78) | 12.5 | 6.25 |
| *K. pneumoniae* (SC 82) | 6.25 | 3.12 |
| *K. pneumoniae* (SC 93) | 6.25 | 3.12 |

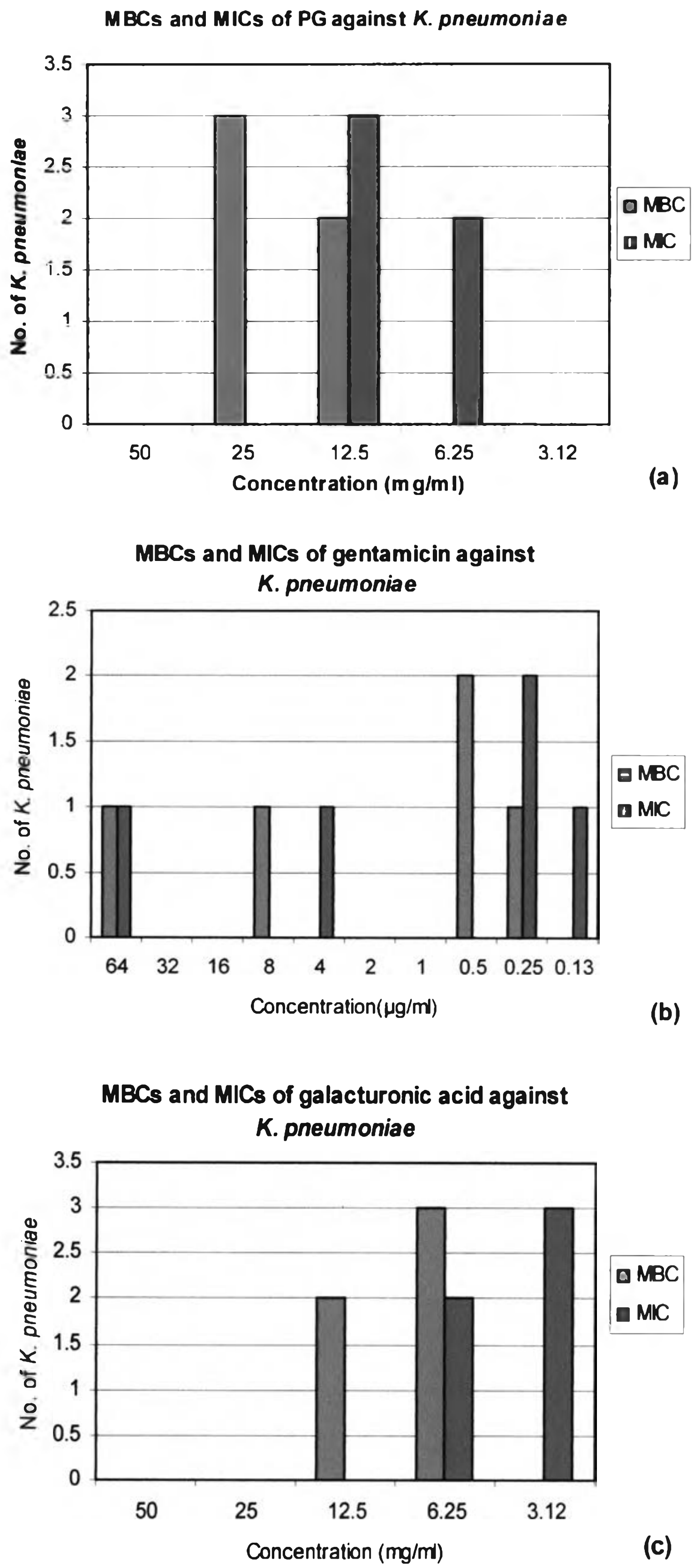



**Figure 9: A summary of MICs and MBCs of (a) polysaccharide gel (PG) Monthong cultivar (b) gentamicin and (c) galacturonic acid against mastitis causing bacteria, *K. pneumoniae*, by broth microdilution test**

**Table16: MICs and MBCs of polysaccharide gel (PG) and gentamicin sulfate against mastitis causing bacteria, *Pseudomonas* spp., by broth microdilution test, SC = subclinical mastitis**

| *Pseudomonas* spp. isolates | PG Monthong (mg/ml) | | Gentamicin sulfate(μg/ml) | |
|---|---|---|---|---|
| | MBC | MIC | MBC | MIC |
| *Pseudomonas sp.* (SC 71) | 12.5 | 6.25 | 1 | 0.5 |
| *Pseudomonas sp.* (SC 74) | 12.5 | 6.25 | 2 | 1 |
| *Pseudomonas sp.* (SC 75)* | 25 | 12.5 | 8 | 4 |
| *Pseudomonas sp.* (SC 76) | 6.25 | 3.12 | 4 | 2 |
| *Pseudomonas sp.* (SC 184) | 12.5 | 6.25 | 1 | 0.5 |
| *Pseudomonas sp.* (SC 190) | 12.5 | 6.25 | 4 | 2 |
| *Pseudomonas sp.* (SC 228) | 12.5 | 6.25 | 2 | 1 |
| *Pseudomonas sp.* (SC 273) | 12.5 | 6.25 | 4 | 2 |
| *Pseudomonas sp.* (SC 360)* | 12.5 | 6.25 | 8 | 4 |
| *Pseudomonas sp.* (SC 694) | >50 | >50 | 2 | 1 |
| *Pseudomonas sp.* (SC 697) | 25 | 12.5 | 2 | 1 |
| *Pseudomonas sp.* (SC 1001) | 12.5 | 6.25 | 4 | 2 |
| *Pseudomonas sp.* (SC 1002) | 25 | 12.5 | 2 | 1 |
| *Pseudomonas sp.* (SC 1021) | 12.5 | 6.25 | 4 | 2 |
| *Pseudomonas sp.* (SC 1135) | 25 | 12.5 | 4 | 2 |

* resistant strains to gentamicin (MBC ≥8 μg/ml)

**Table17: MICs and MBCs of galacturonic acid against mastitis causing bacteria, *Pseudomonas spp.*, by broth microdilution test SC = subclinical mastitis**

| *Pseudomonas* spp. isolates | Galacturonic acid (mg/ml) | |
|---|---|---|
| | MBC | MIC |
| *Pseudomonas sp.* (SC 71) | 12.5 | 6.25 |
| *Pseudomonas sp.* (SC 74) | 6.25 | 3.12 |
| *Pseudomonas sp.* (SC 75) | 50 | 25 |
| *Pseudomonas sp.* (SC 76) | 6.25 | 3.12 |
| *Pseudomonas sp.* (SC 184) | 25 | 12.5 |
| *Pseudomonas sp.* (SC 190) | 12.5 | 6.25 |
| *Pseudomonas sp.* (SC 228) | 50 | 25 |
| *Pseudomonas sp.* (SC 273) | 12.5 | 6.25 |
| *Pseudomonas sp.* (SC 360) | 50 | 25 |
| *Pseudomonas sp.* (SC 694) | 50 | 25 |
| *Pseudomonas sp.* (SC 697) | 6.25 | 3.12 |
| *Pseudomonas sp.* (SC 1001) | 6.25 | 3.12 |
| *Pseudomonas sp.* (SC 1002) | 6.25 | 3.12 |
| *Pseudomonas sp.* (SC 1021) | 12.5 | 6.25 |
| *Pseudomonas sp.* (SC 1135) | 6.25 | 3.12 |

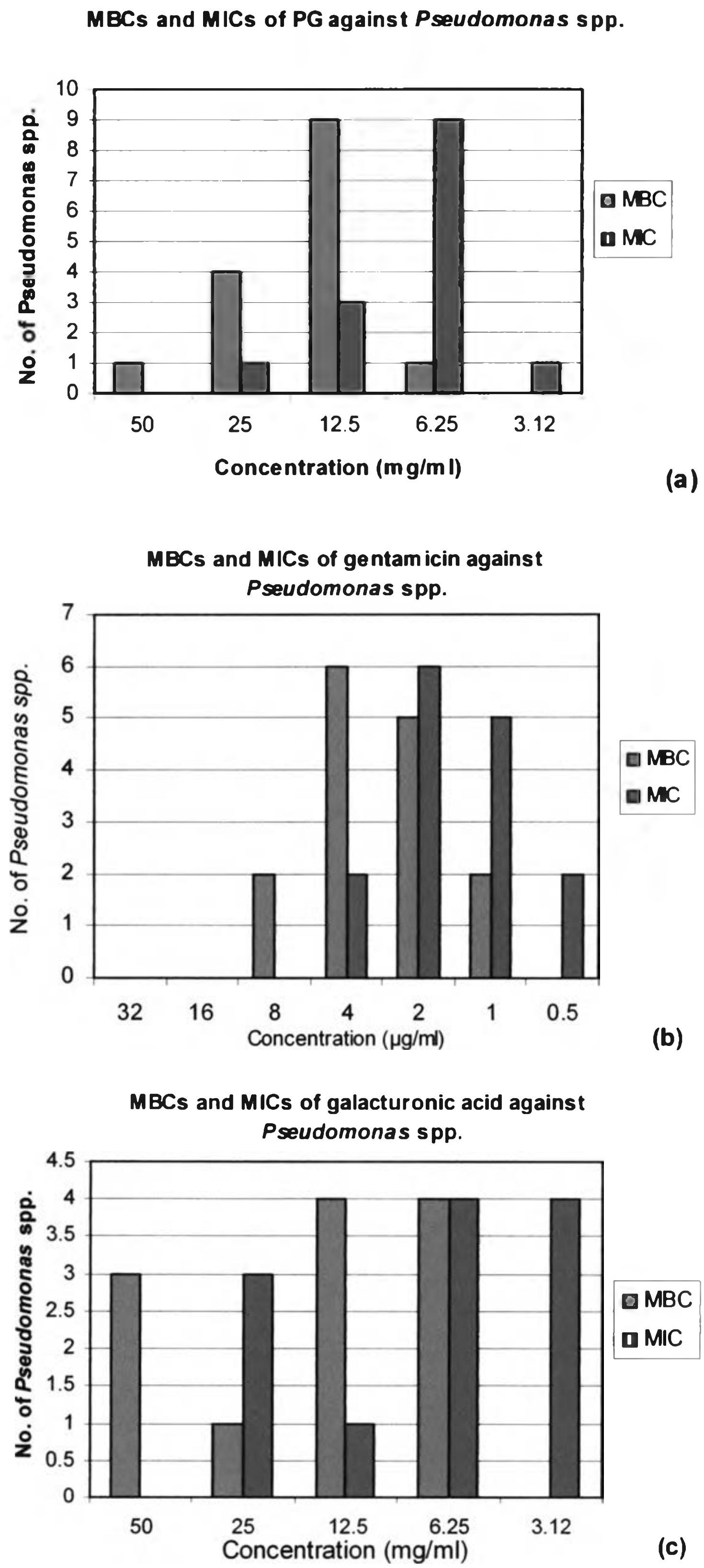



**Figure 10: A summary of MICs and MBCs of (a) polysaccharide gel (PG) Monthong cultivar (b) gentamicin and (c) galacturonic acid against mastitis causing bacteria, *Pseudomonas* spp., by broth microdilution test, SC = subchronic mastitis**

1.2.2 Determination of MIC and MBC of different cultivar of durian rinds (Monthong, Chanee and native from Chumporn province)

In comparison of the antibacterial activity of PG from different cultivar of durian; Monthong (M), Chanee (C) and native cultivar from Chumporn province (Ncv) against *S. aureus* and *E. coli*, it was found that the activity of the PG Monthong was less active than that of Chanee and native cultivar from Chumporn province. Interestingly, PG Chanee and native from Chumporn province were found to be more active than Monthong in inhibiting growth of *S. aureus* (Table 18). Also, PG Monthong, Chanee and native from Chumporn province showed very similar MBC and MIC in inhibitory growth of *E. coli* (Table 18)

**Table 18: MICs and MBCs of polysaccharide gel (PG) from varieties of durian against tested bacteria, by broth microdilution test**

| Bacteria | PG Native cultivar (mg/ml) | | PG Chanee (mg/ml) | | PG Monthong (mg/ml) | |
|---|---|---|---|---|---|---|
| | MBC | MIC | MBC | MIC | MBC | MIC |
| Staphylococci | | | | | | |
| *S. aureus* ATCC 29213 | 25 | 12.5 | 6.25 | 3.12 | 50 | 25 |
| *S. aureus* ATCC 25923 | 12.5 | 6.25 | 6.25 | 3.12 | 50 | 25 |
| *S. aureus* (SP2) | 6.25 | 3.12 | 6.25 | 3.12 | 50 | 25 |
| *S. aureus* (SP23) | 6.25 | 3.12 | 6.25 | 3.12 | 6.25 | 3.12 |
| *Escherichia coli* | | | | | | |
| *E. coli* ATCC 25922 | 12.5 | 6.25 | 12.5 | 6.25 | 12.5 | 6.25 |
| *E. coli* (CM 49) | 25 | 12.5 | 12.5 | 6.25 | 12.5 | 6.25 |
| *E. coli* (CM 85) | 12.5 | 6.25 | 12.5 | 6.25 | 50 | 25 |

1.3 Time kill analysis

1.3.1 PG Monthong (M)

Bactericidal activity of PG Monthong against 6 strains of PG susceptible bacteria, *S. aureus* (SP 2), *S. aureus* (SP 23), *S. aureus* ATCC 25923, *E. coli* (CM 49), *E. coli* (CM 85) and *E. coli* ATCC 25922, was demonstrated by time kill analysis. The PG inhibitory effect on bacterial survival during a 24 hr. period of time at 37°C was analyzed. Bacterial cells were cultivated in NSS, media without PG, media with PG at its MBC and MIC. In NSS, bacteria were normally survived in the static level after incubation. The colony counts of bacteria that cultivated in media with MIC of PG mostly they were slowly declined throughout the incubation time. While, the colony counts of bacteria that cultivated in media with MBC of PG were declined to 0% within 24 hr of incubation. Time kill analysis illustrated that PG at the concentration of 50, 6.25, 25, 12.5, 50 and 12.5 mg/ml produced bactericidal activity in MHB medium against *S. aureus* (SP 2), *S. aureus* (SP 23), *S. aureus* ATCC 25923, *E. coli* (CM 49), *E. coli* (CM 85) and *E. coli* ATCC 25922, respectively, the colony counts were declined to 0% at 8, 2, 24, 12, 16 and 24 hr, respectively (Figure 11 and 15).

1.3.2 PG Chanee (C)

Time kill curves were performed for 6 bacteria, *S. aureus* and *E. coli* in the presence of PG Chanee at their respective MBC and MIC (Figure 12 and 16 ). Killing of *S. aureus* (SP 2) and *S. aureus* (SP 23) began almost immediately in 2 hr. In contrast, killing of *S. aureus* ATCC 25923, *E. coli* (CM 49), *E. coli* (CM 85) and *E. coli* ATCC 25922, was not apparent over the 12 hr of incubation, the colony counts were declined to 0% at 16, 16, 24 and 16 hr, respectively. Control suspensions without PG showed bacterial in viability over the same period.

1.3.3 PG Native cultivar (Ncv) from Chumporn province

Time kill curves were performed for bacterial isolate from cow mastitis, *S. aureus* and *E. coli* in the presence of PG at their respective MBC and MIC (Figure 13 and

17). To obtain the highest antibacterial agent performance it was very important to optimize the contact time between bacteria and PG. Initial counts for control sample were $5x10^5$ CFU/ml. At 12 hr, MBC of PG application produced 1 log reduction of *S. aureus* ATCC 25923, *E. coli* (CM 49) and *E. coli* (CM 85) compared with the control sample and, the colony counts were declined to 0% at 20, 16 and 16 hr, respectively. Killing of *S. aureus* (SP 2) and *S. aureus* (SP 23) and *E. coli* ATCC 25922 began in 2 hr, the colony counts were declined to 0% at 8, 2 and 8 hr, respectively.

1.3.4 Galacturonic acid

Bactericidal activity of galacturonic acid against 6 strains of PG susceptible bacteria, *S. aureus* (SP 2), *S. aureus* (SP 23), *S. aureus* ATCC 25923, *E. coli* (CM 49), *E. coli* (CM 85) and *E. coli* ATCC 25922, was demonstrated by time kill analysis. The PG inhibitory effect on bacterial survival during a 24 hr. period of time at 37°C was analyzed. Bacterial cells were cultivated in NSS, media without PG, media with MBC and MIC of PG. In NSS, bacteria were normally survived in the static level after incubation. The colony counts of bacteria that cultivated in media with MIC of PG mostly they were slowly declined throughout the incubation time, except *S. aureus* (SP 23) the bacterial decrease 5 log after 16 hr incubation. While, the colony counts of bacteria that cultivated in media with MBC of galacturonic acid were declined to 0% within 24 hr of incubation. Time kill analysis illustrated that PG at the concentration of 50, 6.25, 25, 12.5, 50 and 12.5 mg/ml, respectively produced bactericidal activity in MHB medium against *S. aureus* (SP 2), *S. aureus* ATCC 25923, *E. coli* (CM 49), *E. coli* (CM 85) and *E. coli* ATCC 25922, the colony counts were declined to 0% at 16, 24, 12, 24 and 12 hr, respectively (Figure 14 and 18).

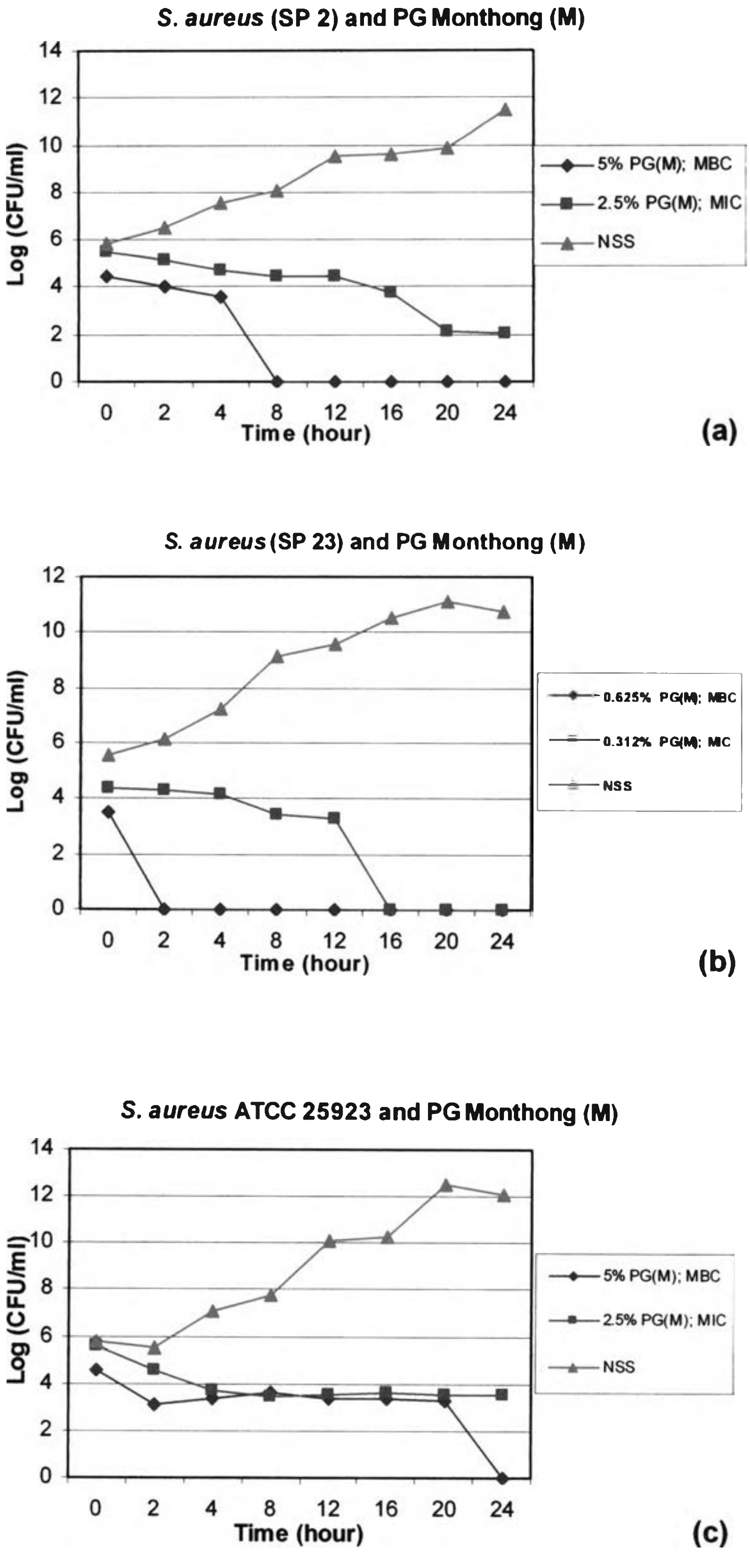



**Figure 11: Time- kill analysis of polysaccharide gel (PG Monthong) in (a) *Staphylococcus aureus* (SP2), (b) *Staphylococcus aureus* (SP 23) and (c) *Staphylococcus aureus* ATCC 25923 in Mueller hinton broth**

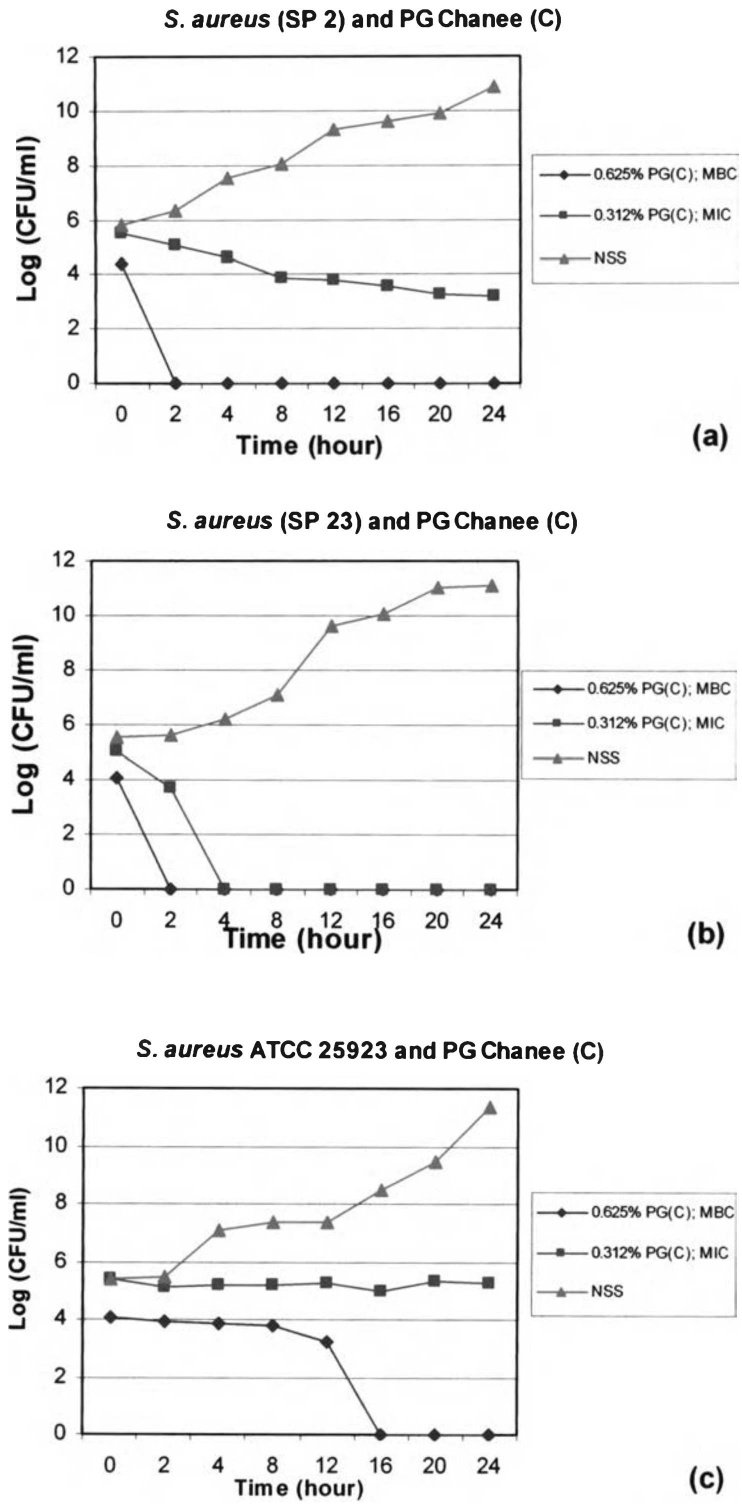
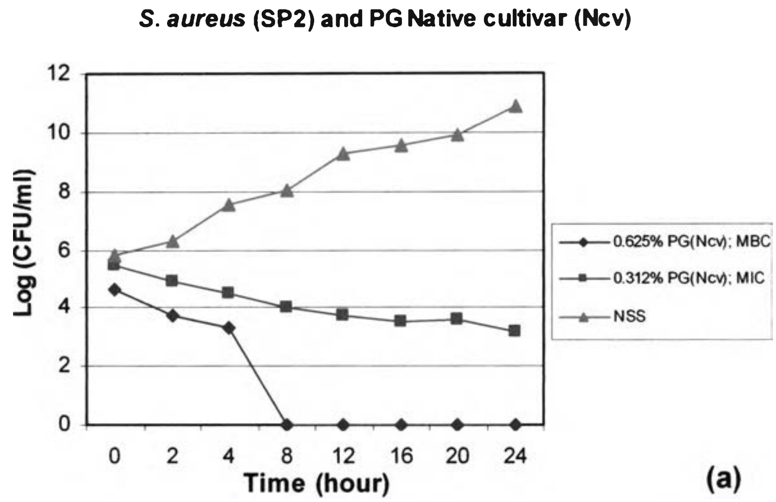
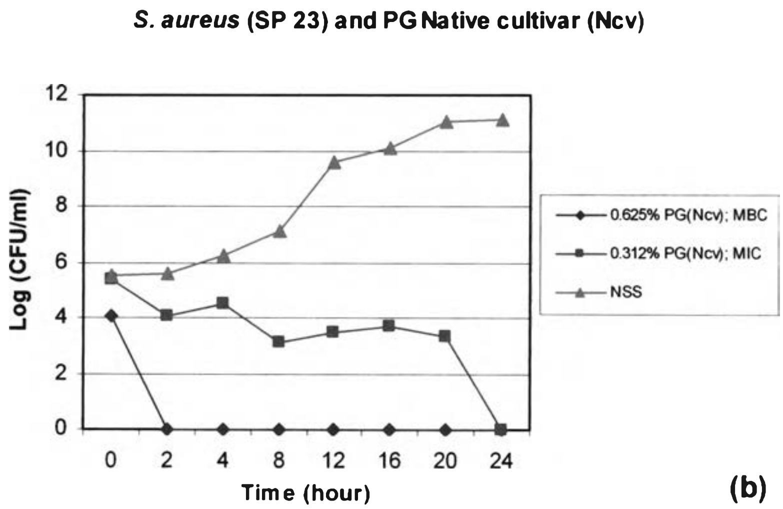


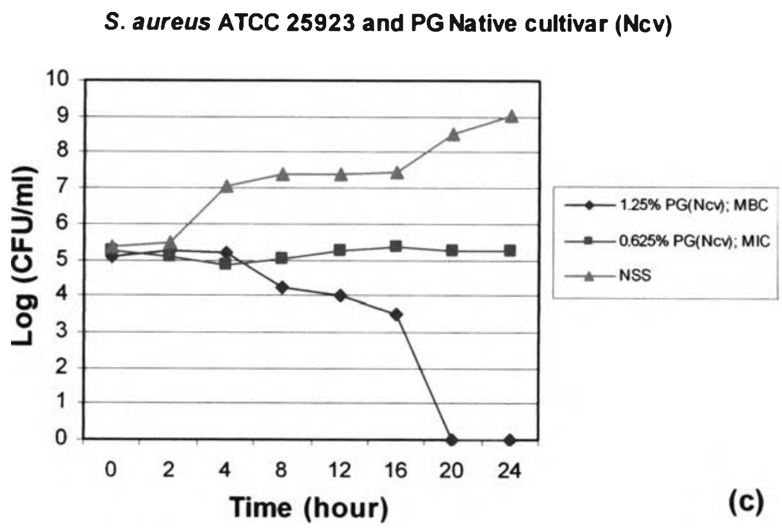

**Figure 12: Time- kill analysis of polysaccharide gel (PG Chanee) in (a) *Staphylococcus aureus* (SP2), (b) *Staphylococcus aureus* (SP 23) and (c) *Staphylococcus aureus* ATCC 25923 in Mueller hinton broth**




(a)

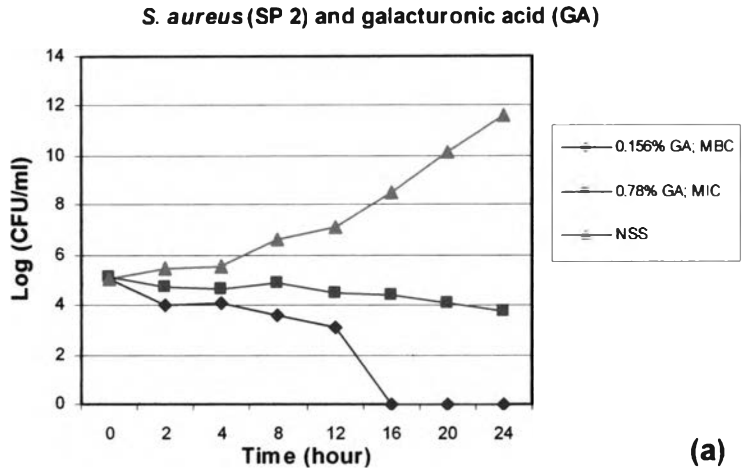



(b)

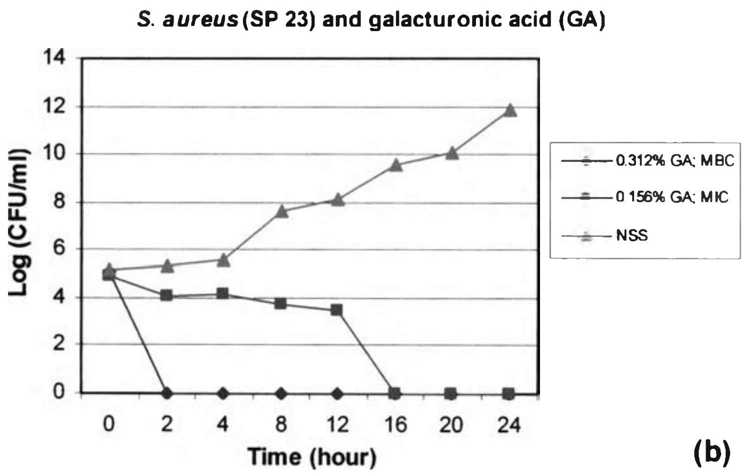



(c)

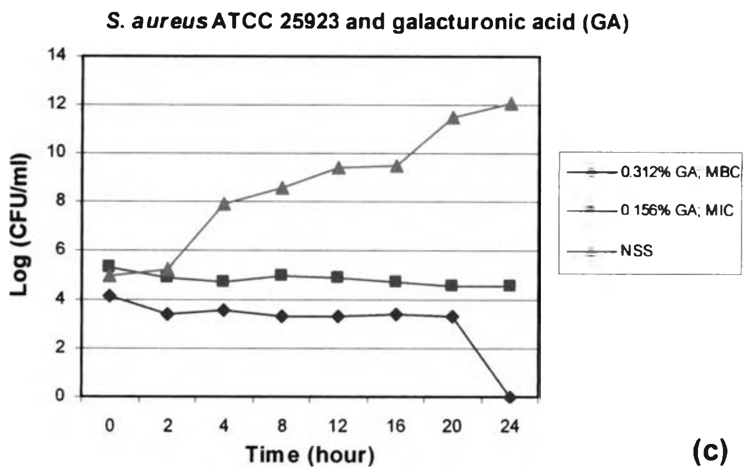
**Figure 13: Time- kill analysis of polysaccharide gel (PG Native cultivar) in (a) *Staphylococcus aureus* (SP2), (b) *Staphylococcus aureus* (SP 23) and (c) *Staphylococcus aureus* ATCC 25923 in Mueller hinton broth**




(a)

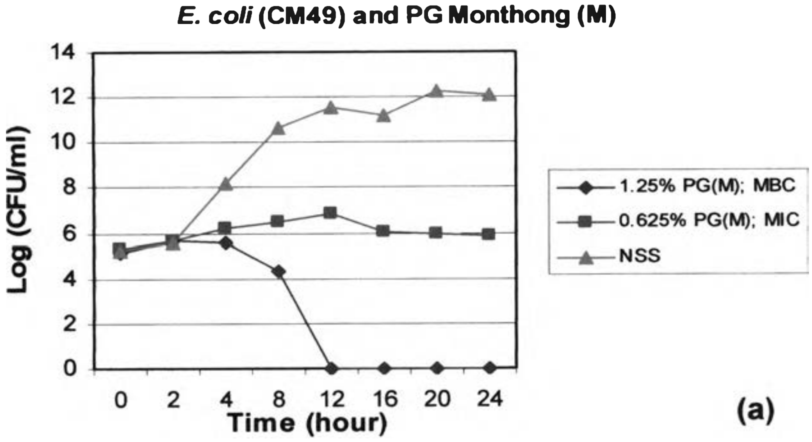



(b)

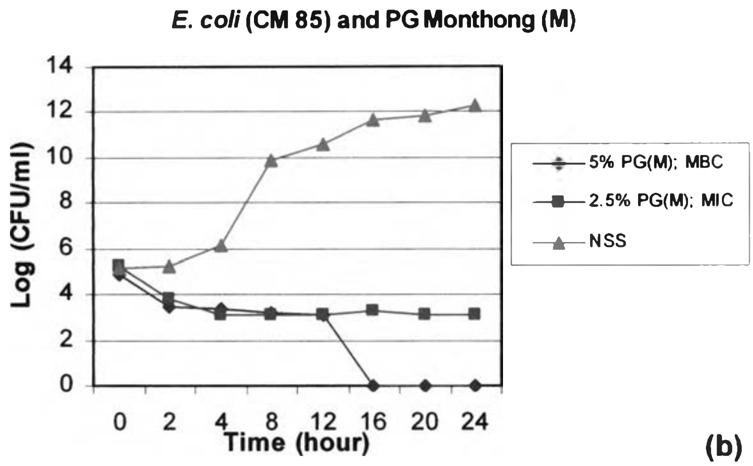



(c)

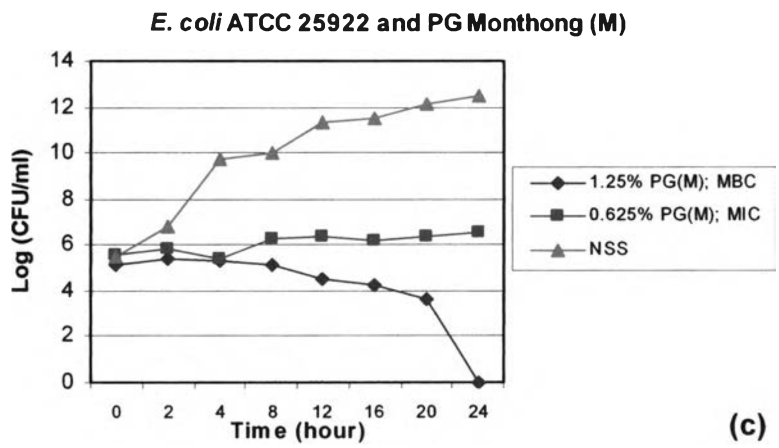
**Figure 14: Time- kill analysis of galacturonic acid in (a) *Staphylococcus aureus* (SP2), (b) *Staphylococcus aureus* (SP 23) and (c) *Staphylococcus aureus* ATCC 25923 in Mueller hinton broth**




(a)

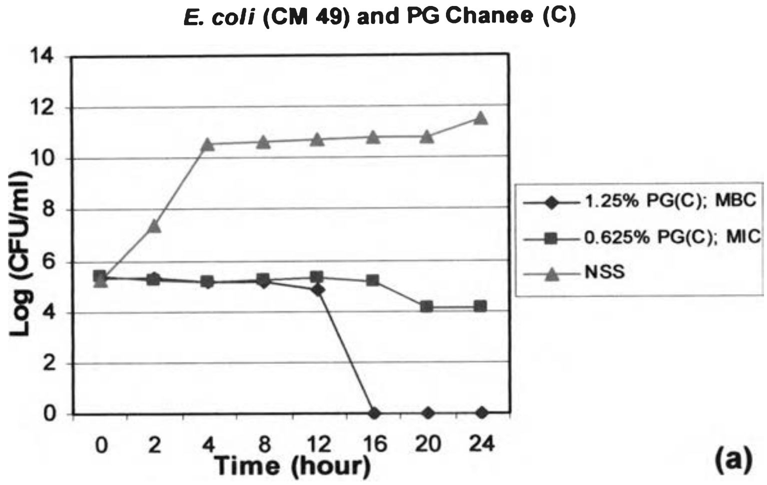



(b)

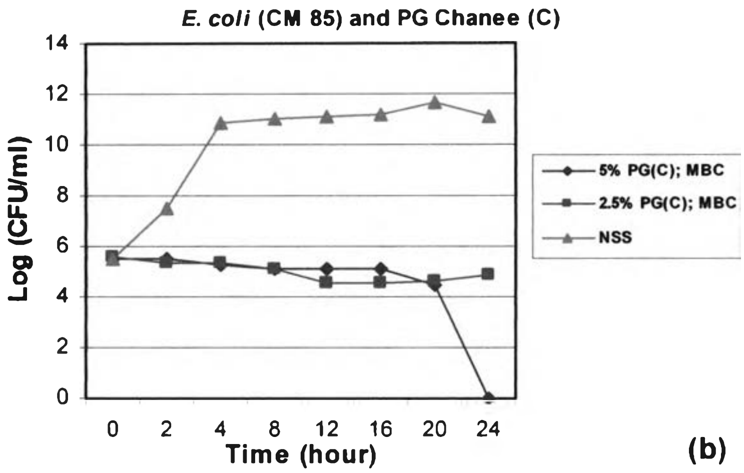



(c)

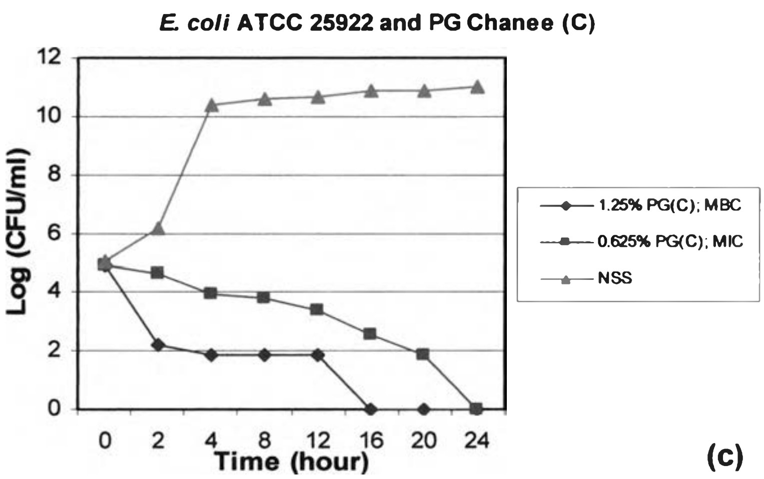
**Figure 15:Time- kill analysis of polysaccharide gel (PG Monthong) in (a) *Escherichia coli* (CM 49), (b) *Escherichia coli* (CM 85) and (c) *Escherichia coli* ATCC 25923 in Mueller hinton broth**




(a)




(b)




(c)

**Figure 16: Time- kill analysis of polysaccharide gel (PG Chanee) in (a) *Escherichia coli* (CM 49), (b) *Escherichia coli* (CM 85) and (c) *Escherichia coli* ATCC 25923 in Mueller hinton broth**

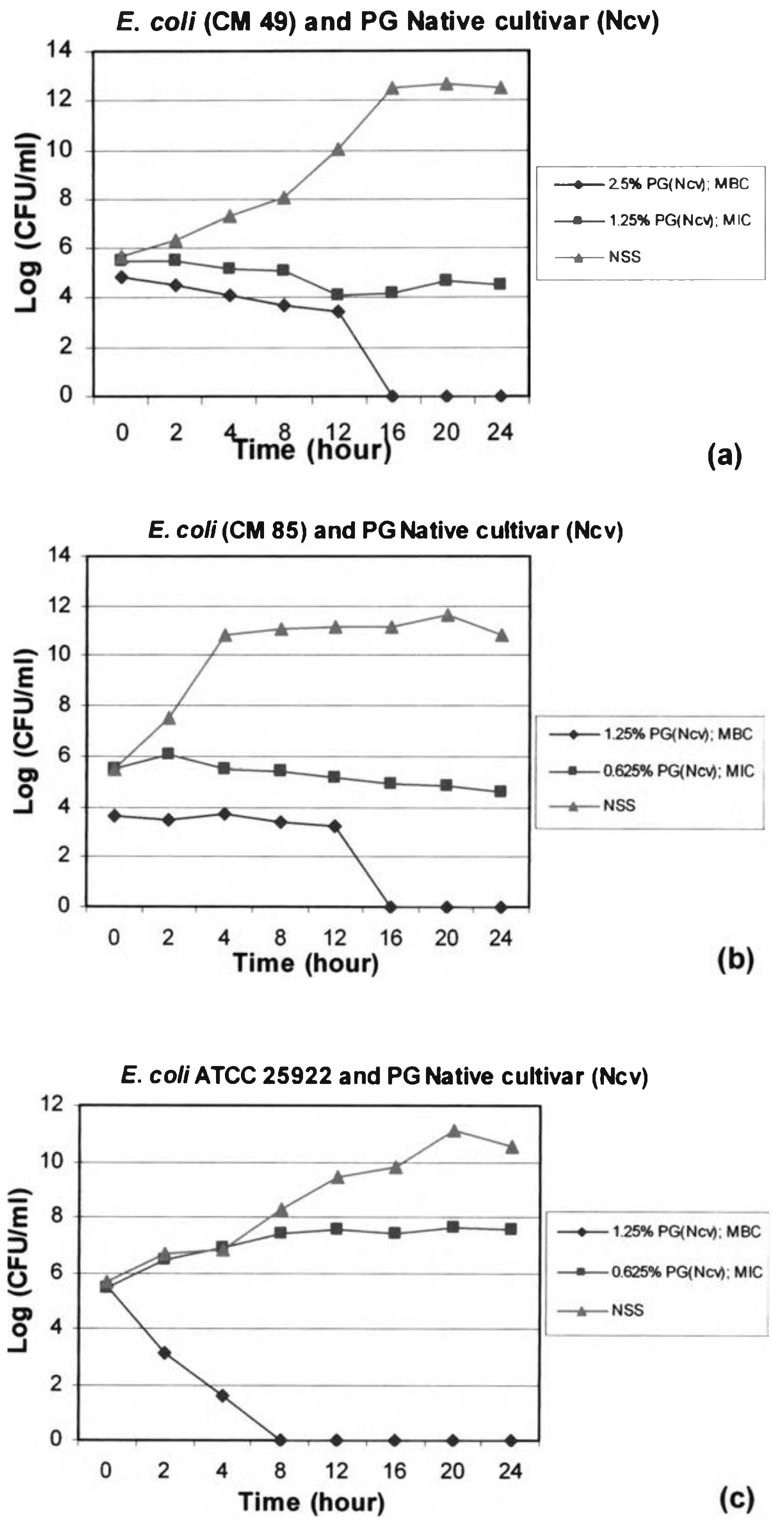



**Figure 17: Time- kill analysis of polysaccharide gel (PG Native cultivar) in (a) *Escherichia coli* (CM 49), (b) *Escherichia coli* (CM 85) and (c) *Escherichia coli* ATCC 25923 in Mueller hinton broth**

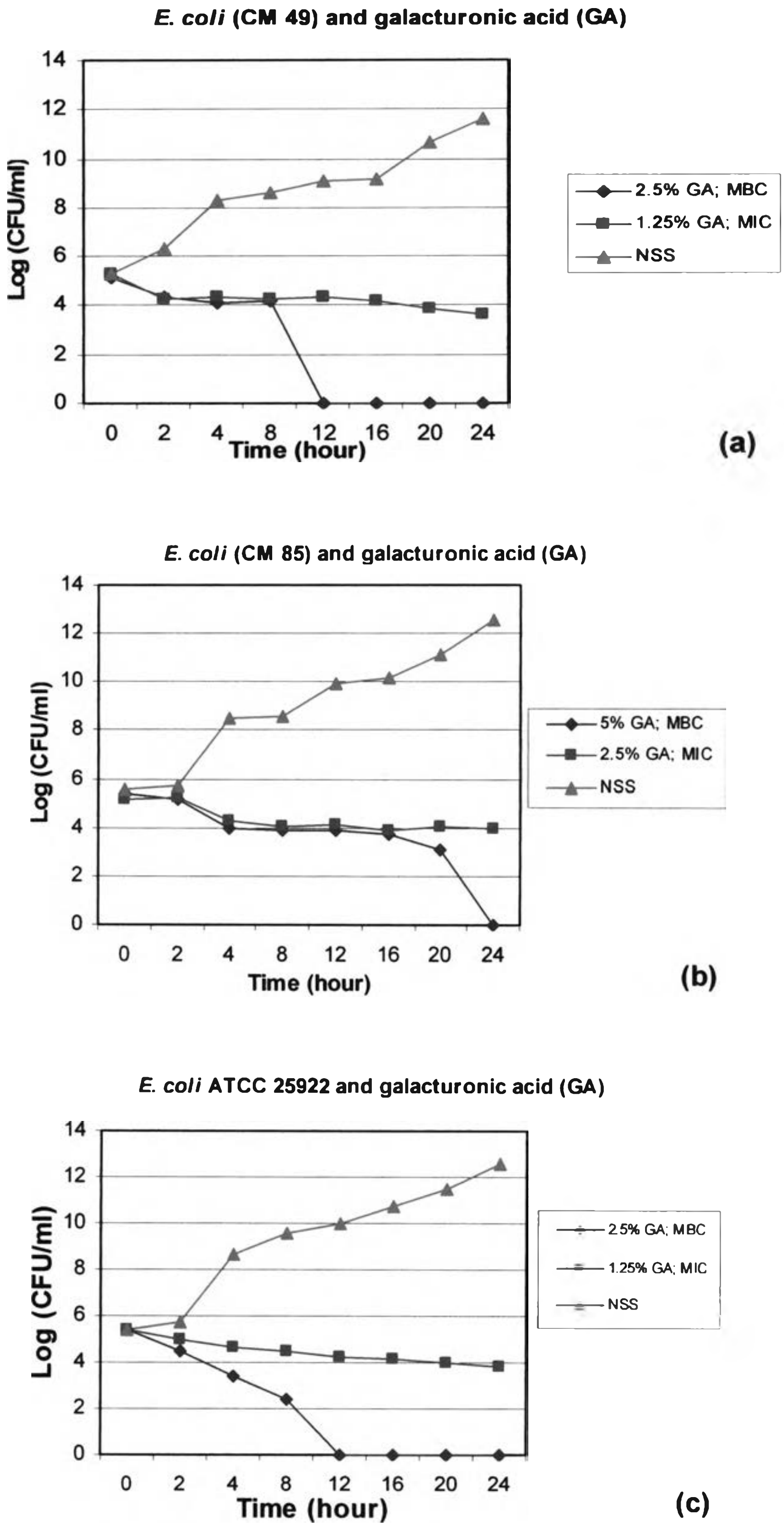



**Figure 18: Time- kill analysis of galacturonic acid in (a) *Escherichia coli* (CM 49), (b) *Escherichia coli* (CM 85) and (c) *Escherichia coli* ATCC 25923 in Mueller hinton broth**

2. The effect of various factors on inhibitory activity of PG

2.1 Temperature effect: Temperature had a substantial influence on the antibacterial activity of PG. At incubation temperature (37°C), PG showed killing effect against *S. aureus* and E. coli after 24 hr incubation. Within the range of 25 to 37°C, PG's bactericidal activity against *E. coli* decreased with decreasing temperature to 25°C (Figure 19 and 20). At incubation temperature (25°C), numbers of colony count of *E. coli* was not declined to zero. Whereas number of colony count of *S. aureus* declined to zero as showed in Figure 20. The results indicated that temperature at 25°C effected in decreasing bactericidal activity of PG against growth of *E. coli* but not effected against *S. aureus*.

2.2 pH effect: The antibacterial activity of PG against *S. aureus* and *E. coli* was also affected by pH, with the greatest activity being found at acidic pH values. Cell number of *S. aureus* ATCC 25923 were inhibited 65.84 % at pH 5.0. Cell number of *S. aureus* (SP2) were inhibited 25.51 % at pH 5. Cell number of *S. aureus* (SP23) were inhibited 36.56 % at pH 5.0 (Figure 21).

Cell number of *E. coli* ATCC 25922 were were inhibited 59.9% at pH 5.0 and 24.66 % at pH 3.0. Cell number of *E. coli* (CM 49) were inhibited 32.1 % at pH 4.0. Cell number of *E. coli* (CM 85) were inhibited 24.29 % at pH 5.0 (Figure 22).

Effect of PG on inhibitory activity of galacturonic acid is also demonstrated in Figure 23 and 24 against *S. aureus* and *E. coli*, respectively.

2.3 Salt effect: The effect of NaCl on the antibacterial activity of polysaccharide gel was shown in Figure 25 and 26. In the control groups (without polysaccharide gel) 100mM NaCl alone effect on *E. coli* (Figure 26). In the presence of PG at its MBC with 25 or 100 mM NaCl, viable cells of *E. coli* were detected in experimental groups after 24 hr. Whereas killing effect of PG against *S. aureus* was not change in the present of NaCl (Figure 25).

2.4 Divalent cations effect: The divalent cations ($CaCl_2$, $BaCl_2$, and $MgCl_2$) at concentrations of 10 and 20 mM did not interfere the bactericidal effect of PG on *S. aureus* (Figure 27, 29 and 31, respectively). The divalent cations at concentration of 10 and 20 mM reduced the bactericidal effect of PG on *E. coli.* The average of *E. coli* cell numbers were inhibited approximately 70.55% to each divalent cations (Figure 28, 30 and 32, respectively). Except for the activity of $ZnCl_2$ on *S. aureus* and *E. coli*, showed that $ZnCl_2$ with MHB or with PG had against *S. aureus* and *E. coli* (Figure 33 and 34).

The effect of mono and divalent cations on percentage inhibitory activity of bacteria is also demonstrated in Table 19.

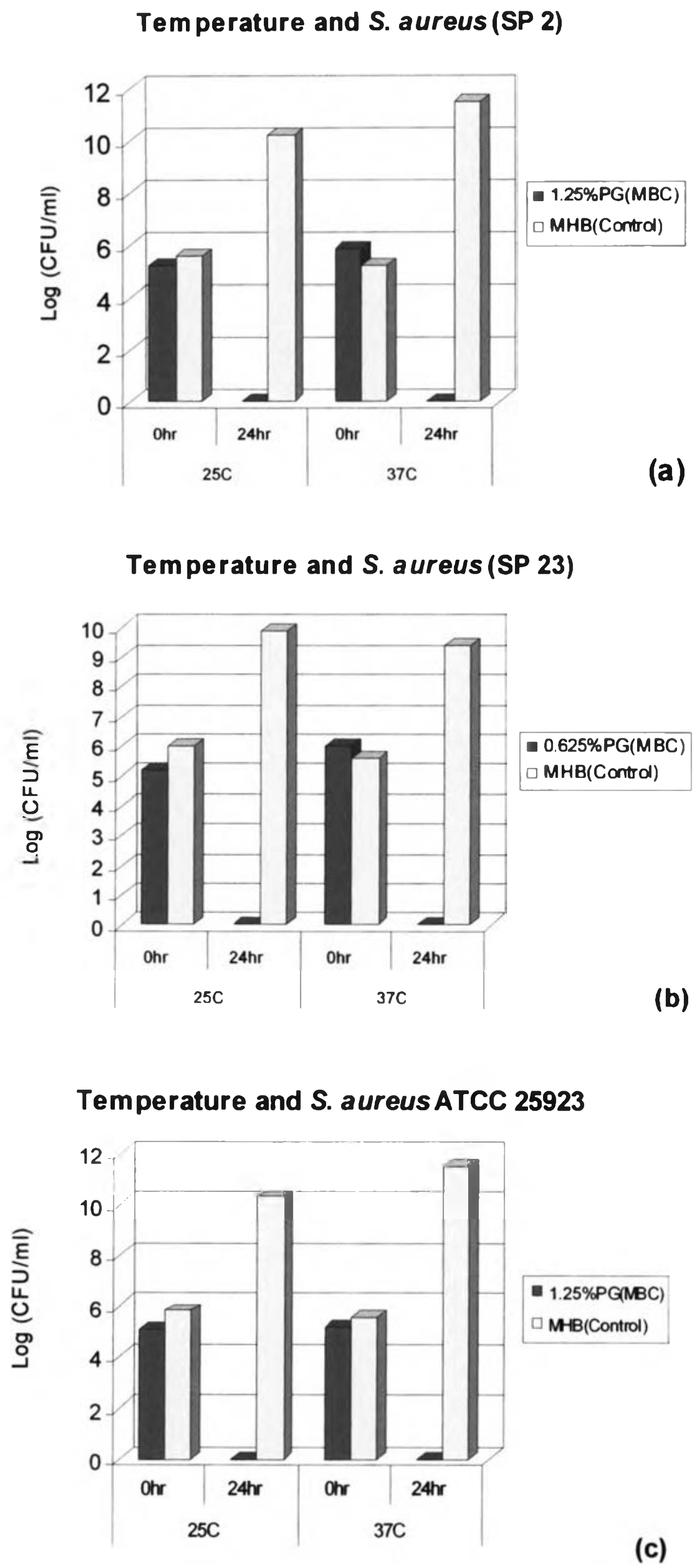



**Figure 19: Effect of temperature on inhibitory activity of PG against (a) *S. aureus* (SP2) (b) *S. aureus* (SP23) and (c) *S. aureus* ATCC 25923 after 24 hours incubation**

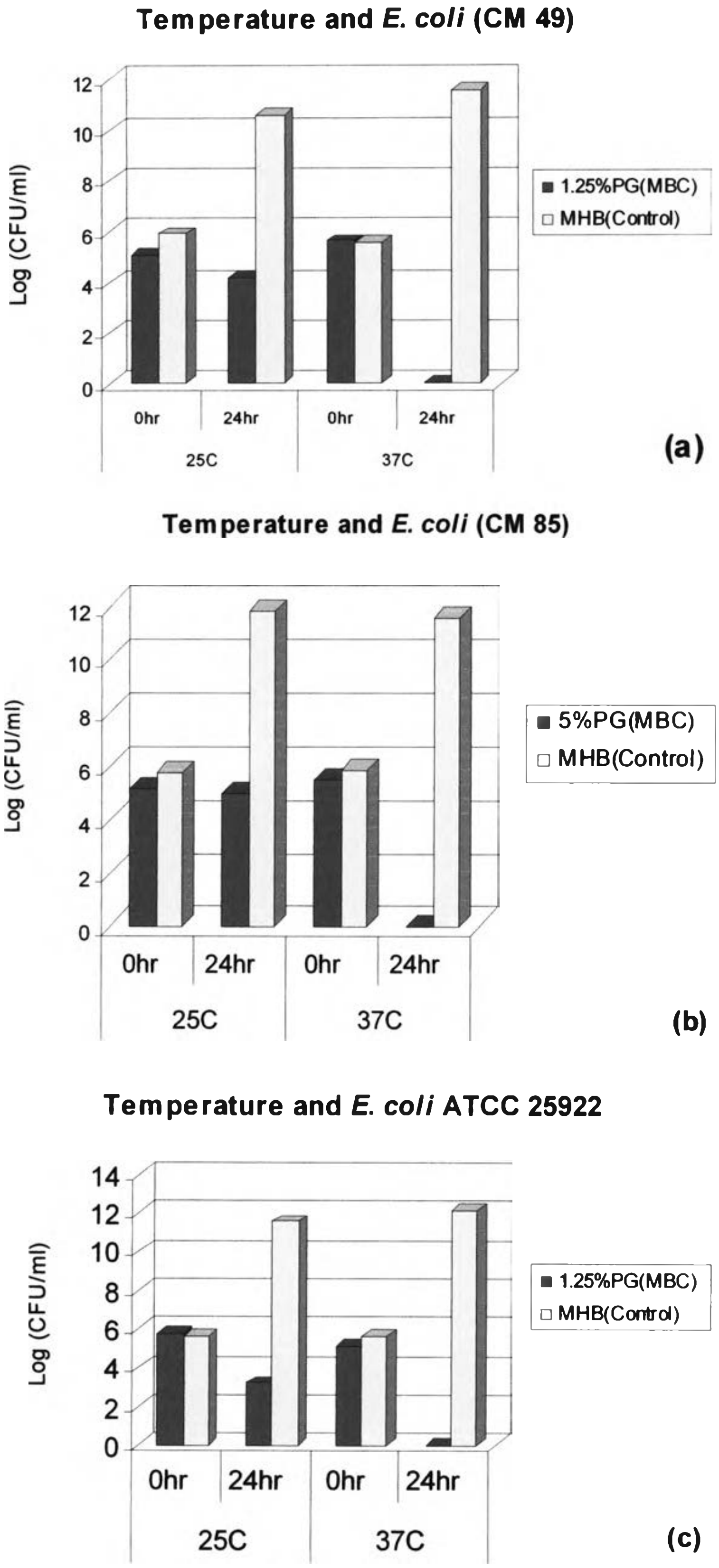



**Figure 20: Effect of temperature on inhibitory activity of PG against (a) *E. coli* (CM49), (b) *E. coli* (CM 85) and (c) *E. coli* ATCC 25922 after 24 hours incubation**

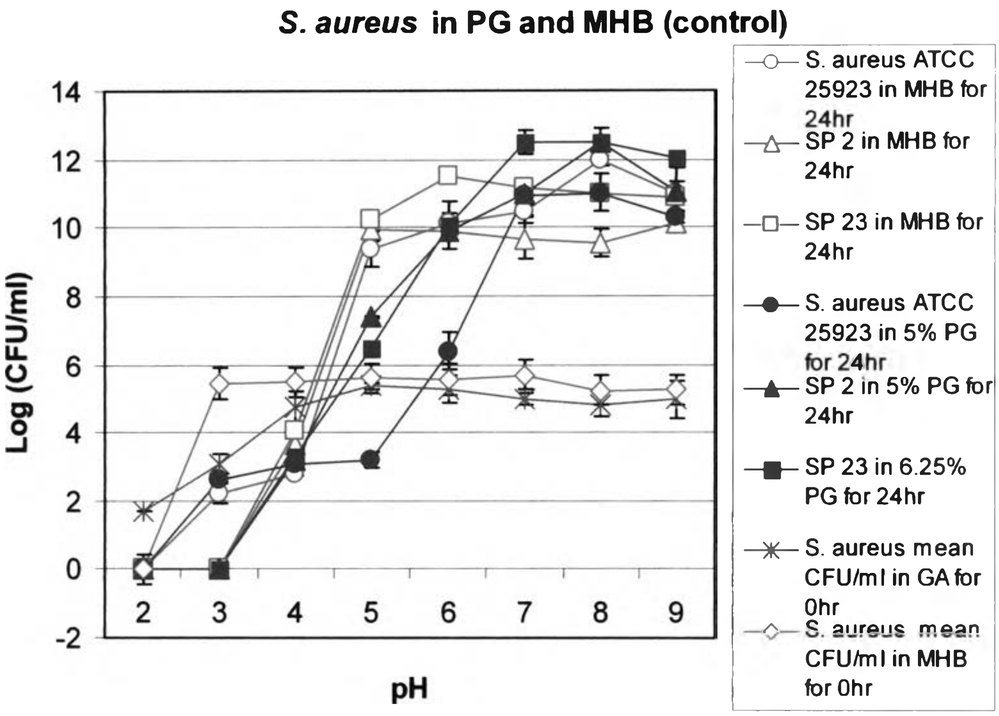



**Figure 21 : Effect of pH on inhibitory activity of PG against *S. aureus* after 24 hours incubation, pH of medium was adjusted by 1M HCl and 1M NaOH**

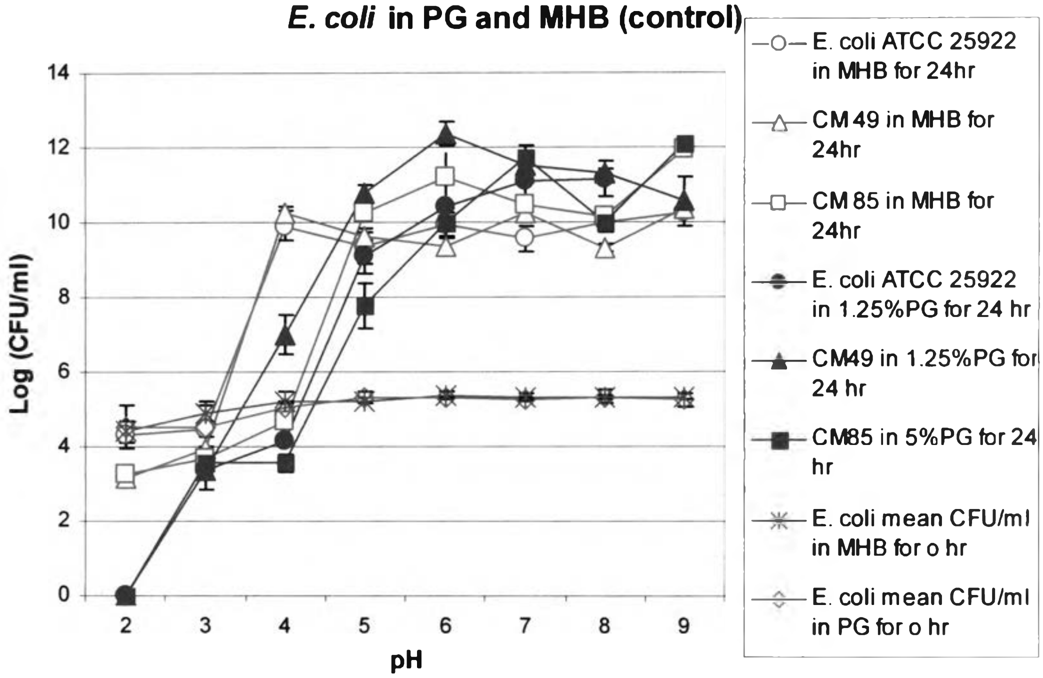



**Figure 22: Effect of pH on inhibitory activity of PG against *E. coli* after 24 hours incubation, pH of medium was adjusted by 1M HCl and 1M NaOH**

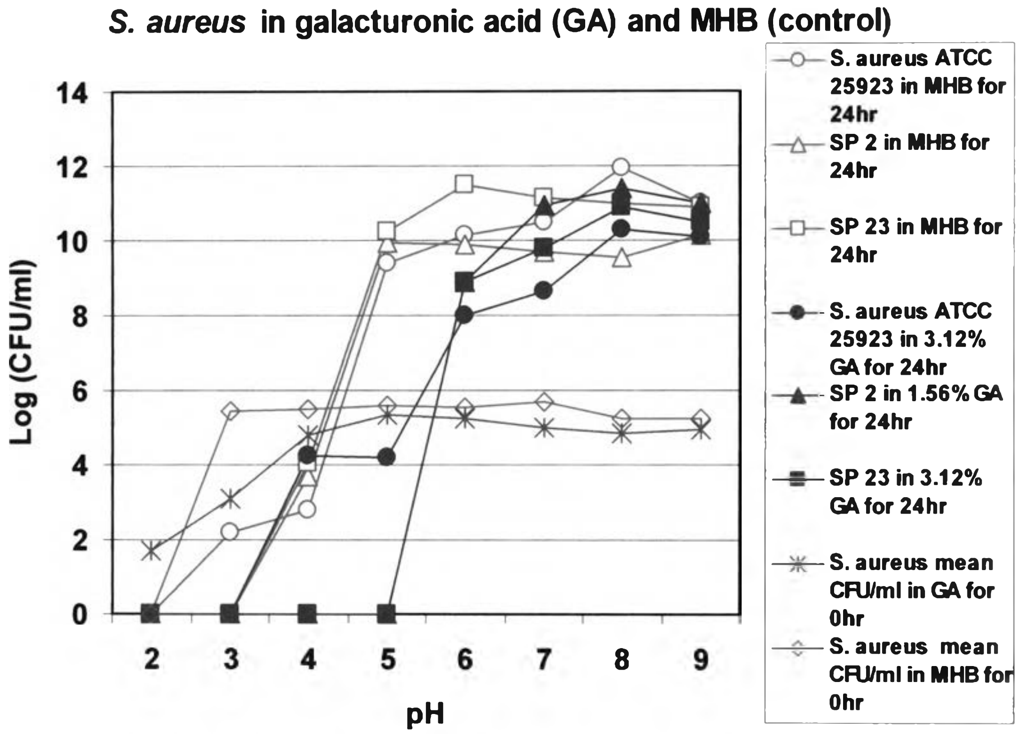



**Figure 23 : Effect of pH on inhibitory activity of galacturonic acid (GA) against *S. aureus* after 24 hours incubation, pH of medium was adjusted by 1M HCl and 1M NaOH**

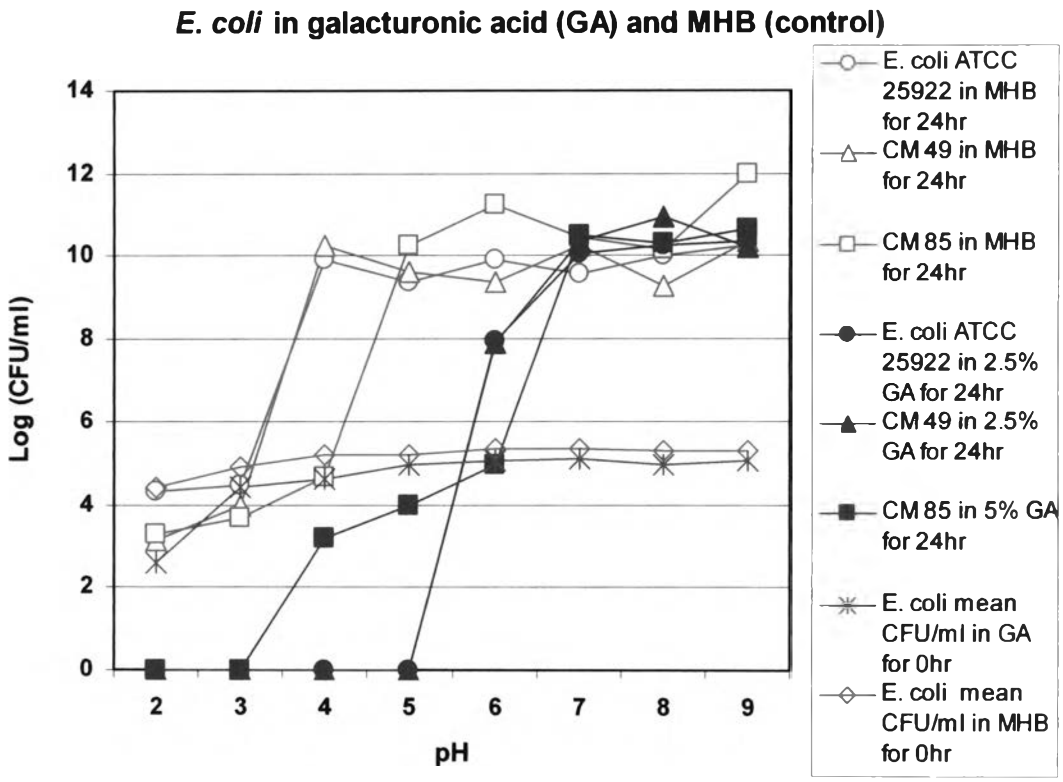
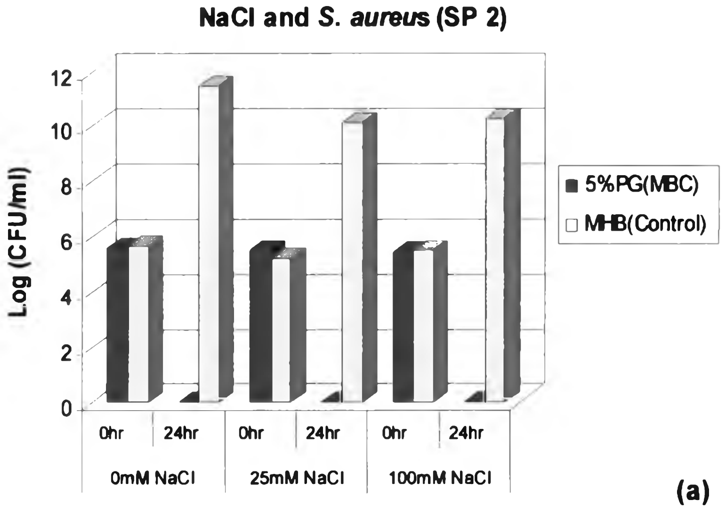
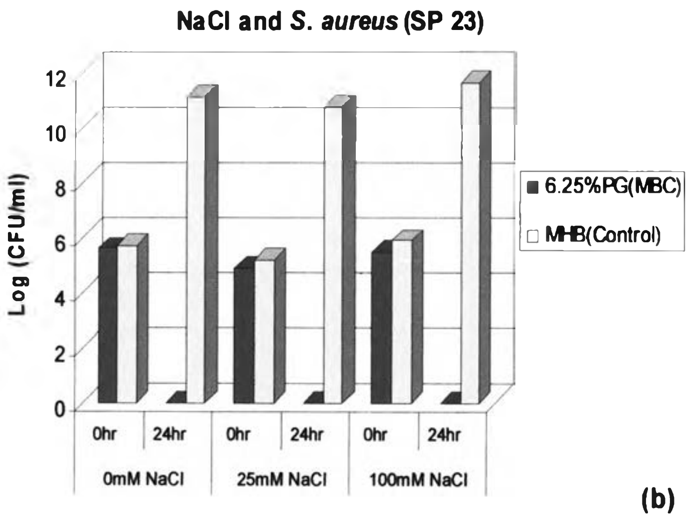


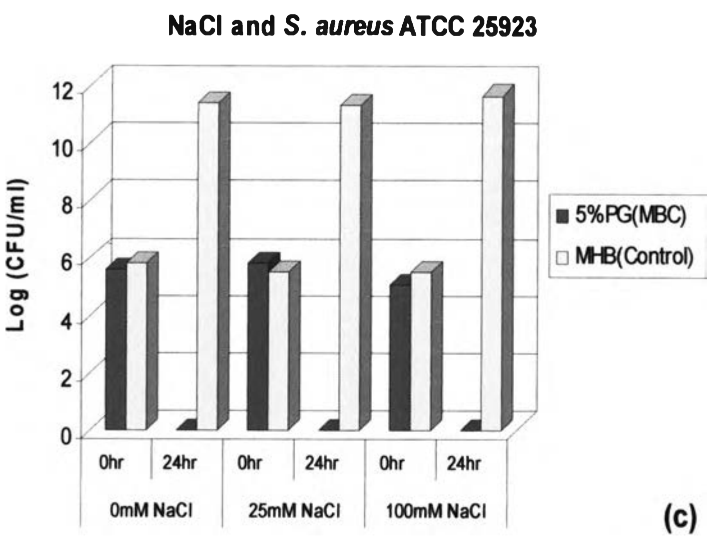

**Figure 24: Effect of pH on inhibitory activity of galacturonic acid against *E. coli* after 24 hours incubation, pH of medium was adjusted by 1M HCl and 1M NaOH**




(a)




(b)




(c)

**Figure 25: Effect of NaCl on inhibitory activity of PG against (a) *S. aureus* (SP2), (a) *S. aureus* (SP23) and (c) *S. aureus* ATCC 25923 after 24 hours incubation**

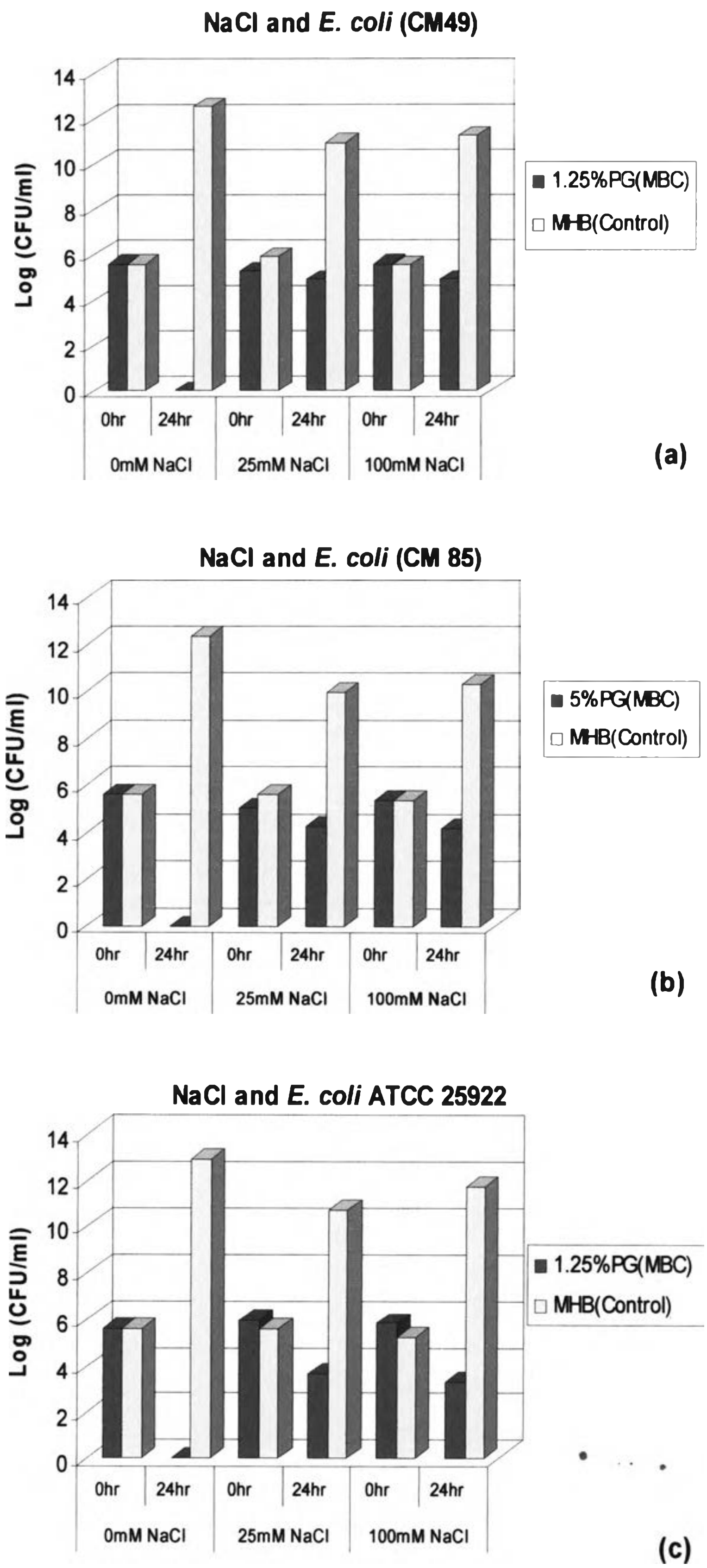



**Figure 26: Effect of NaCl on inhibitory activity of PG against (a) *E. coli* (CM 49), (b) *E. coli* (CM 85) and (c) *E. coli* ATCC 25922 after 24 hours incubation**

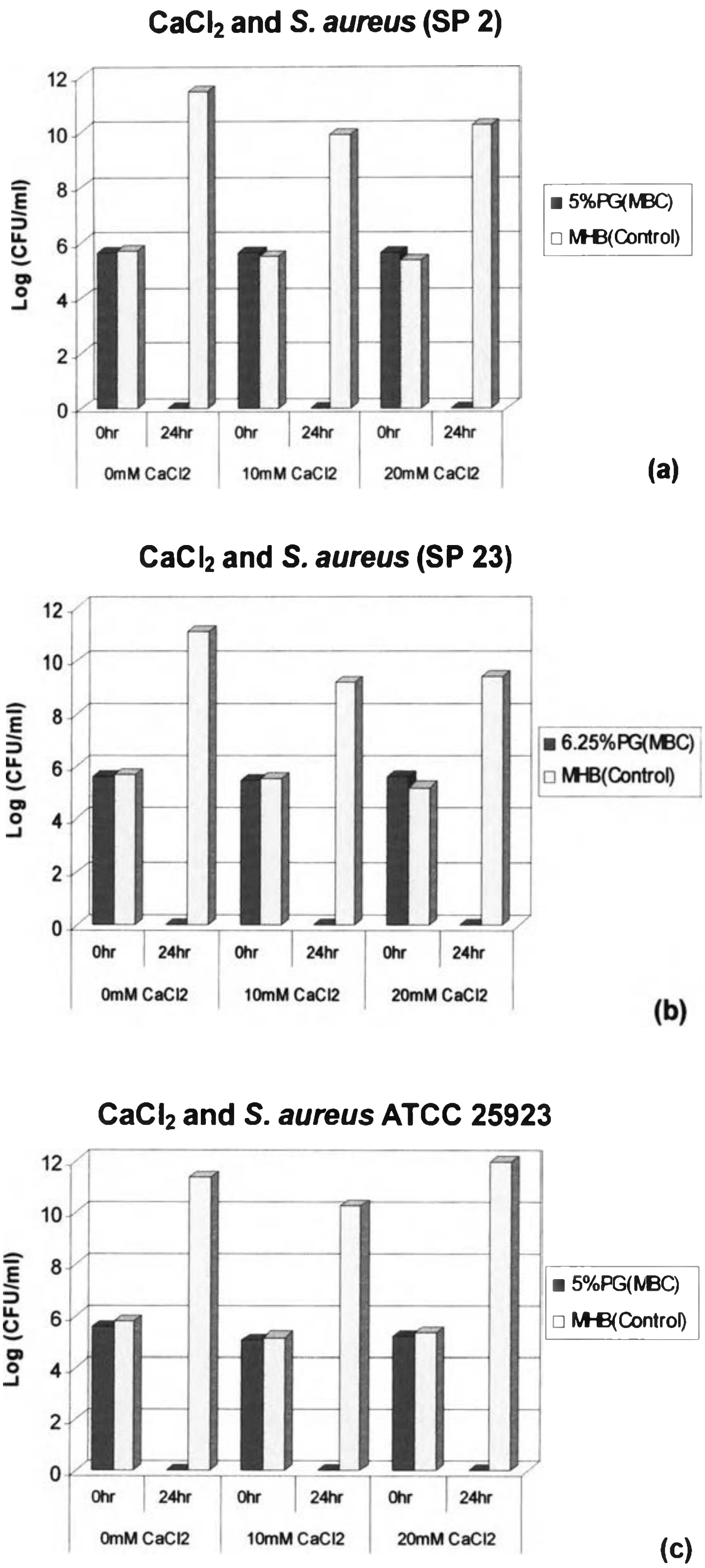



**Figure 27: Effect of $CaCl_2$ on inhibitory activity of PG against (a) *S. aureus* (SP2), (b) *S. aureus* (SP23) and (c) *S. aureus* ATCC 25923 after 24 hours incubation**

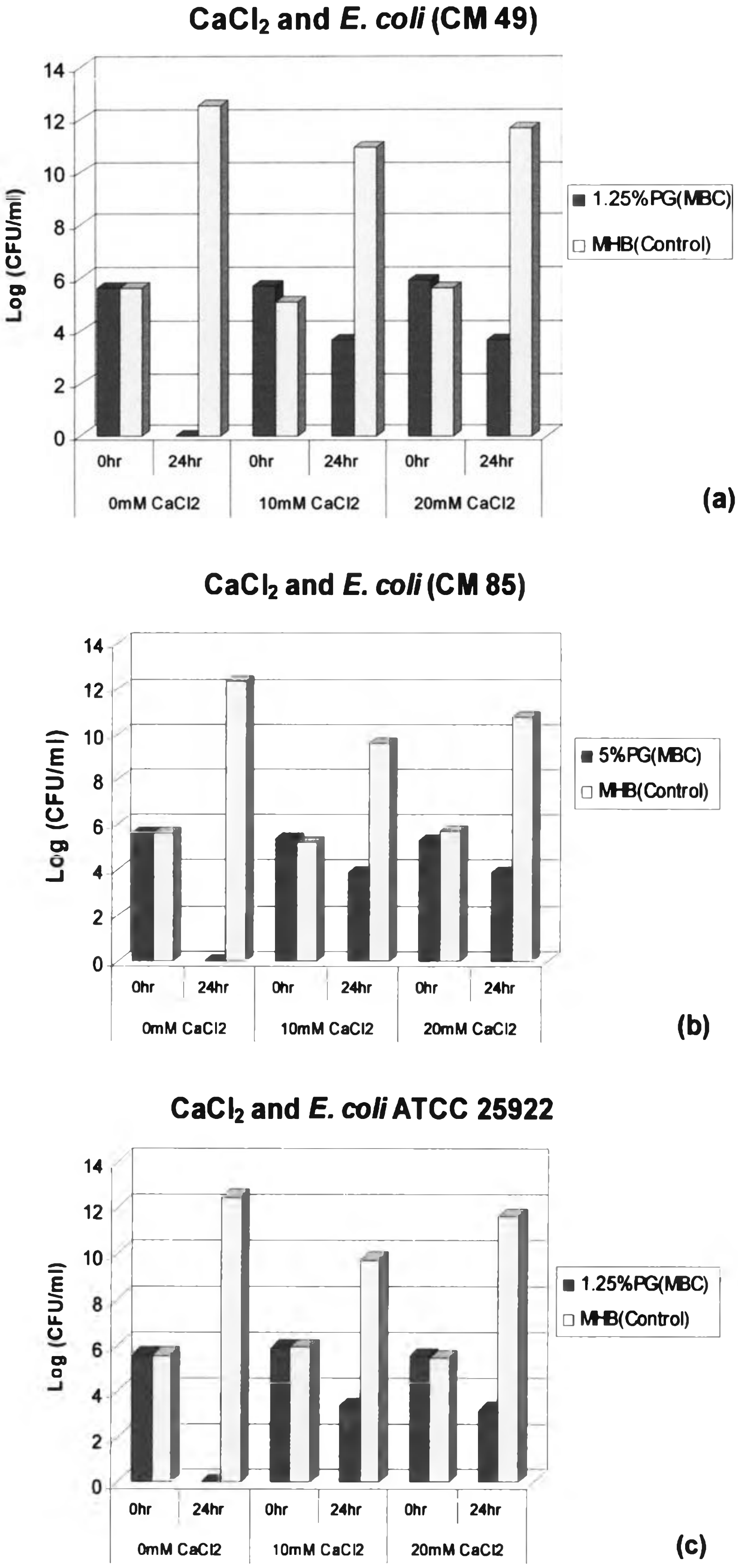



**Figure 28: Effect of $CaCl_2$ on inhibitory activity of PG against (a) *E. coli* (CM 49), (c) *E. coli* (CM 85) and (c) *E. coli* ATCC 25922 after 24 hours incubation**

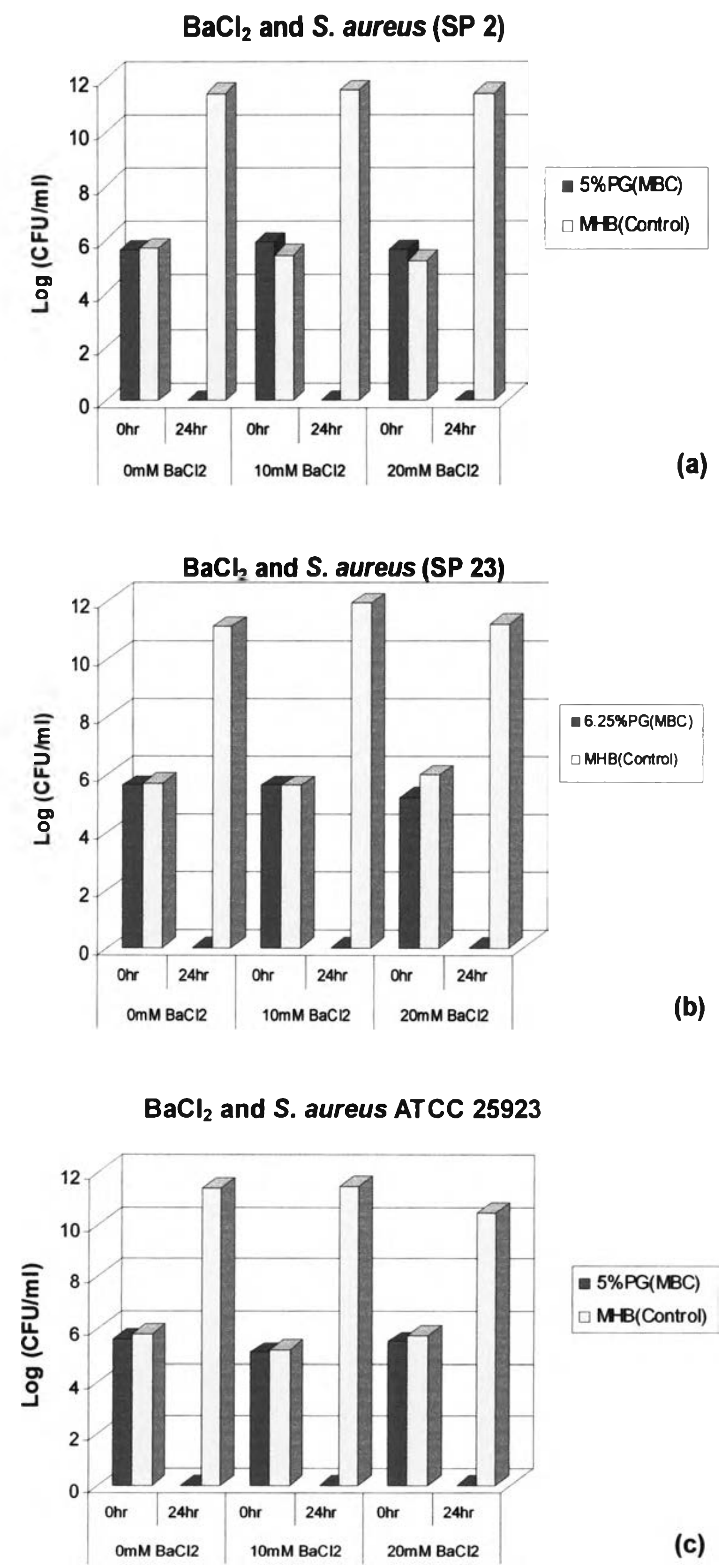
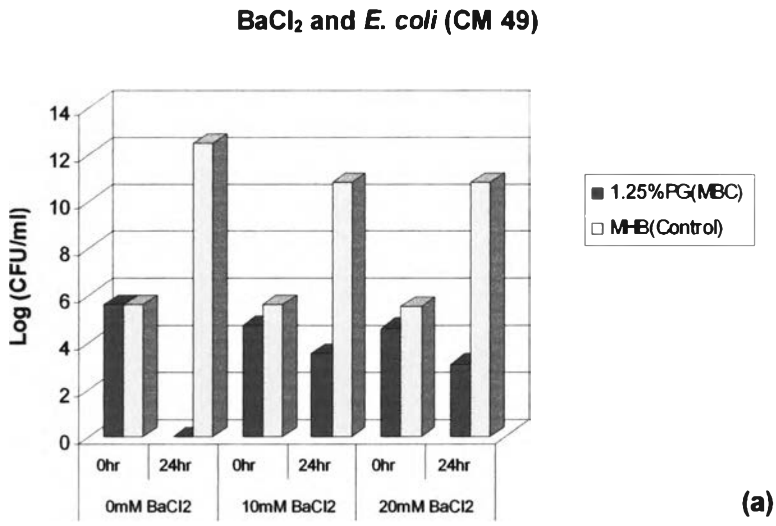
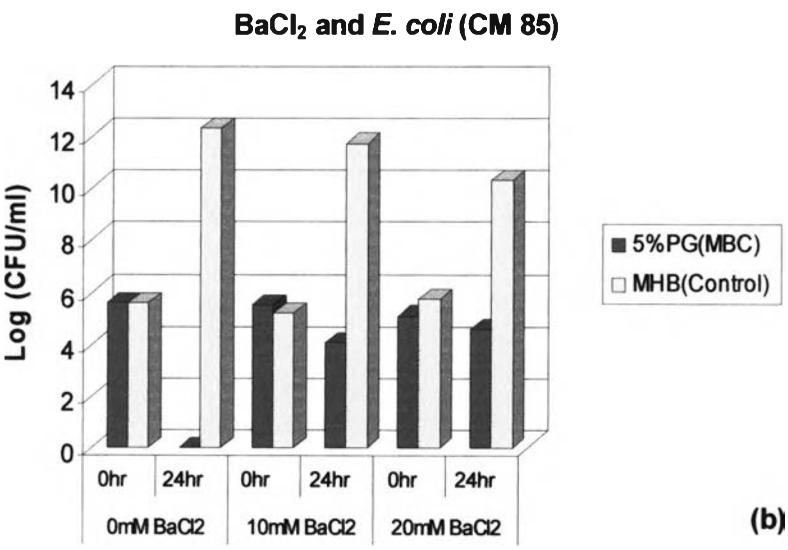



**Figure 29: Effect of $BaCl_2$ on inhibitory activity of PG against (a) *S. aureus* (SP2) (b) *S. aureus* (SP23) and (c) *S. aureus* ATCC 25923 after 24 hours incubation**



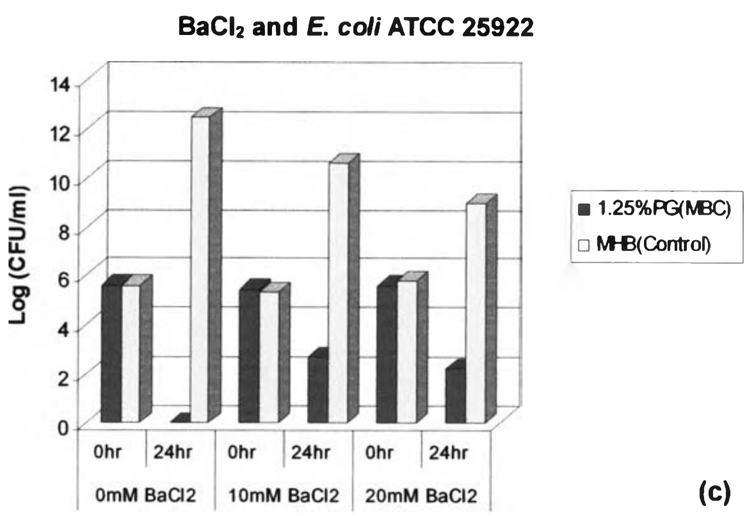




**Figure 30: Effect of $BaCl_2$ on inhibitory activity of PG against (a) *E. coli* (CM 49), (b) *E. coli* (CM 85) and (c) *E. coli* ATCC 25922 after 24 hours incubation**

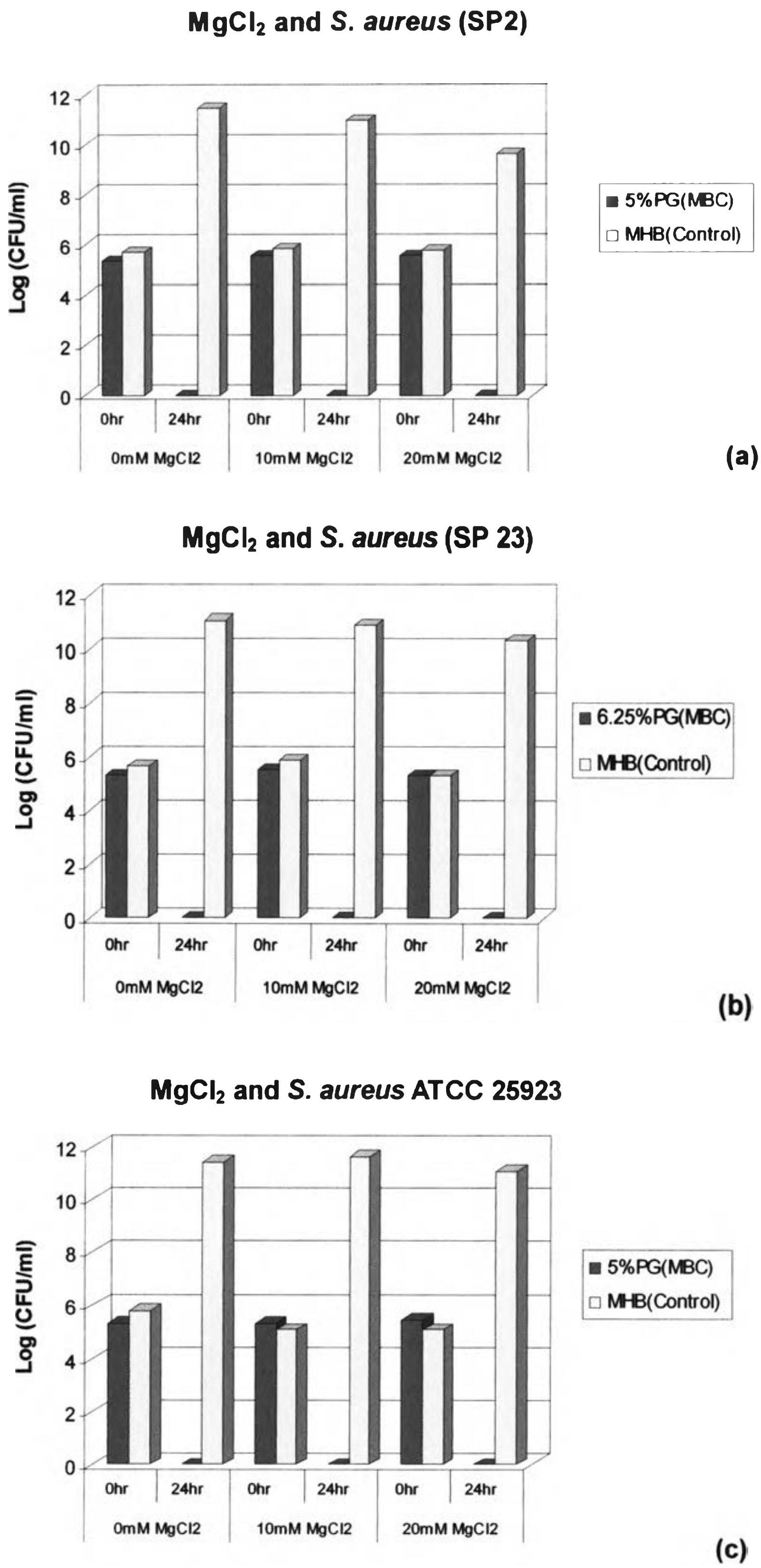



**Figure 31: Effect of $MgCl_2$ on inhibitory activity of PG against (a) *S. aureus* (SP2), (c) *S. aureus* (SP23) and (c) *S. aureus* ATCC 25923 after 24 hours incubation**

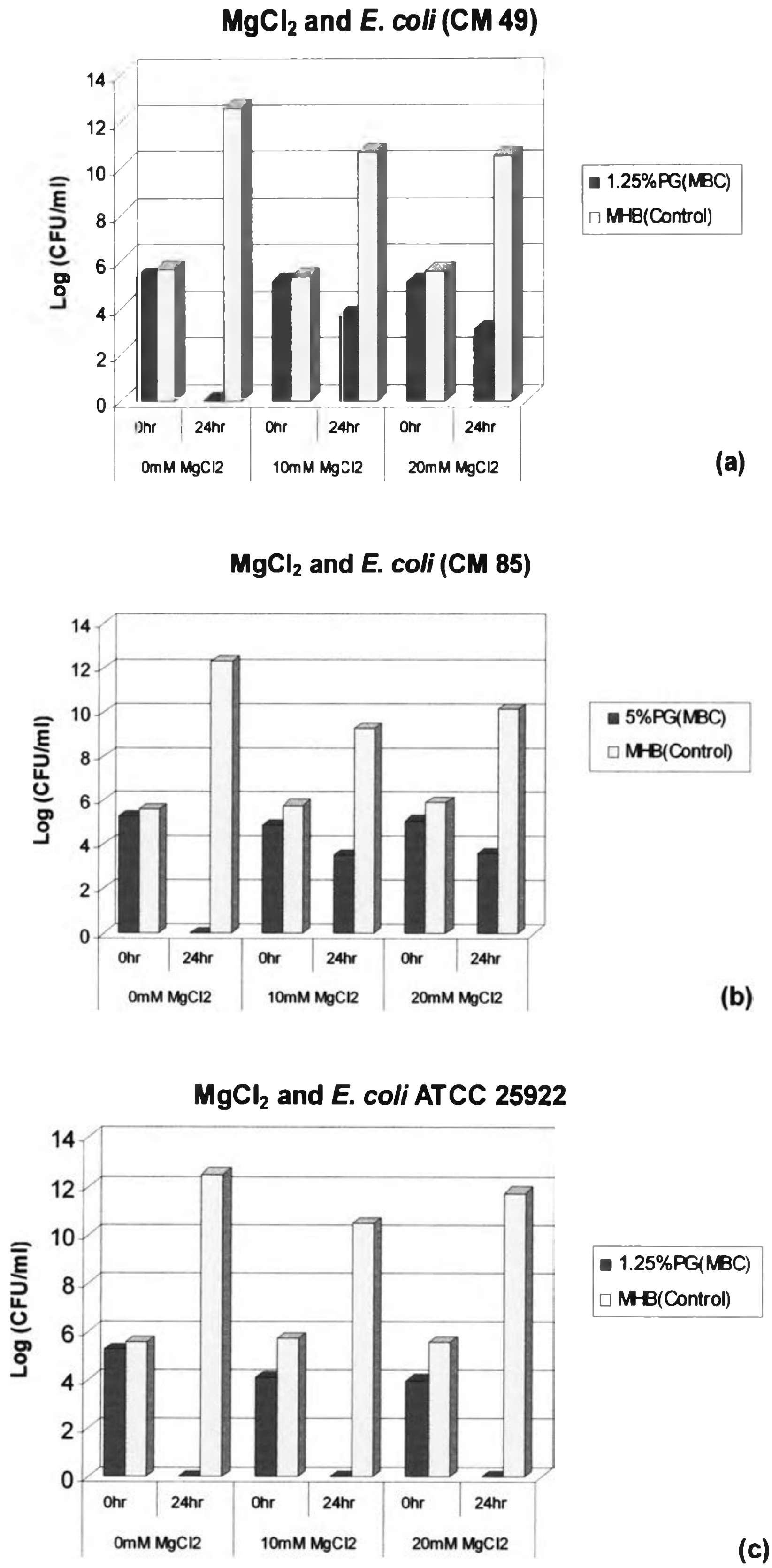
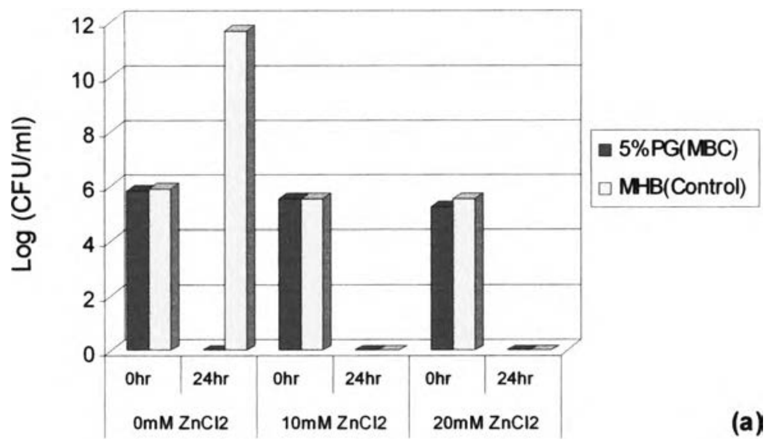


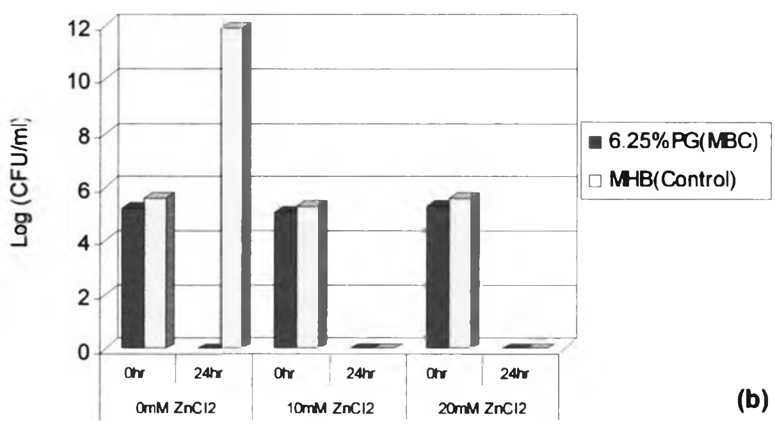

**Figure 32: Effect of $MgCl_2$ on inhibitory activity of PG against (a) *E. coli* (CM 49) *E. coli* (CM 85) and (c) *E. coli* ATCC 25922 after 24 hours incubation**

**$ZnCl_2$ and *S. aureus* (SP2)**



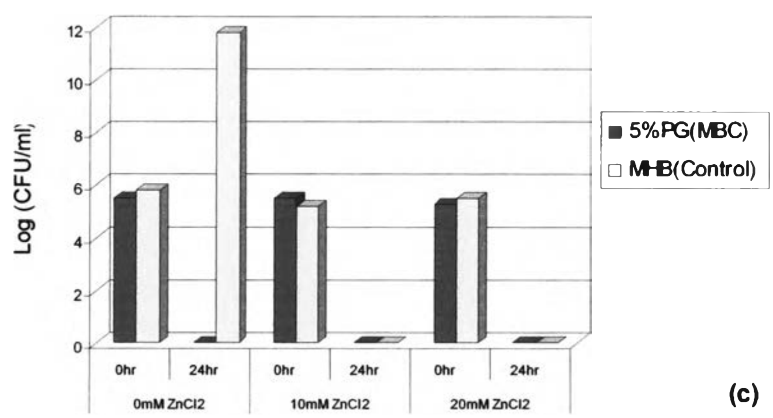

(a)

**$ZnCl_2$ and *S. aureus* (SP 23)**



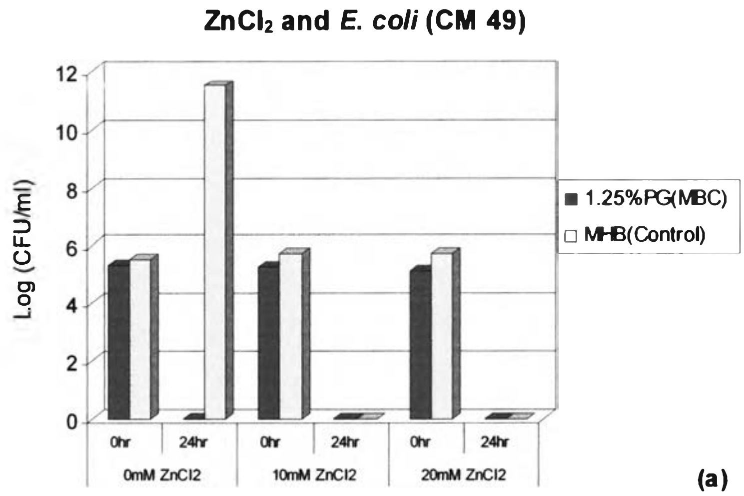

(b)

**$ZnCl_2$ and *S. aureus* ATCC 25923**

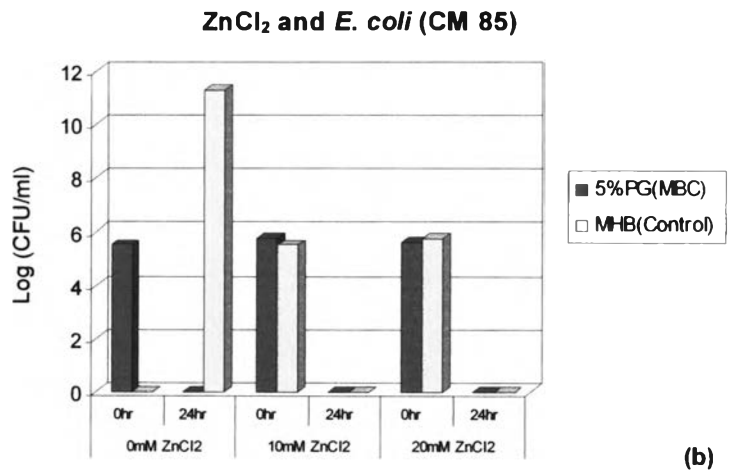



(c)

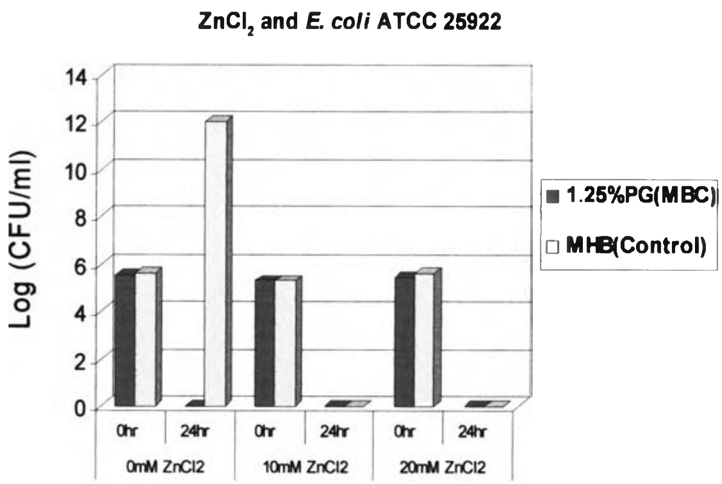
**Figure 33: Effect of $ZnCl_2$ on inhibitory activity of PG against (a) *S. aureus* (SP2), (b) *S. aureus* (SP23) and (c) *S. aureus* ATCC 25923 after 24 hours incubation**




(a)

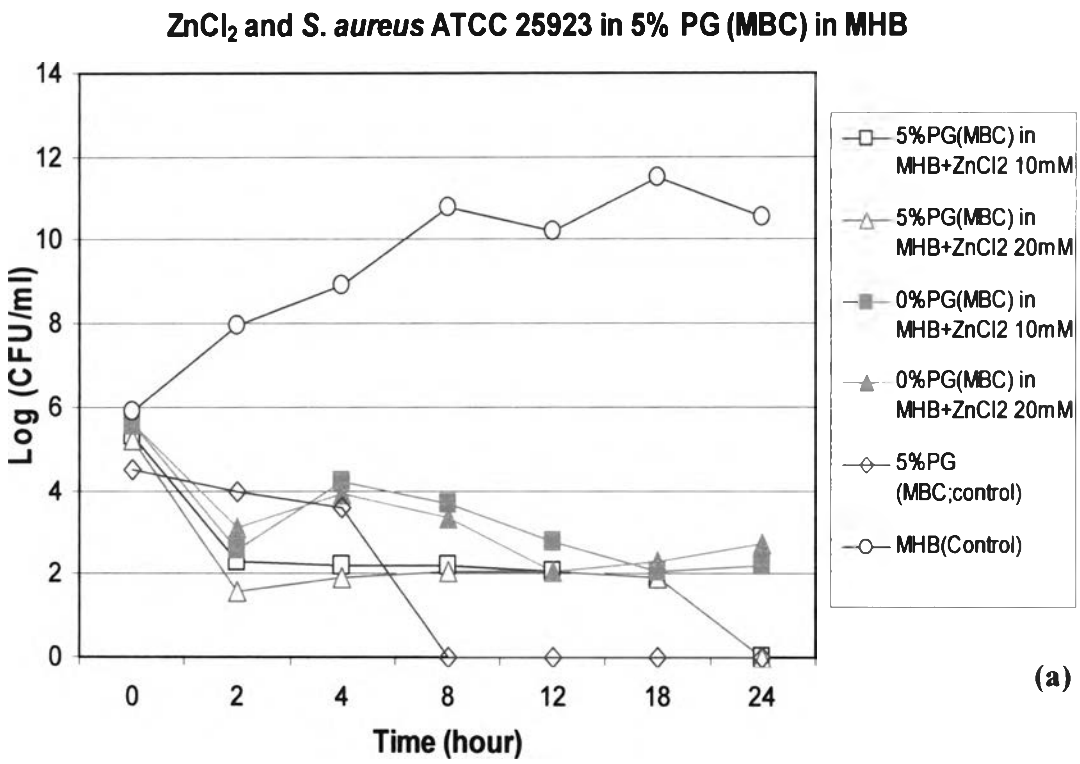



(b)

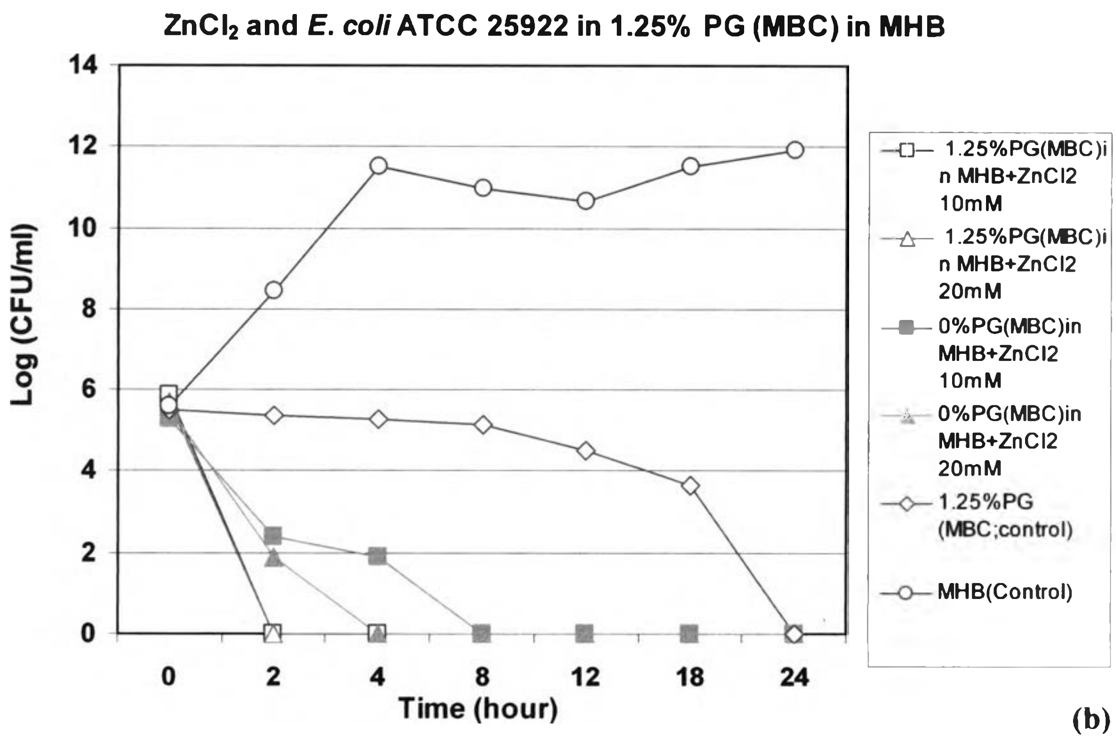



(c)

**Figure 34: Effect of $ZnCl_2$ on inhibitory activity of PG against (a) *E. coli* (CM 49) (b) *E. coli* (CM 85) and (c) *E. coli* ATCC 25922 after 24 hours incubation**




(a)




(b)

**Figure 35: Effect of $ZnCl_2$ on inhibitory activity of PG against (a) *S. aureus* ATCC 25923 and (b) *E. coli* ATCC 25922 after 24 hours incubation**

Table 19 A summary of mono and divalent cations on the inhibitory activity of PG against bacteria

| Cation/ concentration | % Inhibition | | | | | |
|---|---|---|---|---|---|---|
| | *S. aureus* (SP 2) | *S. aureus* (SP 23) | *S. aureus* ATCC 25923 | *E. coli* (CM 49) | *E. coli* (CM 85) | *E. coli* ATCC 25922 |
| $Na^+$ | | | | | | |
| 0mM NaCl | 100 | 100 | 100 | 100 | 100 | 100 |
| 25mM NaCl | 100 | 100 | 100 | 54.57 | 57.57 | 65.42 |
| 100mM NaCl | 100 | 100 | 100 | 56.17 | 59.51 | 71.86 |
| $Ca^{++}$ | | | | | | |
| 0mM $CaCl_2$ | 100 | 100 | 100 | 100 | 100 | 100 |
| 10mM $CaCl_2$ | 100 | 100 | 100 | 66.71 | 59.42 | 65.43 |
| 20mM $CaCl_2$ | 100 | 100 | 100 | 68.38 | 63.85 | 72.66 |
| $Ba^{++}$ | | | | | | |
| 0mM $BaCl_2$ | 100 | 100 | 100 | 100 | 100 | 100 |
| 10mM $BaCl_2$ | 100 | 100 | 100 | 67.47 | 65.53 | 74.71 |
| 20mM $BaCl_2$ | 100 | 100 | 100 | 71.43 | 55.32 | 75.51 |
| $Mg^{++}$ | | | | | | |
| 0mM $MgCl_2$ | 100 | 100 | 100 | 100 | 100 | 100 |
| 10mM $MgCl_2$ | 100 | 100 | 100 | 65.77 | 62.31 | 100 |
| 20mM $MgCl_2$ | 100 | 100 | 100 | 70.98 | 64.45 | 100 |
| $Zn^{++}$ | | | | | | |
| 0mM $ZnCl_2$ | 100 | 100 | 100 | 100 | 100 | 100 |
| 10mM $ZnCl_2$ | 100 | 100 | 100 | 0 | 0 | 0 |
| 20mM $ZnCl_2$ | 100 | 100 | 100 | 0 | 0 | 0 |