# CHAPTER III

# RESULTS

## 1. Isolation of Polysaccharide Gel (PG)

Polysaccharide gel (PG) of durian fruit rinds was isolated and semi-purified by the method of Pongsamart and Panmaung (1998). The dried polysaccharide gel product was ground to fine powder and the polysaccharide gel powder was passed through 60 meshes. The pale brown powder was obtained, final yield of polysaccharide gel (PG) was 6.72%, the polysaccharide gel powder is shown in figure 10. Polysaccharide gel (PG) at 2.5% by weight in distilled water had pH 2.10 ± 0.03 and the viscosity was 528 ± 2.00 cps.

## 2. Properties of Polysaccharide gel (PG)

### 2.1 Viscosity

The product of PG gel powder was swelled and dispersed in water forming a liquid gel. The polysaccharide gel (PG) at concentration 2.5% by weight in water had viscosity 528 ± 2.00 cps.

### 2.2 pH

The pH was measured by using pH meter. PG at 2.5% by weight in water had pH 2.10 ± 0.03. The pH value of PG dispersion at a high concentration was low nearly to the pH 2.00. It was noticed that the pH was depended on the concentration of PG.

### 2.3 Effect of sorbitol on viscosity of PG

PG dispersion at 2.5% by weight concentration was prepared, sorbitol was added in 2.5% PG to make 1 to 10% sorbitol as indicated, and the viscosities of PG mixture with increasing concentration of sorbitol were measured. The viscosity profile of PG was plotted against increasing concentration of sorbitol as shown in figure 11. The viscosity of PG was slowly increased with respected to the increasing concentration of sorbitol. Sorbitol at concentration used in the formulation (5-10%) produced less effect to the viscosity of PG.

## 3. Properties of tea tree oil (TTO)

### 3.1 Compatibility with solubilizer

The emulsifying or solubilizing agents most commonly used are 0.05%-10% Tween 80 (polysobate 80), 0.05%-10% Tween 20 (polysorbate 20), 20%-70% ethanol, and 0.001%-1% DMSO (Beylier, 1979; Walsh and Longstaff, 1987; Patkar, 1993; Peans, 1994). In this study, the stability of the emulsions was determined by mixing with various type of solubilizer such as, 0.5% Tween 20, 10% Tween 20, 0.5% Tween 60, 10% Tween 60, 0.5% Tween 80, 10% Tween 80, 10% Cremophore RH40® and 15% Cremophore RH40®. Each solubilizer was mixed with 1% tea tree oil (TTO). The mixture of TTO in 20% ethanol, 0.1% DMSO and 1% DMSO produced turbid solution, the mixture with 0.5% Tween 20, 0.5% Tween 60 and 0.5% Tween 80 gave less turbid solution except at high concentration produced a pale yellow solution, the mixture of TTO in 10% Cremophore RH40® and 15% Cremophore RH40® were clear solution. The results showed in table 2 indicated that Cremophore RH40® provided a suitable solubilizer in the formulation of antiseptic PG gel product, because of the Cremophore RH40® form a clear solution and the product was not too sticky. In this study, the results indicated that the concentration of 0.5% Tween 80 and 10%-15% Cremophore RH40® were necessarily to maintain the stability of 1 % tea tree oil (TTO) with in emulsion.

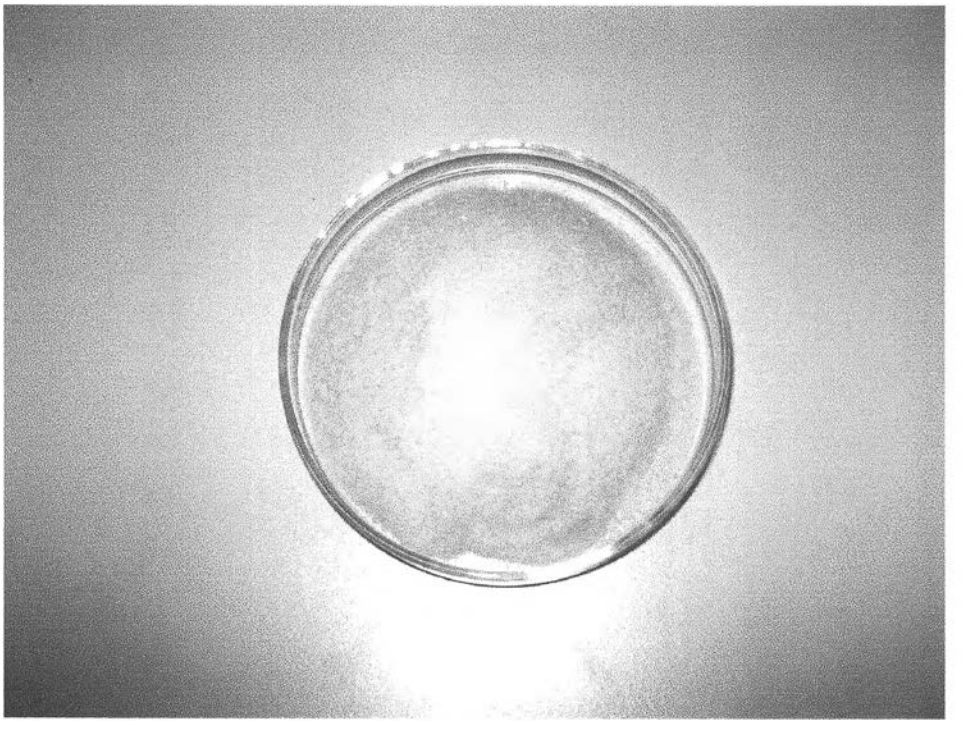


**Figure 10**. Polysaccharide gel (PG) powder product isolated from dried fruit-rinds of durian.

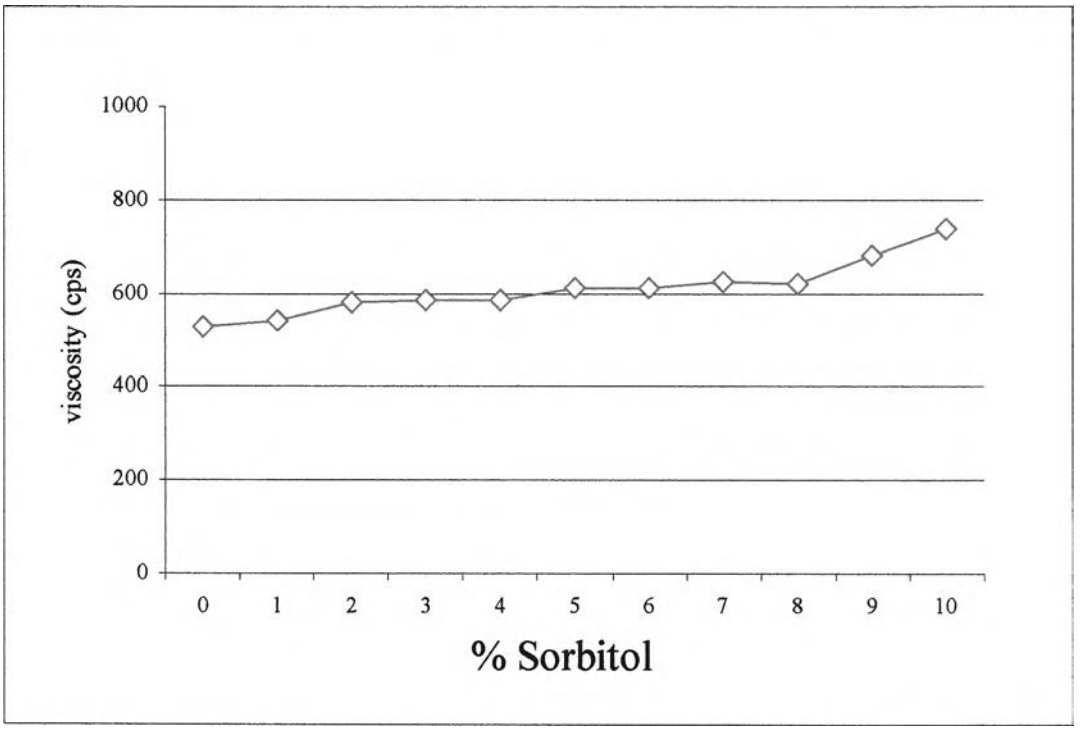



**Figure 11.** Effect of concentrations of sorbitol on the apparent viscosity of polysaccharide gel (PG)

**Table 2.** Compatibility test of 1% Tea tree oil (TTO) in each tested solubilizer.

| Solubilizer | Appearance |
|---|---|
| 20% ethanol | Homogenous, more turbid solution |
| 0.5% Tween 20 | Homogenous, less turbid solution |
| 10% Tween 20 | Homogenous, pale yellow solution |
| 0.5% Tween 60 | Homogenous, less turbid solution |
| 10% Tween 60 | Homogenous, pale yellow solution |
| 0.5% Tween 80 | Homogenous, less turbid solution |
| 10% Tween 80 | Homogenous, pale yellow solution |
| 1.0 % DMSO | Homogenous, more turbid solution |
| 0.1% DMSO | Homogenous, less turbid solution |
| 10% Cremophore RH 40® | Homogenous, clear solution |
| 15% Cremophore RH 40® | Homogenous, clear solution |

## 3.2 Antimicrobial activity test

### 3.2.1 Agar diffusion test

Tea tree oil has inhibitory activity against a wide range of gram-positive and gram-negative bacteria and fungi in this study. The inhibition zone was observed on agar media at tea tree oil concentration 0.312% - 0.625%(v/v) against nine tested bacteria, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* ATCC 12228, *Micrococus luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Proteus vulgaris* ATCC 13315, *Klebsiella pneumoniae* ATCC 10031 and *Pseudomonas aeruginosa* ATCC 9721 and two yeast strains, *Saccharomyces cerevisiae* ATCC 9763 and *Candida albicans* ATCC 10230, respectively. Inhibition zone of sharp and clear margin was obtained. Inhibition of microbial growth of tea tree oil on agar plates is demonstrated in table 3 and figure 12. All strains of test bacteria and fungi were inhibited at the lowest 0.312% concentration to the highest 5% concentrations of tea tree oil (TTO) tested according to this assay.

### 3.2.2 Broth macrodilution test

Minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) values of TTO are shown in table 4, the values showed the lowest concentration of TTO that inhibited a visible growth and bactericidal activity of tea tree oil against bacteria. The results indicated that 9 strains of bacteria, *S. aureus*, *S. epidermidis*, *M. luteus*, *B. subtilis*, *E.coli*, *P. vulgaris, S.typhimurium*, *K. pneumoniae* and *Ps. aeruginosa* were inhibited by 0.078-0.312% TTO and two yeast strains, *Saccharomyces cerevisiae* and *Candida albicans*, were inhibited by 0.156-0.312% TTO. All bacteria were killed at 0.078-0.625% TTO and fungi were killed at 0.312-0.625% TTO.

Figure 13 demonstrated MIC value of tea tree oil against *M. luteus* ATCC 9341, MIC at 0.312% of TTO which was the lowest concentration of tea tree oil that inhibited bacterial growth, no visible growth was obtained at this concentration.

**Table 3.** Antimicrobial activity of tea tree oil against bacteria and fungi by agar diffusion method, nz = no inhibition zone

| % tea tree oil in 0.5% tween 80 | Diameter of inhibition zone (mm), mean (SD ) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aureus* | *S. epidermidis* | *M. luteus* | *B. subtilis* | *E. coli* | *P. vulgaris* | *S. typhimurium* | *K .pneumoniae* | *Ps. aeruginosa* | *C. albicans* | *S. cerevisiae* |
| 5 | 12.17 (0.07) | 12.73 (0.07) | 12.03 (0.04) | 11.13 (0.08) | 10.33 (0.02) | 11.83 (0.02) | 10.85 (0.03) | 10.93 (0.08) | 11.40 (0.02) | 15.94 (0.12) | 13.67 (0.06) |
| 2.5 | 11.27 (0.03) | 11.53 (0.02) | 11.57 (0.02) | 10.23 (0.03) | 9.83 (0.01) | 10.30 (0.02) | 10.50 (0.02) | 10.17 (0.04) | 11.17 (0.05) | 14.33 (0.06) | 13.27 (0.02) |
| 1.25 | 10.97 (0.08) | 12.27 (0.04) | 11.33 (0.01) | 9.83 (0.08) | 9.57 (0.02) | 9.87 (0.01) | 10.10 (0.02) | 10.00 (0.02) | 10.80 (0.07) | 13.37 (0.08) | 12.80 (0.02) |
| 0.625 | 10.53 (0.05) | 11.27 (0.06) | 11.13 (0.02) | 9.70 (0.04) | 9.33 (0.01) | 9.73 (0.01) | 10.10 (0.02) | 9.20 (0.00) | 10.60 (0.07) | 12.16 (0.02) | 12.23 (0.05) |
| 0.312 | 10.47 (0.02) | 10.87 (0.02) | 10.90 (0.01) | 9.63 (0.02) | 9.27 (0.03) | 9.60 (0.00) | 9.53 (0.01) | 9.20 (0.00) | 10.40 (0.06) | 10.90 (0.04) | 11.80 (0.01) |
| Control (0.5%tween80) | nz | nz | nz | nz | nz | nz | nz | nz | nz | nz | nz |

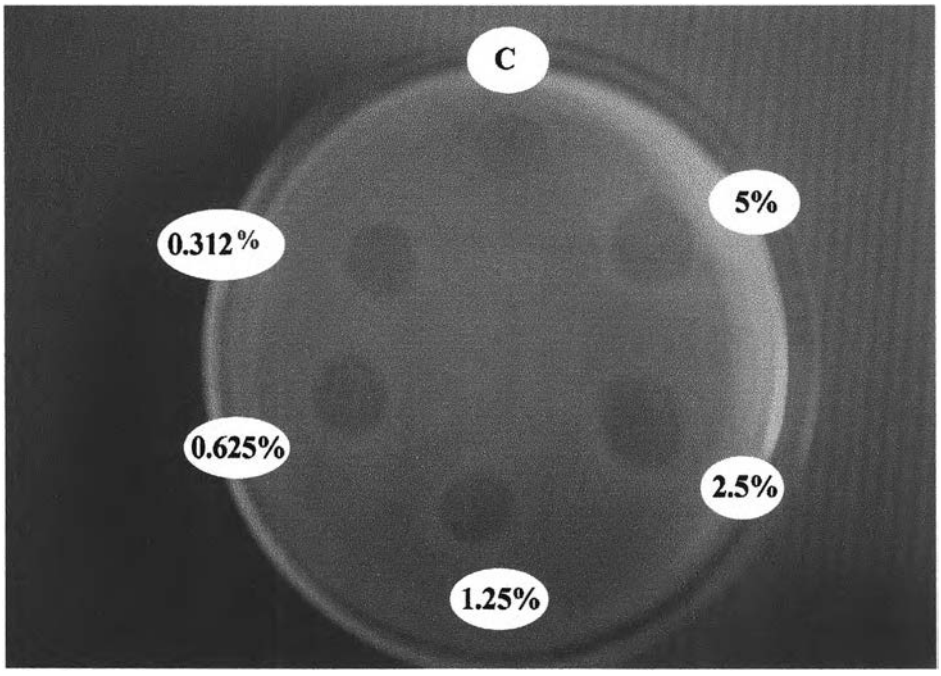



**Figure 12**. Microbiological assay plate of tea tree oil (TTO) against *S. aureus* ATCC 6538P on MHA medium. Concentrations of tea tree oil were 5, 2.5, 1.25, 0.625, and 0.312% (v/v) and control (C) was 0.5% Tween 80.

**Table 4.** Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of tea tree oil against microorganisms.

| Microorganisms | Tea tree oil | |
|---|---|---|
| | MIC (%v/v) | MBC (%v/v) |
| *S. aureus* ATCC 6538P | 0.078 | 0.156 |
| *S. epidermidis* ATCC 12228 | 0.078 | 0.156 |
| *M. luteus* ATCC 9341 | 0.312 | 0.625 |
| *B. subtilis* ATCC 6633 | 0.312 | 0.625 |
| *E. coli* ATCC 25922 | 0.078 | 0.156 |
| *P. vulgaris* ATCC 13315 | 0.312 | 0.625 |
| *S. typhimurium* ATCC 14028 | 0.036 | 0.078 |
| *K. pneumoniae* ATCC 10031 | 0.312 | 0.625 |
| *Ps. aeruginosa* ATCC 9721 | 0.078 | 0.156 |
| *C. albican* ATCC 10230 | 0.156 | 0.312 |
| *S. cerevisiae* ATCC 9763 | 0.312 | 0.625 |


**Figure 13**. MIC assay of tea tree oil by broth macrodilution method using MHB medium against *M. luteus* ATCC 9341. MIC represented the tube contained the lowest concentration of tea tree oil (0.312%) that demonstrated no visible bacterial growth.

**4. Preparation of antiseptic PG gel**

Vitamin E gel prepared from PG (Lertchaiporn, 2003) was used as a control PG gel base in this study. The antiseptic gel preparations were prepared by using the combination of polysaccharide gel, tea tree oil and betel oil as an active antimicrobial agent. In the formulation 1.2% menthol was added for cool sensation. The resulting products of antiseptics PG gel formula showed in table 5, 6 and 7. The antiseptic PG gels products were a clear gel with pale brown color (figure 14) of polysaccharide gel (PG). The antiseptic PG gel products, NO. 12, 33 and 43 were chosen. The finished products were smooth homogenous, pale brown color, flow easily, non-sticky and provided antimicrobial activity. So the formula 12 in table 5 was selected. The formula 33 in table 6 and formula 43 in table 7 were selected by the same reasons.

**5. Stability test of antiseptic PG gel products**

The product was kept at 45°C for 48 hrs and then kept in low temperature at -4°C for 48 hrs (1 cycle). The procedure was performed for 6 cycles. Storing at ambient temperature for 30 days also was used for testing. After testing at many conditions, the characteristics of antiseptic PG gel products were not changed. The viscosities and pH of all formulations were determined and the viscosity and pH of the final products were higher than those freshly prepared. The results of antiseptic PG gel products before and after studies were shown in figure 15 and table 8.

**Table 5**. Formulation of PG gel base of durian polysaccharide gel (PG). TEA = Triethanolamine, DW = distilled water

| Formula | Ingredients (%w/w) | | | | | | | | | | | | | Description of PG gel product (freshly prepared) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PG | Amerchol L-101® | Creophore RH 40® | sorbitol | Propylene glycol | Tween 80 | Glycerin | Menthol | Vitamin E acetate | $CaCl_2$ | Paraben conc. | TEA | DW | |
| NO. 2 PG gel base | 2.5 | 0.5 | 10 | 10 | 5 | 1 | 5 | 0.5 | - | - | 1 | qs.ad. pH 2.8-3.0 | qs.ad. 100 | Texture: non-homogenous<br>Color: creamy white<br>Flow: easy<br>Viscosity: 3150 ± 62.5cps.<br>pH: 2.83 |
| NO. 4 PG gel base | 2.5 | 0.5 | 13 | - | 5 | 1 | 5 | 0.5 | - | - | 1 | qs.ad. pH 2.8-3.0 | qs.ad. 100 | Texture: non-homogenous<br>Color: creamy white<br>Flow: easy<br>Viscosity: 1265 ± 119 cps.<br>pH: 2.89 |
| NO. 12 PG gel base | 2.5 | 0.5 | 13 | - | 15 | - | - | 1.2 | 0.1 | 0.1 | 1 | qs.ad. pH 2.8-3.0 | qs.ad. 100 | Texture: smooth homogenous gel<br>Color: pale brown<br>Flow: easy<br>Viscosity: 2598 ± 230cps.<br>pH: 3.05 |

**Table 6.** Formulation of antiseptic PG gel contained tea tree oil (TTO). TTO = Tea tree oil, TEA = Triethanolamine, DW = distilled water

| Formula | Ingredients (%w/w) | | | | | | | | | | | | | Description of antiseptic TTO-PG gel product (freshly prepared) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PG | TTO | Amerchol L-101® | Creophore RH 40® | sorbitol | Propylene glycol | Tween 80 | Glycerin | Menthol | Vitamin E acetate | $CaCl_2$ | Paraben conc. | TEA | DW | |
| NO. 25 TTO - PG gel | 2.5 | 0.7 | 0.5 | 13 | - | 10 | - | 5 | 1.0 | - | - | 1 | qs.ad. pH 2.8-3.0 | qs.ad. 100 | Texture: non-homogenous<br>Color: creamy white<br>Flow: not easy<br>Viscosity: > 10,000 cps.<br>pH: 2.82 |
| NO. 30 TTO - PG gel | 2.5 | 1.0 | 0.5 | 13 | - | 15 | - | - | 1.0 | 0.1 | 0.2 | 1 | qs.ad. pH 2.8-3.0 | qs.ad. 100 | Texture: smooth homogenous gel<br>Color: pale brown<br>Flow: not easy<br>Viscosity: > 10,000 cps.<br>pH: 2.93 |
| NO. 33 TTO - PG gel | 2.5 | 1.0 | 0.5 | 13 | - | 15 | - | - | 1.2 | 0.1 | 0.1 | 1 | qs.ad. pH 2.8-3.0 | qs.ad 100 | Texture: smooth homogenous gel<br>Color: : pale brown<br>Flow: easy<br>Viscosity: 4365 ± 640 cps.<br>pH: 2.99 |

**Table 7.** Formulation of antiseptic PG gel contained tea tree oil (TTO) and betel oil (BO). TTO = Tea tree oil, BO = Betel oil, TEA = Triethanolamine, DW = distilled water

| Formula | Ingredients (%w/w) | | | | | | | | | | | | | | Description of antiseptic PG TTO/BO gel product (freshly prepared) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PG | TTO | BO | Amerchol L-101® | Creophore RH 40® | Propylene glycol | Sorbitol | Glycerin | Menthol | Vitamin E acetate | $CaCl_2$ | Paraben conc. | TEA | DW | |
| NO. 36 TTO/ BO PG gel | 2.5 | 1.0 | 0.5 | 0.5 | 10 | 15 | 5 | 5 | 1.2 | 0.1 | 0.1 | 1 | qs.ad. pH 2.8-3.0 | qs.ad. 100 | Texture: non-homogenous<br>Color: creamy white<br>Flow: not easy<br>Viscosity: > 10,000 cps.<br>pH: 2.79 |
| NO. 41 TTO/ BO PG gel | 2.5 | 1.0 | 0.5 | 0.5 | 11 | 15 | - | - | 1.2 | 0.1 | 0.1 | 1 | qs.ad. pH 2.8-3.0 | qs.ad. 100 | Texture: smooth homogenous gel<br>Color: creamy white<br>Flow: easy<br>Viscosity: 2150 ± 130 cps.<br>pH: 2.98 |
| NO. 43 TTO/ BO PG gel | 2.5 | 1.0 | 0.2 | 0.5 | 13 | 15 | - | - | 1.2 | 0.1 | 0.1 | 1 | qs.ad. pH 2.8-3.0 | qs.ad. 100 | Texture: smooth homogenous gel<br>Color: pale brown<br>Flow: easy<br>Viscosity: 2778 ± 280 cps.<br>pH: 3.02 |

**Table 8.** Summary of antiseptic PG gel products after stability test

| Antiseptic PG gel preparation | Description of antiseptic gel products | | |
|---|---|---|---|
| | After freshly prepared | After 6 temperature cycling test | After 30 days storage at room temperature |
| (NO.12) PG-gel base | Texture: smooth homogenous gel<br>Color: pale brown<br>Flow: easy<br>Viscosity: 2598 ± 230cps.<br>pH: 3.05 | Texture: smooth homogenous gel<br>Color: pale yellow<br>Flow: not easy<br>Viscosity: > 10,000 cps.<br>pH: 3.01 ± 0.01 | Texture: smooth homogenous gel<br>Color: pale yellow<br>Flow: not easy<br>Viscosity: > 10,000 cps.<br>pH: 3.08 ± 0.01 |
| (NO.33) Tea tree oil - PG gel | Texture: smooth homogenous gel<br>Color: pale brown<br>Flow: easy<br>Viscosity: 4365 ± 640 cps.<br>pH: 2.99 | Texture: smooth homogenous gel<br>Color: pale yellow<br>Flow: not easy<br>Viscosity: > 10,000 cps.<br>pH: 3.03 ± 0.03 | Texture: smooth homogenous gel<br>Color: pale yellow<br>Flow: not easy<br>Viscosity: > 10,000 cps.<br>pH: 3.06 ± 0.01 |
| (NO.43) Tea tree oil/Betel oil-PG gel | Texture: smooth homogenous gel<br>Color: pale brown<br>Flow: easy<br>Viscosity: 2778 ± 280 cps.<br>pH: 3.02 | Texture: smooth homogenous gel<br>Color: pale yellow<br>Flow: not easy<br>Viscosity: > 10,000 cps.<br>pH: 3.01 ± 0.02 | Texture: smooth homogenous gel<br>Color: pale yellow<br>Flow: easy<br>Viscosity: 3285 ± 621cps.<br>pH: 3.07 ± 0.06 |


**Figure 14**. Antiseptic PG gel products after freshly prepared. A= PG-gel base (NO.12), B= Tea tree oil-PG gel (NO.33), C= Tea tree oil/Betel oil-PG gel (NO.43)

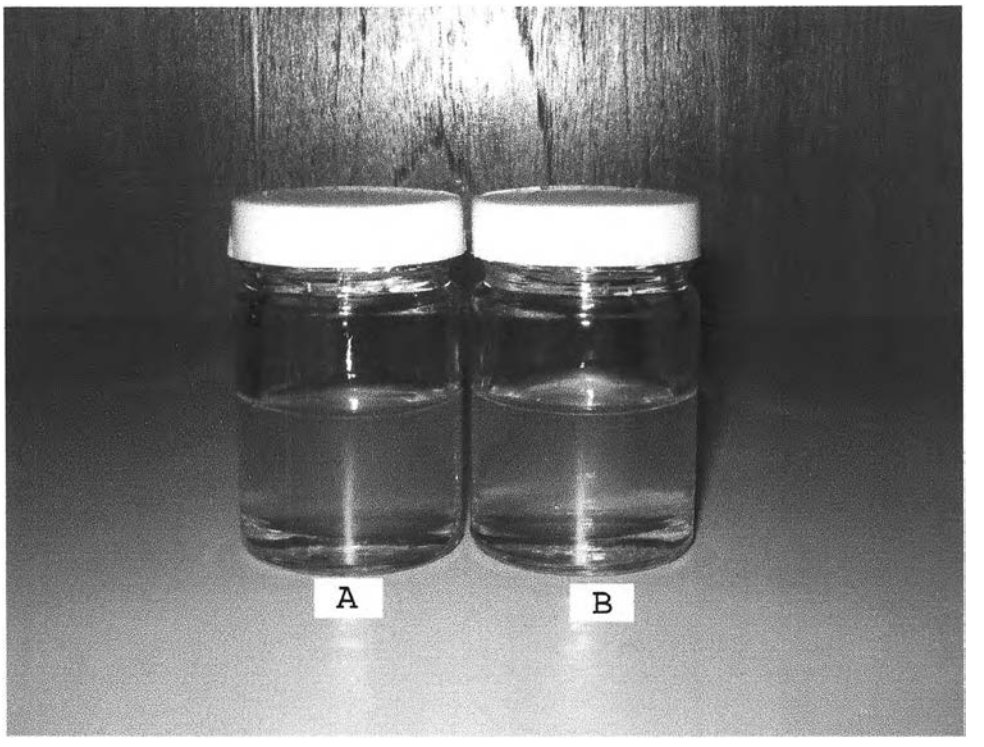



**Figure 15**. Tea tree oil-PG gel antiseptic finished product of formula NO.33 (A: after freshly prepared B: After 30 days storage at room temperature.)

## 6. Efficacy antimicrobial activity of the products antiseptic PG gel

### 6.1 Time-kill analysis

Antimicrobial potency of the finished products of tea tree antiseptic gel preparations were demonstrated against 2 bacterial strains and 1 yeast strain of represent microorganisms by time-kill analysis. Two strains of tested bacteria were *S. aureus* that represent gram-positive bacteria and *E. coli* that represent gram-negative bacteria and a tested yeast strain was *C. albicans* that represent fungi. The microorganisms were cultivated in 50%strength of the products PG-gel base without menthol (Lertchaiporn, 2003), PG-gel base contained menthol (NO.12), tea tree oil-PG gel (NO.33) and tea tree oil/betel oil-PG gel (NO.43), respectively, and NSS was control. Microorganism survival in NSS was determined in comparison with each of finished product as previously described at indicated times for 24 hours. All test microorganisms showed a similar survival pattern by time-kill study as shown in figure 16-18. In NSS, all tested microorganisms were survived in the static level after 24 hrs incubation. Time-kill analysis illustrated that antiseptic gel preparations of tea tree oil-PG gel (NO.33) and tea tree oil/betel oil-PG gel (NO.43) gave bactericidal activity in MHB medium against *S. aureus* and *E. coli*, the colony counts were declined to zero within 15 min (Figure 16, 17).

Antiseptic gel preparations of tea tree oil-PG gel (NO.33) and tea tree oil/betel oil-PG gel (NO.43) were also gave fungicidal activity in Sabouraud dextrose agar (SDA) medium against *C. albicans*, the colony counts was declined to zero within 24 hours (Figure 18).

### 6.2 Agar diffusion method

Antiseptic PG gel products were tested for inhibitory activity by agar diffusion method (Lorian, 1991). The inhibition zones were observed on agar media with the antiseptic PG gel finished products. The result showed inhibitory activity

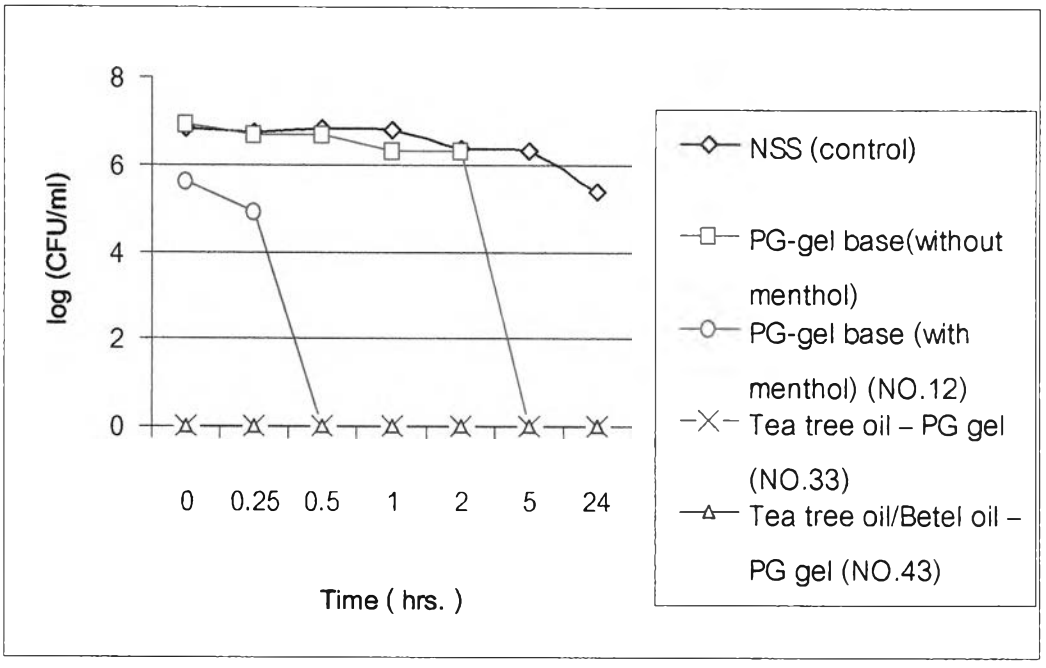



**Figure 16**. Time-kill analysis of antiseptic PG gel products against *S. aureus* ATCC 6538P, normal saline was used as a control.

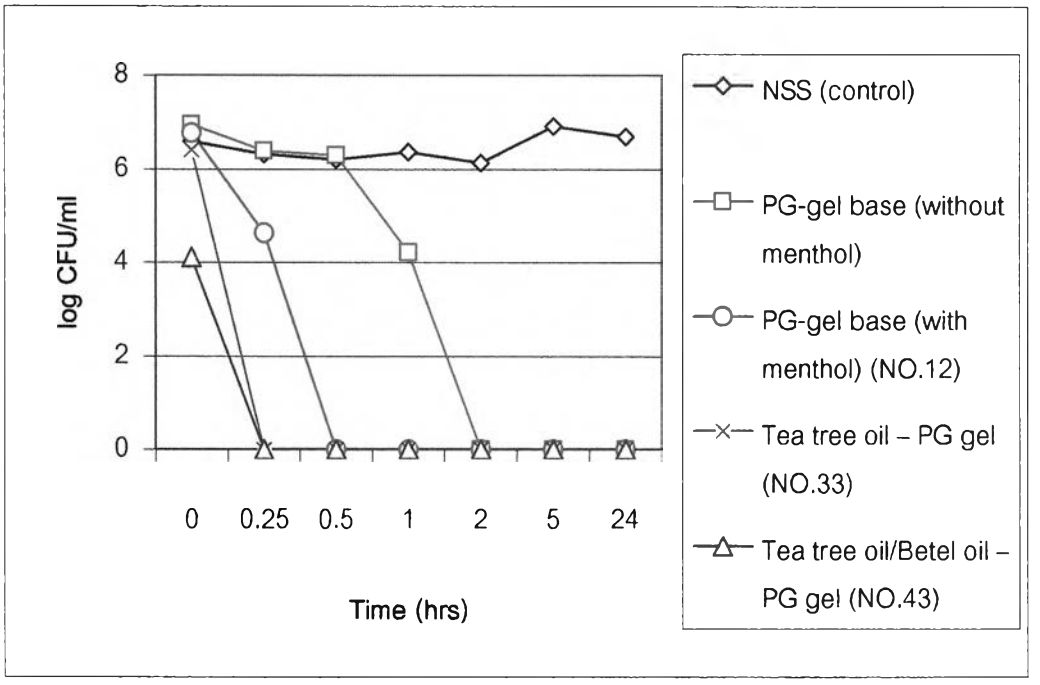



**Figure 17**. Time-kill analysis of antiseptic PG gel products against *E. coli* ATCC 25922, normal saline was used as a control.

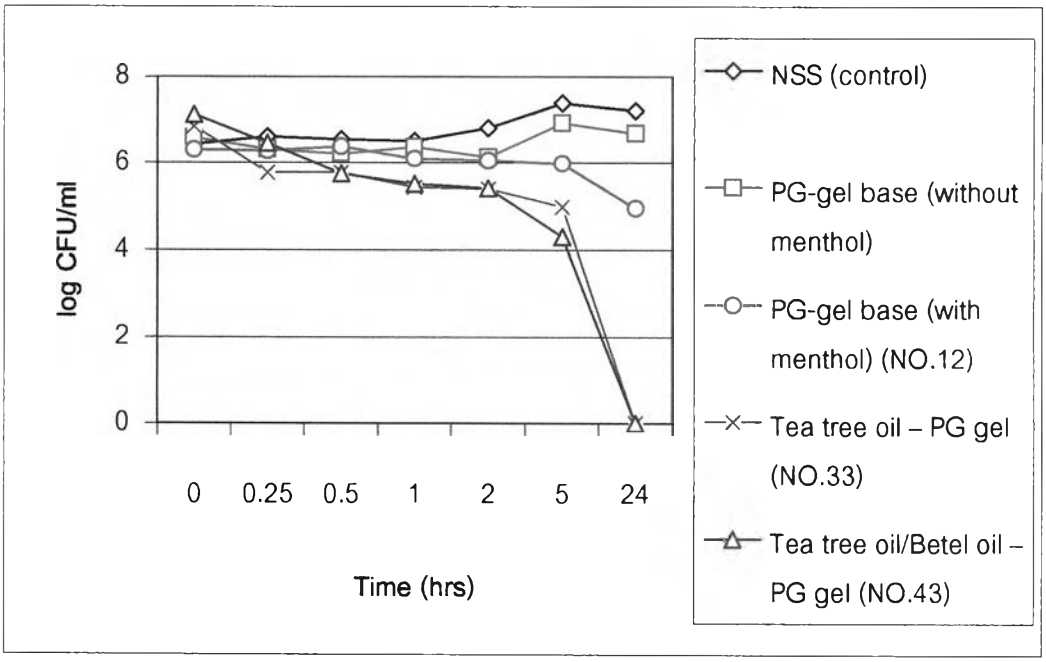



**Figure 18**. Time-kill analysis of antiseptic PG gel products against *C. albicans* ATCC 10230, normal saline was used as a control.

against nine bacteria, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* ATCC 12228, *Micrococus luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Proteus vulgaris* ATCC 13315, *Klebsiella pneumoniae* ATCC 10031 and *Pseudomonas aeruginosa* ATCC 9721 and two yeast strains, *Saccharomyces cerevisiae* ATCC 9763 and *Candida albicans* ATCC 10230, respectively. Inhibition zone of sharp and clear margin was obtained. Inhibition of microbial growth of antiseptic PG gel finished products on agar plates is demonstrated in Table 9, figure 19 and 20.

All ingredients in the formula were also tested for microorganism-free and inhibitory activity against test microorganisms, such as *S. aureus*, *S. epidermidis*, *M. luteus*, *B. subtilis*, *S. typhimurium*, *P. vulgaris*, *K. pneumoniae* and *Ps. aeruginosa*. Inhibition of microbial growth of these ingredients on agar plates is demonstrated in table 10 and figure 21, 22. All ingredients were free of microorganisms and no inhibitory activity against test microorganisms, except for menthol as indicated (table 10 and figure 22).

### 6.3 In-vivo hand washing test

The prevalue and postvalue of colony counts were determined in each of finished products of antiseptic PG gel (NO.33 and 43) and PG-gel base (NO.12). The visible colony counts of microorganisms performed on agar plates after washing hands with tap water and followed by using each antiseptic PG gel product successively as indicated and the inoculated plates were incubated at 37°C, overnight. The score number of microorganisms released was depended on time and finished products tested. The inhibitory activity of tea tree oil-PG gel (NO.33), tea tree oil/betel oil-PG gel (NO.43) and PG-gel base (NO.12) were always better than that of washing with tap water. In the present study, the antimicrobial activity of antiseptic PG gel NO.43 and antiseptic PG gel NO.33 showed considerable inhibition of microbial growth better than washing with tap water as the results illustrated in figure 23 and figure 24.

Table 9. Antimicrobial activity of antiseptic PG gel finished products against microorganisms by agar diffusion method, nz = no inhibition zone

| Microorganisms | Diameter of inhibition zone, mm (Mean ± SD) | | | | | |
|---|---|---|---|---|---|---|
| | NSS | 2.5%PG | (NO.12) PG-gel base contained menthol | (NO.33) Antiseptic PG gel contained 1%tea tree oil | (NO.35) Antiseptic PG gel contained 1.5%tea tree oil | (NO.43) Antiseptic PG gel contained 1%tea tree oil and 0.2% betel oil |
| *S.aureus* | nz | 9.40 ± 0.14 | 9.88 ± 0.41 | 10.70 ± 0.36 | 11.23 ± 0.46 | 12.08 ± 0.22 |
| *S.epidermidis* | nz | 10.27 ± 0.40 | 10.60 ± 0.36 | 10.93 ± 0.06 | 11.00 ± 0.17 | 12.30 ± 0.17 |
| *M.luteus* | nz | 10.03 ± 0.15 | 10.10 ± 0.10 | 10.30 ± 0.10 | 10.60 ± 0.20 | 10.87 ± 0.40 |
| *B.subtilis* | nz | 10.30 ± 0.36 | 10.30 ± 0.36 | 10.43 ± 0.29 | 10.73 ± 0.61 | 11.80 ± 1.35 |
| *E.coli* | nz | 9.80 ± 0.10 | 10.13 ± 0.05 | 10.46 ± 0.35 | 11.63 ± 0.67 | 11.16 ± 0.45 |
| *P.vulgaris* | nz | 9.43 ± 0.32 | 10.87 ± 0.89 | 11.45 ± 0.15 | 12.30 ± 0.62 | 12.36 ± 0.28 |
| *Salmonella typhimurium* | nz | nz | 10.23 ± 0.11 | 10.80 ± 0.36 | 11.03 ± 0.05 | 11.63 ± 0.15 |
| *K.pneumoniae* | nz | nz | 10.53 ± 0.32 | 11.36 ± 0.15 | 12.20 ± 0.53 | 12.43 ± 0.45 |
| *Ps.aeruginosa* | nz | nz | 11.17 ± 0.32 | 12.07 ± 0.15 | 12.30 ± 0.17 | 12.97 ± 0.41 |
| *C.albicans* | nz | nz | nz | 14.25 ± 0.93 | 15.88 ± 0.63 | 17.15 ± 0.59 |
| *S.cerevisiae* | nz | nz | nz | 12.33 ± 0.15 | 12.83 ± 0.84 | 13.63 ± 0.65 |

Table 10. Antimicrobial test of ingredients in PG gel base against microorganisms by agar diffusion method, nz = no inhibition zone

| Microorganisms | Diameter of inhibition zone, mm (Mean ± SD) | | | | | |
|---|---|---|---|---|---|---|
| | $H_2O$ | Propylene glycol | Cremophore RH 40® | Amerchol L-101® | Vit E acetate | Menthol |
| *S.aureus* | nz | nz | nz | nz | nz | 15.70 ± 3.38 |
| *S.epidermidis* | nz | nz | nz | nz | nz | 9.97 ± 0.15 |
| *M.luteus* | nz | nz | nz | nz | nz | 10.23 ± 0.12 |
| *B.subtilis* | nz | nz | nz | nz | nz | 14.90 ± 0.10 |
| *E.coli* | nz | nz | nz | nz | nz | nz |
| *P.vulgaris* | nz | nz | nz | nz | nz | 10.30 ± 0.17 |
| *Salmonella typhimurium* | nz | nz | nz | nz | nz | 11.37 ± 0.31 |
| *K. pneumoniae* | nz | nz | nz | nz | nz | 10.97 ± 0.32 |
| *Ps.aeruginosa* | nz | nz | nz | nz | nz | 11.70 ± 0.85 |
| *C.albicans* | nz | nz | nz | nz | nz | nz |
| *S.cerevisiae* | nz | nz | nz | nz | nz | nz |

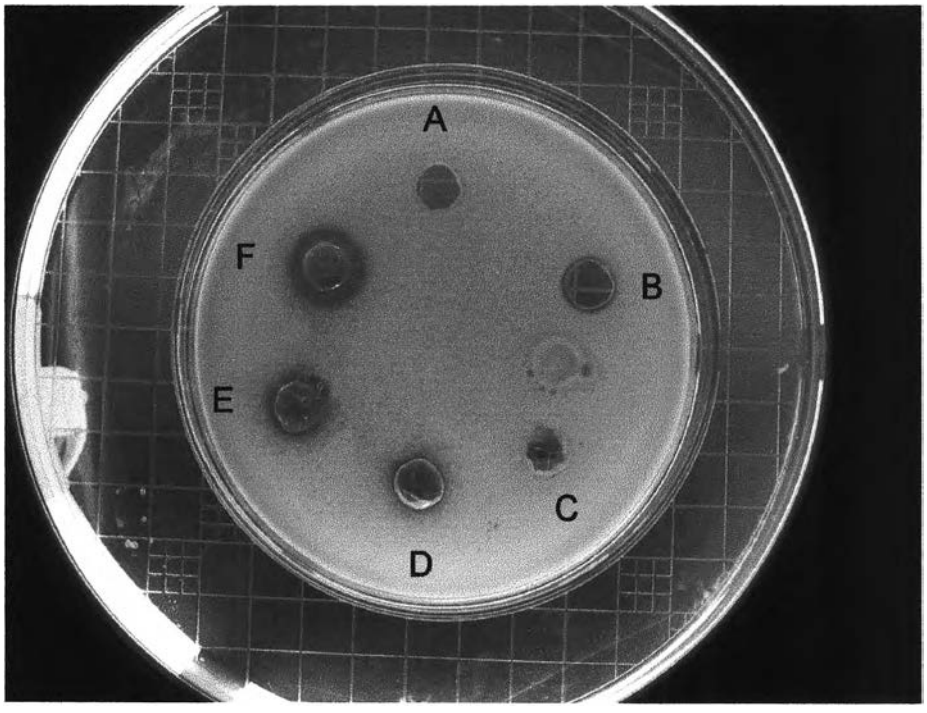



**Figure 19**. Microbiological assay plate against *B. subtilis* ATCC 6633 on Mueller hinton agar (MHA). A, NSS (normal saline); B, 2.5% PG, C: PG-gel base (NO.12); D, 1%tea tree oil-PG gel (NO.33); E, 1.5% tea tree oil-PG gel (NO.35) and F, 1% tea tree oil/0.2% betel oil-PG gel (NO.43).

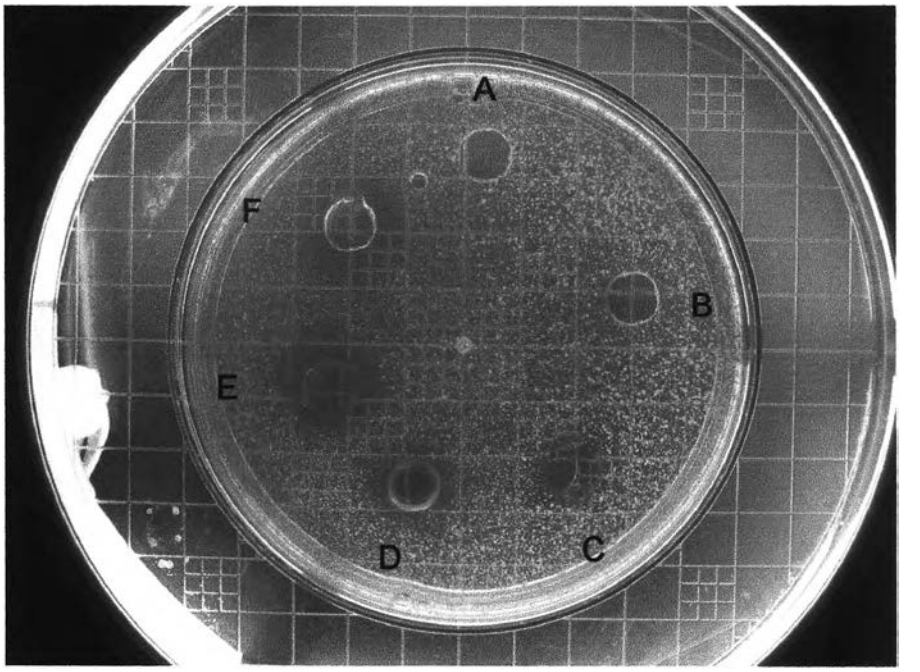



**Figure 20.** Microbiological assay plate against *C. albicans* ATCC 10230 on Sabouraud dextrose agar (SDA). A, NSS (normal saline); B, 2.5% PG, C: PG-gel base (NO.12); D, 1%tea tree oil-PG gel (NO.33); E, 1.5% tea tree oil-PG gel (NO.35) and F, 1% tea tree oil/0.2% betel oil-PG gel (NO.43).

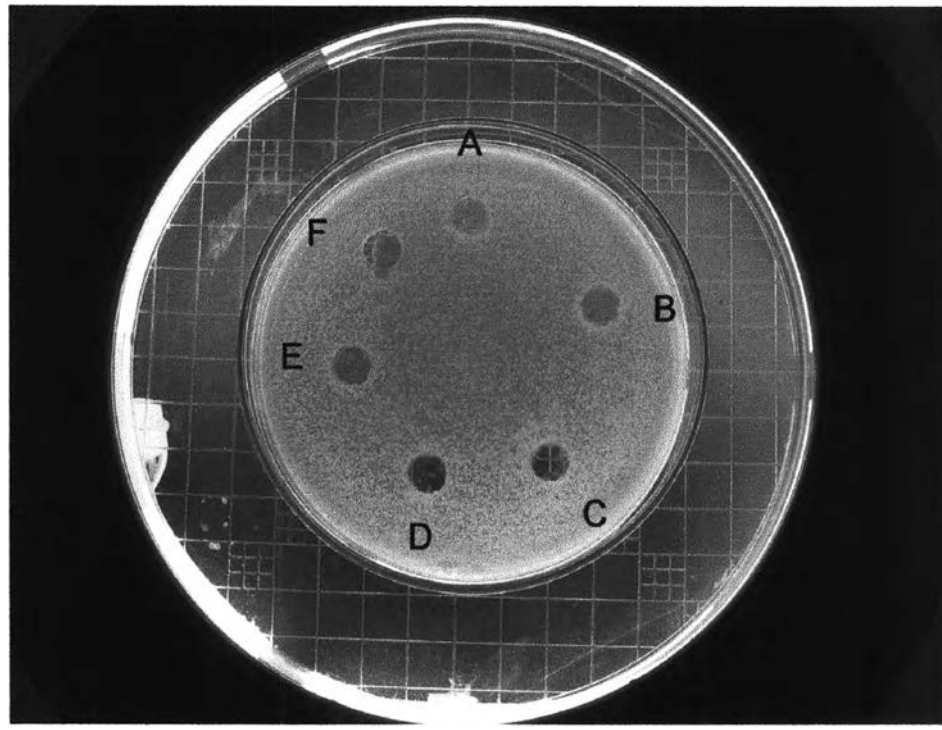



**Figure 21**. Microbiological assay plate against *C. albicans* ATCC 10230 on Sabouraud dextrose agar (SDA). A, sterile water; B, 15% propylene glycol; C, 13% Cremophore RH 40®; D, 0.5% Amerchol L-101®; E, 0.1% vitamin E acetate and F, 1.2% menthol.

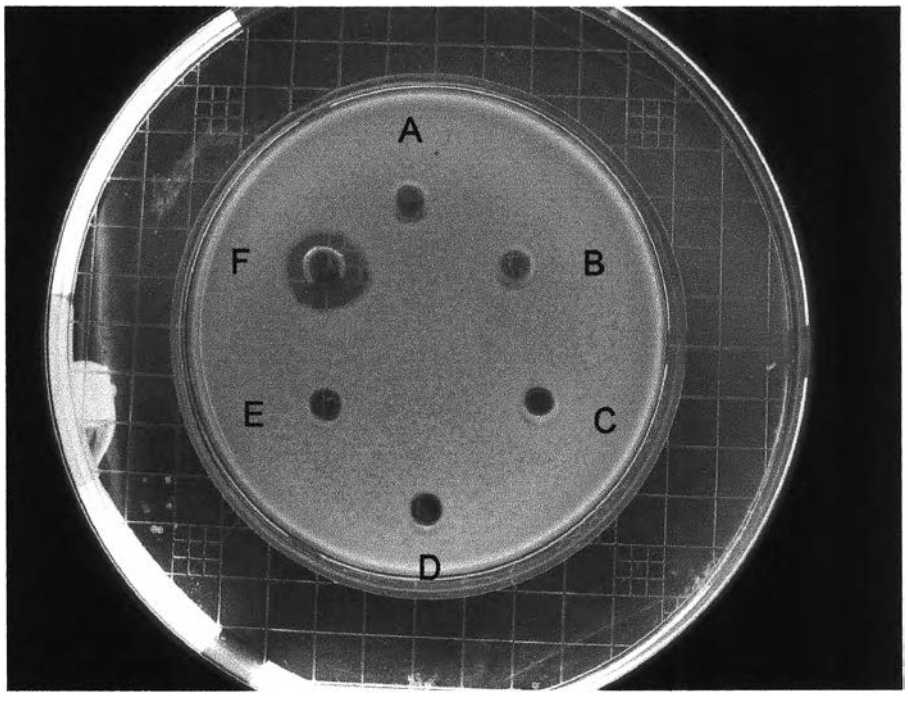



**Figure 22.** Microbiological assay plate against *B. subtilis* ATCC 6633 on Mueller hinton agar (MHA). A, sterile water; B, 15% propylene glycol; C, 13% Cremophore RH 40®;D, 0.5% Amerchol L-101®; E, 0.1% vitamin E acetate and F, 1.2% menthol.

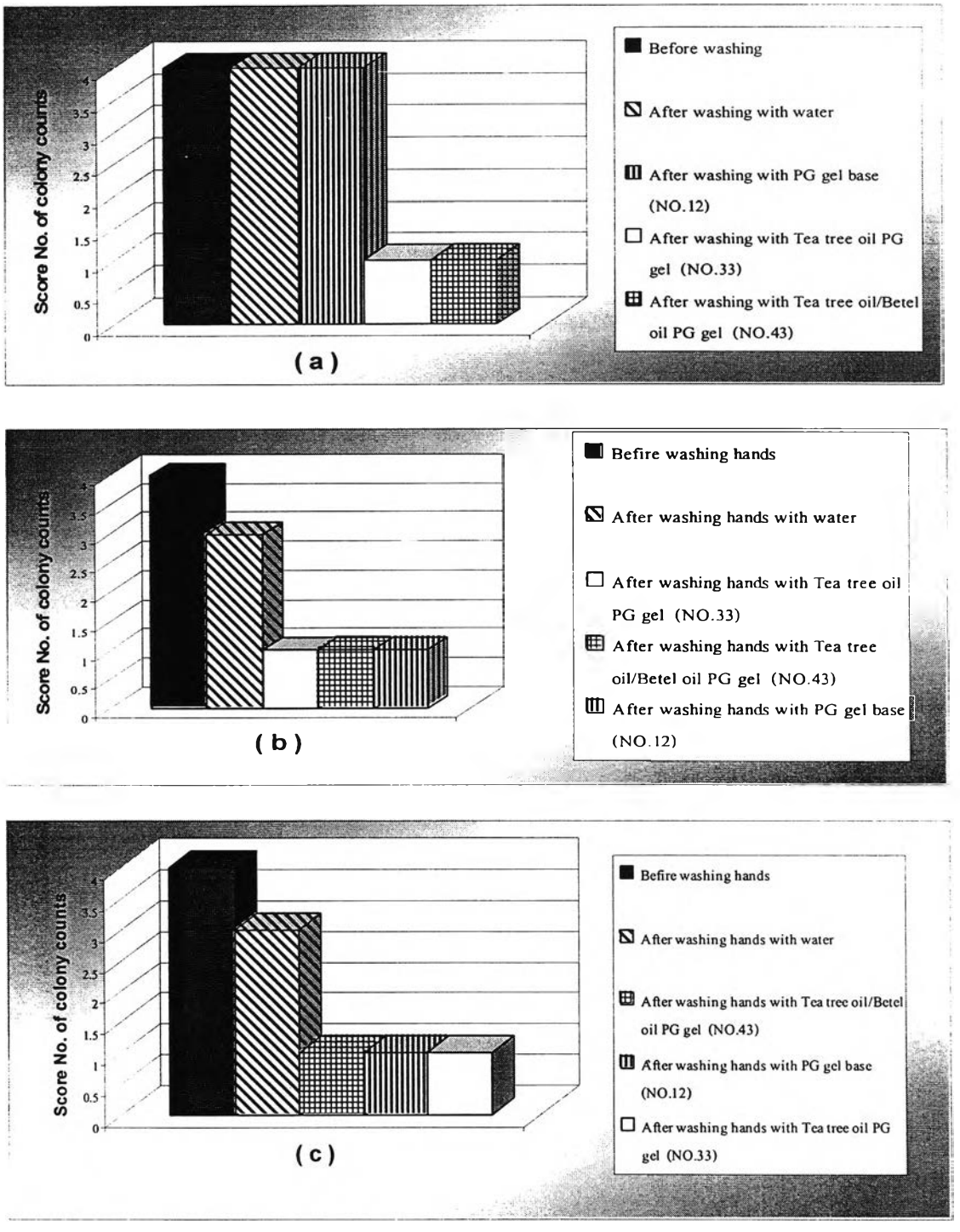



**Figure 23**. In-vivo studies of antiseptic PG gel finished products NO.33 and 43 by hand washing test. (a) = performed by process 1; (b) = performed by process 2; (c) = performed by process 3. The colony counts were performed after incubation the inoculated MHA plates at 37°C for 24 hrs. Score no: 1 = 0 - 50 colony, 2 = 50 -100 colony, 3 = 100 – 200 colony, 4 = >200 colony

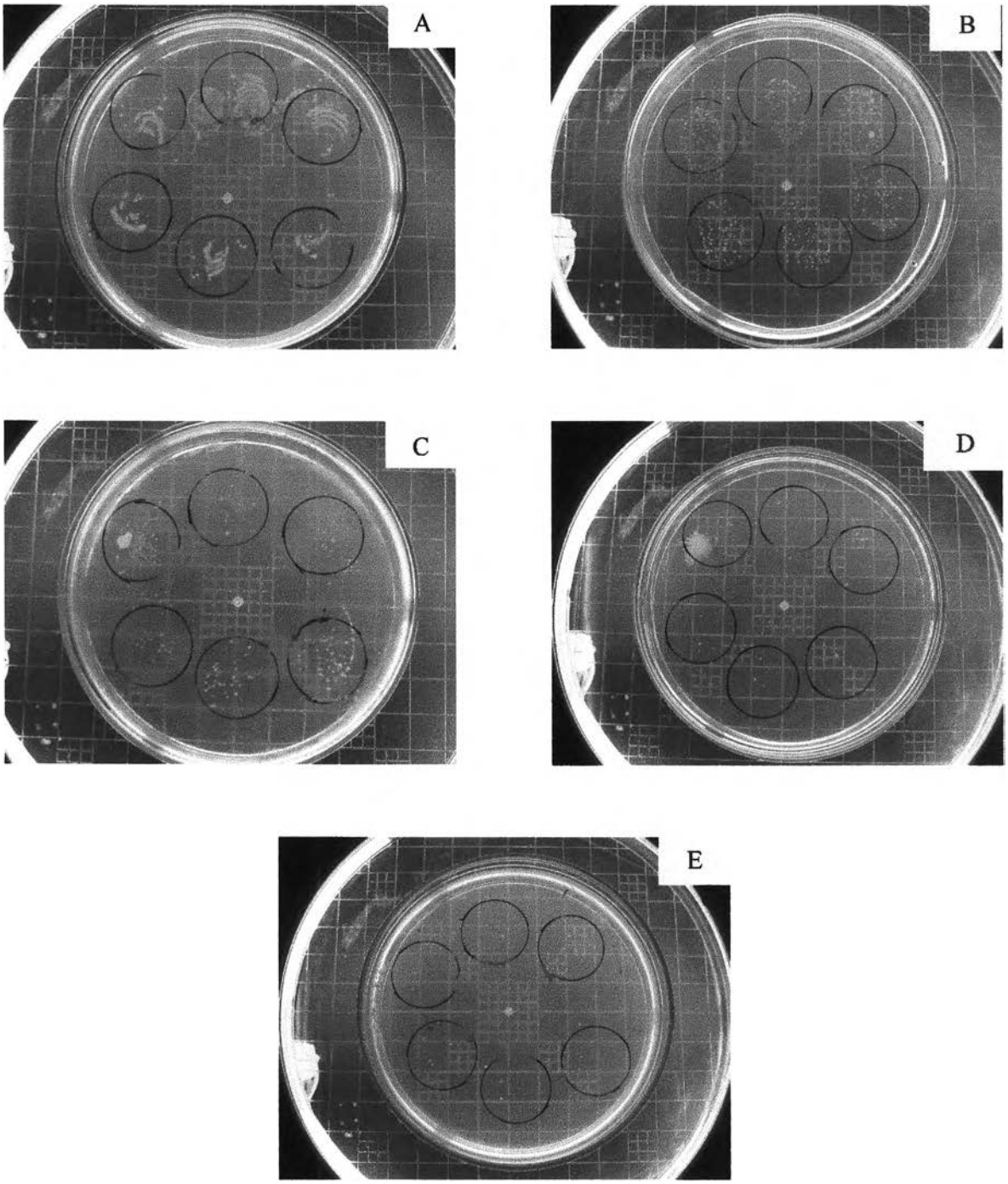



**Figure 24.** The number of colonies of normal flora on hands inoculated by finger pressed on MHA media for 30 seconds and incubated overnight at 37°C. (A = before washing with tap water, B= after washing with tap water, C= after washing PG-gel base (NO.12), D= after washing with antiseptic tea tree oil-PG gel (NO.33) , E= after washing with antiseptic tea tree oil/ betel oil-PG gel (NO.43)).

Antiseptic efficacy of antiseptic PG gel product was evaluated in comparison with gel commercial product in 10 subjects. The antibacterial activity of tree oil PG gel (NO.33), tea tree oil/betel oil PG gel (NO.43) were comparable with gel commercial product, and better than tap water. The score number of bacterial counts after treatment (postvalue) with the antiseptic gel product (NO.33 and 43) and gel commercial product was significantly ($p$ <0.05) lower than that in the prevalue (prior treatment). The antimicrobial activity of antiseptic PG gel NO.43 and antiseptic PG gel NO.33 and gel commercial product showed considerable reduction of hands normal flora after hands washing more than washing with tap water, the results illustrated in table 11 and figure 25.

## 6.4 Perception analysis of antiseptic PG gel products

The perception analysis of antiseptic PG gel product (NO.33 and 43) by volunteers revealed that most of them satisfied with the antiseptic PG gel NO. 33 and NO.43. The results illustrated in figure 26 and figure 27.

Table 11. In-vivo evaluation of antiseptic potency of antiseptic gel products by hand washing test gel Score no: 1 = 0-50 colony, 2 = 50-100 colony, 3 = 100-200 colony, 4 = >200 colony. Data are mean ± SD., n = 10

| Product | The score number of colony counts | | | |
|---|---|---|---|---|
| | Before application | After application 5 min | After application 15 min | After application 30 min |
| Tap water (control) | 3.8 ± 0.32 | 3.3 ± 0.48 | 2.9 ± 0.32 | 2.8 ± 0.42 |
| Gel commercial (clean feel®) | 3.5 ± 0.53 | 2.7 ± 0.83 | 1.6 ± 0.52 | 1.1 ± 0.32 |
| Gel NO.33 (1% TTO) | 3.3 ± 0.49 | 2.3 ± 0.82 | 1.4 ± 0.52 | 1.0 ± 0.02 |
| Gel NO.43 (1%TTO+0.2%BO) | 3.5 ± 0.53 | 2.8 ± 0.63 | 1.5 ± 0.74 | 1.1 ± 0.42 |

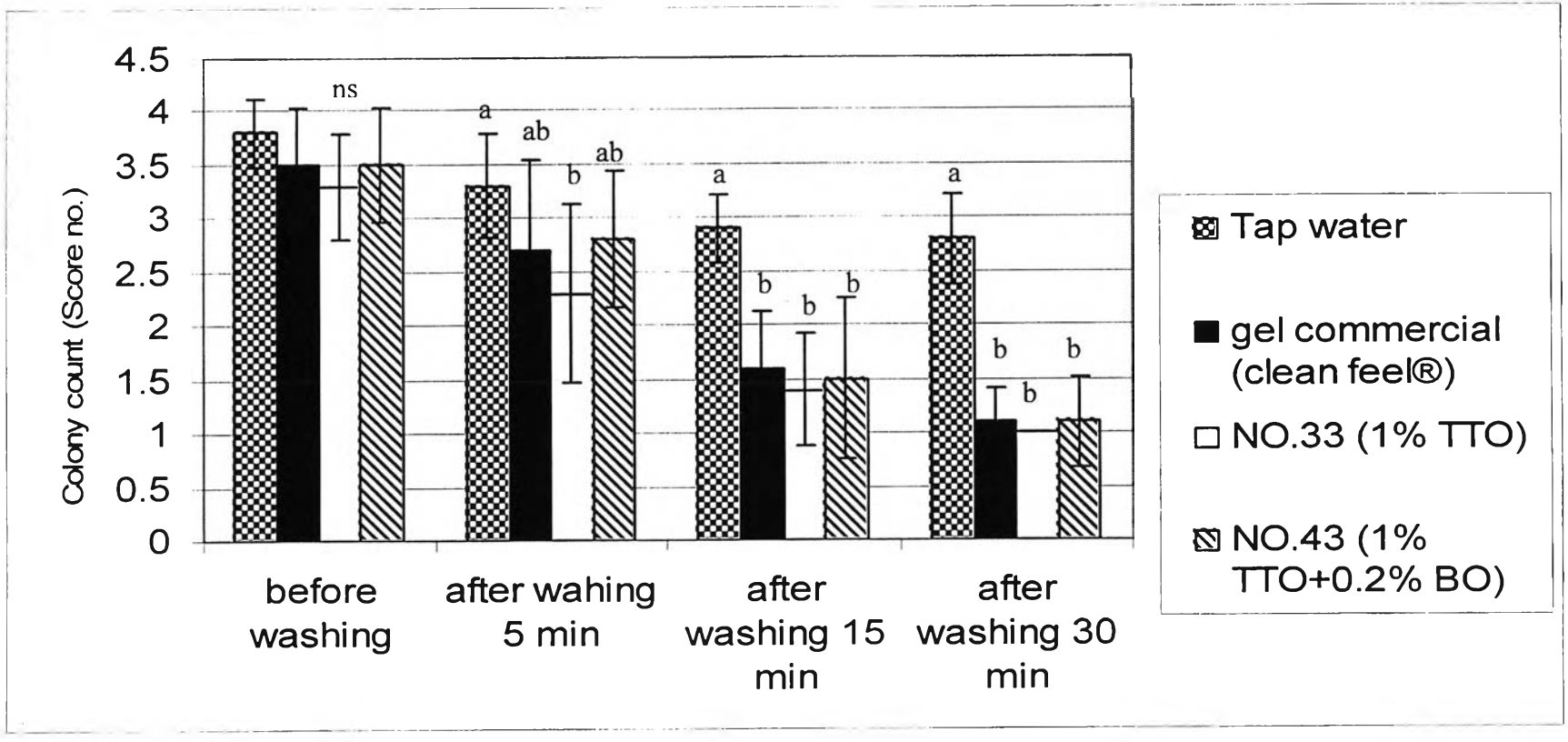
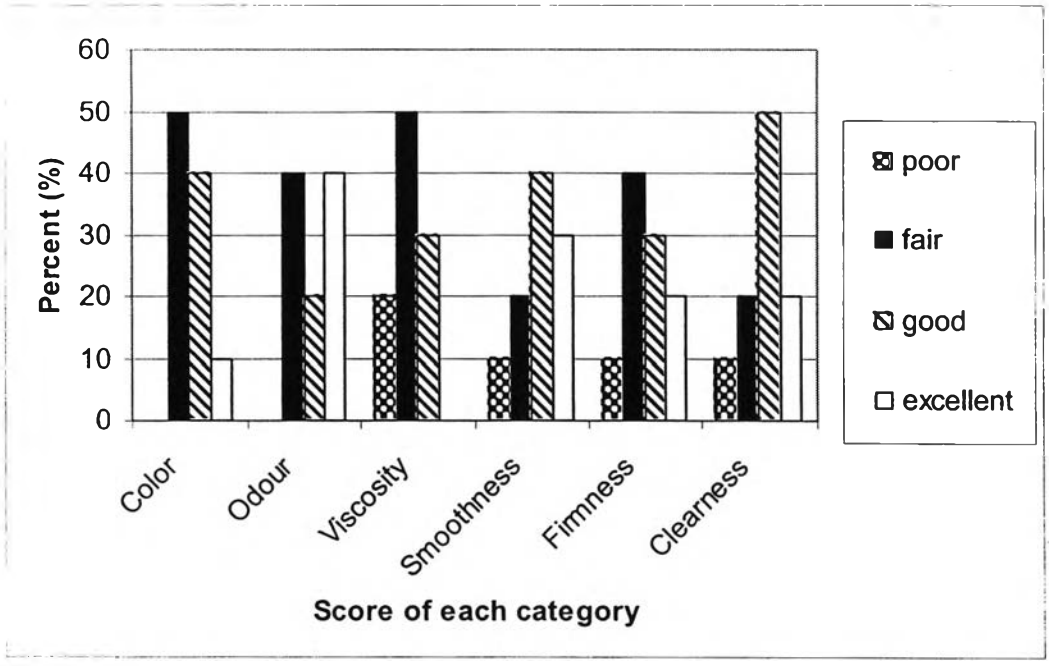
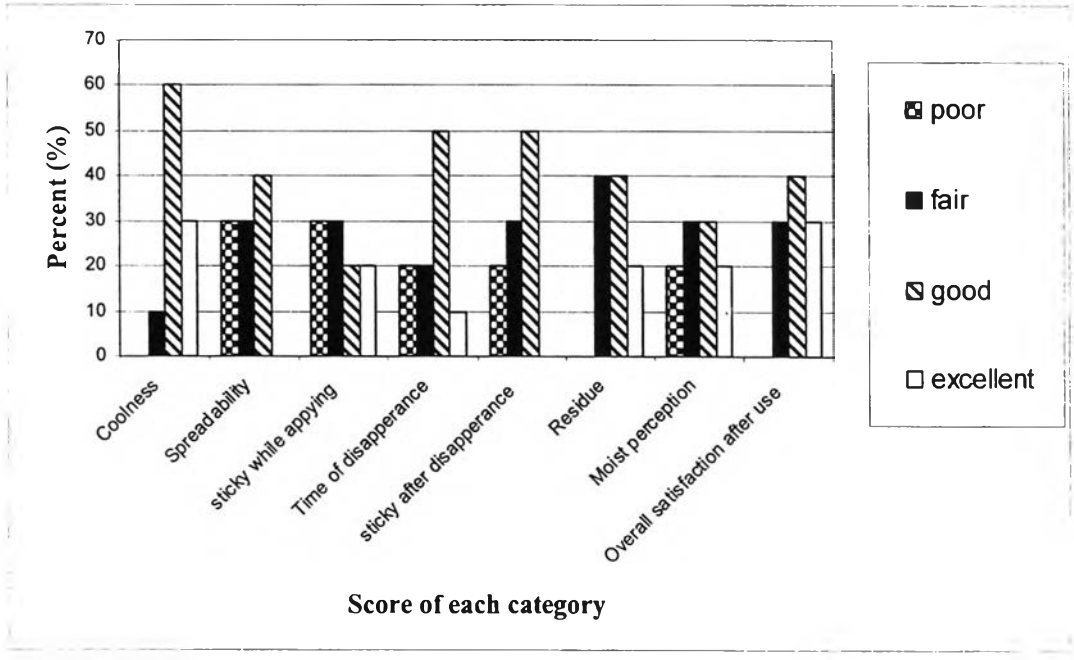



Figure 25. In-vivo evaluation of antiseptic potency by hand washing test of between gel commercial product (clean feel®), antiseptic PG gel NO.33 and NO. 43, tap water was control. The colony counts were determined after incubation the inoculated MHA plates at 37°C for 24 hrs. Score no: 1 = 0-50 colony, 2 = 50-100 colony, 3 = 100-200 colony, 4 = >200 colony. Data are mean ± SD., n = 10 a, b = significant difference ($p$ <0.05).

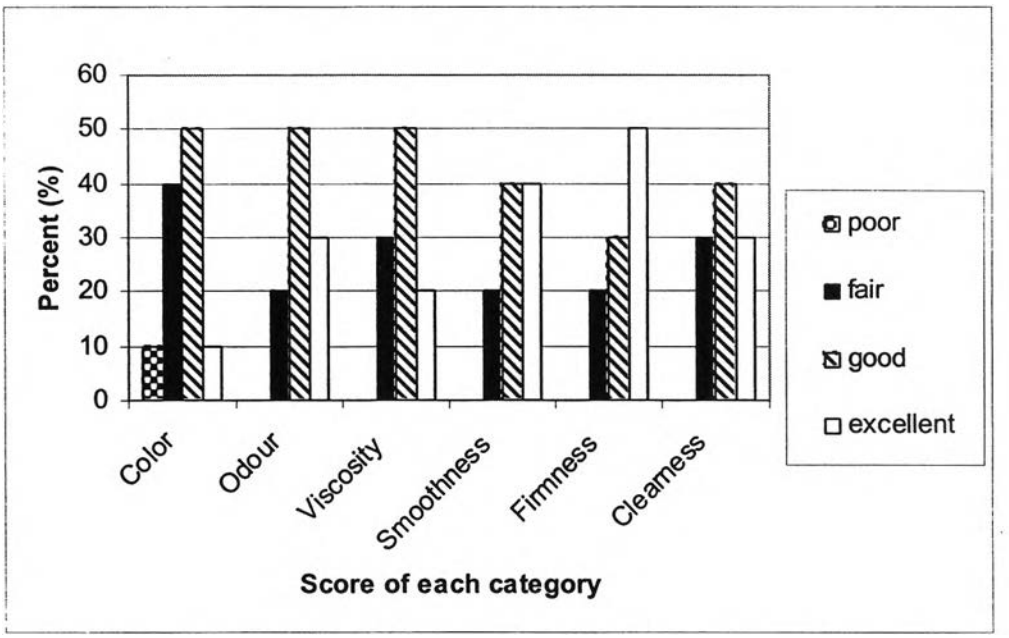



A) Physical appearance

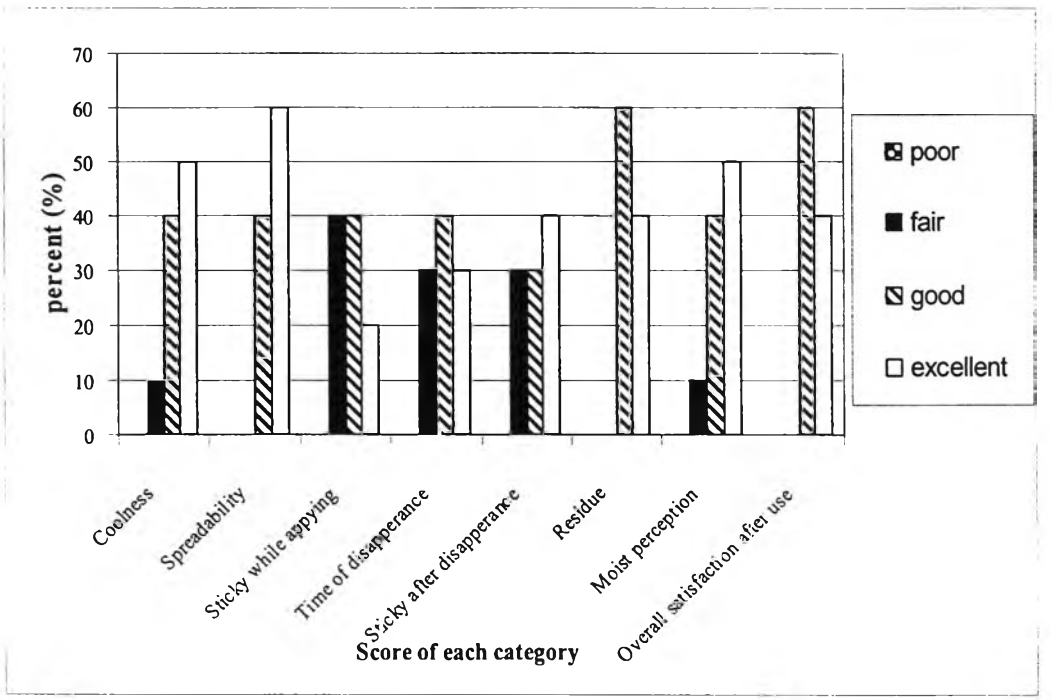



B) After application

Figure 26. Evaluation of subject's perception of tea tree oil-PG gel products (NO.33).




A) Physical appearance




B) After application

Figure 27. Evaluation of subject's perception of tea tree oil/betel oil-PG gel products (NO.43).