CHAPTER IV

DISSCUSION AND CONCLUSION

Discussion:

1. Polysaccharide Gel (PG) from Fruit-Hulls of Durian

Polysaccharide gel (PG) extracted from the fruit-hulls of durian are composed of pectic substance as the principal component and short chain amylose (Hokputsa *et al.*, 2004). The study showed the presence of sugars including fructose 3.7%, arabinose 1.2%, rhamnose 4.8%, xylose 0.4% galactose 4.9%, glucose 20.9%, and galacturonic acids 67.9% in Polysaccharide gel (Greddit, 2002; Hokputsa *et al.*, 2004; Pongsamart and Panmaung, 1998). Percentage yield of purified extract was 6.5% by weight of dried fruit-hulls as creamy white powder. The result of purified PG obtained was about the same as previous study by Greddit, 2002; Hokputsa *et al.*, 2004; Nakchat, 2002. An aqueous dispersion of PG at 3% concentration gave the pH value and apparent viscosity of 2.27 ± 0.01 and 439.67 ± 0.58 cps, respectively.

The polysaccharide gel (PG) has found to be useful for pharmaceutical products such as tablet binder, film coat tablet, suspension, emulsion and gelling, (Pongsamart *et al.*, 1989; Pongsamart *et al.*, 1998; Umprayn *et al.*, 1990). The formulation of PG containing vitamin E or vitamin C or mixture of E and C were studied (Lerchiporn, 2003). Antibacterial activity of polysaccharide gel was investigated. The results showed that PG produced antibacterial activity against strains of gram positive and negative bacteria (Nantawanit, 2001).

The formulation of antimicrobial PG preparation of polysaccharide gel combined with betel vine oil was determined for development anti-acne preparation.

2. Physical properties of Polysaccharide Gel (PG)

The polysaccharied gel was a water soluble acidic polysaccharide. The pH values of 1-6% w/v of PG were slightly decreased, its viscosities were increased with respect to the increasing concentration of PG and most of which is made in reference to previous works (Lertchaipom, 2003). At 4% concentration of PG the viscosity increased more steeply at concentrations higher than 4%. Low pH value at higher concentration was agreed with acidic sugar content, polygalacturonic acid, in the molecule of PG (Hokputsa *et al.*, 2004). The high viscosity was also represent the high molecular weight of longchain polygalacturonan with branches of neutral sugars.

3. Compatibility studies of Polysaccharide Gel (PG)

The effect of acid and base on 3% PG solution in water was determined. Hydrochloric acid was used to adjust the acid pH while sodium hydroxide was used to adjust the basic pH of the PG dispersion. Increasing pH of PG solution resulted in decreasing viscosity. The result was showed that acid pH lower than 2 had less effect on viscosity of PG. At the higher pH from 6.04, PG solution was changed. PG for color was turned into brown color as previously described (Greddit, 2002; Lertchaipom, 2003; Tachatawepisarn, 2003).

Compatibility studies of PG with divalent electrolytes such as $CaCl_2$, $MgCl_2$, $ZnSO_4$ and $FeSO_4$ were investigated. All of divalent cations increased the apparent viscosity of PG solution. $ZnSO_4$ at final concentration of 0.1 M, showed the most effective to PG solution, the highest viscosity of PG was obtained. $FeSO_4$ showed moderate effective but at 0.08 M concentration increased the viscosity of PG solutions very rapidly. $CaCl_2$ and $MgCl_2$ showed less effective to PG solutions compared to the first two electrolytes described. These results correlate well with the previous studies of carrageenans by research of Valenta and Schultz, (2004) indicated that gel formation of carrageenans involves helix formation and gel-strengthening K^+ or Ca^{2+} which not only aid helix formation but subsequently support aggregating linkages between the helices and are so forming junction zones, such structure created viscous gel.

The influence of organic solvents such as ethanol and isopropanol, on the viscosity of PG was demonstrated. The viscosity slightly increased as the solvent concentration was increased form 5 to 15% w/v of the two solvents, and then the viscosity rapidly increased with increasing higher concentration than 15% w/v of the solvents (Lertchaiporn, 2003). However, these organic solvents caused PG precipitated at the concentration higher than 40% w/v. Our results are in agreement with Valenta and Schultz, (2004) they observed the incompatibility of carageenan hydrogels with ethanol, it displays a quite limited compatibility with ethanol of about 30% w/v, according to salting effect.

The different kinds of humectants such as propylene glycol, glycerin and sorbitol have been studied (Capkova *et al.*, 2005). The increasing concentrations of propylene glycol, glycerin and sorbitol resulted in increasing the apparent viscosity of PG. Propylene glycol showed most effect on viscosity values of PG. However, these humectanats showed slightly effect to the viscosity of PG at the concentration used in antimicrobial PG preparation in this study.

Effect of emollient on viscosity of PG was studied by adding amerchol L101to the 3% PG solutions. The viscosity and pH value of PG mixture was increased with increasing concentration of amerchol L101. The viscosity of PG was slightly increased as amerchol L101 concentration increased up to 15% w/v, but at concentration of the amerchol L101 higher than 15% the viscosity of PG increased rapidly. In the antimicrobial PG preparation, amerchol L-101 was used at vary concentration of 0.25%, which was not effect to PG viscosity.

Preservative was also studied by adding an appropriate volume of paraben concentrate to the 3% PG solution. The results showed that preservative produced less effect to viscosity and pH value of PG solution. In the antimicrobial PG preparation uses paraben concentrate only 1% w/v, which slightly change the viscosity and pH value of PG solution (Lertchaiporn, 2003).

4. Effect of buffers on polysaccharide gel (PG)

The influence of citrate buffer and phosphate buffer at different pHs on the apparent viscosity was investigated. The result showed that citrate buffer was not produced viscosity changes of PG. On the other hand phosphate buffer showed highly increased the apparent viscosity of PG, especially at the higher pH values of pH6. The viscosity values of PG in citrate buffer were at ranges of 357.40$\pm$2.05 cps to 210.83$\pm$1.72 cps whereas the viscosity values of PG in phosphate buffer were 910.57$\pm$1.86 to 9839.33$\pm$2.08 cps. The low-ester citrus pectin gels investigated when evaluated in varied pH gels having about 30% soluble solids, show a steady decline in gel strength and gel power as the pH increase from 3, a pH used for low-ester pectin gels grading, to pH 3.6 and above. (El-Nawawi and Heikal, 1995). There was an optimum gellan concentration for minimum gelation time, this optimum being pH dependent (Fonkwe *et al.*, 2003).

5. Determination of free radical scavenging activity using DPPH method

Antioxidant activity of samples were determined by DPPH method. The odd electron of DPPH shows a strong absorption maximum at 517 nm and its solution is deep purple color. As the odd electron of the radical becomes paired off, the absorption strength is decreased. The resulting decolorization is stoichiometric with respect to the number of electrons captured (Blois, 1958).

The data demonstrated that the IC_{50} value varied between 14.81 to 54.72 mg/ml. The highest DPPH radical scavenging properties were found in curcuminoid which obtained from Thai-china flavours and fragrances industry CO., LTD, with an IC_{50} value of 14.81 mg/ml, which could be compared with the synthetic antioxidant agent gallic acid (4.39mg/ml). Whereas betel vine oil and oleoresin of curcumin showed less or no DPPH radical scavenging activity.

Free radical scavenging activity of tested natural products provide beneficial information of natural product that might incorporated in antiacne preparations.

6. Antimicrobial susceptibility test of betel vine oil

Betel vine oil was investigated to evaluate its antimicrobial activity by agar diffusion and broth macrodilution method against bacteria and fungus.

6.1 Agar diffusion

The agar diffusion method used in this study is one of the most often used methods for antimicrobial activity assessment. Betel vine oil has limited solubility in aqueous media. Tween 80, at concentration 0.1%, have been used to improve the distribution of oil within aqueous media without neutralyzing the antimicrobial effect (Opalchenova and Obreshkova, 2003). Inhibition of microbial growth on agar plates of betel oil were demonstrated, the results showed clear inhibition zones on the agar after incubation for 24 or 48 hrs. The zones of inhibition were obtained at the lowest concentration 0.08% v/v of betel vine oil against bacteria, *M. luteus, B. subtilis, P. vulgaris, Ps. aeruginosa* and fungus, *S. cerevisiae*, were ranging from 10.20 (0.20) to 12.53 (1.84) mm (Table 5-7). Diameter of microbial inhibition zone against fungus, *C. albicans* was 11.20 (0.26) mm with 0.16% v/v of the betel vine oil (Table 7). While, inhibition zones obtained at 0.31% v/v concentration betel vine oil against *P. acnes, S. aureus, S. epidermidis, E. coli, Salmonella typhimurium* and *K. pneumoniae* were ranging from 9.67 (0.58) to 11.28 (0.85) mm (Table 5-7). An increment of inhibition zone diameter was produced with respect to increasing concentrations of betel vine oil. Several factors that are relevent for diffusion capacity of meterials in agar plates must be considered, such as the contact between experimental material and agar, molecular weight, size and shape of the antimicrobial agent, load and concentration of test material, agar gel viscosity, and ionic concentration in relation to medium. Furthermore, control and standardization of inoculation density, evaluation of results, selection of agar medium, selection of microorganisms, depth of agar medium, incubation temperature of plates, and reading point of inhibition zones are also restricting factors affecting the dynamics and variability of diffusion tests in an agar medium (Leonardo *et al.*, 2000).

6.2 Broth macrodilution test

The susceptibility of the test bacteria and fungus to betel vine oil at different concentrations was evaluated. Solvent for essential oil such as 1% DMSO had no inhibitory activity against the test organisms. MBCs of betel oil were in ranges of 0.010 to 0.039%. The lowest MIC and MBC values of betel vine oil were 0.010 and 0.020% (v/v), respectively against *B. subtilis* and *C. albicans*. Whereas, MIC and MBC values of betel vine oil were 0.020 and 0.039% (v/v) against *P. acnes, M. luteus, P. vulgaris, Ps. aeruginosa* and *S. cerevisiae*, repectively (Table 10). MIC and MBC were 0.039 and 0.078% (v/v) against *S. aureus, S. epidermidis* and *K. pneumoniae* (Table 10). The highest values of MIC and MBC of betel vine oil were 0.078 and 0.156% (v/v) against *S. typhimurium* and *E. coli*, respectively, (Table 10). It is interesting that *C. albican* was the most sensitive to betel vine oil whereas PG was not effective against this microorganism (Nantawanit, 2001).

Antibacterial effect betel vine oil might be due to the isoeugenol component in essential oil. Isoeugenol has found possess antibacterial activity against *S. aureus* and *E. coli* (Morris *et al.*, 1979). Essential oil of *Piper betle* L. leaves has activity against bacteria and fugus. Its activity may be due to its major constituents, eugenol. Major consittuent has found to showed activity against bacteria (Morris *et al.*, 1979; Ross *et al.*, 1980).

Rasooli and Mirmostafa, (2003) have established that the composition of essential oils will depend on the plant species, the chemotypes, and the clilmatic conditions; therefore, their antimicrobial activities could vary. The study also revealed that the active compound in *P. betle* is hydroxychavicol. The inhibitory action is similarly to phenolic compounds, which are also antimicrobial agents.

Overall results indicated that betel vine oil has potential benefit as an antiseptic or antimicrobial agent for treatment of skin microorganisms associated with acne.

7. Surfactant optimization

In order to be able to prepare a type of pharmaceutical or cosmetic transparent gel, some suitable surfactants needed to be added. These included Cremophor RH-40, Pluronic F-68 and Tween 80. Clear gels can be produced in combinations of oil, water, and high concentrations of certain nonionic surfactants. These combinations resulted in the formation of microemulsions; the semisolid rheology encountered is due to the existence of liquid crystalline phases. Gel characteristics can be varied by adjusting the proportion and concentration of the ingredients (Bouchemal *et al.*, 2004). The clear gels were obtained using Cremophor RH-40, Pluronic F-68 and Tween 80 at concentrations 5, 10 and 15% w/w while at concentration 1% w/w of three surfactant gave cloudy solution. Therefore, Cremophor RH-40, Pluronic F-68 and Tween 80 was used as surfactant in the formulation of antimicrobial PG preparation.

8. Formulation of antimicrobial PG preparation

In the formulation of antimicrobial PG preparation, polysaccharide gel (PG) was used as gelling agent and antibacterial agent (Lertchaiporn, 2003; Nantawanit, 2001). The concentrations of the gelling agents is typically less than 10%, usually in the 0.5% to 2.0% range, with some exceptions (Allen, 2002).

PG at 2.5% w/v concentration was selected due to its effective concentration for inhibiting bacteria growth as well as gelling effect. The formulation was developed with 2.5% PG.

Propylene glycol has been used in a wide variety of pharmaceutical formulations and it is generally regarded as a nontoxic material. In topical preparations, propylene glycol is regarded as minimally irritant although it is more irritant than glycerin (Well, 1982). Propylene glycol is also used in cosmetics and in the food industry acts as humectant and carrier for emulsifers. It is a better general solvent than glycerin and dissolves a wide variety of materials. The concentration used in topical products is about 15%. In the formulation of antimicrobial PG preparation the concentration of 5% propylene glycol was used (Table12 and appendix c). The freshly prepared product was clear solution.

In topical pharmaceutical formulations and cosmetics, glycerin is used primarily for its humectant and emollient properties. Glycerin may also be used in topical at concentrations up to 30% (Aoshima, 2005; Rowe, 2005). Concentration at 5% glycerin was used in the formulation of antimicrobial PG preparation (Table12 and appendix c). The freshly prepared product was transparent gel.

Sorbitol is widely used as an excipient in pharmaceutical formulations. It is used as humectant, plasticizer, tablet and capsule diluent. Sorbitol is additionally used in injectable and topical preparations and therapeutically as an osmotic laxative. (Krogars *et al.*, 2003). For antimicrobial PG preparation at concentration 5 and 10% was used (Table12 and appendix c). The freshly prepared product was cloudy solution.

Amerchol L-101 is an oily liquid used in topical pharmaceutical formulations and cosmetics as an emulsifying agent with emollient properties. Concentration at 0.5 and 0.25% amerchol L-101 was used in the formulation of antimicrobial PG preparation (Table12 and appendix c). The finished product at concentration 0.25% gave clear solution but at concentration 0.5% gave cloudy solution. Though, amerchol L-101 at concentration 0.25% was used for antimicrobial PG preparation at concentration.

Polysorbates are hydrophillic nonionic surfactant used widely as emulsifying agents in the preparation of stable oil-in-water pharmaceutical emulsions. They may also be used as solubilizing agents for a variety of substances including essential oils and oil soluble vitamins, and as wetting agents in the formulation of oral and parenteral suspensions (Rowe, 2005). The Tween 80 is nonionic surfactant that is able to bind phenolic antimicrobial agents such as the thymol (Orafidiya *et al.*, 2001). In the formulation of antimicrobial PG preparation the concentration of 5, 10 and 15% Tween 80 was used (appendix c). The finished product was cloudy solution and separated to two phases during stability tested.

Poloxamer 188 used as an emulsifying agent in intravenous fat emulsions, and as solubilizing agent to maintain the clarity of elixirs and syrups. May also be used as a wetting agent in eye drops, ointments, suppository bases, gels and as tablet binders and coatings. For antimicrobial PG preparation at concentration 5, 10 and 15% was used (appendix c). The freshly prepared product was cloudy solution and was not stable under storage 30 days at ambient temperature and freeze-thaw cycles.

Polyoxyethylene castor oil derivatives are nonionic surfactatnts used in oral, topical and parenteral pharmaceutical formulations. They are also used in cosmetics and animal feeds. They act as emulsifying agent, solubilizing agent and wetting agent. Cremophor RH-40 can be used to solubilize vitamins, essential oil and drugs. From all these considerations it should be concluded that Cremorphor RH-40 at concentration 5 and 10% produced a satisfactory product, transparent gel, good texture and greaseless, was selected in the antimicrobial PG preparation in this study (Table12 and appendix c).

Since microorganisms can reside in the water or the lipid phase or both, the preservative, regardless of its water-oil partition coefficient, should be available at an effective level in both phases. In addition to the stabilization of pharmaceutical preparations against chemical and physical degradation due to changed environmental conditions within a formulation, certain liquid and semisolid preparations must be preserved against microbial contamination. The concentration of preservative required in a preparation depends to a large extent on its ability to interact with microorganisms. In the formulation of antimicrobial PG preparation the concentration of 1% paraben used (Table12 and appendix c).

Triethanolamine is a counteragent to control pH level and fatty acid. In the cosmetic products, it neutralizes cabomer to give cohesive power. One of the suggested approaches in the management and prophylaxis of acne involves binding of free fatty acids in the form of soap with alcoholamines. Triethanolamine is one of the best recognized alcoholamines. Complexation of triethanolamine with anionic polymers decreases its pH (Musial and Kubis, 2004). In the formulation of antimicrobial PG preparation triethanolamine was used at concentration 0.1%(Table12 and appendix c).

In formulation of antimicrobial PG gel contained organic acid ingredient such as lactic acid and/or salicylic acid were prepared. Lactic acid is used in beverages, foods, cosmetics, and pharmaceuticals as an acidifying agent and acidulant. In topical formulations, particularly cosmetics, it is used for its softening and conditioning effect on the skin, and treatment of warts. Lactic acid may also be used in the production of biodegradable polymers and microspheres, used in drug delivery system (Rowe, 2005). Some study has showed that as the lactic acid's concentration increased, the cumulative amount permeated also increased after 24 hours at all pH values (Souria *et al.*, 2003). Some effort has resulted in a novel anti-acne formulation containing salicylic acid and a proprietary synergistic natural complex that delivers keralytic, sebum control and anti-inflammatory effects to control the root causes as well as symptoms of acne. For formulation of antimicrobial PG preparation, precipitate sulphur was used at concentration 0.1-1%. The freshly prepared products were clear gel and was stable under storage 30 days at ambient temperature and freeze-thaw cycles.

Additionally, preparations of PG gel contained antimicrobial agents that contained precipitate sulphur and/or zinc oxide and/or HPMC 4000 were investigated. Sulphur is act as antiacne agent and keratolytic for topical used. Zinc oxide exhibits such antibacterial activity, and previously showed that it acted on gram-positive bacteria more strongly than on gram-negative bacteria (Sawai *et al.*, 1998). In the formulation of antimicrobial PG preparation, precipitate sulphur and zinc oxide was used at concentration 0.1-1%, the freshly prepared products were yellow and white suspension, respectively but all products were unstable due to cake formulation after standed at ambient temperature.

The selected antimicrobial PG preparation with betel vine oil was composed of 2.5% polysaccharide gel (PG), 5% propylene glycol, 5% glycerin, 2% betel vine oil, 0.25% amerchol L101, 10% cremorphor® RH-40, 0.1% triethanolamine and 1% paraben concentrate. The freshly prepared product was transparent gel (Table 12) the products remained unchanges after stability test (Table 15).

9. Physical properties evaluation of the finished products

The pH values and viscosity of the finished products of antimicrobial PG gel of antimicrobial PG gel were measured at ambient temperature. Characteristic of finished products was recorded after freshly prepared and any change was also recorded after stability tested. The pH value of antimicrobial PG preparations with betel vine oil were little changed while the viscosities of the preparations were slightly increased as indicated in Table 15.

Additionally, the preparation of PG gel contained organic acid, the pH values of PG preparations after stability test were slightly decreased, while their viscosities were increased (Table 15).

However, preparation of PG gel contained solid antimicrobial agents, the pH values of formulations contained precipitate sulphur were slightly decreased whereas zinc oxide incorporation resulted in increasing pH values of the product. The viscosities of the preparations of PG gel containing precipitate sulphur and/or zinc oxide were very much increased both after freshly prepared and after stability tested (Table 15). However, unsatisfactory products were obtained.

10. Assessment of antimicrobial PG preparation stability

The formulation of antimicrobial PG preparations using betel vine oil 2% was the selected product due to this formulation produced a satisfactory antimicrobial PG preparations, the product possessed good gel texture and color, good skin-sensation and minimal change occurred after 30 days stand at ambient temperature and after stability test for six freeze-thaw cycles (Table 15 and appendix d).

As regards the preparation of PG gel contained organic acid such as lactic acid and/or salicylic acid were formulated. The freshly prepared products were

(Table 15 and appendix d). After physical stability test, the products remain stable the characteristic of gel products remain the same as after freshly prepared. However, the pH values of PG preparations were slightly decreased and the viscosities were increased after stability tested.

In preparation of PG gel contained solid antimicrobial agents (precipitate sulphur and/or zinc oxide) the formula contained precipitate sulphur gave yellow suspension with high viscosity whereas zinc oxide gave white suspension with high viscosity as well. The observation of physical stability found that solid ingredients induces phase separation in these formulations study (Table 15 and appendix d).

11. Bacterial susceptibility tests of the finished products antimicrobial PG preparation

11.1 Agar well diffusion method

The diameter of inhibition zones from different concentrations (1 and 2%) of betel vine oil in acne gel product were determined in comparison among gel base, betel vine oil (1 and 2%), 2.5% PG and as positive control. The results of inhibition zone diameters of antimicrobial PG preparation containing 2% betel vine oil was range 10.52 (0.08) to 10.95 (0.05) mm against *Propionibacterium acnes, Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively. Whereas PG gel base was used as control showed no inhibition zone (Table 16, Figure 31). As regards, Panoxyl 5® gel (positive control) showed most effective inhibition of bacteria growth, represented by zones of inhibition against *P. acnes*, *S. aureus* and *S. epidermidis*. The antibacterial activity of resulting product showed inhibitory activity (in vitro) against tested bacteria causing acnes and skin diseases. Panoxyl 5® is a chemical antiseptic preparation used for treatment of acne pimples. Resulting product of acne gel made of natural material seemed to provide alternative for treatment of acnes.

11.2 Broth microdilution assay

The MIC and MBC values of different products were also determined against *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. The MIC (MBC) values of antimicrobial PG product contained 1% betel vine oil were 4 (20), 20 (100), 0.80 (4)% of the finished products against *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, respectively. As regards, MIC (MBC) values were 0.8 (4), 4 (20), 0.8 (4) of finished products contained 2% betel vine oil against *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively. The 2.5% PG showed MIC (MBC) values against *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* at 20 (100)% of the finished product. The positive control exhibited the strong antibacterial activity. While PG base preparation did not exhibit visible growth.

Conclusion:

Polysaccharide gel is a water soluble polysaccharide and isolated from fruit-hulls of durian (*Durio zibethinus* Murr.). Creamy white powder was obtained, final yield of polysaccharide gel (PG) was 6.5%. The pH value and apparent viscosity of PG in distilled water at 3% concentration was 2.27 ± 0.01 and 439.67 ± 0.58 cps, respectively.

The apparent viscosity of PG dispersion was higher at acidic pH than that of basic pH. All of divalent cations such as $CaCl_2$, $MgCl_2$, $ZnSO_4$ and $FeSO_4$ highly increased the viscosity but less effected on pH value of PG solution. Organic solvents, ethyl alcohol and isopropyl alcohol at >15-30% increased the apparent viscosity and the higher concentration PG was precipitated in the solvents. Glycerin and sorbitol showed less effect on PG viscosity, but propylene glycol at >15% increased PG viscosity whereas at 15-20% showed less effect on PG viscosity. The viscosity and pH value of PG solution were increased with increasing concentration of amerchol L101. Preservative (paraben concentrated) at the concentration used in the formulation showed less effect on viscosity and pH value of PG solution.

Effect of buffers on polysaccharide gel (PG) was also studied. Citrate buffer at any pH values showed less effect on viscosity while phosphate buffer highly increased the apparent viscosity of PG, especially at the higher pH values of pH6.

Susceptibility test of betel vine oil illustrated that zones of inhibition at the lowest concentration 0.08% v/v of betel vine oil were obtained against bacteria, *M. luteus, B. subtilis, P. vulgaris, Ps. aeruginosa* and fungus, *S. cerevisiae*; at 0.16% v/v against *C. albicans;* at 0.31% v/v against *P. acnes, S. aureus, S. epidermidis, E. coli*, *Salmonella typhimurium* and *K. pneumoniae*. Additionally, MICs and MBCs of betel oil were at ranges 0.010 to 0.039%, and 0.020 to 0.078%, respectively.

The formulation of antimicrobial PG preparations using 2% betel vine oil is, however, considered the better anti-acne PG gel product by its appearance of transparent gel and greaseless. After stability test by 30 days storage at ambient temperature and six freeze-thaw cycles, the antimicrobial PG preparation was well stable, the same appearance as the freshly prepared product. In addition preparations of PG gel containing organic acid were also well stable. Whereas unsatisfactory products separation observed in formulations containing solid antimicrobial agents.

Product of antimicrobial PG preparation containing 2% betel vine oil showed better bactericidal activity against acne causing bacterium, *P. acnes*, than the product containing 1% betel vine oil by in vitro broth microdilution assay.

A natural water soluble polysaccharide from durian fruit-hulls possess gelling and antibacterial properties is suitable for wide use in pharmaceuticals and cosmetics. Combination of antimicrobial polysaccharide gel (PG) with antimicrobial essential oil such as betel vine oil was prepared successfully, the products of antimicrobial PG preparations inhibited killing effect against bacteria causing acnes and skin disease according to this study.