Original Research Article

FORMULATION, CHARACTERIZATION AND DETERMINATION OF ANTIBACTERIAL ACTIVITY OF POMEGRANATE (*Punica granatum*) GEL.

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ABSTRACT

Purpose.
Previous studies have shown that *Punica granatum* (*P. granatum*) extract exhibited antimicrobial activity against a wide range of pathogenic microorganisms. Thus, the aim of study was to formulate and determine the effectiveness of antimicrobial properties of gel containing *P. granatum* methanol extract against selected common skin pathogens including *Staphylococcus aureus*, *Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Candida albicans*.

Methods.
Extraction process utilizes *P. granatum* peel and methanol as solvent. Using the disc diffusion method, different concentrations (25 %w/v, 50 %w/v, 75 %w/v and 100 %w/v) of *P. granatum* extract were initially tested against *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *C. albicans* for their antibacterial activity. Gel formulations were then prepared with varying concentration of the extract as active ingredient. Antimicrobial activity of *P. granatum* gel was determined by using agar well diffusion method. The gel formulations were stored at 4°C, 25°C and 37°C for one month. Evaluation on the pH, physical characteristic and antimicrobial activity were then conducted on the gel formulations.

Results.
Both extract and gel showed highest antibacterial activity against *S. epidermidis* followed by *S. aureus* and then *P. aeruginosa*. Surprisingly no antifungal activity was observed against *C. albicans*. The concentration of extract alone and in gel were directly proportional to antibacterial activity. All gels formulated showed satisfactory physical characteristics even after a month of storage at different temperature. As expected, gel stored at 4°C showed the least decrease in antibacterial activity.

Conclusions.
P. granatum has potential to be formulated as gel to treat bacterial skin infection caused by *S. aureus*, *S. epidermidis* and *P. aeruginosa*. Further studies are required in order to optimize the antimicrobial activity of *P. granatum* gel.

Keywords: Antibacterial Activity, Pomegranate (*Punica Granatum*)

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NOVELTY OF WORK:

Pomegranate or *Punica granatum* is a fruit which posseses many health benefits including anti-microbial properties. However to our best knowledge, there are no commercial products or studies which have attempted to incorporate pomegranate extract into a topical dosage form. Given the current consumer trend

of using natural products as a remedy, this study aims to explore the effectiveness and physical characteristics of pomegranate gel formulations for commercial use.

INTRODUCTION

The skin serves as a protective barrier to its external environment. Once there is breakage of intact skin, various types of pathogen may invade the area and proliferate causing severe infection (8). *S. aureus*, the most common etiology of skin infection and intense inflammatory response, causes Scalded Skin Syndrome, impetigo, folliculitis, carbuncles and furuncles. *S. epidermidis*, typically found in wound infection, is characterized by the presence of erythema and pus at the site of trauma or surgical wound. On the other hand, *P. aeruginosa* infects burn wounds, folliculitis, fingernail infection and secondary infection in people with acne or depilate their leg (13). *C. albicans*, an opportunistic pathogen, causes candidiasis (8).

The standard clinical approach in treating skin infection is using antibiotic therapy ranging from semi solid preparation to oral and intravenous administration depending on the severity of the infection. However the emergence of antibiotic-resistant bacteria is a major concern in antibiotic therapy due to indiscrimniate usage. Sensitivity pattern of *S. aureus* towards several antibiotics has shown that *S. aureus* do not respond adequately to common antibiotics used in practice such as penicillin, ampicillin, tetracycline and chloramphenicol (23). A rise in resistance of *S. aureus*, *Staphylococcus coagulase* negative and *P. aeruginosa* against gentamicin (14). As more bacteria become resistance, there is a real need to seek other alternatives to antibiotics in treating infections. Coupled with the growing trend of consumers shifting from conventional medicine to natural products and evolving technology, natural products may provide the answer in combating antibiotic-resistant bacteria strains (10).

P. granatum, locally known as pomegranate, is widely found in Asia continent particularly Iran, Afghanistan and the Himalayas before it was introduced and cultivated in the Mediterranean region. Previous studies have shown the potential antimicrobial activity of *P. granatum*. Pure compound isolated from *P. granatum* juice has shown significant antibacterial activity against of gram-positive and gram-negative bacteria (15). *P. granatum* extract had been shown to exhibit antibacterial activity against *S. aureus*, *S. epidermidis*, *P. aeruginosa* (1, 2, 16, 19). It is also revealed that *P. granatum* extract exhibited antifungal activity against *C. albicans* (2). Notwithstanding the positive research findings shown by these researchers, no formulation work has been done to develop these untapped resources into a dosage form suitable for patient use.

Gel is a dosage form consisting of liquid gelled by suitable gelling agents (4). Previous studies have been carried out using gel containing various herbal extracts to counter skin infections caused by a number of different bacteria strains (18, 20, 21, 24). Interestingly most studies have shown that the combination of gel and herbal extract is potentially synergistic against skin pathogens. Thus, in this study, gel is considered to be an ideal dosage form for *P. granatum* extract.

MATERIALS AND METHODS

Carbopol 940 was purchased from KOFA Chemical Works (M) Sdn. Bhd. Propylene glycol and methyl paraben was obtained from Euro-Chemo Pharma Sdn. Bhd. Pomegranate fruits were imported from India and they were collected at Masjid India, Kuala Lumpur. *P. granatum* methanolic extract and sterile distilled water were prepared in Cyberjaya University College of Medical Sciences. Glycerine was procured from R&M Marketing, UK. While triethanolamine and gentamicin sulphate was purchased from Becton Dickinson, USA and Atlantic Laboratories Corp. Ltd , Bangkok Thailand, respectively.

Preparation of *P. granatum* methanolic extract

P. granatum methanolic extract was prepared in Cyberjaya University College of Medical Sciences. Eight imported pomegranate weighing between 150 g to 250 g were used in this study. The pomegranate peels weighing 513g was used in the extraction process. It was freeze-dried using liquid nitrogen and ground immediately using a clean mortar and pestle. Peels were soaked in methanol by ratio 1:1 and then transferred into an incubator shaker (Wise Cube, Model WIS-S10) for one hour at 100 RPM. After that, pomegranate extract was filtered by using filter paper. Later the extract was evaporated using a rotary evaporator (Buchirotavapor, Model R-210) for 7 to 8 hours. The final weight of the concentrated methanolic extract was 97.35 g. The extract was converted into powder using a freeze dryer (Freeze dryer, Alpha 1-2LD plus) for 3 days. The weight of the powdered *P. granatum* was 40.69 g. Freeze dried *P. granatum* methanolic extract were then stored in tightly dessicated container at 4°C to maintain its stability prior to use.

Antimicrobial screening of *P. granatum* extract

The extract was tested against *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *C. albicans* using Kirby-Bauer Disc Diffusion method (5, 22). Four different concentrations across the concentration gradient of methanolic extract ie 25 %w/v, 50 %w/v, 75 %w/v, and 100 %w/v were prepared from freeze dried *P. granatum* extract using sterile distilled water as solvent. Mueller Hinton agar was the media used (Thermo Fisher Scientific, Malaysia). Briefly, 6-mm filter papers were impregnated with known concentration of the extract. The suspension of bacteria was spread on medium agar then four discs impregnated with the compound were placed on the agar with certain distance. After that, the plate was placed inverted in incubator at 37°C for 24 hours. The zone of inhibition was determine by measuring its diameter by using a ruler to the nearest mm. The bacterial suspension was standardized by using 0.5 McFarland standard. Gentamicin disc (Oxoid Ltd, UK), 6 mm diameter, were used as positive control (22). The negative control used was disc impregnated with sterile distilled water.

Gel formulation

The gel formulations are shown in Table 1 . The gels prepared in this study were listed in Table 2 which were gel with *P. granatum*, gel without *P. granatum* as negative control and with gentamicin sulphate as positive control.

Table 1 Gel formulation used in study.

Component	**General formulation**	**With *P. granatum***	**Without *P. granatum* (negative control)**	**With gentamicin sulphate (positive control)**
		Amount		
Carbopol 940 (g)	1.5 %	1.5	1.5	1.5
Propylene glycol (ml)	10 %	10	10	10
Glycerine (ml)	2 %	2	2	2
Methyl paraben (g)	0.02 %	0.02	0.02	0.02
Triethanolamine (ml)	q.s.	q.s.	q.s.	q.s.
P. granatum extract (ml)	5 %	5	-	-

Gentamicin sulphate (g)	1 %	-	-	0.1
Distilled water (ml)	q.s. to 100 ml	q.s.	q.s.	q.s.

Table 2 The gel formulations prepared in this study

Type of gel	Name
Gel without extract	Negative control
0.1 %$^{v}/_{v}$ gentamicin sulphate gel	Positive control
Gel incorporated with 5 ml 25 %$^{w}/_{v}$ *P. granatum* extract.	PG25%
Gel incorporated with 5 ml 50 %$^{w}/_{v}$ *P. granatum* extract.	PG50%
Gel incorporated with 5 ml 75 %$^{w}/_{v}$ *P. granatum* extract.	PG75%
Gel incorporated with 5 ml 100 %$^{w}/_{v}$ *P. granatum* extract.	PG100%

Gel preparation

Carbopol 940 was dispersed in 50 ml distilled water. It was kept under magnetic stirrer until a homogenous dispersion was formed. 1 g of methyl paraben was dissolved in 100 ml distilled water in a heated water bath. The solution was later allowed to cool. 2 ml methyl paraben was added to the carbopol dispersion. Then 10 ml propylene glycol 400 and 2 ml glycerin were added to the mixture. Freeze dried *P. granatum* extract was dissolved in 100 ml of distilled water to produce *P. granatum* solution at various concentration (25 %w/v, 50 %w/v, 75 %w/v and 100 %w/v). 5 ml of this solution was added to the gel base mixture. The homogenous dispersion was stirred using a magnetic stirrer. The pH of formulation was then adjusted to a neutral pH by titrating triethanolamine which enhances the gelling properties of carbopol 940. For positive control, gentamicin sulphate for injection was used instead of the extract. For negative control, gel was prepared without *P. granatum* extract.

pH test

Digital pH meter (Terri & William, USA) was used to measure pH of gel. pH measurement was carried out by dipping glass electrode completely into the gel (24). Prior to usage, pH meter was calibrated with standard buffer solution pH 4.00 and pH 7.01. pH measurement was carried out in triplicate and average reading was recorded.

Physical evaluation

P. granatum gel was inspected visually for its colour, homogeneity, consistency, and phase separation (24).

Effect of temperature on *P. granatum* gel

Effect of temperature on a few parameters such as pH, physical characteristic, and antimicrobial activity were evaluated. It was carried out at three different temperatures (4°C, 25°C and 37°C) and stored for one month.

Antibacterial screening of *P. granatum* gel

P. granatum gel formulations were screened for its antibacterial activity against *S. aureus*, *S. epidermidis*, and *P. aeruginosa* using modified agar well diffusion method. *P. granatum* gel is not tested against *C. albicans* as during antimicrobial screening of *P. granatum* extract, the extract did not show any antifungal activity. Marketed dermatological preparation intended for bacterial infection, Begenta (0.1% gentamicin cream) was also being tested against those bacteria in order to compare effectiveness of formulated gel with marketed product. Mueller Hinton was used as medium agar. In this method, inoculum of

microorganisms was mixed with molten agar that was cooled to 45°C. As agar solidified, holes were punched and then loaded with the gel. The agar was incubated in an inverted position at 37°C for 24 hours. The zone of inhibition was later determined.

Statistical analysis

Statistical analysis was performed by using Statistic Package for Social Sciences Programme version 20.0 (SPSS 20.0) and Microsoft Excel 2007. One way ANOVA followed by Tukey test were performed to determine statistical significance ($p<0.05$).

RESULTS

Evaluation of antimicrobial properties of *Punica* granatum methanolic extract

Antimicrobial activity of *P. granatum* extract was expressed as average zone of inhibition exhibited by the extract. Figure 1 shows the average zone of inhibition exhibited by various concentration of *P. granatum* extract against *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *C. albicans*.

S. aureus showed highest sensitivity against gentamicin and lowest sensitivity against 25 %w/v *P. granatum* extract. The zone of inhibition exhibited all concentration of *P. granatum* extract discs against *S. aureus* were significantly lower than gentamicin disc ($p<0.05$). *S. epidermidis* showed the highest sensitivity against 100 %w/v *P. granatum* extract and the lowest sensitivity against 25 %w/v *P. granatum* extract. Surprisingly the zone of inhibition exhibited by 25 %w/v, 50 %w/v, 75 %w/v and 100 %w/v of *P. granatum* extract discs were significantly higher than gentamicin disc ($p<0.05$). 25 %w/v and 50 %w/v *P. granatum* extract exhibit no activity against *P. aeruginosa*. *P. aeruginosa* showed low sensitivity against 75 %w/v and 100 %w/v *P. granatum* extract. *P. aeruginosa* showed the highest sensitivity toward gentamicin disc. Zone of inhibition exhibited by 75 %w/v and 100 %w/v *P. granatum* extract were significantly lower than gentamicin disc ($p<0.05$). 100 %w/v *P. granatum* extract showed the highest antibacterial activity against *S. epidermidis* and the lowest against *P. aeruginosa*. All concentration of *P. granatum* extracts exhibited no antifungal activity against *C. albicans*.

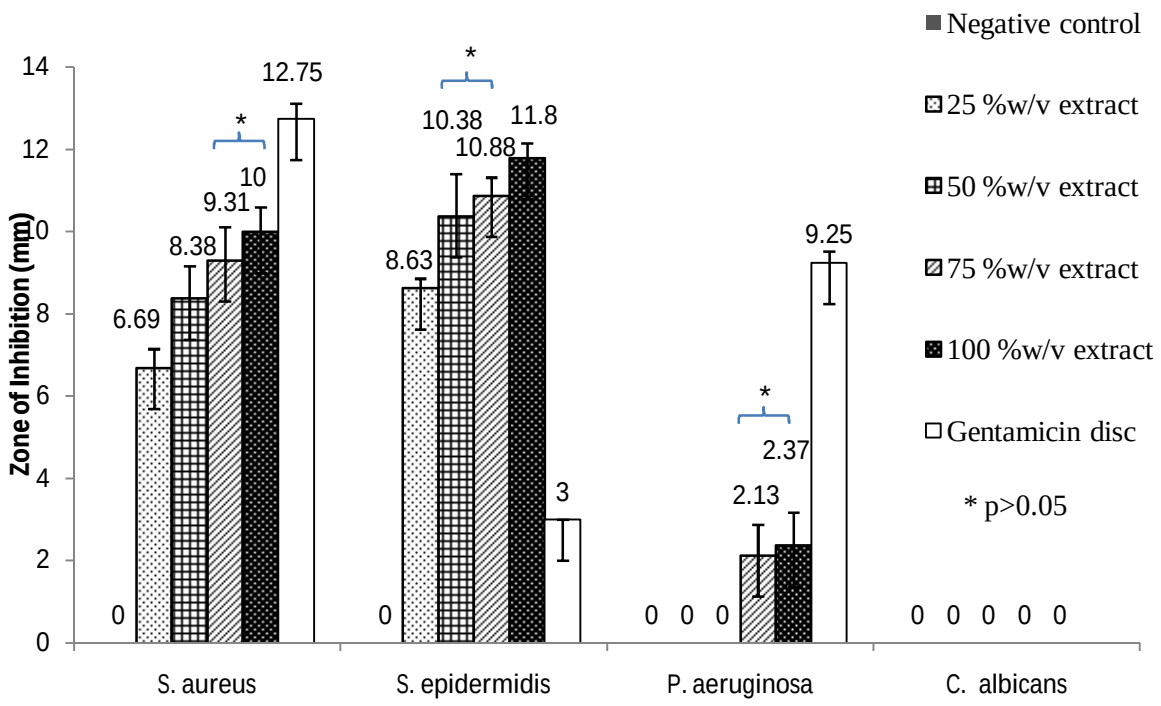

Figure 1 **Zone of inhibition (mm) exhibited by various concentration of *P. granatum* discs against *S. aureus, S. epidermidis, P. aeruginosa* and *C. albicans* (n=8).**

Physical characterization

The physical characteristics and pH of the gel formulations are shown in Table 3. The pH of gel was adjusted to neutral pH so that they are suitable for application on human skin. *P. granatum* gels were characteristically reddish-orange in colour as shown in Figure 2. It was observed that the colour intensity of the gel increases when the higher extract concentration was added.

Table 3 Physical characteristics and pH of *P. granatum* gel formulations

Formulation	Colour	Homogeneity	Consistency	Phase separation	pH (Mean±SD)
Negative control	White	Good	Good	No	7.39 ± 0.02
Positive control	White	Good	Good	No	7.18 ± 0.01
PG25%	Light brownish orange	Good	Good	No	7.20 ± 0.01
PG50%	Brownish orange	Good	Good	No	7.14 ± 0.01
PG75%	Dark brownish orange	Good	Good	No	7.00 ± 0.02
PG100%	Dark brownish orange	Good	Good	No	7.05 ± 0.01

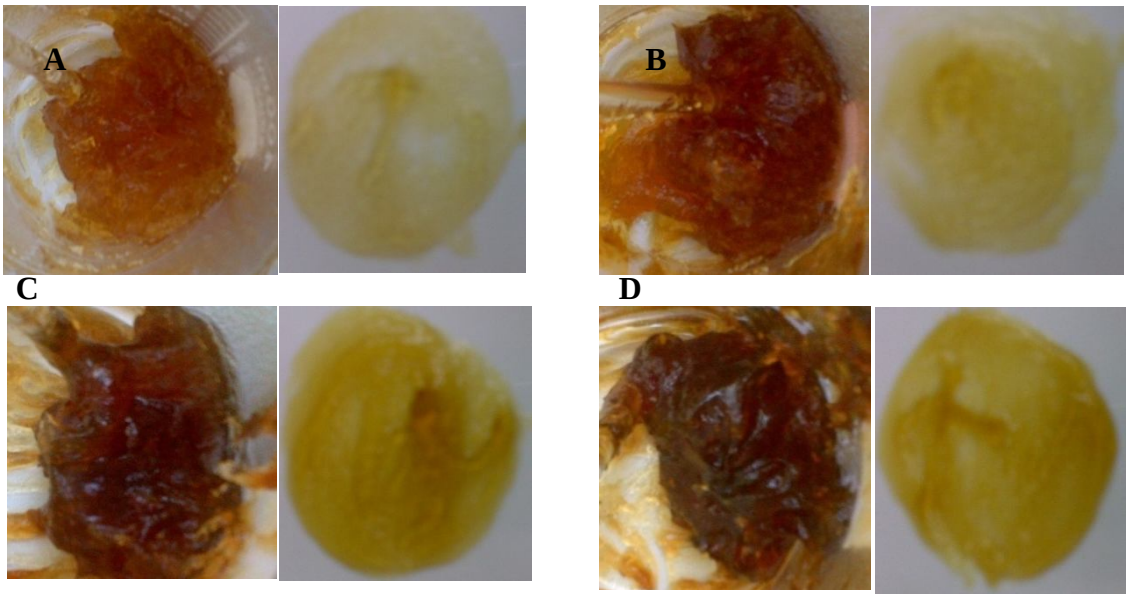

Figure 2 **Physical appearance of *P. granatum* gel of various concentrations; A: PG25%; B: PG50%; C: PG75%; D: PG100%.**

Evaluation of antibacterial activity of *Punica granatum* gel

Zone of inhibition exhibited by all gel formulations against *S. aureus*, *S. epidermidis* and *P. aeruginosa* are shown in Figure 2. *S. aureus* showed highest sensitivity against PG100% gel and lowest sensitivity against PG25% gel. The zones of inhibition exhibited by all *P. granatum* gel formulations against *S. aureus* and *S. epidermidis* were significantly higher than positive control ($p<0.05$). *S. epidermidis* showed highest sensitivity against PG100% gel and lowest sensitivity against PG25% gel. *S. epidermidis* showed no sensitivity towards positive control. The zones of inhibition exhibited by all of *P. granatum* gels against *S. epidermidis* were significantly higher than the marketed product ($p<0.05$). PG25%, PG50% and PG75% gel exhibited no activity against *P. aeruginosa*. *P. aeruginosa* showed low sensitivity against PG100% gel. *P. aeruginosa* showed the highest sensitivity toward marketed product. Zone of inhibition exhibited by PG100% gel against *P. aeruginosa* were significantly lower than marketed product ($p<0.05$).

In comparison to the positive control ie 0.1% gentamicin sulphate gel, *S. aureus* showed highest sensitivity against PG100% gel and lowest sensitivity against PG25% gel. The zones of inhibition exhibited by all of *P. granatum* gels were significantly higher than positive control ($p<0.05$). However marketed product has significantly greater activity against *S. aureus* than *P. granatum* gels ($p<0.05$). *S. epidermidis* showed highest sensitivity against PG100% gel and lowest sensitivity against PG25% gel. *S. epidermidis* showed no sensitivity toward positive control. The zones of inhibition exhibited by all of *P. granatum* gels against *S. epidermidis* were significantly higher than the marketed product. PG25%, PG50% and PG75% gel exhibited no activity against *P. aeruginosa*. *P. aeruginosa* showed low sensitivity against PG100% gel. *P. aeruginosa* showed the highest sensitivity toward marketed product. Zone of inhibition exhibited by PG100% gel against *P. aeruginosa* were significantly lower than marketed product, PG100% gel showed the highest and lowest antibacterial activity against *S. epidermidis* and *P. aeruginosa* respectively. Interestingly, positive control showed a reverse profile where antibacterial activity was the highest and

lowest against *P. aeruginosa* and *S. epidermidis*. However, it was observed that *S. aureus* was more sensitive towards the marketed product compared to PG100% gel. *S. epidermidis* was significantly more sensitive toward PG100% gel compared to marketed product. *P. aeruginosa* was significantly more sensitive against marketed product compared to PG100% gel.

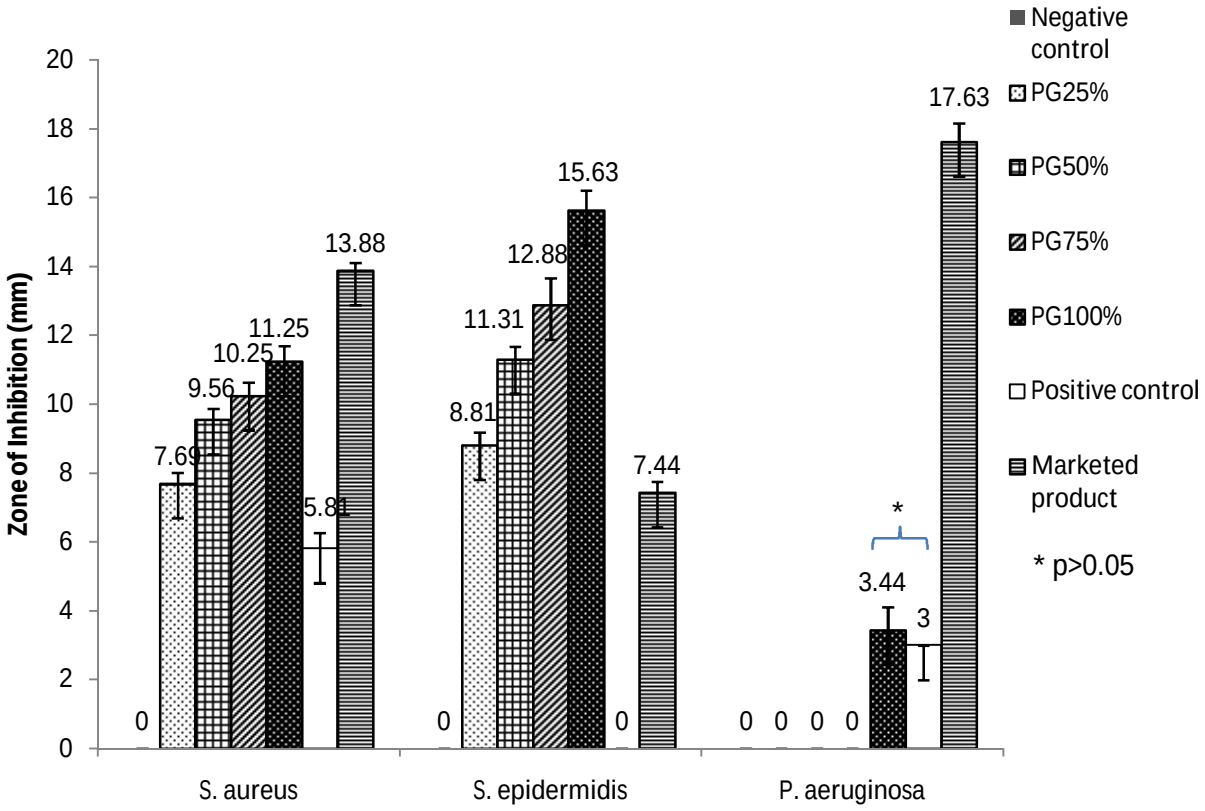

Figure 2 Effect of temperature on characteristic and antibacterial activity of *P. granatum* gel after a month.

P. granatum gels were also evaluated for their physical characteristics and antibacterial activity after a month in storage at different temperatures. All gel formulations were physically characterized. However, for antibacterial activity, only PG100% was tested since it showed the highest activity among all.

Based on Table 4 below, the pH of gel formulations were higher compared to the initial pH during their preparation. The change in pH ranges from 1% to 6% increment. It appears that the pH increase varies according to the extract concentration and is not or minimally affected by the storage temperature. For physical evaluation, no phase separation or change in colour, homogeneity and consistency of different concentration of gels and control at 4°C, 25°C and 37°C for all gel formulations was observed.

Table 4 Mean initial and after a month pH at different temperature.

Type of gel	Initial pH	4°C	25°C	37°C
Control	7.39±0.02	7.62±0.02 (↑ 3.14%)	7.72±0.03 (↑ 4.47%)	7.67±0.01 (↑ 3.79)
PG25%	7.20±0.01	7.62±0.02 (↑ 5.83%)	7.65±0.01 (↑ 6.25%)	7.38±0.02 (↑2.5%)
PG50%	7.14±0.01	7.4±0.01 (↑ 3.61%)	7.33±0.12 (↑ 2.64%)	7.4±0.00 (↑ 3.61%)
PG75%	7.02±0.01	7.4±0.00 (↑ 5.27%)	7.31±0.01 (↑ 4.02%)	7.15±0.04 (↑ 1.81%)
PG100%	7.05±0.01	7.38±0.01 (↑ 4.58%)	7.17±0.02 (↑ 1.67%)	7.16±0.02 (↑ 1.52%)

On the other hand, the zone of inhibition exhibited by PG100% gel against *S. aureus*, *S. epidermidis* and *P. aeruginosa* was reduced regardless of storage temperature. Based on Figure 3, storing the gel formulations at higher temperature seems to decrease the zone of inhibition.

Interestingly, PG100% gel that was stored at 4°C and 25°C retained its antibacterial activity against *S. aureus* and *P. aeruginosa*. The post-storage zone of inhibition was not statistically significant when compared with the pre-storage zone of inhibition ($p>0.05$). The antibacterial activity of PG100% gel stored at 37°C against *S. aureus* was significantly decreased (30.76%). In contrast, gel stored at 25°C showed a significant decrease in its activity against *P. aeruginosa* (56.4%) and a total loss of its activity.

Apart of that, zone of inhibition exhibited by PG100% gel against *S. epidermidis* decreases with increasing storage temperature. Gel stored at 4°C showed the highest antibacterial activity whereas gel stored at 37°C showed the lowest activity. The percentage of reduction of antibacterial activity for PG100% gel stored at 4°C, 25°C and 37°C were about 5.53%, 11.82% and 19.45% respectively.

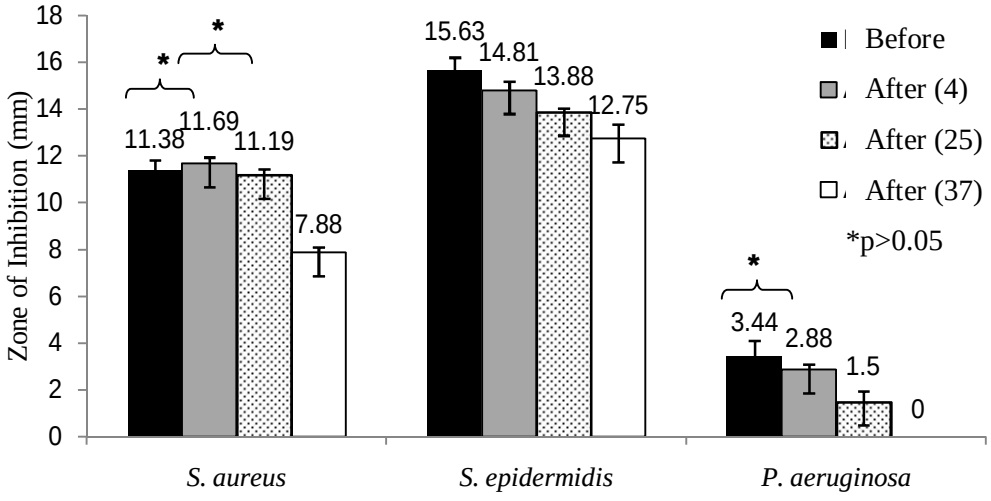

Figure 3 **Zone of inhibition exhibited by PG100% gel against *S. aureus*, *S. epidermidis* and *P. aeruginosa* after a month of different storage temperature (n=8); Before denotes**

freshly prepared gel; After (4) denotes gel after a month of storage at 4°C; After (25) denotes gel after a month of storage at 25°C; After (37) denotes gel after a month of storage at 37°C.

DISCUSSION

Antimicrobial activity of *P. granatum* methanolic extract

Chemical constituent of *P. granatum* responsible for antimicrobial activity are phenolic compound including pelargonidin-3-galactose, cyanidin-3-glucose, gallic acid, quercetin and myricetin (15). It is also has been reported that antimicrobial activity is related to flavonoids compound which is a class of phenolic compound (6). Phenolic compound is a polar compound due to its abundance of polar hydroxyl group. Since the solvent used for extraction of *P. granatum* peel is methanol ie polar solvent, phenolic compounds contained in the peel are able to be dissolved in the methanol. Thus, methanolic extract of *P. granatum* containing phenolic compounds is thought to be responsible for antimicrobial activity.

Generally, it is observed that the antibacterial activity exhibited by *P. granatum* is considerably lower than the ones previously reported (2, 16, 19).The main reason for the low antibacterial of *P. granatum* extract in this study could be due to the fact that the pomegranate fruit used in the previous study was locally-sourced in comparison to imported pomegranate fruit which were used in this study. There is difference in total phenolic content (TPC) of local and imported pomegranates. TPC of local and imported pomegranate had been compared and found that, extract of local *P. granatum* (4.8 GAE mg/g plant extract) has a significantly higher TPC than the extract of imported *P. granatum* (3.8 GAE mg/g plant extract) (9).

A study on the effect of different storage temperature (22°C, 10°C, °C and 5°C) for 16 weeks on TPC of pomegranate fruit showed that the TPC was significantly reduced with longer storage (7). After 16 weeks of storage, all fruits decayed except for fruits stored at 7°C and 5°C. Although storage at low temperature can prevent decay of fruit, storage at low temperature reduced the total phenolic content in fruit especially when stored at 5°C. So, storage at low temperature can also be the factor that cause TPC of imported pomegranate lower than the local ones. Interestingly, the handpicked local pomegranates contained higher TPC due to shorter storage period (9). These two factors could explain the lower TPC of imported pomegranate.

A study claimed that phenolic compound is the active constituent which responsible for antibacterial activity of *P. granatum* extract including pelargonidin-3-galactose, cyanidin-3-glucose, gallic acid, quercetin and myricetin (15). Besides, antimicrobial activity is also related to the presence of flavonoid which is a phenolic compound (6).

The pomegranate used in the studies by Ahmet *et al* (2009), Saad *et al* (2010) and Naziri *et al* (2012) were claimed to be local fruits. So the TPC in the fruits used in the previous study should be higher when compared to imported pomegranate fruit used in the current study. Due to high TPC, antibacterial activity *P. granatum* extract in the previous study is more superior compared to the antibacterial activity of *P. granatum* extract in the current study.

According to Figure 1, *P. granatum* extract did not show any activity against *C. albicans*. This could be due to the fact that the grade of pomegranate cultivars used in the study has no antifungal activity. A previous study conducted by Ahmet *et al* (2009) evaluated antimicrobial activity of six different *P. granatum* cultivars. The finding reveals that different pomegranate cultivars exhibited different pattern of antimicrobial activity against *S. aureus*, *P. aeruginosa* and *C. albicans*. Three of the pomegranate cultivars exhibited no

antifungal activity against *C. albicans*. Thus, it can be hypothesized that the pomegranate cultivar used in the current study is probably one of the cultivars which do not have antifungal activity.

Optimization of *Punica granatum* gel

PG100% gel has inferior activity in treating skin infection caused by *S. aureus* and *P. aeruginosa*. Besides, the gel has no antifungal activity against *C. albicans*. The gel formulation can be optimized so that it will have significant antimicrobial activity in treating skin infection. Optimization of the formulation can be done by varying the carbopol concentration, type of pomegranate cultivar and by using local pomegranate fruit.

One way to optimize the gel is by choosing the right carbopol concentration of the gel as carbopol concentration will affect the release of active constituent. Previous studies have shown that increasing concentration of carbopol increases the viscosity of gel (11,17). As the viscosity of gel increases the percentage of cumulative drug release decrease. Therefore, based on the previous studies it can be hypothesized that increasing concentration of carbopol reduce cumulative release of drug. During preliminary study, carbopol concentration was chosen based on the amount used in a few previous studies. The gel produced was too viscous which means a lesser amount of carbopol can be used to induce gelation. So using lesser amount of carbopol may increase the cumulative release of active ingredient of *P. granatum* from gel matrix. Thus, using less amount of carbopol may potentially increase the antibacterial activity of PG100% gel against *S. aureus* and *P. aeruginosa*.

Secondly, antimicrobial activity of *P. granatum* gel may be enhanced by selecting the right cultivar. Different pomegranate cultivars exhibit varying degree of antimicrobial activity (2). Some of the cultivars have both antifungal and antibacterial activity and some have only one function. The efficacy of antimicrobial activity also varies between cultivars. Therefore, the choice of cultivars is critical. Further research is required in order to determine the range of cultivars available in Malaysia that possess superior antimicrobial activity against specific microorganisms. It is best to choose cultivars with both antifungal and antibacterial activity so that *P. granatum* gel can be multifunctional.

The use of local fruits can influence the antimicrobial activity of *P. granatum* gel against skin infection. As been described earlier, local pomegranate contain higher phenolic compound that responsible for antimicrobial activity (9,15).

Effect of temperature on characteristic and antibacterial activity of *Punica granatum* gel after a month.

In general, increasing the storage temperature causes a decrease in the antibacterial activity of PG100% gel. A possible reason for the reduced of antibacterial activity of gel is the degradation of active constituent responsible for antibacterial activity. Chemical constituent of *P. granatum* responsible for antimicrobial activity is phenolic compound including pelargonidin-3-galactose, cyanidin-3-glucose, gallic acid, quercetin and myricetin (15). Pelargonidin-3-galactose and cyanidin-3-glucose are anthocyanin. A number of studies reported that anthocyanin degrades at high temperature. Previous study showed that degradation of red currant juice anthocyanin takes place at temperature above 30°C (12). Similarly, another previous study revealed that degradation of anthocyanin is 10.7 times faster at 37°C than the 2°C (3).

CONCLUSION

Methanolic extract and gel formulation of *P. granatum* peel have been shown to possess good antibacterial activity against *S. aureus*, *S. epidermidis* and *P. aeruginosa*. The extract, however, did not exhibit any antifungal activity against *C. albicans*. *P. granatum* showed promising activity against *S. epidermidis*

followed by *S. aureus* and finally *P. aeruginosa*. PG100% gel was chosen as the best formulation because after a month of storage at different temperature, it was still homogenous, showed no phase separation, did not change its colour and had good consistency. More importantly, PG100% gel exhibited the highest antibacterial activity. Moving forward, *P. granatum* has the potential to be formulated as gel to treat bacterial skin infection caused by *S. aureus*, *S. epidermidis*, and *P. aeruginosa*. However, further studies are required in order to optimize the antimicrobial activity of *P. granatum* gel.

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