

Original Research Article**FORMULATION AND CHARACTERIZATION OF AN ANTI-BACTERIAL GEL USING TENGGEK BURUNG (*Melicope ptelefolia*)****Afendi Dahlan and Izzah Alia Mohamad Rosli**

Department of Pharmaceutics, Faculty of Pharmacy, Cyberjaya University College of Medical Sciences, Lot 3410 JalanTeknokrat 3, Cyberjaya 63000, Selangor, Malaysia.

ABSTRACT

Introduction: Gram positive bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis* cause various types of infection in human. Due to increased incidence of antibiotic-resistant Staphylococci, natural products were proposed as an alternative to combat this problem. *Melicope ptelefolia* (*M. ptelefolia*) or *tenggek burung*, a local herb found in Malaysia, is traditionally used for various medical purposes including the treatment of inflammation and infection. Previous studies have shown that *M. ptelefolia* exhibited limited anti-bacterial properties. Hence, the aim of this study is to examine the anti-bacterial properties of *M. ptelefolia* extract and gel formulation against *S. aureus* and *S. epidermidis*.

Methods: Different concentrations of methanolic extract of *M. ptelefolia* were used in the disc diffusion method for determining their anti-bacterial properties. Various concentrations of gel were formulated using different amount of extraction from 100%^{v/v} concentration. The gels were then evaluated physically and chemically.

Results: Both extract and gel formulations showed good anti-bacterial activity. All gel formulations showed acceptable physical properties and were stable at various temperatures after prolonged storage.

Conclusions: In conclusion, *M. ptelefolia* is a promising candidate as active ingredient to be developed as a gel for the treatment of infection caused by *S. aureus* and *S. epidermidis*. Further studies in establishing the full spectrum of anti-bacterial activity of *M. ptelefolia* and optimizing of the gel formulation are necessary.

***Corresponding author:** Dr Afendi Dahlan, Cyberjaya University College of Medical Sciences, Lot 3410 Jalan Teknokrat 3, Cyberjaya 63000, Selangor, Malaysia. afendi@cybermed.edu.my.

Novelty of Work: *M. ptelefolia* has wide medicinal properties including anti-microbial activity. Majority of research focuses on determining anti-microbial properties of the plant extract. So far, there is no formulation of gel dosage form that incorporates *M. ptelefolia* has been produced or marketed. Given the popularity of natural product remedies lately, this preliminary study explores the feasibility of *M. ptelefolia* gel formulation as anti-bacterial gel in the future.

INTRODUCTION

Bacterial infection can be caused by a wide range of bacteria, resulting in mild to life-threatening illnesses. The development of antibiotics gives clinician a great tool against bacterial infections (13). Prolonged use of antibiotic, however, may result in the development of resistance by the bacteria strain, leading to reduced treatment effectiveness. In the quest to

find an alternative solution to this issue, researchers have started looking at untapped natural resources from flora and fauna and their therapeutic potential for the treatment of various medical conditions (22).

In this study, *M. ptelefolia* plant from Melicope species was chosen. Generally, different parts of the plant from different Melicope species have shown positive anti-bacterial activity against Methicillin Resistant *Staphylococcus Aureus* (MRSA), *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* (9). In the case of *M. ptelefolia*, various parts of the plant have been used traditionally for centuries as natural remedy for fever, stomach ache, rheumatism and the treatment of wounds and itches (11).

Topical semisolids are pharmaceutical preparation intended to utilize local activity when applied to the skin or mucous membrane. Staphylococci are normal flora that reside the skin. The predominant bacteria specie on the skin is *Staphylococcus epidermidis*, a non-pathogenic strain under normal circumstances, which may cause severe infection if it reaches certain organ sites such as artificial heart valve and prosthetic joint. It is frequently found on the skin compared with its pathogenic relative *S. aureus*. Gel is a topical semisolid dosage form which is aesthetically acceptable for most patients. Gel consists of liquids gelled by means of suitable gelling agents and there are two types of gel: namely, oleo gels and hydrogels. Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids (15).

METHODS

Materials

Carbopol 940 was purchased from KOFA Chemical Works (M) Sdn. Bhd. Propylene glycol and methyl paraben were purchased from Euro-Chemo Pharma Sdn. Bhd. *M. ptelefolia* 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 were purchased from Becton Dickinson, USA and Atlantic Laboratories Corp. Ltd , Bangkok Thailand, respectively.

Preparation of *M. ptelefolia* extract

Leaves of *M. ptelefolia* (5 kg) were collected from Institute of Bioscience University Putra Malaysia (UPM), Serdang, Selangor, Malaysia. Authentication of plants was carried out by a qualified botanist from the faculty of Forestry, UPM where vouchers SK- 2116/13 have been deposited in herbarium.

The freshly collected leaves of *M. ptelefolia* were cleaned and air dried under shade for 48 hours. The dried leaves were then grounded into powder using an electrical grinder. 150 g powder was extracted using 250 ml of methanol where the ratio of solvent to the sample was 5:1. The mixture was allowed to stand for 24 hours to ensure that all solvent and samples were completely homogenized. The macerated mixture was then filtered using filter paper and Buchner funnel. The liquid filtrate was concentrated and evaporated using a rotary evaporator (Buchi R210, Switzerland) under controlled temperature of 55⁰ C and reduced pressure. The resultant extract was then stored in a refrigerator (Mediref Pharmaceutical Refrigerator, Malaysia) at -20[°]C prior to use (21).

Antimicrobial screening of *M. ptelefolia* extract

A concentration gradient of methanolic *M. ptelefolia* extracts were prepared from concentrated liquid. Four different concentrations of methanolic extracts 25%^{v/v}, 50%^{v/v},

75%^{v/v} and 100%^{v/v} were prepared from concentrated liquid extract of *M. ptelefolia*. The most effective concentration was then determined by comparing the results of the zone of inhibition. Zone of inhibition measured in four different concentrations against *S. aureus* and *S. epidermidis*. These tests were carried out by using disc diffusion method. These bacteria were chosen for their potential to cause skin infection.

Disc diffusion method is a method using paper disc which will be impregnated with different concentration of chemical or solvent to determine the resistance of an organism towards different concentration of the solution. Mueller Hinton agar plates were prepared and the test microorganisms were inoculated using spread plate method with a L-rod shape stick. The sterile filter paper were then impregnated with a drop of plant extract by using a micropipette and were left dried. Standard gentamicin disc was used as positive control to ensure the activity of standard antibiotic against the test organism. Steriled distilled water was used as negative control. When applying the disc, each disc was pressed down to ensure complete with contact and even distributed with agar surface. Next, the agar plate was inverted and stored in incubator of 37°C for 24 hours. After 24 hours of incubation, each plate was examined. The diameter of zones of inhibition was measured. The test was carried out in triplicates (9). The concentration having the biggest zone of inhibition was taken to incorporate in the gel base.

Preparation and evaluation of *M. Ptelefoliagel*

Briefly, 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. Required volume of *M. ptelefolia* extract was added to 100 ml of distilled water to produce *M. ptelefolia* solution at various concentrations (2%^{v/v}, 4%^{v/v}, 6%^{v/v}, and 8%^{v/v}). 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 disc was used. For negative control, gel was prepared without *M. ptelefolia*. Table 1 below shows the various gel formulations.

Table 1. Various gel formulations for the experiment.

Extract concentration	Gel Formulation				
	0% ^{v/v}	2% ^{v/v}	4% ^{v/v}	6% ^{v/v}	8% ^{v/v}
Carbopol	1 g	1 g	1 g	1 g	1 g
Glycerin	2 ml	2 ml	2 ml	2 ml	2 ml
Propylene Glycol	10 ml	10 ml	10 ml	10 ml	10 ml
Methyl Paraben	3 ml	3 ml	3 ml	3 ml	3 ml
100 % Extract of <i>Melicope ptelefolia</i>	-	2 ml	4 ml	6 ml	8 ml
Distilled water	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml

Antimicrobial screening of *M. ptelefolia* gel

The anti-bacterial activity of various gel formulations was determined by using modified agar well diffusion method. *S. aureus*, *S. epidermidis* and Mueller Hinton agar was used in this method. Modified agar well diffusion method is different from disc diffusion method as the

bacteria inoculum were mixed with the agar base and solidified before being incubated. Wells were punched into the agar and were filled with the gel sample. The anti-bacterial activity of gel was then determined.

Statistical analysis

Statistical analysis was performed by using the SPSS version 20 and Microsoft Excel 2010. One-way ANOVA followed by post-hoc 'Tukey's test' were conducted to determined significance between groups. p value less than 0.05 was accepted as significant.

RESULTS

Evaluation of anti-bacterial properties of *M. ptelefolia* methanolic extract

The methanolic extract of *M. ptelefolia* were prepared and tested at four different concentrations (25% v/v, 50% v/v, 75% v/v, and 100% v/v) using disc diffusion method against *S. aureus* and *S. epidermidis*. The anti-bacterial activity was determined by calculating the average of zone of inhibition of bacterial growth around the disc. Figure 1 shows the zone of inhibition exhibited by various concentration of methanolic extract of *M. ptelefolia* against *S. aureus*. All extracts displayed good anti-bacterial activity against gram positive strain *S. aureus*. 25% v/v plant extract was less sensitive with compared with higher concentrations but it appears at 50% v/v and 75% v/v, the activity surprisingly were not significantly different from 25% v/v extract. 100% v/v concentration exhibited the highest zone of inhibition which shows that the *S. aureus* were susceptible to growth inhibition by the plant extracts at highest concentration.

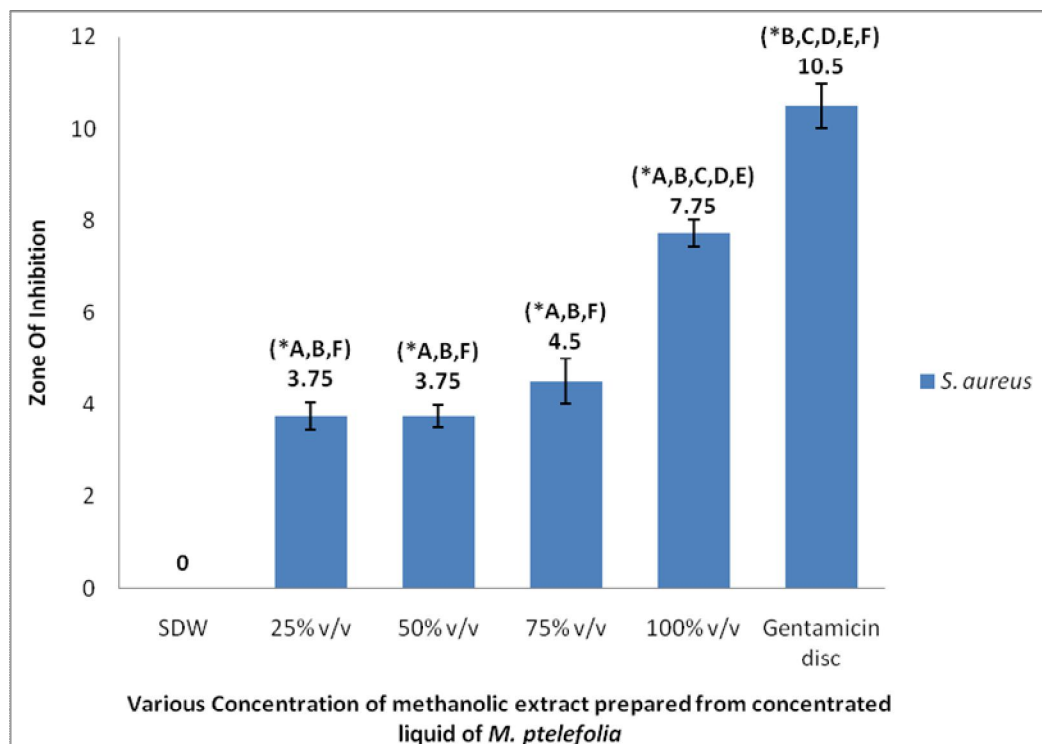


Figure 1 Zone of inhibition exhibited by various concentration of methanolic extract prepared from concentrated liquid of *M. ptelefolia* against *S. aureus* A) Positive control B) Negative control C) 25% v/v extract D) 50% v/v extract E) 75% v/v extract F) 100% v/v extract (* denotes p < 0.05).

Figure 2 shows the zone of inhibition exhibited by various concentration of methanolic extract of *M. ptelefolia* against *S. epidermidis*. All extracts displayed good anti-bacterial activity against *S. epidermidis*. 25%^{v/v} plant extract was less sensitive with compared with higher concentrations but it appears at 50%^{v/v} and 75%^{v/v}, the activity surprisingly were not statistically different from 25%^{v/v} extract. 100%^{v/v} extract exhibited the highest zone of inhibition which shows that the *S. epidermidis* was sensitive at the highest concentration. However, interestingly, *S. epidermidis* exhibited more susceptibility by displaying greater diameter of zone of inhibition (5.00 ± 0.40 mm to 10.25 ± 0.63 mm) compared to *S. aureus* (3.75 ± 0.25 mm to 7.75 ± 0.48 mm).

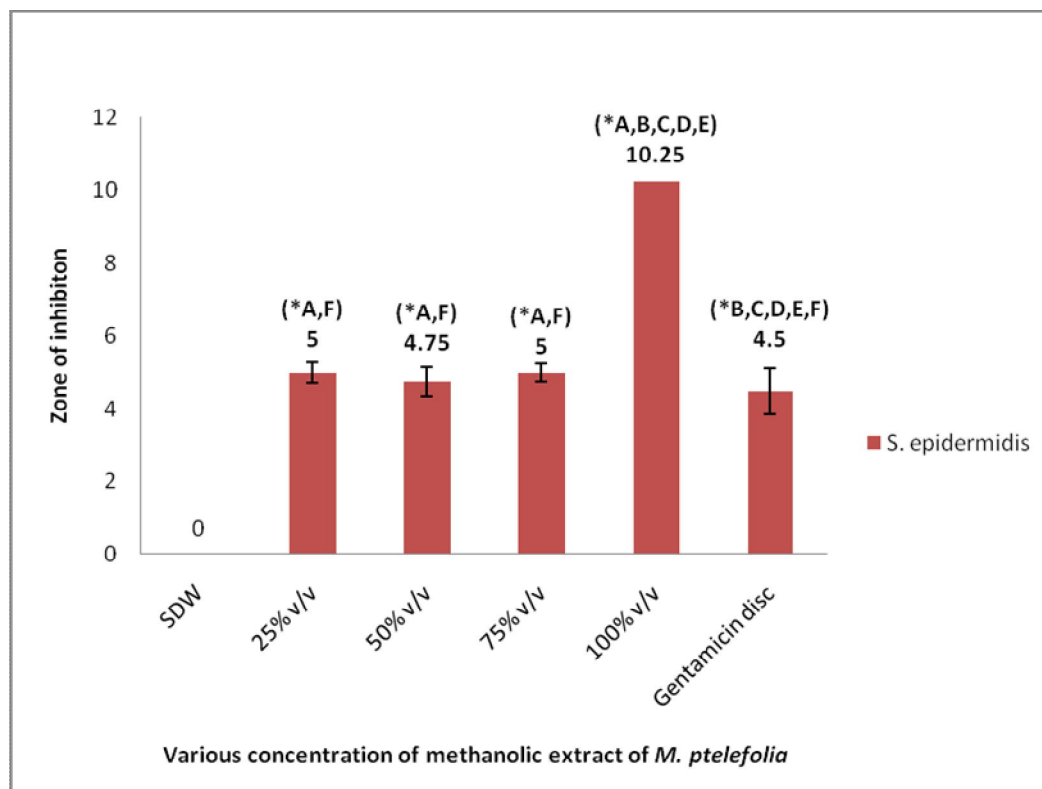


Figure 2 Zone of inhibition exhibited by various concentration of methanolic extract prepared from concentrated liquid of *M. ptelefolia* against *S. epidermidis* A) Positive control B) Negative control C) 25%^{v/v} extract D) 50%^{v/v} extract E) 75%^{v/v} extract F) 100%^{v/v} extract (* denotes $p < 0.05$).

Figures 3 and 4 show the zone of inhibition for *S. aureus* and *S. epidermidis* upon exposure to various concentrations of *M. ptelefolia* extract, respectively.

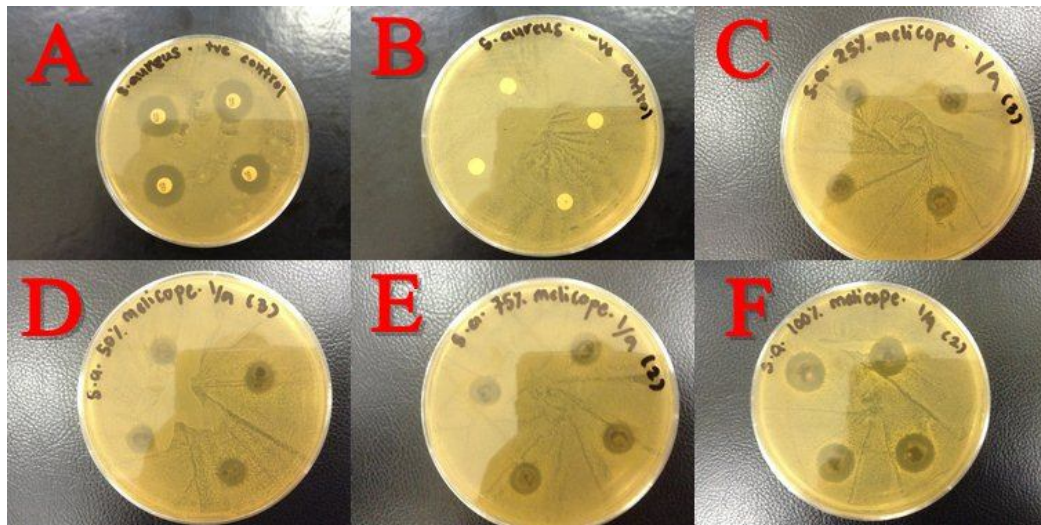


Figure 3 Zone of inhibition of *S. aureus* on exposure to A) Positive control, B) Negative control, C) 25%^{v/v} extract, D) 50%^{v/v} extract E) 75%^{v/v} extract and F) 100%^{v/v} extract.

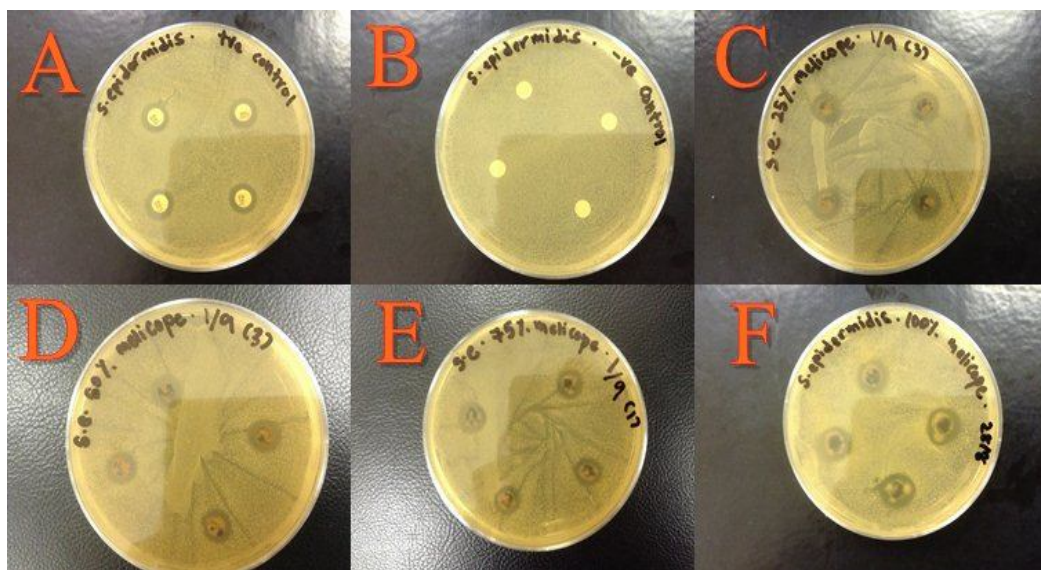


Figure 4 Zone of inhibition of *S. epidermidis* on exposure to A) Positive control, B) Negative control, C) 25%^{v/v} extract, D) 50%^{v/v} extract E) 75%^{v/v} extract and F) 100%^{v/v} extract.

Gel formulation and preparation

For physical appearance, the higher the concentration of extract incorporated in the formulation, the more intense the colour of the gel produced. All gel formulations showed semi-solid properties and smelled plant fragrance. All gels were non-greasy when applied on the skin and was easily removed when washed with water. For accelerated stability testing, although there were fluctuation in pH, but still there was no obvious change on the homogeneity and colour for all the tested formulation from day 1 to 30 at 4°C, 25°C and 37°C. The homogeneity of tested formulation was satisfactory and there was absence of

foreign particles. Other state of physical appearance were maintained the same for all formulations. For the type of smear testing revealed that all the formulation was non-greasy and easily removed by water. Table 2 shows pH of each types of gel for accelerated stability testing.

Table 2 pH of various gel formulations upon one month storage at different temperatures

Types of gel	Initial pH	pH after one month storage		
		25°C	37°C	4°C
Gel base	7.41	6.31	7.10	7.14
2% Gel	7.09	7.51	7.35	7.34
4% Gel	7.20	7.24	7.35	7.17
6% Gel	7.05	7.21	7.03	7.31
8% Gel	7.03	7.36	7.42	7.43

Evaluation of anti-bacterial activity of *M. ptelefolia* gel

Figure 5 shows zone of inhibition exhibited by various gel formulations against *S. aureus*. All gel formulations displayed good anti-bacterial activity against *S. aureus*. As expected, 2% gel was less sensitive compared to the higher concentrations and 8% gel exhibited the greatest bacteria inhibition. Interestingly, there is no statistical difference between 6% and 8% gels.

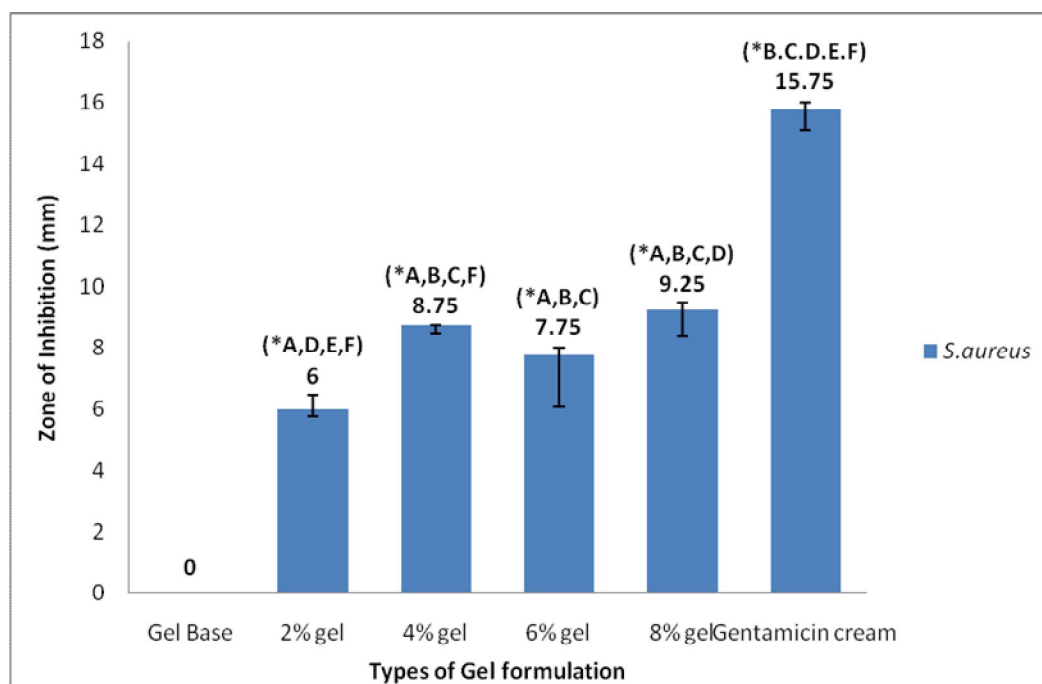


Figure 5 Zone of inhibition exhibited by various formulations of gel against *S. aureus*. A) Positive control B) Negative control C) 2% gel D) 4% gel E) 6% gel F) 8% gel (* denotes $p < 0.05$).

Figure 6 shows zone of inhibition exhibited by various gel formulations against *S. epidermidis*. 2% gel was less sensitive compared to the higher concentrations. 8% gel exhibited the highest zone of inhibition. Although there is no direct relationship between the gel extract concentration and the anti-bacterial activity, interestingly 4% and 8% gels had significantly higher anti-bacterial activity when compared with the positive control ($p < 0.05$).

6% gel, on the other hand, was as effective as the positive control ($p > 0.05$). However, in comparison between both strain, *S. epidermidis* exhibited higher susceptibility (8.50 ± 0.29 mm to 13.50 ± 0.65 mm) to the *M. ptelefolia* gel formulations compared to *S. aureus* (6.00 mm to 9.25 ± 0.25 mm).

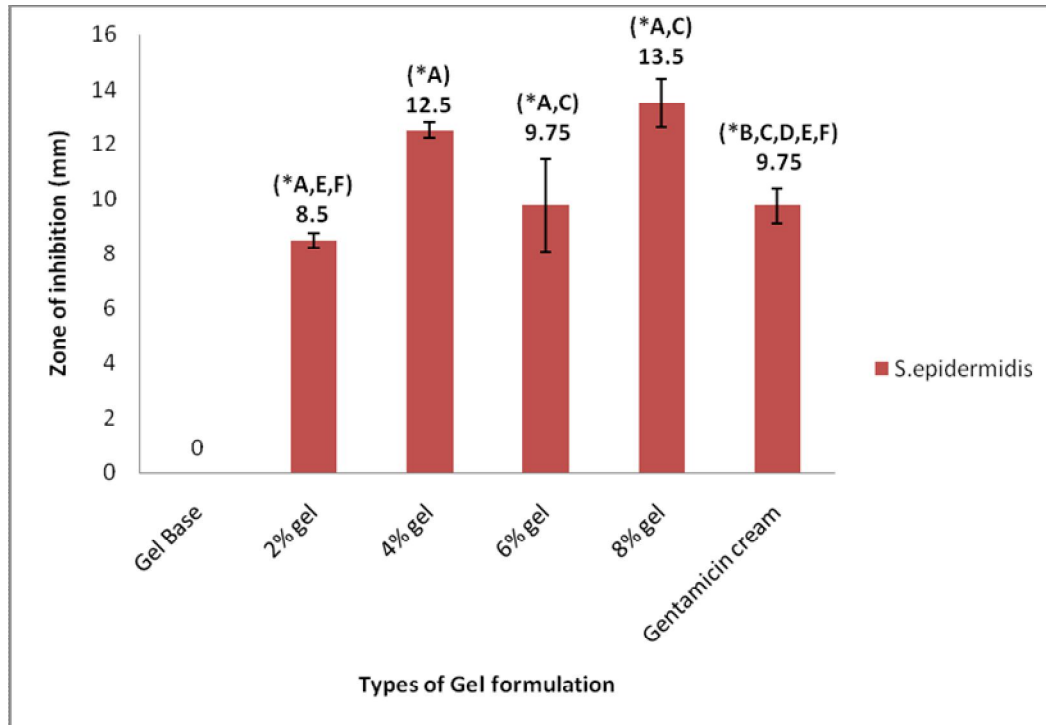


Figure 6 Zone of inhibition exhibited by various formulations of gel. A) Positive control B) Negative control C) 2% gel D) 4% gel E) 6% gel F) 8% gel (* denotes $p < 0.05$).

Figures 7 and 8 show the zone of inhibition for *S. aureus* and *S. epidermidis* upon exposure to various concentrations of gel formulation containing *M. ptelefolia*, respectively.

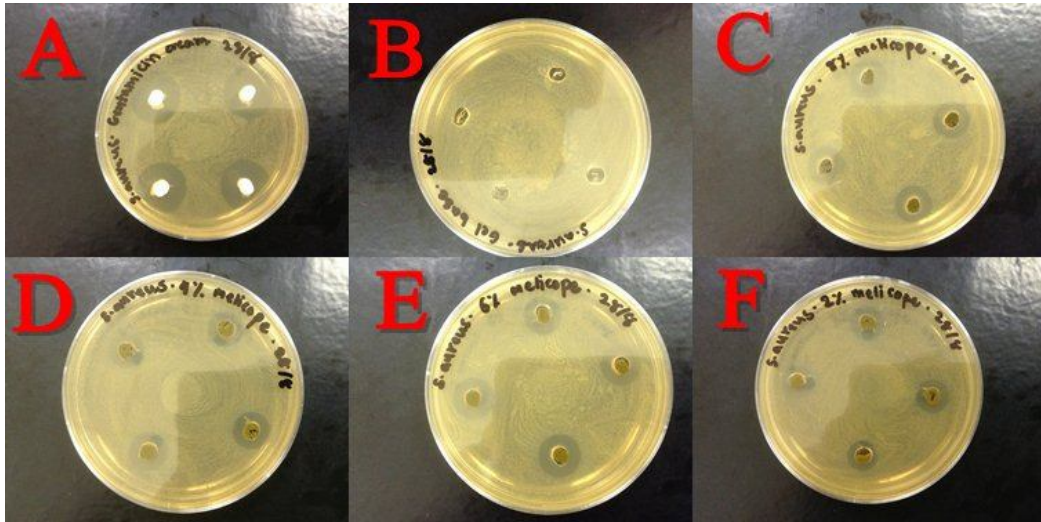


Figure 7 Zone of inhibition of *S. aureus* on exposure to A) Positive control, B) Negative control, C) 2%^{v/v} gel, D) 4%^{v/v} gel, E) 6%^{v/v} gel and F) 8%^{v/v} gel.

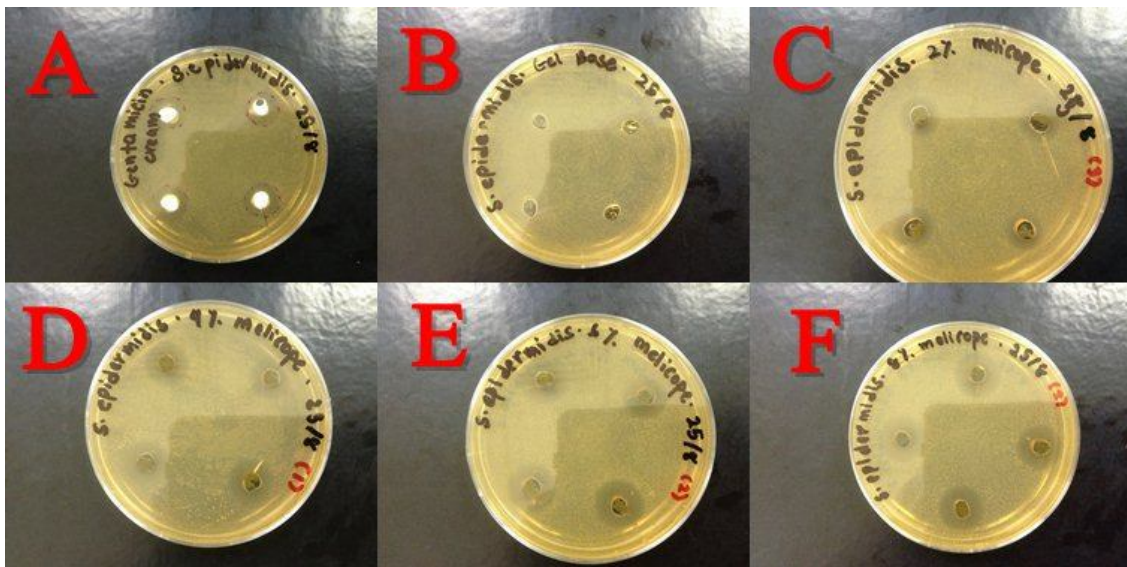


Figure 8 Zone of inhibition of *S. Epidermidis* on exposure to A) Positive control, B) Negative control, C) 2%^{v/v} gel, D) 4%^{v/v} gel, E) 6%^{v/v} gel and F) 8%^{v/v} gel.

DISCUSSION

Evaluation of anti-bacterial properties of *M. ptelefolia* methanolic extract

All methanolic extract prepared from concentrated liquid of *M. ptelefolia* displayed good anti-bacterial activity against *S. aureus* and *S. epidermidis*. There are several phytochemicals, which can be isolated from various parts of the plant such as leaves, roots and stems, includesesquiterpenes, lactone and flavonoids. These phytochemicals are responsible for the anti-bacterial properties of this plant.

In this study, as expected, the highest extract concentration used (100%^{v/v}) exhibited the largest zone of inhibition in comparison with the lower extract concentration. In other words, there is a direct relationship between the extract concentration and the anti-bacterial activity.

This could be due to the fact that high extract concentration possesses a high content of active metabolite which in turn leads to greater potential in inhibiting the growth of the bacteria strain and vice versa (19).

It was observed that there is a significant difference in the antibacterial activity between the extract and the positive control. This was expected as concentration of the extract has not been optimized prior to this study (1). In addition to flavonoids, saponins and sesquiterpenes lactone, secondary metabolites such as alkaloids and steroids have also been reported to exert anti-microbial activity against a range of bacteria (2).

Between *S. aureus* and *S. epidermidis*, *M. ptelefolia* appears to be more effective against *S. epidermidis* than *S. aureus*. The variations in the susceptibilities of these organisms also could be attributed to the intrinsic properties of these organisms, which could be related to the permeability of their cell surface to the extracts (16).

Evaluation of anti-bacterial properties of gel containing *M. ptelefolia*

All gel formulations containing different amount of 100% v/v concentration of *M. ptelefolia* extract showed anti-bacterial activity towards *S. aureus* and *S. epidermidis*. Consistent with the earlier observation for extract, gels containing high concentration of plant extract exhibited a higher anti-bacterial activity when compared with gels with lower concentration. A decrease in anti-bacterial activity was seen when a low volume of extract i.e. 25% v/v was incorporated in the formulation of the gel. This could be attributed to the dilution factor of the extract. Therefore, smaller volumes of the extract are expected to contain less amount of the active component which results in the reduced anti-bacterial activity of the gel (7)(25).

Viswanad et al (2012) showed that gel formulations containing *Samara indica* possessed anti-bacterial effect on both gram-positive and gram negative bacteria. In addition, Patel et al, (2011) also found that gel formulated using various herbs extract as active ingredient exhibited good anti-bacterial activity.

To benchmark anti-bacterial activity of *M. ptelefolia* gel formulations against a commercial antibiotic product, gentamicin antibiotic cream was used as positive control as no commercial antibiotic gel was available locally. Gentamicin, an aminoglycosides antibiotic with a broad spectrum anti-bacterial activity, is highly effective in inhibiting certain bacteria strain. As expected, gentamicin antibiotic cream has a greater anti-bacterial activity to *S. aureus* compared to the extract. However, in the case of *S. epidermidis*, surprisingly *M. ptelefolia* gel formulations at 4% and 8% concentrations possess significantly better antibacterial activity when compared with the gentamicin cream. This is a proof of concept in showing that gel formulation containing *M. ptelefolia* can be used as a therapeutic alternative to commercial antibiotics such as gentamicin. The issue with prolonged use of antibiotic cream relates to increased incidence of bacterial resistance. Hence, producing antibiotics from natural products may contribute to the reduction of bacterial resistance (22).

Evaluation of *M. ptelefolia* gel formulation

Physicochemical properties determine the bioavailability and drug penetration of a semi-solid dosage form (3). All gel formulations were evaluated for their physicochemical properties such as homogeneity, pH, stability study, physical appearance, type of smear and removal test (6). In this study, all gel formulations were found to have constant pH, homogenous, non-greasy, easily removed after the application and showed no sign of physical changes after long storage condition with different temperature. In other words, this gel formulation met the minimum physicochemical requirements for a pharmaceutical semi solid product.

Satpathy(2011) reported that a suitable range of pH for skin is 6.7-7 in relation to the development of herbal gel formulations containing essential oil of *Piper betle*. Furthermore, most of the anti-bacterial soap or product should have a pH ranging from 5.8 to 8.9 (5). Since pH of formulations in this study was reported to be between 6 and 8, this means the gel formulation is in conformance with the industry standards for topical preparations.

Other test such as homogeneity testing, test for physical appearance such as colour in terms of intensity, state and odour has reported to have the same properties compared to studies by other researcher. Study by Satpathy *et al* (2011) and Pandey *et al* (2011) revealed the same properties in the homogeneity testing, physical appearance when other plant extract is used in the preparation of gel. The colour of the gel preparation in this study was dependant on the concentration of the plant extract. The higher the concentration, the darker the green colour of the gel. All gel formulation has the fragrance of the plant extract. According to Pandey *et al* (2011), the gel formulations should have the characteristic odour of extract incorporated.

The gel formulations were non-greasy and easy to remove by water because they were hydrogel formulations. Hydrogel formulation is made up of approximately 96% of water according to a study by Peppaset *al* (2011). Hence removal of the gel in the event of an emergency such as over applying or wrongly applying the gel would not be a problem for patients.

For the accelerated stability testing, the character of the gel did not deviate from the initial value. The gel formulations were stable at 4°C, 37°C and 25°C indicating the stability of the product. As a general rule, products which are stable at various temperatures usually exhibit a long shelf-life (19). The antimicrobial screening for the gel was only performed after one month. The results showed that the gel still exhibited antimicrobial activity. Handali *et al* (2011) said that a stable formulation still can exert their activity even after prolonged storage period.

CONCLUSION

Different concentration (25% v/v , 50% v/v , 75% v/v and 100% v/v) of *M. ptelefolia* methanolic extract exhibited relatively good anti-bacterial activity against gram-positive bacteria strains *S. aureus* and *S. epidermidis*. There is a direct relationship between the plant extract concentration and the anti-bacterial activity. When incorporated into gel formulations, *M. ptelefolia* has shown to be equally effective against both organisms at different concentrations. However, the dose-effectiveness relationship was not apparent for the gel formulations. The gel formulations showed good physicochemical and stability characteristics. Additionally, the gel formulations had retained its anti-bacterial activity post accelerated stability studies. As a conclusion, *M. ptelefolia* has been shown as a promising candidate for optimization and development as an anti-bacterial gel formulation. More research is required in understanding the effect of excipients on potency of *M. ptelefolia* and subsequently in verifying its anti-bacterial activity *in vivo*.

ACKNOWLEDGEMENT

Our deepest appreciation goes to Cyberjaya University College of Medical Sciences for funding this research.

REFERENCES

1. Alabi, O.A., Haruna, M.T., Anokwuru, C.P., Jegede, T., Abia, H., Okegbe, V.U., and Esan, B.E. 2012. Comparative studies on antimicrobial properties of extracts of fresh and dried leaves of *Carica papaya* (L) on clinical bacterial and fungal isolates. *Advances in Applied Science Research* **3**(5): 3107-3114.
2. Aliyu, A.B., Musa, A.M., Abdullahi, M.S., Ibrahim, H., and Oyewale, A.O. 2011. Phytochemical screening and anti-bacterial activities of *Vernonia ambigua*, *Vernonia blumeoides* and *Vernonia ocephala* (Asteraceae). *Acta Poloniae Pharmaceutica Journal* **68**(1): 67-73.
3. Aulton, Michael E. *Pharmaceutics : the design and manufacture of medicines*. 2007
Edinburgh ; New York : Churchill Livingstone.
4. British Pharmacopoeia (2008) Topical Semi-solid preparations. United Kingdom; Pharmacopoeia Commission Secretariat.
5. Chandrasekaran, C. V., Gupta, A., Agarwal, A. (2011). In Vitro modulation of LPS/calcimycin induced inflammatory and allergic mediators by pure compounds of *Andrographis paniculata* (King of Bitters) extract. *International Immunopharmacology* **11**(3): 79-84.
6. Chhetri, H.P., Yogol, N.S., Sherchan, J., Anupa, K.C., Mansoor, S., Thapa, P. (2010). Formulation and evaluation of antimicrobial herbal ointment. *Journal of Science, Engineering and Technology* **6**(1). 102-107.
7. Gidwani, B., Alaspure, R.N., Duragkar, N.J., Singh, V., Rao, S.P., Shukla, S.S. (2010). Evaluation of a novel herbal formulation in the treatment of eczema with *Psoralea corylifolia*. *Iranian Journal of Dermatology* **13**(3): 122-127.
8. Handali, S., Hosseini, H., Ameri, A., Moghimipour, E. (2011). Formulation and evaluation of an anti-bacterial cream from *Oxalis corniculata* aqueous extract. *Jundishapur Journal of Microbiology* **4**(4): 255-260.
9. Hashim, N.M., Rahmani, M., Ismail, H.B.M., Sukari, M.A., and Lian, G.E.C. 2009. Biological activities of four *Melicope* species. *Sains Malaysiana* **38**(5): 767-771.
10. Jafari, B., Ebadi, A., Aghdam, B.M., and Hassanzade, Z. 2012. Anti-bacterial activities of lemon grass methanol extract and essence on pathogenic bacteria. *American-Eurasian Journal of Agriculture and Environment Science* **12**(8): 1042-1046.
11. Karim, M.S., Nasouddin, S.S., Othman, M., Mohd Adzahan, and Hussin, S.R. 2011.
12. McFarland Standards. 2000. Preparation of routine media and reagents used in antimicrobial susceptibility testing. 1-4.
13. Murray, J. F. 1998. Tuberculosis and HIV infection. *International Union Against Tuberculosis and Lung Disease* **65**: 335-342.
14. Murray, Patrick R. 2005. Medical microbiology. Philadelphia : Elsevier Mosby.
15. N.A. Peppas, P. Buresa, W. Leobandunga, H. Ichikawa. Hydrogels in pharmaceutical formulations. *European Journal of Pharmaceutics and Biopharmaceutics* **50** (2000) 27-46.
16. Ojo, O.O., Ajayi, A.O., and Anibijuwon, I.I. 2007. Anti-bacterial potency of methanol extracts of lower plants. *Journal of Zhejiang University* **8**(3): 189-91.
17. Pandey, A., Jagtap, J. V., & Polshettiwar, S. A. (2011). Formulation and evaluation of in vitro antimicrobial activity of gel containing essential oils and effect of polymer on their antimicrobial activity. *International Journal of Pharmacy and Pharmaceutical Sciences*, **3**(1), 234-237.
18. Patel, N. A., Patel, M., & Patel, R. P. Formulation and Evaluation of Polyherbal Gel for Wound Healing. *Intl. R. J. of Pharmaceutics* 2011 **1**(1): 15-20.

19. Sainuddin, T. (2010). Formulation and pharmacological evaluation of herbal gel of *Pothos scandes* linn. *Journal of Pharmaceutical Sciences*1(2): 1-12.
20. Satpathy, B., Sahoo, M., Sahoo, P., & Patra, S. R. Formulation and evaluation of herbal gel containing essential oils of *piper betle* against skin infecting pathogens. *International J. Res. Pharm. Sci.*, 2011 2(3): 373-378.
21. Sulaiman, M.R., Mohd Padzil, A., Shaari, K., Khalid, S., Mohamad, A.S., Ahmad, S., Akira, A., Israf, D., Lajis, N. (2011). Antinociceptive activity of *Melicope ptelefolia* ethanolic extract in experminetal animals. *Journal of Biomedical Biotechnology*, 2010: 37-64.
22. Sule, A., Ahmed, Q.A., Samah, O.A., Omar, M.N. (2011). Screening for anti-bacterial activities of *Andrographispaniculata* extracts on skin disease causing pathogenic bacteria. *Journal of Medicinal Plants Research*5(1): 7-14.
23. Viswanad, V., Aleykutty, N. A., Jayakar, B., Zacharia, S. M., & Thomas, L. (2012). Development and evaluation of antimicrobial herbal formulations containing the methanolic extract of *Samadera indica* for skin diseases. *Journal of advanced pharmaceutical technology & research*, 3(2), 106.
24. WHO., 2000. *General guideline for methodologies on research and evaluation of traditional medicine*.
25. Dahlan, A. & Mohd Razali, M.A. (2015) Formulation, characterization and determination of antibacterial activity of pomegranate (*Punica granatum*) gel. *Journal of Applied Pharmacy*, 7(1): 463-476.