IJPSR (2014), Volume 5, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 March 2014; received in revised form, 11 April 2014; accepted, 22 June 2014; published 01 September 2014

FORMULATION AND CHARACTERIZATION OF AN ANTI-BACTERIAL CREAM USING HEMPEDU BUMI (*ANDROGRAPHIS PANICULATA*)

A. Dahlan [*1], W. S. Yip [1] and K. Y. Pin [2]

Department of Pharmaceutics, Faculty of Pharmacy [1], Cyberjaya University College of Medical Sciences, Lot 3410 Jalan Teknokrat 3, Cyberjaya 63000, Selangor, Malaysia.
Herbal Technology Centre, Medicinal Plant Division [2], Forest Research Institute Malaysia, 52109, Kepong, Selangor, Malaysia.

Keywords:

Staphylococcus aureus, Skin infection, anti-bacterial, *Andrographis paniculata*, hempedu bumi, cream, formulation

Correspondence to Author:
Dr. Afendi Dahlan

Assistant Professor,
Department of Pharmaceutics,
Faculty of Pharmacy, Cyberjaya University College of Medical Sciences, Lot 3410 Jalan Teknokrat 3, Cyberjaya 63000, Selangor, Malaysia.

E-mail: afendi@cybermed.edu.my

ABSTRACT: *Staphylococcus aureus* (*S. aureus*), a bacterium that causes skin infection as a result of skin colonization, is becoming increasingly resistant to many commonly used antibiotics. *Andrographis paniculata* (*A. paniculata*) or hempedu bumi, traditionally used for the treatment of inflammation and infection, in extract form has been shown to exhibited anti-bacterial activity against various bacteria. This study aims to examine the anti-bacterial properties of *A. paniculata* towards *S. aureus* and formulate *A. paniculata* into cream to determine its anti-bacterial properties. Plant extraction using water was performed at Forest Research Institute Malaysia. Different concentrations (5%, 25%, 50%, 75%, and 100%) of aqueous extract of and cream containing *A. paniculata* were used in the disc diffusion method for determination of antibacterial activity towards *S. aureus*. The creams were evaluated physically and chemically. Microbiological pre- and post-testing were carried out for both extract and cream formulations to determine their anti-bacterial properties. Both extract and cream containing *A. paniculata* showed good anti-bacterial properties against *S. aureus*. The anti-bacterial activities were proportional to the concentration of extract alone and in the cream. All cream formulations showed satisfactory physical properties with a smooth texture, emollient, non-greasy, and easy to remove with water. The creams also showed good stability in various temperatures after prolonged storage. It is concluded that *A. paniculata* has the potential to be developed as a cream for skin infections caused by *S. aureus*. Future research is required to confirm the effectiveness of the *A. paniculata* cream formulation.

INTRODUCTION: Plants produce a diverse range of bioactive molecules that make them rich sources of different types of medicine. Many medicinal plant species worldwide are used in traditional medicine as a treatment for skin diseases caused by fungi and bacteria [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(9).3633-40

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(9).3633-40

Andrographis paniculata (*A. paniculata*), locally known as hempedu bumi or Bile of Earth, is a plant which can be found in abundance in Malaysia.

It has been previously shown to exhibit a wide range of pharmacological effects in animals such as anti-diabetes, antidiarrhoeal, anti-viral, anti-inflammatory, hepatoprotective, anti-malarial, anti-HIV, anti-cancer, anti-venom and immune stimulatory [2]. The main chemical constituents of *A. paniculata* which is diterpenoids and flavonoids are believed to be responsible for most of the biological activities of this plant [3]. *A. paniculata* is considered beneficial to the skin and is used both

externally and internally for the treatment of skin diseases [4].

Staphylococcus aureus (*S. aureus*) causes a variety of skin infections.[5] These infections are characterized by the formation of pus containing blisters, which often begin in hair follicles and spread to adjoining tissues. There are a few types of *S. aureus* infections, including folliculitis, furuncles, carbuncles, mastitis, impetigo, and cellulitis.[6] The common treatments for *S. aureus* skin infection include administration of antibiotics and surgical.

Most research focuses on determining anti-bacterial properties of crude extract of *A. paniculata*. So far no pharmaceutical cream dosage form that incorporates *A. paniculata* has been produced or marketed. There is a clear gap between research and product commercialization. Plant-based medicine can be a viable alternative to antibiotics subject to proper investigation and appropriate documentation. Therefore, this study aimed to determine the antibacterial activity of cream formulations containing *A. paniculata* against *S. aureus*.

MATERIALS AND METHODS:

Andrographis paniculata:

Selection of Plant: *A. paniculata* plants with an annual growth of 0.60-0.70 m in height were selected in which the mature leaves were used. The leaves were dark green in color and free of crops and free from insecticides and pesticides.

Collection of Plant: Fresh plant materials leave (75 kilograms) (kg) of *A. paniculata* were procured from the botanical garden of Forest Research Institute of Malaysia (FRIM), Kuala Lumpur, Malaysia.

Plant Identification: Specimen sample was authenticated by Dr. Richard Chung (Taxonomist, FRIM) and deposited (sample number: PID 180712-12) in the herbarium of FRIM, Malaysia. *A. paniculata* is from the family of Acanthaceae with the scientific name of *A. paniculata* (Burm. *f.*) Wall. *Ex* Nees. Its local name includes hempedu bumi and akar cerita. The collector for the fresh plant materials leaves was En Mat Asri Ngah Sanah with the collection number of FRI 38708 from Perlis.

Preparation of Plant Sample Material: Fresh plant leaves of *A. paniculata* were air dried at room temperature for five days to remove excess water in the leaves. After five days, the leaves of *A. paniculata* were dried in a Protech laboratory dryer (LDD-720) from Germany at 37 °C for seven days to remove water further and prevent the growth of fungus on the surface of the leaves. The final weight for the oven-dried *A. paniculata* leaves is 20 kg. The preparation was conducted by Ethno Herbs Resources Sdn. Bhd.

Preparation of Aqueous Extract: The preparation of aqueous extract of *A. paniculata* was conducted at Herbal Technology Centre (HTC), Forest Reserve Institute of Malaysia, Kuala Lumpur. Three major processes were involved, including the extraction, concentration and freeze-drying processes for the preparation of aqueous extract into the powdered form. These processes were carried out using automated equipment on an industrial scale and in compliance with Good Manufacturing Practices (GMP). Briefly, the dried leaves of *A. paniculata* were first ground into smaller fragments of leaves using a grinder (EXTA, model GETF-1200) to increase the surface of the leaves and to remove their moisture.

Extraction: The extraction process was carried out by using an EXTA extractor (EETF-1600) with aqueous or reverse osmosis (R.O.) water at 60°C. A ratio of 1:20 was used for the determination of R.O. water required for the extraction process. The extraction took three days to be completed, which was then followed by extract concentration.

Concentration: The extract was transferred into an EXTA evaporator (EETF-950) to concentrate the extract from 400 liters to 14 liters. The temperature was maintained at 60 °C under vacuum. 2 liters of the aqueous concentrated extract was then stored at -18°C before using. The remaining aqueous extract was freeze-dried under vacuum using a Millrock freeze dryer (85XL) for 48 hours. 0.82 kg of powdered *A. paniculata* was obtained and stored in at room temperature before use.

Antimicrobial Screening of Extract and Cream Formulation: Aqueous extract of *A. paniculata* leaves was evaluated for *in-vitro* antibacterial activity against skin diseases, causing positive

bacteria strain *S. aureus* using the disc diffusion method at five different concentrations of the extract.

Preparation of Aqueous Extracts: Various concentrations of aqueous *A. paniculata* extracts prepared from both concentrated liquid extract and powder. The two different types of aqueous extracts were evaluated for *in-vitro* antibacterial activity. All procedures involved in this preparation were done under strict aseptic conditions to avoid contamination of the extracts. All glassware including beaker, volumetric flask, dropper, measuring cylinder, pipette, conical flask, laboratory glass bottles were autoclaved at 121°C for 20 min before use.

Preparation of different concentration of extracts from the concentrated liquid extract of *A. paniculata:* Five different concentrations of aqueous extracts 5% v/v, 25% v/v, 50% v/v, 75% v/v and 100% v/v (percentage of volume over volume) were prepared from concentrated liquid extract of *A. paniculata*. Normal saline solution was used as a buffer solution.

Agar Disc Diffusion Method: *S. aureus* stock cultures with ATCC number 25923 (Thermo Fisher Scientific, Malaysia) was initially grown on nutrient agar plates or blood agar at 37 °C and incubated for 24 hours followed by transferring them onto blood agar using the streak-plate technique. The inocula were prepared in Mueller-Hinton agar (100 mm) at a density adjusted to a 0.5 McFarland turbidity standard for the disc diffusion method. Gentamicin 6-mm discs (Becton Dickinson, USA) were used as a positive control in this study.

For negative control, 10% aqueous Dimethyl sulfoxide (DMSO) and normal saline were prepared. The diameter of the zone of inhibition was visually measured using a ruler.

Modified Agar Well Diffusion Method: Modified agar well diffusion method was performed for extract cream formulations, as described previously [7]. Mueller-Hinton agar and *S. aureus* were used as mentioned earlier. 8 mm diameter wells were punched into the agar and filled with the cream formulations followed with 24-incubation at 37 °C. Standard gentamicin cream and blank oil-in-water (O/W) cream (without any active ingredient) were used as positive and negative controls, respectively.

Preparation of Dried Filter Paper Discs: *A. paniculata* extracts prepared from concentrated liquid extracts and powdered were initially passed through a 0.45 mm membrane filter. Whatman no. 1 filter paper was used to prepare discs with 6 mm in diameter using a single hole puncher. The discs were placed in a sterile covered petri dish and sterilized in an oven at 160 °C for an hour.

The sterile filter discs of 6mm were then impregnated with 10µL of the aqueous extracts prepared from both concentrated liquid extract and powder *A. paniculata* at a various concentration (5, 25, 50, 75 and 100%$^{v}/_{v}$). The cover of the Petri dishes was closed immediately once the impregnation process done was to minimize the risk of contamination. The discs were later allowed to dry at room temperature for an hour before transferring them onto the Mueller-Hinton agar.

Preparation and Evaluation of *Andrographis paniculata* Cream:

Cream formulation: An O/W cream formulation was used based on the previous study.[8] The formula for the various creams is shown in **Table 1**. The oily phase components such as stearic acid, white beeswax, cetyl alcohol, mineral oil, and stearyl alcohol (R&M) was purchased from Dynamic Sains Sdn. Bhd. For the aqueous phase components, propylene glycol, methylparaben, and propylparaben were purchased from Euro-Chemo Pharma Sdn. Bhd. while triethanolamine was sourced from Becton Dickinson, USA.

Briefly, the components of the oily and aqueous phases were weighed and dissolved in the water bath at 75 °C separately. The aqueous phase was gradually added to the oily phase and stirred continuously until the mixture cooled, and the cream was transferred into a plastic container and labeled accordingly.

Physical Characterization: In addition to the antimicrobial tests as described earlier, each of cream was subjected to visual inspection, pH test using a digital pH meter (Terri + William, USA) and also basic rheological studies.

TABLE 1: FORMULATION OF VARIOUS CREAMS

Components	(% w/w)	With *A. paniculata*	Without *A. paniculata*	Without preservatives
		Grams (g)	Grams (g)	Grams (g)
Oily phase:				
Stearic acid	2.5	1.5	1.500	1.500
White beeswax	1.5	0.9	0.9	0.9
Stearyl alcohol	5.0	3.000	3.000	3.000
Cetyl alcohol	6.5	3.900	3.900	3.900
Mineral oil	5.0	3.000	3.000	3.000
Aqueous phase:				
Propylene glycol (PG)	5.0	3.000	3.000	3.000
Triethanolamine	2.0	1.200	1.200	1.200
Methyl paraben	0.01	0.006	0.006	-
Propyl paraben	0.04	0.024	0.024	-
Aqueous extract of *A.paniculata*	5.0	3.000	-	3.000
Water	Up to 100%	40.5	43.470	40.500
Total		60.000	60.000	60.000

Statistical Analysis: Statistical analysis was performed using the Statistic Package for Social Sciences program version 19 (SPSS 19.0) and Microsoft Excel 2007. ANOVA followed by posthoc 'Tukey's test' were conducted to determine the significant difference between groups. A p-value of less than 0.05 was accepted as statistically significant.

RESULT AND DISCUSSION:

Evaluation of Anti-bacterial Properties of *A. paniculata* aqueous extract: The polar aqueous extracts prepared from the concentrated liquid extract and powder *A. paniculata* were investigated at five different concentrations (5%, 25%, 50%, 75%, and 100%) using disc diffusion method against *S. aureus*. Based on **Fig. 2**, all polar aqueous extracts displayed good antibacterial activity against gram-positive strain *S. aureus* ($p<0.05$). However, *S. aureus* was less sensitive to the 5% polar aqueous plant extract (2.08 ± 0.67 mm) compared to higher concentrations. 100% polar aqueous extract exhibited the highest zone of inhibition (17.08 ± 0.67 mm) which shows that the Gram-positive *S. aureus* strain was susceptible to growth inhibition by the plant extracts at high concentration.

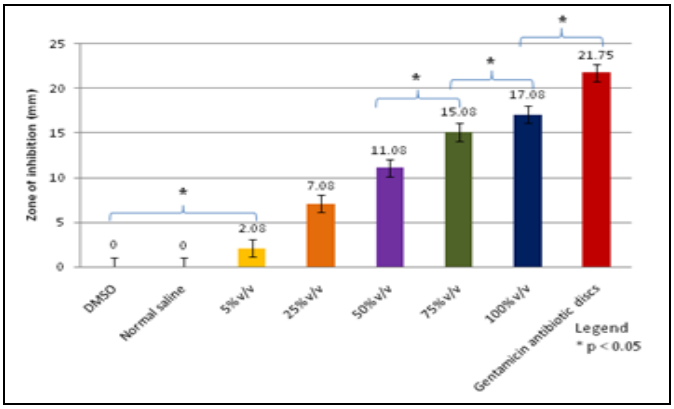

FIG. 2: ZONE OF INHIBITION EXHIBITED BY VARIOUS CONCENTRATIONS OF POLAR EXTRACTS PREPARED FROM CONCENTRATED LIQUID OF *A. PANICULATA* (n=3)

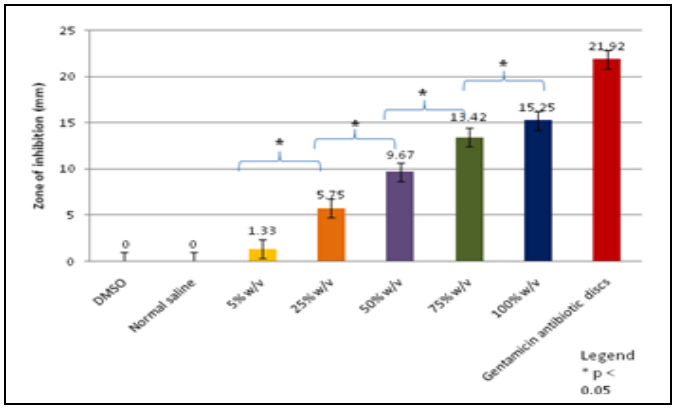

FIG. 3: ZONE OF INHIBITION EXHIBITED BY VARIOUS CONCENTRATIONS OF POLAR EXTRACTS PREPARED FROM POWDER *A. PANICULATA* (n=3)

Fig. 3 shows the zone of inhibition exhibited by various concentrations of polar extracts prepared from powder *A. paniculata*. All polar aqueous extracts displayed positive anti-bacterial activity against Gram-positive strain *S. aureus* ($p<0.05$). However, in comparison with the results using liquid extracts, polar extracts of *A. paniculata* prepared from concentrated liquid extract displayed a higher anti-bacterial activity with greater diameter of inhibition zones (2.08 ± 0.67 mm to 17.08 ± 0.67 mm) compared to the polar extracts of powder *A. paniculata* (1.33 ± 0.49 mm to 15.25 ± 0.62 mm).

Based on this study, all polar aqueous extracts prepared from both concentrated liquid extract and powder *A. paniculata* displayed antibacterial activity against gram-positive bacteria strain *S. aureus* ($p<0.05$). Active plant constituent such as the andrographolide is mostly concentrated in the leaves, which is very bitter and exhibits natural anti-bacterial properties. There are many other phytochemicals which can be isolated from the plant, especially the leaves, roots and stems including sesquiterpenes, lactones, and flavonoids that may contribute to the anti-bacterial properties of the plant.

Although, both polar extracts prepared from concentrated liquid and powder *A. paniculata* displayed antibacterial activity against Gram-positive strain *S. aureus*, they still possess a lower anti-bacterial activity compared to gentamicin antibiotic discs, the positive control ($p<0.05$). However, the anti-bacterial activity for both concentrated liquid and powder extract in this study were lower than the one reported in an earlier study [9]. This might be due to the differences between the maturity and the origin of the plant used.

Characterization of Cream Formulation: Table 2 shows the 11 cream formulations which have been formulated for this study. The cream formulated with *A. paniculata* displayed different green intensity depending on the extract concentration (shown in **Fig. 4** below). Generally, the higher the concentration of extract, the higher the green intensity of the cream produced.

TABLE 2: VARIOUS CREAM FORMULATIONS AND THEIR NAME

S. no.	Types of creams / Formulation	Name for each cream
1	$^{O}/_{W}$ cream without extract	Control
2	5% extract $^{O}/_{W}$ cream	Fa5%
3	25% extract $^{O}/_{W}$ cream	Fa25%
4	50% extract $^{O}/_{W}$ cream	Fa50%
5	75% extract $^{O}/_{W}$ cream	Fa75%
6	100% extract $^{O}/_{W}$ cream	Fa100%
7	5% extract $^{O}/_{W}$ cream without preservative	Fb5%
8	25% extract $^{O}/_{W}$ cream without preservative	Fb25%
9	50% extract $^{O}/_{W}$ cream without preservative	Fb50%
10	75% extract $^{O}/_{W}$ cream without preservative	Fb75%
11	100% extract $^{O}/_{W}$ cream without preservative	Fb100%

Table 3 shows the pH of the cream formulations. The pH of the cream formulations appears to be more alkaline, going across the concentration gradient.

TABLE 3: MEAN pH OF CREAM FORMULATIONS (n=3)

Formulations	pH (Mean ± SD)
Control	7.28 ± 0.00
Fa5%	7.55 ± 0.00
Fa25%	7.62 ± 0.00
Fa50%	7.79 ± 0.00
Fa75%	8.02 ± 0.00
Fa100%	8.22 ± 0.00
Fb5%	7.54 ± 0.00
Fb25%	7.61 ± 0.00
Fb50%	7.78 ± 0.00
Fb75%	8.02 ± 0.00
Fb100%	8.21 ± 0.00

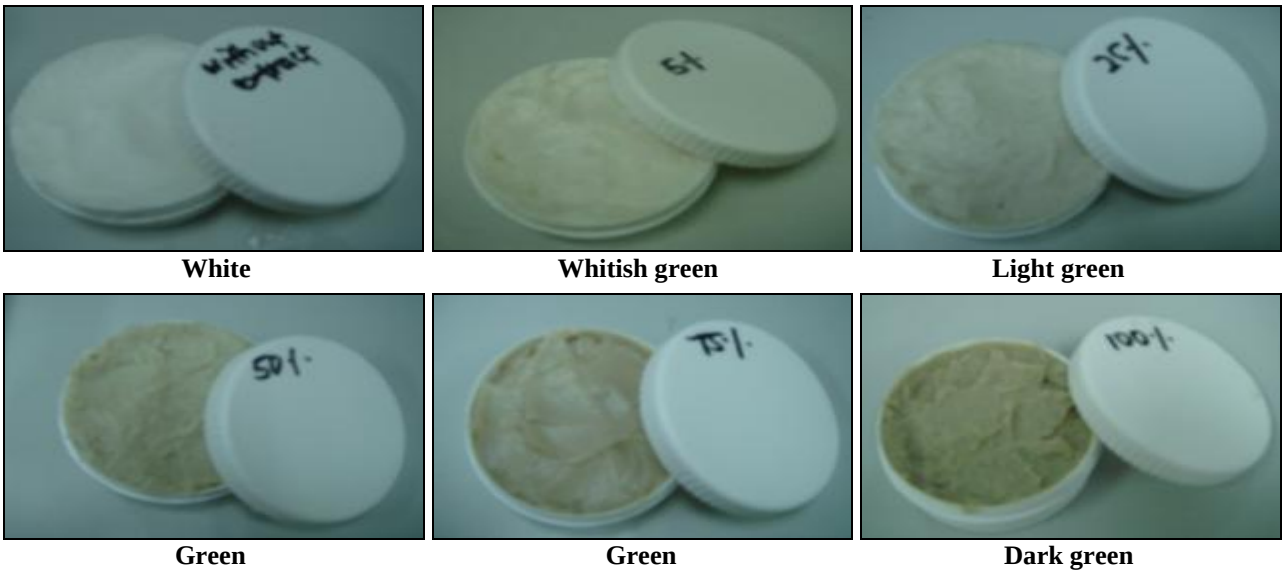

FIG. 4: DIFFERENT COLOURS OF CREAM EXHIBITED BY DIFFERENT CONCENTRATION OF EXTRACTS

Evaluation of Anti-bacterial Properties of Cream Formulations containing *A. paniculata*: All of the *A. paniculata* creams with and without preservatives displayed antibacterial activity against Gram-positive strain *S. aureus* ($p<0.05$) (as shown in **Fig. 5** and **6**. As expected, *S. aureus* was less sensitive to the 5% *A. panicul*ata cream compared to the higher concentrations. 100% *A. paniculata* cream exhibited the highest zone of inhibition, which shows that the Gram-positive strain was susceptible to growth inhibition by the plant extracts at high concentration when incorporated into the cream. Both extract alone and cream exhibited good anti-bacterial activity, although it was slightly reduced in the form of cream.

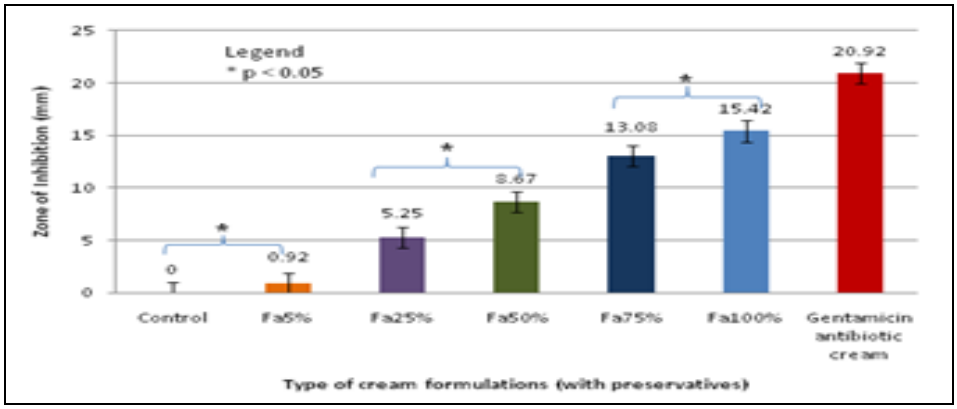

FIG. 5: ZONE OF INHIBITION EXHIBITED BY CREAM FORMULATIONS (WITH PRESERVATIVES) WITH DIFFERENT CONCENTRATION OF EXTRACT (n=3)

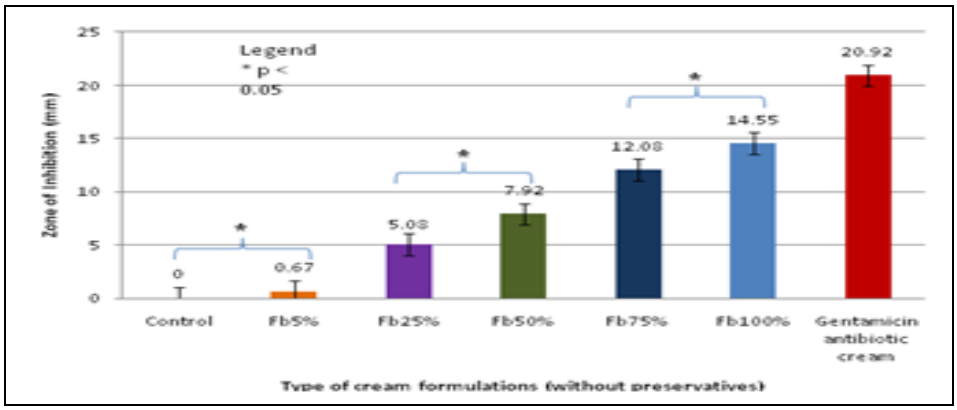

FIG. 6: ZONE OF INHIBITION EXHIBITED BY CREAM FORMULATIONS (WITHOUT PRESERVATIVES) WITH DIFFERENT CONCENTRATION OF EXTRACT (n=3)

Effect of Extract Concentration on the Anti-bacterial Activity of *A. paniculata* Cream: Based on this study, the higher the concentration of the extract incorporated in the cream, the higher the anti-bacterial activity ($p<0.05$). Based on **Fig. 7** below, there is a decrease of anti-bacterial activity when a lower concentration of the extract was incorporated into the cream when compared to liquid extract alone, and this may be due to the dilution effect of the extract.

A lower concentration of extract contains a lower amount of active components, which results in a reduction in the antibacterial activity of the cream.[8] A study on the formulation and evaluation of an anti-bacterial cream using *Oxalis corniculata* (common yellow woodsorrel) polar aqueous extract showed there was an increased anti-bacterial activity of the cream when a higher concentration of the extract was used [10].

In formulating commercial anti-bacterial cream, a high concentration of extract may be incorporated into the preparation of semisolid dosage form to treat adult skin infection caused by *S. aureus* while a lower concentration of extract may be more suitable for children because both high and low extract concentration have been shown to exhibit anti-bacterial properties when incorporated into cream.

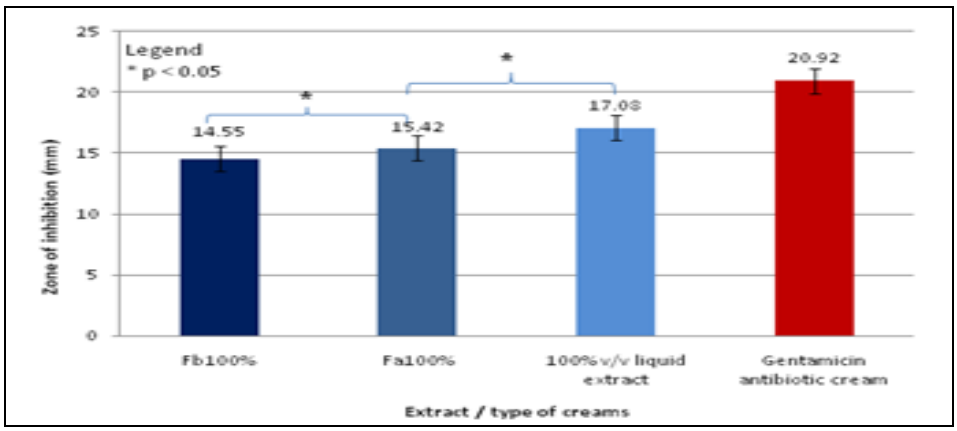

FIG. 7: ZONE OF INHIBITION EXHIBITED BY 100% POLAR AQUEOUS EXTRACT AND DIFFERENT TYPE OF CREAMS (n=3)

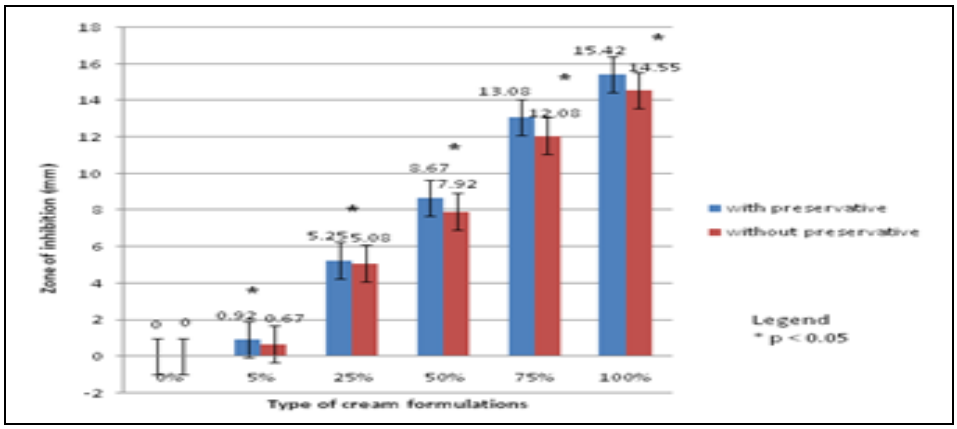

FIG. 8: ZONE OF INHIBITION EXHIBITED BY TWO *A. PANICULATA* CREAM FORMULATIONS (WITH AND WITHOUT PRESERVATIVES) WITH DIFFERENT CONC. OF EXTRACT

Effect of Preservative on the Anti-bacterial Activity of *A. paniculata* Cream: The zone of inhibition exhibited by cream with preservatives is greater than the zone of inhibition exhibited by the creams without preservatives ($p<0.05$). As expected, creams containing preservatives have higher antibacterial activity. Figure 8 below shows the zone of inhibition exhibited by both cream formulations with preservatives and without preservatives.

The preservatives methylparaben and propylparaben, both act as an antimicrobial preservative [11]. Although the antibacterial activity is statistically different ($p<0.05$), this result confirmed that *A. paniculata* on its own still possesses good anti-bacterial properties.

CONCLUSION: To our best knowledge, this is the first study to incorporate *A. paniculata* into a topical cream. In this study, different concentrations (5%, 25%, 50%, 75% and 100%v/v) of aqueous extracts of *A. paniculata*, prepared from both concentrated liquid and powder, exhibited anti-bacterial activity towards *S. aureus*. There seems to be a correlation between the concentration of the aqueous extract and its anti-bacterial properties.

The anti-bacterial activity increases as the concentration of polar aqueous extract increases. Cream formulations using *A. paniculata* were shown to be a potential candidate for anti-bacterial cream at concentration of 100%v/v. The presence of preservative in the formulation contributes to the anti-bacterial properties. Moving forward, more extensive *in-vitro* and *in-vivo* research is required to evaluate the antibacterial activity of the plant to reach commercialization.

ACKNOWLEDGEMENT: The authors wish to acknowledge Cyberjaya University College of Medical Sciences for the research grant provided for this research.

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Chanda S and Baravalia Y: Novel leads from herbal drugs for infectious skin diseases. Applied Microbiology and Microbial Biotechnology Journal 2010; 2(3): 451-57.
2. Singha PK, Roy S and Dey S: Protective activity of andrographolide and arabinogalactan proteins from *Andrographis paniculata* Nees against ethanol-induced toxicity in mice. Journal of Ethnopharmacology 2007; 11(1): 13-21.
3. Parichatikanond W, Suthisisang C, Dhepakson P and Herunsalee A: Study of anti-inflammatory activities of the pure compounds from *Andrographis paniculata* (burm.f.) nees and their effects on gene expression. International Immunopharmacology 2010; 10(2): 1361-73.
4. Sule A, Ahmed QA, Samah OA and Omar MN: Bacteriostatic and bactericidal activities of *Andrographis paniculata* extracts on skin disease-causing pathogenic bacteria. Jo of Medicinal Plants Research 2011; 5(1): 7-14.
5. Rayner C, Munckhof WJ: Antibiotics currently used in the treatment of infections caused by *Staphylococcus aureus*. International Medical Journal 2005; 35(2): 83-16.
6. McCaig LF, McDonald LC, Mandal S and Jernigan DB: *Staphylococcus aureus*-associated skin and soft tissue infections in ambulatory care. Emerging Infectious Diseases 2006; 12(11): 1715-23.
7. Phanse M, Kulkarni P, Kewatkar S, Lande M, Bhujbal S and Chaudhari P: Evaluation of the anti-inflammatory activity of herbal gel formulation. Journal of Natural Product and Plant Resources 2011; 1(2): 25-28.
8. Gidwani B, Alaspure RN, Duragkar NJ, Singh V, Rao SP and Shukla SS: Evaluation of a novel herbal formulation in the treatment of eczema with *Psoralea corylifolia*. Iranian Journal of Dermatology 2010; 13(3): 122-27.
9. Sule A, Ahmed QA, Samah OA and Omar MN: Bacteriostatic and bactericidal activities of *Andrographis paniculata* extracts on skin disease-causing pathogenic bacteria. Journal of Medicinal Plants Research 2011; 5(1): 7-14.
10. Handali E, Sawarkar HA, Khadabadi SS, Mankar DM, Farooqui IA and Japtap NS: Development and biological evaluation of herbal anti-bacterial gel. International Journal of PharmTech Research 2010; 2(3): 2028-31.
11. Das S, Haldar PK and Pramanik G: Formulation and evaluation of herbal gel containing *Clerodendron infortunatum* leaves extract. International Journal of PharmTech Research 2011; 3(1): 140-43.

How to cite this article:
Dahlan A, Yip WS and Pin KY: Formulation and characterization of an anti-bacterial cream using Hempedu bumi (*Andrographis paniculata*). Int J Pharm Sci & Res 2014; 5(9): 3633-40. doi: 10.13040/IJPSR.0975-8232.5(9).3633-40.