FORMULATION AND EVALUATION OF CREAM FROM STEM BARKS OF *PITHECELLOBIUM JIRINGA*

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Abstract

The demand of herbal cream has increased rapidly in the market due to the herbs' natural content which does not pose any side effects on the human skin. In the present study, we investigated the ethyl acetate extract of *Pithecellobium jiringa* (*P. jiringa*) stem barks as antibacterial cream. The antioxidant potential of the *P. jiringa* extract was evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Six formulations of antibacterial creams (oil in water) were prepared and labelled as F1 to F6. All formulations were characterized by different parameters such as pH, spread ability and stability. These formulations were then investigated for their antibacterial activity against several bacteria such as *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Klebsiella pneumonia (K. pneumonia) and Bacillus cereus (B. cereus)* by using agar disc diffusion technique. The DPPH scavenging activity showed moderate radical scavenging activity with IC_{50} value was 46.25 µg/mL. Among the six formulations, F6 showed the good spread ability, homogeneity, appearance and pH. The formulation did not show any phase separation and was easy to remove. The F6 formulation also showed no irritations on skin such as redness, edema, or erythema in irritancy studies. In microbiological assay, F6 formulation exhibited the largest zone inhibition against *Staphylococcus aureus* which was 11.52 mm at the concentration of 2.0% w/v. Therefore, the study suggests that the F6 can be safely used for the consumers that have skin irritations caused by bacteria.

Keyword: anti-allergic, antimicrobial activity, formulation, herbal cream, *Pithecellobium jiringa*

Introduction

Herbal plants possess various therapeutic properties, such as antioxidant, anti-inflammatory, antiseptic, antibacterial and many more. Furthermore, some studies have found that certain plants have an antimicrobial effect against bacteria to protect from skin problems (Budhiraja et al., 2014). The present inventions improve the disadvantages of the formulation available in the market. The invented formulation by using plant extract has a synergistic effect. Therefore, this will enhance the antimicrobial activity with less side effects or toxicity. Extracts from natural plant and their derived products have usually incorporated in the form of emulsions (cream) in pharmaceutical and cosmetics formulations and preparations because of their beneficial and therapeutic properties (Smaoui et al., 2012). Emulsion such as cream can be used as a carrier of the nutrients from the natural plant extracts (Rathore et al., 2014). The capacity of the skin to absorb oils and some chemical compounds is limited. However, the skin is responsive to surface medication such as herbal extracts (Rathore et al., 2014). Therefore, herbal antimicrobial cream is much safer to be used than synthetic one.

Chemical ingredients in synthetic cosmetic products can give negative effects to the human body such as skin and physical outlook. Therefore, the natural ingredients from extraction of

medicinal plants have been used as an alternative source to overcome this problem (Budhiraja et al., 2014). Natural ingredients are acceptable in faith that they are much safer with fewer side effects than the synthetic product due to its natural origin (Bhide and Nitave, 2015). Furthermore, one of the advantages of herbal cosmetics is that they have a wider range of people acceptability (Dubey et al., 2014). This is because herbal cosmetics are suitable for all types of skin. It does not matter if you have normal or sensitive skin, you can also use them without worrying that it will degrade your skin condition (Joshi and Pawar, 2015). The natural content in the herbs can provide nutrients and other useful minerals to the body. Besides, herbs are not that expensive. In fact, it is more affordable to be compared to the synthetic ones (Joshi and Pawar, 2015). Other than that, we can make the medicinal plant such as *P. jiringa* more useful by commercializing it. Instead of discarding the unwanted part of the *P. jiringa*'s plant, we can use it to formulate herbal cream.

P. jiringa, has South-East Asian origin and belongs to the family of Leguminosae (Adriani et al., 2015). It is locally known as jering, as well as djengkol in Indonesia (Bakar et al., 2012; Muslim and Majid, 2010). It has been used traditionally for food and traditional medicine for treatment of various diseases including enuresis, hypertension and diabetes (Yanti et al., 2016). There is an increasing scientific evidence that *P. jiringa* possesses an antimicrobial agent. Bakar et al. (2012), it showed a remarkable antibacterial activity of *P. jiringa* extracts against *Trichophyton rubrum* and *Microsporum canis*. Moreover, recent studies have found that *P. jiringa* is rich in saponins, flavonoids, tannins and terpenoids which serve as antibacterial, antibiotic, anti-inflammatory and antioxidant (Adriani et al., 2015; Hussin et al., 2018; Luthfi et al., 2016). Thus, the objectives of this research are to formulate *P. jiringa* cream, and to evaluate its physical properties and antibacterial activity.

Materials and Methods

Sample Preparation

The stem barks of *P.jiringa* were collected from Jerantut, Pahang. The washed stem barks were air dried and ground to powder form. This powder was soaked for 48 hours in hexane and then concentrated to dryness by rotary evaporator. The residue of *P.jiringa* stem barks was finally extracted in ethyl acetate. The dried ethyl acetate extracts were used for further analysis.

Antioxidant Activity of *Pithecellobium jiringa*

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of ethyl acetate extract was investigated following a method described by Brand-Williams et al. (1995) with slight modifications. The stock solution of 1 mg/ml *P. jiringa* extracts was prepared in ethyl acetate. Each of the reaction mixtures contained 3 mL of 0.1 mM methanolic solution of DPPH and 3 mL of extract at different concentrations (40-250 µg/mL). Controls containing ethyl acetate instead of samples were made. Ascorbic acid was used as a reference standard. Absorbance was recorded at 517 nm after 30 minutes in dark using UV-Vis spectrophotometer. The scavenging activity were calculated using Equation 1:

$$\% \text{ Scavenging activity} = [(A517_{control} - A517_{sample}) / A517_{control}] \times 100 \quad \text{Equation 1}$$

Formulation of *P. Jiringa Cream*

Method by Mishra et al. (2014) was applied in the formulation of *P.jiringa* cream. The emulsifier (stearic acid) and oil soluble components (cetyl alcohol and white oil) were dissolved in the oil phase (Part A) and heated in water bath at 75 ºC. The preservatives and other water-soluble components (triethanolamine, shea butter, polysorbate 20, EDTA and ethyl acetate extract of *P. jiringa)* were dissolved in the aqueous phase (Part B) and heated in water

bath at 75 ºC. After heating, the aqueous phase was added in portions to the oil phase with continuous stirring. Rose oil was added when the temperature dropped to 45 ºC. This solution was stirred continuously until cool and congealed. Different batches of cream formulation were prepared by varying its mass ratio for all of the ingredients.

Evaluation of Cream

Type of Emulsion Using Dye Test

The scarlet red dye was mixed with the cream. A drop of the cream was placed on a microscopic slide, then it was covered with a cover slip and examined under a microscope. If the disperse globules appear red and the ground is colourless, the cream is O/W type. The reverse condition occurs in W/O type cream (the disperse globules appear colourless in the red ground) (Mishra et al., 2014).

Physicochemical properties of the cream

The method or type of observation used for all formulations for different parameters were shown in **Table 1** (Mishra et al., 2014).

Table 1 Parameters for analysis of physical properties of *P. Jiringa's* cream

Parameters	Method/Observation
pH	About 0.5 g of the cream was dissolved in 50 mL of distilled water and pH was measured by pH meter
Homogeneity	The visual appearance and touch affinity were observed whether good or satisfactory
Appearance	The colour of cream pearl essence and roughness were observed and graded.
Spread ability	Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked
After peel	Rubout included spread ability and wetness. A fixed amount of cream was applied on the dorsal skin surface of human volunteer and the properties were observed.
Type of smear	After the application of the cream, the type of film or smear formed on the skin was checked
Removal	The ease of removal of the cream applied was examined by washing the applied part with tap water

Irritancy test

An area of $1cm^2$ (number space unit) on the dorsal left-hand surface was marked and cream was applied to the specified area. The irritancy, erythema and edema were checked for regular intervals up to 24 hours and reported (Mishra et al., 2014).

Microbiological assay

Nutrient agar was used for microbiological analysis on the four pure cultures of bacteria. All media were prepared and sterilized on different petri dishes that followed the manufacturer's specifications. The bacterial suspension was standardized spectrophotometrically to final optical densities (OD) of 0.5 by using UV-Vis at 600 nm (Hussin et.al, 2018). These standardize OD values of inoculums were corresponding to the concentration of $1 – 1.5 \times 10^8$ CFU/mL (Paper, 2015).

Microbial analysis was carried out for all the formulations (Mishra et al. 2014). Nutrient Agar (NA) were inoculated with *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumonia* (*K.pneumonia*) and *Bacillus cereus* (*B. cereus*) by using cotton buds and the plates were dried for five minutes. Then, all discs with different concentrations of F1 to F6 (0.5% (w/v) and 2% (w/v)) were aseptically transferred to the inoculated agar plates and incubated for 24 hours at 37 ºC.

The inhibition activity of microbes was measured on the diameter of the clear zone around the disc (mm). *Ampicillin* (10 µg) was used as a standard and solvent as a negative control. All determinations were carried out in duplicate. Pure culture of *E. coli*, *S. aureus*, *K. pneumonia* and *B. cereus* were obtained from Biology Laboratory 3 in Universiti Teknologi MARA Pahang.

Result and Discussion

Antioxidant activity of *Pithecellobium Jiringa*

DPPH is a stable free radical at room temperature and has the ability to accept an electron or hydrogen radical to become a stable diamagnetic molecule (Ansari *et al.,* 2013). The reaction of DPPH radicals with suitable reducing agents were caused to lose its color stoichiometrically and the number of electrons consumed was measured spectrophotometrically at 517 nm (Mishra *et al.*, 2014). The color of DPPH was reduced significantly by ethyl acetate fractions and the results of DPPH radical scavenging activity were shown in **Table 2**. The decrease of DPPH in the amount of absorbance of the DPPH are associated with phenolic compounds which are due to scavenging of the radical by donation of hydrogen (Riaz *et al*., 2011). Research done by Hussin *et al.* (2018) indicated that the ethyl acetate extract of stem bark from *P. jiringa* has an abundance of phenolic compounds such as flavonoids, tannins, saponins and terpenoids. Ethyl acetate extract at concentration 200 µg/mL presented the highest DPPH scavenging activity (91.88%) while the lowest at 50 µg/mL (54.8%).

The IC_{50} value is referring to the concentration of a substrate or antioxidant that causes the reduction of free radical DPPH about 50% (Yanti *et al.*, 2015). From this study, IC_{50} value of ethyl acetate extract was 46.25 µg/mL which showed moderate radical scavenging activity (Ahmad & Abdullah, 2013). IC_{50} value for ascorbic acid was 11.00 µg/mL. The value was in the range of ascorbic acid IC_{50} value at 11.50 µg/mL that was found by Muthukumarasamy (2016).

Table 2 DPPH assay [IC_{50} value for ascorbic acid = 11.0 µg/ml] of ethyl acetate *P. jiringa's* stem bark extracts

Concentration of ethyl acetate extract (µg/mL)	% inhibition of DPPH (%)
0	0
50	54.80
100	84.87
150	90.22
200	91.88
IC_{50} (µg)	46.25

Formulation of Cream

Cream made from ethyl extract of *P.jiringa's* stem bark were formulated based on **Table 3**.

Table 3 Amounts of ingredients (g) used in formulations 1 to 6

Ingredients	Formulation number					
	F1	F2	F3	F4	F5	F6
Ethyl acetate extract	0.200	0.500	0.500	0.500	0.500	0.500
Stearic acid	1.000	1.000	1.000	1.200	1.000	1.200
Triethanolamine	0.135	0.135	0.135	0.160	0.160	0.165
Rose water	0.300	0.300	0.300	0.400	0.400	0.400
White oil	0.350	0.350	0.350	0.300	0.300	0.300
Shea butter	1.000	1.000	1.000	1.200	1.200	1.200
Cetyl alcohol	-	0.250	0.200	0.150	0.100	0.100
Polysorbate 20	0.020	0.020	0.020	0.020	0.020	0.020
Ethylenediamine tetraacetic acid (EDTA)	0.010	0.010	0.010	0.010	0.010	0.010
Water	Qs	Qs	Qs	Qs	Qs	Qs

Evaluation of Herbal Cream

The dye test showed that all formulations were type O/W type emulsion cream. Therefore, the prepared cream could be easily removed with plain water which gives better customer compliance (Bhide and Nitave, 2015). Formulation F5 and F6 showed high stability in O/W emulsion. While, the other formulations were not stable and resulted in breakdown of the emulsion when stored for a long period of time.

The pH value is important to determine the stability of emulsions. Moreover, the changes in pH implies the occurrence of chemical reaction that can give an image on the quality of final product (Smaoui et al., 2017). The pH of the cream formulations F1, F2, F3 were in the range of 3.8 – 4.5 as shown in Table 4. While, the pH of F4, F5, F6 in the range of 5.6 – 6.0 were suitable pH for skin. Research done by Smaoui et al. (2017) found that the pH of human skin normally ranges from 4.5 to 6.0.

All the cream formulations had no effect on skin irritation and allergic sensitization which were shown in **Table 4**. They also did not show any redness, edema, inflammation and irritation during irritancy study. Therefore, it indicated these formulations were safe to be applied on the skin.

Table 4 Results pH and adverse effect of formulations

Property	Formulation number					
	F1	F2	F3	F4	F5	F6
pH	4.4	3.94	3.81	5.61	5.53	5.61
Adverse effect						
Irritant	NIL	NIL	NIL	NIL	NIL	NIL
Erythema	NIL	NIL	NIL	NIL	NIL	NIL
Edema	NIL	NIL	NIL	NIL	NIL	NIL

Microbiological Assay

Antibacterial assay of all cream formulations of F1 – F6 containing ethyl acetate extracts of *P. jiringa* were assessed against *S. aureus*, *K. pneumonia*, *B. cereus* and *E. coli*. The effectiveness of various cream formulations based on diameter of inhibition zone (mm) were demonstrated in **Table 5**. From the table, F6 formulation had the largest zone of inhibition against the four types of bacteria. At concentration of 2.0 % w/v, the value of the inhibition zone was 11.52 mm against *S. aureus* and 11.45 mm against *B. cereus*. *S. aureus* and *B. cereus* were more susceptible to the cream formulations which were knowingly responsible for the infections of skins (Eichie et al., 2011).

Table 5 Antibacterial activities of various cream formulations on few types of bacteria at several concentrations

Bacteria	**Concentration (% w/v)**	**Diameter of Inhibition Zone, mm Formulation**						
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**Control Ampicillin**
Escherichia coli	0.5	7.46± 0.34	7.67±0.33	7.60±0.56	7.98±2.79	7.58±0.60	7.00±1.41	11.97± 0.18
	2.0	7.75± 0.99	7.92±0.54	7.84±0.66	10.17± 1.74	11.35± 0.50	11.42± 0.55	
Staphylococcus aureus	0.5	6.00± 0.00	7.00±1.41	8.20±0.28	7.50±2.12	7.00±1.41	8.23±1.03	12.89± 0.30
	2.0	8.11± 0.14	7.13±1.59	8.25±0.35	8.74±0.52	8.33±0.18	11.52± 0.68	
Bacillus cereus	0.5	7.92± 0.12	7.98±0.60	8.28±0.40	8.48±0.52	8.35±0.64	10.87± 0.19	14.14± 0.16
	2.0	9.27± 1.11	10.55± 0.71	10.58± 0.74	10.94± 1.22	10.95± 1.21	11.45± 0.64	
Klebsiella pneumonia	0.5	6.00± 0.00	6.60±0.85	7.00±1.41	7.03±1.45	7.03±1.45	7.00±1.41	11.24± 0.94
	2.0	7.00± 1.41	8.35±1.85	8.28±1.09	6.79±1.12	6.00±0.0	8.25±3.18	

The ethyl acetate extract from the stem bark of *P.jiringa* was abundant with saponins, flavonoids, tannins and terpenoids (Hussin et al. 2018). Flavonoids have multiple biological activities such as anti-oxidation, anti-inflammation, anticancer and cardiovascular protection (Xie et al., 2015). Saponins served as a major component as antimicrobial secondary metabolites (Sule et al., 2011). In the research conducted by Parekh and Chanda (2007), it was affirmed that tannins were found to react with proteins in order to give the typical tanning effect that was crucial for the treatment of different illnesses. Terpenoids were also included among compounds that were biologically active, hence aided the antimicrobial activities of the formulated cream of *P. jiringa* stem bark (Sule et al., 2011).

Conclusion

In conclusion, the ethyl acetate extract of *P. jiringa* stem bark showed a good antioxidant activity. At the concentration of 200 µg/mL, ethyl acetate extracts of *P. jiringa* stem bark exhibited the highest DPPH scavenging activity which was 91.88%. The ethyl extracts of *P.*

jiringa stem bark had a moderate radical-scavenging activity. Cream formulation of F4, F5 and F6 have pH range 5.6 – 6.0 which was suitable for skin. All formulations did not show any sign of skin irritation, edema, or erythema. F6 cream formulation was the greatest inhibition zone at 11.52 mm and 11.45 mm against *S. aureus* and *B. cereus*. Therefore, the cream formulation of F6 was the best formulation to be used compared to other formulations due to its good pH, stable for a long time, no sign of skin irritation and showed the greatest inhibitions towards bacteria.

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Conflict of interests

Author hereby declares that there is no conflict of interests with any organization or financial body for supporting this research.

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