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Quality assessment of ointment incorporated with *Piper sarmentosum* (Kaduk) leaves extract

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Abstract. Plant based medication is known as one of the alternative methods in wound management with no or less side effects, as well as less expensive. Various products related to wounds are on the market mostly consist of various unnatural chemicals. Previous preliminary study and ethnopharmacological evidence of *Piper sarmentosum* (Kaduk) also showed that the plant is a good candidate to treat wounds. Thus, this project aims to evaluate an ointment incorporated with the *P. sarmentosum* aqueous extract as an alternative to treat wounds. Two type of ointments; hydrocarbon (oleaginous; ointment 1) and water removeable (oil-in-water; ointment 2) that incorporated with the plant extract were further assessed for its organoleptic, physicochemical properties, as well as its stability at different storage conditions. The findings showed that ointment 1 exhibited good physicochemical properties with a strong scent of extract. However, this ointment did not inhibit *E. coli*, *S. aureus* and *B. subtilis* in the antimicrobial test. The stability study that carried out for three weeks consecutively showed that ointment 1 (hydrophilic) was stable under chill storage condition (5 °C) and room temperature (25 °C) without phase separation, as well as insignificant change of odour, texture and pH value. However, the formulation was physically unstable at high temperature (45 °C). Meanwhile, ointment 2 (oil-in-water) significantly inhibited *E. coli*, *S. aureus* and *B. subtilis* possibly due to presence of parabens in the formulation., not melted at high temperature (45 °C), stable in pH, non-greasy and water-washable. However, the formulation was physically unstable, that it showed phase separation after heating, and harden in texture when compared to the commercial healing ointment (Brand: Zam Buk). Findings from this study could be an initial step for the future development of the effective, affordable and safe wound healing medication by related industries.

1. Introduction

Nowadays, the popularity and demand for natural products has increased significantly not only in the field of cosmetic but also in pharmaceutical industry worldwide [1]. Pathare & Wagh reported that approximately 80% of the world populations are presently making use of herbal medicine for primary health [2], and in some cases as alternative to commercial drugs [3]. Apparently, increasing use of herbal medicines is due to several perceptions such that better efficacy, safer, poses minimal side effects and more affordable [2, 4].

Ointments are considered as the simplest and convenient medication dosage form with high compliance and flexibility [5]. Ointment is used externally and differs from creams due to its greasy base texture. The ointment base mostly is anhydrous and immiscible with skin secretions [6]. There are two types of ointments; unmedicated and medicated. Unmedicated ointment is commonly used for physical effects such as protectants or lubricants, while medicated ointment contains active ingredient(s) or a mixture of medicaments mixed with ointment base. Ointment bases can be classified



into four common groups of hydrocarbon bases, absorption bases, water-removable bases and water-soluble bases. These ointment bases can be applied either pharmaceutically or as active ingredient vehicles. Each has features that are very unique and distinctive.

Most people use creams for various purposes. However, creams can leave the skin feeling drier than before, while ointments work better on dry skin conditions, such as psoriasis. It occlusive which is trapping and absorbing moisture much more slowly into the skin over time [7]. Thus, able to keep the skin moist for longer periods of time and promote complete absorption medication and penetrate deep into the skin. Ointment are less likely to cause an allergic reaction compared with cream and better to use on sensitive skin since many creams are manufactured with sensitizing preservatives and stabilizers to extant shelf life that may cause irritant or allergic reaction [8]. Due to these advantages, ointments are widely employed as the media for transdermal drug or cosmetic delivery and have potential application on skin-based diseases as well as for wound healing [9, 10]. Piper species have been reported to be one of the effective medicinal plants used in folk medicine or as natural remedies to treat various ailment and relief discomforts [11]. *Piper sarmentosum* Roxb. is a member of Piperaceae family and the leaves is commonly known as Wild Betel or “Kaduk” in Malay. Other than Peninsular Malaysia, *P. sarmentosum* is widely distributed over Thailand, India, Laos, Cambodia, Vietnam, Filipina and Indonesia [12, 13].

In both Peninsular Malaysia and Thailand, the leaves of *P. sarmentosum* are usually cooked and eaten as vegetables or to impart flavour to local cuisine [13]. It is also traditionally used to treat pains in bones and relief discomfort such as headache, fever, toothache, cough and asthma [14, 15]. Decoction of the leaves is used to treat malaria, where the crushed leaves are mixed with water and used for bath to treat kidney stone and urination difficulty [16-18]. Referring to various ethnopharmacological reports of *P. sarmentosum*, the plant has been reported exhibiting anti-inflammatory and anti-nociceptive activities [19, 20]. However, to date, there is no reports of this plant on wound healing activity. A preliminary study carried out by Abdullah [21] using the extracts in *in vivo* tests showed a significant wound healing activity after 14 day of treatment. This indicated that the plant species has a vast potential to be developed into various natural-based products, particularly as the wound healing agents. Therefore, this present study aimed to formulate a herbal topical ointment incorporated with *P. sarmentosum* extract that has a potential as an effective, safe and affordable wound medication.

2. Materials and Method

2.1. Plant material

Fresh, mature leaves of *Piper sarmentosum* were collected in Arau, Perlis, Malaysia in June 2020. The plant has been authenticated by a botanist from Universiti Kebangsaan Malaysia Herbarium, Selangor, where its voucher specimen (UKMB40387) has been deposited.

2.2. Sample collection and preparation

The leaves were washed thoroughly with a tap water to remove all the soil sediments and foreign particles. The leaves were then distributed evenly on tray and dried using oven at 40 °C for 1 week. Upon completion of drying, the dried leaves were ground into powder using a blender and were kept in a zip lock bag for subsequent aqueous extraction. *P. sarmentosum* powder was decocted using sterile distilled water at a ratio of 1:20. Approximately 50 g of the sample was added with 1000 ml of sterile distilled water and heated to 80 °C for 3 hours using stirring hotplate. The mixture was filtered, sent to be freeze-dried and was kept at 4 °C until further use.

2.3. Formulation of herbal topical ointment

The ingredients used in the formulations were based on different ointment bases, which were hydrocarbon (oleaginous) and water removable (oil-in-water) bases [22]. The composition of the formulae to produce 100 g of ointment were presented in Table 1. Prior to the formulation process, all

equipment including beakers, measuring cylinder, glass rod, spatula, distilled water, glass petri dishes were autoclaved at 121°C for 15 minutes.

Table 1. Formulation of incorporated *P. sarmentosum* extracts in hydrocarbon (oleaginous) and water removable (oil-in-water) bases.

Hydrocarbon (oleaginous) base (Ointment 1)		Water removable (oil-in-water) base (Ointment 2)	
Ingredient	amount (w/w; g)	Ingredient	amount (w/w; g)
Wool fat	5	Methylparaben	0.025
Cetostearyl alcohol	5	Propylparaben	0.015
Hard paraffin	5	Sodium lauryl sulfate	1
White soft paraffin	5	Propylene glycol	12
		Stearyl alcohol	25
		White petroleum	25
		Purified water	37
Active	X	Active	X

2.4. Organoleptic and physicochemical analysis

The formulated ointments were assessed and characterized for basic organoleptic properties such as appearance, colour, odour, homogeneity, spreadability and immediate after feeling such as stickiness and greasiness. These characteristics were mainly determined through sensory evaluation.

Texture profile analysis was performed using texture analyser (Brookfield CT3 Version 2.1) to evaluate the hardness, adhesiveness and spreadability, while the spreadability test was performed using Fixture Dual Extrusion Cell (TA-DEC), with a conical probe of angle 45°. In the present study, the texture profile of commercialised ointment (Brand: Zam-Buk) was determined and used as a control. The colour profile of incorporated ointments and ointment bases in the form of L*, a* and b* were determined using chromameter (Kinolta Minolta), while the viscosity of ointments was performed using Sine-wave Vibro Viscometer SV10/SV-100 viscometer with concentric cylinder spindle to determine the viscosity of the different ointment formulations.

2.5. Antimicrobial assay of the formulated ointment

Common bacteria species that can be found on skin and could cause infection on skin were used to determine the antibacterial property of the incorporated ointments. The assay was conducted using the plate agar diffusion method against *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) which were obtained from the Faculty of Agro-based Industry's culture collection. The strains were sub-cultured by dipping an inoculating loop into glycerol stock bacteria and streaked on Muller Hinton Agar (MHA). Agar medium was autoclaved after being prepared according to the manufacturer's instructions. The plates were incubated in incubator at 37 °C for 18 hours.

After 18 hours, three to five well-isolated colonies of the standard microorganism from Muller Hinton Agar were suspended using inoculating loop in falcon tube that containing Nutrient Broth (NB). The falcon tube sealed with parafilm and incubate in incubator shaker at 37° C with 160 rpm for 24 hours. A sterile cotton swab was streaked over the surface of the agar plates. The inoculated plates were then left to dry at room temperature. The dried inoculated plates were used for the agar well-diffusion assay.

Five wells with diameters of 5 mm each were made by perforating on the MHA plate using a sterile cork borer. The cut pieces of the agar were removed. 70 µg of *P. sarmentosum* ointments, base ointments and commercial healing ointment (Brand: Zam-Buk) was filled into each well using sterile micropipette tips. An ointment base without active ingredient (*P. sarmentosum* extract) was used as a negative control and a commercial healing ointment (Brand: Zam-Buk) was used as a positive control.

The plates were incubated at 37 °C for 18 h. The detected diameters of the inhibition zones were measured using a ruler to the nearest millimeter.

2.6. Accelerated stability test of the formulated ointment

The accelerated stability test was performed under 3 different storage conditions of 5±1 °C (refrigerator), 25±1 °C (room temperature), and 45 °C (oven). 45 g of sample which was filled in the petri dish kept under the conditions for 28 days. The tested parameters including texture, colour, pH, odour and phase separation was observed and evaluated for any changes on every 7-days interval. This study was carried out in accordance with International Council for Harmonization (ICH) guideline and World Health Organization (WHO) stability guideline [23].

2.7. Statistical analysis

Data were collected in triplicate and expressed as mean±standard deviation (SD). Differences between means were determined using two-way analysis of variance (ANOVA) of IBM SPSS Software (Version 21). At 5% significant level, p -value less than 0.05 ($p<0.05$) was considered statistically significant.

3. Results and Discussion

A topical herbal ointment was formulated by manual mixing of all ingredients. The basic ingredients used in the formulation of ointment bases were based on the literature and the amount of the active incorporated in the formulation was based on the preliminary study by Abdullah [21]. The selected two formulations using different bases were prepared and the composition were given in Table 1 in Section 2.3.

The organoleptic properties of the incorporated ointment were given in Table 2. Based on the result, the incorporated ointment 1 showed favourable organoleptic properties compared to the incorporated ointment 2. The incorporated ointment 1 exhibited good appearance regarding its smooth and homogenized texture. However, the incorporated ointment 2 exhibited the presence of tiny residue, flaky and separated phase between the aqueous and wax phases after heating. The incorporated ointment 1 demonstrated lighter brownish colour and stronger pungent *P. sarmentosum* extract scents, compared to the incorporated ointment 2. The pungent smell of *Piper* species is attributed to the presence of alkaloid compound known as piperine [24]. Besides, the incorporated ointment 1 showed good spreadability, softer and moisturized, greasy and not water removable. However, the incorporated ointment 2 are non-greasy, easily removed from the skin by washing and aesthetical pleasing.

Table 2. Organoleptic characteristics of the incorporated ointments.

Characteristic	Incorporated ointment 1 (oleaginous base)	Incorporated ointment 2 (oil-in-water base)
Colour	Very light brown	Light brown
Odour	Strong scent of the extract	Light scent of the extract
Appearance	Smooth	Present of tiny residue
Texture	Oily and slippery	Hard and flaky
Immediate after feel	Medium stickiness and greasiness	Not- greasy and non-sticky on application
Spreadability	Easy to be spread	Hard to be spreaded
Homogeneity	Homogenous	Separate phase

The hardness and adhesiveness attributes of both ointments were measured using texture analyser and summarised in Table 3.

Table 3. Texture profile analysis of the incorporated and commercial ointments.

Parameters	Oleaginous base (ointment 1)	Oil-in-water base (ointment 2)	Commercial ointment
Hardness (g)	37.00 ± 3.00	321.33 ± 89.90	29.00 ± 1.00
Adhesiveness (mJ)	0.43 ± 0.32	0.07 ± 0.06	0.33 ± 0.15

Colour profiles of the bases and incorporated ointments were determined using a chroma meter with respect to the L*, a*, b* parameter. The L* value indicates lightness, the positive a* value defines redness, while the negative a* defines greenness. Furthermore, the positive b* value determines yellowness, while the negative b* value exhibits bluish of the samples tested and summarised in Table 3.

Table 4. Physicochemical characteristics and antimicrobial test of ointments stored at 25°C over 14 days.

Ointment	Storage temperature (25°C)											
	Antimicrobial test				Hardness (g)	Physicochemical characteristics						
	Inhibition zone diameter (mm)					Colour analysis			pH	Viscosity (Pa.s)	Odour	Phase separation
	Day	Bacteria strain				L*	a*	b*				
	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. aureus</i>									
Commercial ointment	1	6.70	-	-					5.5 - 6.3	47.47		
	7	7.00	-	-								
	14	8.00	6.17	7.33								
Oleaginous base (ointment 1)	1	-	-	-		37.49	-0.43	4.91				
	7	-	-	-								
	14	-	-	-								
Incorporated ointment 1	1	-	-	-	57 ^b	35.08 ^a	-0.18 ^b	6.87 ^b	5.53 ^a	58.77 ^b	No	No
	7	-	-	-	29 ^a	36.81 ^b	-0.60 ^a	5.32 ^a	5.93 ^a	35.53 ^a	Yes	No
	14	-	-	-	37 ^a	36.74 ^b	-0.45 ^a	5.66 ^a	6.53 ^b	42.27 ^a	Yes	No
Oil-in-water base (ointment 2)	1	9.30	-	-		51.51	0.59	7.48				
	7	16.67	-	-								
	14	13.67	-	-								
Incorporated ointment 2	1	9.00	-	-	404	44.05 ^b	0.99	9.71 ^c	6.3	8.9 ^b	No	Yes
	7	5.33	-	-	404	45.59 ^c	0.60	9.18 ^b	6.33	5.84 ^a	Yes	Yes
	14	11.00	6.00	14.33	321	42.24 ^a	1.25	8.49 ^a	6.33	5.33 ^a	Yes	Yes

Values are presented in mean (n=3). The values followed by different superscript letter (a, b, c, and d) showed statistically significant (p<0.05) by Duncan's multiple range test.

In this study, the pH value of the formulated ointments was 5.53±0.404 for incorporated ointment 1 and 6.30±0.36 for incorporated ointment 2 which within the range that compatible with skin and considered safe to be applied topically. Nonetheless, the incorporated ointment 1 having the pH nearly to 5.0 which is recommended for wound healing. According to Naranje et al. [25], an alkaline pH in the wound bed can contribute to creating an unsuitable environment for healing by encouraging the growth of pathogenic bacteria. In contrast, an acidic pH environment helps in wound healing by controlling wound infections, increasing antimicrobial activity, altering protease activity, releasing oxygen, reducing toxicity of bacterial end products, and enhancing epithelization and angiogenesis. pH

value is one of the critical factors involved in the wound healing process due to that skin requires acidic environment to encourage healing but could not be too acidic as this can interrupt the healing process. The high acidic condition could cause endothelial cell damage and degrade the epidermal-dermal junction, which eventually destroys the newly forming tissues [26, 27].

The viscosity value of the ointment bases and incorporated ointments were specifically determined using viscometer presented in Table 4. Most ointments are intended to be thick when standing to prevent them from flowing away from the intended area of use. This present study showed that the incorporated ointment 1 having nearly value viscosity of the commercial ointment which are 58.77 ± 7.67 and 47.47 ± 0.25 , respectively. On a side note, the viscosity of incorporated ointment 2 was lower due to its ingredients that contain water, thus reduce the viscosity of the ointment formulation.

Antibiotic ointments are widely used for preventing wound infections. It is a fairly common wound management practice in clinic and personal wound care [28]. Agar well diffusion method was performed to determine the antibacterial activity of the incorporated ointment towards *E. coli*, *S. aureus* and *B. subtilis*. The antibacterial activity was assessed based on the diameter of halozones observed. The findings showed that the ointment base 1 and incorporated ointment 1 did not exhibit any significant zone of inhibition, while ointment base 2 and incorporated ointment 2 inhibited a significant activity against the tested bacteria as shown in Table 4.

The effects exhibited by the ointment 2 could be contributed by the presence of parabens in the formulation, which act as preservatives and commonly used in cosmetic products, foods and drugs. According to US Food and Drugs Administration [29], parabens used in cosmetics to prevent the growth of harmful bacteria and mold, in order to protect both the products and consumers. Despite many are concerned about the safety of methylparaben particularly its link to cancers, the FDA and other researchers have not found any conclusive evidence, though there have been cases of individuals who have had negative reactions. From the findings, it can be seen that the ointment base 1 and incorporated ointment 1 did not exhibit antibacterial activity on tested bacteria. Presumptions was made such that the extract itself does not exhibit antibacterial property or the concentration of extract incorporated was insufficient to inhibit the tested bacteria [30-32]. In addition, there has been very limited studies on antibacterial properties on *P. sarmentosum* aqueous extract. Several reports stated that aqueous extract of medicinal plants exhibited the least significant antibacterial activity against *E. coli* and *S. aureus* when compared with extract using organic solvent [33-35]. Handali et al. [36] also reported that increasing concentration of herbal extract will significantly affect the antibacterial activity.

4. Conclusion

It can be concluded that the incorporated ointment 1 exhibited good organoleptic and physicochemical characteristics aromas well as having a strong scent of the extract compared with the incorporated ointment 2. This ointment also exhibited good stability in term of the texture, pH and no sign of phase separation at 5°C and 25°C conditions. Even though this ointment melted when stored at 45°C, it turned back to solid when left in room temperature. However, improvements are needed to enhance a pleasant aroma of the ointment by incorporating fragrance and increase its antimicrobial properties using natural preservatives. In addition, the stability of the ointment 2 could be improved with the use of phosphate buffer solution instead of water. The developed topical herbal ointment incorporated with *P. sarmentosum* aqueous extract is potentially effective, safer and affordable for wound management.

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