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Formulation and Antimicrobial Screening of *Piper sarmentosum* Cream against *Staphylococcus aureus*

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ABSTRACT

Piper sarmentosum, known as 'kaduk' has been traditionally used in different parts of the world to cure many diseases and ailments. It contains alkaloids and has been reported to possess many pharmacological properties like anti-cancer, anti-hyperglycaemic, anti-tuberculosis, antioxidant, and antimalarial. This study aimed to evaluate the formulation of *P. sarmentosum* cream and exploring the antimicrobial properties in different types of cream formulation before *in vivo* study. The leaves extract of *P. sarmentosum* was obtained from the cold-soaked methanolic extraction method, evaporated, and dried to produce the powdered extract. Then, it was diluted into four different concentrations, 25% w/v, 50% w/v, 75% w/v, and 100% w/v for *Staphylococcus aureus* antimicrobial screening. Based on the *S. aureus* antimicrobial screening, four types of creams were formulated (Cream A: cream base without *Piper sarmentosum* extract; Cream B: *Piper sarmentosum* extract (5%) only; Cream C: *Piper sarmentosum* extract (5%) with parabens preservatives; Cream D: *Piper sarmentosum* extract (5%) with vitamin E) and evaluated for their physical appearance, pH, stability study, and antimicrobial activity against *S. aureus*. As a result, 100% w/v concentration of the *P. sarmentosum* extract showed the highest result in the zone of inhibition (5.50 mm ± 0.03) towards *S. aureus* and was selected for cream formulation. In evaluating their physical appearance, all formulated creams showed high homogeneity and consistency with no phase separation and pH between 7.2 – 8.0 ± 0.07. On stability study, all creams with three different temperatures of 4°C, 27°C, and 37°C for 30 days show no colour

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changes, high homogeneity, and consistency with any phase separation. The results of antimicrobial screening for all formulated creams, show that Cream D has the highest zone of inhibition towards *S. aureus* (5.53 mm ± 0.25), followed by Cream C (4.43 mm ± 0.13). In conclusion, *P. sarmentosum* cream formulation showed high stability properties and possessed anti-microbial properties suggesting its potentials for wound healing cream.

Keywords: Cream formulation, methanolic extraction, *Piper sarmentosum*, *Staphylococcus aureus*

INTRODUCTION

Piper sarmentosum (*P. sarmentosum*), also known as 'kaduk' belongs to the Piperacea family. It is widely found in tropical countries in Southeast Asia, northeast India, and China (Karthigeyan et al., 2004). *Piper sarmentosum* has been used as a traditional remedy, in treating diabetes mellitus, cough, toothache, fungal infection on the skin, asthma, and inflammation of the pleura (Rahman et al., 2011). Chan and Wong (2014) reported that the leaves of *P. sarmentosum* contains phenylpropanoids, phenylpropanoyl amides, dihydro-flavones, and some essential oils. In addition, alkaloids isolated from *P. sarmentosum* leaves, 1-allyl-2, 6-dimethoxy-3 and 4-methylenedioxybenzene, have been claimed to show antibacterial activities against *Escherichia coli* and *Bacillus subtilis*.

The microorganisms commonly found in the wound infection are *Staphylococcus aureus* and beta-hemolytic *Streptococcus*, identified as a "transient flora" of the skin. Currently, *S. aureus* is overwhelmingly the most prominent cause of skin infections. This bacteria is usually harmless to human skin, but it can be infectious when there are injuries in the skin such as abrasions, cuts, surgical incisions wounds or indwelling catheters (Rain, 2005).

The cream, defined as a semi-solid emulsion, is applied to the skin. There are two types of cream: oil-in-water (o/w) cream and water-in-oil (w/o) cream. The oil-in-water emulsions are the most useful water-washable bases, while water-in-oil emulsions are used as an emollient and cleansing agent (Das et al., 2014).

Nowadays, the widespread belief that "green medicine" (plant-based products) are effectively safe to be used and less expensive. Therefore, the study of active compounds from natural resources has been actively pursued as it is essential in treating diseases (Ab Rahman et al., 2014). The situation prompts the concern in searching for natural compounds that could mimic the effects of synthetic cream but with fewer side effects. Although *P. sarmentosum* is commonly used as folk medicine, there is still little scientific investigation of its potential and preparation in being established as a treatment cream. Therefore, this study intends to evaluate the cream formulation and anti-microbial properties of formulated cream contain *P. sarmentosum* leaf extraction.

METHODS

Sources of *Piper sarmentosum*

The identified plant material was confirmed and validated by a botanist from Institute of Bioscience, Universiti Putra Malaysia (UPM), Serdang, Selangor. The voucher (SK 2100/15) has been deposited at the herbarium in UPM for future reference, while two kilograms of the leaf powder of *P. sarmentosum* was used for this study.

Preparation of *Piper sarmentosum* Leaves Methanolic Extract

The collected samples were washed with tap water and dried in the oven for 48 hours. The dried leaves were macerated to a fine powder before being soaked in methanol at 1:10 (Wong & Kitts, 2006). The mixture was then filtered and concentrated using rotavapor (Büchi®, Germany) to remove methanol. The concentrated extract was further dried using a freeze drier to remove residual methanol. Finally, the powder form of the extract was kept in vials with a tight cap before being stored in a –20°C refrigerator until further use (Fernandez et al., 2012).

In-vitro Antimicrobial Screening

In-vitro antimicrobial activity was examined for methanolic extractions and cream formulations. Prior to sensitivity testing, the *S. aureus* (ATCC11632) bacteria strains, Mueller Hinton (MH) agar (Pronadisa, Europe), and Mueller Hilton broth (Oxoid™, United Kingdom) were prepared. All the equipment used throughout the process was autoclaved at 120°C for 20 minutes to minimize contamination. Kirby-Bauer disc diffusion method was applied as an antimicrobial screening procedure. The bacteria strain *S. aureus* was cultured onto a blood agar plate and incubated at 37°C for 24 hours before conducting sensitivity testing. A single colony was then selected and cultured in 5 mL Mueller Hinton broth. The broth was adjusted to 0.5 McFarland standard using a UV-Vis spectrophotometer (Eppendorf BioSpectrometer®, Germany). Then, bacterial suspension turbidity was adjusted to the McFarland equivalence turbidity standard to produce bacterial counts in an expected range. The spectrophotometer was set at 600 nm or 625 nm, and sterile saline was used as the control.

Antimicrobial Screening of Local *Piper sarmentosum* Leaves Methanolic Extract

Piper sarmentosum methanolic extract powder was used to prepare four different concentrations of the extract. Purified distilled water was used to dissolve the powder extract to make the different concentrations of 25% w/v, 50% w/v, 75% w/v, and 100% w/v solution of the *P. sarmentosum* extract. Then, different solution concentrations were put into vials and kept at -4°C prior to use. Twenty (20) µL from each different concentration solution were used to impregnate a blank sterilized filter paper disc before being dried in a 37°C incubator for 24 hours prior to use for antimicrobial screening. Agar plates were inoculated with a standardized inoculum of

S. aureus strains. Then, filter paper discs (6 mm diameter), containing the test compound at the desired concentration, are placed on the agar surface. The Petri dishes are incubated at 37°C for 24 hours. Microbial growth was determined by measuring the diameter of the zone of inhibition of the extract using a transparent ruler in millimetres (mm).

Zone of inhibition (mm) = Diameter of microbial growth – Diameter of disc

Note. Diameter of disc is 6 mm

Preparation, Formulation, and Evaluation of *Piper sarmentosum* Cream Extract

From the antimicrobial screening results, the best extract concentration (100% w/v) that gave the highest resistance toward *Staphylococcus aureus* was chosen to be formulated into 60 g cream.

Oil in water creams was formulated based on the previous study (Gidwani et al., 2010). The extraction seeds of *Psoralea corylifolia* were substituted with *P. sarmentosum* extracts. First, oil in water cream without *P. sarmentosum* extracts was formulated, followed by the formulation of cream with *P. sarmentosum* extract without paraben preservatives. Then, creams with *P. sarmentosum* extract with paraben and vitamin E preservatives were formulated.

The formulation process was started by dissolving the oily and aqueous phase in a separate beaker in water bath at 75°C. The oil phase consisted of emulsifiers and other components and the extraction of *P. sarmentosum* (phase A). The aqueous phase was then dissolved, and water was added up to 100% of the overall formulation. Then, both phase A and phase B were heated up to 75°C using a water bath. After completing the heating process, it was followed by stirring the added oily phase into the aqueous phase until the mixture cools down. Then, the label formulated cream was transferred into a plastic container.

Evaluation of *Piper sarmentosum* Cream

In the antimicrobial test, as explained earlier, each cream has to undergo a visual inspection and pH test using a digital pH meter and rheological studies (Dahlan et al., 2014). All the formulated creams were evaluated for their colour, homogeneity and consistency, and phase separation. In addition, the appearance and presence of any aggregates were tested for all the formulated creams. The formulated creams' pH was measured three times using electronic pH meter (Mettler Toledo AG, United States of America). Stability studies were conducted for all types of the formulated creams. The evaluated parameters under stability studies include physical appearance and the pH of the cream. The stability studies were carried out at three different conditions with different temperatures, which are 4°C, 27°C, and 37°C for one month (International Conference of Harmonization [ICH] guidelines) (Dixon, 1998). The stability studies were conducted right after the completion of the formulation process.

Evaluation of Antimicrobial Properties of *Piper sarmentosum* Cream

The antimicrobial screening was conducted for the formulated cream to determine the ability of the cream to inhibit bacterial growth and to compare the activity of the extract in pure form and dosage form. The studies were carried out as same as the screening procedure. In addition, different types of cream were tested for antimicrobial activity by using the disc diffusion method. In this study, gentamicin antibiotics cream was used as the positive control. Meanwhile the negative control is the formulated cream without *P. sarmentosum* extract. The sensitivity of *S. aureus* strains towards *P. sarmentosum* extract cream was calculated by measuring the diameter (mm) of the zone of inhibition. The reading was taken at the end of 24 hours after incubation in a 37°C incubator. The plate with zone of inhibition (no growth around the disc) was sensitive to bacteria strain. The tests were conducted in triplicate for each formulation to ensure reproducibility. Appendix 1 shows the general formula based on two phases, which are oily and aqueous phases with different types of the formulated *P. sarmentosum* cream.

Statistical Analysis

Statistical analysis was performed using the Microsoft Excel 2007 and Statistic Package for Social Sciences version 25 (SPSS 25.0, 2017). ANOVA tests were used to identify the significant difference between groups followed by a post-hoc Tukey's test. The statistical significance was accepted when the *p*-value is less than 0.05.

Appendix 1
The general formula and different types of the formulated Piper sarmentosum *cream*

	General formula	Cream A	Cream B	Cream C	Cream D
Components	% w/w	Weight (g)			
Oily phase					
Stearic acid	2.5	1.500	1.500	1.500	1.500
White beeswax	1.5	0.900	0.900	0.900	0.900
Stearyl alcohol	5.0	3.000	3.000	3.000	3.000
Cetyl alcohol	6.5	3.900	3.900	3.900	3.900
Mineral oil	5.0	3.000	3.000	3.000	3.000
Aqueous phase					
Propylene glycol	5.0	3.000	3.000	3.000	3.000
Triethanolamine	2.0	1.200	1.200	1.200	1.200
Methyl paraben	0.01	0.006		0.006	
Propyl paraben	0.04	0.024		0.024	
Piper sarmentosum extract	5.0	3.00	3.00	3.00	3.00
Vitamin E	0.05				0.03
Water	Up to 100%				
Total (g)		60.00	60.00	60.00	60.00

RESULTS AND DISCUSSION

Evaluation of Antibacterial Properties of Local *Piper sarmentosum* Leaves Methanolic Extract

Four different concentrations (25% w/v, 50% w/v, 75%w/v, and 100% w/v) of the local *P. sarmentosum* leaves methanolic extract were tested against *S. aureus* using the Kirby-Bauer disc diffusion method. The antimicrobial activity of *P. sarmentosum* leaves extract could be seen through the average zone of inhibition of bacterial growth around the disc exhibited by various concentrations of *P. sarmentosum* extract against *S. aureus* strain. Figure 1 shows the average zone of inhibition exhibited by various concentrations of *P. sarmentosum* extract against *S. aureus*.

Figure 1 shows the zone of inhibition exhibited by various concentrations of methanolic extract of local *P. sarmentosum* leaves against *S. aureus*. There were significantly (p<0.05) lower inhibition of all the concentrations of *P. sarmentosum* extract discs against *S. aureus* compared with gentamicin antibiotic disc. Even though an increasing trend of the zone of inhibition could be seen between all the extraction concentrations, they are no significant differences between the groups. The minimum antimicrobial activity was exhibited by 25% w/v, while the highest was exhibited by 100% w/v concentration of *P. sarmentosum* extract. Figure 1 also shows that the sterilized distilled water does not affect the antimicrobial activity against *S. aureus*.

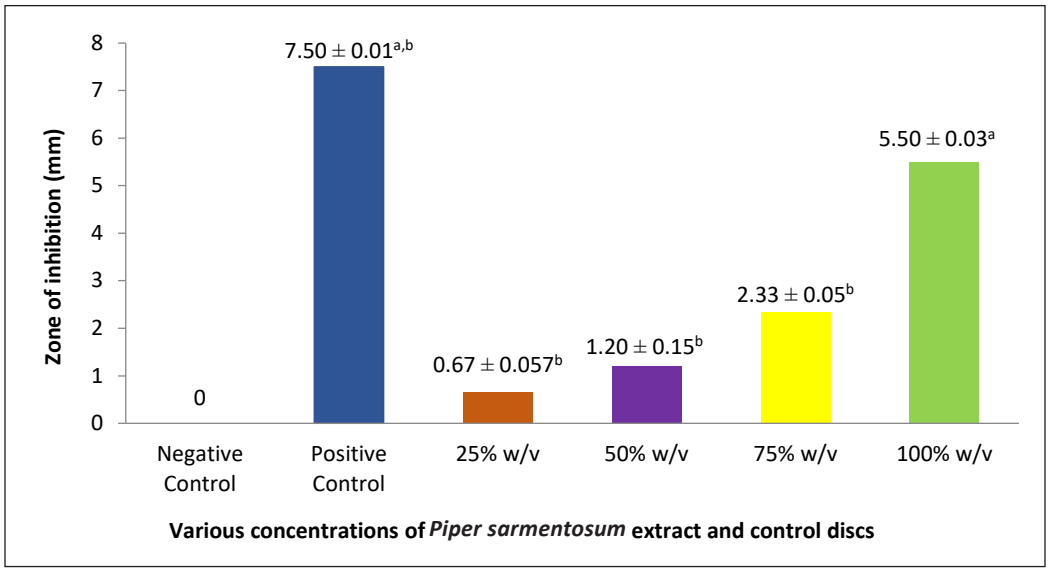

Figure 1. Values of zone of inhibition in sterilized distilled water discs (negative control), gentamicin antibiotic discs (positive control), 25% w/v, 50% w/v, 75% w/v, and 100% w/v concentrations of *Piper sarmentosum* extract discs against *Staphylococcus aureus* (n = 3). Each bar represents the mean ± SEM of the zone of inhibition

Note. [a]significant compared with the negative control while [b]significant compared with the positive control group (p<0.05)

Preparation, Formulation, and Evaluation of Local *Piper sarmentosum* Leaves Extract Cream

Based on the highest antimicrobial activity exhibited against *S. aureus*, the cream was formulated by incorporating 100% w/v of methanolic extract of *P. sarmentosum* leaves. These active ingredients were chosen due to higher inhibition against microbial growth that influenced the wound healing process. Different types of formulated cream were listed in Table 1.

Table 1
Type of creams and name for each cream

No.	Formulation	Name for each cream
1	Cream without *Piper sarmentosum* extract with parabens preservatives	Cream A
2	Cream *Piper sarmentosum* extract without parabens preservatives	Cream B
3	Cream *Piper sarmentosum* extract with parabens preservatives	Cream C
4	Cream *Piper sarmentosum* extract with vitamin E preservatives	Cream D

Evaluation of *Piper sarmentosum* Cream

Piper sarmentosum creams were evaluated based on the colour, homogeneity, consistency, and phase separation. Creams with *P. sarmentosum* extraction has dark green colour. All the formulated creams showed slightly different colour intensity and presented as homogenous semi-solid preparation shown in Table 2.

The pH formulations creams appear to become more alkaline across the concentration gradient, shown in Table 3.

Table 4 shows the condition of the creams after a month stored at different temperatures. There were no changes in colour, homogeneity, consistency, and phase separation of different creams with different temperatures (4°C, 27°C, and 37°C).

There was a slight change in the temperature of the cream within one month. Table 5 exhibits the pH's depreciation percentages of the formulated cream compared to the initial pH.

Evaluation of Antimicrobial Properties of *Piper sarmentosum* Creams

Different types of the formulated cream were tested using the antimicrobial activity against *S. aureus*. This study used the marketed dermatological semi-solid dosage of Diprogenta cream (gentamicin antibiotic cream) as a positive control. Therefore, it is important to compare the effectiveness of the formulated creams against a marketed product. Figure 2 shows the mean zones of inhibition exhibited by the different types of creams.

The methanolic extract was used in this study to obtain the pure extract of *P. sarmentosum* leaves. The methanolic extract method was chosen in this study because this method has been proven by Obeidat et al. (2012) to provide antimicrobial activity expression of the plant's extract. The efficacy of the plant extraction process was dependent on the solvent of extraction. Nayak et al. (2009) reported that both methanol and acetone were proven to be

Table 2
Physical appearance evaluation of Piper sarmentosum *cream*

Formulation	Colour	Homogeneity	Consistency	Phase separation
Cream A 	Whitish creamy	Good	Good	No
Cream B 	Light green	Good	Good	No
Cream C 	Green	Good	Good	No
Cream D 	Dark green	Good	Good	No

strong solvents in extracting inhibitory substances from medicinal plants. Lattanzio et al. (2006) also described that the phenolic and flavonoid compounds present in the extracts from various medicinal plants possess antimicrobial activity. Fernandez et al. (2012) presented their findings on *P. sarmentosum* leaves extraction with various phytochemicals except for saponins. Flavoinds and alkaloids are secondary metabolites classified as chemical classes that are generally activated with antimicrobial activities and soluble in polar solvents.

Table 3
The mean pH of the prepared cream formulations

Formulation	pH (Mean ± SEM)
Cream A	7.28 ± 0.01
Cream B	7.93 ± 0.01
Cream C	7.87 ± 0.01
Cream D	8.01 ± 0.07

Based on a preliminary investigation on antibacterial properties of methanolic extract of local *P. sarmentosum* leaves, 100% w/v of *P. sarmentosum* leaves extract was incorporated into a cream formulation. The cream is chosen as the semi-solid

Table 4
Condition of the various type formulated creams

Formulation	Temperature	Colour	Homogeneity	Consistency	Phase separation
Cream A	4°C	White creamy	Good	Good	No
	27°C	White creamy	Good	Good	No
	37°C	White creamy	Good	Good	No
Cream B	4°C	Light green	Good	Good	No
	27°C	Light green	Good	Good	No
	37°C	Light green	Good	Good	No
Cream C	4°C	Creamy dark green	Good	Good	No
	27°C	Creamy dark green	Good	Good	No
	37°C	Creamy dark green	Good	Good	No
Cream D	4°C	Creamy dark green	Good	Good	No
	27°C	Creamy dark green	Good	Good	No
	37°C	Creamy dark green	Good	Good	No

Table 5
Mean initial and final pH ± SEM and depreciation percentage after a month stored at different temperatures

Formulation	Initial pH	4°C	27°C	37°C
Cream A	7.28 ± 0.01	7.28 ± 0.00	7.29 ± 0.00	7.28 ± 0.01
		0.00%	0.14%	0.00%
Cream B	7.93 ± 0.01	7.87 ± 0.02	7.86 ± 0.01	7.92 ± 0.01
		0.76%	0.88%	0.13%
Cream C	7.87 ± 0.01	7.86 ± 0.01	7.87 ± 0.01	7.86 ± 0.05
		0.13%	0.00%	0.13%
Cream D	8.01 ± 0.01	7.95 ± 0.01	7.93 ± 0.01	7.94 ± 0.03
		0.75%	1.00%	0.90%

dosage in this study because it is easy to apply and well-absorbed by the skin. The formulation's vehicle is crucial in selecting the dosage as it will affect the delivery rate of the active pharmaceutical ingredients and the efficacy of the dosage. The active ingredient in creams is dissolving typically in an oil and water emulsion. The proportions of oil and water are approximately equal. Therefore, the creams are suitable for moistening the skin or exudative skin lesions (Chanwitheesuk et al., 2007).

The pH test of each formulated creams showed pH ranging from 7.0 to 8.0. In order to prevent or reduce skin irritation of topical and transdermal systems, it is important to maintain the pH of the topical formulations near the skin pH (Paudel et al., 2010). Besides that, it is also important to maintain the skin barrier function and defend against infections and diseases (Schmid-Wendtner & Korting, 2006). Cream with *P. sarmentosum* extract was evaluated for its stability at three different temperatures of

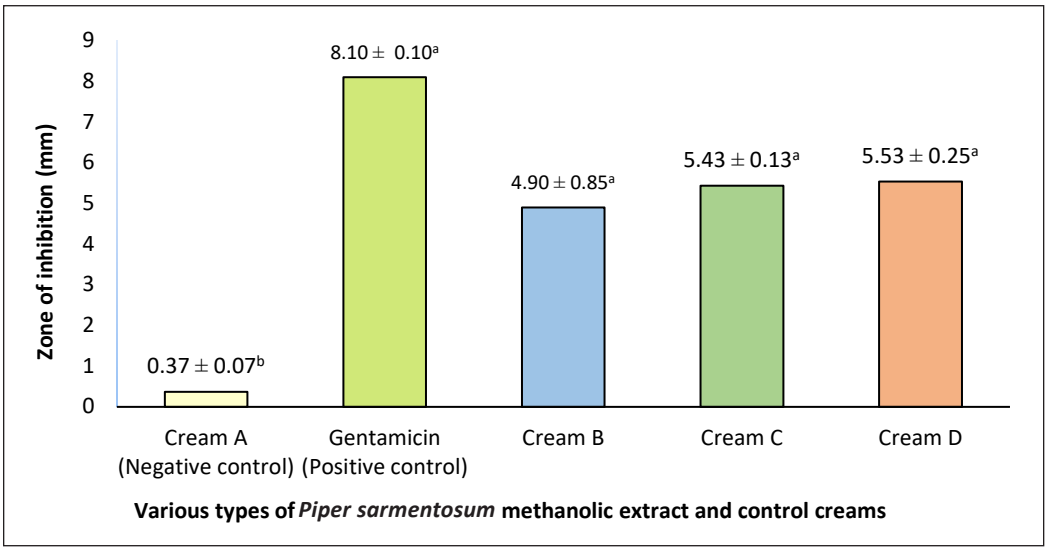

Figure 2. The zone of inhibition of the cream formulated with *Piper sarmentosum* methanolic extracts (Cream B: 4.90 ± 0.85; Cream C: 5.43 ± 0.13, and Cream D: 5.53 ± 0.25) were compared to the positive control cream (Gentamicin: 8.10 ± 0.10) and the negative control cream (Cream A: 0.37 ± 0.07), respectively. Each bar represents the mean ± SEM of the zone of inhibition

Note. [a]significant compared with the negative control while [b]significant compared with the positive control group (p<0.05)

4°C, 27°C, and 37°C for 30 days. Moreover, all the formulations are almost consistent in terms of their pH, colour, homogeneity, consistency, and phase separation even after a prolonged storage period at three different conditions (4°C, 27°C, and 37°C). All these tests are components of stability testing. The pH levels of the creams were in the range of 7.00 to 8.00 even after 30 days of stability testing. There were no colour changes while the creams' homogeneity and consistency remained stable (physical evaluation after formulation). The pH depreciation percentage of all creams showed less than 1.00% when compared to the initial pH. The results showed no indication of instability either in discolouration or a noticeable change in the formulated creams. The stability study passes the criteria stated in the United State Pharmacopeia (USP) for Pharmaceutical Compounding-Non-Sterile Preparation (Allen, 2012).

In this study, all the formulated creams containing *P. sarmentosum* extract showed growth inhibition towards *S. aureus*. In contrast, Cream A (without *P. sarmentosum* extract), which acts as a negative control, shows no zone inhibition towards *S. aureus*. Cream C (cream *P. sarmentosum* extract with paraben preservative) showed a higher average zone of inhibition when compared to Cream B (cream *P. sarmentosum* extract without preservative) and Cream D (cream *P. sarmentosum* extract with vitamin E as preservatives). Meanwhile, marketed gentamicin antibiotic cream, which acts as a positive control, showed the highest average zone of inhibition towards tested bacteria

strains. Based on a previous study conducted by Sahu et al. (2016), the preliminary investigation on the antimicrobial activity of *P. sarmentosum* showed that the methanolic extract of *P. sarmentosum* have the highest zone of inhibition towards Gram-positive bacteria (*S. aureus* and methicillin-resistant *Staphylococcus aureus* [MRSA]). Fernandez et al. (2012) indicated that the presence of flavonoids and alkaloids in the crude extract of *P. sarmentosum* is responsible for its antimicrobial activity.

The presence of secondary metabolites in the *P. sarmentosum* leaves extract may contribute to antimicrobial properties. Phenolics and flavonoids present in medicinal plants possess antimicrobial activity. The leaves of *P. sarmentosum* showed tannic acid, gallic acid, and quercetin in phenolics and flavonoids analysis. These compounds showed a mechanism of action towards the antibacterial activity of *P. sarmentosum* leaves. The mechanism of action, such as cytoplasmic membrane function, nucleic acids, and energy metabolism, acts by antimicrobial activities of naringin, quercetin, and rutin against human pathogenic microbes. A study by Syed et al. (2016) showed gallic acid and naringin in *P. sarmentosum* leaves in their antimicrobial activity.

In this study, a gentamicin antibiotic disc was used as the positive control, which exhibited higher antimicrobial activity against *S. aureus* strain with a larger zone of inhibition as compared to 100% w/v extract of *P. sarmentosum* leaves ($p<0.05$). Gentamicin antibiotic is an established antimicrobial agent used in clinical settings. Thus, it showed remarkable antimicrobial properties in this study. This conventional antibiotic was used as the positive control because of its higher inhibition of bacterial growth. Various established researches and lab works have been carried out to test the efficacy of the substances and drugs as antimicrobial agents. Gentamicin is an aminoglycoside antibiotic used to treat many types of bacterial infections, particularly those caused by Gram-negative organisms, including *Pseudomonas*, *Proteus*, and Gram-positive *Staphylococcus* (Ghashghaei & Emtiazi, 2013).

Fernandez et al. (2012) indicated that the presence of flavonoids and alkaloids in the crude extract of *P. sarmentosum* is responsible for its antimicrobial activity. The other three concentrations (25% w/v, 50% w/v, and 75% w/v) did not show a clear zone of inhibition of growth towards *S. aureus*. Based on a study conducted by Rain (2005), the antimicrobial activity of *P. sarmentosum* leaves showed a zone of inhibition against *S. aureus* (9 mm) with a concentration of 2000 µg/disc. This study did not mention the concentration of the extract before it was impregnated into the discs. Our findings were in line with a previous study by Fernandez et al. (2012), which stated that the antimicrobial activity of *P. sarmentosum* leaves would show better results at higher concentrations. Through our findings, concentrations less than 100% w/v may be insufficient to show antimicrobial activity. It is also important to note the possibility of contamination,

which may cause the inability of the three concentrations below 100% w/v (25% w/v, 50% w/v, and 75% w/v) to show clear zones of inhibition towards *S. aureus*.

The *P. sarmentosum* leaves could contribute to the antibacterial activity by the mechanical action of these compounds. Recent studies by Cushnie and Lamb (2005) found that antimicrobial activity of naringin, quercetin, and rutin against human pathogenic microbes showed mechanism actions of energy metabolisms, nucleic acid synthesis, and cytoplasmic membrane function. In addition, the leaf and fruit of *P. sarmentosum* were found to have gallic acid that contributes to the antibacterial activity of extraction against *S. aureus*. Paudel et al. (2010) reported that gallic acid exhibited potent activity against fungal and human pathogenic bacteria.

CONCLUSION

The present study found that *Piper sarmentosum* leaf methanolic extract is a potential antimicrobial agent as it showed antimicrobial properties against *Staphylococcus aureus* in the *in-vitro* antimicrobial assay. The cream formulation possesses anti-microbial properties showed a similar effect as in the antimicrobial screening of extraction. This study exhibited that the *P. sarmentosum* leaf could be safely used to treat in various diseases by increasing physical appearance, homogeneity, and consistency in stability studies. However, the previous study did not continue for further isolation of alkaloids. The same alkaloids are essential in exhibiting the significant antimicrobial properties of *P. sarmentosum* extract, which need further investigation in the future.

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REFERENCES

Ab Rahman, M. R., Abdul Razak, F., & Mohd Bakri, M. (2014). Evaluation of wound closure activity of *Nigella sativa*, *Melastoma malabathricum*, *Pluchea indica*, and *Piper sarmentosum* extracts on scratched monolayer of human gingival fibroblasts. *Evidence-Based Complementary and Alternative Medicine*, *2014*, 190342. https://doi.org/10.1155/2014/190342

Allen, L. V. (2012). Basics of compounding: Implementing United States pharmacopeia chapter <795> pharmaceutical compounding – Nonsterile preparations, Part 4. *International Journal Pharmacology Compound*, *16*(1), 64-68.

Chanwitheesuk, A., Teerawutgulrag, A., Kilburn, J. D., & Rakariyatham, N. (2007). Antimicrobial gallic acid from *Caesalpinia mimosoides* Lamk. *Food Chemistry*, *100*(3), 1044–1048. https://doi.org/10.1016/j.foodchem.2005.11.008

Chan, E. W. C., & Wong, S. K. (2014). Phytochemistry and pharmacology of three *Piper* species: An update. *International Journal of Pharmacognosy*, *1*(9), 534–544.

Cushnie, T. P. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, *26*(5), 343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002

Dahlan, A., Yip, W. S., & Pin, K. Y. (2014). Formulation and characterization of an anti-bacterial cream using hempedu bumi (*Andrographis paniculata*). *International*

Journal of Pharmaceutical Sciences and Research, *5*(9), 3633–3640.

Das, T., Debnath, J., Nath, B., & Dash, S. (2014). Formulation and evaluation of an herbal cream for wound healing activity. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(2), 693–697.

Dixon, J. R.(1998). The international conference on harmonization good clinical practice guideline. *Quality Assurance Journal*, *6*(2), 65-74. https://doi.org/10.1080/105294199277860

Fernandez, L., Daruliza, K., Sudhakaran, S., & Jegathambigai, R. (2012). Antimicrobial activity of crude extract of *Piper sarmentosum* against methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Vibrio cholera* and *Streptococcus pneumoniae*. *European Review for Medical and Pharmacological Sciences*, *16*(3), 105-111.

Ghashghaei, S., & Emtiazi, G. (2013). Increasing the antibacterial activity of gentamicin in combination with extracted polyphosphate from *Bacillus megaterium*. *Journal of Applied Microbiology*, *114*(5), 1264–1272. https://doi.org/10.1111/jam.12142

Gidwani, B., Alaspure, R., Duragkar, N., Singh, V., Rao, S. P., Shukla, S., & Pawar, S. (2010). Evaluation of a novel herbal formulation in the treatment of eczema with *Psoralea corylifolia*. *Iranian Journa of Dermatology*, *13*(4), 122-127.

Karthigeyan, K., Sumathi, R., Jayanthi, J., Diwakar, P. G, & Lakra, G. S. (2004). *Piper sarmentosum* Roxb. — An addition to the flora of Andaman Islands. *Current Science*, *87*(2), 141–142.

Lattanzio, V., Lattanzio, V. M. T., & Cardinali, A. (2006). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. *Phytochemistry: Advances in Research*, *661*(2), 23–67.

Nayak, B. S., Sandiford, S., & Maxwell, A. (2009). Evaluation of the wound-healing activity of ethanolic extract of *Morinda citrifolia* L. leaf. *Evidence-Based Complementary and Alternative Medicine*, *6*, 608202. https://doi.org/10.1093%2Fecam%2Fnem127

Obeidat, M., Shatnawi, M., Al-alawi, M., Al-Zu`bi, E., Al-Dmoor, H., Al-Qudah, M., El-Qudah, J., & Otri, I. (2012). Antimicrobial activity of crude extracts of some plant leaves. *Research Journal of Microbiology*, *7*(1), 59-67. https://doi.org/10.3923/jm.2012.59.67

Paudel, K. S., Milewski, M., Swadley, C. L., Brogden, N. K., Ghosh, P., & Stinchcomb, A. L. (2010). Challenges and opportunities in dermal/transdermal delivery. *Therapeutic Delivery*, *1*(1), 109–131. https://doi.org/10.4155%2Ftde.10.16

Rahman, N., Noor, K., Hlaing, K., Suhaimi K., Kutty M., & Sinor, M. (2011). *Piper sarmentosum* influences the oxidative stress involved in experimental diabetic rats. *The Internet Journal of Herbal and Plant Medicine*, *2*(1), 1-8.

Rain, N. (2005). *In vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Tropical Biomedicine*, *22*(2), 165–170.

Sahu, T., Patel, T., Sahu, S., & Gidwani, B. (2016). Skin cream as topical drug delivery system: A review. *Journal of Pharmaceutical and Biological Sciences*, *4*(5), 149-154.

Schmid-Wendtner, M.-H., & Korting, H. C. (2006). The pH of the skin surface and its impact on the barrier function. *Skin Pharmacology and Physiology*, *19*(6), 296–302. https://doi.org/10.1159/000094670

Syed, A. R. F., Sijam, K., Omar, D., Zafri, M., & Wahab, A. (2016). Identification of phenolic compounds and evaluation of antibacterial properties of *Piper sarmentosum* Roxb. against

rice pathogenic bacteria. *Malaysian Journal of Microbiology*, *12*(6), 475–484.

Wong, P. Y. Y., & Kitts, D. D. (2006). Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) extracts. *Food Chemistry*, *97*(3), 505–515. https://doi.org/10.1016/j.foodchem.2005.05.031