Formulation and evaluation of antibacterial gel incorporated with *Stachytarpheta jamaicensis* crude ethanolic leaf extract against *Staphylococcus aureus*

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Abstract

Stachytarpheta jamaicensis (sentimento) is utilized by locals from Maasin, Iloilo as an open wound poultice for its antibacterial properties. This study aimed to formulate and evaluate an antibacterial gel incorporated with *S. jamaicensis* leaf extract against *Staphylococcus aureus*. The gel's antibacterial activity was compared to that of a negative control (gel base) and a positive control (hand sanitizer) using the agar well diffusion method. The gel was found to be stable in all physicochemical parameters evaluated (pH, viscosity, spreadability, centrifugation, and mechanical vibration) except for viscosity. The hand sanitizer exhibited the highest zone of inhibition (6.17 ± 0.29 mm) followed by the gel (4.67 ± 0.29 mm). Although not comparable to the positive control, the gel exhibited antibacterial activity. Thus, the drug delivery system effectively delivered the extract's active ingredient. However, it can not be used as a hand sanitizer with its current extract concentration.

Introduction. - Plant species traditionally used as an alternative medicine to address various illnesses and diseases have been widely investigated through phytochemical screenings. Those proven to contain phytochemical constituents whose functions coincide with their intended use are then subjected to antibacterial and anti-inflammatory tests, among others [1]. Once their efficacy has been tested in the laboratories, they can now be incorporated into medicinal preparations such as syrups, tablets, capsules, and topical formulations for mass production and commercial sale [1].

One such plant is the *Stachytarpheta jamaicensis*, locally known as kandikandilaan or sentimento, a flowering plant that belongs to the family of *Verbenaceae*. This plant can be found thriving in the tropical forests of the Americas, and the subtropical forests of Asia and Africa. It has numerous medicinal benefits in infectious and chronic health systems [2]. In the Philippines, there is an abundance of *S. jamaicensis* where locals use its leaves as a poultice in treating open wounds, for it is known to have antibacterial properties [3,4].

Since Abadilla et al. [5] have already developed an ointment using *S. jamaicensis* leaf extracts, this study formulated and evaluated a gel. Gel formulations are generally preferred over other topical semisolid preparations because they stay longer on the skin, have a higher viscosity, are more bioadhesive, and cause less irritation [6]. In addition, gel formulations are moisturizing, water-dependent, have a smooth application, and release active ingredients more effectively [6,7].

According to Taylor and Unakal [8], *Staphylococcus aureus* is a common bacteria usually found in the skin of most healthy humans, since *S. aureus* is one of the standard components of the human's environment and normal flora. According to the study of Jacopin et al. [9], a significant number of community-acquired and hospital-acquired diseases are triggered by commensal bacteria such as *Escherichia coli*, *Staphylococcus aureus*, or *Streptococcus pneumoniae* which can also be opportunistic pathogens.

With this, an antibacterial gel incorporated with *S. jamaicensis* crude ethanolic leaf extract was formulated and evaluated. If proven effective, the antibacterial gel may be commercialized to produce a sanitizer affordable for the masses and address the necessity of discovering new drug delivery systems for herbal medicine.

Thus, the study aimed to formulate and evaluate an antibacterial gel incorporated with *Stachytarpheta jamaicensis* (sentimento) crude ethanolic leaf extract against *Staphylococcus aureus.* The specific objectives of this study were to:

(i) Formulate an antibacterial gel incorporated with *S. jamaicensis* crude ethanolic leaf extract against *S. aureus*;

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(ii) Evaluate and compare the results of the physicochemical tests of the formulated gel before and after accelerated stability testing;

(iii) Evaluate the antibacterial activity of the formulated gel against *S. aureus* by measuring its zone of inhibition using the agar well diffusion method; and

(iv) Determine if there is a significant difference between the antibacterial activity of the formulated gel with the gel base without the extract as the negative control and a commercially available hand sanitizer as the positive control.

Methods. - The methodology is divided into four (4) parts: extraction, gel formulation, physicochemical evaluation, and antibacterial evaluation. The formulated gel's physicochemical properties and antibacterial activity against *Staphylococcus aureus* alongside the gel base (negative control) and a commercially available sanitizer (positive control) were evaluated.

Collection and Identification of Samples. A random sampling method was employed in the leaf collection in a lot located at Brgy. Daja, Maasin, Iloilo, at 10°53'45.3"N, 122°24'43.5"E on November 27, 2020. A 36-sq. meter (6 m by 6 m) main plot was established that was further divided into 36 1-square meter subplots with dimensions of 1 m by 1 m. Each subplot was labeled with numbers from 1 to 36, and 9 subplots were randomly chosen as sampling sites. Sentimento plants with green leaves and bright even colors were uprooted and then verified as *S. jamaicensis* by the Department of Agriculture in Sta. Barbara, Iloilo.

Extract Acquisition. The collected *S. jamaicensis* leaves were washed under running tap water and rinsed with distilled water. The leaves were oven-dried for 48 hours [4], pulverized using a blender, and sifted with sieves mesh numbers 5 and 10 [10]. Fifty (50) grams of the leaf powder was mixed with 500 mL of 70% ethanol [11,12] and was sonicated using an ultrasonic cleaner (42 kHz, 135 W; Branson Ultrasonic Corporation, USA) for 60 minutes. The mixture was filtered twice [4] using a vacuum pump and was subjected to a rotary evaporator (Biobase IKA RV8-S099) at 40 °C with 150 revolutions per minute (rpm) for 10 hours [13]. The aqueous extract with a concentration of 100 mg/mL was then used for the antibacterial gel formulation for better solubility with the gel base.

Antibacterial Gel Formulation. To formulate the gel, propylene glycol, an antifreeze and anti-melting preservative, was added to enhance its stability [14]. Glycerin was also added to help the gel stay on the skin for a prolonged period [15]. Five (5) grams of carbomer 934 (1%), 35 mL of propylene glycol (7%), and 35 mL of glycerin (7%) were dispersed using a hot plate with a magnetic stirrer in 410 mL of distilled water. The mixture was allowed to rest for 60 minutes for the carbomer to hydrate and swell [16].

The initial mixture was neutralized with 2 mL of triethanolamine to attain the desired pH of 8.0 [16]. Forty (40) milliliters of the formulation was then set aside in a beaker at room temperature until use, while 460 mL was incorporated with the leaf extract. Five (5) mL of the leaf extract [4] was diluted with 5 mL of polysorbate 20, which also improves the gel's stability [17]. The leaf extract and polysorbate 20 mixture was then added to the carbomer mixture. The final concentration of the extract in the carbomer mixture was 106 mg/mL.

Physicochemical Evaluation of Gel. The tests suggested by the Food and Drug Administration (FDA), the United States Pharmacopeia (USP), and the Brazilian Health Surveillance Agency (ANVISA) were conducted with the formulated antibacterial gel [18].

pH. The pH of the formulated antibacterial gel was measured using a digital pH meter. The electrode was dipped into the antibacterial gel and left for 10 minutes at room temperature before pH reading [19,20]. The measurement was carried out in triplicates and the average of the three readings was recorded to ensure accuracy.

Viscosity. The viscosity of the antibacterial gel was determined using a viscometer at 25 °C with a spindle speed of 12 rpm [21]. The measurement was carried out in triplicates and the average of the three readings was recorded to ensure accuracy.

Spreadability. The parallel-plate method was used to measure the spreadability of the formulated gel [22]. Spreadability was calculated using the formula:

$$S = \frac{M \cdot L}{T}$$

Where:
S = Spreadability (g · cm/s)
M = Weight (g) tied to the upper slide
L = Length (cm) moved by the glass slide
T = Time (s) it took to separate the upper and lower slides

The measurement was carried out in triplicates and the average of the three readings was recorded to ensure accuracy.

Centrifugation Test. Five (5) grams of the antibacterial gel were subjected to a centrifuge at a cycle of 3000 rpm for 30 minutes at room temperature [23] to observe the occurrence phase separation.

Mechanical Vibration Test. Five (5) grams of the antibacterial gel were transferred to a test tube and subjected to a vortex shaker for 10 seconds to observe the occurrence of phase separation [24].

Stability Test. The formulated antibacterial gel underwent a hot and cold temperature cycling adopted from Krongrawa et al. [22]. It was placed alternately at 4 ± 1 °C and 45 ± 1 °C for 24 hours each for 6 cycles. The pH, viscosity, and spreadability were measured, and centrifugation and vibration testing were conducted in the post-stability test antibacterial gel.

Antibacterial Evaluation of Gel. Samples obtained from the *S. aureus* Tryptic Soy Broth (TSB) subculture from the Philippine Biobank Facility in University of the Philippines Los Baños were inoculated to the surface of the Mannitol Salt Agar (MSA) using the quadrant streaking method. The plate was then incubated for 24 hours at 35 °C. Large, bright yellow, and opaque isolated colonies of *S. aureus* were inoculated to 15 mL of TSB and incubated [23]. Tryptic Soy Broth (TSB) was then added to reduce and achieve the turbidity of 0.5 McFarland standard [23].

Using a sterile blue micropipette tip, three Mueller Hinton Agar (MHA) plates were each punctured to create three uniformly sized wells. Pure colonies of *S. aureus* from the TSB were then inoculated and swabbed to the three MHA plates [23]. Treatments were then dispensed using a micropipette into the wells according to their labels with a uniform amount of 1 mL. The plates were then incubated for 24 hours at 37 °C [23]. The antibacterial activity of the formulated gel, gel base without leaf extract (negative control), and a commercially available hand sanitizer (positive control) were evaluated by measuring the zone of inhibition [23].

Data Analysis. For calculations, p-values were calculated using R (v4.04, GNU GPL v2). Paired t-test was then performed to determine if there is a significant difference between the mean pH, viscosity, and spreadability of the formulated gel obtained before and after accelerated stability testing. One-way ANOVA test with the statistical significance set at 5% was then used to determine if there is a significant difference between the antibacterial activity of the gel base, formulated gel, and commercially available hand sanitizer against *S. aureus* based on their generated zones of inhibition, and post-hoc analysis was evaluated using Tukey HSD test.

Safety Procedure. Proper protective equipment was worn throughout the conduct of the data gathering to avoid sample and bacterial contamination. Working areas were disinfected with 70% ethanol. All chemical wastes were handled according to their respective safety data sheet, placed inside empty water bottles, and were disposed of by the personnel of the school. Biological materials such as cultures and contaminated glassware were autoclaved before disposal.

Results and Discussion. - The data from the agar well diffusion assay were statistically analyzed using one-way ANOVA to determine if there is a significant difference between the zones of inhibition generated. Paired t-test was used to determine if there is a significant difference among the pH, viscosity, and spreadability values acquired.

Physicochemical Evaluation. For the physicochemical evaluation, three parameters, namely the pH, viscosity, and spreadability of the gel, were assessed. Each parameter was then statistically analyzed through paired t-tests set at 0.05 alpha with n=3 trials. With this, the p-values of the pH, viscosity, and spreadability are 0.11, 5.17 x 10^{-2}, and 0.23, respectively. The paired t-test showed that there is no significant difference in the formulated gel before and after accelerated stability testing (AST) in terms of pH and spreadability, indicating stability and good quality of the formulated gel in these parameters. Meanwhile, a significant difference in the viscosity of the formulated gel was established before and after stability testing, indicating that the formulated gel is not of good quality in terms of this parameter. No phase separation was observed in the formulated gel following the centrifugation and mechanical vibration tests before and after stability testing, indicating stability and retained homogeneity of the formulated gel.

The formulated gel is slightly runny, immediately dries after spreading on the skin, has a chartreuse color, and has a smooth and somewhat heavy feel. The chartreuse color of the gel is due to the dark green color of the extract used.

Stability studies on pharmaceutical gels are done to determine if a formulation stored in a specific container is capable of retaining its physical, chemical, and microbiological properties, as well as evaluate the effect of the environmental factors on the formulation [24].

Topical treatments usually have an acidic pH, since an acidic environment improves the release of oxygen in wounded or affected tissues, hence aiding in the healing of the wounds [25]. The formulated gel had an acidic pH due to its main component being carbomer, which is an acid-based polymer [26]. Although the pH of the formulated gel is slightly lower than that of the skin which is 4.1 to 5.8 [27], it was not acidic enough to cause skin irritation, therefore safe to use [28].

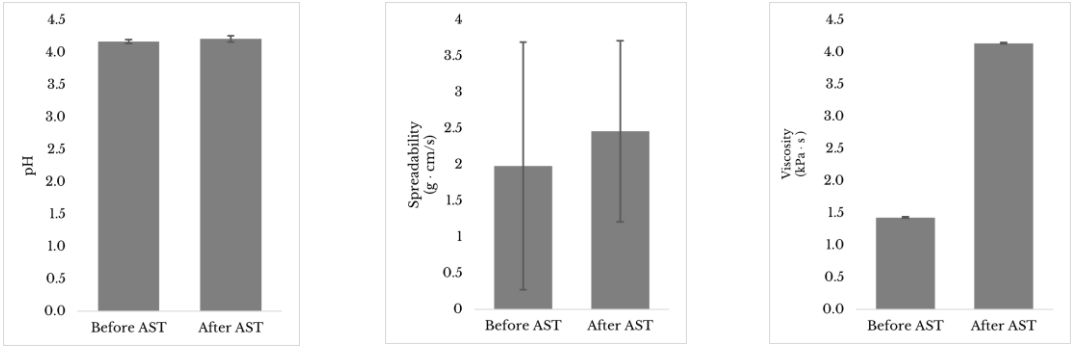


Figure 1. Results of the physicochemical evaluation. Data are expressed in terms of mean ± standard deviation.

Viscosity is important in evaluating a gel formulation because it affects the spreadability and release of the active ingredient. Spreadability aids the ability of the gel to be uniformly applied to the skin [16]. The spreadability of the formulated gel before stability testing was low therefore not ideal [29,30]. The low pH attained by the formulated gel caused a decrease in its viscosity [30]. The viscosity of a gel is highest at its gelling point [32], which in the case of the gelling agent used, carbomer, is 8 [16]. With this, it could be inferred that the low pH of the gel affected its viscosity. The decrease in viscosity then caused an increase in the gel's spreadability [16]. The formulated gel is therefore favorable for wound healing in terms of its pH [28]. However, the formulated gel still requires a lower viscosity and, consequently, a higher spreadability in order to improve in these parameters [16,29,30].

Antibacterial Evaluation. The One-way ANOVA test conducted on the results of the agar well diffusion assay showed that there is a significant difference between the formulated gel and the positive control. It is significantly different in favor of the positive control (refer to Figure 2).

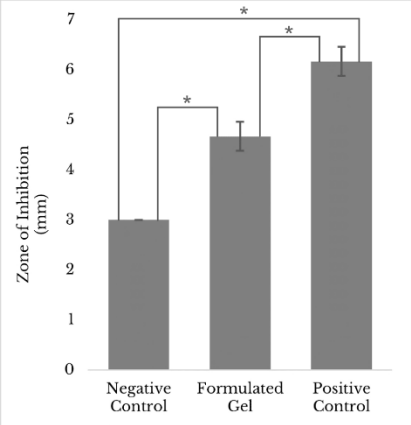


Figure 2. Results of the antibacterial evaluation. Data are expressed in terms of mean ± standard deviation.

A higher concentration of the extract may have achieved the same effectivity as the commercially available hand sanitizer which corresponds to the findings of Ruma and Zipagang [33], which states that higher concentration extract results in better effectivity in bacterial growth inhibition.

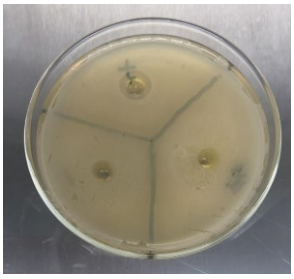
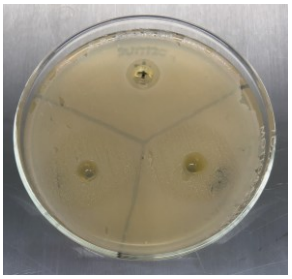
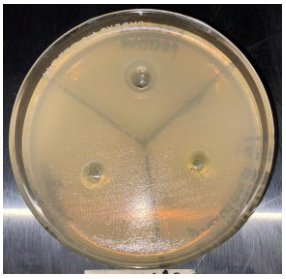
Although the formulated gel is effective as an antibacterial gel, it still has areas of improvement and the findings of this study may be different to future research. Therefore it is prescribed that more studies be conducted that would improve the formulated gel's antibacterial activity and physicochemical properties.

This indicates that the positive control is a more effective antibacterial formulation in comparison to the formulated gel. The commercially available hand sanitizer used for the positive control is alcohol-based therefore is more effective compared to the formulated gel which is water-based. This difference in formulation provides the commercially available hand sanitizer with more efficacy attributed to its alcohol content. The formulated gel is not as effective as the commercially available hand sanitizer because of the concentration of the crude ethanolic leaf extract of *S. jamaicensis*, which was based on the minimum inhibitory concentration (MIC) test of Idu et al. conducted in 2007 [4].

Limitations. The data gathering was conducted for two months. Within those two months, the period between the acquisition of the extract and gel formulation was a month. Hence, the quality of the extract may also have been compromised, particularly the antibacterial activity. Despite the setback, the findings of this research may help future studies in improving the formulation and discover the most effective concentration for antibacterial inhibition. Furthermore, the commercially available hand sanitizer used as a positive control has a different formulation as to that of the formulated gel, which may have affected its diffusion to the agar in the antibacterial evaluation.

Conclusion. - The formulated gel with *S. jamaicensis* crude ethanolic leaf extract has antibacterial activity against *S. aureus*. However, due to it having a significantly smaller zone of inhibition, it is not comparable to that of the commercially available sanitizer.

Table 1. Zones of inhibition generated on the MHA plates after incubation.

	Replicate No. 1	Replicate No. 2	Replicate No. 3
MHA Plates			

Recommendation. - To further improve the results of the study, it is recommended to perform the MIC test before proceeding with gel formulation since the literature where the MIC was based may be outdated. It is also recommended to use a higher concentration of *S. jamaicensis* extract or incorporate the extract of another plant that exhibits antibacterial properties to investigate synergistic effects to attain a higher antibacterial activity. Isolation of known phytochemicals associated with the antibacterial activity of *S. jamaicensis*, such as tannins and saponins [33], can be done to further improve its bactericidal effect. With this, it is recommended that the positive control would be the gel base incorporated with a known antibiotic with the same concentration as the extract. Furthermore, it is recommended to perform a microbial load count on the gel to determine its degree of microbial contamination. It is also recommended to use a paddle attachment in formulating the gel base in addition to the overhead stirrer to thoroughly mix the gel and reduce the formation of bubbles. Moreover, it is recommended to measure the physicochemical properties in regular intervals during AST to be able to plot a trend line that monitors the state of the gel throughout the stability testing. Lastly, it is recommended to perform the accelerated stability testing for a longer period to identify the limit of the gel and to determine its expiration date.

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References

[1] Bhalodia N, Shukla V. 2011. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: An ethnomedicinal plant. J Adv Pharm Technol Res. 2(2): 104. doi: 10.4103/2231-4040.82956.

[2] Naik ND, Suresh B, Priyanka MP, Geethanjali B, Yamini K, Malothu R. 2012. Formulation and evaluation of herbal ointment consisting *Sida spinosa* leaves extracts. Pharm Biol. 2(2): 40–42.

[3] Ololade ZS, Ogunmola OO, Kuyooro SE, Abiona OO. 2017. *Stachytarpheta jamaicensis* leaf extract: chemical composition, antioxidant, anti-arthritic, anti-inflammatory and bactericidal potentials. Int J Sci. 6(4): 119–125.

[4] Idu M, Omogbai EKI, Aghimien GE, Amaechina F, Timothy O, Omonigho SE. 2007. Preliminary phytochemistry, antibacterial properties and acute toxicity of *Stachytarpheta jamaicensis* (L.) Vahl. Leaves. Trends Med Res. 2: 193–198. doi: 10.1155/2016/7842340.

[5] Abadilla SC, Bonete TM, Carinugan RJ. 2009. Wound healing and antibacterial property of vervain ointment from *Stachytarpheta jamaicensis* (L) Vahl (*Verbanaceae*). University of the Philippines Manila. Available from: https://bit.ly/3bk5Ic3.

[6] Loganathan VM, Animaran S, Jaswanth A, Sulaiman A, Sivaprasadha RMV, Senthil KB, Rajaseskaran A. 2001. The effects of polymers and permeation enhancers on releases of flurbiprofen from gel formulations. Indian J Pharm Sci. 63(3): 200–204.

[7] Chang RK, Raw A, Lionberger R, Yu L. 2012. Generic Development of Topical Dermatologic Products: Formulation Development, Process Development, and Testing of Topical Dermatologic Products. AAPS J. 15(1): 41–52. doi: 10.1208/s12248-012-9411-0.

[8] Taylor TA, Unakal CG. 2020. *Staphylococcus aureus*. StatPearls Publishing LLC.

[9] Jacopin E, Lehtinen S, Débarre F, Blanquart, F. 2020. Factors favouring the evolution of multidrug resistance in bacteria. J R Soc Interface. 17(168): 20200105. doi: 10.1098/rsif.2020.0105.

[10] Deli M, Ndjantou EB, Ngatchic-Metsagang JT, Petit J, Njintang-Yanou N, Scher J. 2019. Successive grinding and sieving as a new tool to fractionate polyphenols and antioxidants of plants powders: Application to *Boscia senegalensis* seeds, *Dichrostachys glomerata* fruits, and *Hibiscus sabdariffa* calyx powders. Food Sci Nutr. 7(5): 1795–1806. doi: 10.1002/fsn3.1022.

[11] Rozianoor M, Nadia K, Nurdiana S. 2014. *Stachytarpheta jamaicensis* ethanolic leaf extract as wound healer on alloxan-induced diabetic sprague dawley rats. Indian J Biotechnol. 9: 460–466. doi: 10.1155/2016/7842340.

[12] Balamurugan V, Sheerin F, Velurajan S. 2019. A guide to phytochemical analysis. Int J Adv Res Innov Ideas Educ. 5: 236–245.

[13] Yen MP, Tang G. 2019. JoVE Science Education [Internet]. Massachusetts: J Vis Exp. Available from: https://bit.ly/2IAOFqr.

[14] Aiyalu R, Govindarjan A, Ramasamy A. 2016. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. Braz J Pharm Sci. 52(3): 493–507.

[15] Dashnau JL, Nucci NV, Sharp KA, Vanderkooi JM. 2006. Hydrogen Bonding and the Cryoprotective Properties of Glycerol/Water Mixtures. J Phys Chem B. 110(27): 13670.

[16] Dantas MGB, Reis SAGB, Damasceno CMD, Rolim LA, Rolim-Neto PJ, Carvalho FO, Almeida JRGS. 2016. Development and evaluation of stability of a gel formulation containing the Monoterpene Borneol. Sci World J. 2016: 1–4.

[17] Ross ARS. 2005. Application of surface activity in therapeutics. Surface Activity in Drug Action. 233–293.

[18] Food and Drug Administration (FDA). 2014. Minimum number of sample units required for each test analysis. Alabang, Muntinlupa City, Philippines.

[19] Queiroz MBR., Marcelino NB, Ribeiro MV, Espindola LS, Cunha F, Silva MV. 2009. Development of gel with *Matricaria recutita* L. extract for topic application and evaluation of physical-chemical stability and toxicity. Lat Am J Pharm. 28(4): 574–579.

[20] Viswanad V, Jayakar B, Thomas L, Aleykutty N, Zacharia S. 2012. Development and evaluation of antimicrobial herbal formulations containing the methanolic extract of *Samadera indica* for skin diseases. J Adv Pharm Technol. 3(2): 106. doi: 10.4103/2231-4040.97285.

[21] Nayak SH, Nakhat PD, Yeole PG. 2005. Development and evaluation of cosmeceutical hair styling gels of ketoconazole. Indian J Pharm Sci. 52: 231–33.

[22] Krongrawa W, Limmatvapirat S, Pongnimitprasert N, Meetam P, Limmatvapirat C. 2018. Formulation and evaluation of gels containing coconut kernel extract for topical application. Asian J Pharm Sci. doi: 10.1016/j.ajps.2018.01.005.

[23] Tendencia EA. 2004. Disk diffusion method. In Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment (pp. 13–29). Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

[24] Bajaj S, Singla D, Sakhuja N. 2012. Stability testing of pharmaceutical products. J Appl Pharm Sci. 2:. 129–138. doi: 10.7324/JAPS.2012.2322.

[25] Hunt TK, Beckert S. 2005. Therapeutical and practical aspects of oxygen in wound healing. In: Lee B (ed) The Wound Management Manual. McGraw-Hill Medical, New York.

[26] Caluya ED. 2017. Wound healing potential of the crude leaf extract of *Stachytarpheta jamaicensis* Linn. Vahl (Kandikandilaan) on induced wounds in rats. 5(1). 375-381.

[27] Proksch E. 2018. pH in nature, humans and skin. J Dermatol. 45(9): 1044–1052. doi: 10.1111/1346-8138.14489.

[28] Lambers H, Piessens S, Bloem A, Pronk H, Finkel P. 2006. Natural skin surface pH is on average below 5, which is beneficial for its resident flora. Int J Cosmet Sci. 28(5): 359–370.

[29] Singh VK, Singh PK, Sharma PK, Srivastava PK, Mishra A. 2013. Formulation and evaluation of topical gel of acelofenac containing piparine. Indo Am J Pharm. 2013: 3(7).

[30] Shukr MA, Metwally GF. 2013. Evaluation of Topical Gel Bases Formulated with Various Essential Oils for Antibacterial Activity against Methicillin Resistant *Staphylococcus aureus*. Trop J Pharm Res. 12(6): 877–884.

[31] Mahalingam R, Li X, Jasti BR. 2008. Semisolid dosages: ointments, creams, and gels. Pharmaceutical Manufacturing Handbook, 267–312.

[32] Maslii Y, Ruban O, Kasparaviciene G, Kalveniene Z, Materiienko A, Ivanauskas L, Mazurkeviciute A, Kopustinskiene DM, Bernatoniene J. 2020. The Influence of pH Values on the Rheological, Textural and Release Properties of Carbomer Polacril® 40P-Based Dental Gel Formulation with Plant-Derived and Synthetic Active Components. Molecules (Basel, Switzerland). 25(21): 5018. doi: 10.3390/molecules25215018.

[33] Ruma OC, Zipagang TB. 2015. Determination of secondary metabolites and antibacterial property of extract from the leaves of *Stachytarpheta jamaicensis* (L.) Vahl. J Med Plants Stud. 3(4): 79–81.